2017

ISCE/APACE

The joint meeting of the 33rd annual meeting
of the ISCE and the 9th meeting of the APACE

Kyoto, Japan
23-27 August, 2017

Contents

Abstracts

*Note: Some abstracts are adjusted to the format for editorial reasons.

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SYNTECH

Venue

Wagenkan, Fukakusa-campus, Ryukoku University
67 Tsukamoto-cho,Fukakusa,Fushimi-ku,Kyoto 612-8577

Location & Access
http://www.ryukoku.ac.jp/english2/about/location.html

Social Program

- Banquet -

18:30- August 26, Saturday

Genji Ballroom, Hotel Granvia Kyoto 3F

JR Kyoto Station, Karasuma Chuo-guchi, Shiokoji-sagaru,
Karasuma-dori, Shimogyo-ku, Kyoto 600-8216
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Meinwald Symposium

Sponsored by ChemTica International, Bedoukian Research Inc., and Fuji Flavor Co. Ltd.

PROGRAM

13:30-13:35	**Walter S. Leal**, University of California-Davis ***Happy birthday, Jerry!***
13:35-13:40	*Congratulations, Jerry! A short video message*
13:40-14:00	**Jonathan Gershenzon**, Max Planck Institute for Chemical Ecology ***For love of ecological chemistry***
14:00-14:05	*Recognizing Jerry for all his contribution to science*
14:05-14:25	**Kenji Mori**, University of Tokyo ***Sharing my joy of making molecules with others: Synthetic chemistry of juvenile hormones***
14:25-14:30	*e-Celebrating Jerry Meinwald's 90th Birthday*
14:30-14:50	**Sean Brady**, The Rockefeller University ***Natural products from uncultured bacteria***
14:50-14:55	*Greetings, Jerry! Happy Birthday from…*
14:55-15:15	**Coby Schal**, North Carolina State University ***Adaptive gustatory changes confer behavioral resistance to toxic baits and alter mate choice***
15:15-15:20	*Hello, Jerry! I wish you a very, very happy birthday!*
15:20-15:35	BREAK
15:35-15:55	**Walter S. Leal**, University of California-Davis **The gospel of chemical ecology, according to Jerry**
15:55-16:15	**Glenn D. Prestwich,** The University of Utah

	Jerry made it possible: Fifty years of cross-disciplinary experiences
16:15-16:35	**Martin Burke**, University of Illinois at Urbana-Champaign ***Making molecular prosthetics***
16:35-16:40	Jerrold Meinwald, Cornell University ***Closing remarks***
16:40-17:15	Concert

Program overview

August 23 (Wed.)

12:00-18:00	Registration	
10:30-12:00	APACE Executive meeting	Room: TBA
12:00-13:30	ISCE Executive meeting	Room: TBA
13:30-17:15	Symposium to Celebrate the 90th Birthday of Prof. Jerrold Meinwald	Room: B201
17:30-19:30	Welcome Reception	Room: 1F Lounge

August 24 (Thu.)

TBA	Registration	
9:00-9:20	Opening Ceremony	Room: B201
9:20-10:10	*ISCE Silver Medal Award* Bark beetle pheromone and insect hydrocarbon production **Gary J. Blomquist**	Room: B201
10:10-10:30	Coffee Break	
10:30-11:20	*APACE Lifetime Achievement Award* Collaborative chemical ecology of Asian pest fruit flies - Biological and socio-economic perspectives **Keng Hong Tan** Collaborative chemical ecology of Asian pest fruit flies - Semiochemical perspectives **Ritsuo Nishida**	Room: B201
11:20-12:10	*ISCE Silverstein-Simeone Lecture Award* Perfume as dark matter: Unveiling the explanatory power of floral scent **Robert A. Raguso**	Room: B201
12:10-13:20	Lunch (Poster viewing)	
13:20-13:50	*ISCE Early Career Award* How plant viruses use chemistry to manipulate hosts and vectors **Kerry Mauck**	Room: B201

Session	Session 1 Plant-animal interactions	Session 2 Characterization of semiochemicals: analysis and synthesis	Session 3 Microbial chemical ecology
Room	B101	B102	B103
Organizer	**Gary W. Felton** (Pennsylvania State Univ., USA) **Kotaro Konno** (National Agriculture and Food Research Organization, Japan)	**Paulo H. G. Zarbin** (Federal Univ. of Parana, Brazil) **Jun Tabata** (National Agriculture and Food Research Organization, Japan)	**Paolina Garbeva** (The Netherlands Institute of Ecology, the Netherlands) **Ikuro Abe** (Univ. of Tokyo, Japan)
13:50-14:20	*Keynote lecture* Microbes mediate plant perception of herbivores **Gary W. Felton**	*Keynote lecture* Recent results in pheromone synthesis **Kenji Mori**	*Keynote lecture* The chemical ecology of plant-animal-microorganism interactions **Robert R. Junker**
14:20-14:35	*Keynote lecture* Miracle maize is toxic to three different herbivore feeding guilds **Dawn S. Luthe**	GC/FT-IR analysis of a novel 2,4,6,9-tetraene occurring in a female pheromone gland of *Parasemia plantaginis* (Lepidoptera: Arctiidae) **Testu Ando**	*Keynote lecture* Activated chemical defense of Japanese marine sponge *Discodermia calyx* **Toshiyuki Wakimoto**
14:35-14:50		Cytochrome P450s are involved in iridoid biosynthesis in leaf beetles **Nanxia Fu**	
14:50-15:05	*Keynote lecture* Why are terrestrial ecosystems green? Why are anti-nutritive and inducible defenses effective?: A novel parameterized food web model and its implications to chemical ecology and plant-herbivore interactions **Kotaro Konno**	Elucidating the biosynthesis of the aphid sex pheromone **Suzanne J. Partridge**	*Keynote lecture* Volatile affairs in microbial belowground interactions **Paolina Garbeva**
15:05-15:20		Anti-aphrodisiac pheromones of *Heliconius erato phyllis* and *Heliconius ethilla narcaea* (Nymphalidae, Heliconiinae) **Paulo H. G. Zarbin**	

15:20-15:40	Coffee Break		
15:40-15:55	Mechanisms underlying cardenolide sequestration in a highly adapted herbivorous insect **Fee L. E. M. Meinzer**	*Keynote lecture* An explosion of diversity in cerambycid beetle pheromones: Identifications and syntheses **Jocelyn G. Millar**	Comparison of secondary metabolic gene evolution in microorganisms from different extreme environments **Dana Ulanova**
15:55-16:10	Dynamics and origin of cytokinins involved in plant manipulation by a leaf-mining insect **David Giron**		Roles of the DSF-family quorum sensing signals in cell-cell communication **Yinyue Deng**
16:10-16:25	Indirect manipulation of plant induced defenses by parasitoids of caterpillars **Ching-Wen Tan**	Host plant affects the sexual attractiveness of the female white-spotted longicorn beetle, *Anoplophora malasiaca* **Nao Fujiwara-Tsujii**	Post-translationally modified quorum sensing pheromone from *Bacillus subtilis* subsp. *natto* **Masahiro Okada**
16:25-16:40	Honeybees modulate dance communication in response to nectar toxicity and demand **Ken Tan**	A shift in the paradigm of arthropod cuticular lipids: Isolation, structural elucidation, and asymmetric synthesis of an unusual tetraterpenoid hydrocarbon from the springtail *Hypogastrura viatica* (Hexapoda: Collembola) **Jan E. Bello**	Terpenes as *lingua franca* between fungi and bacteria **Ruth L. Schmidt**
16:40-16:55	Being punctual: The coordination of a plant-pollinator interaction by the circadian clock **Myles Fenske**	Identification of the aggregation pheromone of the American cockroach **Yukihiro Nishimura**	
16:55-18:55	Poster Session 1		Venue: 2F

August 25 (Fri.)

TBA	Registration	
9:00-9:40	*Plenary 1* Chemical ecology from calling females to talking plants **James H. Tumlinson**	Room: B201
9:40-10:20	*Plenary 2* Factors controlling the composition of the microbiome of the agricultural pest *Spodoptera littoralis* **Wilhelm Boland**	Room: B201
10:20-10:40	Coffee Break	
10:40-11:20	*Plenary 3* Molecular phenology: '*in natura*' analyses of gene functions **Hiroshi Kudoh**	Room: B201
11:20-12:00	*Plenary 4* Chemical ecology in China in the last decade **Yongping Huang**	Room: B201
12:00-13:20	Lunch (Poster viewing)	

Session	Session 4 Plant perception and response	Session 5 Pheromone communication	Session 6 Aquatic chemical ecology
Room	B101	B102	B103
Organizer	**Consuelo De Moraes** (ETH Zürich, Switzerland) **Naoki Mori** (Kyoto Univ., Japan)	**Hidefumi Mitsuno** (Univ. of Tokyo, Japan) **Takeshi Sakurai** (Univ. of Tokyo, Japan) **Takeshi Fujii** (TUAT, Japan) **Zsolt Kárpáti** (Hungarian Academy of Sciences, Hungary)	**Michiya Kamio** (Tokyo Univ. of Marine Science and Technology, Japan) **Kye Chung Park** (The New Zealand Institute for Plant & Food Research Limited, New Zealand)
13:20-13:50	*Keynote lecture* Priming of plant defenses by an insect pheromone **Mark Mescher**	*Keynote lecture* Modes and mechanisms of evolution of insect olfaction **Teun Dekker**	*Keynote lecture* Cracking the code: Understanding the qualitative and quantitative properties of waterborne chemical cues that control prey risk assessment **Marc Weissburg**
13:50-14:05	Chemical cues from beneficial entomopathogenic nematodes enhance plant protection against herbivores **Anjel Helms**	Two hundred million years and four types of pheromones: A phylogenetic perspective on moth pheromone diversity and evolution **Christer Löfstedt**	*Keynote lecture* Chemical defense of sea hares: Sequestration and secretion of algal metabolite in ink and skin **Michiya Kamio**
14:05-14:20	Selective adaptation within the chemosensory system of the leaf beetle, *Chrysomela lapponica*, following host plant shift **Antje Burse**	Candidate cells producing alkenyl sex pheromones in moths **Takeshi Fujii**	
14:20-14:35	Natural variation of phytoalexin sakuranetin production in rice cultivars **Atsushi Ishihara**	Age-dependent plasticity in the sexual signal of a noctuid moth **Rik Lievers**	*Keynote lecture* Extracellular recordings from the brain cells of the New Zealand paddle crab, *Ovalipes catharus*, and evaluation of olfactory-active compounds

14:35-14:50	Effect of warming on VOC-mediated plant-insect interactions in high attitude alpine meadow ecosystems of the Himalayas **Joyshree Chanam**	Does divergent selection predict sexual selection in the adaptive radiation of the tropical butterfly genus *Melinaea*? **Melanie McClure**	**Kye Chung Park**
14:50-15:10	Coffee Break		
15:10-15:25	*Keynote lecture* Ligands seeking receptors: A multipronged approach in legumes to discover how plants perceive herbivore attack **Eric Schmelz**	How pheromone binding proteins sustain sexual behavior initiation in *Bombyx mori*? **Qun Liu**	A coral control acquisition of *Symbiodinium* using lectins **Mitsuru Jimbo**
15:25-15:40		In vivo functional analysis of genes involved in sex pheromone detection in the silkmoth *Bombyx mori* **Takeshi Sakurai**	Chemoreception of crabs and the development of baits for trap fishing and eradication **Miguel Vazquez-Archdale**
15:40-15:55	Induced foliar volatile production in response to the herbivore elicitor *N*-linolenoyl L-glutamine maps to a single QTL in maize **Alisa Huffaker**	Molecular basis of alarm pheromone detection in aphids **Guirong Wang**	Some like it cold: Antarctic marine chemical ecology **Conxita Avila**
15:55-16:10	The leucine-rich repeat receptor-like kinase OsLRR-RLK1 in rice functions as an early regulator in plant-herbivore interactions **Yonggen Lou**	Insect odorant receptor-based biosensor -a proof of concept using pheromone receptors and its application to general odor sensing- **Hidefumi Mitsuno**	Investigating the chemical landscape of microalgae cultures to mitigate pond crashes **Carolyn L. Fisher**
16:10-16:25	Volatiles from the giant knotweed, *Fallopia sachalinensis*, induced by the Japanese beetle, *Popillia japonica*, attract conspecific females **Koji Noge**	Odor detection using an insect olfactory receptor reconstructed in bilayer lipid membrane **Nobuo Misawa**	
16:25-17:05	*APACE Young Scientist Award Lecture* Identification of pheromone-like compounds of the flower bug *Orius sauteri* **Takuya Uehara**		Room: B201

	Flavone exposure improves the insecticide resistance and fecundity of *Spodoptera litura* through different cytochrome P450 monooxygenases **Kai Lu**	
17:05-19:05	Poster Session 2	Venue: 2F
19:05-19:35	ISCE/APACE Business Meeting	Room: TBA

August 26 (Sat.)

TBA	Registration		
Session	Session 7 Multitrophic interactions	Session 8 Semiochemicals in social interactions	Session 9 Chemical ecology of forest ecosystem
Room	B101	B102	B103
Organizer	**Ted Turlings** (Univ. of Neuchâtel, Switzerland) **Junji Takabayashi** (Kyoto Univ., Japan) **Yooichi Kainoh** (Univ. of Tsukuba, Japan)	**Toshiharu Akino** (Kyoto Institute of Technology, Japan) **Patrizia d'Ettorre** (Univ. of Paris 13, France)	**Zhen Zhang** (Chinese Academy of Forestry, China) **Junheon Kim** (National Institute of Forest Science, Korea) **Kiyoshi Nakamuta** (Chiba Univ., Japan)
9:00-9:30	*Keynote lecture* Exploiting the chemical ecology of tritrophic interactions for crop protection **Ted Turlings**	*Keynote lecture* Collective control of colony development through socially exchanged fluids **Adria C. LeBoeuf**	*Keynote lecture* Trap design factors and deployment methodologies: Effect on forest Coleoptera **Jeremy D. Allison**
9:30-9:45	Crop domestication in peppers: Consequences for direct and indirect plant defense **Michael Garvey**	*Keynote lecture* Sex and parental care: How pheromones regulate family life **Sandra Steiger**	Identification of pheromones of the potentially invasive beetles *Callidiellum villosulum* and *Allotraeus asiaticus* **Jacob D. Wickham**
9:45-10:00	Temporal dynamics of herbivore-induced volatiles provide robust indirect defense in nature **Youngsung Joo**		Use of pheromones for detection and monitoring of native and invasive cerambycid beetles **Jocelyn G. Millar**

10:00-10:15	Larval parasitoid wasp *Lytopylus rufipes* needs both background leaf volatiles and herbivore-induced plant volatile for searching a host **Chia-Ming Liu**	Chemical tactic of juvenile orchid mantis for capturing honeybee **Takafumi Mizuno**	Volatiles released by Cerambycidae beetles used as chemical clues by the pine wood nematode to identify the insect vector inside the dead pine host trees **Luis F. Bonifácio**
10:15-10:30	*Helicoverpa zea* gut-associated bacteria indirectly induce defenses in tomato by triggering a salivary elicitor(s) **Jie Wang**	Queen fire ant inhibits (primer pheromone) female sexual development, but newly-mated female sexuals need a quick colony foundation start **Robert K. Vander Meer**	Identification and field attraction test of aggregation pheromone of *Monochamus saltuarius*, insect vector of pine wood nematode in Korea **Il-Kwon Park**
10:30-10:50	Coffee Break		
10:50-11:05	Herbivore induced plant volatiles affect entomopathogens infectivity **Laila Gasmi**	Social and physiological factors affecting queen-worker pheromone interactions in honeybees **Abraham Hefetz**	Contact sex recognition pheromone of the juniper bark borer, *Semanotus bifasciatus* Motschulsky (Coleoptera: Cerambycidae) **Xiang-bo Kong**
11:05-11:20	Yeast-insect interactions in a tephritid fruit fly pest **Paul Cunningham**	Response profiles of sensory neurons in basiconic sensilla to cuticle hydrocarbons, key semiochemicals for nestmate discrimination in Japanese carpenter ant *Camponotus japonicas*. **Hidehiro Watanabe**	Odorant receptors as molecular markers for pheromone use in the longhorned beetles (Coleoptera: Cerambycidae) **Robert F. Mitchell**
11:20-11:35	Plant cell-wall degrading enzymes improve endophytism of entomopathogenic *M. brunneum* in potato plants **Anant V. Patel**	Ants eavesdropping on the variational trail pheromone in termites leads to signal arms race between the predator and the prey **Ping Wen**	Function of pheromone binding proteins in olfactory recognition of two sympatric *Dendrolimus* **Sufang Zhang**
11:35-11:50	Earwigs (*Labidura riparia*) mimic rotting-flesh odor to deceive vertebrate predators **John A. Byers**	Multifunctional roles of soldier pheromone in a termite **Yuki Mitaka**	Stress-induced host tree chemistry benefits fungus farming by ambrosia beetles **Christopher Ranger**
11:50-12:05	Subtropical plant-insect-parasitoid tri-trophic interactions under elevated CO_2 and temperature **Papitchaya Teawkul**		Test trial for controlling Japanese oak wilt using living trees or mass accumulated oak logs with the aggregation pheromone and kiromone of the Ambrosia Beetle, of *Platypus quercivorus* (Coleoptera, Platypodidae) **Masahiko Tokoro**

12:05-13:10	Lunch		
Session	Session 10 Plant-plant communication	Session 11 General chemical ecology	Session 12 Chemical ecology of invasive species
Room	B101	B102	B103
Organizer	**Kaori Shiojiri** (Ryukoku Univ., Japan) **Richard Karban** (UC Davis, USA)	**Junji Takabayashi** (Kyoto Univ., Japan) **Jeremy N. McNeil** (Western Univ., Canada)	**Jocelyn G. Millar** (UC Riverside, USA) **Kiyoshi Nakamuta** (Chiba Univ., Japan) **David M. Suckling** (The New Zealand Institute for Plant & Food Research Limited, New Zealand)
13:10-13:25	*Keynote lecture* Ecological implications of flowering communication **Ariel Novoplansky**	Spatial distribution of floral scent luring blow fly pollinators into inner cavity of *Rafflesia cantleyi* **Suk Ling Wee**	*Keynote lecture* Searching for Achille's heel: Chemical ecology for invasive species suppression **David M. Suckling**
13:25-13:40		A sesquiterpene attractive to male and female Oriental fruit fly, *Bactrocera dorsalis* **Alvin Kah-Wei Hee**	
13:40-13:55	*Keynote lecture* Insect herbivory selects for volatile-mediated plant communication in *Solidago altissima* **Aino Kalske**	Ecological contexts of mosquito odorant receptor function **Jonathan D. Bohbot**	Correlation of flower color polymorphism and defense phenotype of the invasive weed *Solanum elaeagnifolium* **James Sims**
13:55-14:10		What are stink bug male-produced pheromones doing on eggs? Or not. **Jeffrey R. Aldrich**	Genetic diversity, metabolic variation and functional life-history syndromes suggest multiple mechanisms facilitating invasion in a Brassicaceae species **Lisa J. Tewes**

14:10-14:25	The role of kin discrimination in interspecific competition in *Plantago asiatica* **Akira Yamawo**	Defense allocation upon multiple stresses: Impacts of drought stress on performance of caterpillars and induced defense responses in tomato **Po-An Lin**	Chemotypes in *Erodium cicutarium* (Geraniaceae) of native and invasive origin and effects of plant competition on offspring terpene profiles **Elisabeth J. Eilers**
14:25-14:40	Conversion of green leaf volatiles for processing the information from surrounding environments in tomato leaves **Koichi Sugimoto**	Secretion of isoflavones from soybean roots and their degradation dynamics in the rhizosphere **Akifumi Sugiyama**	Host selection by the olfactory system in *Drosophila suzukii*: Can flies discriminate among fruits by smell? **Claire Duménil**
14:40-15:00	Coffee Break		
15:00-15:15	Pest management using plant-plant signalling mediated by mint volatiles **Satoru Sukegawa**	Mechanisms of resistance to bufadienolide toxins in toad-eating snakes **Alan H. Savitzky**	Olfactory responses of winter morph spotted wing *Drosophila* (*Drosophila suzukii*) to volatile semiochemicals **Danielle Kirkpatrick**
15:15-15:30	Applying plant-plant communication on rice field **Kaori Shiojiri**	The chemical analysis of bufadienolides in defensive glands of the Asian *Rhabdophis* snakes. **Tatsuya Yoshida**	Identification of self-destructive defense system using a hemolymph enzyme, mandelonitrile oxidase, from the invasive millipede, *Chamberlinius hualienensis* **Yuko Ishida**
15:30-15:45	Action of avenacins in oat roots as allelochemicals **Tetsu Tsurushima**	Evolution of chemical mimicry in cuckoo wasp **Thomas Schmitt**	Identification of antennal olfactory receptor neurons and corresponding active compounds in tomato-potato psyllid, *Bactericera cockerelli* **Kye Chung Park**
15:45-16:00	*Keynote lecture* The language of plant communication **Richard Karban**	The biochemical mechanism underlying sex pheromone evolution in *Nasonia* **Joachim Ruther**	Red-necked longhorn beetle, *Aromia bungii*, an invasive pest of Rosaceae trees: Present status of distribution and damage and monitoring trials in Japan **Hiroe Yasui**
16:00-16:15		Where do they come from?: Can we use naturally occurring stable isotopes to better understand seasonal migration of insects **Jeremy N. McNeil**	Mating disruption of a Japanese gypsy moth, *Lymantria dispar japonica* **Hiroyuki Minegishi**

18:30-	Banquet (Venue: Hotel Granvia Kyoto)

August 27 (Sun.)

TBA	Registration		
Session	Session 13 Ecological omics: genome to the field	Session 14 Plants, microorganisms: next generation insecticides	Session 15 Utilization of semiochemicals in pest management
Room	B101	B102	B103
Organizer	**Gen-ichiro Arimura** (Tokyo Univ. of Science, Japan) **Wilhelm Boland** (MPI Chemical Ecology, Germany)	**Kazuhiko Matsuda** (Kindai Univ., Japan) **Ke Dong** (Michigan State Univ., USA)	**Junwei (Jerry) Zhu** (USDA-ARS, USA) **Tom Baker** (Pennsylvania State Univ., USA) **Rikiya Sasaki** (Fuji Flavor Co., Ltd., Japan)
9:00-9:30	*Keynote lecture* Glucoside transporters in leaf beetle defence: A proteomics approach **Wilhelm Boland**	*Keynote lecture* Selective toxicity profile of plant-based natural products **Jeffrey R. Bloomquist**	*Keynote lecture* Predicting the success of mating disruption **Larry Gut**
9:30-9:45	*Keynote lecture* Deciphering allopolyploidy-mediated innovations in plant defense metabolism against insects using structural metabolomics **Emmanuel Gaquerel**	*Keynote lecture* Modulation of ligand-gated chloride channels by fungal metabolites produced in response to plant factors **Kazuhiko Matsuda**	Mating disruption and aerial releases of sterile codling moth in New Zealand: Is local eradication possible? **David M. Suckling**
9:45-10:00			Designing a mega-dispenser for sex pheromone mating disruption **Tom Baker**

10:00-10:15	*Keynote lecture* Multiple omics analysis of shikonin production system in *Lithospermum erythrorhizon* **Kazufumi Yazaki**	*Keynote lecture* Molecular basis of pyrethrum repellency in *Drosophila melanogaster* **Ke Dong**	Codling moth mating disruption 25 years on: How is it working, what's changed, what's new and what is still needed **Don Thomson**
10:15-10:30			Mating disruption of codling moth with reduced sex pheromone load dispensers **Alex Il'ichev**
10:30-10:50	Coffee Break		
10:50-11:05	*Keynote lecture* Omics everywhere: How about in chemical ecology? **Ivan Galis**	Effects of floral scents and their memories on feeding preference of the fly **Mamiko Ozak**i	Methyl benzoate is a natural, plant-based, and green pesticide for sustainable agriculture **Aijun Zhang**
11:05-11:20		Bioactivities of cardanol derivatives isolated from *Anacardium occidentale* (Cashew) nut shell liquid against *Tribolium castaneum* Hebst (Coleoptera: Tenebrionidae) and *Sitophilus oryzae* L. (Coleoptera: Curculionidae) **Thomas Buxton**	Production of moth sex pheromones in an oil crop **Bao-Jian Ding**
11:20-11:35	NPR1-mediated immune system in the model monocot plant *Brachypodium distachyon* **Takuya Uemura**	Asian soybean rust-induced metabolites in a resistant soybean cultivar Hougyoku (PI 224270): Structure determination and antifungal activity evaluation **Ryu Nakata**	Electrospun mesofibers in precision viticulture: Joint integrated pest management on *Lobesia botrana* (Lep.: Tortricidae), and *Grapholita molesta*, in Germany and Brazil **Simone S. Langner**
11:35-11:50	CRISPR/Cas9 mediated three PBP genes knock out in *Spodoptera litura* resulting in low responses of sex pheromone **Shuang-Lin Dong**	Elucidating the target of communesins, fungal metabolites acting as insecticides **Akira Noguchi**	Discoveries of novel long-lasting repellents against biting flies on livestock animals **Junwei (Jerry) Zhu**
11:50-12:05	Field transcriptomics: Integration of transcriptomics and meteorology **Atsushi J. Nagano**	Development of CO_2-releasing formulations for the control of soil-borne insect pests **Anant V. Patel**	Phagostimulants for the Asian citrus psyllid also elicit volatile release from citrus leaves **Stephen L. Lapointe**

12:05-13:00	Closing Ceremony (Travel & Poster Awards)	Room: B201

13:30-	Excursion

Award lectures

ISCE Silver Medal Award

Bark beetle pheromone and insect hydrocarbon production

Gary J. Blomquist
Department of Biochemistry and Molecular Biology, University of Nevada, USA

ISCE Silverstein-Simeone Lecture Award

Perfume as dark matter: Unveiling the explanatory power of floral scent

Robert A. Raguso
Department of Neurobiology and Behavior, Cornell University, USA

ISCE Early Career Award

How plant viruses use chemistry to manipulate hosts and vectors

Kerry Mauck
Department of Entomology, University of California, USA

APACE Lifetime Achievement Award

Collaborative chemical ecology of Asian pest fruit flies - Biological and socio-economic perspectives

Keng Hong Tan
Mobula Research, Malaysia

Collaborative chemical ecology of Asian pest fruit flies - Semiochemical perspectives

Ritsuo Nishida
Kyoto University, Japan

APACE Young Scientist Award

Identification of pheromone-like compounds of the flower bug *Orius sauteri*

Takuya Uehara
Institute of Agrobiological Sciences, National Agriculture and Food Research Organization, Japan

Flavone exposure improves the insecticide resistance and fecundity of *Spodoptera litura* through different cytochrome P450 monooxygenases

Kai Lu
College of Life Sciences, Fujian Agriculture and Forestry University, China

Bark beetle pheromone and insect hydrocarbon production

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Our understanding of bark beetle chemical communication underwent a paradigm shift in the 1990s when it became recognized that most bark beetle pheromones are produced *de novo* by beetle tissue, and only a few arise from direct hydroxylation of host tree precursors. Midgut tissue was shown to be the major site of pheromone production. Mmicroarrays, real time PCR and RNA-seq has allowed us identify, express and characterize many of the key reactions in bark beetle pheromone biosynthesis, including the first animal geranyldiphosphate synthase and key cytochromes P450. The majority of the steps in ipsdienol and ipsenol production in Ips spp. and verbenol, exo-brevicomin and frontalin production in the mountain pine beetle have been characterized and some have been put to commercial use to produce enatiomerically pure pheromone components.

Insect cuticular hydrocarbons function extensively in chemical communication, and a few examples will be discussed. The terminal steps in hydrocarbon production have been determined over the past few years. All insects possess one or two cytochromes P450 in the CYP4G family, and CYP4Gs from the housefly, honeybee, *Anopheles gambiae* and the mountain pine beetle have been expressed as fusion proteins with cytochrome P450 reductase and assayed. It appears that an acyl-CoA reductase converts very long chain fatty acyl-CoAs to alcohols, which are then oxidized to aldehydes and oxidatively decarbonylated to hydrocarbon and carbon dioxide by CYP4Gs. The oenocytes have high levels of CYP4Gs, and RNAi knockdown in Drosophila oenocytes markedly reduced hydrocarbon production resulting in desiccation. In several species, high levels of CYP4Gs are expressed in antennae, suggesting that they may have a role in clearing of pheromones and phytochemicals. Several CYP4Gs convert C10 alcohols and aldehydes to hydrocarbon one carbon shorter, providing further evidence for a novel function for CYP4Gs.

Perfume as dark matter: Unveiling the explanatory power of floral scent

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For at least half a century it has been acknowledged that complex ecological interactions across the biosphere are driven, in part, by biogenic chemical toxins, signals and cues. Semiochemicals are known to mediate nuanced multi-trophic interactions in diverse natural biomes (e.g. coral reefs, grasslands, rainforests) as well as human-designed or modified habitats (e.g. agro-ecosystems, aquacultural systems), and thus play prominent roles in the concept structure underpinning those fields. One exception to this chemically-informed paradigm is the study of plant-pollinator interactions, which traditionally has been dominated by optimal foraging theory and other density-dependent ecological models and more recently has embraced community and landscape level network analyses with no conceptual space for chemically mediated processes. This bias persists in the study of plant-pollinator evolution, in which seminal papers on floral isolation, reinforcement and adaptive radiation continue to focus primarily on floral form and color as phenotypic features.

Here I outline the evidence for direct and indirect roles of floral volatiles as "ghost variables" that shape plant-pollinator interactions in ecological and evolutionary time. In each case study, I emphasize the complementary, additive or synergistic ways that floral scent interacts with visual or morphological traits to attract or filter floral visitors. At the autecological scale, single volatiles can be important drivers across the full spectrum of plant-pollinator interactions, from pollen-based foraging by solitary bees at strawberry flowers (2-phenylethanol) to hawkmoth-mediated floral isolation between *Ipomopsis* species driven by indole. The information content of floral scent may be intimately tied with important nutritional resources, such that nitrogenous aldoximes may provide an honest index for nectar amino acids in hawkmoth-pollinated *Oenothera* flowers and 8C alcohols and ketones may mislead fungal-breeding flies to visit and pollinate brood site-deceptive *Dracula* orchids. At the community level, floral volatiles have been ignored in the search for factors that contribute to plant-pollinator networks and link strength, including flowering time, abundance and trait mismatches. Nevertheless, scent-swapping experiments temporarily shift visitation patterns despite unchanging floral color and structure, underscoring the importance of scent in floral constancy by pollinators. Multivariate approaches reveal striking non-random associations between floral scent and color when parameterized in the context of insect visual perception, further reinforcing the primacy of multimodal flower learning by pollinators. Finally, at the population level, large scale studies reveal departures from the straightforward expectations of pollination syndromes or phylogenetic diversification, suggesting that variation in scent composition is strongly influenced by abiotic factors and natural enemies as well as pollinators, consistent with the Geographic Mosaic Theory of Coevolution.

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ISCE Award-3

How plant viruses use chemistry to manipulate hosts and vectors

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A primary means by which parasites alter the interactions of their hosts with other organisms, and thereby influence transmission, is via effects on sensory cues produced by infected hosts. As a result, selection may be expected to favor parasites that influence cues in ways that significantly enhance their own fitness. Given that contacts with insects are critical for the fitness of vector-borne pathogens, such as plant viruses, we may hypothesize that these organisms are frequently under strong selective pressure to elicit (or maintain) host chemical phenotypes that are conducive to transmission. We explored this hypothesis using several species and genotypes of aphid-vectored plant viruses in the family Bromoviridae. Using multiple host-virus pathosystems, we have identified conserved, cross-species patterns of virus effects on host chemical phenotypes, characterized the mechanistic basis for these changes, and produced empirical estimates of enhancements to transmission resulting from changes in sensory cues. Importantly, our work demonstrates that virus effects on host chemistry and vector behavior are unlikely to be simple by-products of virus pathology. Instead, virus effects on specific host cues, and subsequent behavioral responses of vectors, exhibit congruence across virus transmission mechanisms and appear to track with virus local adaptation to specific hosts. This suggests that multi-host viruses experience trade-offs in their ability to manipulate all susceptible hosts, and all host-vector combinations, with significant consequences for virus fitness in agricultural and wild plant communities. Beyond our direct explorations of virus-host-vector interactions, we have also demonstrated the virus effects on sensory cues can benefit pathogen fitness indirectly via effects on non-vector organisms (specialist herbivores and predators) that are capable of influencing pathogen dynamics by altering the survival of infected hosts and behavior of insect vectors. This work reveals the importance of studying pathogen influence on sensory cues in a community context that includes additional drivers of pathogen evolution, which could act to reinforce, or counter, selection pressures imposed by vectors.

Collaborative chemical ecology of Asian pest fruit flies – Biological and socio-economic perspective

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Pest fruit flies in the Asia Pacific region are species of two genera, *Bactrocera or Zeugodacus*, belonging to the tribe Dacini (Tephritidae:Diptera). Many species are either methyl eugenol (ME)-sensitive, namely, *B. carambolae*, *B. dorsalis*, and *B. umbrosa*, or raspberry ketone (RK)-sensitive namely *B. albistrigata*, *Z. caudatus*, *Z. cucurbitae* and *Z. tau*. They act as exclusive pollinators for wild orchids, especially the *Bulbophyllum* species. These Dacini attracting -daciniphilous orchid flowers release specific attractants which act as a floral reward in order to attract fruit flies as vectors for pollination – this chemo-ecological research with Ritsuo Nishida is ongoing. These attractants are i) ME – found in *Bulbophyllum cheiri, Bu. elevatopunctatum* and *Bu. vinaceum*, ii) RK -found in *Bu. econutum*, *Bu. emiliorum* and *Bu. praetervisum*, and iii) zingerone -found in *Bu. baileyi, Bu. macranthum*, and *Bu. patens*. These floral attractants are consumed by the visiting fruit flies to be either bio-transformed into sex pheromone components (which also act as an allomone to deter predation) or sequestered as a pheromone booster to attract conspecific females for mating – thereby, acting as a floral synomone. Daciniphilous *Bulbophyllum* flowers have also evolved specific dynamic mechanisms to temporally trap an attracted fly for pollinia removal or deposition during pollination. Video footage of these fascinating mechanisms will be shown.

Ongoing area wide control or eradication of certain pest fruit fly species coexisting with endemic daciniphilous orchids may inadvertently lead to a ‘pollinator limitation’ for the *Bulbophyllum* species. This will affect the biodiversity of wild orchids in the rainforest. Thus, a good fruit fly pest management program should take into account the conservation and sexual reproduction of the daciniphilous orchid species in endemic areas.

The *Bactrocera dorsalis* species complex consists of >80 sibling taxa, of which eight (namely, *B. dorsalis, B. papayae, B. invadens, B. philippinensis, B. carambolae, B. caryeae, B. kandiensis and B. occipitalis*) are highly invasive, polyphagous and considered key pests causing many socio-economic problems. The discovery of identical sex pheromone components in *B. dorsalis* and *B. papaya*e post ME-consumption initiated an international effort to resolve the taxonomic status related to the *B. dorsalis, B. papayae, B. invadens, B. philippinensis* sibling taxa. In 2015, based on FAO/IAEA sponsored integrative chemo-ecological, phylogenetic, cytogenetic, interbreeding and morphometric investigations conducted by many international research institutes, these four taxa were eventually synonymized into a single biological species --*B. dorsalis*. This synonymization has assisted in reducing strict quarantine and food security procedures, and also enabled fair international trade of fruit commodities, especially for agricultural countries. The remaining four of the eight taxa listed above, i.e*., B. carambolae, B. caryeae, B. kandiensis* and *B. occipitalis* may also be incipient taxa of *B. dorsalis* as indicated by some preliminary chemo-ecological, phylogenetic and interbreeding investigations. Therefore, their taxonomic status also warrants further in-depth studies and needs to be resolved based on robust scientific evidence.

Collaborative Chemical Ecology of Asian Pest Fruit Flies – Semiochemical Perspectives

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There are a number of serious fruit fly pests of the tribe Dacini, such as the oriental fruit fly (*Bactrocera dorsalis*) and melon fly (*Zeugodacus cucurbitae*), which infest various agricultural crops of economic importance particularly in the tropical and subtropical regions centered Southeast Asia, often causing quarantine problems. Males of these pest species show strong affinity either to methyl eugenol (ME) or cue-lure (CL) (or its deacetyl derivative, raspberry ketone (RK)), and these attractants have been used as selective mass trapping and monitoring agents in the fruit fly eradication programs. However, it was not known why only males are so strongly attracted and voraciously feed on the lure chemicals. Under a collaborative project with Keng Hong Tan in Malaysia, we demonstrated that males of *B. dorsalis* acquire ME from various plant sources as a sex pheromone precursor and store the metabolites to rectal secretory organ to attract conspecific females. The ME metabolites also played an ecological role as an allomone to repel predators.

We have been seeking new attractants for pest species which respond neither to ME nor CL/RK, particularly those endemic agricultural pests, by observing the flies' natural interactions with plants and also by analyzing specific chemicals the wild male flies accumulate in their rectal glands. Orchid flowers of many *Bulbophyllum* species attract *Bactrocera* fruit fly males by emitting specific volatiles for pollination, in the rain forests of Southeast Asia. Several species produce either ME or RK, but some species use zingerone (ZN) to attract both ME-sensitive and RK-sensitive males. ZN was subsequently found to be a specific male attractant for *B. jarvisi* in Australia. A specific attractant was isolated and characterized as 3-hydroxy-α-ionone from a variety of eggplants where *B. latifrons* males congregate and compulsively feed on. Several 3-oxygenated α-ionone analogs were developed as monitoring agents for *B. latifrons* in Okinawa, Japan. Furthermore, wild *Bactrocera* males that have fed on attractants from certain plant sources selectively accumulated/biotransformed compounds in the rectal gland possibly for sex pheromone to attract females. A sesquiterpene hydrocarbon - β-caryophyllene, was identified from the rectal gland only in wild *B. correcta* males. It was found to be more attractive than ME, and thus, serves as a new monitoring agent for the species in Thailand.

Our collaborative work on fruit flies offers various semiochemical perspectives including pheromone, allomone, kairomone and synomone, which may be useful for controlling those key pest species of high economic importance. Further studies will provide a clue to understanding their adaptive radiation mechanisms in the interactions between fruit flies and plants especially centered in Southeast Asia.

Identification of pheromone-like compounds of the flower bug *Orius sauteri*

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The flower bug *Orius sauteri* is a tiny predatory stinkbug that occurs throughout Japan, and is a promising indigenous natural enemy for micro-pests such as thrips. It is easily bred on so-called 'insectary plants,' such as buckwheat (*Fagopyrum esculentum*) and okra (*Abelmoschus esculentus*). However, it is difficult to recruit *O. sauteri* from these insectary plants to infested crops and to keep them on the crops. We aim to control their attraction, aversion, and settlement behavior with natural chemical substances that they utilize.

First, we established gas chromatography with electroantennographic detection (GC-EAD), which is a method for screening semiochemicals that control their behavior based on their antenna response. Females were immersed in hexane and extracted in an ultrasonic bath for 5 min. The male antenna response to the extract was recorded, and this demonstrated that the male antenna perceives at least two components in the extract. Gas chromatography-mass spectroscopy (GC-MS) showed that these EAD-active components were C8 unsaturated aldehydes.

Next, we observed the behavioral responses of the bug to these compounds. Adult males were released on a Petri dish (φ 90 mm), allowed to walk there spontaneously and exposed to these components. The bugs showed distinct behavioral featrures, such as mounting another individual and bending the copulatory organ, and jumping vigorously inside the Petri dish. The activity of the bugs increased greatly after exposure to these components compared to air. These results suggest that these components are *O. sauteri* pheromones, although their functions are still unclear.

Flavone exposure improves the insecticide resistance and fecundity of *Spodoptera litura* through different cytochrome P450 monooxygenases

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In respond to damage by toxic secondary metabolites from their host plants, phytophagous insects developed a series of detoxification and adaptation mechanisms to protect themselves. Although it is known that herbivorous insects have evolved the ability to up-regulate the expression of cytochrome P450 monooxygenases (CYP450s) to metabolize plant allelochemicals, how herbivorous insects precisely respond to specific secondary metabilites to elevate counterdefense capability remains obscure.

Here we studied the larval insecticide resistance and adult fecundity of the polyphagous pest *Spodoptera litura* feeding on artificial diets with different flavone levels. *S. litura* larvae exposed to low concentrations of flavone (0-1 mg g^{-1}) did not inhibit their growth and development. However, *S. litura* larvae gained significantly more weight on the flavone-contained diet compared to normal diet after topical application of chlorpyrifos. Furthermore, the mortality of *S. litura* pretreated with flavone was significantly lower than control after insecticide application. Transcripts coding for two P450s detoxification enzymes (*CYP6AE43* and *CYP6AE44*) in midgut and fat body of *S. litura* were significantly upregulated after flavone exposure. Injection of double-stranded RNA (dsRNA) of *CYP6AE43* or *CYP6AE44* into *S. litura* larvae successfully reduced the expression levels of target genes, and resulted in higher mortality rates of chlorpyrifos-exposed larvae pretreated with flavone. Meanwhile, the expression of these two P450s was also significantly induced by chlorpyrifos application, suggesting there existed obvious cross-resistance between flavone and chlorpyrifos for *S. litura*. These results demonstrated that the resistance of *S. litura* to chlorpyrifos is elevated by flavone exposure through induction of *CYP6AE43* and *CYP6AE44* expression.

Interestingly, flavone exposure significantly enhanced the fecundity of *S. litura* adult females. The expression of a P450 (*CYP314A1*), catalyzes the conversion of ecdysone to 20-hydroxyecdysone (20E), was significantly induced by flavone. RNA inteference-mediated knockdown of *CYP314A1* severely decreased 20E titre, and impaired the inductive effects of flavone on the fecundity. Injection of 20E into *CYP314A1*–dsRNA-injected females did not increase *CYP314A1* expression level, but markedly relieved the negative effects on female fecundity. These results suggested that flavone stimulates 20E synthesis to enhance the fecundity of *S. litura*.

Keywords: *Spodoptera litura*; flavone; insecticide resistance; fecundity; P450

Plenary lectures

Plenary 1

Chemical ecology from calling females to talking plants

James H. Tumlinson
Center for Chemical Ecology, Department of Entomology, Penn State University, USA

Plenary 2

Factors controlling the composition of the microbiome of the agricultural pest *Spodoptera littoralis*

Wilhelm Boland
Max Planck Institute for Chemical Ecology, Germany

Plenary 3

Molecular phenology: ‘*in natura*’ analyses of gene functions

Hiroshi Kudoh
Center for Ecological Research, Kyoto University, Japan

Plenary 4

Chemical ecology in China in the last decade

Yongping Huang
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Chemical ecology from calling females to talking plants

James H. Tumlinson

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In nature volatile organic compounds (VOC) abound in air, soil and water, and their use as chemical signals by a broad array of organisms has been well documented. The first identification of a VOC as a signal that transmitted information between individual animals occurred a little over 50 years ago, with the identification of the sex pheromone from female silkworm moths as (10*E*,12*Z*)-hexadeca-10,12-dien-1-ol. Initially, it was theorized that each insect species would produce and use a single, unique VOC as its specific signal to promote mating communication between the sexes and exclude all other species. This theory was quickly revised when Silverstein, Wood and colleagues identified a multicomponent, synergistic blend of compounds serving as the pheromone of the bark beetle *Ips confusus*. Subsequently, Roelofs and others showed that blends were the rule, rather than the exception in numerous species of moths and other insects. Since the bombykol discovery analytical techniques and instrument sensitivity have greatly improved, and thousands of biologically active VOC have been identified, and their roles in signaling mechanisms have been investigated and elucidated. In plant-insect interactions it has long been recognized that floral VOC play key roles in attracting pollinators. However, the importance of volatile chemical signals in plant defenses against herbivores only became evident in the 1980s, after Price pointed out the importance of tritrophic interactions among plants, insect herbivores and natural enemies of the herbivores. Subsequently, several reports showed the importance of herbivore-induced plant VOC as host location cues for parasitoids and predators of the herbivores. More recently, it has been clearly demonstrated that plants can detect and respond to VOC, and that this ability is an important defensive mechanism. Plants exposed to VOC from neighbors damaged by insect herbivores can prime their defenses to respond more quickly and strongly if they are subsequently attacked by the herbivores. Thus, herbivore-induced VOC not only attract natural enemies of the herbivores, but also act as “alarm” signals for neighboring plants and/or as intra plant signals between damaged leaves and leaves distal to the site of damage. Farmer and Ryan demonstrated that plant hormones, ethylene and methyl jasmonate, travel through the air to activate plant defensive responses. The six-carbon aldehydes, alcohols and hexenyl esters, called green leaf volatiles (GLV), as well as methyl salicylate and indole, have also been shown to act as airborne plant-plant signals. Thus, our understanding of the complexity in chemical ecology has evolved and increased markedly. Yet, we clearly don’t fully comprehend the complexity of the vast chemical signaling networks, employing VOCs, in nature. Continued exploration and elucidation of these intricate networks will yield many more exciting and important discoveries.

Factors controlling the composition of the microbiome of the agricultural pest *Spodopteta littoralis*

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Microbes that live inside insects play critical roles in host nutrition, physiology, and behavior. Using ribosomal tag pyrosequencing of DNA and RNA, we investigated biodiversity and activity of gut microbiotas across the holometabolous life cycle of *Spodoptera littoralis*, a notorious agricultural pest worldwide. Proteobacteria and Firmicutes dominate but undergo a structural "metamorphosis" in tandem with its host. Enterococcus, Pantoea and Citrobacter were abundant and active in early-instar, while Clostridia increased in late-instar. Interestingly, only enterococci persisted through metamorphosis. We combined pyrosequencing of amplified 16S rRNA genes with *in vivo* stable isotope probing (Pyro-SIP) to unmask metabolically active bacteria in the gut of S. littoralis. With ^{13}C glucose as the trophic link, Pyro-SIP revealed that a relatively simple but distinctive gut microbiota co-developed with the host, both metabolic activity and composition shifting throughout larval stages. Pantoea, Citrobacter and Clostridium were particularly active in early-instar, likely the core functional populations linked to nutritional upgrading. Enterococcus was the single predominant genus in the community over the whole developmental cycle. We have engineered a GFP-tagged species of *E. mundtii*, to track how it colonizes and adapts to the conditions in the intestine of *S. littoralis*. The promoter ermB was the most effective, exhibiting the highest GFP fluorescence intensity; the reporter organism survived and proliferated in the intestinal tract of the insect and gave first access to transcriptomic data from the microbes of different areas of the insect gut. Insect produced 8-hydroxyquinolinic acid (8-HQA) and the microbial cationic protein mundticin were identified as important factors that determine the composition of the microbiome during the development of the insect.

Plenary-3

Molecular phenology: '*in natura*' analyses of gene functions

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Recently, importance of studying organisms in their natural habitats has been emphasized in the areas of biology that aim to understand functions of genes as well as other molecular and cellular processes. The aim of the talk is to introduce molecular phenology, a methodologically and conceptually new approach for the understanding of organismal seasonality [1]. One of the recent advances in the study of plant phenology is the combined applications of molecular biology and statistical modelling in natural habitats. This type of analyses of gene regulation in natural habitat is referred as *in natura* study. Aims of *in natura* study are not only to validate gene regulatory responses identified in laboratory conditions but also asking novel questions that are specific to natural conditions, such as how the gene regulatory system actually behave in natural conditions, how useful environmental cues are extracted from noisy signals, and how gene regulatory system is robust against fluctuations of factors other than the responding signal. For example, seasonal changes of temperature exist as a long-term trend. Actual changes of temperature involve day-and-night, day-by-day, and week-by-week fluctuations. Therefore, to know the season from temperature, plants have to remember the long term trend of past temperature. We conducted 'molecular phenology' study on a homolog of *Flowering Locus C* (*FLC*) in a natural population of *Arabidopsis halleri* subsp. *gemmifera* [2]. By analysing the relationship between the gene expression and past temperature, we revealed that this key gene for flowering-time control is regulated in response to temperature over the past six-weeks. Then, we further conducted seasonal transcriptome analyses using RNA-seq technique in the natural population for two years by visiting the field site at one-week intervals. Approximately 15%-20% of analysed genes showed seasonal patterns. Especially, flowering-time, circadian clock, and defence related genes showed specific seasonal patterns. Our study demonstrates that *in natura* study provides us more comprehensive understanding of gene functions.

References:

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Chemical ecology in China in the last decade

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The Chinse Association of Chemical Ecology (CACE) is one of the 18 divisions of Ecological Society of China (ESC). ESC is founded in 1979 and has more than 7,800 members. CACE has more than 500 members from different universities and research institutions in China. CACE is an organization that facilitates academic exchange and promotes the understanding of the relationship among organisms and their environment. CACE will organize conference once every two years. Many of the world-renown researchers have been invited to participate the conference series. CACE members research fields encompass traditional semiochemical identification and application, aquatic chemical ecology, microbio-chemoecology of insects and their environment, insect plant interactions and plant allelopathy, molecular chemical ecology, receptors and olfaction, mechanisms of orientation to odors, and the application of newly emerged genome editing in Chemical Ecology. The most recent research progresses of the CACE members will be summarized in this talk.

Keynote lectures
&
Oral presentations

Session 1
Plant-animal interactions

S1-K-01	Microbes Mediate Plant Perception of Herbivores	**Gary W. Felton**
S1-K-02	Miracle maize is toxic to three different herbivore feeding guilds	**Dawn S. Luthe**
S1-K-03	Why are terrestrial ecosystems green? Why are anti-nutritive and inducible defenses effective?: A novel parameterized food web model and its implications to chemical ecology and plant-herbivore interactions	**Kotaro Konno**
S1-O-01	Mechanisms underlying cardenolide sequestration in a highly adapted herbivorous insect	**Fee L. E. M. Meinzer**
S1-O-02	Dynamics and origin of cytokinins involved in plant manipulation by a leaf-mining insect	**David Giron**
S1-O-03	Indirect manipulation of plant induced defenses by parasitoids of caterpillars	**Ching-Wen Tan**
S1-O-04	Honeybees modulate dance communication in response to nectar toxicity and demand	**Ken Tan**
S1-O-05	Being punctual: The coordination of a plant-pollinator interaction by the circadian clock.	**Myles Fenske**

Session 2
Characterization of semiochemicals: analysis and synthesis

S2-K-01	Recent results in pheromone synthesis	**Kenji Mori**
S2-K-02	An explosion of diversity in cerambycid beetle pheromones: Identifications and syntheses	**Jocelyn G. Millar**
S2-O-01	GC/FT-IR analysis of a novel 2,4,6,9-tetraene occurring in a female pheromone gland of *Parasemia plantaginis* (Lepidoptera: Arctiidae)	**Tetsu Ando**
S2-O-02	Cytochrome P450s are involved in iridoid biosynthesis in leaf beetles	**Nanxia Fu**
S2-O-03	Elucidating the biosynthesis of the aphid sex pheromone	**Suzanne Partridge**
S2-O-04	Anti-aphrodisiac pheromones of *Heliconius erato phyllis* and *Heliconius ethilla narcaea* (Nymphalidae, Heliconiinae)	**Paulo H. G. Zarbin**
S2-O-05	Host plant affects the sexual attractiveness of the female white-spotted longicorn beetle, *Anoplophora malasiaca*	**Nao Fujiwara-Tsujii**
S2-O-06	A shift in the paradigm of arthropod cuticular lipids: Isolation, structural elucidation, and asymmetric synthesis of an unusual tetraterpenoid hydrocarbon from the springtail *Hypogastrura viatica* (Hexapoda: Collembola)	**Jan Edgar Bello**
S2-O-07	Identification of the aggregation pheromone of the American cockroach	**Yukihiro Nishimura**

Session 3
Microbial chemical ecology

S3-K-01	The chemical ecology of plant-animal-microorganism interactions	**Robert R. Junker**
S3-K-02	Activated chemical defense of Japanese marine sponge *Discodermia calyx*	**Toshiyuki Wakimoto**
S3-K-03	Volatile affairs in microbial belowground interactions	**Paolina Garbeva**
S3-O-01	Comparison of secondary metabolic gene evolution in microorganisms from different extreme environments	**Dana Ulanova**

S3-O-02	Roles of the DSF-family quorum sensing signals in cell-cell communication	**Yinyue Deng**
S3-O-03	Post-translationally modified quorum sensing pheromone from *Bacillus subtilis* subsp. *natto*	**Masahiro Okada**
S3-O-04	Terpenes as *lingua franca* between fungi and bacteria	**Ruth Schmidt**

Session 4
Plant perception and response

S4-K-01	Priming of plant defenses by an insect pheromone	**Mark Mescher**
S4-K-02	Ligands seeking receptors: A multipronged approach in legumes to discover how plants perceive herbivore attack	**Eric Schmelz**
S4-O-01	Chemical cues from beneficial entomopathogenic nematodes enhance plant protection against herbivores	**Anjel M. Helms**
S4-O-02	Selective adaptation within the chemosensory system of the leaf beetle, *Chrysomela lapponica*, following host plant shift	**Antje Burse**
S4-O-03	Natural variation of phytoalexin sakuranetin production in rice cultivars	**Atsushi Ishihara**
S4-O-04	Effect of warming on VOC-mediated plant–insect interactions in high altitude alpine meadow ecosystems of the Himalayas	**Joyshree Chanam**
S4-O-05	Induced foliar volatile production in response to the herbivore elicitor *N*-linolenoyl L-glutamine maps to a single QTL in maize	**Alisa Huffaker**
S4-O-06	The leucine-rich repeat receptor-like kinase OsLRR-RLK1 in rice functions as an early regulator in plant-herbivore interactions	**Yonggen Lou**
S4-O-07	Volatiles from the giant knotweed, *Fallopia sachalinensis*, induced by the Japanese beetle, *Popillia japonica*, attract conspecific females	**Koji Noge**

Session 5
Pheromone communication

S5-K-01	Modes and mechanisms of evolution of insect olfaction	**Teun Dekker**
S5-O-01	Two hundred million years and four types of pheromones: A phylogenetic perspective on moth pheromone diversity and evolution	**Christer Löfstedt**
S5-O-02	Candidate cells producing alkenyl sex pheromones in moths	**Takeshi Fujii**
S5-O-03	Age-dependent plasticity in the sexual signal of a noctuid moth	**Rik Lievers**
S5-O-04	Does divergent selection predict sexual selection in the adaptive radiation of the tropical butterfly genus *Melinaea*?	**Melanie McClure**
S5-O-05	How pheromone binding proteins sustain sexual behavior initiation in *Bombyx mori*?	**Qun Liu**
S5-O-06	In vivo functional analysis of genes involved in sex pheromone detection in the silkmoth *Bombyx mori*	**Takeshi Sakurai**
S5-O-07	Molecular basis of alarm pheromone detection in aphids	**Guirong Wang**
S5-O-08	Insect odorant receptor-based biosensor -a proof of concept using pheromone receptors and its application to general odor sensing-	**Hidefumi Mitsuno**
S5-O-09	Odor detection using an insect olfactory receptor reconstructed in bilayer lipid membrane	**Nobuo Misawa**

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S6-K-02	Chemical defense of sea hares: Sequestration and secretion of algal metabolite in ink and skin	**Michiya Kamio**
S6-K-03	Extracellular recordings from the brain cells of the New Zealand paddle crab, *Ovalipes catharus*, and evaluation of olfactory-active compounds	**Kye Chung Park**
S6-O-01	A coral control acquisition of *Symbiodinium* using lectins	**Mitsuru Jimbo**
S6-O-02	Chemoreception of crabs and the development of baits for trap fishing and eradication	**Miguel Vazquez-Archdale**
S6-O-03	Some like it cold: Antarctic marine chemical ecology	**Conxita Avila**
S6-O-04	Investigating the chemical landscape of microalgae cultures to mitigate pond crashes	**Carolyn L. Fisher**

Session 7
Multitrophic interactions

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S7-O-01	Crop domestication in peppers: Consequences for direct and indirect plant defense	**Michael Garvey**
S7-O-02	Temporal dynamics of herbivore-induced volatiles provide robust indirect defense in nature	**Youngsung Joo**
S7-O-03	Larval parasitoid wasp *Lytopylus rufipes* needs both background leaf volatiles and herbivore-induced plant volatile for searching a host	**Chia-Ming Liu**
S7-O-04	*Helicoverpa zea* gut-associated bacteria indirectly induce defenses in tomato by triggering a salivary elicitor(s)	**Jie Wang**
S7-O-05	Herbivore induced plant volatiles affect entomopathogens infectivity	**Laila Gasmi**
S7-O-06	Yeast-insect interactions in a tephritid fruit fly pest	**Paul Cunningham**
S7-O-07	Plant cell-wall degrading enzymes improve endophytism of entomopathogenic *M. brunneum* in potato plants	**Anant V. Patel**
S7-O-08	Earwigs (*Labidura riparia*) mimic rotting-flesh odor to deceive vertebrate predators	**John A. Byers**
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Session 8
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S8-O-01	Chemical tactic of juvenile orchid mantis for capturing honeybee	**Takafumi Mizuno**
S8-O-02	Queen fire ant inhibits (primer pheromone) female sexual development, but newly-mated female sexuals need a quick colony foundation start	**Robert K. Vander Meer**

S8-O-03	Social and physiological factors affecting queen-worker pheromone interactions in honeybees	**Abraham Hefetz**
S8-O-04	Response profiles of sensory neurons in basiconic sensilla to cuticle hydrocarbons, key semiochemicals for nestmate discrimination in Japanese carpenter ant *Camponotus japonicas*	**Hidehiro Watanabe**
S8-O-05	Ants eavesdropping on the variational trail pheromone in termites leads to signal arms race between the predator and the prey	**Ping Wen**
S8-O-06	Multifunctional roles of soldier pheromone in a termite	**Yuki Mitaka**

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S9-O-01	Identification of pheromones of the potentially invasive beetles *Callidiellum villosulum* and *Allotraeus asiaticus*	**Jacob D. Wickham**
S9-O-02	Use of pheromones for detection and monitoring of native and invasive cerambycid beetles	**Jocelyn G. Millar**
S9-O-03	Volatiles released by Cerambycidae beetles used as chemical clues by the pine wood nematode to identify the insect vector inside the dead pine host trees	**Luis F. Bonifácio**
S9-O-04	Identification and field attraction test of aggregation pheromone of *Monochamus saltuarius*, insect vector of pine wood nematode in Korea	**Il-Kwon Park**
S9-O-05	Contact sex recognition pheromone of the juniper bark borer, *Semanotus bifasciatus* Motschulsky (Coleoptera: Cerambycidae)	**Xiang-bo Kong**
S9-O-06	Odorant receptors as molecular markers for pheromone use in the longhorned beetles (Coleoptera: Cerambycidae)	**Robert F. Mitchell**
S9-O-07	Function of pheromone binding proteins in olfactory recognition of two sympatric *Dendrolimus*	**Sufang Zhang**
S9-O-08	Stress-induced host tree chemistry benefits fungus farming by ambrosia beetles	**Christopher Ranger**
S9-O-09	Test trial for controlling Japanese oak wilt using living trees or mass accumulated oak logs with the aggregation pheromone and kiromone of the Ambrosia Beetle, of *Platypus quercivorus* (Coleoptera, Platypodidae)	**Masahiko Tokoro**

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S10-K-02	Insect herbivory selects for volatile-mediated plant communication in *Solidago altissima*	**Aino Kalske**
S10-K-03	The language of plant communication	**Richard Karban**
S10-O-01	The role of kin discrimination in interspecific competition in *Plantago asiatica*	**Akira Yamawo**
S10-O-02	Conversion of green leaf volatiles for processing the information from surrounding environments in tomato leaves	**Koichi Sugimoto**
S10-O-03	Pest management using plant-plant signalling mediated by mint volatiles	**Satoru Sukegawa**
S10-O-04	Applying plant-plant communication on rice field	**Kaori Shiojiri**
S10-O-05	Action of avenacins in oat roots as allelochemicals	**Tetsu Tsurushima**

Session 11
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S11-O-01	Spatial distribution of floral scent luring blow fly pollinators into inner cavity of *Rafflesia cantleyi*	**Suk Ling Wee**
S11-O-02	A sesquiterpene attractive to male and female Oriental fruit fly, *Bactrocera dorsalis*	**Alvin Kah-Wei Hee**
S11-O-03	Ecological contexts of mosquito odorant receptor function	**Jonathan D. Bohbot**
S11-O-04	What are stink bug male-produced pheromones doing on eggs? Or not.	**Jeffrey R. Aldrich**
S11-O-05	Defense allocation upon multiple stresses: Impacts of drought stress on performance of caterpillars and induced defense responses in tomato	**Po-An Lin**
S11-O-06	Secretion of isoflavones from soybean roots and their degradation dynamics in the rhizosphere	**Akifumi Sugiyama**
S11-O-07	Mechanisms of resistance to bufadienolide toxins in toad-eating snakes	**Alan H. Savitzky**
S11-O-08	The chemical analysis of bufadienolides in defensive glands of the Asian *Rhabdophis* snakes.	**Tatsuya Yoshida**
S11-O-09	Evolution of chemical mimicry in cuckoo wasp	**Thomas Schmitt**
S11-O-10	The biochemical mechanism underlying sex pheromone evolution in *Nasonia*	**Joachim Ruther**
S11-O-11	Where do they come from?: Can we use naturally occurring stable isotopes to better understand seasonal migration of insects.	**Jeremy N. McNeil**

Session 12
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S12-O-02	Genetic diversity, metabolic variation and functional life-history syndromes suggest multiple mechanisms facilitating invasion in a Brassicaceae species	**Lisa J. Tewes**
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S12-O-04	Host selection by the olfactory system in *Drosophila suzukii*: Can flies discriminate among fruits by smell?	**Claire Duménil**
S12-O-05	Olfactory responses of winter morph spotted wing *Drosophila* (*Drosophila suzukii*) to volatile semiochemicals	**Danielle Kirkpatrick**
S12-O-06	Identification of self-destructive defense system using a hemolymph enzyme, mandelonitrile oxidase, from the invasive millipede, *Chamberlinius hualienensis*	**Yuko Ishida**
S12-O-07	Identification of antennal olfactory receptor neurons and corresponding active compounds in tomato-potato psyllid, *Bactericera cockerelli*	**Kye Chung Park**
S12-O-08	Red-necked longhorn beetle, *Aromia bungii*, an invasive pest of Rosaceae trees: Present status of distribution and damage and monitoring trials in Japan	**Hiroe Yasui**
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Session 13
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S13-K-02	Deciphering allopolyploidy-mediated innovations in plant defense metabolism against insects using structural metabolomics	**Emmanuel Gaquerel**
S13-K-03	Multiple omics analysis of shikonin production system in *Lithospermum erythrorhizon*	**Kazufumi Yazaki**
S13-K-04	Omics everywhere: How about in chemical ecology?	**Ivan Galis**
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S14-K-03	Molecular basis of pyrethrum repellency in *Drosophila melanogaster*	**Ke Dong**
S14-O-01	Effects of floral scents and their memories on feeding preference of the fly	**Mamiko Ozaki**
S14-O-02	Bioactivities of cardanol derivatives isolated from *Anacardium occidentale* (Cashew) nut shell liquid against *Tribolium castaneum* Hebst (Coleoptera: Tenebrionidae) and *Sitophilus oryzae* L. (Coleoptera: Curculionidae)	**Thomas Buxton**
S14-O-03	Asian soybean rust-induced metabolites in a resistant soybean cultivar Hougyoku (PI 224270): Structure determination and antifungal activity evaluation	**Ryu Nakata**
S14-O-04	Elucidating the target of communesins, fungal metabolites acting as insecticides	**Akira Noguchi**
S14-O-05	Development of CO_2-releasing formulations for the control of soil-borne insect pests	**Anant V. Patel**

Session 15
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S15-O-01	Mating disruption and aerial releases of sterile codling moth in New Zealand: Is local eradication possible?	**David M. Suckling**
S15-O-02	Designing a mega-dispenser for sex pheromone mating disruption	**Tom Baker**
S15-O-03	Codling moth mating disruption 25 years on: How is it working, what's changed, what's new and what is still needed	**Don Thomson**
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S15-O-06	Production of moth sex pheromones in an oil crop	**Bao-Jian Ding**
S15-O-07	Electrospun mesofibers in precision viticulture : Joint integrated pest management on *Lobesia botrana* (Lep.: Tortricidae), and *Grapholita molesta*, in Germany and Brazil	**Simone S. Langner**
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S1-K-01

Microbes mediate plant perception of herbivores

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Plants are subject to attack by an onslaught of microbes and herbivores, yet are able to specifically perceive the threat and mount appropriate defenses. Plants have evolved two primary defense pathways: one regulated by jasmonic acid (JA), which defends against herbivorous insects, the other by salicylic acid (SA), which responds to microbial pathogens and is frequently antagonistic with JA. Chewing herbivores cause massive damage when crushing plant tissues with their mandibles, thus releasing an array of specific cues that may be perceived by the plant, which mobilizes plant defenses.

While specific cues in the oral secretions of herbivores such as caterpillars and beetles trigger plant defenses, we have found that bacteria associated with these secretions can trigger the SA pathway, which benefits the herbivore by suppressing JA regulated defenses. These results reveal a new strategy for how herbivores evade plant defenses by using symbiotic bacteria that deceive the plant into perceiving a herbivore threat as microbial, thus resulting in suppression of plant defenses against herbivores.

In another recent study, we have found that insect parasitoids that parasitize caterpillars may indirectly have a strong impact on plant defenses. Along with injecting an egg inside the caterpillar, the parasitoid injects symbiotic polydnaviruses, which disable the caterpillar’s immune system. As part of this immunosuppression, one component in the caterpillar’s saliva known to trigger plant defenses is nearly completely suppressed. These striking findings indicate that a symbiotic virus produced in parasitoids not only causes a massive suppression of the caterpillar’s immune system, but also suppresses the plant’s immunity or defenses against herbivores.

These findings indicate that microbes are the “hidden” players in mediating plant-herbivore interactions. Our evidence from several plant-herbivore systems indicates that insect-associated microbes can have a profound effect on the ability of a plant to perceive herbivores and thus trigger plant defenses.

S1-K-02

Miracle maize is toxic to three different herbivore feeding guilds

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The maize inbred line Mp708 developed by conventional plant breeding displays a suite of resistance traits to three types of insect pests: whorl, phloem and root feeding herbivores. Initially, Mp708 was bred to have superior resistance to feeding by fall armyworm (FAW, *Spodoptera frugiperda*) and other lepidopterans. Resistance to these caterpillars is a quantitative trait regulated by several genes. One gene contributing to FAW resistance encodes the cysteine protease Mir1-CP. This protease accumulates in the maize whorl in response to herbivory and is toxic to FAW at 0.6 ppm. Mir1-CP permeabilizes the caterpillar peritrophic matrix (PM) and specifically attacks the PM protein, Insect Intestinal Mucin (IIM). The combination of a damaged PM and the caterpillar's attempts to repair it, severely stunts growth.

Further studies indicated that Mir1-CP is localized in the thick-walled sieve elements of the maize phloem and constitutively present in roots. Root removal prior to herbivory reduces the accumulation of Mir-1CP in the whorl suggesting that it is transported from the roots to whorl via the vascular system. Because of Mir1-CP's location in the phloem, we examined the resistance of Mp708 to the corn leaf aphid (CLA, *Rhopalosiphum maidis*). Mp708 limited the aphid population and deterred their settling. Transcripts of *mir1* increased within 1 hour of infestation and recombinant Mir1-CP was toxic to CLA. This process appeared to be regulated by ethylene and not jasmonic acid (JA) signaling pathways.

The localization of Mir1-CP in maize roots, prompted us to ask if Mp708 was resistant to root-feeding herbivores. Studies suggested that Mp708 was more resistant to western corn rootworm (WCR, *Diabrotica virgifera virgifera*) than one of its parental lines, Tx601. Significantly fewer WCR larvae were recovered from Mp708 than Tx601 and weighed less than those recovered from Tx601. Mp708 has mechanically stronger nodal roots that are more resistant to cutting than Tx601. Constitutively elevated levels of JA and defense transcripts regulated by JA implicate this pathway in Mp708's resistance phenotype. Furthermore, *mir1* transcripts increased dramatically in response to WCR infestation implicating Mir1-CP in resistance.

Mp708 was developed from landraces of maize that likely originated in Mesoamerica, where many phytophagous maize pests including WCR, FAW and CLA have originated. One could speculate that multiple generations of selection for adequate yield despite intense insect pressure led to the incorporation of multiple resistance traits into these landraces, which ultimately were incorporated into Mp708 by selective breeding.

S1-K-03

Why are terrestrial ecosystems green? Why are anti-nutritive and inducible defenses effective?: A novel parameterized food web model and its implications to chemical ecology and plant-herbivore interactions

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Despite decades of chemical, physiological, and molecular studies on proximate mechanism of plant-herbivore interactions, there remain some very important but unanswered questions related plant-herbivore interactions. For example, chemical ecology has revealed perfect adaptations of specialist herbivores to plant defenses. Then, why are terrestrial ecosystems still green with vegetation and only a small part (<10%) of the total plant matter is consumed by herbivores annually, which means herbivore density is consistently low and stable? This plant-rich pattern called “The Green World” is common and persistent around the world wherever and whenever (the Paleozoic to the present) precipitation and temperature is adequate. Nonetheless, the mechanism underlying this pattern has not been explained. Another unanswered question is about the function of anti-nutritive plant defenses such as protease and amylase inhibitors, oxidized phenolics, tannin, iridoid aglycone, deaminases, low nutritive value, etc,. Anti-nutritive defenses reduce digestion and/or uptake of nutrients, and thus slowdown the growth rate, but do not normally kill herbivores. Although so-called “anti-nutritive defenses” are very common among plants, the reason why they decrease the damage by herbivores and protect plant is not well explained; if they decrease the digestion/absorption rates of nutrients into half, herbivore may eat twice to fulfil the necessity, which means that the damage of the plant may become twice if so-called “anti-nutritive defenses” are present, and they cannot function as defenses any more, which is a paradox.

In order to solve these unanswered questions, I created, for the first time, a fully-parameterized mathematical food-web model describing three-trophic-level systems that can predict the biomass density of herbivores **h** (kg protein/m^3) and carnivores **c** from factors such as nutritive values of plants n_p (kg protein/m^3), herbivores n_h, and carnivores n_c, searching efficiency (volume) of carnivores S (m^3/m^{3}day=/day), eating efficiency (speed) of herbivores e_h (m^3/m^{3}day=/day) and carnivores e_c, respiratory decreases in biomasses, d_h (kg/kg day=/day) and d_c, absorption efficiencies α_h (ratio) and α_c, and probabilities of carnivores preying on herbivores or carnivores, P_{hc} (ratio) and P_{cc}. The model predicts a stable convergent equilibrium with biomass densities of herbivore $\mathbf{h}=n_c\{(1-\alpha_c)P_{cc}(\alpha_h e_h n_p - n_h d_h)+2d_c P_{hc}\}/2\alpha_c P_{hc}^2 n_h S$ (kg/m^3) and carnivore $\mathbf{c}=n_c(\alpha_h e_h n_p - n_h d_h)/P_{hc} n_h S$ (kg/m^3) with low herbivore biomass **h** sufficient to keep the world green provided; intraguild predation exists but is rarer than interguild predation ($P_{hc}>P_{cc}>0$); plants are less nutritive than animals ($n_p<n_h,n_c$); and carnivore searches huge volume daily while eating speed of herbivore is limited ($S>>e_h$). These conditions are well-realized in above-ground terrestrial ecosystems where plant-rich “green worlds” are common, versus animal-rich belowground and aquatic ecosystems where some conditions are not realized. The **h** and **c** calculated from our model agreed well with those from empirical observations in forest ecosystems, where both **h** and **c** are within an order of magnitude of 100 mg (fresh biomass/m^2 forest), and in savannah ecosystems. The model predicts that plant nutritive value n_p, digestibility of plants by herbivores α_h, and herbivore eating speed e_h are positively correlated with **h** and the intensity of herbivory, which theoretically explains the out-door defensive effects of plant anti-nutritive or quantitative defenses. The model also suggests the consequences of sporadically high or low nutritive values of plants on intensity of herbivory, which may be important to understand the functions of inducible defenses.

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Mechanisms underlying cardenolide sequestration in a highly adapted herbivorous insect

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Sequestration of ingested host plant toxins is a highly specialized strategy of herbivorous insects to profit from plant chemical defense compounds. The storage in specialized structures brings two advantages: the toxins are removed from sensitive body tissues and sequestered compounds can act as predator deterrents. The large milkweed bug, *Oncopeltus fasciatus* (Hemiptera: Lygaeidae), is a prime example for handling host plant derived cardenolides. On one hand, the species evolved insensitive Na,K-ATPases that can no longer be inhibited by the toxins. On the other hand, ingested toxins are efficiently enriched in a specialized thoracic storage compartment and discharged upon attack for the bug's defense. Chemical analysis of the sequestered defense fluid shows that polar cardenolides are preferentially and rapidly transferred into the storage compartment in a time dependent manner. Yet, polar cardenolides are not able to cross membranes passively on a large scale. Therefore we hypothesized that active transport mediated by carrier proteins plays a role. This hypothesis is supported by the observed efficient sequestration of ouabain, a polar cardenolide, when injected into the hemolymph. In addition, the uptake is not impaired by high amounts of natural cardenolides already present in the storage compartment.

One of the rare examples for carrier proteins known from mammalian systems to transport cardenolides are ATP-binding cassette transporters of the subfamily B (ABCB transporters). We therefore investigated whether they could also be major players in the sequestration of cardenolides in *O. fasciatus*. By bioinformatic analysis of a transcriptome of *O. fasciatus* two ABCB full-transporters (ABCB1 and ABCB2) could be identified. Tissue specific expression analysis and histological investigations support their potential role in protecting the sensitive nervous tissue against cardenolides. Even more strikingly a combination of RNAi mediated gene silencing of the transporters and cardenolide injection resulted in a drastically reduced sequestration efficiency. Thus, our results provide evidence that (i) cardenolide sequestration in *O. fasciatus* relies on active transport mechanisms, (ii) ABCB proteins are major players in this process and (iii) their direct involvement in the transport of polar cardenolides.

Dynamics and origin of cytokinins involved in plant manipulation by a leaf-mining insect

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Several herbivorous insects and plant-associated microorganisms control the phytohormonal balance, thus enabling them to successfully exploit the plant by inhibiting plant defences and withdrawing plant resources for their own benefit. The leaf-mining moth *Phyllonorycter blancardella* modifies the Cytokinin (CK) profile of mined leaf-tissues and the insect symbiotic bacteria *Wolbachia* is involved in the plant manipulation to the benefit of the insect host. To gain a deeper understanding into the possible origin and dynamics of CKs, we conducted an extensive characterization of CKs in larvae and in attacked apple leaves. Our results show that mines are enriched in CKs both on green and yellow leaves, allowing insects to control their nutritional supply under fluctuating environmental conditions. The spatial distribution of CKs within attacked leaves show that plant manipulation is strictly limited to the mine suggesting the absence of CK translocation from distant leaf areas towards the insect feeding site. Analyses revealed that major CK types accumulating in mines and larvae are similar to what is observed for most gall-inducers, suggesting that strategies underlying the plant manipulation may be shared between herbivorous insects with distinct life histories. They further show that CKs are detected in the highest levels in larvae reinforcing our hypothesis that CKs accumulating in the mines originate from the insect itself. Presence of bacteria-specific methylthio-CKs is consistent with previous results suggesting that insect bacterial symbionts contribute to the observed phenotype. Our study provides key findings towards the understanding of molecular mechanisms underlying this intricate plant-insect-microbe interaction.

Keywords: Cytokinins, Insect bacterial symbionts, Leaf-miners, Phytohormones, Plantinsect-microbe interactions, Plant manipulation

S1-O-03

Indirect manipulation of plant induced defenses by parasitoids of caterpillars

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Plants possess diverse defense mechanisms against herbivores that include defensive proteins and secondary metabolites that impair herbivore growth and survival. The oral secretions (regurgitant and saliva) of insect herbivores play a crucial role in providing cues that trigger plant defense responses. However, we contend that the interaction between plant and insect is considerably more complex because other trophic levels are involved in nature. Our research focuses on how the third trophic level (parasitoid) influences plant perception of the insect's oral cues. The main hypothesis is that parasitized (P-) caterpillars induce lower plant defense responses than healthy (H-), non-parasitized caterpillars. Consequently, this suppression of defenses can benefit both the plant and parasitoid by minimizing plant defense costs and providing a better host quality for the parasitoid. *Helicoverpa zea* caterpillars were parasitized by its solitary parasitoid, *Microplitis croceipes.* Glucose oxidase (GOX) and ATP hydrolyzing enzymes (ATPases), two important salivary cues that mediate plant defense expression, were dramatically affected in P-caterpillars compared to H-caterpillars. Tomato (*Solanum lycopersicum*) plants fed by P-caterpillars had significantly lower induced defense responses compared to plants fed on H-caterpillars. In bioassays, *H. zea* caterpillars grew larger on P-caterpillar treated plants. In addition, the plants damaged by P-caterpillars have higher fitness (flower number and fruit weight) than those damaged by H-caterpillars. These results support our hypothesis that plants can distinguish feeding between healthy and parasitized caterpillars and alter their defense response accordingly. This study provides novel evidence of how the third trophic level influences plant perception of insect herbivores, and new perspective of mutualism between plants and parasitoids.

Honeybees modulate dance communication in response to nectar toxicity and demand

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Some honeys such of that of the Thunder God Vine *Trypterygium wilfordii* contain alkaloids that are mildly toxic to honeybees (*Apis* spp). Given a choice, foragers prefer non-toxic honey to that of *T. wilfordii*, but only if there are no alternative nectar sources. Here we show that foragers collecting *T. wilfordii* honey modulate their recruitment dances depending on the availability of alternatives. When alternative nectar sources are available, dancers decrease the frequency of waggle dances and increase the frequency of negative tremble dances. Further, the waggle dances are less precise than normal. These changes reduce recruitment. However when there are no alternative nectar sources available, foragers collecting *T. wilfordii* honey perform near-normal dances. Because dance behaviour is dependent on what alternative food sources are available, changes in the bees' behaviour is not due to the nectar's toxicity *per se*. This conclusion is supported by the fact that the same changes in dance behaviour can be induced by including the toxic component of *T. wilfordii* nectar in sucrose syrup in low amounts. We conclude that modulation of in-hive communication serves to protect the colony from death caused by the collection of high quantities of toxic food while preventing starvation when no other food is available.

Keywords: *Apis cerana*, waggle dance, communication, foraging tradeoffs

Being punctual: The coordination of a plant-pollinator interaction by the circadian clock.

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Petunia flowers emit scents at night[1], which attract nocturnal pollinators such as *Manduca* hawkmoths. Using these organisms as models, we analysed their respective circadian clocks' involvement in regulating this nocturnal meeting. In *Petunia*, we show that a core clock component, LATE ELONGATED HYPOCOTYL (LHY), transcriptionally represses genes involved in a key floral volatile metabolic pathway during the daytime, restricting scent production to the night[2]. This work was accomplished in transgenic *Petunia* lines using a combinatorial analysis of gene expression (qPCR), volatile collection (GC-MS), and protein-DNA interaction (EMSA). In *Manduca*, using a combination of actograms, behavioural choice assays, and electroantennograms, we show that that both the clock and light are critical for moth foraging activity, and that sensitivity to floral odours is possibly modulated by the clock. Altogether, our experiments highlight the critical role that the clock plays in organizing interactions between plant and pollinator.

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S2-K-01

Recent results in pheromone synthesis

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The female-produced sex pheromone of the pineapple mealybug *Dysmicoccus brevipes* (Cockerell) [Homoptera: Pseudococcidae] was identified as (*anti*-1,2-dimethyl-3-methylenecyclopentyl)acetaldehyde on the basis of its spectral analysis and racemic synthesis. Its absolute configuration is now established as 1*R*,2*S* by synthesizing its enantiomers and comparing their chiroptical as well as biological properties with those of the natural pheromone.

The synthesis started from (±)-2,3-dimethyl-2-cyclopenten-1-ol. Its asymmetric acetylation with vinyl acetate and lipase AK afforded the corresponding (*R*)-acetate and the recovered (*S*)-alcohol. The (*S*)-alcohol was acetylated, and the resulting (*S*)-acetate was subjected to the Ireland-Claisen rearrangement to give (*R*)-(1,2-dimethyl-2-cyclopentenyl)acetic acid. Further functional group transformation and chromatographic separation furnished (1*R*,2*S*)-(-)-(1,2-dimethyl-3-methylenecyclopentyl)acetaldehyde. Similarly, (*R*)-1-acetoxy-2,3-dimethyl-2-cyclopentene yielded (1*S*,2*R*)-(+)-isomer. The levorotatory (1*R*,2*S*)-synthetic pheromone was identical with the natural one on the basis of its specific rotation and pheromone activity. Details of the synhthesis will be reported in *Tetrahedron*. K.M.'s thanks is due to Dr. J. Tabata for the identification and bioassay of the synthetic materials.

An explosion of diversity in cerambycid beetle pheromones: Identifications and syntheses

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Great progress has been made in the chemical ecology of longhorned beetles (Coleoptera: Cerambycidae) over the past 15 years, including the identification of volatile sex and aggregation pheromones for numerous species, and a better understanding of the interaction of pheromones with host plant volatiles. To date, pheromones have been formally identified from ~100 species. Likely pheromones have been tentatively identified from numerous other species based on their strong responses to test compounds in field bioassays. We and others had hypothesized that pheromone structures appeared to be highly conserved within closely related taxonomic groups (genera and tribes), based on the fact that only ~15 compounds were known or likely pheromone components for >> 100 species. Furthermore, some of these 15 compounds were homologs (e.g., 3-hydroxyalkan-2-ones of 6, 8, or 10 carbons). However, in the past several years, an increasing number of entirely new pheromone structures have been identified from various species, including saturated and unsaturated straight-and branched-chain aldehydes, a pyrrole, a pyrone, an alkyl-substituted tetrahydropyran, an alkene, several oxygenated monoterpenoids, and a new hydroxyketone motif. The identification and syntheses of representatives of these new structures will be described, along with a revised hypothesis relating the chemistry to the phylogeny of the family.

S2-0-01

GC/FT-IR analysis of a novel 2,4,6,9-tetraene occurring in a female pheromone gland of *Parasemia plantaginis* (Lepidoptera: Arctiidae)

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IR spectrometer is useful to determine functional groups of an unknown compound, but its utilization for pheromone researches is very limited because of its low sensitivity. Meanwhile, a new type of GC/FT-IR (Discover IR, Spectra Analysis) has been developed by applying a system with a cooled and turned disk, where compounds elute from a capillary GC column are fixed and irradiated with an IR beam. This FT-IR measurement is carried out sensitively as well as GC-MS. We had successfully used it to identify a terminal conjugated dienyl pheromone produced by the nettle moth.[1] As a next challenge, it was tried to apply it for structure determination of a tetraene found in a female pheromone gland of *Parasemia plantaginis*, the wood tiger moth, in the family Arctiidae. Noteworthily, 15 subspecies of this species have been recorded in Northern Hemisphere. We are interested in their mating communication systems and started to investigate the sex pheromone from the females distributed in Japan and Finland.

GC-EAD analysis of a pheromone extract of the Japanese subspecies showed four EAG-active components (**I** – **IV**). GC-MS analysis utilizing synthetic standards revealed their chemical structures of C_{21} unsaturated hydrocarbons as follows; 3,6,9-triene for **I** (most abundant component), 4,6,9-triene for **II**, and 1,3,6,9-tetraene for **III**. The mass spectrum of **IV** indicated it also to be a C_{21} tetraene, but its retention time on GC is much longer than that of the 1,3,6,9-tetraene suggesting a longer conjugated system than a conjugated diene. Since **IV** is expected to include two double bonds at the 6- and 9-positions in common with other three components, we tentatively assigned a novel 2,4,6,9-tetraenyl structure for it. Then, GC/FT-IR analysis was conducted in order to determine configuration of the double bonds at the 2- and 4-positions. While the content of **IV** is very low, its IR spectrum with absorption at 3019 cm^{-1} due to C-H stretching of two (*Z*)-double bonds at the 6,9-dienyl moiety was successfully recorded. This spectrum also showed characteristic weak absorptions at 993 and 942 cm^{-1} due to C-H bending of one (*E*)-double bond at the 2,4-dienyl moiety. Intensities of these absorptions excluded the possibility of the (*E,E*)-conjugated system, and the (*Z,Z*)-conjugated system was denied because of no absorption around at 3040 cm^{-1}. As a result, we estimated (2*Z*,4*E*,6*Z*,9*Z*)-configuration for **IV**. This GC/FT-IR experiment also confirmed (*Z*)-double bonds of **I** – **III**.

Similar analytical data was obtained from the extract of a Finnish subspecies. Furthermore, (2*Z*,4*E*,6*Z*,9*Z*)-2,4,6,9-henicosatetraene was synthesized utilizing a double Wittig reaction. It showed a EAG activity and all of its chemical data coincided well with those of the natural component **IV**.

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S2-O-02

Cytochrome P450s are involved in iridoid biosynthesis in leaf beetles

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Iridoids are a variety of natural derived cyclopentapyran ring monoterpenoids with a range of pharmacological properties. However, the extremely low production has greatly hampered their popularization. Biotechnology-based production has been proposed as a potential alternative option. Therefore, their biosynthesis pathway in Madagascar periwinkle, *Catharanthus roseus*, the iridoids-producing plant, has been intensively studied. Conversely, attention to another iridoids-producing organism, juvenile mustard leaf beetle has been limited. *Phaedon cochleariae* (Chrysomelidae, Chrysomelina), a specialized herbivore, *de novo* synthesize chrysomelidial to protect themselves from predators. Previous studies indicated that early stage precursors geraniol and glucosidically bounded 8-hydrogeraniol are accumulated firstly in fat body tissue and then transferred via the hemolymph into the glandular reservoir for further conversion into the biological active iridoids. It's hypothesized that, like its plant counterpart, *P.cochleariae* recruits cytochrome P450 monooxygenases to catalyze geraniol to 8-OH-geraniol. However, whether it's the case remains exclusive. Based on combined proteomic and transcriptome analysis, we showed that *P.cochleariae* possesses 87 cytochrome P450s in the fat body tissue. Furthermore, RNAi and metabolites analysis revealed one cytochrome P450 is involved in *P.cochleariae* chrysomelidial biosynthesis. These findings broaden our knowledge on the crucial enzymes responsible for iridoids biosynthesis, which are important catalysts for industrial iridoids production.

Elucidating the biosynthesis of the aphid sex pheromone

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Aphids (Homoptera: Aphididae) are a prolific pest of agricultural and horticultural crops in temperate climate regions around the globe, causing damage both directly and indirectly through feeding and plant virus transmission, respectively [1]. A sex pheromone blend, comprising a mixture of the iridoids (4a*S*,7*S*,7a*R*)-nepetalactone (I) and (1*R*,4a*S*,7*S*,7a*R*)-nepetalactol (II) and common to most aphid species, is released by female oviparae to attract conspecific males [2]. Compounds I and II are also found in many plant species, e.g. *Nepeta* spp. (Lamiaceae) and *Catharanthus* spp. (Apocynaceae), and there is considerable research into the plant-derived biosynthesis of I and II due to their positioning as precursors to many pharmacologically active compounds, including the indole alkaloids [3].

To test the hypothesis that aphids biosynthesize I and II *de novo* similarly to that elucidated for plants, isotopic labelling techniques have been deployed with oviparous pea aphids, *Acyrthosiphon pisum*. Deuterium-labelled putative sex pheromone precursors have been synthesized, and sex pheromone components released by *A. pisum* following incubation with the precursors have been collected by dynamic headspace collection. The collected pheromone components have been analysed by gas chromatography coupled to mass spectrometry (GC-MS), to confirm the presence and position of labelling.

Successful deuterium incorporation in the sex pheromone components has been demonstrated on a highly precursor-specific basis, with initial results providing a basis for further strategic labelling. With the position and extent of labelling providing information on the biosynthetic route to I and II, and on the mechanisms underlying their formation, results have allowed a comparison to the biosynthetic mechanisms to I and II in plants and provided an indication as to the route by which aphids produce these compounds.

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S2-O-04

Anti-aphrodisiac pheromones of *Heliconius erato phyllis* and *Heliconius ethilla narcaea* (Nymphalidae, Heliconiinae)

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Lepidoptera of the *Heliconius* genus are known for association with passion vine. In subfamily Heliconiinae the interspecific communication through sex pheromones benefits the sexual encounter, the court, the protection of offspring and newly mated female. This work studied the abdominal scent glands that are present in male valves, which volatiles are transferred during copulation to the abdominal scent gland of the female, an inflatable bag, displayed during the courtship behavior and defense situations. The results of morphological studies took to a new description of the genitalia and glandular structures of males and females, which may be related to the dissipation of volatiles. The male gland of the two subspecies is in the middle portion of the inner region of the valve, as an inflated translucent bag formed by a very fine and delicate tissue, covered by long bristles. The female gland of the two subspecies is composed of one bag present dorsally and laterally in the final portion of the abdomen, and is composed of a very delicate tissue, with translucent projections on the surface of its lobes. In *H. erato phyllis* female gland the lobes are vertically smaller and the translucent projections closer than in *H. ethilla naracaea*, and the *H. erato phyllis* male gland present glandular tissues with a darker coloration. The compounds 1hexen-3-one, 1-octen-3-one, (*E*)-β-ocimene, 3-undecanone, and (*E*)-2 3-dihydrofarnesoic acid were identified in the abdominal scent glands bouquet of *H. erato phyllis*. A sixth compound was additionally detected and its structure is proposed as an unsaturated methyl branched macrolide, a new natural product. The synthesis of the macrolide is underway in order to confirm the proposed structure. This compound elicits the greatest electroantennogram response, and may be the compound that is most important for identifying males. Bioassays with virgin females proved the anti-aphrodisiac function of odoriferous bouquet of *H. erato phyllis* male valve. The compounds (*E*)-β-ocimene, (*Z*)-henicos-9-ene, henicosane, ethyl (*Z*)-octadec-9-enoate, (*Z*)-tricos-9-ene, icosan-1-ol, butyl (*Z*)-octadec-9-enoate and docosan-1-ol were identified in the abdominal scent glands bouquet of *H. ethilla narcaea*. Electroantennographic and bioassays analyzes are in progress to confirm the biological function.

S2-0-05

Host plant affects the sexual attractiveness of the female white-spotted longicorn beetle, *Anoplophora malasiaca*

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Anoplophora malasiaca (Coleoptera: Cerambycidae) is a serious pest of horticultural crops, such as citrus, apple, and pear, as well as street trees, such as the oriental plane tree and willow. This species has a very wide host plant range, which includes 108 known species of trees belonging to 73 genera. The control of this species is highly desirable in Japan, as is the case with *A. glabripennis* and *A. chinensis* in North America and Europe.

Recently, extensive progress has been made in understanding their mate location and recognition system in adults collected from mandarin orange (MO), blueberry (BB), and willow (WI). Males are attracted to volatile chemicals from wounded plants of their respective host species that are released close to a female dummy. We examined which chemicals attract mate-seeking males in MO, BB and WI population, respectively. These chemicals are equally emitted from artificially wounded branches and branches damaged by the adults' feeding. Additionally, volatiles from their original host plants attract males but not females. Therefore, we hypothesized that these volatiles may indicate the presence of conspecific females. Because many of their hosts are sympatric, these beetles may encounter potential mates on other host plant species in addition to their original host. Understanding the differences in responses among populations could provide substantial insights into the background and mechanisms of natural host shifts. When a male *A. malasiaca* encounters a female, a mating sequence occurs. Female elytra extracts include contact sex pheromone which evoke male mating behavior, and the components have already been identified in females of an MO population.

We previously observed abdominal-bending behaviors of males from the three wild populations (MO, BB, and WI) toward glass dummies coated with elytra extracts from females of the three populations. During the experiments, we observed that considerable numbers of males in the BB and WI populations turned away from dummies coated with the extract of the MO population's females immediately after touching their antennae to the dummies. Because all three of the female populations produced contact sex pheromones on their elytra, the females of the MO population were predicted to possess extra chemicals that repelled the males of the other two populations.

To avoid complications from only using field-collected individuals, we also designed experiments with laboratory-reared individuals. They were fed the same diet (a mulberry leaf-based artificial diet) during their larval stage and were then fed WI or MO after adult emergence (WI-fed and MO-fed individuals). Same mating behaviour bioassays were conducted on laboratory-reared individuals, and rejection responses of WI-fed male to MO-fed females were also observed.

β-Elemene was identified as a key component that was only found in mandarin MO-fed females and induced a rejection response in WI-fed males. Our results represent the first example of a female-acquired repellent against conspecific males of different host plant populations, indicating that the host plant greatly affects the female's sexual attractiveness.

S2-O-06

A shift in the paradigm of arthropod cuticular lipids: Isolation, structural elucidation, and asymmetric synthesis of an unusual tetraterpenoid hydrocarbon from the springtail *Hypogastrura viatica* (Hexapoda: Collembola)

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Insect lipids are generally composed of a complex blend of simple aliphatic compounds biosynthesized via the fatty acid synthase pathway and are known to have numerous functions in insect behavior. Although closely related to insects, very little is known of the lipids of springtails (Collembola). Previous studies of collembolan lipids have identified unusual natural products that differ from insect lipids in both structural complexity and biosynthetic origin [1]. Here we will report a new class of tetraterpenoid compounds isolated from the cuticular lipid extracts of *Hypogastrura viatica* (Hexapoda: Collembola.) This highly branched tetraterpenoid compound, containing five chiral centers and unusual connectivity, was structurally elucidated via GC-MS, high-resolution mass spectrometry, micro-chemical derivatization techniques, as well as 1-D and 2-D NMR spectroscopy. Chemical synthesis of the core segment of the molecule was performed to confirm the structure. Asymmetric synthesis of the molecule's core segment, coupled with chiral-GC analysis of both the synthetic and natural core segments, gave insight into the stereochemistry of this novel arthropod lipid. We also report the structural elucidation of several other unusual tetraterpenoid and tetraprenylsesquiterpenoid compounds from the lipid extracts of the springtails *Podura aquatica, Xenylla grisea,* and *Anurophorus laricis*. Our result marks a shift in the paradigm of arthropod cuticular lipids. Tetraterpenes with unprecedented biosynthesis appear to replace fatty acid derived hydrocarbons as the major constituents of cuticular lipids for several species of springtails from different phylogenetic lineages.

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S2-O-07

Identification of the aggregation pheromone of the American cockroach

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The American cockroach, *Periplaneta americana*, is well-known for the production of its sex pheromone, periplanones-A and -B, used in the courtship communication (Persoons et al., 1979). It is also reported that the insect uses an aggregation pheromone to locate a shelter (*e.g.* Bell et al., 1972). However, the structure of the aggregation pheromone of *P. americana* has been unknown for almost five decades probably because of behaviourally undiscriminating assay methods and difficulty in separating a minute amount of the pheromone from miscellaneous chemicals in excreta. We focused on the attractiveness for nymphs in T-maze olfactometer (Sakuma and Fukami, 1975). We obtained active materials from 176 kg of frass and isolated six new compounds as the aggregation pheromone of the species. The major component of the pheromone is an isocoumarin periplanolide (PLD)-1 or 8-hydroxy-5,7-dimethyl-3-(1-methylpropyl)-isochroman-1-one with its minor homologue PLD-2. The planar structures of the compounds were estimated based on the extensive 2D-NMR, and the absolute configurations were established by the subsequent chiral specific HPLC analyses and ECD spectroscopy with reference to stereo-specifically synthetized materials (Nishimura et al., 2015). The olfactometer assay on *P. americana* nymphs revealed that the isolated stereoisomers of synthetic PLDs demonstrated almost the same attractant activity in ED50 values at 2.4 - 3.5 pg (PLD-1) and 1.9 - 4.6 pg (PLD-2), as that of natural products at 4.4 pg (PLD-1) and 2.2 pg (PLD-2), respectively.

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The chemical ecology of plant-animal-microorganism interactions

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Plants and animals intimately interact with bacteria and other microorganisms and are thus well adapted to these organisms. I am testing the hypothesis that non-pathogenic bacteria are not only passive commensalists but massively interfere with animal-plant interactions and thus may pursue their own interests. I will present results from ongoing projects testing the influence of bacteria on plant-animal interactions. We found that animals behaviourally respond to bacteria-induced changes in plants’ scent emissions and that bacteria associated with animals directly affect the behaviour of their hosts. Additionally, we showed that plant volatiles shape bacterial communities by either serving as a carbon source and thus facilitate growth of some strains or by inhibiting the growth of other strains. These results strongly suggest that bacteria are of strong ecological and evolutionary importance in animal-plant interactions.

S3-K-02

Activated Chemical Defense of Japanese Marine Sponge *Discodermia calyx*

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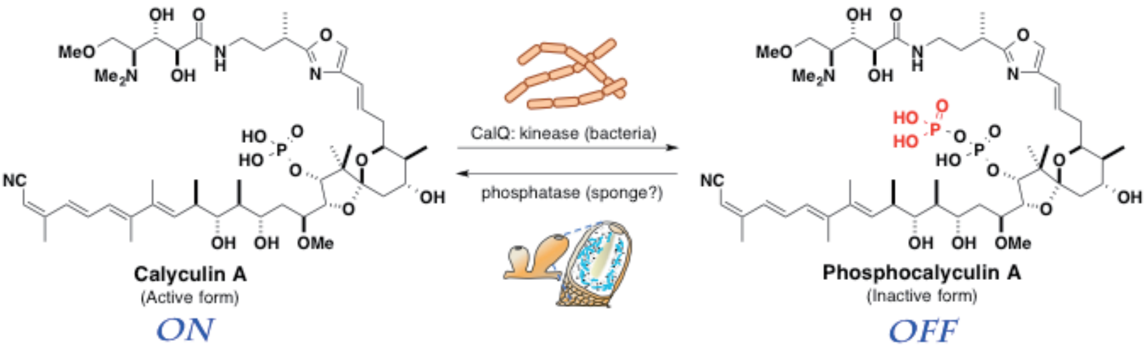
Marine sponges are prolific sources of highly potent cytotoxic compounds, some of which are promising as anticancer drugs. However, the potent cytotoxicity would be harmful to the host sponge, because marine sponges are also animals. Therefore, it is likely that the self-resistance or activated defense systems underlie their biosynthetic mechanism. Wound-activated chemical defense systems are prevalent among terrestrial plants and algea.[1] The precursor molecule and the activating enzyme are compartmentalized in the tissue, which is readily disrupted by wounding to accomplish chemical defense. The similar activated defense strategy has also been found in a few marine sponges. The same strategy in marine sponges was first reported for the isoxazoline alkaloids in the genus *Aplysina*.[2] This defense mechanism might be more widespread in marine sponges than currently reported, but overlooked due to restricted methodological approaches as well as a lack of knowledge about the biosynthetic pathways of defensive chemicals. In this study, we obtained the biosynthetic gene cluster of natural phosphatase inhibitor, calyculiln A from the metagenomic DNA of Japanese marine sponge *Discodermia calyx* and the deactivating enzyme was found to be clustered and encoded in the sponge symbiont.[3] In the ecological context, the sponge symbiont 'Entotheonella' produces phosphocalyculin A as a protoxin, which is likely to be less toxic to the host sponge cells. Once the sponge tissue is wounded, such as by predators, phosphatase and phosphocalyculin A are decompartmentalized to generate calyculin A in a quick and site-specific manner. This activated system meets both requirements: to avoid self-toxicity and to function as a chemical defense for a sessile animal. It is noteworthy that the activity of the phosphatase inhibitor calyculin A itself is regulated by enzymatic phosphorylation and dephosphorylation in the sponge-microbe association.



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Volatile affairs in microbial belowground interactions

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Many secondary metabolites have been reported to be involved in the microbial interactions. One group of secondary metabolites produced by soil and plant-associated microorganisms but largely unexplored to date, are the volatile organic compounds (VOCs). Microbial VOCs (mVOCs) are typically small compounds (up to C15) with low molecular mass (< 300 Da), high vapour pressure, low boiling point, containing a lipophilic moiety. These properties facilitate evaporation and diffusion above-ground, as well as below-ground through both water- and gas-filled pores in soil and rhizosphere environments.

mVOCs were often considered to be side products of primary metabolism, but our recent finding revealed that many mVOCs demonstrate biological activity [1,2,3,4] and the production of certain bacterial VOCs is depended on the GacS/GacA two-component regulatory system [5]. Hence, these findings disagree with the viewpoint that mVOCs are a waste products.

Here I will present our recent discovery about the role of mVOCs in the belowground microbe-microbe and microbe-plant interactions. Furthermore I will indicate challenges in studying belowground mVOCs mediated interactions and opportunities for practical applications.

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S3-O-01

Comparison of secondary metabolic gene evolution in microorganisms from different extreme environments

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Microorganisms from extreme environments present a potential source for novel bioactive secondary metabolites. These metabolites are thought to have an important role as signals in communication of microbial communities. Less is known about the effect of extreme environmental conditions on the evolution of these compounds. In this study, we performed *in silico* analysis of genomes of archaea and bacteria from hypersaline, high-temperature and alkaline environments in order to screen for the presence of gene clusters for secondary metabolite biosynthesis. Next, we analyzed the phylogeny of these genes, as well as selected housekeeping genes. Finally, considering that mutation rates may be related to the selective pressure we studied the mutational rates of secondary metabolic and housekeeping genes in context of environmental background. In this presentation, we report the results and discuss the implications of our study.

S3-O-02

Roles of the DSF-family quorum sensing signals in cell-cellcommunication

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Quorum sensing (QS) is a cell-to-cell communication mechanism that is widely employed by bacterial cells to coordinate communal behavior, such as the regulation of virulence, biofilm formation, motility and antibiotic production. QS involves the production and detection of diffusible signal molecules and the initiation of appropriate responses in a cell density-dependent manner. The original concept of QS was developed based on N-acylhomoserine lactone (AHL) signal molecules, which are constitutively produced at basal levels until they reach a critical threshold concentration and then bind to and activate a cognate receptor to control target gene expression. Recently, diffusible signal factor (DSF)-type QS signals have been recognized as another important and common type of QS system. DSF, which was originally identified in *Xanthomonas campestris* pv. *campestris*, is involved in regulating biofilm dispersal, motility and virulence. Accumulating evidence suggests DSF-type signals are widespread in bacteria and play an important role in cell-cell communication. Besides the significance on intraspecies signaling, our studies also showed that the DSF-family signals appear their critical importance roles through interspecies and inter-kingdom communication. It was revealed that *Candida albicans* germ tube formation was strongly inhibited by exogenous addition of physiological relevant level of both DSF and BDSF. Moreover, BDSF from *Burkholderia cenocepacia* and *Stenotrophomonas maltophilia* was found to be involved in modulation of virulence, antibiotic resistance and persistence of *Pseudomonas aeruginosa* in the cystic fibrosis airway. Recently, we have demonstrated that DSF and its structurally related molecules could be used to induce bacterial antibiotic susceptibility. Exogenous addition of DSF or its structural analogues could significantly increase the antibiotic susceptibility of *Bacillus cereus*, possibly through reducing drug-resistant activity, biofilm formation and bacterial fitness. The synergistic effect of DSF and its structural analogues with antibiotics on *B. cereus* is dosage-dependent. Combination of DSF with gentamicin showed an obviously synergistic effect on *B. cereus* pathogenicity in an in vitro model. We also found that DSF could increase the antibiotic susceptibility of other bacterial species, including *Bacillus thuringiensis*, *Staphylococcus aureus*, *Mycobacterium smegmatis*, *Neisseria subflava* and *Pseudomonas aeruginosa*. These findings indicate a promising potential of using DSF-type molecules as novel adjuvants to conventional antibiotics for treatment of infectious diseases caused by bacterial pathogens. In combination, our results have well established the roles of the DSF-family signals not only in regulation of a range of biological functions through intraspecies signaling, but also through interspecies and cross-kingdom communication, suggesting the vital role of these signals in microbial community.

S3-O-03

Post-translationally Modified Quorum Sensing Pheromone from *Bacillus subtilis* subsp. *natto*

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Quorum sensing is a cell density dependent genetic regulation system widely observed in bacteria. In quorum sensing, extracellular signaling molecules, named quorum sensing pheromones, are constantly being secreted and accumulate in the environment. When the concentration of the pheromone reaches threshold levels; i.e. bacteria exist in high cell density, bacteria coordinate their behavior in response to the pheromone by altering their gene expression. The quorum sensing pheromones are responsible for a variety of characteristic phenomena in bacteria, such as bioluminescence, antibiotic production, secretion of virulence factors, biofilm formation, conjugation, sporulation, and competent cell formation, and so on.

Bacillus subtilis secretes ComX pheromone under the control of quorum sensing system to stimulate natural genetic competence. Previously, our studies demonstrated that ComX pheromone is a ribosomal oligopeptide biosynthesized from precursor peptide ComX through post-translational modification and processing of N-terminal region. Striking diversity is observed in the amino acid sequence of the ComX pheromone variants in *Bacillus* model strains, but each possesses an isoprenylated tryptophan residue at either the 3rd or the 4th position from the C-terminal end. The tryptophan residue is modified with either a geranyl or a farnesyl group at its γ-position, resulting in the formation of a tricyclic structure with a newly formed pyrrolidine ring resembling proline. The isoprenylation was confirmed to be essential for competent cell formation in *B. subtilis*.

B. subtilis subsp. *natto* is closely related to *B. subtilis* model strains and is used in the fermentation of natto, which is a highly sticky traditional Japanese food made from soybeans. The subsp. *natto* also possesses the homologous genes, *comQnatto* and *comXnatto*, and previous studies indicated that the genes are involved in the production of highly sticky poly-γ-glutamate. Therefore, ComXnatto pheromone possessing an isoprenylated tryptophan residue is predicated to stimulate the production of poly-γ-glutamate, but ComXnatto pheromone has not been identified until now. In addition, ComXnatto contains a tryptophan residue at neither the 3rd nor the 4th from the C-terminal end, but the 54th of the 73 amino acid residues, unlike other ComX pheromones.

Our recent studies have revealed that ComXnatto pheromone is a hexapeptide having an amino acid sequence of Lys-Trp-Pro-Pro-Ile-Glu, corresponding to the 53rd to the 58th residue of the precursor peptide ComXnatto. The 2nd tryptophan residue corresponding to the 54th residue of ComXnatto is post-translationally modified with a farnesyl group, similar to other ComX pheromones. This is the first report of quorum sensing pheromone from *B. subtilis* subsp. *natto*.

In addition, ComXnatto pheromone was demonstrated to stimulate the production of poly-γ-glutamate. Notably, the 20th tryptophan residue from the C-terminus of ComXnatto is farnesylated, and C-terminal residues as well as N-terminal residues of the precursor are processed in the biosynthesis of ComXnatto pheromone. These results suggested that, in general, post-translational isoprenylation of tryptophan residue could occur not only near the C-terminal end, but also at other positions of the precursor peptide.

S3-O-04

Terpenes as *lingua franca* between fungi and bacteria

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Interactions and communication among organisms are essential for the functioning of any ecosystem. It is recognized that in the ecosystem soil bacteria and fungi communicate with each other over long distances via the exchange of volatile organic compounds (VOCs). However, the molecular responses by bacteria to fungal VOCs remain unknown. Here we perform transcriptomics and proteomics analyses of *Serratia plymuthica* PRI-2C exposed to VOCs emitted by the fungal plant pathogen *Fusarium culmorum*. We find that the bacterium responds to fungal VOCs with changes in gene and protein expression related to motility, signal transduction, energy metabolism, cell envelope biogenesis, and secondary metabolite production. Metabolomic analysis of the bacterium exposed to the fungal VOCs and heterologous co-expression of a terpene synthase and a methyltransferase revealed the production of the unusual terpene sodorifen in response to fungal VOCs. These results strongly suggest that terpenes are important compounds in the long-distance communication between fungi and bacteria and set the basis for further studies on the role of terpenes in plant-microbe interactions.

S4-K-01

Priming of plant defenses by an insect pheromone

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It is increasingly clear that plants can sense and respond to environmental odor cues in a variety of ecological contexts. For example, the priming of plant defenses by the damage-induced volatile emissions of neighboring plants has been shown to be widespread. Some of our recent work has shown that defense priming can also be mediated by the perception of odor cues from plant antagonists themselves. Specifically, we documented enhanced anti-herbivore defenses in tall goldenrod plants, *Solidago altissima,* exposed to the putative sex pheromone of a specialist herbivore, the goldenrod gall fly, *Eurosta solidaginis*. Building on this finding, our ongoing work is exploring the mechanisms of priming in this system as well as the implications of priming for plant ecology and the evolutionary ecology of the association between *S. altissima* and *E. solidaginis*. This talk will address recent work documenting the specific chemical cues responsible for defense priming in this system, as well as recent findings regarding the broader effects of priming on *S. altissima* communities.

S4-K-02

Ligands seeking receptors: a multipronged approach in legumes to discover how plants perceive herbivore attack

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Herbivore-induced biochemical defense responses in plants have been intensively investigated for nearly 50 years. In addition to highly conserved master regulators such as wound-inducible jasmonate hormones, plants selectively perceive and respond to herbivory through the recognition of specific molecules, termed Herbivore Associated Molecular Patterns (HAMPs). Plants within the Phaseolineae subtribe, such as *Phaseolus* and *Vigna*, recognize Lepidoptera herbivory through the guarded perception of inappropriate fragments of chloroplastic ATP synthase γ-subunits, termed inceptin-related peptides, present at femptomolar levels within insect oral secretions. To gain a molecular level understanding how plants recognize herbivores through the perception of inceptin (In), we are pursuing three parallel approaches to discover the In receptor (InR). These include 1) large scale transcriptomic and genomic sequencing, 2) mass spectrometry based proteomic detection of InR candidates following ligand affinity enrichment and 3) forward genetics via quantitative trait loci (QTL) mapping. Firstly, using common bean (*Phaseolus vulgaris*) elicited with In we identified 61 receptor-like kinases (RLKs) and receptor-like proteins (RLPs) that displayed increased transcript abundances. In a signal auto-amplification model, inceptin-elicited RLK and RLP transcripts exist as receptor candidates. An additional effort is the identification of InR candidates via genomic sequencing of naturally occurring *P. vulgaris* germplasm containing extreme In-response variants. Secondly, peptide baits derived from biotin conjugated to the N-terminal of In were infiltrated into *P. vulgaris* leaves and a streptavidin affinity column was used to enrich for InR candidates identified by mass spectrometry. In a third approach, we utilized responses of diverse cowpea (*Vigna unguiculata*) germplasm to inceptin-related peptide variants. Using plant responses to C-terminal truncated inceptin (In^{-A}) as a mappable trait, biparental populations and a diversity panel-based genome wide association study (GWAS) identified a single shared QTL. RLPs associated with the genetic locus have detectable expression in leaves and display high homology within In-responsive legumes yet comparatively low homology to RLPs in non-responsive legumes. Approaches to validate InR candidates include heterologous expression in plants that do not respond to inceptin-related peptides such as *Nicotiana benthamiana* and *Arabidopsis thaliana*. Likewise virus-induced gene silencing (VIGS) is being pursued in *P. vulgaris* to validate the inhibition of In-induced responses. Historically, attempts to successfully validate the identity of a single HAMP receptor have been elusive. Using a variety of approaches in *P. vulgaris* and *V. unguiculata* we have generated a greatly narrowed candidate list of genes likely to encode the InR. Given that inceptin-related peptides occur in the oral secretions of all leaf-feeding Lepidoptera larvae tested to date, heterologous expression of the InR in plants exists as a testable strategy to confer enhanced perception of herbivory and improved defenses for crop protection.

S4-O-01

Chemical cues from beneficial entomopathogenic nematodes enhance plant protection against herbivores

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Chemical cues mediate many ecological interactions, especially among plants and invertebrates. Previous studies have documented both animal and plant responses to chemical compounds, including insect attraction to host-plant odors and plant defense priming in response to herbivore-associated cues. In addition to plants and herbivores, herbivore natural enemies also play an important ecological role and provide another source of chemical information in the environment. Some natural enemies, for example, emit compounds that deter foraging herbivores. In this study, we explored the ecological significance of chemical cues from natural enemies for plant protection against herbivores. More specifically, we investigated whether chemical cues from beneficial entomopathogenic nematodes (EPN), an important natural enemy in agroecosystems, influence plant defense responses or herbivore behavior.

Our findings indicate that EPN chemical cues influence both performance and preference of the specialist herbivore, Colorado potato beetle (CPB, *Leptinotarsa decemlineata*) feeding on its host plant, potato (*Solanum tuberosum*). CPB larvae feeding on plants exposed to EPN cues consumed less leaf tissue and gained less mass compared to larvae on control plants; and EPN-exposed plants exhibited higher levels of salicylic acid and expression of the pathogen-resistance gene PR1. Additionally, female CPB laid fewer eggs on plants in the presence of EPN compared to controls. Together, these findings suggest that plants respond to beneficial nematodes by inducing systemic defense responses, and herbivores respond by avoiding EPN cues. This work has important implications for pest management in agroecosystems, as EPN natural enemies both directly protect plants by killing insect pests, and indirectly reduce herbivore damage through chemical warning cues to plants and herbivores.

Selective adaptation within the chemosensory system of the leaf beetle, *Chrysomela lapponica*, following host plant shift

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The adaptation of herbivorous insects to plant metabolites promotes though diet conservatism, but does not lead inevitably to evolutionary dead ends. For example, host plant shifts have occurred during the evolutionary history of the specialist leaf beetle *Chrysomela lapponica*. This species forms geographically separated populations that colonize either willow (*Salix* spp.; Salicaceae) or birch (*Betula* spp.; Betulaceae) in the Eurasian Palearctic. The recent reconstructions of the host-shift history of the *C. lapponica* populations disentangled that willow is the ancestral feeding niche and that the transition to Betulaceae occurred several times independently. We have tested the hypothesis if a host plant shift of *C. lapponica* is accompanied by the modulation of the chemosensory repertoire between *Salix*- and *Betula*-adapted populations found in the Altai region of Kazakhstan. By using RNA-sequencing we identified at first 113 chemosensory candidate genes in *C. lapponica*: 31 odorant binding proteins (OBPs), 12 chemo sensory proteins, 4 sensory neuron membrane proteins, 41 olfactory receptors (ORs), 8 gustatory receptors, and 17 ionotropic receptors. We further studied the differential expression of the identified genes in the antennae, the major olfactory organ, of male and female adult beetles either adapted to birch or willow. Between the sexes we did not find significant differences but between the two populations. As a result, OBPs and ORs were differentially expressed. Among the antennal chemosensory genes the minus-C OBP ClapOBP27 was upregulated significantly in both sexes of the willow-feeders. In comparison, the two minus-C OBPs, ClapOBP20 and ClapOBP02, and the OR ClapOR17 were upregulated significantly in both sexes of the birch-feeders. We analyzed further the volatile bouquet of the host plant species, *Salix* sp. and *Betula rotundifolia*. With the exception of salicylaldehyde, both plant species shared a qualitative similar volatile pattern. However, they appeared to differ in the quantity of their emitted compounds, e.g. regarding the terpenoids (*E,E*)-α-farnesene (high in willow) or 4,8-dimethylnona-1,3,7-triene (high in birch). Our subsequent homology modeling experiments of the identified OBPs and related docking studies demonstrated that all the investigated plant volatiles may bind to the odorant binding proteins with varying affinity due to individual differences in polarity and the architecture of the ligand binding cavities. Based on our result we suggest that the host plant shift of *C. lapponica* was facilitated by the similarity in the volatile bouquet of the host plants. We further assume that the broad ligand binding abilities of the investigated OBPs can contribute to tolerating novel host plants.

Natural variation of phytoalexin sakuranetin production in rice cultivars

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Rice plants accumulate at least 20 species of phytoalexins in response to pathogen attack. These include diterpenoids such as oryzalexins, momilactones, and phytocassanes, as well as the flavonoids sakuranetin and naringenin. Jasmonic acid (JA), an endogenous plant signaling molecule, is a potent inducer of phytoalexin production in rice. Numerous cultivars of rice have been developed in the rice-cultivating areas around the world. However, the diversity in the phytoalexin production has not been fully investigated.

To provide new insight into the diversity of rice phytoalexin production, we analyzed cultivars in the World Rice Core Collection provided by the Genebank, National Institute of Agrobiological Sciences, and the National Plant Germplasm System of the USDA. To induce phytoalexin production, leaf segments from the third leaves of rice seedlings were floated in a 1 mM JA solution. The accumulation of sakuranetin, together with its precursor naringenin, was analyzed by LC-MS/MS.

The amounts of sakuranetin and naringenin varied, with different cultivars predominantly accumulating sakuranetin, naringenin, or neither of these compounds. Sakuranetin is synthesized from naringenin by the enzyme naringenin 7-*O*-methyltransferase (NOMT). NOMT enzymatic activity and accumulation of *NOMT* transcripts were analyzed in sakuranetin- and naringenin-accumulating cultivars. *NOMT* expression levels roughly correlated with the accumulation of sakuranetin. In a phylogenetic tree of *NOMT* genes from different rice cultivars, sequences from naringenin-accumulating cultivars formed two distinct clusters, suggesting that the differences in *NOMT* expression are attributable to the *NOMT* gene sequence itself, and that mutations leading to low *NOMT* expression occurred at least twice in the course of rice evolution.

Model rice cultivars Nipponbare and Kasalath are sakuranetin- and naringenin-accumulating, respectively. Analyses of chromosome segment substitution lines and backcross inbred lines indicate that the chromosomal region harboring *NOMT* is responsible for the differential phytoalexin accumulation between these cultivars. Sakuranetin-accumulating cultivars are distributed in all of the rice-cultivating areas in the world, whereas naringenin-accumulating cultivars are not found in East Asia.

Sakuranetin showed strong antifungal activity against *Magnaporthe grisea*, the causal agent of rice blast, whereas naringenin inhibited the growth of bacterial pathogens. Therefore, sakuranetin and naringenin may play distinct ecological roles in the interaction of rice with pathogens. It is noteworthy that rice bacterial diseases are more prevalent in tropical Asia.

S4-O-04

Effect of warming on VOC-mediated plant–insect interactions in high altitude alpine meadow ecosystems of the Himalayas

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Global average temperature has been increasing at an unprecedented rate since the early 20th century, and predicted to increase further by 2-3°C in the next century. The effect of this climate warming on plant–insect interactions could be profound. While numerous studies have reported change in phenological patterns and range shifts that break the synchrony between plants and their insect partners, relatively less is known about the effects on the chemical compounds that mediate interactions between plants and their associated insects. Warming could affect biosynthesis and volatility of Volatile Organic Compounds (VOCs), which are crucial in pollinator attraction significantly alter interspecies interactions. At present, there is relatively little known about warming effects on plant–insect interactions, especially in tropical alpine ecosystems, which are reported to be highly susceptible to global warming.

We investigated the effect of warming on the floral VOCs of four plant species in the alpine meadows of the Himalayas by conducting *in situ* warming experiments as well as comparison across an elevation gradient (3000, 3500, and 4000m asl), which act as natural warming gradient experiments, as temperature decreases with increase in elevation. We used warming chambers called Open Top Chambers which increase the ambient temperature by 2-3°C, similar to predicted temperature increase in the next century, to simulate future conditions. VOCs were collected using PDMS silicon tubing method which has been especially developed for field - based volatile collection, and analysed in a TD-GCMS. Only those compounds which are present in more than 95% of the samples in each species are selected for comparative analysis.

Our results show that floral VOCs of conspecific plants (*Anemone obtusiloba*) from warming chambers and control plots form distinct clusters in non-metric dimensional scaling (NMDS) analysis. Floral VOC samples of the four species of plants viz., *Bistorta vivipara* (Polygonaceae), *Fragaria nubicola* (Rosaceae), *Anemone obtusiloba* (Ranunculaceae) and *Lysimachia prolifera* (Primulaceae) collected across an elevation gradient also showed intraspecies variation in floral volatile profiles and formed distinct clusters in NMDS analysis. This significant separation in floral VOC clusters in each species is attributed to variations in presence and relative proportions of only a few compounds, even though 18 to 25 compounds were used in the analysis of the different species. Investigations on the effect of this changed VOC profiles due to warming on insect response is presently underway.

This study will contribute to our understanding of the effects of future climate warming on plant–insect interactions in tropical alpine meadows, such as those of the Himalayas, which are less studied. Additionally, we also hope that it will help focus current research attention to the practical ways of data collection in remote field conditions, its challenges and solutions.

Induced foliar volatile production in response to the herbivore elicitor *N*-linolenoyl L-glutamine maps to a single QTL in maize

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Plants perceive and respond to conserved biochemical patterns known as Herbivore-Associated Molecular Patterns (HAMPs) present in insect oral secretions. The most broadly active and widely occurring HAMPs present in lepidopteran larvae are the fatty acid-amide conjugates (FACs). Ironically, while common in mediating plant-herbivore interactions, FAC elicitation varies greatly among and even within plant species. First discovered in maize (*Zea mays*) seedling bioassays, FACs such as volicitin and *N*-linolenoyl-L-glutamine (Gln-18:3) are potent inducers of herbivore-associated foliar volatile emissions that indirectly defend plants via recruitment of parasitic wasps. To understand mechanisms of Gln-18:3 signaling and perception in maize, we utilized a forward genetics approach to identify loci associated with FAC-elicited plant responses. We discovered a maize variety that is insensitive to Gln-18:3 but maintains functional emission of herbivore-associated volatiles after treatment with a naturally occurring plant-derived signal, Plant Elicitor Peptide 3 (ZmPep3). To identify quantitative trait loci (QTLs) associated with Gln-18:3 elicited volatiles, we analyzed a recombinant inbred line (RIL) population created with a second maize inbred harboring functional Gln-18:3 sensitivity. Over 300 members of the RIL population were analyzed for induced volatile emission upon treatment with Gln-18:3 and ZmPep3. Use elicitor-induced volatiles for metabolite-based QTL (mQTL) mapping revealed a single mQTL significantly associated with emission of both terpenes and green leafy volatiles (GLVs) specifically after treatment with Gln-18:3. Candidate genes underlying the mQTL locus have been identified and their potential role in modulating sensitivity to Gln-18:3 are currently being examined.

S4-O-06

The leucine-rich repeat receptor-like kinase OsLRR-RLK1 in rice functions as an early regulator in plant-herbivore interactions

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Leucine-rich repeat receptor-like kinases (LRR-RLKs), the largest subfamily of RLKs, by perceiving and transmitting various internal and external signals, play important roles in plant growth, development and defense responses against biotic and abiotic stresses. However, their functions in herbivore-induced plant defenses are largely unknown. Here, we cloned a novel rice plasma membrane-localized LRR-RLK gene *OsLRR-RLK1*, whose expression level was induced by infestation of the striped stem borer (SSB) *Chilo suppressalis* but not affected by infestation of the brown planthopper (BPH) *Nilaparvata lugens*. OsLRR-RLK1 functions upstream of mitogen-activated protein kinase (MPK) cascades. Silencing of *OsLRR-RLK1* decreased SSB-elicited transcript levels of three defense-related MPKs and six defense-related WRKY transcription factors, and levels of jasmonic acid (JA), jasmonoyl-isoleucine (JA-Ile) and ethylene, which in turn reduced the activity of trypsin protease inhibitors and the resistance of rice to SSB. On the other hand, silencing of *OsLRR-RLK1* enhanced BPH-induced transcript levels of three defense-related MPKs and three defense-related WRKY transcription factors, and levels of JA, JA-Ile, ethylene and H_2O_2, which subsequently increased the resistance of rice to BPH. The data suggest that OsLRR-RLK1 acts as an early factor which perceives specific herbivore-associated signals and thus initiates specific and appropriate defense responses.

S4-O-07

Volatiles from the giant knotweed, *Fallopia sachalinensis*, induced by the Japanese beetle, *Popillia japonica*, attract conspecific females

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Plants are known to respond to attacks by herbivores and pathogens, and environmental stresses. When plants are attacked by herbivores, they emit characteristic volatile blends which function as cues for predators and parasitoids of the herbivores to locate their prey and hosts. We have reported that phenylacetonitrile (PAN) and some terpenoids, such as (*E*)-β-ocimene, are produced and emitted from the leaves of the giant knotweed, *Fallopia sachalinensis* (Polygonaceae), when they are infested by the Japanese beetle, *Popillia japonica* (Coleoptera: Scarabaeidae) [1]. We usually found the beetles aggregated loosely on beetle-infested leaves under natural conditions, indicating that the beetle-induced volatiles act as kairomones for *P. japonica*. The volatile emission is also induced by application of methyl jasmonate (MeJA), but not by mechanical damage, suggesting that the production of these volatiles is triggerd by biotic stimulation. Using MeJA as an exogenous inducer of volatile production, we have been reported that PAN is synthesized from phenylalanine which is also induced by either herbivory or MeJA and that a cytochrome P450, CYP71AT96, catalyzes the conversion of phenylacetaldoxime, an intermediate of PAN biosynthesis, to PAN [2,3]. However, in spite of the advances in biosynthetic study of PAN in *F. sachalinensis*, the biological role of herbivore-induced plant volatiles including PAN in the interaction between *F. sachalinensis* and *P. japonica* has not yet determined. Thus, in this study we planned to investigate the function of the volatiles and the Japanese beetle-derived elicitors that induce volatile production in *F. sachalinensis*. In laboratory conditions, female beetles showed a significant preference for MeJA-treated leaf when they encountered either MeJA-treated leaf or intact leaf, but males did not. These results suggest that the females could recognize the conspecific beetle-induced volatiles as host location cues, which corresponds with field observations. Males can be attracted to a sex pheromone released from females, which may lead to food finding. Water extract of *P. popillia* abdomen elicited volatile production in *F. sachalinensis*, whereas water extracts of head and thorax did not affect *F. sachalinensis*. When the abdomen extract was boiled, its elicitor activity was largely inactivated. Fractionation of *P. popillia* abdomen water extract by gel filtration chromatography revealed that there were a proteinous elicitor and low-molecular-weight elicitor in *P. popillia*. The former elicited production of (*E*)-β-ocimene and PAN, whereas the latter induced only (*E*)-β-ocimene production, suggesting that these two different types of elicitors have different modes of action in plant .

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S5-K-01

Modes and mechanisms of evolution of insect olfaction

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While the wiring of the olfactory circuitry of insects is increasingly understood, how the layout translates into preference and what are the major determinants through which coding and preference evolve have been less clear. Although olfactory receptors are readily conceivable hotspots for evolutionary change, and indeed they appear evolutionarily dynamic, are their shifts at the basis of niche differentiation, or they more frequently posthoc mechanism to provide a better olfactory fit to an insect species' new dietary niche. What are other mechanisms through which olfactory preference may shift.

Here we explore correlates of shifts in olfactory preference using *Drosophila* [2,5] and moth pheromone communication [1,3,4,6] as models. We will identify mechanisms that are not directly linked to olfactory receptors that can act as major hotspots for saltational shifts in preference. These include shifts in relative abundance of receptor neurons, and the sensilla in which they are expressed, and shifts in the expression pattern of receptors across neurons. While the molecular underpinnings still require further elucidation, these mechanisms provide potential corridors for rapid changes in an insect's olfactory preference.

(This paper reflects the collective contributions of many colleagues. Rather than a very long list of co-authors, I will highlight their contributions during my talk. Co-authors are also reflected in some key papers cited below).

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Two hundred million years and four types of pheromones: A phylogenetic perspective on moth pheromone diversity and evolution

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Communication depends on the mutual understanding of senders and responders. Divergence in a communication system requires matching evolution of signal and response. The Lepidoptera (moths and butterflies) are the second largest group of insects with ca. 160,000 described species. In moths (ca. 140,000 species), mate communication is mediated by the use of female-produced long-range sex pheromones building on chemically conserved structures. Still more or less species-specific blends have evolved by selection for efficient mate finding and reproductive isolation. Butterflies (ca. 20,000 species), on the other hand, appear to have lost female-produced pheromones as part of their long-range attraction mate communication system. However, they have not completely abandoned chemical communication: during courtship they use chemically diverse male-produced pheromones, which have been selected as sexual ornaments, aphrodisiacs or anti-aphrodisiacs.

Four major types of moth sex pheromones may be recognized based on biosynthetic characteristics and mapped onto a phylogenetic hypothesis of Lepidoptera. Superficially, it appears that major pheromone types are very stable and unlikely to change evolutionarily but upon closer examination, variations on the major themes show interesting patterns, which mirror critical events in lepidopteran evolutionary history. I will discuss the evolution of the different pheromone types, the adaptive value of pheromone chemistry, major and minor transitions in pheromone chemistry and the corresponding changes in matching pheromone receptors.

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S5-O-02

Candidate cells producing alkenyl sex pheromones in moths

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Sex pheromones secreted by female moths are crucial for mating with conspecific males. With respect to the chemistry of sex pheromones, the majority of moth species use aliphatic compounds with a terminal functional group (type I), whereas moths in the second largest group use alkenyl compounds with no terminal functional group (type II). The type-I pheromones are biosynthesized via palmitoyl-CoA with unique enzymes such as fatty acyl-CoA Δ11-desaturases and reductases. In this type, all biosynthetic reactions are assumed to occur in the pheromone gland. In contrast, type-II pheromones/precursors are considered to be biosynthesized from essential fatty acids (linoleic and linolenic acids) in the "oenocytes" in the abdomen and transported via hemolymph to the pheromone gland, where epoxidation of the precursors by P450 mono-oxygenase occurs in some species. It is interesting that two divergent types of pheromones, which are produced via independent biosynthetic pathways, evolved in moths despite that sex pheromone communication systems are under strong stabilizing selection due to its importance in maintaining conspecific mating.

Recently, we found transcripts of Δ11-desaturase gene in the pheromone gland of the giant looper *Ascotis selenaria*, in which desaturation step is not involved in the pheromone biosynthesis. Moreover, when we analyzed the hemolymph of the Asian corn borer *Ostrinia furnacalis* and the silk moth *Bombyx mori*, both of which secrete type-I pheromones, we found trace of alkenyl sex pheromones/precursors in the hemolymph of *O. furnacalis* whereas none in that of *B. mori*. These findings imply that the common ancestor of moths had a potential to produce both types of pheromones, and most extant species still retain this potential. To test this possibility, we investigated the presence of "oenocytes", cells involved in the production of alkenyl sex pheromones, in moths that produce type-I pheromones.

In the lichen moth *Eilema japonica* and the fall webworm *Hyphantria cunea*, both of which secrete alkenyl sex pheromones, we found a translucent organ associated with trachea. Observation of this organ under dark field microscopy with DAPI and Nile red staining revealed that this organ consists of cells exhibiting the features of adipocytes. Interestingly, in *O. furnacalis* and *B. mori*, the species that produce type-I pheromones, a similar organ associated with trachea was found in the abdomen of female *O. furnacalis* whereas no such organ was found in the abdomen of *B. mori*. Because the presence of this organ in the abdomen coincides with the presence of alkenyl sex pheromones/precursors in the hemolymph, we considered that these cells are involved in the production of type-II alkenes. The role of this organ in the production of alkenyl sex pheromones will be discussed.

Age-dependent plasticity in the sexual signal of a noctuid moth

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The level and extent of plasticity in sexual signals is crucial to understand how signals evolve under a scenario of stabilizing selection, and how different environmental forces may drive the evolution of sexual signals. In moths, females produce species-specific sex pheromones and these sexual signals seem to be under stabilizing selection, which limits evolutionary change. Female moths with less attractive pheromone blends experience a delay of mate acquisition and consequently suffer a reproductive cost. If the female pheromone signal is honest, the pheromone blend could provide males information on the female's deteriorating quality as she ages. Alternatively, if signal production is cheap, aging females could adjust their sexual signal to increase their attractiveness. We hypothesized that virgin females adjust their sexual signal, depending on their age, thereby masking their deteriorating quality. To test this hypothesis, we used disposable SPME fibers to measure within-individual changes in the pheromone blend of aging females. We found that older females have increased levels of acetates in their pheromone blend, which increases the chance of attracting a conspecific male and decreases the chance of attracting a heterospecific male. To determine if the adjustments were costly, we investigated the interaction of pheromone production with calling intensity, and the effect on male preference, mating success, longevity, fertility and fecundity. We also tested whether age-dependent plasticity in the pheromone composition was heritable. Our findings suggest that age-dependent plasticity could play an important role in the evolution of sexual communication by promoting diversification of the pheromone blend.

S5-O-04

Does divergent selection predict sexual selection in the adaptive radiation of the tropical butterfly genus *Melinaea*?

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While divergent selection can drive speciation, understanding the factors that facilitate or constrain this process remains a major goal in evolutionary research. Reproductive barriers, such as those mediated by visual and chemical signals, are considered to play an important role in speciation and adaptive radiation. However, whether these barriers occur early on in the speciation process remains unclear. Adaptive radiations, whereby a group undergoes rapid diversification, offer an interesting comparative framework along the speciation continuum.

We investigate this in the warningly coloured and mimetic butterfly genus *Melinaea* (Nymphalidae: Ithomiini), using 5 young taxa (< 1 mya) distributed along a speciation continuum and ranging from subspecies to full species. Our data shows clear phenotypic differentiation (colour pattern and pheromones) between all taxa, despite the lack of genetic differentiation between subspecies (using 12 microsatellite markers). This indicates that despite being of recent origin, there is strong selective pressure for differentiation. Mate choice experiments also show strong assortative mating within species, and even between some subspecies. Our results suggest that even in the early stage of speciation, reproductive isolation can be nearly complete due to prezygotic factors. We discuss possible explanations for variable progress toward speciation. Whether these conclusions are repeatable across taxa remains unclear, but we are presently testing this using replicated Ithomiini butterfly taxa, as the tribe as a whole has undergone simultaneous rapid and extensive adaptive radiations.

How pheromone binding proteins sustain sexual behavior initiation in *Bombyx mori*?

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Pheromone binding proteins (PBPs) are thought to transport the hydrophobic pheromones through the sensillar lymph to the dendritic membrane of the sensory neurons in antenna. In the silkworm, *Bombyx mori*, three PBPs have been demonstrated, among which *BmPBP1* is supposed to transfer the main sex pheromone bombykol [(10E,12Z)-hexadecadien-1-ol]. For comparison, functions of the other two PBPs, as well as the transportation of the other pheromone component, bombykal [(10E,12Z)-hexadecadienal] are still largely unknown. To explore the relationship between three PBPs and two pheromone components, transgenic CRISPR/Cas9 technique was applied to obtain three different silkworm mutants, respectively knocking out PBP1, PBP1-PBP2 and PBP1-PBP2-PBP3. From single sensillum recording results, the olfactory neuron of *PBP1* mutant presented a stronger response to bombykol than wild type, while the response to bombykol in *PBP1-PBP2* mutant disappeared, suggesting that PBP2 would make the compensation in absence of PBP1. From mating behavior assay, when exposed to bombykol and 11:1 mixture of bombykol and bombykal, the mating behavior of *PBP1-PBP2* mutant increased, while the mating behavior of *PBP1-PBP2-PBP3* mutant decreased, suggesting that PBP3 could compensate the absence of PBP1 and PBP2. To explore the compensation mechanism in *PBP1* mutant, RNAseq, far-western blotting combined with mass spectrometry were successively conducted. One potential protein named transaldolase which interacted with the residual PBP1 in *PBP1* mutant was figured out to possibly make the compensation. A speculated model for the compensation effect was further put forward.

In vivo functional analysis of genes involved in sex pheromone detection in the silkmoth *Bombyx mori*

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Male moths locate their mates using species-specific sex pheromones emitted by conspecific females. One striking feature of sex pheromone detection in male moths is the high degree of specificity and sensitivity at all levels from primary sensory processes to behavior. So far, extensive research has elucidated the molecular mechanisms of pheromone reception that involve several molecular components, such as pheromone binding proteins (PBP), olfactory receptor co-receptor (Orco) family proteins, and sex pheromone receptor proteins. However, causal relationship between the molecular function of these genes and sex pheromone response in *in vivo* is still largely unknown. In this talk, recent work that addresses this question using a genetically tractable moth species the silkmoth, *Bombyx mori*, will be presented.

In the silkmoth, female moths emit two pheromone components, bombykol [10,12-(*E*,*Z*)-hexadecadien-1-ol] and bombykal [10,12-(*E*,*Z*)-hexadecadienal]. Male moths detect sex pheromones by the trichodeum sensilla on the antennae. A single type of PBP (BmPBP1) was reported to be expressed in sensillum lymph of the trichodeum sensilla, and thought to contribute to sensitivity of pheromone detection by enhancing solubility of hydrophobic pheromone molecules into sensillum lymph. Then, pheromone molecules are detected by sex pheromone receptors BmOR1 and BmOR3, which are narrowly tuned to bombykol and bombykal, respectively, and mutually exclusively expressed on dendritic membrane of a pair of olfactory receptor neurons (ORNs) in the trichodeum sensilla. *BmOrco* is expressed in most ORNs and thought to be essential for formation of functional pheromone/odorant receptor complexes on dendritic membrane of ORNs. To examine *in vivo* function of these genes, we generated *BmPBP1*, *BmOR1*, or *BmOrco* gene knockout silkmoth lines by transcription activator-like effector nuclease-mediated gene targeting. From electrophysiological analysis of male antennae, we found: (1) antennal response of *BmPBP1*-knockout males to both bombykol and bombykal was significantly reduced compared to those of wild type males, suggesting that BmPBP1 is important for sensitive detection of both pheromone components but not for discrimination of pheromone components. (2) *BmOR1*-knockout male antennae showed no response to bombykol, while they showed normal response to bombykal, confirming that BmOR1 is the receptor that mediates only bombykol response. (3) *BmOrco*-knockout male antennae lost electrophysiological response to most of tested odorants including bombykol and bombykal, suggesting that BmOrco is necessary for formation of functional pheromone/odorant receptors as in other insect species. Based on these results, we will discuss about molecular mechanisms underlying highly specific and sensitive sex pheromone detection in moths.

Molecular basis of alarm pheromone detection in aphids

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The sesquiterpene, (*E*)-β-farnesene (EBF) is the alarm pheromone for many species of aphids. When released from aphids attacked by parasitoids or predators, it causes dispersion of other closely feeding aphids. Up to now, the molecular basis of EBF detection is largely unknown in insects. Here we demonstrate that ApisOR5, a member of the large superfamily of odorant receptors, is allocated in a large placoid sensillum neuron on the 6th antennal segment and mediates EBF reception in *Acyrthosiphon pisum*. ApisOR5 confers response to EBF when co-expressed with Orco, an obligated odorant receptor, both *in vitro* (*Xenopus* oocytes) and *in vivo* (*Or67d*$^{\mathrm{GAL4}}$ knock-in neuron) expression systems. The repellent behaviour of *A. pisum* to EBF is abolished after knocking down the *A. pisum* odorant binding proteins mediating EBF perception (ApisOBP3 and ApisOBP7) or ApisOR5 by RNA interference. Furthermore, odorants that can activate ApisOR5, such as geranyl acetate, significantly repel *A. pisum* as well as EBF. The characterization of EBF receptor allows high-throughput screening of aphid repellents, providing the necessary information to develop new strategies of aphids control at the molecular level. In addition, dsRNA-mediated gene silencing of olfactory pathway components is a promising tool to functionally characterize these proteins and represents an important potential strategy for insect pest biological control.

Keywords: aphid, (*E*)-β-farnesene, odorant receptor, odorant-binding protein, repellent

S5-O-08

Insect odorant receptor-based biosensor -a proof of concept using pheromone receptors and its application to general odor sensing-

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Highly sensitive and selective odorant sensing technology is required for various applications such as food administration, rescue of human life, and risk management. Although current practical odorant sensors have been fabricated using sensing technologies based on metal-oxide semiconductors and quartz crystal microbalances, it is difficult to develop the odorant sensors with high sensitivity and selectivity. In contrast, insects can sensitively and selectively detect various types of environmental odorants. Therefore, in order to provide innovative sensing technology we attempt to develop cell-based odorant sensors focusing on insect's odorant receptors (ORs), which are expressed in olfactory sensory neurons, for selectively detecting various types of environmental odorants.

First, we used Sf21 cell lines expressing sex pheromone receptors from silkmoth, *Bombyx mori*, along with co-receptor (Orco) and GCaMP3 to evaluate the performances as odorant sensor elements. The cell lines exhibited the sensitivity at the scale of several tens of parts per billion (ppb) in solution and the selectivity that can detect pheromone components in accordance with the selectivity intrinsic to the expressed receptors. In addition, they detected pheromone components without variable responsiveness over at least 2 months, thereby overcoming the short life span of biosensors for practical use [1]. Therefore, we established the basic methodology for creating odorant sensor elements based on Sf21 cell lines co-expressing ORs, Orco, and GCaMP.

Next, for development of odorant sensor elements sensitive to general odorants, we used two *Drosophila melanogaster* ORs (Or56a; geosmin, Or13a; 1-octen-3-ol), Orco, and GCaMP6s to establish the Sf21 cell lines. Or56a and Or13a cell lines selectively responded to geosmin and 1-octen-3-ol by exhibiting the increased fluorescence intensities. Both cell lines dose-dependently detected target odorants with lower detection limits of 100 nM, which corresponds to ten and several ppb in solution. Interestingly, Or56a cell line was able to specifically detect geosmin even in the presence of background odors such as orange flavor. Thus, the methodology is available for development of practical odorant sensor elements.

Finally, we tried to construct a compact odorant sensor array chip that discriminate odorants as fluorescence patterns. We used biocompatible anchor for membrane (BAM) to attach four cell lines with BmOR1, BmOR3, Or56a, and Or13a onto a glass chip. Using this array chip, we demonstrated that the chip can detect four odorants (bombykol, bombykal, geosmin, and 1-octen-3-ol) as a pattern of increased fluorescence intensity [2]. These results represent the first step towards practical cell-based odorant sensors that enable us to discriminate various types of odorants with high degree of sensitivity and selectivity.

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Odor detection using an insect olfactory receptor reconstructed in bilayer lipid membrane

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This paper describes a membrane protein-based odor sensor using an olfactory receptor (OR) with the co-receptor (Orco) reconstructed in artificial cell membranes formed in an electric circuit. As a biohybrid odor sensor, we used a mosquito's OR to obtain high sensitivity and selectivity to octenol.

Life's olfactory perception has high sensitivity and selectivity to the target odor at the molecular-level system. Even with the use of current technologies, it is still difficult for us to realize such a sophisticated compact system as an odor sensor. Here, we propose an odor detection system based on integration of biological olfaction and artificial electronics.

We prepared the artificial cell membranes using droplet contact method that gave easily bilayer lipid membranes (BLMs) at the interface of two aqueous droplets submerged in organic solvent containing lipid molecules. For the direct detection of octenol gas from atmosphere, the stable air-liquid interface uncovered with organic solvent was formed by using agarose gel as a model of mucus in our OR-BLM system. The reconstruction of OR and Orco into BLM was carried out by fusion of proteoliposomes purified from membrane fraction of transformed cells expressing the OR with Orco. The OR-BLM system was built between two electrodes of patch-clamp recordings. We additionally downsized the patch-clamp amplifier, and integrated the OR-BLM system with electrical devices including electrodes, amplifier, wireless equipment, and battery. Furthermore, we prototyped a locomotive robot combined with the compact octenol sensor whose output signals triggered a motion of the robot.

Gas chromatographic analyses of agarose gel exposed to octenol gas showed that the agarose gel more efficiently absorbed octenol than aqueous solution covered with organic solvent. In the presence of octenol gas, the biohybrid odor sensor output current signals that were due to the ORs' specific response to octenol. Finally, we succeeded in demonstration of robotic motion activated by signals from the odor sensor mounted on the robot. Hence, we expect that this biohybrid odor sensing will be a key technology for the future machine olfaction.

Cracking the code: Understanding the qualitative and quantitative properties of waterborne chemical cues that control prey risk assessment

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Water born chemical deterrents are ubiquitous in aquatic and marine systems, where they exert important direct effects on prey species, such as changes in habitat use, feeding, or other behaviors. These changes to focal prey species also have cascading effects to the other organisms that interact with the focal prey, such as those prey's resources (e.g. trophic cascades). In particular, behaviorally mediated trophic cascades are as, or perhaps more important, than those produced by direct consumption. Unfortunately, we have little understanding of how prey encode the risk associated with a particular predator, and of the chemical nature of such water born deterrents. This is a significant source of uncertainty given that the response intensity of prey species to these chemical cues is dependent on the perceived risk. Our work on predator detection in various marine systems has provided insights into the properties of chemical cues used by prey to perceive risk. This includes identifying, for the first time, the signal molecules in diet dependent waterborn chemical cues. Using our work, as well as observations from other systems, I will discuss the qualitative and quantitative properties of chemical cues used by prey to assess risk, and how the physical environment modulates the effectiveness of chemical cue perception in natural systems. I will discuss the consequences of these effects on the range and intensity of both direct and cascading interactions mediated by chemical cues, and the ecological implications for systems in which these waterborn chemical cues are important. Although methodology is not a specific focus, I will discuss novel approaches to characterizing the nature of waterborne deterrents that have, until now, defied characterization.

Chemical defense of Sea hares: sequestration and secretion of algal metabolite in ink and skin

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Sea hares(Opisthobranchia: Anaspidea) live in marine benthic communities where they have relatively specialized diets, concentrating on macro algae. They are mobile but slow and have a soft body without a shell for protection. Consequently, they chemically defend themselves against a range of predators by sequestering secondary metabolites from their dietary red algae and mobilizing them into their skin and digestive glands. In addition to these constitutive chemical defenses in their skin and tissues, sea hares also have a behaviorally activated chemical defense, ink secretion, which is released when they are attacked by predators. Ink secretion is composed of two co-released glandular products: ink, which is a dense, dark purple product released from the ink gland, and opaline, which is a translucent whitish product released from the opaline gland. To understand the mechanisms of the chemical defense, molecules in ink secretions that alters feeding behavior of predators have been identified in *Aplysia californica*. An case is the amino acid components of ink and opaline, which function as a deterrent against spiny lobsters by stimulating appetitive feeding responses of predatory spiny lobsters, a process called phagomimicry. Another case is molecules generated by escapin. Escapin is an L-amino acid in ink. When ink and opaline are co-secreted, escapin oxidizes L-lysine, which is present in high millimolar amounts in opaline. This reaction produces three molecules: hydrogen peroxide, ammonia and the alpha-keto acid of lysine. The alpha-keto acid exists as an equilibrium mixture of imine, enamine and other forms in an aqueous solution, and these compounds react with hydrogenperoxide to produce another set of compounds. Addition to these, the opaline alone and ink alone deter predators. To identify deterrent molecules in ink we used bioassay guided fractionation and identified two purple pigments, aplysiovioline and phycoerythrobilin, as the deterrent. Whitish ink from *A. juliana* that does not contain these purple pigment does not deter spiny lobsters that are deterred by purple ink. This result supports that the purple pigments are the deterrent purple ink of sea hares in. Survey of these two purple pigments in 7 sea hare species showed that the purple pigments are common component of their ink except *A. juliana* and common chemical defense in sea hares. Metabolomic analysis based on NMR data showed that the *A. juliana* sequesters DMSP and acrylate from its dietary green algae and release them when they are exposed to physical stresses that mimic biting of predator. These molecules may contribute the chemical defense of *A. juliana*.

Extracellular recordings from the brain cells of the New Zealand paddle crab, Ovalipes catharus, and evaluation of olfactory-active compounds

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The electrophysiological activities of brain cells receiving signals from antennular olfactory receptor neurons (ORNs) were monitored and the activities of olfactory-active compounds were evaluated in the New Zealand paddle crab, *Ovalipes catharus* (White), using an extracellular recording technique. We hypothesized that the olfactory activities of different chemical stimuli can be evaluated by examining the responses of antennular ORNs in the brain of *O. catharus* using the extracellular recording technique. Characteristic action potentials were observed from various parts in the brain. Typically, action potentials of a few different classes of amplitudes with aperiodic time intervals were observed at the circumferential areas of both the olfactory lobe (antenna 1 neuropil) and antenna 2 neuropil. In contrast, action potentials with even amplitudes and periodic time interval were mostly recorded at the further inside of these neuropils. Using this technique, the responses of antennular ORNs to various chemical stimuli could be displayed in the circumferential region of the olfactory lobe in the brain. The stimulation of antennules with amino acids, sugars, uridine diphosphate or uridine triphosphate exhibited excitatory electrophysiological responses in a dose-dependent manner in the olfactory lobes of both males and females. In behavior bioassays, male *O. catharus* displayed sexual attraction to an HPLC fraction of urine extract collected from premolt stage females. When antennules were stimulated with the urine extract fraction excitatory responses were only observed in the olfactory lobe of the males. The sexual dimorphism in the electrophysiological responses to the urine extract, together with the behavioral attraction of conspecific males, may indicate the presence of sex-specific semiochemicals in the premolt female urine of *O. catharus*.

S6-O-01

A coral control acquisition of *Symbiodinium* using lectins

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The corals thrive in tropical and subtropical areas. They symbiose with *Symbiodinium* and obtains photosynthates from *Symbiodinium* as carbon source, and symbiosis between corals and *Symbiodinium* is indispensable to their survival. Many corals acquire *Symbiodinium* each generation. This acquisition strategy is reasonable for their survival, since *Symbiodinium* spp. are classified by clades and each clade *Symbiodinium* has different property like temperature tolerance, efficiency of nutrition transfer to host and so on. However, corals harbor only limited clades of *Symbiodinium*, and the acquisition mechanism is not fully known. It was previously proposed that corals acquire *Symbiodinium* by using nitrogen-containing compounds or light. These cues attract Symbiodinium, but the selective acquisition of *Symbiodinium* by corals does not seem to explain. One of other candidate is glycans or lectins, because *Symbiodinium* acquisition by a coral *Fungia scutaria* was inhibited by carbohydrates and glycosidase. Since lectins are a group of carbohydrate-binding proteins, and known as a decorder of carbohydrates, we hypothesize that coral lectins involve to selective acquisition of *Symbiodinium*. In this study, we tested this hypothesis using larvae of a coral *Acropora tenuis*, which larvae has no *Symbiodinium*, and *Symbiodinium* strain NBRC102920. NBRC102920 acquisition of *A. tenuis* larvae was inhibited by D-galactose, *N*-acetyl-D-glucosamine (GlcNAc), and *N*-acetyl-D-galactosamine (GalNAc), and we purified corresponding lectins. First, we identified GalNAc-binding lectin. Two components were purified at 29.0 and 14.6 kDa. A component at 29.0 kDa has similarity to Tachylectin-2 (TL-2), which function in opsonization or in the blocking of adhesion of pathogens to the host cells, and the component was named as AtTL-2. The acquisition of *Symbiodinium* was inhibited by anti-TL-2 antibody. Secondly, GlcNAc-binding lectin was purified by GlcNAc-fixed Sepharose 6B. The lectin fraction contained major two components at 23.7 and 15.0 kDa. The N-terminal amino acid sequences of these components were closely related each other. The *de novo* sequencing of the lectin fraction revealed that the lectin fraction mainly contains a gene product s130_g38.t1, and the component was named as ActL. The lectin fraction also contained AtTL-2. The deduced amino acid sequence of the main component had no similarity to deposited sequences in SwissProt, but the sequences were widely spread in the cnidarians. The lectin ActL was distributed in the nematocysts, and was released to surrounding environment. Moreover, lectin fraction in a capillary attracts *Symbiodinium* NBRC102920, and inhibited by GlcNAc. Interestingly, the more intensely the lectin fraction binds to *Symbiodinium*, the more *Symbiodinium* acquire to a *A. tenui*s larvae, suggesting that the lectin fraction components contribute to selective acquisition of *Symbiodinium* by *A. tenuis* in early stage.

Chemoreception of crabs and the development of baits for trap fishing and eradication

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Traps are a common and energy saving method to harvest crustaceans, but their effectiveness is heavily dependent on the quality of the bait used. Traditionally, bait has mostly consisted of frozen fish. Studying the chemoreceptive properties of swimming crabs has been useful to increase bait effectiveness. For instance, saccharides are as important as amino acids in crab chemoreception, and their addition to fish bait can considerably affect crab catches. Bait combinations of fish and sugarcane have a synergistic effect that can double the catch of two commercial, and invasive, swimming crabs (*Portunus pelagicus* and *Charybdis japonica*), and this new baiting method has been valuable to increase the attractive power of commercial and eradication traps. Using fisheries by-products (heads, skins and viscera) as bait instead of high-quality fish might be possible if this waste is made more palatable by adding attractants. Mincing is a simple way to process this waste, and by enclosing it in a porous membrane (polyethylene/polyester teabag), which ensures the rapid diffusion of soluble substances, it might be used as bait. In addition, this method is suitable for mixing other ingredients and supplements into the mince. Fishing trials with traps were conducted using three bait treatments: fish (100g of mackerel), mince in teabag (100g of minced mackerel) and mince plus sugar in teabag (80g of minced mackerel plus 20g of sugar). The counts of crabs in the fish and mince teabag treatments were similar; but adding sugar reduced the number of some of the by-catch crab species while the number of commercial crabs (*P. pelagicus*) remained unaffected. Thus, the selectivity of bait might be manipulated through the addition of attractants or repellents. This could lead to the development of species-specific bait in the future, and artificial bait holds considerable promise. Artificial bait also has the advantage that it is solid, cleaner and easier to handle, and can be stored for prolonged periods of time at room temperature; therefore, eliminating the need for frozen storage. To substitute frozen fish, two solid artificial baits were developed that differed in some of their components, but were similarly composed of starch (34%) and fish waste (30%). The artificial baits were tested against fish bait (100g mackerel) and mince (100g minced greenling heads placed in teabags) during fishing trials with traps. The model species was the Sand crab (*Ovalipes punctatus*), which showed a clear preference for fish bait (42% of the catch). The other three treatments, minced fish (23.4%) and the artificial baits (16% and 19%) showed only minor differences. While the design of the artificial bait for this crab species can be improved, the test showed promising results. When the catch returns are calculated with basis on fish content, the artificial bait showed clearly higher efficiency (88-100 crabs/kg of fish waste) than the whole fish bait (69 crabs/kg of fish). These results suggest there is potential for making artificial bait that is cheap and easy to store and handle, and which recycles waste and conserves valuable fisheries resources.

S6-O-03

Some like it cold: Antarctic marine chemical ecology

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The structure of marine benthic ecosystems is determined by both environmental and biological factors. Among the biological ones, the ecological relationships (symbiosis, competition, predation), the life-cycles, the phylogenetic history, and the colonization rates of the organisms are crucial for their survival. In contrast to what happens in other regions of the planet, the Antarctic ecosystems are ruled by a strong environmental stability, only comparable to that observed in caves or abyssal regions, and thus the interactions between organisms become a very important factor in structuring these communities. Many of these interactions are regulated by natural products. To date, less than 3% of the ≈24 000 marine reported compounds originates from higher latitudes, despite the fact that a huge portion of global shallow water habitat is found around the Antarctic continent. The reasons for this asymmetry are probably related to the difficult access, extreme climate, and the still prevailing theory that polar systems should be less chemically productive. Since 1998, the Antarctic research projects ECOQUIM, ACTIQUIM, and DISTANTCOM aimed at gaining a better understanding of the diversity and structure of Antarctic benthic marine communities, both at biological and chemical levels. To do so, among other aspects, we studied the ecological activity of the marine natural products from benthic organisms, by carrying out *in situ* chemical ecology experiments. Our chemical ecology studies on marine benthic organisms include, among others, repellence, toxicity, and antifouling activity assays, as well as the identification of the natural products and their potential bioactivities. A few selected examples are presented here on how marine benthic organisms use natural products in their interactions with others, including sponges, soft corals, molluscs, bryozoans, and tunicates. Over the years, our studies have diversified to include other related topics in marine ecology. Therefore, we also study the trophic relations and symbiosis in shallow Antarctic benthic communities. As further related topics arise, our team is also producing new methodologies to answer new questions about marine benthic invertebrates.

Investigating the chemical landscape of microalgae cultures to mitigate pond crashes

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Open ponds are likely to succumb to unpredictable, devastating crashes as a result of algal predation by one or several deleterious species. Developing methodology to monitor, circumvent, and ultimately prevent predation of algae will increase biomass production, drive down costs for algae farmers, and reduce the risk involved with algae cultivation, making it more favorable for investment by entrepreneurs and biotechnology companies. We are interested in identifying signature chemicals to aid in predator identification, subsequent treatment, and algal protection from predatory species to prevent pond crashes.

It is well understood that deleterious species produce various volatile organic compounds (VOCs) as specific biomarkers of their predatory action [1]. We have surveyed and compared the production of algal volatile organic compounds (AVOCs) by several strains of microalgae, including *Nannochloropsis salina*, *N. oceanica, N. gaditana, Dunaliella tertiolecta, Emiliania huxleyi, Phaeodactylum tricornutum*, and others. Additionally, we have identified specific AVOC differences for microalgae in the presence and absence of rotifer predation. Interestingly, several AVOCs are decreased between strains of microalgae in the presence of active predation from rotifers and a select few are also increased in abundance. These upregulated AVOCs will aid in specific predator detection and treatment and will be added to a growing AVOCs library to be used in the development of a chemical monitoring system for algae farmers to use on their raceway ponds.

Previous studies have shown that bacteria have a positive impact on microalgae culture [2] and optimizing this relationship could help strengthen the algal pond community and protect against pond crashes. We have isolated microbial consortia from the pilot scale production ponds, and other sources that have all shown specific, but unexplained, protection against rotifer predation. Currently, we are in the process of investigating the method by which these bacterial species are selectively killing algal predators. To do this, we are analyzing secreted secondary metabolites using Liquid-Chromatography Mass Spectrometry (LCMS) and we are also sampling the AVOCs from algae cultures treated with and without protective microbial consortia. Collectively, our data will contribute to the growing knowledge of the chemical ecology that defines pond communities, helps algae thrive, and allows survival in the presence of algal predators.

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Exploiting the chemical ecology of tritrophic interactions for crop protection

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Plants have the keen ability to respond to attacks by herbivores or pathogens attack with the production of various defense chemicals. A typical response to herbivory is the production and release of volatile organic compounds. These volatiles may serve a number of functions, such as direct toxic and repellent effects on herbivores, indirect defense through the attraction of natural enemies of the herbivore, and the volatiles can serve as signals that warn undamaged plant parts of incoming attack. It is widely recognized that plant-produced volatiles have great potential for application in agriculture. The use of companion or sentinel plants that are highly responsive or that release specific volatiles show great promise and some successes have already been booked, in particular in the context of the so-called "push-pull" strategy. Another intriguing prospect is the development of sensors that can capture the information contained in the volatile signals. For now, this may seem farfetched, but sensor technology is advancing rapidly. I will discuss the various ways in which herbivore- and pathogen-induced plant volatiles may be exploited for crop protection, with special emphasis on efforts to fully understand the specificity of inducible volatile blends.

S7-O-01

Crop domestication in peppers: consequences for direct and indirect plant defense

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Plant breeding has improved traits such as fruit flavor and yield, but may have compromised direct and indirect defenses to herbivores compared to their wild ancestors. For example, cultivated plants may have lost or reduced foliar toxins, making them more susceptible to insect pests. Domesticated plants also might be subject to changes in the amount and composition of volatile organic compounds, impairing their ability to attract natural enemies. However, this perception that domesticated plants are more vulnerable to herbivores compared to their wild counter parts might not always be true. We present evidence that domestication of *Capsicum annuum* (the sweet bell pepper) from its wild progenitor, *Capsicum annuum* var. *glabriusculum*, has resulted in no loss of resistance to the insect herbivore, *Manduca sexta* (the tobacco hornworm). Caterpillars performed equally well on the domesticated and wild pepper. Subsequent behavioral choice assays with one of the main natural enemies of *M. sexta*, the parasitoid wasp *Cotesia congregata*, showed that parasitoids preferentially chose herbivore-damaged domesticated sweet pepper plants over their wild relative. To investigate this tri-trophic interaction further, we plan to analyze constitutive and herbivore-induced plant volatiles across numerous cultivated pepper varieties and wild accession lines to determine differences between domesticated and cultivated volatile blends. We also aim to assess the efficiency of *C. congregata* to parasitize *M. sexta* on these plants. However, our current results imply that domestication in peppers has not decreased direct resistance to herbivorous insects, and possibly has improved volatile attraction to recruit natural enemies.

S7-0-02

Temporal dynamics of herbivore-induced volatiles provide robust indirect defense in nature

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Plants emit volatile blends specific to particular herbivore interactions, which predators and parasitoids learn to associate with prey, increasing herbivore mortality and thereby plant fitness in a phenomenon termed indirect defense. Herbivore-induced plant volatile blends commonly include both rapid, transient green leaf volatiles (GLVs) and delayed, enduring sesquiterpenes. A few laboratory studies indicate that insects can use plant volatiles to time behavior, but it is not known whether and how the temporal dynamics of plant volatile blends influence their function in indirect defense. We characterized the activity of the native herbivores *Manduca sexta* and *Tupiocoris notatus* and their predators, *Geocoris* spp., on their host plant *Nicotiana attenuata* in their natural habitat. Diurnal predator activity only partially overlapped with variable herbivore activity, and herbivore attack at the beginning or end of the photophase elicited plant volatile blends with distinct GLV and sesquiterpene profiles. In field trials, day-active *Geocoris* spp. predators preferred morning- over evening-typical GLV blends. Using plants genetically transformed so as to be unable to produce specific volatiles, we found that GLVs increased predation after dawn elicitations, whereas sesquiterpenes increased predation after dusk elicitations in field trials. We conclude that predators respond to temporal differences in plant volatile blends, and that the different dynamics of specific volatiles permit effective indirect defense despite variable herbivore activity in nature.

Larval parasitoid wasp *Lytopylus rufipes* needs both background leaf volatiles and herbivore-induced plant volatile for searching a host

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Lytopylus rufipes (Hymenoptera: Braconidae) is a larval parasitoid of the oriental fruit moth (*Grapholita molesta*) (Lepidoptera: Tortricidae). Larvae of *G. molesta* usually remain concealed in new shoots of its host plant, so olfactory cues may play important roles for females *L. rufipes* to search their host larvae. To confirm this hypothesis, the volatiles extracts (hexane solution) were collected from shoots of healthy and host-infested pear (*Pyrus pyrifolia* var. culta Kosui), and were treated on filter papers and subjected to a two-choice bioassay against *L. rufipes* in a 4-arm olfactometer. A significant difference ($p < 0.01$) was obtained in olfactory response between extracts of healthy plant (EHe) and host-infested plant (EHI) suggests some attractive compounds were involved in the EHI. GC-MS analyses of EHe and EHI revealed that (*Z*)-3-hexenyl acetate, linalool, (*E*)-β-ocimene, and (*E*,*E*)-α-farnesene existed in volatiles with different proportions between EHe and EHI. With a higher ratio in EHI, (*E*,*E*)-α-farnesene was regarded as a candidate of herbivore-induced plant volatile (HIPV), whereas the other 3 components were regarded as background leaf volatiles (BLVs). When the attractiveness and dose-response of (*Z*)-3-hexenyl acetate, linalool, and (*E*,*E*)-α-farnesene were tested singly in the bioassays against hexane (solvent control), no significant attraction could be observed in all volatile treatments with all tested dosages. On the contrary, when (*E*,*E*)-α-farnesene was mixed with (*Z*)-3-hexenyl acetate or with both (*Z*)-3-hexenyl acetate and linalool at the ratio of EHI to make 2-component or 3-component volatile mixtures, females *L. rufipes* showed positive responses to both mixtures. These results suggest the combination between BLVs and HIPV is important for attracting female *L. rufipes* to it host rather than a single component. Further experiments on (*E*)-β-ocimene should be continued in assessing the attractive and/or synergistic effect when combining with other volatile components. Responses of female *L. rufipes* to BLVs and/or HIPVs emitted from other host plants (e.g. peach) are also interesting information in this wasp.

S7-O-04

Helicoverpa zea gut-associated bacteria indirectly induce defenses in tomato by triggering a salivary elicitor(s)

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Insect gut-associated microbes modulating plant defenses have been observed in beetles and piercing-sucking insects, but the role of caterpillar-associated bacteria in regulating plant induced defenses has not been adequately examined. We identified bacteria from the regurgitant of field-collected *Helicoverpa zea* larvae using 16S ribosomal RNA (rRNA) gene sequencing and matrix-assisted laser desorption/ionization time of flight (MALDI-TOF) mass spectrometry. A combination of biochemical, molecular, and confocal electron microscopy methods were used to determine the role of caterpillar-associated bacteria in mediating defenses in *Solanum lycopersicum* (tomato). Laboratory-reared *H. zea* inoculated with one of the bacteria identified in field-collected *H. zea*, *Enterobacter ludwigii*, induced expression of the tomato defense-related enzyme polyphenol oxidase and genes regulated by jasmonic acid (JA), whereas the salicylic acid (SA)- responsive pathogenesis-related gene was suppressed. Additionally, saliva and its main component glucose oxidase from inoculated caterpillars played an important role in elevating tomato anti-herbivore defenses. However, there were only low detectable amounts of regurgitant or bacteria on *H. zea*-damaged tomato leaves. Our results suggest that *H. zea* gut-associated bacteria indirectly mediate plant–insect interactions by triggering salivary elicitors. These findings provide a proof of concept that introducing gut bacteria to a herbivore may provide a novel approach to pest management through indirect induction of plant resistance.

Herbivore induced plant volatiles affect entomopathogens infectivity

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Tritrophic interactions involving insect pests, their natural enemies and plant hosts are getting an increasing interest as important factors in pest management. In response to herbivory attacks, plants activate a wide range of defences that aim to reduce their damages, including the release of herbivore induced plant volatiles (HIPVs). Through the past few years, studies have been focusing in the capacity of HIPVs to prime plants against insect herbivores, as well as their role as repellents of those herbivores and attractants of their parasitoids. However, so far there is no reports about the possible influence of these plant secondary metabolites on the interaction herbivore pests - entomopathogens.

In the present work, we aim to assess the effect of three relevant HIPVs, indole, linalool and (Z)-3-hexenyl acetate, in the tolerance of the polyphagous pest *Spodoptera exigua* to viral and bacterial entomopathogens. Exposure of larvae to indole or linalool increased the susceptibility of *S. exigua* larvae to its nucleopolyhedrovirus (SeMNPV) in terms of mortality and median survival time. The increase in susceptibility was the result of synergism between the baculovirus and the volatiles. In contrast, no effect has been detected when larvae were exposed to the (Z)-3-hexenyl acetate. Moreover, larval susceptibility to the *B. thuringiensis* increased in an additive manner when larvae were exposed to indole. No effect has been detected in the case of linalool or (Z)-3-hexenyl acetate exposure. Furthermore, our results show that the indole and linalool resulted in changes in the gene expression pattern of the exposed insects. This result suggests that volatiles might also manipulate the insect physiology, making the insects more susceptible to the invading pathogens and open new ways for a more efficient integrated pest management.

Yeast-insect interactions in a tephritid fruit fly pest.

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Yeast-insect interactions have been well characterized in drosophilid flies, but not in tephritid fruit flies, which include many highly polyphagous pest species that attack ripening fruits. Using the Queensland fruit fly (*Bactrocera tryoni*) as our model tephritid species, we identified yeast species present in the gut of wild collected larvae and found two genera, *Hanseniaspora* and *Pichia* were frequently present. Focussing on the two most commonly occurring yeast species in our study, we found that a fruit-agar media inoculated with one species, *Pichia kluyveri*, resulted in odour emissions that increased the attraction of adult female flies, whereas inoculation with the other species, *Hanseniaspora uvarum*, produced odours that strongly deterred flies and lead to decreased oviposition. We then carried out larval development trials, and discovered that the fruit-agar media inoculated with the 'deterrent odour' yeast species, *H. uvarum,* resulted in significantly faster larval development and higher survival to pupation, compared to media inoculated with the 'attractive odour' yeast species, *P. kluyveri ,* and a yeast free control media. Investigating a possible explanation for our seemingly counterintuitive finding (that the odours of a beneficial yeast should deter, rather than attract, adult flies), we showed that offspring fitness for female *B. tryoni* flies ovipositing into substrates that were already infested with fruit fly larvae was significantly lower than for flies that oviposited into uninfested substrates: thus adult flies may have evolved detection and avoidance responses to *H. uvarum* volatiles as a cue indicating infested fruits. GC-MS analysis investigated volatiles in *H. uvarum* and *P. kluyveri* that might be responsible for the yeast-specific olfactory responses of adult flies, and showed significant quantitative differences in volatile emissions between the two yeasts. Field trials using synthetic blends revealed that combining fruit ripening odours with particular yeast volatiles significantly increased attraction to traps, indicating that some, but not all, yeast volatiles may prove to be important components of odour lures used in tephritid "attract and kill" programs.

S7-O-07

Plant cell-wall degrading enzymes improve endophytism of entomopathogenic *M. brunneum* in potato plants

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Tritrophic interactions between crops, microorganisms and insects are highly diverse and not fully understood. Potential biocontrol microorganisms such as endophytic entomopathogenic fungi can strongly modify the interaction of insect pests with crops, paving the way for a novel plant protection measure. However, the transfer of this knowledge to practical applications, such as improved endophytism by means of formulation has not been widely exploited, yet.

Inspired by penetration mechanisms of plant pathogenic fungi, we aimed at enhancing *Metarhizium brunneum* strain Cb15 endophytism in potato plants through supplementation of plant cell-wall degrading enzymes pectinase and cellulase or corresponding substrates into beads.

Pectin beads containing *M. brunneum* mycelial biomass with (i) cellulose, and (ii) cellulose and cellulase were applied to potato tubers and incubated at 18-23 °C with a LD cycle of 16:8 for 21 days in the greenhouse. Plant roots, shoots and tubers were surface-sterilized prior to re-isolation of *M. brunneum.* Additional verification of re-isolated *M. brunneum* was conducted with qPCR. Pectinase and cellulase activity were determined by analysis of reducing sugars formed as a result of enzymatic degradation of corresponding substrates. Spore formation on the surface of beads was analyzed by resuspension of beads in 0.1 % Tween 80 and counting of propagules with a Thoma cell counting chamber. Statistical analysis was carried out with the software SPSS Statistics v.23 with a significance level of $P < 0.05$.

After application of pectin/cellulose beads to potato tubers, *M. brunneum* was re-isolated from roots, tubers, and shoots in 75.0 ± 9.4 %, 33.3 ± 9.8 %, and 29.2 ± 15.1 % of samples, respectively. Pectinolytic and cellulolytic enzymes were successfully induced by addition of corresponding substrates, but activity levels were low and no correlation between enzymatic activity and fungal penetration was found. However, incorporation of cellulase into beads led to a substantial increase in plant penetration by 25.0 % in roots, 54.2 % in tubers, and 16.6 % in shoots. This was accompanied by a 3.0-fold enhanced spore formation on the surface of beads to $1.91 \times 10^8 \pm 0.26 \times 10^8$ per bead. Finally, a stronger root development of treated plants was observed indicating a fertilizing effect mediated by the formulation.

This study will pave the way to utilization of enzymes in crop-microorganism-insect interactions in field applications.

S7-O-08

Earwigs (*Labidura riparia*) mimic rotting-flesh odor to deceive vertebrate predators

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Many insects repel predators with caustic chemicals, while insects mimicking odors of wastes/dead insects to fool predators have not been documented. We found that the shore earwig, *Labidura riparia* (Dermaptera: Labiduridae) when bitten by anole lizards, *Anolis carolinenesus*, spits a rotting-flesh odor that deceives these insectivores into rejecting prey. Once a lizard attacked and rejected an earwig, the lizard did not attack another earwig during several weeks despite consuming other prey, indicating associative learning after one trial. The fetid odor was found in the head-prothorax containing salivary glands of both male and female earwigs and was comprised of ~100 ng dimethyl disulfide and ~600 ng dimethyl trisulfide. Nymphs had <5 ng of either compound. Adults also spit odorous sulfides after prolonged attacks by harvester ants *Pogonomyrmex rugosus* that were only deterred by the earwig's forceps. Sulfides released by the earwig are similar to odors of carrion/feces, which may be innately repulsive to some vertebrate predators. The mean initial discharge percentage (IDP) of sulfides from a cohort of earwigs was 62%; however, IDPs of individuals were highly variable (3-99%; mean 57%). The discharge refill time (DRT) to refill 50% of the earwig's allomone reservoir was estimated at 13 h. A positive relationship in sulfide amounts with body weight was found only in females in 2009, suggesting metabolic cost tradeoffs were revealed when sulfide content was half that in 2010. This is the first report of insects releasing sulfur-containing compounds that may mimic carrion-fecal odors as a deceptive defense against vertebrate predators.

S7-O-09

Subtropical plant-insect-parasitoid tri-trophic interactions under elevated CO_2 and temperature

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The climate change and extreme weather affect plants and animals have been documented extensively over the past years. In tropical and subtropical regions shown only limited understanding of how these factors effect on plant chemistry and insect interactions. The purpose of this research is to address the consequences of elevated CO_2 and temperature on tri-trophic system (plant-herbivore-parasitoid) in sub-tropical regions by using greenhouse system. The experiment was set up, ambient CO_2 (500 ppm) and elevated CO_2 (1000 ppm), at ambient temperature 24/19 °C (day/night) and elevated temperature 29/26 °C (day/night). *Brassica oleraces* var. italica primary and secondary substaces were quantifed six weeks after seedling, after that insect performance bioassays were conducted. *Spodoptera litera* (Fabricius) larvae were fed directly on these plant until developed to pupae. The parasitoid *Snellenius manilae* (Ashmead) were fed on second instar *S. litura* larvae under the same condition as plant grown. Developmental and parasitism rate were analyzed. The results show that CO_2 elevation is the main factor that led to changed in plant performance and foliar quality; leaf area, fresh-dry weight and organic available protein, nitrogen, and carbohydrate contents were affected. Besides, the elevated temperature seems to shorten *S. litura* duration and increase the larval relative growth rate. Parasitism rate of *S. manilae* was high at elevated temperature; however their development durations were reduced. The interaction of temperature and CO_2 was found in development of *S. litura* and *S. manilae.* In connection with top-down control of herbivore by parasitoids, it is possible that, *S. manila* may get a benefit from abundance of host and asynchrony between host - parasitoid might not occur or be less severe.

Keyword: Tri-trophic system, Temperature, Climate change, Natural enemy

S8-K-01

Collective control of colony development through socially exchanged fluids

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Intra-specific fluid exchange often occurs in fitness-relevant contexts like mating, mate-choice or parental care, where a nominally important substance is exchanged (e.g. genetic material, food), but other more manipulative components are exchanged as well. Social insects frequently engage in oral fluid exchange–trophallaxis–wherein they pass the contents of their social stomach(s) between adults, and between adults and larvae. Although normally considered a simple food-sharing mechanism, we hypothesized that endogenous components of trophallactic fluid might underlie a novel means of communication. Through mass spectrometry and RNAseq analyses in the ant *Camponotus floridanus*, we found that trophallactic fluid contains a contingent of specific proteins, hydrocarbons, microRNAs, and juvenile hormone, an important insect growth regulator. When nursing workers' trophallactic fluid was supplemented with juvenile hormone, the larvae they reared more often completed metamorphosis and became larger workers. Phyloproteomic comparison of trophallactic fluid across two other ant species and the honey bee revealed that many proteins are regulators of growth and development. Specifically, we found a recently expanded clade of socially-transmitted enzymes that appear to regulate hormone levels in trophallactic fluid. Together, these results suggest that trophallaxis underlies a private communication channel that can have multiple phenotypic consequences including direction of larval development, and provide a new mechanism for how social insect colonies regulate community development through collective decision-making over the social network.

Sex and parental care: How pheromones regulate family life

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Scientists have long been fascinated by the fact that parents of some animals go to great lengths to increase survival and growth of their offspring, usually at a cost to their own survival and reproduction. They invest time and energy to feed their young, to carry them around, or to protect them from parasites, desiccation and other environmental hazards. However, the classical view that saw the family as harmoniously bound radically changed during the last decades and the current view is that families are rife with evolutionary conflicts of interest. For example, due to differences in the strength of sexual selection and certainty of parentage between the sexes, males have usually a greater potential for increasing reproductive success through attempting to gain additional mating opportunities than females, and the benefits of providing care are often lower for males. But why do males provide care at all and how is biparental care coordinated between the sexes? I will present new insights into family life evolution, thereby highlighting the central role of pheromones. I will show which important function pheromones can play in the evolution of male care and how chemical communication can resolve conflicts between the parents by regulating mating and parental care behavior. Finally, I will discuss how the evolution of parental care generates the potential to influence offspring phenotype by means of semiochemicals.

S8-O-01

Chemical tactic of juvenile orchid mantis for capturing honeybee

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It had been believed that juvenile orchid mantes made flower-visiting insects misidentify the mantes as real flowers by means of their flower-like appearance to capture them. Nevertheless, it was not well known where juvenile orchid mantes usually ambush and what kinds of insects they actually captured. Our field survey revealed that the juvenile female orchid mantes ambushed not on flowers but on leaves and frequently hunt oriental honeybees, while the adult female mantes ambushed on flowers and attacked butterflies more frequently than honeybees. Most of captured honeybees approached the juvenile mantes from the front as if to be captured. We therefore hypothesized that juvenile orchid mantes might attract oriental honeybees by chemical cues in addition to their flower-like appearances. Gas chromatography-mass spectrometry analyses revealed that the mandibular adducts of only the juvenile female mantes contained 3-hydroxyoctanoic acid (3HOA) and 10-hydroxy-(*E*)-2-decenoic acid (10HDA), both of which are also features of the pheromone communication of the oriental honeybee. We also successfully detected 3HOA emitted in the head space air only at the time when the juvenile mantes were attempting to capture their prey. Field bioassay showed that the oriental honeybee predominantly preferred to visit dummies impregnated with a mixture of the appropriate amounts and ratios of 3HOA and 10HDA. We therefore conclude that the juvenile orchid mantes utilize these hydroxy acids as allelochemicals to trick and attract oriental honeybees.

S8-O-02

Queen fire ant inhibits (primer pheromone) female sexual development, but newly-mated female sexuals need a quick colony foundation start

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When winged female sexuals are produced in a fire ant colony a queen produced primer pheromone inhibits the sexual's development, e.g., wing loss, ovariole development and pheromone production. This prevents competition for colony resources. If winged sexuals are removed from the influence of the queen, they will become disinhibited and lose their wings (dealation) and start producing eggs. The time to dealation is days to weeks. The normal process is where winged sexuals go on mating flights, mate, return to the ground, where they remove their wings within minutes. The total female sexual mating flight time is only about 60 minutes. The rapid dealation after mating is more than simply removal from the influence of the queen. This presentation will show evidence for an evolved male/female sexual mechanism to accelerate alate dealation and initiation of the typical physiological changes associated with newly mated queens.

S8-O-03

Social and physiological factors affecting queen-worker pheromone interactions in honeybees.

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The production of caste specific pheromones in the queen honeybee is not hard-wired, since workers that activate their ovaries, e.g., under queenless conditions, produce concomitantly these pheromones. In queens, the pheromones not only signals “I am present”, which consequently drives workers to refrain from reproduction, but also provide workers with information regarding the queen quality. To test whether workers can discern queen quality we performed preference test in which workers were free to choose between two compartments, each housing different types of queens. Workers preferred the more fecund queens, a trait that was apparently mirrored by her pheromone production. Preferred queens had greater amounts of both the queen mandibular and Dufour’s glands pheromones.

In workers, pheromone production has an important role in the establishment of reproductive dominance. When rendered queenless, workers first start to produce the mandibular queen bouquet, which signals their higher position in hierarchy. Secondly, concomitant with ovary activation, Dufour’s gland queen-characteristic esters are produced. Although the co-occurrence of Dufour’s ester and ovary activation suggests common regulation, inhibition of ovary activation with juvenile hormone did not affect ester production. Such an uncoupling indicate that these processes are independently regulated.

Reproductive dominance and esters production seem to be regulated also by biogenic amines. Octopamine significantly reduced ovarian activation in workers, while simultaneously increasing ester production in the Dufour’s gland, while dopamine had no apparent effect on both these parameters. Chlorpromazine, an antagonist of dopamine inhibited both ovarian activation and ester production, and the inhibition was age-dependent.

In conclusion, while ovary activation and royal pheromones are tightly associated, their expression seems to be independently regulated. This indicates a higher order common regulator, the nature of which is to be explored.

S8-O-04

Response profiles of sensory neurons in basiconic sensilla to cuticle hydrocarbons, key semiochemicals for nestmate discrimination in Japanese carpenter ant *Camponotus japonicas*.

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Japanese carpenter ants *Camponotus japonicas* are eusocial insects and discriminate between nestmates and non-nestmates by perception of colony-specific composition of 18 kinds of cuticular hydrocarbons (CHCs). These substances are received by sensory neurons in antennal basiconic sensilla. It has been revealed that sensory neurons in basiconic sensilla exhibited strong responses to CHCs extracted from non-nestmates but did scarcely to those extracted from nestmates [1]. On the other hand, it recently revealed in different carpenter ants that CHC extracts from both nestmates and non-nestmates induced strong responses in sensory neurons in single basiconic sensilla with different patterns [2]. Consequently, it is controversial how the antennal sensory neurons respond to CHCs and how CHCs is coded in its sensory system. Because single basiconic sensilla contain more than 130 sensory neurons and some CHCs are commercially unavailable, previous results were leaded from mass responses of many sensory neurons to mixture of CHCs extracted from ants. To reveal the neural mechanisms underlying nestmate discrimination in more detail, it is necessary to analyse response properties of each sensory neuron to a given component of CHCs. First, we newly synthesized the commercially unavailable CHCs of *C. japonicas*. Then we recorded mass responses of multiple sensory neurons from single basiconic sensilla to each of CHCs. Generally, each of mass-responses consisted of responses from 10-20 different sensory neurons. Subsequently, we segregated them based on the shapes of impulses. In this study, we successfully recorded 107 sensory neurons from 9 different basiconic sensilla. In the result, a given CHC elicited simultaneously excitatory responses from multiple sensory single sensilla. Response patterns and magnitudes of activated sensory neurons in single sensilla were different one another. In addition, each CHC activated different sets of sensory neurons in single sensilla. Response spectra analysis revealed that there are “generalist” and “specialist” types in sensory neurons; the former excited to most tested CHCs, whereas the latter excited to cognate sets of CHCs. Furthermore, with quantitative analysis of response intensities to different CHCs, we classified 107 sensory neurons to several groups based on the similarities of response spectra. These analyses suggested that responses of the “specialist” sensory neurons are correlated with chemical structures of CHCs, such as chain-lengths and loci of double-bonded carbons. These results strongly suggested that the ant discriminate compositions of 18 kinds of CHCs based on the firing patterns of sensory neuron populations.

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Ants eavesdropping on the variational trail pheromone in termites leads to signal arms race between the predator and the prey

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Background: Predators may eavesdrop on their prey using innate signals of varying nature. In regards to social prey, most of the prey signals are derived from social communication and may therefore be highly complex. The most efficient predators select signals that provide the highest benefits. *O. transversa* selected the trail pheromone of termites as kairomone in several species of fungus-growing termites (Termitidae: Macrotermitinae: *Odontotermes yunnanensis*, *Macrotermes yunnanensis*, *Ancistrotermes dimorphus*). **Objectives**: Here, we aimed to investigate the conflict of the use of eusocial prey signals by the termite-raiding ant *Odontoponera transversa*. **Methods:** We used GC-MS quantification, trapping bioassay, trail-following bioassays and motion analysis methods. **Findings and Conclusions:** The most commonly predated termite, *O. yunnanensis*, was able to regulate the trail pheromone component ratios during its foraging activity. The ratio of the two trail pheromone compounds were correlated with the number of termites in the foraging party. (3*Z*)-Dodec-3-en-1-ol (DOE) was the dominant trail pheromone component in the initial foraging stages when fewer termites were present. Once a trail was established, (3*Z,6Z*)-dodeca-3,6-dien-1-ol (DDE) became the major recruitment component in the trail pheromone and enabled mass recruitment of nestmates to the food source. Although the ants could perceive both components, they revealed stronger behavioural responses to the recruitment component, DDE, than to the common major component, DOE. In other words, the ants use the trail pheromone information as an indication of suitable prey abundance, and regulate their behavioural responses based on the changing trail pheromone component. The eavesdropping behaviour in ants therefore leads to an arms race between predator and prey where the species specific production of trail pheromones in termites is targeted by predatory ant species.

S8-O-06

Multifunctional roles of soldier pheromone in a termite

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Division of labor in eusocial insects is characterized by efficient communication systems based on pheromones. Among eusocial insects, termites have evolved specialized sterile defenders, called soldiers. Because they are incapable of feeding themselves, it has been suggested that soldiers are taken care of by workers and emit the pheromone arresting workers. However, such a soldier pheromone has not been identified in any termite species, and the details of the soldier-worker interaction remain unknown. Here, we identified a soldier-specific volatile sesquiterpene as a releaser pheromone arresting workers, which also acts as a primer pheromone regulating soldier differentiation and an anti-pathogenic agent in a Japanese subterranean termite *Reticulitermes speratus*. Gas chromatography and mass spectrometry analysis and optical rotation analysis revealed that (−)-*β*-elemene is the major component of soldier extract. Authentic standard of (−)-*β*-elemene exhibited an arrestant activity to workers and inhibited the differentiation from workers to soldiers. This compound also has inhibitory effects on the growth of entomopathogenic fungi (*Beauveria bassiana* and *Metarhizium anisopliae*). These suggest that (−)-*β*-elemene originally functioned only as a fungistatic agent derived from soldiers in *R. speratus*, and then came to be used for the soldier recognition and as an inhibitory pheromone. Our study provides novel evidence supporting the multifunctionality of termite soldier pheromone and provides new insights into the role of soldiers and the evolutionary mechanisms of termite pheromone compounds.

Trap Design Factors and Deployment Methodologies: Effect on Forest Coleoptera

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In addition to the threats posed by native insect pests, the introduction and establishment of non-native forest insects is one of the most significant threats to forest ecosystems globally. The introduction of non-native species to forest ecosystems, continues to occur at a constant or increasing rate, particularly for bark and woodboring beetles. Attractant-baited flight intercept traps are a critical component of operational surveys, and monitoring and management programs that target native and non-native forest Coleoptera. Our ability to develop and optimize flight intercept traps for operational trapping programs targeting forest Coleoptera is limited by the absence of a mechanistic understanding of how and why the most effective trap design varies with target taxa and how the community sampled varies among intercept trap designs. A series of field trapping experiments were conducted to document the effect of flight intercept type and design factors on the abundance and diversity of forest insects captured. Additionally, meta-analyses were used to examine patterns in effects across guilds and families of forest Coleoptera. In general these studies observed that the abundance of target taxa (Cerambycidae) varied with intercept trap type (in general panel traps were superior to multiple-funnel traps), bark beetles and woodborers were captured in higher numbers in traps treated with a surface treatment to make them slippery than in untreated traps, panel and multiple-funnel traps equipped with a wet cup outperformed traps with a dry cup, and black traps were superior to white and clear traps. The community of beetles captured in the field trapping experiments was analysed to determine if these trap type and design factors influenced community composition. These analyses demonstrated that there is a significant difference in the community of forest beetles captured by the different intercept trap designs, by traps treated with a surface treatment and untreated traps, and by traps with a modified large diameter bottom funnel and traps without a modified bottom funnel. There was no difference in the community of beetles captured by traps with a wet or dry collection cup. Differences in the silhouette presented are often assumed to be the mechanism for the patterns of differential trap capture observed among intercept trap designs. Clear, black and white panel traps were baited with host volatiles and monochamol in central Louisiana and northern Ontario to examine the effect of silhouette prominence on the capture of *Monochamus* spp. Finally, CO2 was used as a surrogate for pheromone and was released from a point source located on different intercept traps. A LI-COR gas analyser was used to measure CO2 at five height levels, five widths, and seven distances (175 measurements per trap) downwind. Heat maps of CO2 concentration will be constructed for each trap design to visualize differences in plume structure of the different intercept trap designs.

Identification of pheromones of the potentially invasive beetles *Callidiellum villosulum* and *Allotraeus asiaticus*

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Callidiellum villosulum (Coleoptera: Cerambycidae: Cerambycini) is a potentially invasive beetle that has been intercepted multiple times in wood trunks exported to the United States for the manufacture of artificial Christmas trees. Despite being a secondary pest for China fir (*Cunninghamia lanceolata*) and Japanese cedar (*Cryptomeria japonica*), it has potential to be a pest of living arborvitae (*Thuja occidentalis* 'Emerald Green'), a popular landscaping tree in the United States. Its congener *Callidiellum rufipenne* is invasive to North Carolina and several northeastern states in the US. Both sexes of both species are attracted to 3-hydroxyhexan-2-one (3R*) and a pyrrole synergist, 1-(1H-pyrrol-2-yl)-1,2-propanedione. While male *C. rufipenne* produce copious amounts of 3R* and minor components, the pyrrole synergist was not detected in volatiles from male beetles. In its native range in Guangxi province in China, *C. villosulum* occurs in China fir forests with the other cerambycids *Allotraeus asiaticus*, *Semanotus* spp. and *Xylotrechus buqueti*. In this system, 3R* + pyrrole are also attractants for *X. buqueti*, while the pyrrole is a potent attractant for *A. asiaticus* and attracts *Semanotus* spp. To identify possible pheromone components, live beetles were collected in flight intercept traps baited with 3R* + pyrrole. GC-MS analysis of the extracts of headspace volatiles collected from aerations conclusively demonstrated that male *C. villosulum* produce both 3R* + pyrrole, while male A. asiaticus produce only the pyrrole. Interestingly, both species also produced a pyrrole analog, which is being further investigated. This discovery will help to optimize traps for the detection of *C. villosulum* and *A. asiaticus* at ports of entry in the US and other countries.

S9-O-02

Use of pheromones for detection and monitoring of native and invasive cerambycid beetles

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Cerambycid beetles are some of the most important invasive insect species, both because they are readily transported to new areas of the world by international commerce, and because some species, such as the Asian longhorned beetle *Anoplophora glabripennis*, the peach red neck longhorn *Aromia bungii*, and a number of *Monochamus* species have the potential to cause tremendous damage in newly invaded areas. Up until ~2000, pheromones had been identified from less than 10 cerambycid species, and the prevailing view seemed to be that volatile pheromones were unlikely to play a major role in the chemical ecology of cerambycids. However, research over the past 12 years has diametrically changed that view, because it has become clear that numerous cerambycid species use pheromones for mate location and/or aggregation. For some species, these pheromones are active alone, whereas for others, the pheromones are strongly synergized by host plant volatiles. Equally as important, in parallel with the identification of new semiochemicals, the traps, lures, and protocols for effectively detecting and sampling cerambycid beetles have evolved dramatically. Here, we will summarize factors that are key to developing effective methods of monitoring cerambycid beetles, and discuss several recent examples of how newly identified pheromones have been exploited for detection and management of invasive and native pest cerambycid species. Conversely, the possible exploitation of cerambycid pheromones for monitoring rare and endangered cerambycid species will be described.

Volatiles released by Cerambycidae beetles used as chemical clues by the pine wood nematode to identify the insect vector inside the dead pine host trees

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The pine wilt disease (PWD), caused by the nematode *Bursaphelenchus xylophilus*, is responsible for the death of millions of conifer trees worldwide. This disease was detected in the early 20th century in Japan, spreading to Korea, China and to Europe, when in 1999 was detected in Portugal, killing *Pinus pinaster* trees. The affected trees are colonized by dozens of Coleoptera species, mainly scolytids, buprestids and cerambycids, however the insect-vectors used by the nematode to exit the dead pines and infect healthy ones are in all situations exclusively cerambycids from the genus *Monochamus*, and in Portugal there is only one insect-vector *Monochamus galloprovincialis*. To understand how the nematode identifies this species among the hundreds of insects colonizing each pine tree, the chemical volatiles released by the different stages of development of the insect vector and the other cerambycid species *Arhopalus syriacus* and *Pogonocherus perroudi*, which are present in the same pines at the same time, were identified and compared. Volatile compounds were isolated either by hydrodistillation, by organic solvent (ethanol and pentane) or by solid phase micro extraction (SPME), and analyzed by Gas Chromatography-Mass Spectrometry (GC-MS) for volatiles identification and by Gas Chromatography (GC), for their quantification. Each species and life stage showed a particular volatiles profile and some were elected as potential clues for the vector identification´s by the nematode.

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Key words: Pine wilt disease, *Bursaphelenchus xylophilus*, Cerambycidae, Gas Chromatography.

Identification and field attraction test of aggregation pheromone of *Monochamus saltuarius*, insect vector of pine wood nematode in Korea

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In this study, we isolated and identified an aggregation pheromone from *Monochamus saltuarius*, the major insect vector of the pine wood nematode in Korea. Adult male of *M. saltuarius* produces 2-undecyloxy-1-ethanol, which is known to be an aggregation pheromone in other *Monochamus* species. We performed field experiments to determine the attractiveness of the pheromone and other synergists. More *M. saltuarius* adult beetles were attracted to traps baited with the pheromone than to unbaited traps. Ethanol and (-)-α-pinene interacted synergistically with the pheromone. Traps baited with pheromone+(-)-α-pinene+ethanol were more attractive to *M. saltuarius* adults than traps baited with pheromone, (-)-α-pinene, or ethanol alone. Ipsenol, ipsdienol, and limonene were also identified as synergists of the aggregation pheromone for *M. saltuarius* adults. Our results suggest that a combination of aggregation pheromone and synergists could be very effective for monitoring and managing *M. saltuarius.*

Key Words: Aggregation pheromone; 2-Undecyloxy-1-ethanol; *Monochamus saltuarius*; Field test; Synergist

S9-O-05

Contact sex recognition pheromone of the Juniper bark borer, S*emanotus bifasciatus* Motschulsky (Coleoptera: Cerambycidae)

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Juniper bark borer, *Semanotus bifasciatus* (Motschulsky), is a destructive stem borer pest to *Platycladus orientalis* and causing severe damage to some old Chinese Arbor-vitae plants. We extracted the cuticular hydrocarbons from live *S. bifasciatus* female and male elytra with the solventless solid-phase microextraction (SPME) technique. Gas chromatography (GC) of the SPME samples compared with hexane extracts resulted in similar profiles. SPME-GC-mass spectrometry (MS) analyses demonstrated that the female elytra always had higher levels of 11-methylpentacosane (11-Me-C_{25}) and 11-methylheptacosane (11-Me-C_{27}) as well as lower levels of 11-methylhexacosane (11-Me-C_{26}). The three homologous series of methyl branched alkanes from the elytra of the *S. bifasciatus* were identified as contact sex recognition pheromones by behavioral assays. Live males can quickly recognize, locate, and attempt to copulate with live female once the adult females were introduced into the bioassay arena (Petri dish, ϕ150mm). The successful response percentage of male attempting copulation with live female within 60 seconds amounts to 94%, in contrast, only 39.02% to dead, unwashed female, which was freezed to death in the icebox for 5 min. Males in the arena spent 9.14 seconds attempting copulation with decoys (2 mL centrifuge tube) coated with 11-Me-C_{25}, the successful response percentage was 13.22%, 14.81 seconds with 29.63% successful response to 11-Me-C_{26}, and 19.05 seconds with 37.74% successful response to 11-Me-C_{27}. All tested males showed no responses to 13-methylpentacosane (13-Me-C_{25}), 13-methylhexacosane (13-Me-C_{26}), and 13-methylheptacosane (13-Me-C_{27}) in single or different combinations behavioral treatment, which shared the same retention times as the corresponding 11-Me-C_{25}, 11-Me-C_{26}, 11-Me-C_{27}, respectively. The males were presented with one of several mixtures of 11-Me-C_{25}, 11-Me-C_{26}, and 11-Me-C_{27} applied in hexane to decoys. Males demonstrated better responses (53.34%) to 1:2 ratio of 11-Me-C_{25} to 11-Me-C_{27} than any other treatments. This is the first contact sex recognition pheromone identified in the *S. bifasciatus*.

Keywords *Semanotus bifasciatus*, *Platycladus orientalis*, contact pheromone, 11-methylpentacosane, 11-methylheptacosane, 11-methylhexacosane

S9-O-06

Odorant receptors as molecular markers for pheromone use in the longhorned beetles (Coleoptera: Cerambycidae)

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The odorant receptors (ORs) are a diverse and rapidly-evolving family of genes, and a primary means by which insects recognize volatile compounds. Each OR is sensitive to a specific compound or class of compounds that evoke behavior, and thus, can provide a direct window into the chemical ecology and evolution of insects. We are currently describing the odorant receptors of the Cerambycidae, or longhorned beetles, a family of wood-boring insects that includes many damaging pests of forests. Subfamilies and genera of longhorned beetles often produce similar or identical pheromone structures (“motifs”), which in turn are likely detected by lineages of conserved or homologous OR genes. Hence, the OR genes associated with a known motif in one species may act as a genetic marker to reveal the production of pheromone in another. Here, we test that hypothesis by comparing the odorant receptor suites of six cerambycid species that produce two different pheromone motifs: the alkanediols, represented by 2,3 hexanediol (*Megacyllene caryae* and *Tragosoma* spp.); and the hydroxyethers, including 4-(heptyloxy)butanol and its aldehyde (*Anoplophora glabripennis*) and 2-(undecyloxy)ethanol (*Monochamus mutator*, *M. notatus*, and *M. scutellatus*).

S9-O-07

Function of pheromone binding proteins in olfactory recognition of two sympatric *Dendrolimus*

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Dendrolimus are serious coniferous pests in China. *Dendrolimus houi* and *Dendrolimus kikuchii* (Lepidoptera: Lasiocampidae) are two closely related and sympatric pests in southwestern China, and the pheromone compounds of these two species include cis-trans-isomers. Pheromone-binding proteins (PBPs) are believed to bind and primarily recognize pheromone components, thus PBPs are indispensable for insects during pheromone recognition. Here, we mainly focused on the functions of PBPs in pheromone recognition and reproductive isolation of *D. houi* and *D. kikuchii*.

Firstly, based on the next-generation sequencing of the antennal transcriptome of these two moths, we identified the major olfactory genes in these two species and compared the sequence with other insects. Secondly, we analyzed the ligand binding affinity of the PBPs from *D. houi* and *D. kikuchii*, and found that these two moth species used different strategies to recognize their pheromone, we also found that the four PBPs have different *cis-trans* isomer discrimination abilities. Thirdly, we studied PBPs functions in *D. tabulaeformis*, another sister moth species of *D. houi* and *D. kikuchii*. The results indicated that *D. tabulaeformis* recognize their own pheromone components with affinities negatively correlated to the ratios, similar with that of *D. kikuchii*. We deduce that “negative ratios recognition” is an important manner for insect pheromone components. Pheromone recognition mechanism explanation of *D. houi* and *D. kikuchii* will be the base to advance the control efficiency of these two pests using pheromones.

Stress-induced host tree chemistry benefits fungus farming by Ambrosia beetles

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As true fungus-farming insects, ambrosia beetles tunnel deep into trees to cultivate their nutritional fungal symbiont. Many species of ambrosia beetles attack a wide diversity of tree genera, but host quality often plays an important role such that physiologically-stressed trees are preferentially attacked over healthy trees. Foundresses also readily colonize stressed hosts, while superficial tunneling and abandoned galleries are associated with vigorous trees. Ethanol is induced within trees subjected to a variety of stressors and is a crucial attractant and boring cue for ambrosia beetles. While the antimicrobial properties of ethanol have been known and exploited by humans for centuries, ethanol also stimulates the growth of some microorganisms purportedly by acting as a carbon source. Thus, we hypothesized that ambrosia beetles specifically attack trees and tissues containing ethanol because it benefits their fungus farming practices.

We conducted a series of experiments to characterize the effects of ethanol on growth of ambrosia beetle fungal symbionts and unwanted "weedy" pathogens. Ethanol promoted the growth of *Ambrosiella* spp. fungal symbionts and inhibited *Aspergillus* sp. and *Penicillium* sp. Complementary field experiments demonstrated that ambrosia beetles readily attacked, established their fungal gardens, and produced offspring in galleries created in trees irrigated with varying concentrations of ethanol solutions. Beetles also attacked trees baited with ethanol lures, but did not establish their fungal gardens or produce offspring in tissues absent of ethanol. The influence of ethanol on ambrosia beetles colonizing a sawdust-based artificial diet will also be discussed.

We propose that ambrosia beetles use ethanol as a form of chemical pest management to suppress microbial competitors (e.g. *Aspergillus*, *Penicillium*) that are passively introduced during tunnel excavation and can devastate fungal gardens. Notably, if the fungal symbionts promptly become established within the tunnels, then they can outcompete secondary microorganisms. We also propose that stress-induced ethanol indirectly benefits ambrosia beetle colonization success since foundresses do not begin ovipositing eggs until their fungal symbiont is established and growing within tunnels. The energetic costs associated with tunnel/gallery excavation and the challenges of establishing fungal gardens have conceivably resulted in foundresses carefully selecting a host tree chemistry ideal for fungal farming and rearing offspring.

Test trial for controlling Japanese oak wilt using living trees or mass accumulated oak logs with the aggregation pheromone and kiromone of the Ambrosia Beetle, of *Platypus quercivorus* (Coleoptera, Platypodidae) .

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Oak wilt has increased explosively in Japan, killing oak trees on a massive scale. This tree disease is caused by attacks of the ambrosia beetle *Platypus quercivorus,* (Maruyama) (Coleoptera: Platypodidae) which vectors a pathogenic fungus, *Raffaelea quercivora*, responsible for Japanese oak wilt. In order to attract, capture and kill the ambrosia beetle, we isolated the main component of the aggregation pheromone of the beetle *P. quercivorus* from volatiles in the boring frass produced by unmated males using GC-EAD. The pheromone was identified as (1*S*,4*R*)-*p*-menth-2-en-1-ol (Quercivorol) by comparison of authentic compounds using GC-MS. Despite identification of Quercivorol as the aggregation pheromone, we were unable to capture large numbers of the beetles, after having tried several types of traps.

We thus developed a more effective decoy tree method, which uses the synthetic pheromone to attract the beetles to live oak trees inoculated with a fungicide that destroys their food supply and starves them. The synthetic pheromone used together with a boring treatment to stimulate (= the release of Kairomone from oak trees) attracts beetles in sufficient numbers. Since the male beetles bore into the sapwood of vaccinated oak trees (Decoy trees) and release natural aggregation pheromone, the decoy trees attract more beetles, and suffer mass attack. This combination of the use of fungicide-vaccinated live oak trees and synthetic quercivorol has proven to be an effective method for capturing the beetles and controlling their number. In this decoy tree method holes are drilled into the trunks of living trees in order to stimulate the discharge of kairomone and to improve the effectiveness of the synthetic pheromone. The results of a field trap test show that the most consistently effective kairomone was ethanol. We can attract more beetles using the synthetic pheromone and mass accumulated oak logs.

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S10-K-01

Ecological implications of flowering communication

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The timing of reproduction is a critical determinant of fitness, especially in organisms inhabiting seasonal environments. Flowering synchrony can increase reproductive fitness by enhancing pollinator attraction and probability of outcrossing, and by reducing seed predation. Increasing evidence suggests that inter-plant communication plays important roles in plant functioning and performance. We tested the hypothesis that besides abiotic cues such as photoperiod, temperature and humidity, flowering timing can involve communication between neighboring plants. Here we show that soil leachates from plants growing under flowering-inductive conditions accelerate flowering and decreased allocation to vegetative organs in target plants growing under non-inductive conditions. The results suggest that besides endogenous signaling and external abiotic cues, flowering acceleration and synchrony may involve inter-plant cuing mediated by root exudates. Further work is underway to decipher the identity and mode of operation of the involved vectors and to explore the ecological implications of inter-plant flowering communication. The study of this phenomenon is expected to illuminate neglected aspects plant interactions and reproductive biology, and to provide novel opportunities for controlling the timing of plant reproduction in agricultural settings.

S10-K-02

Insect herbivory selects for volatile-mediated plant communication in *Solidago altissima*

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Plants commonly respond to insect herbivory by altering the emission of volatile organic compounds (VOC's). This altered emission can attract mutualists attacking herbivores or function as means of communication between branches or stems of the same plant genotype. More recently the role of VOC's in transfer of information between different genotypes within a population has gained more attention. Plant communication can be beneficial under herbivore pressure if it results in more even distribution of damage in a population. Here we compared the herbivore-induced changes in VOC emission from *Solidago altissima* originating from long-term replicate experimental populations where insect herbivory was suppressed (H-) or not (H+). VOC profiles did not differ between the two origins overall, but the damaged plants from H+ plots converged in their emission of sesquiterpenes, i.e. they became more similar when damaged, whereas the plants from H- plots did not. We then tested the effect of damage to a focal plant on defense induction in the neighboring plants and discovered that the plants from the two origins differed in their response to non-self plants: plants from the H- plots induced a stronger resistance to herbivores when the focal plant was of the same genotype whereas H+ plants induced resistance equally to all genotypes, self and non-self. Genetic distance among non-self plants did not explain the differences in induction of resistance. Our results suggest that release from selection by herbivores has resulted in more diffuse induction of VOC's and private channels of chemical communication within genotypes instead of among genotype communication. Insect herbivores may therefore be important drivers of the evolution of plant-plant communication.

The language of plant communication

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My talk will consider 5 questions:

1. What is communication? I use this term to refer to release of a cue by a sender that is perceived by a receiver and causes the receiver to change its phenotype.
2. Do plants communicate? Sagebrush plants exposed to cues from experimentally clipped neighbors experienced less herbivory throughout the season. A meta-analysis revealed that this was not unusual.
3. How does this compare to animal perception and response? Plants are composed of redundant units that sense their environment but are not mobile. They can tolerate some loss of tissue. As a result, they alter their allocation of resources rather than fleeing from danger. They are better able to multi-task and can collect more accurate cues before responding.
4. What are the costs and benefits of responding to cues? Sagebrush that was exposed to experimental cues of herbivory experienced higher survival of seedlings and more branching and flowering. These benefits may come at the cost of less over-topping growth.
5. How specific (private) is communication? Some cues are very general, others very specific. Sagebrush responds more effectively to cues from self tissue, from kin, from individuals of a similar chemotype, and from geographically local individuals. This specificity may provide clues about the language of communication.

The role of kin discrimination in interspecific competition in *Plantago asiatica*

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Some plant species discriminate between kin and non-kin neighbours through root chemicals or leaf volatiles, and alter root growth or leaf position in response, thereby potentially minimising competition among kin. The plasticities through kin-discrimination may alter the outcomes of interspecific competition. Here we report that the genotype of a conspecific neighbour determines the outcome of interspecific competition through phenotypic plasticity in a perennial plant, *Plantago asiatica* (Plantaginaceae). We investigated effects of genotype of neighbor sibling or non-sibling *P. asiatica* plants on competition with *Trifolium repens* plants. Growth, seed production and plasticities of *P. asiatica* plants in each condition were estimated. Growth and seed production of *P. asiatica* were heavily reduced when grown with *Trifolium repens.* However, sibling *P. asiatica* plants inhibited the shoot growth of *T. repens* plants by placing leaves towards *T. repens* plants. As a result, seed production of paired *P. asiatica* plants competing with *T. repens* was greater when they were siblings than when they were unrelated. These results suggest that the genetic spatial structure within a species influences outcomes of interspecific competition through genetic-structure-dependent plasticity. In addition, we found that the germination behaviour of *P. asiatica* seeds also change in response to genetic similality of neighbouring conspecies seed and presence of *T. repens* seed. We investigated the timing of emergence of *P. asiatica* seed while manipulating the presence of *T. repens* seed and the relatedness of neighbouring *P. asiatica* seed. As a result, when encountering a *T. repens* seed, a *P. asiatica* seed emerged faster and synchronous in the presence of a sibling seed than in the presence of a non-sibling seed. To identify possible cues, we conducted water extract experiments the following: 1) sibling seed + DW, 2) non-sibling seed + *T. repens* extract, 3) sibling seed + *T. repens* extract, and 4) sibling extract + *T. repens* extract. The *P. asiatica* seed emerged faster in 3) and 4) conditions than the 1) and 2) conditions. These results indicate that *P. asiatica* seeds integrate information about the relatedness of conspecific neighbour seeds and the presence of other species, likely by sensing water-soluble chemicals. The acceleration of emergence and synchronous-emergence in the presence of *T. repens* may support the competitive advantage in seedling.

S10-O-02

Conversion of green leaf volatiles for processing the information from surrounding environments in tomato leaves

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Volatile organic compounds are used as a communication tool among organisms. Plants are one of the major sources of volatiles, which are used for attraction of pollinators and carnivorous natural enemies of herbivores, and repellent of herbivores. Further, they are used as alarm signal by surrounding plants. In the context of alarm signal between plants, we previously reported that tomato leaves specifically accumulate a glycoside, (*Z*)-3-hexenyl vicianoside (HexVic), as a defensive compound against insect herbivore, *Spodoptera litura*, when the plants were exposed to volatiles emitted from *Spodoptera*-infested tomato plants. The aglycone of HexVic was (*Z*)-3-hexenol, one of the common green leaf volatile compounds emitted from herbivore-infested plants, indicating that the aglycone was derived from airborne (*Z*)-3-hexenol. However it remains to be answered that whether the glycosylation system is conserved against various volatile alcohols and conserved among various plant species. To address this question, we analyzed glycosylation of various green leaf volatiles in tomato and glycosylation of (*Z*)-3-hexenol among different species of plants.

When we exposed other green leaf volatile compounds, (*Z*)-3-hexenal or (*Z*)-3-hexenyl acetate, to tomato leaves, the exposed plants also accumulated HexVic like they did when exposed to (*Z*)-3-hexenol. When we exposed (*E*)-2-hexenol or *n*-hexanol, the plants accumulated the compounds corresponding to (*E*)-2-hexenyl vicianoside or *n*-hexyl vicianoside, respectively.

All the plants we tested (Poaceae, Cucurbitceae, Favaceae, Malvaceae, Brassicaceae, Plantaginaceae, Lamiaceae, Solanaceae, and Asteraceae) had ability to convert (*Z*)-3-hexenol into (*Z*)-3-hexenyl glycosides, even though there were differences in sugar moiety, e.g. Brassicaceae plants exclusively accumulate (*Z*)-3-hexenyl glucoside rather than HexVic. Among plants, we found that a wild species of tomato, *Solanum pennellii*, had extremely low ability to synthesize HexVic after (*Z*)-3-hexenol exposure. Based on this difference, we found a single locus involved in HexVic biosynthesis through the screening of low HexVic-accumulating plants among recombinant inbred lines of *S. lycopersicum* × *S. pennellii*.

Our results suggested that conversion of volatile compounds into their glycosides is conserved system among various plant species. Additionally, plants have an ability to convert different green leaf volatiles into their glycosides. These results implied that plants are probably able to process various kinds of volatile alcohols as information through glycosylation. Additionally, plants can apply other metabolic processes, probably reduction and hydrolysis, to process aldehyde or ester volatiles before glycosylation.

S10-0-03

Pest management using plant-plant signalling mediated by mint volatiles

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"Plant-plant signaling" is a type of natural plant communication in which plants eavesdrop on volatile organic chemicals emitted from neighboring plants to boost their defense responses. Lamiaceae have been classically used as companion plants that assist in repelling pests, in which especially mint volatiles have been noted to manage interactions of plants with arthropods and microorganisms. However, not much is known yet about the plant-plant signalling via mint volatiles. Here we present a novel ability of mint volatiles acting as "plant-plant signals" for powerful pest control of agriculture crops. We assessed 12 varieties of *Mentha* spp. to explore their ability to induce defence responses in neighboring legume plants. We found that *Mentha* x *piperita* cv. Candy (candy mint) was the variety that most strongly enabled activation of the expression of defence genes (trypsin inhibitor [*TI*] and *PR1*), thereby leading to acquired resistance to pests and pathogens. The activated expression statuses of *TI* and *PR1* were maintained no longer than 8 days after discontinuing mint volatile communication carried out for 7 days. The mechanism of the mint volatile-mediated enhancement of gene expression was likely based on histone acetylation, an epigenetic basis of memory for plant defence. Field assays showed valuable pest management of soybean plants when co-cultivated with candy mint and when pre-cultivated with the mint. Together, the findings suggest a pest control system relying on mint companion plants could be commercially useful for pest control in agriculture.

Applying plant-plant communication on rice field

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When intact plants receive volatiles of damaged plant, the intact plants become resistant against herbivores. The phenomena is called “Plant-plant communication”. We have been applying the phenomena on weed-crop communication. We have already reported that soybean plants which received volatiles from cut goldenrods got less damage than unexposed soybean plants, moreover the exposed plants produces more seeds than unexposed ones.

In this presentation, I will talk our rice filed experiments for 4 years. We conducted over 2 years (2012 and 2013), in which half of the seedling rice plants were exposed to volatiles from rice filed surrounding weeds over 2–3 weeks, while the other half remained unexposed. After 2 months, the number of damaged leaves of the exposed rice plants were less than the unexposed ones. Moreover, the harvests of the exposed rice plants were higher than the unexposed one. In next 2 years (2015, 2016), we used goldenrod and Japanese pampas grass as exposing volatiles. The rice plants exposed volatiles from cut goldenrod got more damaged by herbivores than controls. While, rice plants which were exposed volatiles from cut Japanese pampas grass got a little bit less damages than control ones. According to results, we suggest that the combination of weed species and crop species are important in plant communication.

Action of avenacins in oat roots as allelochemicals

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Wild oat has been known as the most problem plants against wheat and other cereals. It has been thought that some chemicals released from oat roots might cause this phenomenon. Fay & Duke (1977) showed that scopoletin was released from roots of 3,000 *Avena* accessions. They reported that scopoletin would be allelochemicals of oat. On the other hand, avenacins have been demonstrated to act as defencing factors for invading microbes in oat roots (Osbourn et al. 1994). It was also shown that avenacins were released into the soil around roots of oat (Carter et al. 1999). Antifungal activity in root extracts and root exudate of oat (*Avena stringosa*) was examined in this study. Oat seeds that husked, and sterilized, were germinated on wet filter paper for one day and then grown onto plastic mesh that was floated on water in plastic container [13 x 9 x 7 (height) cm] for 9 days at 22 °C. Their roots were cut from seedlings and homogenized in liquid N_2. Their iced powders of homogenates were extracted with MeOH and the extract was filtrated. The filtrate was used as root extract. Water in the container was evaporated and dissolved in MeOH. This was used as root exudate. The root extract and the root exudate were spotted on a silica gel TLC plate. The plate was developed with $CHCl_3$-MeOH-H_2O (13:6:1, v/v/v). After removal of the solvent from the plate, antifungal bioassay on the plate using conidia of *Colletotrichum orbicular* was performed. Main antifungal compounds in the root extract and the root exudate were confirmed as avenacins, not scopoletin. Next, phytotoxic activity in root extract of oat was examined. Root extract was charged width of 2 cm on the TLC plate. The plate was developed with the solvent. After removal of solvent from the plate, 1% agar solution at 40 °C was uniformly sprayed on the plate. After 1 h, germinated seeds of lettuce and Chinese spinach were placed covering the complete surface of the plate, respectively and their plates were incubated at 28 °C for three days. The growth of their seedlings was strongly inhibited at the position of avenacins on both plates, but not inhibited at the position of scopoletin. At last, allelopathic effect of oat using wild-type or *sad 1* mutant (non-production of avenacins) was examined against the growth of rice roots. Seven germinated seeds of wild type oat and *sad1* mutant oat were lined on band of 1% agar (width of 1 cm) over wet filter paper (5 cm x 10 cm) of lower half part in the square plastic box [10 x 10 x 1 cm (height)], respectively. Their boxes were incubated at 22 °C adding 1 mL water every 24 h for 4 days. Every one Seedling of rice (*Oryza sativa* var. Nipponbare) with root of length of 0.5~0.8 cm was placed between oat seedlings of five day old on agar band in the box and the box was incubated at 28 °C for 3 days. Roots of rice seedlings between seedlings of wild-type oat were inhibited greater than them between seedlings of *sad 1* mutant oat. This result suggests that avenacins secreted from roots of wild-type oat might inhibit the growth of neighboring plants of oat. Avenacins are released from oat roots are more likely to act as allelochemicals.

S11-O-01

Spatial distribution of floral scent luring blow fly pollinators into inner cavity of *Rafflesia cantleyi*

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Recent chemical analysis of floral scent has confirmed that *Rafflesia* is indeed a ‘carrion flower’ as the flowers emit predominantly the oligosulphides, the common compounds which were reported to attract carrion insects. However, a few questions remain unanswered: with respect to the relatively large flowers, which part(s) of the flower is responsible for the floral emission of the putrid scent of *Rafflesia*? We sampled floral odour from various parts of freshly bloomed flower via dynamic headspace and thermo-desorption GC-MS. Results showed a spatial pattern in floral scent distribution, with a higher content of floral volatiles from the inner structures within the cavity compared to external structures of the flower. The results also corroborated with the landing frequency of blow flies on the flower. Scanning electron microscope examination revealed the presence of microstructure responsible for scent production and emission in *Rafflesia cantleyi*.

S11-O-02

A sesquiterpene attractive to male and female Oriental fruit fly, *Bactrocera dorsalis*

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The Oriental fruit fly, *Bactrocera dorsalis* Hendel (Diptera: Tephritidae) is one of the most destructive and highly invasive pest species. It has been recorded or intercepted in almost all tropical and subtropical regions in the world. Although current control of this pest species has been centred on the application of a toxicant-baited potent male lure, methyl eugenol, the search for the silver bullet in managing the destructive gravid females has been elusive to date. Hitherto, the control of female fruit flies has been largely limited by application of protein bait that also attracts many other non-target arthropod species. Thus, in addressing the progress of current fruit fly control methods, it is highly desirable and economical that an ideal fruit fly lure be attractive to both sexes. In our search for new attractants, we have discovered a particular sesquiterpene compound found in certain host fruits that is attractive to both male and female *B. dorsalis*. Interestingly, this compound has also been observed to significantly attract more gravid females rather than virgin females. Contrastingly, wind tunnel assays revealed significantly lower attraction of gravid females to calling males as compared with virgin females during peak courtship period at dusk. These results suggest the possibility that mating may also induce a switch in preference to host fruit odours rather than to conspecific males. Further, laboratory bioassays also revealed the sustained sesquiterpene attractancy to the gravid female even after 5 days following successful initial copulation.

S11-O-03

Ecological contexts of mosquito odorant receptor function

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It is an unfortunate but well-known fact that mosquitoes primarily rely on olfaction to locate a variety of resources. For example, 1-octen-3-ol and indoles have long been associated with animal host and oviposition site locating behaviours. Yet, the behavioural significance and precise ecological role of these known mosquito attractants remain obscure. To complicate the matter, these odorants are produced by microorganisms and plants encountered by mosquitoes in a variety of situations. Blood-feeding mosquitoes rely on a group of evolutionarily conserved odorant receptors for the detection of these ubiquitous odorants. Homologous receptor genes were recently identified in a strict nectar-feeding mosquito species, which we functionally characterized using the two-electrode voltage clamp of *Xenopus* oocytes. We show that these conserved receptors exhibit similar tuning properties to those from blood-feeding mosquitoes suggesting a role outside animal-host seeking and perhaps in multiple ecological contexts.

What are stink bug male-produced pheromones doing on eggs? Or not.

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Egg parasitoids are effective biological control agents against stink bugs because they kill pentatomid pests before plant damage occurs. The present study using eggs of the stink bugs *Euschistus conspersus* and *Halyomorpha halys*, and the native North American parasitoids, *Telenomus podisi* and *Trissolcus erugatus*, was an extension of earlier research on scelionid/pentatomid egg parasitism [1] The primary objective of the present study was to identify the volatiles from the surface of stink bug eggs, and bioassay crude egg extracts and the compounds identified from egg extracts. The pentatomid bugs and scelionid wasps were maintained by established methods, gram quantities of host eggs were extracted by immersion of eggs in solvent for 5 min, extracts were analyzed by GC-MS, and natural and synthetic egg volatiles were tested in a Y-tube olfactometer using young mated female parasitoids [2]. The genital capsules (terminal segment) of wild *E. conspersus* and *H. halys* males were also extracted as for eggs, and the extracts were analyzed by GC-MS. The main component of the male-produced aggregation pheromone of *E. conspersus*, methyl (2*E*,4*Z*)-2,4-decadienoate, was the major volatile identified from extracts of *E. conspersus* eggs. In contrast, for *H. halys*, the sesquiterpenoid compounds that comprise the male-produced aggregation pheromone of this species were not detected on their eggs; however, hexadecanal, octadecanal, and eicosanal were identified in extracts of *H. halys* eggs. Methyl (2*E*,4*Z*)-2,4-decadienoate and tridecane were the main volatiles from genital capsules of *E. conspersus* males; whereas genital capsule extracts of *H. halys* males contained the $C_{16,18,20}$ aldehydes that were found on *H. halys* eggs, but not the male-produced sesquiterpenoid aggregation pheromone of *H. halys*. Both *Tr. erugatus* and *Te. podisi* females were attracted to extracts of *E. conspersus* eggs in laboratory olfactometer tests, and to synthetic methyl (2*E*,4*Z*)-2,4-decadienoate. On the contrary, female *Tr. erugatus* and *Te. podisi* wasps were repelled both by extracts of *H. halys* eggs, and by a blend of the aldehydes identified from *H. halys* eggs. These results suggest that methyl (2*E*,4*Z*)-2,4-decadienoate is transferred to conspecific females during mating, eggs are subsequently contaminated with this pheromone compound during oviposition, and parasitoids then exploit this pheromone compound as a kairomone to find host eggs. Similarly, $C_{16,18,20}$ aldehydes present on *H. halys* eggs may be transferred from males during mating, but the possibility that *H. halys* females themselves are the source of the $C_{16,18,20}$ aldehydes cannot yet be excluded. In conclusion, there must have been some positive function associated with the methyl ester pheromones transferred to females during mating that, at least in evolutionary history, outweighs the disadvantage of attracting egg parasitoids. Perhaps sesquiterpenoid stink bug pheromones, which are produced from cells in the abdominal sternum, evolved subsequently to the methyl ester-type pheromones under parasitoid pressure toward an enemy-free space.

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Defense allocation upon multiple stresses: impacts of drought stress on performance of caterpillars and induced defense responses in tomato

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The ability for plants to respond accurately upon specific stressor or stresses combination is important to the survival of plants. Water stress and herbivory often co-occur as two of the most important stressors affecting survival and distribution of plants. The ability for plant to respond to single stressor, either herbivory or water stress, are well studied. However, how water stress affects the ability for plant to respond against insects are less addressed. Using Tomato, *Solanum lycopersicum* L. cv. Better Boy, and two pest species, tomato fruitworm, *Helicoverpa zea*, and tobacco hornworm, *Menduca sexta*, we study the impact of drought stress on (1) plant primary metabolites, (2) herbivore performance, and (3) plant defensive response against insects. Drought stress were divided into three levels: severe, moderate and well-watered. Insect damages were introduced after the establishment of drought stress. Primary metabolites, and activity of two defensive proteins, polyphenol oxidase and protease inhibitors, were analysed to determine the nutritional status and defensive responses of treated plants. Drought stress lead to slight changes in primary metabolite compositions in tomato leaf tissue. Moderate drought stress has a positive impact on generalist pest species *H. zea* on tomato leaf, whereas, specialist pest *M. sexta* are negatively affected by drought stress. Severe drought stress increases the constitutive defensive response of tomato leaf, but decreases the inducibility of defensive response. Well-watered tomato have a lower constitutive defensive response, and a higher inducibility of such defense. We observed altered defense allocation patterns in plant challenged by insect under different drought severity. In conclusion, herbivore of different host specificity were indirectly affected by drought stress differently. The results suggested that drought stress differentially alters the dynamics of plant-insect interaction among insect herbivores, potentially having a broader impact on community and ecosystem dynamics.

S11-O-06

Secretion of isoflavones from soybean roots and their degradation dynamics in the rhizosphere

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Isoflavones play important roles in the communication between plant and soil microbes in rhizosphere [1]. Soybean secretes daidzein, genistein and their glucosides into the rhizosphere, which attract rhizobia to form nodules and possibly modulate the rhizosphere microbial communities. In hydroponic culture soybean secrete higher amount of daidzein during the vegetative growth than during reproductive stages [2]. Despite their important roles of isoflavones, little is known about the biosynthesis, secretion and fate of isoflavones in field-grown soybeans. In this study, we analysed isoflavone contents, the expression of isoflavone biosynthesis genes, and the degradation dynamics of isoflavones in field conditions.

In leaves and roots, isoflavone contents and composition did not change during the crop growth, but the expression of *UGT4*, an isoflavone-specific 7-*O*-glucosyltransferase, and of *ICHG* (Isoflavone conjugates hydrolysing beta-glucosidase) was decreased during the reproductive stages. Isoflavone contents were higher in rhizosphere soil than bulk soil both vegetative and reproductive stages in the field growth soybean. In rhizosphere soil, isoflavone contents did not decrease at reproductive stage, suggesting either higher secretion of isoflavone at reproductive stages in field condition, or secreted isoflavones are rather stable in rhizosphere.

We then analysed the degradation dynamics of daidzein and its glucosides in order to develop a model for predicting rhizosphere isoflavone contents using the amount of isoflavones secreted in hydroponic culture. Isoflavone glucosides such as malonyldaidzin and daidzin were degraded much faster than daidzein, with degradation rate constants of 8.51 d^{-1} for malonyldaidzin and 11.6 d^{-1} for daidzin. In contrast, the degradation rate constant for daidzin was 9.15×10^{-2} d^{-1}. The rhizosphere isoflavone degradation model suggested that secretion of isoflavones into the rhizosphere is higher during vegetative stages than during reproductive stages in field-grown soybean as well as hydroponic culture.

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S11-O-07

Mechanisms of resistance to bufadienolide toxins in toad-eating snakes

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Most toads (Bufonidae) are defended by cardiotonic steroids known as bufadienolides (BDs), which are synthesized from cholesterol in abundant granular glands of the skin. BDs are chemically and pharmacologically similar to the cardenolides (CDs) of plants, which figure prominently in many plant-insect chemical interactions. Although BDs are lethal to most potential predators, a number of snakes are capable of surviving the lethal effects of occasional toad consumption. Furthermore, a few taxa have evolved diets rich in toads and some sequester the toxins, which can be redeployed for the snakes' defense. Like CDs, the BDs of toads target the Na^+/K^+-ATPases (NKAs) of cell membranes, which are responsible for maintaining intra-and extracellular ion concentrations. As with cardenolide-eating insects, it has been reported that a number of bufophagous (toad-eating) snakes possess mutations of the first extracellular loop of NKA, at least in the commonly studied paralog encoded by *ATP1a3*, which confer target-site insensitivity to BDs. However, our recent studies reveal that such mutations are not limited to snakes that consume toads, even occasionally, but rather occur repeatedly across the phylogeny of snakes and in some cases occur in large and trophically diverse clades. This suggests that additional physiological mechanisms may also contribute to the survival of BDs, especially in in specialized bufophagous species that experience chronic exposure to the toxins. We have investigated several potential physiological mechanisms that may contribute to BD resistance and find that bufophagous snakes differ from non-bufophagous species in several respects. In a study of one genetically resistant dietary generalist we find that *ATP1a3* is expressed at especially high levels in cardiac muscle in comparison with several other tissues, including kidney, and expression increases in cardiac muscle in response to challenge with the BD gamabufotalin. We also find that the NKAs of cardiac muscle and kidney in genetically resistant species are less senstive to challenge with a cardenolide (used as a surrogate for a BD in an *in vitro* assay) than are the NKAs of other tissues or of a non-resistant species. Finally, we find that a specialized bufophagous snake responds to cardiotonic steroid exposure with lower corticosterone and higher aldosterone levels than does a generalist predator with genetic resistance. Collectively, these results suggest that varied physiological mechanisms may operate in tandem with target-site resistance to confer increased resistance to BDs in some species of snakes, including those that prey heavily on toads.

S11-O-08

The chemical analysis of bufadienolides in defensive glands of the Asian *Rhabdophis* snakes.

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The Japanese snake *Rhabdophis tigrinus* has defensive glands on its neck called nuchal glands. The major chemical components of the glands are steroidal toxins known as bufadienolides (M.W., 398 ~ 434). *Rhabdophis tigrinus* sequesters the toxins from toads it consumes as prey. The nuchal glands have been reported from about ten species of Asian *Rhabdophis* snakes but chemical components of the glandular fluid have not been investigated in all species except for *R. tigrinus*. The Chinese snake *R. pentasupralabialis,* which has nucho-dosal glands, mainly eats earthworms while *R. tigrinus* eats frogs.

As a result of LCMS analysis, we revealed that the snakes had some heavier bufadienolides (M.W., 448 ~ 564) than *R. tigrinus* had. Then we purified four bufadienolides from the glandular fluid of the snakes. Through NMR spectroscopic analysis, we identified one of them as a bufadienolide xyloside reported from the North American firefly *Lucidota atra* and the others also had same planer structures as bufadienolides from *L. atra*. These results suggested that *R. pentasupralabialis* would prey on Chinese fireflies closely related to *L. atra* and sequester bufadienolides from the fireflies in the glands.

We also conducted chemical analysis on the glandular fluid from ten species of other *Rhabdophis* and related snakes and compared with bufadienolides from *R. tigrinus* and *R. pentasupralabialis.* The analysis showed that the glandular fluid from all species contained bufadienolides and *R. nuchalis,* which mainly eats earthworms, also had bufadienolide identified from *R. pentasupralabialis.* It implies that the origin of bufadienolides sequestered in the glands may have shifted from toads to fireflies after the shift of prey in the course of the evolution of *Rhabdophis* species.

S11-O-09

Evolution of chemical mimicry in cuckoo wasp

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Parasitism is a non-mutual interaction between species where the parasites benefits from the host. There are different forms of parasitism, one of which is cleptoparasitism where one species exploits resources of another species as found in brood parasitism. In this case the parasites' offspring are raised by the host. Thereby, the parasite reduces their energy cost for nest production and providing resources which leads to a reduced fitness of the host. This selects for strategies used by the host to counteract parasitism. In contrast, parasitoids evolve strategies to develop undetected by the host such as through mimicry. These strategies are recognized in birds, fish and insects. The most prominent example is the cuckoo who lays colour adapted eggs unnoticed in the nest of the host. Whereas parasitisation in birds are heavily investigated, brood parasitism in insects does not attract as much attention. A cleptoparasitic family in the Hymenoptera is the Chrysididae (cuckoo wasps). There are parasites of species from different insect families and many of them lay their eggs in the host nests during the provisioning phase of the host. In this case the detection of the parasitisation process is prevented by a chemical adjustment of the cuticular hydrocarbon profile (CHC) of the parasite to the host (chemical mimicry). This chemical adjustment was shown in a few selected species in the family of the cuckoo wasps. Therefore, we will investigate whether chemical mimicry is a general strategy evolved in chrysidids. By analysing and comparing the CHC profiles of parasites and their hosts from various species within the family of the Chrysididae, we will also understand whether host changes in the phylogeny could be partly explained by similarities of CHC profiles between hosts from different taxa.

The biochemical mechanism underlying sex pheromone evolution in *Nasonia*

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Speciation in insects is typically accompanied by a diversification of sex pheromones. The genetic and biochemical mechanisms underlying pheromone diversification, however, are only poorly understood in most taxa. Males of all species of the parasitic wasp genus *Nasonia* use (4*R*,5*S*)-5-hydroxy-4-decanolide (*RS*) as component of their sex pheromone while only *N. vitripennis* (*Nv*), employs additionally (4*R*,5*R*)-5-hydroxy-4-decanolide (*RR*) [1]. Three genes coding for the NAD^+-dependent short-chain dehydrogenases/reductases (SDRs) NV10127, NV10128, and NV10129 are linked to the ability of *Nv* to produce *RR*. Here we show by assaying recombinant enzymes that SDRs from both *Nv* and *N. giraulti* (*Ng*), the latter a species with only *RS* in the pheromone, epimerise *RS* into *RR* and *vice versa* with (4*R*)-5-oxo-4-decanolide occurring as an intermediate [2]. *Nv*-derived SDR orthologues generally had higher epimerisation rates, which were also influenced by the availability of the co-factor NAD^+. Semiquantitative protein analyses of the pheromone glands by tandem mass spectrometry revealed that NV10127 as well as NV10128 and/or NV10129 were more abundant in *Nv* compared to *Ng*. Hence, an interplay of differential expression patterns and SDR epimerisation rates on the ancestral pheromone component *RS* accounts for the evolution of a novel pheromone phenotype in *Nv*.

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S11-O-11

Where do they come from?: Can we use naturally occurring stable isotopes to better understand seasonal migration of insects

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Currently there is no effective long-term strategies to manage insect outbreaks caused by migratory species. One major hurdle in developing such strategies is the fact that in many species, we have no clear idea about the origin of immigrants. Do they originate from different places each year based on prevailing climatic conditions or do most come from a specific geographic region each year?

For example, the true armyworm *Pseudaletia unipuncta* is a sporadic pest in eastern North America and management decisions for outbreaks are based on the pheromone and/or light trap catch data from late spring, which provides an intervention window of less than three weeks.

We will present first the results of a pilot study undertaken to determine the feasibility of using stable isotopes of hydrogen (δ^2H, $\delta^{13}C$) to determine the origin of true armyworm adults captured during the three annual flight periods in London, Ontario in 2016. We will then show data examining differences in intra-and inter-year patterns obtained from samples collected in London in previous years, and discuss how such information may be used in management strategies.

Searching for Achille’s Heel: Chemical ecology for invasive species suppression

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Globalisation has tremendously accelerated natural biogeographic processes. Many formerly limited pest species are experiencing new and expanded geographical ranges, predator-free space, and exploiting new hosts. In some cases, such as the giant willow aphid in New Zealand, where honeybees and wasps now exploit a massive new honeydew resource along rivers, entire ecosystems can be affected, including unexpected consequences. Not all such insect taxa have been studied by chemical ecologists with an eye for application. Chemical ecology has demonstrably contributed to many applications of attractants in traps for pests and a more limited range of control tactics, sure as mass trapping, lure and kill and mating disruption for pest management. Alternative tools, such as mating disruption and mass trapping have frequently been investigated, but actually the number of products available to the farmer for pest control is more limited. New applications of other pheromones, such as pheromones of social insects have been demonstrated, but the real opportunity that has hardly been touched thus far, is in biosafety for biological control agents. Classical biological control, where a natural enemy is moved into a country, relies on host range testing but can be informed by chemical ecology. In the case of a mealybug parasitoid, its’ attraction to the host mealybug sex pheromone illustrates the close co-evolutionary basis for predicting that other organisms will not be at risk, since mealybugs use separate compounds for each species and the parasitoid relies on the sex pheromone for host location. A better understanding of insect-insect and insect-plant interactions will likewise reduce risks of non-target effects in classical biological control and expedite the safe release of natural enemies. Thus applications of chemical ecology can be considered in a wide range of areas, and suitable formulations or application systems need to be developed, and new knowledge such as the chemical basis for host specificity where this exists, applied to re-risk classical biological control. Failure to keep pace with globalisation of pests is otherwise a risk leading to increased use of insecticides, and the loss of integrated pest management systems.

S12-O-01

Correlation of flower color polymorphism and defense phenotype of the invasive weed *Solanum elaeagnifolium*

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Solanum elaeagnifolium is a perennial weed native to Southwestern United States and Mexico, and have successfully invaded worldwide. The species predominantly produce anthocyanin rich blue flowers; however, also produces white color morphs in low frequencies. Most studies on flower color polymorphism have focused on pollinator preference and plant fitness, and have overlooked whether there is also variation in defense traits. The persistence of white flowers among the sea of blue morphs in many invasive populations suggests that there could be some ecological benefit of these morphs, thereby preventing them from being competed out of the population. To examine this, using multiple invasive populations of *S. elaeagnifolium* from the Greek city of Thessaloniki, fitness and herbivory levels of blue and white morphs were measured and compared. The blue morphs across multiple locations scored better in fitness traits than their white counterparts; however, they were also found to have higher floral and foliar herbivore damage-suggesting that plants with white flowers are more defended than the those with blue flowers.

To test this in detail, we then characterized both floral and foliar defences including leaf trichomes, floral and foliar volatiles, phytohormones, and components of the floral anthocyanin biosynthetic pathway-also considered as direct chemical defense. Biochemical analysis of the pathway revealed that white flowers accumulated a flavanol precursor of anthocyanins, myricetin, suggesting a breakdown in this pathway. And, myricetin when added to Solanaceae specialist *Manduca sexta* caterpillar diet, was also found to cause inhibitoryeffects on caterpillar growth. This was followed by other feeding studies with larvae to determine the impact of these traits on herbivore performance. We find that defensive traits are consistently elevated in the white morphs and that both larval growth is increased and adult *M. sexta* more frequently oviposit on blue plants rather than the white morphs. Taken together, our data shows that there is a strong correlation between flower color polymorphism and plant defences in this species. We are currently exploring the molecular basis of thepolymorphism by sequencing the genes involved in the anthocyanin pathway, followed by controlled pollinations to examine the mode of inheritance.

S12-O-02

Genetic diversity, metabolic variation and functional life-history syndromes suggest multiple mechanisms facilitating invasion in a Brassicaceae species

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Understanding the mechanisms underlying successful invasion of plant species is one of the major challenges in invasion biology. Some invaders might possess chemical or physiological traits that provide them with advantages over congeners in their native or novel range, regarded as preadaptation. Other individuals might quickly adapt to changes in environmental conditions, causing post-invasion alterations in resource allocation and thus shifts in various plant traits. The perennial *Bunias orientalis* L. (Brassicaceae) is native in Western Asia and Southeastern Europe but has expanded its range westwards within the Eurasian continent over the past decades, particularly conquering disturbed, nutrient-rich habitats. We explored multiple traits related to plant chemistry, resistance, growth and reproduction to evaluate mechanisms that may facilitate the invasion success in this species. Therefore, we grew plants of native, invasive and exotic (i.e., non-invasive range expanders) populations under controlled conditions.

A metabolomics approach using ultra high pressure liquid chromatography coupled with time-of-flight mass spectrometry (UHPLC-ToF-MS) disclosed a remarkably high chemical variation in the total metabolite as well as in glucosinolate composition of leaves within populations. Genome-wide fingerprinting of amplified fragment length polymorphisms (AFLP) of the same individuals revealed a high correlation between chemical and genetic distance matrices. Moreover, a positive relationship between measures of genetic and metabolic diversity of populations and a tendency for reduced genetic diversity in exotic populations could be shown.

From plants grown in a field common garden under high or low nitrate availability, various traits were investigated over two years. Suites of correlated traits were grouped as functional units that were differently affected by population origin and fertilisation. Plants from native populations had shorter leaves and were less attacked by antagonists than non-native populations, pointing to an ‘evolution of increased competitive ability’ scenario, although the glucosinolate concentrations did not differ. Most strikingly, highly efficient nitrate allocation to growth and reproduction traits became evident in the second year and was found only in plants from invasive populations.

Our results suggest multiple mechanisms to facilitate invasion in this species simultaneously. High within population variation in glucosinolate concentrations might impede adaptations of (novel) antagonists and provide *B. orientalis* with advantages, while other defensive traits might have shifted in the novel range in favour of growth and reproduction in non-native plants. A higher colonisation potential of invasive compared to exotic, potentially leading-edge populations might be explained by a higher genetic diversity, providing increased opportunities for physiological responses to novel environments. The use of chemical analyses in combination with the investigation of various other life-history traits presents a powerful approach to disentangle mechanisms potentially underlying successful plant invasion.

Chemotypes in *Erodium cicutarium* (Geraniaceae) of native and invasive origin and effects of plant competition on offspring terpene profiles

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Biological invasions pose extensive threats to biodiversity and entail severe economic consequences. The invasion of new territories is often accomplished by specific chemical-ecological characteristics, raising the competitive ability of invaders above the one of natives. For instance, a higher chemical diversity has been found in leaves of exotic compared to native populations in a few species within the Asteraceae, indicating a broadening of defense means. Moreover, plants may contain specific chemicals (i.e., terpenes) preventing neighboring competitors from germination (allelopathy). The production of these chemicals should be enhanced when competition with neighboring plants is high. Furthermore, the new foreign habitat of invaders may be exempt from specialist herbivores, leading to a decreased need for defence and allowing an increased investment in size or fecundity.

The common stork's-bill *Erodium cicutarium* (L.) L'Hér. ex Aiton (Geraniaceae) is an invasive annual herb with major impact on biodiversity and agriculture. Its native range comprises Europe, North Africa and Western Asia, but it meanwhile established a global distribution. Although the plant it is rich in various secondary metabolites, particularly phenolic compounds and terpenes, current research on invasion mechanisms of *E. cicutarium* focused only on ecological traits but neglected the plant chemistry. We compared terpene concentrations in leaves and flowers as well as growth and seed production of *E. cicutarium* from different origin and different intensity levels of competition for light at source sites, when plants were grown under common conditions. We hypothesized that plants of native and invasive origin contain distinct chemical profiles (i.e., express chemotypes). In line with prevailing invasion ecology hypotheses, we expected plants of invasive populations to produce more biomass and seeds and to contain lower terpene concentrations but a higher terpene diversity.

We indeed found that flowers and leaves of *E. cicutarium* express distinct terpene profiles that depend on the origin of seeds. Interestingly, we found higher terpene concentrations and a higher terpene diversity in leaves of native compared to invasive plants. Furthermore, the foliar monoterpene concentration was increased in plants from high competition sites, indicating an involvement in allelopathy. Biomass production was related to both, origin and competition, and plants with high competition background produced more flowers and seeds. The results of this study offer novel insights into the chemo-ecological traits of an important invasive plant and provide first evidence for terpene-based chemical adaptation to the environment that may be fundamental for its competitive ability.

S12-O-04

Host selection by the olfactory system in *Drosophila suzukii*: Can flies discriminate among fruits by smell?

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Insect species have evolved different degrees of host specialization to optimize their survival and reproductive success. Overcoming plant host defences is metabolically costly, so only a minority of insect species, referred to as generalists, are able to cope with a broad range of plant hosts. How generalist insects recognize their hosts is still unclear. Do they recognize a small set of ubiquitous host-derived volatiles, or are there other olfactory mechanisms related to host-specific blend recognition? To understand how a generalist insect processes plant olfactory cues, we investigated how host volatiles are encoded by the peripheral olfactory system located in the antennae, using gravid female *Drosophila suzukii* Matsumura, the Spotted-Wing Drosophila (SWD), as the model system. SWD larvae are generalist frugivores, adult females laying eggs under the skin of ripening thin-skinned fruits of more than 50 plant species belonging to several families. It is a widespread and invasive agricultural pest, which causes significant crop losses: fruits are damaged by larval feeding and by infection with pathogens through the oviposition puncture. New semiochemical tools may provide alternative approaches to controlling this notorious pest, as pesticide use on fruit harvests is often non-permissible.

Dynamic headspace collection was used to collect the volatile organic compounds (VOCs) from six potential host fruits representing different plant families: strawberry and raspberry (fam. Rosaceae), blueberry (Ericaceae), grape (Vitaceae), orange (Rutaceae) and tomato (Solanaceae). VOC extracts were presented to SWD antennae using coupled gas chromatography-electroantennography (GC-EAG) experiments. VOCs with EAG-activity were identified by coupled GC-mass spectrometry (GC-MS) and GC peak enhancement with authentic standards. Attraction to fruits was assessed in a wind tunnel. Furthermore, in separate studies, olfactory receptor neuron (ORN) classes on the antenna and maxillary palps of SWD were characterized using single sensillum recording with a panel of key ligands identified previously for the closely related species *D. melanogaster*. The six fruit headspaces were then tested on these ORN classes to determine which ones are tuned to which fruit odour.

To date, we have shown that gravid female SWD show different degrees of attraction to their hosts and no attraction to the non-hosts (orange and tomato). Only a few antennally active VOCs are shared by the four hosts, indicating that each fruit type releases a specific volatile blend that is detectable by gravid females. In addition, host fruits activate different, but to a certain degree overlapping, sets of ORNs, which suggests that SWD can differentiate among host fruits by olfaction. The identification of key ligands for the specific ORNs responding to host or non-host VOCs may permit the development of attractants and repellents, thereby increasing control efficiency and specificity for *Drosophila suzukii.*

S12-O-05

Olfactory responses of winter morph spotted wing Drosophila (*Drosophila suzukii*) to volatile semiochemicals

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The invasive frugivore, spotted wing Drosophila (*Drosophila suzukii*), has rapidly become a devastating global pest causing significant damage to small and stone fruit throughout North America, South America, and Europe. Most research to date has focused on the damaging summer morph, with little research on the overwintering morph, equipped with a darker body and longer wings than the summer morph. We compared antennal response of winter and summer morphs to six ecologically-relevant life-related odorants using electroantennography, revealing morph-specific sensitivity to three of the compounds tested. Our results provide insights into how the different morphs of this invasive insect respond to environmental and chemical cues, with implications for season-long monitoring of this pest.

Identification of self-destructive defense system using a hemolymph enzyme, mandelonitrile oxidase, from the invasive millipede, *Chamberlinius hualienensis*

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The invasive millipede, *Chamberlinius hualienensis*, originally from Hualien, Taiwan, invaded Okinawa Island, Japan in 1983. Large swarms of the millipedes enter houses and sometimes cause train delays. The millipede species has been expanding its habitat yearly because there are no predators in Japan. The millipede exudes irritant chemicals such as benzoyl cyanide as a defensive secretion. Although it has been thought that this defensive chemical was converted from mandelonitrile, the biocatalyst has not been identified for 40 years.

On the basis of biochemical, molecular biological, and chemical ecological analyses, we identify the novel hemolymph enzyme, mandelonitrile oxidase (ChuaMOX) (EC 1.1.3.49), which stoichiometrically catalyzes oxygen consumption and synthesis of benzoyl cyanide and hydrogen peroxide from mandelonitrile. The enzymatic activity is suppressed at a blood pH of 7, and the enzyme is segregated by membranes of defensive sacs from mandelonitrile which has a pH of 4.6, the optimum pH for ChuaMOX activity. In order to produce benzoyl cyanide, strong body muscle contractions are necessary. Taken together, we propose that, to protect its swarm, the sacrificial millipede attached by large enemies applies a self-destructive defense strategy—the endogenous rupturing of the defensive sacs to mix ChuaMOX and mandelonitrile at an optimum pH [1].

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S12-0-07

Identification of antennal olfactory receptor neurons and corresponding active compounds in tomato-potato psyllid, *Bactericera cockerelli*

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The tomato-potato psyllid, *Bactericera cockerelli* (Hemiptera, Triozidae), feed on a number of plant species of Solanaceae, giving damages to potato industry by causing significant loss in potato yields and transmitting *Liberibacter* bacterium causing zebra chip disease. The behavioral studies on *B. cockerelli* have indicated that olfactory signals are involved in their host location. Our scanning electron microscope observation exhibited the presence of numerous nanoscale pores on the cuticular surface of placoid sensilla on the antennal flagellomeres of *B. cockerelli*, indicating that their main function is olfactory. Using single sensillum recording technique, we have then identified the olfactory receptor neurons (ORNs) in the placoid sensilla of male and female *B. cockerelli*, respectively, and characterized the responses of these ORNs to a panel of 35 host and non-host plant volatile compounds. These ORNs displayed highly specialized responses to a narrow range of compounds such as linalool, nonanal, and some green leaf volatiles in a dose-dependent manner. The profile of ORNs and corresponding olfactory active compounds were not different between males and females. Taken together, it is likely that these compounds have behavioral activities relating to the host location of *B. cockerelli*.

S12-O-08

Red-necked longhorn beetle, *Aromia bungii*, an invasive pest of Rosaceae trees: Present status of distribution and damage and monitoring trials in Japan

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The invasive wood-boring beetle *Aromia bungii* (Faldermann) (Coleoptera:Cerambycidae) is attacking cherry blossom and plum trees in Japan. It is native to China, Korea, Mongolia, and eastern Russia, but it recently invaded and became established in Japan and several countries in Europe, including Italy (2013) and Germany (2012). Since first being reported in Japan in 2012 in Aichi prefecture, by 2016 it had been reported from more than 7 prefectures. The adult is glossy black with a red thorax and the body length is ~3 to 4 centimeters. The lifecycle is 2-3 years, with larvae attacking the trunk and larger branches. Its host plants are mainly Rosaceae, including cherry blossom tree, peach, plum, and apricot, but also willow, mulberry, oak, and other species. It has rapidly become a major problem in both ornamental and important horticultural fruit trees. Methods of detection and monitoring this invasive pest are urgently needed to prevent spread, and to hopefully eradicate this pest. We observed the adults' behaviors, and found that activity peaked during the daytime. Females are attracted to males, suggesting that they use a male-produced volatile pheromone for mate location. Analysis of the volatiles from males showed several male specific compounds, but only one elicited responses from female antennae in GC-EAD assays. The EAG-active compound was field-tested in China in the beetles' native range, and in Japan in a newly-infested region where the beetle is already causing substantial damage. Fluon® coated black flight-intercept panel traps were hung in a grid orientation with alternating treatments and controls. Traps were baited with the synthetic compound (50 mg in 1 mL isopropanol), control (1 mL isopropanol), or with no lures at all (N=4). Traps were deployed from July to early August 2016, trap catches were counted daily, and traps and lures were replaced weekly. Traps baited with the test compound captured significantly more *A. bungii* than the two control treatments ($P<0.0001$ for both). Although trapping protocols have not been optimized, identification of the pheromone should provide a valuable tool for use in ongoing worldwide efforts to detect and eradicate this economically important invasive species.

Mating disruption of a Japanese gypsy moth, *Lymantria dispar japonica*

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The Asian gypsy moth (AGM) is a serious threat to the countries that the AGM did not naturally distributed, because of female flight and their apparently wider host range than the European gypsy moth (EGM), *Lymanria dispar*. Invasion and establishment of the AGM would have the potential to seriously affect their agricultural and forest resources, even their landscapes. Because of these threats, vessels departing from east Russia, northeast China, Korea and Japan to Canada, Chile, New Zealand and USA have to be inspected and must obtain pre-departure certification of AGM-free.

Mating disruption (MD) has been practically used to slow the spread of the EGM in the USA. Any trial of MD, however, has not been tested for the AGM. Therefore, we tested MD with synthetic pheromone for the Japanese gypsy moth (JGM), *Lymanria dispar japonica*, one of subspecies of the AGM and a target of inspection. We checked whether MD inhibited attraction to pheromone-baited traps and mating success of tethered females in the hardwood forests in the Matsudo campus of Chiba University. The JGM is known to be attracted to (+)-disparlure [(7*R*, 8*S*)-cis-7, 8-Epoxy-2-methyloctadecane].

High-density polyethylene tubes were loaded with 1 g of the synthetic (±)-disparlure, and the dispensers were hung from tree branches at a height of 1.5 m. Numbers of dispensers were changed by the area of the forest to adjust the amount of pheromone to about 90 g or 15 g per hectare in a high-dose MD forest and in a low-dose MD forest, respectively. The dispensers were deployed in July and August in 2014, 2015, and 2016.

The monitoring traps placed in the high-dose MD forest captured no males, but many male moths were captured in the corresponding traps in the control forest. Even in the low-dose MD forest significantly fewer male moths than the corresponding traps in the control forest were captured.

Eggs oviposited by tethered females placed in the MD forest and untreated forest were collected 24 h after the placement of tethered females and kept at the room temperature for 30 days and thereafter checked for the embryonation of eggs to determine the mating of tethered females. No tethered females placed in the high-dose MD forest mated, although about 45 % of tethered females mated in the control forest, suggesting that MD strongly inhibited mating of the JGM. In the low-dose MD forest about 10 % of the tethered females mated.

These results suggest mating disruption reduce the population of the JGM and trials of MD on an expanding scale in regulated areas are desired.

Glucoside transporters in leaf beetle defence: a proteomics approach

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Leaf beetles occur worldwide; some of them are even economically important pests of agriculture. They raise broad ecological interest because of their host plant preferences and varying modes of life. Their larvae have developed an impressive repertoire of toxins and repellents to defend themselves against predators. Upon attack, the larvae discharge small droplets from specialized glandular reservoirs on their back (e.g. *Chrysomela populi*). The reservoirs represent “bioreactors” performing all late reactions of the toxin-production, starting from plant-derived or de novo synthesized glucosides. The import of the plant-derived glucosides relies on a sophisticated transport system. Physiological studies revealed a functional network of transporters guiding the plant derived glucosides through the larval body into the defensive system. As transporters usually represent trans-membrane proteins, we developed a protocol for the isolation and identification of these proteins from membranes of the defensive system. After cell lysis and differential centrifugation proteins were separated by SDS-page followed by in-gel digestion and nanoLC-HDMS^{E} analysis. From more than 2000 proteins of glandular tissue, bioinformatic criteria revealed several ABC-transporters and candidates from the solute carriers (SLC) that mediate facilitative diffusion of their substrates. Expression analysis of these candidate sequences revealed for some of them high expression in the defensive glands compared to other larval tissues. A subsequent lack-of-function screening by using RNAi revealed in particular one ABC-transporter and an SLC2-candidate being involved in the uptake of salicin from hemolymph into the secretory cell. Heterologous expression of these transporters in frog oocytes confirmed salicin as a substrate. A functional model of the transport network in the leaf beetle *C. populi* has be developed and will be presented.

Strauss, W. Boland, A. Burse. ABC transporter functions as a pacemaker for the sequestration of plant glucosides in leaf beetles. (**2013**) eLife http://dx.doi.org/ 10.7554/eLife.01096 .

S13-K-02

Deciphering allopolyploidy-mediated innovations in plant defense metabolism against insects using structural metabolomics

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Interaction with insects is one of the many selection pressures thought to have sculpted plant specialized metabolism. The overarching objective of our research is to understand the defensive function against insects of some of these specialized metabolites as well as the evolutionary history of their biosynthetic pathways. At the heart of my research agenda lies the idea that such process, can be moved up to an unprecedented level through innovations in structural metabolomics and in the bioinformatics integration of heterogeneous omics data.

Half of the species of the genus *Nicotiana*, including many of the model species we use in plant-insect interaction studies, are allopolyploids of different ages and for some of them, the closest progenitor genomes have been phylogenetically mapped. One of the current research lines of our group is to explore “transgressive” defense metabolism characters that emerged in certain of these allopolyploidy events and to characterize their importance for the increased resistance against native insect herbivores.

In this talk, I will articulateMS metabolomics developments to improve the exploration of intra- and inter-species metabolic variations in the context of plant-insect interactions [1, 2]. Most particularly, I will present MetCirc, an R package [3], which allows computing metabolic trees to explore metabolic innovations. In this context, I will illustrate how this approach is currently incorporated in the exploration of the leaf surface metabolomes of *Nicotiana* allopolyploids of the section Repandae.

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Multiple omics analysis of shikonin production system in *Lithospermum erythrorhizon*

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Studies on model plants have provided fundamental biological information on the transcriptome, proteome, metabolome, and phenome in the past decade. In recent years, those omics studies have been prevailed depending on the remarkable development of sequencing technology as next generation sequencer and a variety of MS analytical technologies that are equipped to liquid chromatography. Due to the availability of those new technologies, transcriptomics, proteomics, and metabolomics have been applied to many non-model plant species, in which their genome sequences are not available. It is to be emphasized that plant specialized (secondary) metabolites are not commonly produced by all plants, but their occurrences are often limited to a couple of particular plant species, in which complicated biosynthetic pathways and accumulation machineries are specifically involved in those particular compounds. For instance, morphine is only synthesized by a couple of *Papaver* species, and nicotine is restricted to several *Nicotiana* species, and thus the biosynthetic pathways and the accumulation mechanisms should be studied in those species.

As those non-model plants, we use a perennial herbal plant, *Lithospermum erythrorhizon* (Boraginaceae), which produces lipophilic antimicrobial compounds, shikonin derivatives, at the root bark. These naphthoquinone compounds are red pigments and show sublimation property and thus recognized as a chemical barrier for the root against soil-borne microorganisms. One important feature is that these hydrophobic metabolites are secreted out from the epidermal cells of *L. erythrorhizon* root. To uncover the molecular mechanism on secretion of lipophilic compounds, we have applied omics studies to the shikonin production systems, i.e. cell cultures, hairy root cultures, and intact plants of *L. erythrorhizon*.

Shikonin biosynthesis is strictly inhibited by light irradiation. Utilizing these characteristics we obtained triple positive clones that are highly expressed in shikonin-producing cells (more than three times higher than shikonin-non-producing cells), and expected to be involved in shikonin production (biosynthesis and accumulation) by de novo sequence of RNA-Seq using HiSeq 2000 by Ilumina. Ca. two hundred million reads were analyzed to obtain ca. 254,000 contigs, out of which 247 genes were detected as being specific for shikonin-producing cells.

Subsequently, we prepared protein fractions from shikonin-producing cell cultures (production medium M9 in dark), and as the negative controls, shikonin-non-producing cells in M9 medium under illumination and shikonin-non-producing cells in Murashige-Skoog's medium. From those protein samples, we carried out both quantitative and qualitative proteome analyses, where RNA-Seq data were used as reference because the genome sequence is not available for this plant. Out of ca. 3,200 fragments, 272 fragments were identified as being specific for shikonin-producing cells.

In the symposium, strong candidate genes involved in shikonin production are introduced, and a comparison between transcriptome and proteome data is also discussed. I will discuss the possibilities for further utilization of these omics data, as well.

Omics everywhere: How about in chemical ecology?

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The introduction of omics methods provided enormous possibilities but also challenges in every biological field. In chemical ecology, integration of transcriptomics and metabolomics data provided especially useful tools for finding many novel metabolites in plant defense against various herbivores. Starting with a single gene and its profound regulation upon methyl jasmonate treatment in tobacco cells, the role of phenolamides in plant response to herbivores has been uncovered in our work over the last decade. The MYB8 transcription factor is a master regulator of phenolamide biosynthesis, which strongly accumulate after wounding and herbivore attack in tobacco plants. Our previous transcriptomic and co-expression analyses revealed several new tobacco genes involved in PA biosynthesis [1,2,3], as well as another branch of MYB8 function in wound-induced lignification process [4]. More recently, PAs, their function, and several new biosynthetic genes have been revealed in rice [5,6], demonstrating a wide radiation of PAs and their biosynthetic pathways in plant evolution. Using similar approaches, our work is now focusing on rice and sorghum to seek a full spectrum of metabolic defences in these monocot crop models with highly contrasting growth and environmental requirements. Similar to direct defense metabolites, current technologies also allowed studying profiles of volatile organic compounds (VOCs) at unbiased level. Interestingly, generalist and specialist herbivores induced different amplitudes of VOCs in rice that seemed to correlate with the composition of the oral secretions of these insects, supporting the specialist/generalist paradigm. In conclusion, while new technologies proved very useful in our work, this presentation eventually demonstrates that even more questions remained unanswered. It is the real challenge in this work to select proper targets and directions, out of vast amount of omics data, and promote their efficient use in ecology field.

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S13-O-01

NPR1-mediated immune system in the model monocot plant *Brachypodium distachyon*

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Plants induce the salicylic acid (SA)-mediated immune response against pathogenesis. It appears that in *Arabidopsis*, the dicot model plant, nonexpresser of PR genes 1 (NPR1) acts as a master regulator of the immune response through SA-dependent interaction with disease-related transcription factors (TFs) for defense gene activations, but the nature of this regulator remains unclear in monocots. In the current study, we therefore explored the novel *Brachypodium distachyon* NPR1 gene. The expression of *BdNPR1* was induced in response to methyl salicylate along with that of *BdPR1*, indicating that this NPR1 is primarily functional for the regulation of *BdPR1*. To elucidate the molecular mechanism of BdNPR1-dependent immunity, we used the AlphaScreen system to screen 553 *Arabidopsis* TFs that are able to interact with BdNPR1. Since this system enabled identification of disease-related TGA transcription factors as possible candidates of BdNPR1-interacting proteins, we then explored *B. distachyon* TGA homologue proteins that were able to interact with BdNPR1. Moreover, *in vivo* function of BdNPR1 is going to be assessed using BdNPR1 overexpression lines and *bdnpr1* knockout, and will be discussed.

S13-O-02

CRISPR/Cas9 mediated three PBP genes knock out in *Spodoptera litura* resulting in low responses of sex pheromone

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The custom-design bacterial CRISPR/Cas9 system has been recently used in some insects, indicating a powerful technique for studies on gene function. However, its use in lepidopteran pests is scare. Here, we reported an efficient and heritable gene mutagenesis analysis in an important lepidopteran pest, *Spodoptera litura*, using the CRISPR/Cas9 system. By using this system, we successfully obtained the homozygous *S. litura* strain by targeting each of the three pheromone binding protein (PBP) genes, which allowed us to elucidate the role and relative importance of PBP genes in the perception of the female sex pheromones. By co-injection of Cas9 mRNA and sgRNA into *S. litura* eggs, highly efficient chimera mutation in PBPs were detected both in injected eggs (39.1 % - 51.5 %) and in the resulting individual moths (63.2 % - 85.0 %). We used the mutant moths as parents to obtain the G1 offspring. And the homozygous mutants of three different PBP genes were obtained by Mendel's law in G2 generation. The EAG recordings and behavioral experiment indicated that PBP1 and PBP2 play more important roles than PBP3 in the perception of female sex pheromones. In addition, our study provides a useful methodology with the CRISPR/Cas9 system for gene *in vivo* functional study, particular for lepidopteran pests in which the RNAi approach is not efficient.

S13-O-03

Field transcriptomics: Integration of transcriptomics and meteorology

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Recent advances in plant molecular biology have revealed large effects of the circadian clock, organism age, and environmental stimuli on transcriptomes under simple, controlled laboratory conditions. However, the factors that control transcriptomes under natural conditions are largely unknown. We have developed statistical models using extensive field transcriptome data and the corresponding meteorological data. We showed that the transcriptome dynamics of rice leaves in a paddy field were mainly governed by ambient temperature and endogenous diurnal rhythms, as well as by plant age and solar radiation. We also found diurnal gates for environmental stimuli, detected associations between the thresholds for plant response to solar radiation and signal-to-noise ratios for day-length change, and predicted transcriptomes under given environmental conditions. Our models comprehensively describe transcriptome dynamics under complex field conditions and will help researchers to translate the vast molecular knowledge amassed in laboratories into solutions to problems in agricultural and natural environments.

S14-K-01

Selective toxicity profile of plant-based natural products

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Growing resistance to pyrethroid insecticides poses an increased risk for disease vector control world-wide. Thus, alternative compounds are urgently needed and natural products are an attractive source of new chemistries. Accordingly, we are examining the mosquitocidal activity of plant-based natural products to *Aedes aegypti* and *Anopheles gambiae*, as well as investigating their modes of action in various physiological preparations. Compounds found in essential oils from rosemary (camphor and 1,8-cineole), thyme (thymol and *para*-cymene), and anise/fennel (*trans*-anethole) were toxic to *Ae. aegypti* adults at doses (3-50 µg/mg), similar to published toxicities for the cabbage looper larvae, *Trichoplusia ni.* It was noted that thymol caused a sluggish form of paralysis in treated mosquitoes, while *trans*-anethole caused twitching of the extremities. On homomultimeric *Anopheles gambiae* GABA receptor-chloride channel complex expressed in *Xenopus laevis* oocytes, thymol acts as a positive allosteric modulator, increasing the potency and maximal effectiveness of applied GABA, consistent with the sluggish paralysis it elicits in exposed insects. The neural basis of excitation by *trans*-anethole awaits further investigation. Other studies investigated matrine; a bioactive component extracted from *Sophora flavescens* that is used as the main ingredient in Chinese bio-pesticidal products. Matrine caused flaccid paralysis in headless fourth instar larvae of *Ae. aegypti* (50% paralysis in 5 hours at 8 ppm) and was toxic to adult females by contact (topical LD_{50} = 258 ng/mg). Adult toxicity was increased about 2-fold by pretreatment with piperonyl butoxide. Interestingly, this compound was much less effective on *Drosophila melanogaster* in either glass contact or feeding bioassays. Adult mosquito knockdown was rapid, with little or no expression of hyperactivity or hyperexcitability. Matrine (ca. 1 mM) had effects similar to thymol on expressed *An. gambiae* GABA receptors, and reduced EPSP amplitude at the *Musca domestica* neuromuscular junction, without any evidence of neuroexcitation or membrane depolarization. These physiological actions are sufficient to explain the whole animal intoxication by matrine, but require relatively high concentrations to manifest themselves. Overall, the data indicate that some natural products have consistent effects across insect species, while others differ considerably.

S14-K-02

Modulation of ligand-gated chloride channels by fungal metabolites produced in response to plant factors

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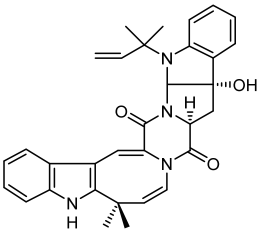
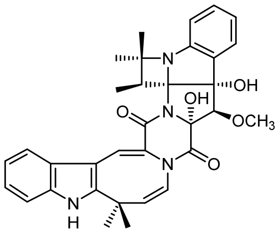
Fungi produce a variety of secondary metabolites in response to environmental changes as well as constitutes in culture media. Some fungi produce non-selective toxins, while others produce compounds that exhibit selective toxicity to invertebrates, plants and microorganisms. An example is the production of indole alkaloids produced by some fungi in okara, soybean pulp, medium. Dr. Hideo Hayashi, now Emeritus Professor of Osaka Prefecture University found that *Penicillium simplicissimum* produced indole alkaloids named okaramines in the okara medium as insecticidal compounds. The okaramines possess eight-membered azocine rings as a structure essential for the insecticidal activity. Motivated by this discovery, Dr. Hayashi explored insect regulators by use of the okara medium and isolated asperparalines, chrodrimanins, etc. as those killing or paralyzing the larvae. In constrast with consequtive isolations of these compounds, their mechanisms of actions were unknown.

The onset of the intoxicated larval symptoms in response to the fungal products was observed within a few hours afger administration. Hence, it was postulated that the compounds may target nervous system. The whole-cell patch-clamp electrophysology was employted to test okaramines on the silkworm larval neurons. It was found that okaramines induced inward currents when applied alone. The current-voltage relationship indicated that the okaramine-induced currents were mediated by chloride ions and indeed the currents were blocked by insecticide fipronil known to block ligand-gated chloride channels. The Resistant-to-dieldrin (RDL) γ-aminobutyric acid (GABA)-gated chloride channels and glutamate-gated chloride channel (GluCl) of the silkworm larvae were expressed in *Xenopus laevis* oocytes to test okaramines on the expressed chloride channels. Okaramines selectively activate GluCl with potency showing a high correlation with the insecticidal activity. They were inactive on human GABA and glycine-gated chloride channels, suggesting selectivity for insects. In this talk, the author will present these results and recent findings.



Okaramine A



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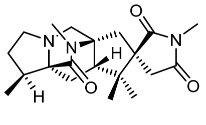

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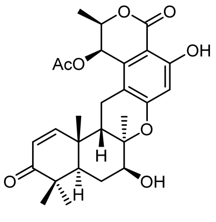

Chrodrimanin B

Molecular basis of pyrethrum repellency in *Drosophila melanogaster*

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Pyrethrum is a botanical insecticide from *Chrysanthemum* specie. It exerts its toxic effect by modifying the function of voltage-gated sodium channels, which are critical for electrical signaling in the insect nervous system. Besides its insecticidal activity, pyrethrum is also an insect repellent. However, the molecular basis of pyrethrum repellency remains unknown. We found that fruit flies (*Drosophila melanogaster*) exhibit avoidance to pyrethrum in T-maze and two-choice assays establishing *D. melanogaster* as a model to explore the molecular basis of pyrethrum repellency. Spatial repellency evoked by pyrethrum was abolished in two mutants of the olfactory co-receptor, Orco, demonstrating that pyrethroid repellency is Orco-dependent. Furthermore, single sensillum recording (SSR) revealed that pyrethrum activated six specific odorant receptors in six different types of olfactory sensilla in adult antennae. Our study provided new insight into the molecular basis of pyrethrum repellency.

S14-O-01

Effects of floral scents and their memories on feeding preference of the fly

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The blowfly, *Phormia regina*, is a nectar feeder insect. When the fly feeds on the nectar in a flower, its olfactory organs, antennae and maxillary palps, are exposed to the floral scent. In this situation, the fly perceives sweet taste of the nectar and at the same time particular scent of the flower, olfactory information of which affects the feeding preference. With a wide scale survey of floral scents of 50 different plant species, we categorized them into three groups, based on their increasing/decreasing effects on the proboscis extension reflex (PER) threshold concentration of sucrose, by which we knew whether the fly innately likes, ignores or dislikes them. Moreover, memory of feeding experiences with the floral scents variously influenced the PER threshold. Considering their effects on the PER threshold of sucrose both with and without the dietary experiences, we could categorize the effects of 50 tested scents into 16 of the 27 theoretically predicted types. Thus, we found the scent of Narcissus, for example, which mainly contains D-limonene, strongly depressed the appetite via antennae and particular glomeruli in the antennal lobe, and that the information route was changed by feeding experience with this sent.

Here in this presentation, considering D-limonene has oral toxicity that make bitter taste sensation in the blowfly. We discuss interactive development of chemical strategies between plants and animals about a secondary metabolite of plant, which can target not only gustatory but also olfactory sensing systems to make the insects say NO!

Bioactivities of cardanol derivatives isolated from *Anacardium occidentale* (Cashew) nut shell liquid against *Tribolium castaneum* Hebst (Coleoptera: Tenebrionidae) and *Sitophilus oryzae* L. (Coleoptera: Curculionidae)

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Sitophilus oryzae and *Tribolium castaneum* are major insect pests of stored grains in tropical regions of the world. Though several methods especially contact insecticides and insecticidal fumigants are used in the management of these pests, other alternatives are also strongly required. The use of botanicals in stored product protection is practiced recently. During our study on insecticidal activities of some Ghanaian plants, cashew nut shell liquid (CNSL) was found to show insecticidal and progeny growth and development inhibition activities against these insects. Therefore, this study was done to isolate and determine the active compounds from it.

Insecticidal activity was determined by dipping adult insects into 2g/mL equivalent of the extracts and isolated compound. The growth and development inhibition was determined by dipping same age larva into isolated sample and incubating for six weeks. Insect culture and treatments were kept under controlled conditions of 27 ±2 ^{o}C temperature, 70 % relative humidity and 12 L: 12D photo regime

Bioassay guided isolation led to the isolation of three cardanol derivatives from hexane soluble layer of CNSL and their structures were determined by Proton and Carbon -13 Nuclear Magnetic Resonance (NMR) together with Gas chromatography-Mass Spectrometry (GC-MS) to be (*Z*)-3-(8- pentadecenyl)phenol **(1)**, (8*Z*,11*Z*)-3-(8,11-pentadecadienyl)phenol **(2)** and (8*Z*,11*Z*,14*Z*)-3-(8,11,14-pentadecatrienyl)phenol **(3)**.

Of the three compounds, compound **3** with a yield of 36.55 mg in the 1 g equivalent of extract, showed the highest insecticidal activity with LC_{50} values of 60.36 mg/mL (95% CL = 64.52 – 126.41) against adult *S. oryzae* and 47.75 mg/mL (95% CL = 24.42 – 93.37) against adult *T. castaneum.* For the progeny growth and development inhibition bioassay, against *T. castaneum,* 46.7 ± 4.4 % of larvae were recorded dead and 20.7 ± 6.7 % adults without deformities emerged after the six-week period of studies for compound **3**. On the other hand, the control recorded no larval mortality and 100.0 ± 0.0 % of adult without deformities emerged.

Cardanol can be considered effective in the management of *T. castaneum* based on the results of our study.

S14-0-03

Asian soybean rust-induced metabolites in a resistant soybean cultivar Hougyoku (PI 224270): structure determination and antifungal activity evaluation

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Asian soybean rust (ASR) caused by *Phakopsora pachyrhizi* (Syd. & P. Syd.) is a devastating foliar disease in soybean (*Glycine max* (L.) Merr.) that can drive significant yield losses. In order to control ASR, the *P. pachyrhizi* resistance (R) gene (*Rpp*) provides a useful tool. While *Rpp* mapping and molecular breeding have been conducted, the investigation *P. pachyrhizi*-induced specialized metabolites in soybean have been poorly described. In this study, we analyzed the metabolites of a resistant cultivar Hougyoku (PI 224270) challenged by *P. pachyrhizi* infection and evaluated the biochemical activities. Induced metabolites in Hougyoku was analyzed by LC-MS and compared with those in susceptible cultivars (Tamahomare, Enrei and Fukuyutaka) by PCA. Four metabolites were consistently increased in Hougyoku following infection. Induced metabolites isolated and identified by LC-IT-TOF-MS, NMR and CD include; (6a*S*, 11a*S*)-glyceollin II, (6a*S*, 11a*S*)-glyceollin III, afrormosin (6,4'-dimethoxy-7-hydroxyisoflavone) and the novel compound (6a*S*, 11a*S*)-13-hydroyxyglyceollin II. Subsequently, antifungal activity against *P. pachyrhizi* was evaluated. (6a*S*, 11a*S*)-Glyceollin II and (6a*S*, 11a*S*)-glyceollin III showed strong inhibition of sporulation in detached leaf assays and moderate inhibition of the spore germination in water agar assays. On the other hand, clear activity was not observed on afrormosin and (6a*S*, 11a*S*)-13-hydroyxyglyceollin II. (6a*S*, 11a*S*)-Glyceollin II and (6a*S*, 11a*S*)-glyceollin III are established inducible antibacterial and antifungal metabolites, termed phytoalexins, in soybean and other legume plants. In addition to understanding pathogen defense at the R gene level, a foundational knowledge of defense-related metabolomes and underlying pathways could further advance the development of improved soybean rust resistance.

Elucidating the target of communesins, fungal metabolites acting as insecticides

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Communesins are indole alkaloids produced by several fungal species. They were first described as compounds inhibiting the growth of P-388 cancer cells, and later found as insecticidal metabolites of *Penicillium expansum* MK-57. While total syntheses of communesins have been achieved, their mode of actions on insects remains unknown. Hence, we have investigated the actions of communesin A and B on the silkworm (*Bombyx mori*) larval neurons using whole-cell patch-clamp electrophysiology since they exhibit toxicity shortly after administration to the silkworm larvae.

When applied alone, communesin A and B induced inward currents in a concentration-dependent manner in the silkworm neurons. The communesin-induced currents were blocked by fipronil, a phenylpyrazole insecticide that selectively blocks ligand-gated chloride channels. Also, the reversal potential for the communesin-induced current was similar to an equilibrium potential calculated from intracellular and extracellular chloride ion concentrations supporting that the current is mediated by chloride ion.

GABA-gated chloride channels (GABACls) and L-gluatamate-gated chloride channels (GluCls) are pentameric ligand-gated chloride channels that are expressed abundantly in the central nervous system of insects. Therefore, communesin A and B were applied to *Xenopus laevis* oocytes expressing the *B. mori* GABACl and GluCl. As a result, both communesins induced inward currents in oocytes expressing GluCl and the potency to activate the GluCl (communesin A < communesin B) was in accord with the order of insecticidal potency on the silkworm larvae, suggesting that GluCl is a major target of communesins.

Development of CO_2-releasing formulations for the control of soil-borne insect pests

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Since effective synthetic chemicals used by conventional farmers in the past to control various soil-borne insect pests of worldwide relevance, e.g. wireworms or the western corn rootworm, have been phased out or are no longer marketed, the control options for conventional as well as organic farmers are strongly limited. Therefore, alternative control strategies based on biological control agents including semiochemicals are urgently needed.

Root-feeding herbivores generally use CO_2 gradients in soil to locate their host plants. Given the property of baker's yeast (*Saccharomyces cerevisiae*) to convert sugars into CO_2, it finds application in biological pest control, especially in the form of so-called "Attract-and-Kill" co-formulations. In the project ATTRACT a formulation based on starch and baker's yeast which releases CO_2 over several weeks and which is thus attractive towards soil-dwelling insect pests was developed [1]. In the EU project INBIOSOIL the attractive formulation was upgraded with a *Metarhizium brunneum* isolate, which effectively kills wireworms. This co-formulation acts as a micro-fermenter multiplying the entomopathogenic fungus, when applied into the soil, thus reducing the dose/hectar and costs. A 10 to 100 fold reduction of the initial biomass concentration in the formulation had no impact on the performance of the formulation. Field trials in 2015 and 2016 demonstrated a mean control efficacy of about 50% [2]. This talk will focus on the development of the CO_2-releasing beads and the technical aspects related to the development and the scale-up of the "Attract-and-Kill"-formulation.

Our formulation will pave the way towards novel "Attract-and-Kill" strategies in pest control. Based on the biocompatibility of all ingredients, the "Attract-and-Kill" technology has a great potential to be adapted for the control of various arthropod pests all around the world.

[1] Humbert P, Vemmer M, Giampà M, Bednarz H, Niehaus K, Patel AV (2017) *World J Microbiol Biotechnol*, *33*(4), 71.
[2] Brandl MA, Schumann M, French BW, Vidal S (2016) *J Insect Behav*, *29*(4), 395-414.

Predicting the success of mating disruption

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Examining the relative efficacy of mating disruption reveals that there is a great deal of variability in the susceptibility of species to this control tactic. For some species, such as redbanded leafroller and oriental fruit moth, treatment with pheromone alone is often sufficient to mitigate crop damage. Other pests, such as the codling moth and certain leafrollers, appear to be more "difficult" to control using only mating disruption. What is the explanation for where a given pest falls on the scale bounded by “easy” versus “difficult” to disrupt? Our research suggests that ease of disruption is related to a species susceptibility to competitive versus non-competitive mechanisms. Easy to disrupt species, like OFM, are amenable to non-competitive mechanisms, while disruption of more difficult species may only be achieved competitively. It appears there are fundamental differences in the capacities of species to become adapted and/or habituated when exposed to high doses of synthetic pheromone. In addition, pheromones vary considerably with respect to rates of evaporation, dispersion in air, and adsorption onto solid surfaces. Collectively, differences in these properties can have profound effects on the longevity and movement of pheromones in the environment. We propose that it is these basic differences in the properties of moths and their pheromone that make some species more susceptible than others to mating disruption. Basic knowledge of the causes and of the phylogeny of the easy to disrupt trait could direct the pheromone industry toward suitable target species and away from those unlikely to yield successful products in the long run.

Mating disruption and aerial releases of sterile codling moth in New Zealand: Is local eradication possible ?

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[2] *The New Zealand Institute for Plant & Food Research Limited, PB 4704, Christchurch 8140, and Better Border Biosecurity, New Zealand*

[3] *The New Zealand Institute for Plant & Food Research Limited, Havelock North, New Zealand*

[4] *M3 Consulting Group, Phoenix, Arizona, U.S.A*

[5] *Okanagan-Kootenay Sterile Insect Release Program, 1450 K.L.O. Road, Kelowna, BC, V1W 3Z4, Canada*

The Sterile Insect Technique developed against codling moth in Canada is now being tested in combination with mating disruption to determine the feasibility of local eradication, and to assess the potential for wider use in the New Zealand apple industry. Our project, supported by the International Atomic Energy Agency, is at the forefront of efforts to deliver the Sterile Insect Technique in new ways. The insects arrive in the isolated Central Hawkes Bay orchards after under 35 hours travel time from the *Okanagen-Kootenay Sterile Insect Release* factory in British Columbia, Canada. Weekly releases have been conducted for three years over 230-370 ha, where we are testing the concept that tactical combination of highly effective mating disruption technology, an export programme of selective insecticides and sterile insects can drive the population to local extinction, if we consistently overflood the very low residual wild population of moths. The local population on two ~100 ha properties has been driven as low as 2-4 moths per 100 ha per year caught in pheromone traps. A low cost remotely-controlled fixed-wing airframe can deliver sterile insects to cover 100 ha of in 10 minutes, with preset swathes controlled by GPS coordinates via a laptop. By commencing population assessments in peri-urban areas, and later developing spatially-explicit population models of codling moth population suppression based on pheromone trap catch, in future we hope to estimate the feasibility of wider eradication for the benefit of growers and exporters and the community interested in developing and maintaining alternatives to pesticide use.

S15-O-02

Designing a mega-dispenser for sex pheromone mating disruption

T. C. Baker

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Male moths flying in wind respond with upwind surges to individual strands of pheromone emanating from point source dispensers and they respond to the pockets of cleaner air between the pheromone strands by initiating cross-wind casting flight. Time-averaged emission rates from pheromone mating disruption dispensers are instructive for calculating field longevity of dispensers and understanding emission levels field-wide that produce desired levels of mating impairment and damage reduction. However, such dispenser emission rate measurements are not relevant to optimizing the ability of each dispenser to manipulate male moth behavior as far downwind as possible. Widely spaced, high-emission-rate pheromone mating disruption dispensers should be designed so that they can have the farthest downwind “reach”, e.g., with the strongest possible downwind strands so that each dispenser can get males to “lock on” to a pheromone plume from far away and begin dosing and habituating themselves with repetitive contact with pheromone strands as they fly upwind in the plume.

Codling moth mating disruption 25 years on: How is it working, what's changed, what's new and what is still needed

Don Thomson

Pacific Biocontrol Corp., Vancouver, WA., USA

Codling moth *Cydia pomonella* pheromone was identified over 45 years ago. Subsequent research led to the commercialization of mating disruption technologies over 25 years ago. Initial adoption was slow and difficult but codling moth mating disruption is now integral to pest management systems in pome fruit around the world. Mating disruption technologies have improved substantially in the last 25 years to ensure season long release of pheromone. Despite these improvements in dispenser technologies, codling moth mating disruption is not a stand alone technology and requires close monitoring and supplemental controls to ensure growers get acceptable levels of control. Many growers still struggle to achieve a satisfactory level of control. Why? Due to extensive research, we now better understand the mechanisms of codling moth mating disruption. The physiological and subsequent behavioural responses to pheromone by codling moth seem to be different than for other insects such as oriental fruit moth *Grapholita molesta*. How differences in physiological and behavioural responses by insects to their pheromones and the impact on the efficacy of mating disruption will be discussed. Pest management systems and technologies have changed dramatically over the last 25 years. Orchard architecture has completely changed with high density plantings creating more open and exposed environments. Contact insecticides have been largely replaced by more expensive technologies that need to be ingested and require careful attention to timing and coverage. Given what is known about the physiological response of codling moth to its pheromone, this shift away from contact insecticides has made it more difficult for some growers to use mating disruption cost effectively. More research is still needed on the physiological and behavioural responses of codling moth to pheromone and how dispensing systems can deliver pheromone more effectively.

Mating disruption of Codling moth with reduced sex pheromone load dispensers.

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Codling moth (CM) *Cydia pomonella* L. (Lepidoptera: Tortricidae) is a major pest of pome fruit worldwide, and infests commercial apple, pear, quince and nashi orchards in Australia. Sex pheromone mediated mating disruption (MD) with standard registered dispensers of Isomate C-S® (Bioglobal Ltd., Wacol, Australia) applied at the registered rate of 1000 dispensers per hectare successfully controls low populations of CM. If CM mating disruption is based on a competitive mechanism [1,2] the number of sex pheromone point sources would be more important for successful disruption than the sex pheromone load in the point sources (dispensers). The use of dispensers with reduced load of sex pheromone active ingredient, but applied at the same registered dispenser density (point sources per hectare), should therefore be as effective for MD as the application of the standard registered dispensers with the full load of sex pheromone active ingredient.

Season-long completely randomised replicated field trials were established in a large commercial apple orchard in Greater Shepparton region of northern Victoria to ensure consistent management practices across all treatments, plots and replicates without any insecticide spraying against CM. Analysis of CM catches during the season, and fruit damage at harvest time, revealed that MD treatments with 25% and 50% load of CM sex pheromone plus 75% and 50% dodecanol respectively did not provide effective control of the low initial population of CM . Only dispensers with 50% load of sex pheromone plus 50% linalool were as effective as the registered Isomate C-S® dispensers with 100% load of sex pheromone when all dispensers were applied at the same standard registered rate (point source density). These results support the theory that MD of CM is based on a competitive mechanism where the number of sex pheromone point sources is more important for successful MD than the sex pheromone load in the point sources. Application of MD dispensers with reduced sex pheromone load will be economically advantageous for fruit growers who would like to use cost effective pest control technology while protecting the environment by reducing pesticide pressure in orchards.

[1] Miller J.R. et al (2006) J Chem Ecol 32: 2089-2114

[2] Miller J.R. et al (2006) J Chem Ecol 32: 2115-2143

S15-0-05

Methyl benzoate is a natural, plant-based, and green pesticide for sustainable agriculture

Aijun Zhang and Yan Feng

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A floral fragrance, methyl benzoate, has been discovered as a new, natural, plant-based, green pesticide against many insect pests, including the brown marmorated stinkbug, *Halyomorpha halys*, diamondback moth, *Plutella xylostella*, tobacco hornworm, *Manduca sexta,* as well as spotted wing drosophila, *Drosophila suzukii*. Based on our laboratory data, it is 5 – 20 times more toxic than some conventional pyrethroid and organic pesticides available on the market. Methyl benzoate is a volatile component naturally-occurring in fermented apple juice and many plants, approved by the US Food and Drug Administration and the European Union as a food-grade ingredient, and widely used in food industries and perfumeries. It is cheap, commercially available, and environmental friendly. Methyl benzoate has grerat potential to be an excellent alternative tool to conventional synthetic pesticides in agriculture, therefore, protecting our natural ecosystem, public health, human communities, and animal welfare.

Production of moth sex pheromones in an oil crop

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Application of synthetic sex pheromones for mating disruption is an environmentally friendly method for pest control and one of the foremost alternatives to the use of conventional pesticides. The high cost and the environmental concerns associated with the synthetic pheromone production, however, prevents the use of this technique in many crops and against many pest insects. By expressing the necessary and sufficient biosynthetic genes of insect and plant origin, we have previously made plants produce monounsaturated alcohols, which after acetylation proved to be efficient for trapping of target insects [1]. We aim to produce moth pheromone intermediates in oil crop seeds, which can be extracted and then reduced to fatty alcohols that are either used directly, or oxidized to the corresponding aldehyde or acetylated, and then used to control pest insects. We generated transgenic lines of *Camelina sativa*, namely “high myristate + BarCpaE11”, “high myristate + BarAveΔ11”, and “high palmitate + BarAtrΔ11”. These lines produce (11*E*)-tetradecenoic acid (E11-14:Acid), a mix of E11-14:Acid and Z11-14:Acid, and Z11-16:Acid respectively, precursors of the pheromones. We planted the seeds from selected lines in the field and harvested in summer 2016. We extracted the seed oil using heptane, and isolated target compounds. For the downstream processing, we took the oil product from the ¨high palmitate + BarAtrΔ11¨ line and converted the Z11-16:Acid into target alcohol and the corresponding acetate and aldehyde by simple chemical approaches, obtaining two batches of products with 38% and 83% purity, respectively. To validate the biological activity of this seed oil-derived pheromone, we have formulated sex pheromone baits according to the reported optimal pheromone composition in the diamondback moth, *Plutella xylostella*, a worldwide pest on cruciferous vegetable crops. The field trapping experiments revealed that the *Camelina*-derived pheromones are as attractive as the synthetic pheromone, regardless of the degree of the purity.

[1] Ding, B.-J. *et al.* (2014) Nat Commun 5:3353 doi: 10.1038/ncomms4353

Electrospun mesofibers in precision viticulture : Joint Integrated Pest Management on *Lobesia botrana* (Lep.: Tortricidae), and *Grapholita molesta*, in Germany and Brazil

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Agricultural technology may gain more public interest when in the middle of the 21st century the world population reaches the ten billion mark and then vociferously demands to be sufficiently fed. Quite unfortunately, weeds, insects, fungi and bacteria are challenging us by claiming their share. Additionally, the development of resistant strains is a worldwide, fundamental phenomenon pointing out that purely toxicological approaches in plant protection cannot be effective forever. Thus, new approaches have to be developed in the near future. Insect pheromones are known to disrupt natural mating communication in insects if broadcasted in sufficient concentration over cultivated fields during critical time periods of the pest mating season. In field trials in Germany, we explored novel insect pheromone dispenser types and found electrospun mesofibers within the diameter range of 600 to 1400 nm to be quite effective dispensers. In the case of the grape vine moths *Lobesia botrana* and *Eupoecilia ambiguella* (Tortricidae), mesofibers charged by electrospinning with 33% of (*E,Z*)-7,9-dodecadienyl acetate will disrupt mating communication within the boundaries of the vineyard. The effect will last for seven weeks and then has to be renewed for the 2nd and 3rd flight period of the moths. The mesofibers used in the experiments are equally effective compared to the older ampoule type marketed by BASF or the newer spaghetti type dispensers by SHIN ETSU. There are no environmental concerns against mesofibers since both the Ecoflex® polyester and the pheromone are equally biodegradable and are no environmental burden. Moreover, the synthesis of both components, polymer and pheromone, is possible using renewable resources.

Similarly, our Brazilian colleagues recently found the Oriental fruit moth *Grapholita molesta* (Lep.: Tortricidae) to be lured and thus quantitatively monitored by their sex specific pheromone ISCAlure Grafolita® embedded in nanofibers of the PCL/PEG type, when exposed in commercial peach orchards near Porto Alegre in Brazil.

However, specific experience in engineering is required for putting the pheromone filled mesofibers mechanically into the vineyards. We solved this challenge by integrating the mesofibers into the holding ropes an employing a modified commercially available cultivating machine doing the weeding, pruning and binding operations simultaneously from one platform. Future refinements call for automatic deployment of the prefabricated mesofibers.

S15-O-08

Discoveries of novel long-lasting repellents against biting flies on livestock animals

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Biting flies are the most serious livestock pest fly species that feed mainly on cattle and cause significant economic losses up to 2 billion dollars' losses annually in US cattle industry. They also attack pet animals and humans in cosmopolitan areas while livestock animals are not available, or in recreation areas, such as beaches. Using repellents against biting insects, particularly for mosquitoes, has been demonstrated effectiveness and widely accepted by the public. Repellents have also been applied to prevent biting fly attack on livestock animals, but with only relatively short-period effectiveness. Recently, we have discovered one natural product, coconut oil, which can provide up to 2-week antifeedancy in the lab bioassays. The compositional compounds of coconut oil and its derivatives have been identified and tested with some strong activity not only against biting flies, but also to ticks and bedbugs. Here, I reported our preliminary findings from this multi-institutional collaborative project carried out both indoors and in the field.

Phagostimulants for the Asian citrus psyllid also elicit volatile release from citrus leaves.

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Chemical cues that elicit orientation by the Asian citrus psyllid, *Diaphorina citri* Kuwayama (Hemiptera: Liviidae), are of great interest because it is the primary vector of the causal pathogen of citrus greening disease. We identified an optimal blend ratio of formic and acetic acids that stimulated substrate probing by *D. citri* [1]. We then applied geometric mixture designs and response surface modeling to identify and optimize a 3-component blend that further increased the number and size of salivary sheaths produced by *D. citri* on a wax substrate (SPLAT™) containing a 3.5:1.6:1 blend of formic acid, acetic acid and p-cymene, respectively [2]. A probing assay was developed that allows differentiation between attraction by olfactory cues and phagostimulatory effects produced by tastants. No evidence was found for remote orientation by *D. citri* adults by olfaction to the phagostimulant blends. Increased probing in response to the presence of phagostimulants in the wax matrix occurred only after contact with the substrate. Yellow wax beads, with or without odorants or tastants, attracted more *D. citri* adults and received more probes compared with white wax beads. Yellow beads containing the 3-component phagostimulant blend were probed by *D. citri* 2 to 3 times more often compared with yellow beads alone. The phagostimulant effect was also tested by covering wax beads containing the 3-component blend with a plastic film to minimize olfaction or contact chemoreception by antennation or tarsal chemoreceptors. The plastic film did not affect the probing response suggesting that chemosensation responsible for the phagostimulant response is associated with chemosensory receptors present on psyllid mouthparts. Salivary sheaths produced in wax beads containing the phagostimulant blend were 4.5 times longer than sheaths produced in beads without tastants. We also described the release of volatile organic compounds by citrus plants after brief (1 h) exposure to volatilized formic acid. Exposure dramatically altered the volatile profile released from citrus and resulted in modified settling of *D. citri* adults. A larger number of adult psyllids settled on the susceptible *Citrus macrophylla* in cages compared with a resistant accession of *Poncirus trifoliata.* After exposure of the *P. trifoliata* accession to formic acid, no such preference was observed. Psyllids also preferentially fed on citrus leaf area topically treated with dilute formic acid compared with an untreated area of the same leaf. Continued examination of this phenomenon may result in an improved trap, an attract-and-kill product, or enhanced means of managing *D. citri* and citrus greening disease.

[1] George J, Robbins PS, Alessandro RT, Stelinski LL, Lapointe SL (2016) Chem Senses 41:325-338

[2] Lapointe SL, George J, Hall DG (2016) J Chem Ecol 42:941-951

Poster presentations

Underlined poster numbers: student presentations

S1-P-001	What triggers an insect herbivore to adopt a new host plant that has not co-evolved with the insect?	**Sylvester Atijegbe**
S1-P-002	Fetid and fragrant: What VOCs attract beetle and fly pollinators to two thermogenic Australian Araceae?	**Thomas Sayers**
S1-P-003	Identification of host fruit volatiles from sea buckthorn (*Hippophae rhamnoides*) biologically active to sea buckthorn fly (*Rhagoletis batava*)	**Vincas Būda**
S1-P-004	A transfer protein Nl19025 plays an important role in the interaction of the brown planthopper *Nilaparvata lugens* with rice	**Jiamei Zeng**
S1-P-005	When life gives you sugar: Metabolic fates of phytoanticipins in phloem feeding insects	**Michael L. A. E. Easson**
S1-P-006	Elucidation of triadic relationship among feeding behavior of *Zizeeria maha* (Kollar) (Lepidoptera: Lycaenidae), its host plant (*Oxalis corniculata* L.) quality, and light environment	**Mei Yamaguchi**
S1-P-007	Identification and functional research of chemosensory genes in three rice planthoppers	**He Peng**
S1-P-008	Flower VOCs' perception: Specialization sign in generalist pollinator	**Laima Blažytė-Čereškienė**
S1-P-010	Plant utilization by phloem feeder insects: Secondary metabolites interacts in primary metabolism	**Osnat Malka**
S1-P-011	Evolution of multidrug resistance transporters in highly specialized herbivores	**Paulina Kowalski**
S1-P-012	Involvement of larval frass volatiles in host selection by female adults of *Pieris rapae*	**Yukiko Okumura**
S1-P-013	Glycine betaine as an oviposition stimulant for the common grass yellow butterfly, *Eurema mandarina*, in *Albizia julibrissin*	**Toshiki Ohashi**
S1-P-014	Effects of mating on host selection of female small white butterfly *Pieris rapae* (Lepidoptera: Pieridae)	**Hisashi Ômura**
S1-P-015	Why must *Aphis gossypii* Glover change the host plant?	**Ippei Niwata**
S1-P-016	Evolution of induced response to herbivory and competition in invasive goldenrod	**Akane Uesugi**
S1-P-017	Synergistic defensive function of raphides and chitinase/chitinase-like protein from *Streptomyces griseus* and yam (*Dioscorea japonica*) leaf extract through the needle effect	**Nobuhiro Matsumoto**
S1-P-018	Fluorescence from flower pollen and anther as a visual cue in plant-pollinator	**Shinnosuke Mori**
S1-P-019	Feeding stimulants for a larva of *Graphium doson*	**Yusuke Yoshimoto**
S1-P-020	Olfactory cues and honeybee foraging in response to flower scent compounds	**Dong In Kim**
S1-P-021	Tissue specific fine tuning of toxin resistance and overall activity of the Na,K-ATPase in milkweed bugs	**Susanne Dobler**
S1-P-022	Modification of interactions between a whitefly vector and host plants by a semipersistent plant virus	**Fengming Yan**
S1-P-023	The cytotoxicology of momordicins on *Spodoptera litura* ovary cell line SL-1 and *Ostrinina furnacalis* hemocytes Ofh cells	**Huan Liu**
S1-P-024	Resistance/susceptibility of 11 tea plant cultivars to the tea green leafhopper determined by EPG technique and Its correlation with the defensive substances in the tea leaves	**Baoyu Han**
S1-P-025	Biotic and abiotic factors influencing the feeding behavior of *Cacopsylla pruni*, the vector of European Stone Fruit Yellows (ESFY)	**Jannicke Gallinger**
S1-P-026	Identification of oviposition stimulants of the leaf-mining moth *Acrocercops transecta*	**Shota Shimoda**
S1-P-027	How does an invasive plant affect a native plant and associated butterflies across altitude?	**Chuan-Kai Ho**

S1-P-028	Genetic variations of Kanzawa spider mites in their levels of detoxification and defense induction in plants	**Rika Ozawa**
S1-P-029	Identification and investigation of compounds induced in apple by insect attack	**Yuuki Tetsumoto**
S1-P-030	The scent of male and female flowers in the *Glochidion–Epicephala* brood-site pollination mutualism in India	**Renee M. Borges**
S1-P-031	Effect of inhibitory substances contained in calcium carbonate wettable powder on oviposition of peach fruit moth, *Carposina sasakii* (Lepidoptera: Carposinidae)	**Haruna Kazama**
S1-P-032	*Bt* rice provides ecological resistance to non-target planthoppers	**Yunhe Li**
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S2-P-034	Evaluation of male and female extracts upon *Opsiphanes invirae* Hubner (Lepidoptera: Nymphalidae) antennae	**Antônio Santana**
S2-P-035	Absolute configuration of pheromones of the bumblebee wax moth, *Aphomia sociella*	**Irena Valterová**
S2-P-036	Multidimensional chromatographic techniques applied to chemical ecology	**Eduardo Mateus**
S3-P-038	Antifungal effects of volatile organic compounds emitted during infection of barley roots by fungal pathogens	**Caroline De Clerck**
S3-P-039	Biflavones of *Cycus revoluta* induce differentiation of filamentous *Nostoc cyanobacteria* into motile hormogonia	**Hiroaki Nishizuka**
S3-P-040	Diacylglycerols isolated from *Cycus revoluta* coralloid root and analogous anacardic acid derivatives induce morpho-differentiation of filamentous *Nostoc cyanobacteria* into motile hormogonia	**Hiroaki Nishizuka** **(Yasuyuki Hashidoko)**
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S4-P-043	Silencing of the gibberellin biosynthesis-related gene *OsGAS1*decreases the resistance of rice to the brown planthopper *Nilaparvata lugens*	**Ting Luo**
S4-P-044	Mechanism of heavy-metal tolerance in shade plant *Aucuba japonica* and the effects of endophyte	**Kouhei Doyama**
S4-P-045	Root endophytic *Chaetomium cupreum* enhanced tolerance of aluminum in *Miscanthus sinensis* and produced a siderophore	**Toshikatsu Haruma**
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S4-P-047	Insights into rice (*Oryza sativa* L.) root growth promotion mediated by β-1,3/1,6-glucans present in yeast (*Saccharomyces cerevisiae*) cell walls	**Naoki Mori**
S4-P-048	Making sense of contact: The role of a single gustatory receptor in host plant identification in the specialised poplar leaf beetle (*Chrysomela populi*)	**Antje Burse**
S4-P-049	Metabolic changes in *Oryza sativa* accompanying acquisition of drought tolerance by acetic acid treatment	**Shunsaku Isaji**
S4-P-050	Evolutionary changes in defensive secondary metabolism in the genus *Hordeum*	**Naoki Ube**
S4-P-051	Mechanisms of gustatory receptor gene for host selection in swallowtail butterflies	**Katsuhisa Ozaki**
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S5-P-053	Identification and expression profile analysis of chemosensory protein genes in grasshopper, *Oedaleus asiaticus* (Orthoptera: Acridoidea)	**Baoping Pang**
S5-P-054	Bees eavesdrop upon informative and persistent signal compounds in alarm pheromones	**Zhengwei Wang**
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S5-P-056	Morphology and physiology of antennal lobe projection neurons in the hawkmoth *Agrius convolvuli*	**Takuya Nirazawa**

S5-P-057	Comparative analysis of the sex pheromone biosynthesis related genes between the *Spodoptera exigua* and *S. litura*	**Ya-Nan Zhang**
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S5-P-060	Production rhythm, isolation and field evaluation of sex pheromone from calling females in *Diaphanina angustalis* (Lepidoptera: Crambidae), a medicinal plant moth	**Tao Ma**
S5-P-062	Identification of female sex pheromones of rare moth species: Perspective for conservation biology	**Hideshi Naka**
S5-P-064	Identification of inhibitory compounds against sex pheromone communication of light brown apple moth, *Epiphyas postvittana*	**Gwang Hyun Roh**
S5-P-065	Female sex pheromone of *Athetis lepigone* (Lepidoptera: Noctuidae): Identification and field evaluation	**Qi Yan**
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S5-P-068	Application of the sex pheromone and plant volatiles for mass trapping of the diamond back moth, *Plutella xylostella* L. (Lepidoptera: Plutellidae), in the Southern Vietnam	**Chau Nguyen Quoc Khanh**
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S5-P-078	Mechanosensory and chemosensory cues mediate the sex pheromone-induced upwind orientation behaviour in walking Indian meal moth	**Shin Tejima**
S5-P-079	Identification of olfactory-related genes in the brownwinged green bug, *Plausia stali*	**Kiyoko Taniai**
S5-P-080	Functional characterization of a Niemann–Pick type C2 protein in the parasitoid wasp *Microplitis mediator*	**Yong-Jun Zhang**
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S7-P-087	Chemical components involved in the cross-stimuli modulation of olfactory response of a generalist parasitic wasp *Aphidius gifuensis*	**Hiroyuki Takemoto**
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S11-P-113	Morphology and ultrastructure of olfactory sensilla in the antennae and maxillary palps of spotted wing drosophila, *Drosophila Suzukii* (Matsumura, 1931) (Diptera, Drosophilidae)	**Seon Ah Jeong**
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S15-P-171	Analysis, screening and field evaluation of volatiles emitted from tea plants infested by *Ectropis obliqua* larvae	**Jin Zhang**
S15-P-175	A review and recent developments in research of chemical communication in *Sitona* spp. (Coleoptera: Curculionidae): Pheromones and plant volatiles	**Zoltán Imrei**
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What triggers an insect herbivore to adopt a new host plant that has not co-evolved with the insect?

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Wiseana (Lepidoptera: Hepialidae), also known as porina, are endemic insects that are major pasture pests in New Zealand. Porina are a complex of seven closely related but poorly-defined species [1] that are difficult to distinguish using adult morphological characters. They are univoltine, nocturnal and fly from spring to autumn, with flight times varying between species, locality and year [2]. The damaging larval stage is subterranean and causes pasture damage from autumn to spring, depending on the locality and year. The larvae feed on the surface at night, removing all herbage nearest the burrow entrance by severing tillers, leaves and stems near ground level and dragging foliage into their tunnels where it is eaten [3]. Despite their impact on exotic pastures, porina are not known to cause significant damage in their native habitats. To improve management of porina in pastures, it is important to determine why porina cause considerable damage in exotic pastures. Apart from obtaining general knowledge on the host plant preferences of porina, our study also hopes to shed light on the question why some insect species become invasive in their native range. A study was conducted to identify the role physical traits and nutritional phytochemicals play in porina larval preference on five native (*Festuca actae*, *Acyphylla squarrosa*, *Poa cita*, *Chionochloa rubra*, *Phormium tenax*) and two exotic (*Lolium perenne* x *Lolium multiflorum* and *Trifolium repens*) host plants. Primary metabolome profiling of native and exotic hosts was done using GC-MS, carbon and nitrogen were quantified with a CN analyser and silica content was measured by inductively coupled plasma optical emission spectrometry. Acid detergent fibre and neutral detergent fibre were analysed using the method of [4]. Our results show that the native host plants had higher carbon and lower nitrogen contents, while the reverse was the case for the exotic host. The acid detergent fibre content was between 34-42% for the natives and 18-20% for exotic hosts; the neutral detergent fibre content ranged between 50-70% and 22-33% for exotics and natives, respectively. The exotics were also found to be high in amino acids and sugar compounds. Against our expectation, the analyses revealed that the pasture grass *Lolium perenne* x *Lolium multiflorum* contained most silica, which is associated with insect resistance, while native *Chionochloa rubra* and *Phormium tenax* contained least. These differences could partly explain the damage caused by porina on exotic pastures when compared to the native hosts.

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S1-P-002

Fetid and fragrant: What VOCs attract beetle and fly pollinators to two thermogenic Australian Araceae?

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There is significant worldwide interest in the chemical ecology of pollination systems of Araceae[1], highlighted by its use as a model to investigate the evolution of foral volatiles driven by pre-existing bias of pollinators[2]. The family Araceae contains the greatest number of thermogenic species, which is assumed to be important in pollination biology by enhancing scent dispersal[3]. Little is known, however, about the chemical ecology and prevalence of thermogenesis in Australian *Typhonium*, the predominately endemic and largest genus of Araceae in Australia. This study investigated thermogenesis and the chemical ecology of plant-pollinator interactions in two Australian aroids, *Typhonium angustilobum* and *Typhonium wilbertii*, which share similar distributions in tropical far north Queensland. Insect visitors were comprehensively sampled, and a range of floral traits were characterised, including floral morphology, trapping mechanisms, thermogenic capacity, and floral volatile compounds (using solid-phase microextraction and gas chromatography mass spectrometry). Both species are highly thermogenic, the appendix of each inflorescence on average heating more than 11 °C above non-thermogenic tissue temperatures during the pistillate phase of anthesis. The timing of peak thermogenesis is consistent between the two *Typhonium* species occurring during dusk. During this pistillate phase, inflorescences release strong odours that help to deceptively lure and capture pollinators within the floral chamber. Both species attract saprophagous pollinators, however, *T. angustilobum* almost exclusively captures Coleoptera (Staphylinidae, Hydrophilidae, and Scarabaeidae) whilst *T. wilbertii* almost exclusively captures Diptera (Sphaeroceridae, Sepsidae, Psychodidae, and Phoridae). Associated with differences in pollinator, floral volatile composition also differs markedly between species. The peculiar musty, fetid odour of *T. angustilobum* is dominated by a complex blend of sesquiterpenes, primarily bicyclogermacrene, whereas the sweet, dung-like odour of *T. wilbertii* consists of skatole and *p*-cresol, along with varying proportions of terpenes and aliphatics. Skatole and *p*-cresol are typical of dung-like aromas in species in the Aroideae subfamily, and are strongly correlated with beetle and fly pollination in deceptive systems[1,2]. The potential role of complex blends of sesquiterpenes and other compound groups in the attraction of saprophagous insects within deceptive systems is less understood. These findings suggest that specific compounds or compound combinations may be more effective at attracting a particular insect assemblage. Differences in the floral morphology and trapping technique between the two species may further influence the trapping and release of particular insect groups.

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S1-P-003

Identification of host fruit volatiles from sea buckthorn (*Hippophae rhamnoides*) biologically active to sea buckthorn fly (*Rhagoletis batava*)

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In Europe, economic demand of sea buckthorn (*Hippophae rhamnoides*) berries is growing permanently, because of its wide use in cosmetics, food and medicine industry. Besides, sea buckthorn shrubs can grow in poor soil, and can be planted in eroded fields. Sea buckthorn berries are damaged by non-native pest sea buckthorn fly, *Rhagoletis batava*. After maggot feeding and development inside of a berry, it loses its nutritional and economical value. In this way *R. batava* can destroy about 80-100% of harvest in organic farms. In Europe this pest appeared and started to spread in 2009-2013.

The fruit flies, as many other plant pests, use their host plant volatiles to detect berries. Data on the structures of these compounds could be useful for the fruit fly monitoring or even biological control.

Both ripe and unripe sea buckthorn berries were extracted by hydrodistillation. Linked gas chromatographic and electroantennographic detection (GC-EAD) analysis of berries extracts and *R. batava* males and females revealed 23 bioactive compounds. Synthetic analogues of the compounds are under the process of laboratory and field tests to find out which of them can affect *R. batava* behaviour. Data on the compounds involved in host plant recognition and on their behavioural effect will be presented.

S1-P-004

A transfer protein Nl19025 plays an important role in the interaction of the brown planthopper *Nilaparvata lugens* with rice

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It has been well documented that oviposition by herbivorous insects can elicit defense responses in plants. Yet, the mechanism underlying these phenomena remains largely unknown. The brown planthopper (BPH), *Nilaparvata lugens* (Stål), is one of the most important pests in rice and its oviposition has been reported to induce defenses in rice. To elucidate chemical signals secreted by BPH during egg-deposition that elicit defenses in rice, we cloned a gene *Nl19025* which encodes an egg shell-forming protein. Sequence analysis revealed that Nl19025 is a high-abundant transfer protein. Bioassays found that knockdown of *Nl19025* (injecting double stranded RNA of *Nl19025* (*dsNl19025*)) decreased the survival rate, feeding capacity and fecundity of BPHs. On the other hand, the accumulation of salicylic acid and H_2O_2 in rice infested by gravid BPH females injected with *dsNl19025* was significantly higher and lower, respectively, than those in rice infested by gravid BPH females injected with double stranded RNA of *GFP*. The results suggest that Nl19025 plays important roles not only in the growth and development of BPH, but in BPH-rice interactions as well.

When life gives you sugar: Metabolic fates of phytoanticipins in phloem feeding insects

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Many piercing-sucking insects of the order Hemiptera are worldwide crop pests which can cause severe agricultural losses. While sometimes not visually obvious, the damage inflicted by these insects via phloem feeding can nevertheless cripple a healthy plant through the introduction of viruses and the deposition of sugar-rich honeydew on the leaves, leading to the growth of sooty-mould. Plants are however not entirely without defense to this specific type of feeding, and load phloem tissue with phytoanticipins implicated in defense, for example cyanogenic glycosides and glucosinolates. In addition to those chemical defenses, phloem-feeding insects must also overcome the non-defense related challenges presented by a phloem sap-based diet, and can circumvent the extremely high osmotic potentials via biosynthesis of sugar oligomers. Here we report the discovery of a connection between osmoregulation and detoxification of plant chemical defenses in several phloem feeding insects. Metabolic approaches identified modified plant glycosides in the honeydew of both whiteflies and aphids feeding on various host plants and artificial diets containing the abovementioned phytoanticipins. Modified metabolites are stable to the respective plant enzymes responsible for activation of these defense compounds. Interestingly, it was shown that several phloem feeding insects are capable of producing these modified glycosides, indicating a general method of modification/detoxification.

S1-P-006

Elucidation of triadic relationship among feeding behavior of *Zizeeria maha* (Kollar) (Lepidoptera: Lycaenidae), its host plant (*Oxalis corniculata* L.) quality, and light environment.

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Plants are unable to migrate from one place to another except for seed dispersal. Because of this, differences in environmental properties may lead to differences in plant physicochemical qualities among plant individuals or patches, even among the leaves in the same plant individual. Likewise, since it is difficult for small neonate larvae to move from a leaf to another, differences in plant physicochemical properties may affect growth of the larvae. The aim of this study was to experimentally elucidate insect-plant-environment interaction, by using *Z. maha* larvae (a specialist), *O. corniculata* (a host plant), and light intensity (an environmental factor).

Experiments were conducted in both field and laboratory. In the field, seven different patches of *O. corniculata* with different light intensities were selected as study areas, and physiochemical properties of the plants and soils were analyzed. Feeding bioassays against *Z. maha* larvae were also conducted with the leaves collected from the above areas. In laboratory, *O. corniculata* plants were cultivated from seeds under a controlled light intensity. After the true leaves came out, plants were placed under conditions of either high light or low light intensities. Leaves were collected periodically, subjected to feeding tests and to analyses of oxalic acid which was identified as a larval feeding stimulant of *Z. maha* (Yamaguchi et al. 2016). Correlations were analyzed among the light intensities, leaf chemical contents, and larval feeding.

In the field, differences in leaf content of organic acid and phenolics were detected among the study areas, with positive correlations to light intensity. Feeding bioassays with the field collected leaves showed a positive correlation of leaf consumption to the organic acid content. In laboratory, oxalic acid content decreased in the leaves under low light condition whereas that under the high light condition stayed steady. Leaves under the high light condition were consumed significantly more than those under the low light intensity. These results demonstrated a triadic relationship among *Z. maha* larvae, *O. corniculata*, and light intensity as follows; an increase of light intensity induces *O. corniculata* to raise the production of oxalic acid in the leaves, which stimulates *Z. maha* larvae to feed more on the plants under light condition. This is the first demonstration of three components interaction (light-plant-herbivore) with a concrete semiochemical, oxalic acid, as a key compound.

S1-P-007

Identification and functional research of chemosensory genes in three rice planthoppers

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Three rice planthoppers (The brown planthopper (BPH), *Nilaparvata lugens*, the small brown planthopper (SBPH), *Laodelphax striatellus* and the whitebacked planthopper (WBPH) *Sogatella furcifera*) are notorious pests causing a huge rice loss in Asia every year. They not only damage rice plant by sap-sucking but also transmitting different plant viruses. Chemoreception play a crucial role in locating and selecting rice plant, which have been identified three decades ago. But its molecular mechanism is rarely unknown.

In this study, based on the WBPH, SBPH and BPH transcriptome or genome, we first annotated massive genes encoding for several families of chemosensory genes, odorant binding proteins (OBPs), odorant receptors (ORs), gustatory receptors (GRs) and ionotropic receptors (IRs). Second, we found a variety of positive selective orthologous genes by using phylogenetic analysis (positive analysis). For example, SfurOBP11, NlugOBP8 and LstrOBP2 of the three rice planthoppers displayed >85% identities.

Fluorescence *in situ* hybridization results showed that the three OBPs located in sensilla chaetica and sensilla auricillica of both nymphs and adults. Further, We used ligand-binding experiments to measure odorant substrate spectrum of three OBPs *in vitro*, SfurOBP11, NlugOBP8 and LstrOBP2. Results revealed that purified recombinant these three OBPs bound several rice plant volatiles. Finally, RNAi treatment by using the same siRNA sequence successfully decrease the mRNA level of the three OBP genes to the level of 20-30%. Subsequently rice selection test unveiled the three OBPs play key roles in locating rice host plant.

Taken together, these findings will be helpful for determining the potential physiological functions of chemosensory genes of rice planthoppers, which in turn may help to discover new targets for broad specrum behavioral antagonists for use as control strategies.

S1-P-008

Flower VOCs' perception: specialization sign in generalist pollinator

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Oedemera (Coleoptera: Oedemeridae) beetles are known as generalist pollinators for many plant species including wild strawberries *Fragaria vesca* and *F. viridis*. The aims of the present study were to determine if sympatric strawberry species *F. vesca* and *F. viridis* that co-flower and co-occur in the same habitat were visited by *Oedemera* beetles equally or not, and to reveal the floral VOCs emitted by the strawberries that are perceived by the *Oedemera* beetles.

In the test locality *F. vesca* and *F. viridis* flowers were visited by two *Oedemera* species, *O. lurida* and *O. virescens*. Specimen collection indicated that *O. lurida* beetles significantly preferred *F. viridis* flowers towards *F. vesca* ones, whereas *O. virescens* beetles tended to prefer *F. vesca* towards *F. viridis*.

GC-EAD analysis revealed three volatile compounds of *F. vesca* and four compounds of *F. viridis* flowers which evoked antennal responses in *O. lurida* beetles. Three compounds (nonanal, phenylacetaldehyde, and unknown compound) were common both for *F. viridis* and *F. vesca* flowers, and one compound (1,4-dimethoxybenzene) was characteristic for *F. viridis* flowers only. Thus, *O. lurida* beetles are capable to distinguish between flowers of the two strawberry species based on the floral volatiles.

Antennae of *O. virescens* beetles responded to higher number of compounds present in *F. vesca* flowers. Three compounds were the same as those elicited antennal response of *O. lurida*, (nonanal, phenylacetaldehyde, and the unidentified compound) and five compounds (acetic acid, 3-ethyl-4-methyl-1-pentanol, decanal, (*E*)-2-nonenal, and linalool) were different. This indicates, that *F. vesca* flowers were perceived by *O. virescens* and *O. lurida* differently, based on the number of EAD-active VOCs, i.e. certain specialization signs in the two generalist pollinators are evident.

Discussion on the role of EAD-active VOCs released by strawberry flowers in mutualistic insect-plant interaction will be presented.

Plant utilization by phloem feeder insects: Secondary metabolites interacts in primary metabolism

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The importance of plant secondary metabolites in determining patterns of host plant utilization is well established in herbivore arthropods, especially on chewing insects. As a matter of fact, the involvement of plant secondary metabolites in determining specific insect-plant interactions in phloem-feeding insects is questioned and controversial. At the same time, phloem feeding insects face a unique challenge when feeding on their host plants, as they are required to overcome the high osmotic potential of the phloem sap by converting sucrose into various oligosaccharides which are excreted as honeydew. Therefore, traits involved in sugar metabolism, have the potential to play an important role in host-plant adaptation in phloem-feeding insects, and might be under strong selection pressure. Taken together, we pose here the hypothesis that research on phloem-feeding-insects interactions with their plant hosts, should focus mainly on primary (sugar and amino-acid) metabolism and its interface with defensive secondary metabolic pathways. In this study, we zoomed-in on the interaction between glucosinolates producing plants and few hemipteran models belonging to the phloem-feeding guild: whiteflies, aphids and mealybugs. Honeydew analysis indicated that all analyzed members were capable of conducting a novel biochemical modification which involve the sequential additions of multiple glucose moieties to glucosinolates. This modification was found to be unique and was not detected in the frass of other analyzed groups such as leaf-chewers. It is proposed, that the presence, simultaneously, in the gut lumen of phloem feeders, of both glucosinolates (or other defensive glucoside conjugates) and high-levels of non-assimilated glucose (derived from the hydrolysis of sucrose), allowed phloem-feeding insects to evolve an efficient, elegant and energetically cheap solution to handle two major feeding limitations that interfere with successful host utilization: the control of osmotic pressure and the need to detoxify plant defensive chemistry.

Evolution of multidrug resistance transporters in highly specialized herbivores

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Multidrug resistance (MDR or ABCB) transporters belong to the large ATP-binding cassette (ABC) protein family. These transmembrane transporters are involved in detoxification mechanisms by mediating the translocation of xenobiotics across different cellular barriers and membranes. Notably, ABC subfamily B transporters have been described as essential elements of the Blood Brain Barrier (BBB) protecting the (central) nervous system against neurotoxic phytochemicals.

To shed light on the role of MDR proteins in highly specialized herbivores we focus on innovative methodologies to characterize the function and substrate specificity of the leaf beetle's *Chrysochus auratus* ABCB transporters (*Ca*_ABCB). *C. auratus* is specialized to incorporate host plant-derived cardenolides in its own anti-predator defense strategy.

We have taken a widely used in vivo expression system, based on Baculovirus-infected insect cells and optimized various assays to reveal the transporter interaction with low and high permeability compounds: (i) vesicular uptake assays using membranes prepared from *Ca*_ABCB-expressing cells, (ii) ABCB ATPase activity assays, (iii) transporter efflux studies with cells expressing heterologous *Ca*_ABCB.

As indicated for ABCB transporters in other organisms we found that cardenolides consistently stimulate *Ca*_ABCB ATPase activity to a high degree whereas specific inhibitors block the ATPase activity. Interestingly, the three ABCBs identified in the transcriptome of *C. auratus* show distinct tissue distribution and substrate specificity. We conclude that MDR transporters play a key role in the adaptation of the beetles to their poisonous host plants. Further protein biochemical investigations of insect ABC transporters are important to understand the evolution of MDR proteins and to determine whether they constitute a general detoxification mechanism in insect herbivores.

Involvement of larval frass volatiles in host selection by female adults of *Pieris rapae*

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Phytophagous insects are considered to use plant volatiles for their host finding and location; however, few investigations have been conducted on the involvement of plant volatiles in host location by butterflies. A previous study[1] clarified that mated females of small white butterfly *Pieris rapae* utilize the odor of their hosts, and we have identified several cabbage plant volatiles as their host-finding cues. In addition to the location of host plants, phytophagous insects can also recognize the conditions of host plants by perceiving olfactory cues such as larval frass odor.[2] In the present study, we assessed the effects of conspecific larval frass odor on the host location by *P. rapae* females.

With virgin and mated *P. rapae* females reared in our laboratory, we conducted (1) two-choice bioassays using potted cabbages in the experimental arena, in which females were able to utilize visual cues, and (2) two-choice bioassays using an olfactometer, in which no visual cues were available. In the former experiments with visual cues, both virgin and mated females visited an intact cabbage significantly more frequently than an infested cabbage with larval frass (after 2-day infestation by 3–5 late-instar larvae), and slightly more frequently selected an intact cabbage than an intact cabbage placed with larval frass. These results indicate that conspecific larval frass has weak repellency to *P. rapae* females. In the latter experiments without visual cues, both virgin and mated females were significantly attracted to intact cabbage odor and weakly repelled by larval frass odor as compared with the odorless air plume. However, they did not discriminate between the intact cabbage odor and a mixture of intact cabbage and larval frass odors. These results suggest that *P. rapae* females tend to avoid larval frass odor, but the repellent effect of frass odor is overcome by the attractancy of intact cabbage odor. Our chemical analyses revealed that larval frass odor consisted mostly of aliphatic sulfides and thionitriles, in which dimethyl disulfide was a predominant component. *P. rapae* females may recognize the presence of conspecific larvae via these sulfur-containing volatiles and avoid to oviposit on heavily-infested cabbages.

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Glycine betaine as an oviposition stimulant for the common grass yellow butterfly, *Eurema mandarina*, in *Albizia julibrissin*

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The common grass yellow *Eurema mandarina* is a Fabaceae-feeding pierid butterfly widely distributed in the temperate region of Japan. In Hiroshima, female adults exploit on *Albizia julibrissin* and *Lespedeza cuneata* as the primary hosts. We have identified D-(+)-pinitol as one of the oviposition stimulants of *E. mandarina* from these hosts.[1] However, the oviposition-stimulatory activity of D-pinitol is weak at the natural concentration although this cyclitol at the concentration of 0.5% induced a maximum response from approximately 55% females. The neutral/amphoteric (N) fractions of both hosts, which contained D-pinitol, elicited approximately 10% oviposition responses from females, while the neutral/amphoteric/basic (N+B) fractions elicited approximately 50% responses. These results suggest that the basic (B) fractions have other oviposition stimulants that act synergistically with D-pinitol.

In this study, we attempted to identify basic substances that stimulate oviposition of *E. mandarina* from *A. julibrissin*. The methanolic extract of fresh leaves was subjected to fractionation to prepare its aqueous layer. The B fraction was yielded from this aqueous layer by reversed-phase flash column chromatography and ion-exchange chromatography. HR-ESI-MS and NMR analyses revealed that *N*,*N*,*N*-trimethylglycine (glycine betaine: GB) was contained as a major substance in the B fraction. Females exhibited oviposition responses to authentic GB, at concentrations over 0.001%, with a peak response (45%) to 0.1% of GB. The binary mixture of GB (0.1%) and D-pinitol (0.5%) elicited 73% female responses. Because fresh leaves of *A. julibrissin* contained approximately 0.01% of GB on average, we concluded that GB induces the oviposition of *E. mandarina* together with D-pinitol.

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Effects of mating on host selection of female small white butterfly *Pieris rapae* (Lepidoptera: Pieridae)

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Mating greatly affects the host selection behavior of phytophagous insects, which is often associated with an increase or decrease in their sensitivity to particular chemicals.[1] In several species of moths, post-mating changes in behavioral and sensory responses to olfactory cues for mating and host selection have been reported. Females of small white butterfly *Pieris rapae* (Lepidoptera: Pieridae) were found to initiate an olfactory-guided host-finding behavior after mating.[2] However, it remains unclear (1) which plant volatiles are involved in their host selection and (2) whether mating changes sensory responses of females.

Here, we investigated the post-mating changes in behavioral and antennal responses of *P. rapae* females to host plant volatiles. We identified 15 compounds from the headspace odor of intact cabbage plants, and prepared its synthetic blends by mixing authentic standards. In two-choice bioassays using artificial plant models, mated females visited the model scented with 15-, 9-, or 6-component synthetic blend of cabbage plant volatiles more frequently than the unscented control, whereas virgin females did not exhibit this preference. Since single compounds and the 3-component blend did not induce preferential visiting, mated females apparently utilized complex odor blends as their host-finding cue. Moreover, 2- to 4-day-old mated females visited the models, scented or unscented, more frequently than did their virgin counterparts. These results indicated that mating enhanced the host-finding behavior of young females and their responsiveness to plant volatiles. Gas chromatography–electroantennographic detector analysis revealed that 8 of the 15 compounds in the cabbage plant volatiles elicited responses from female antennae. However, significant post-mating and age-dependent changes in antennal responses were not detected. Since female peripheral (antennal) sensitivity to volatiles remained practically unchanged after emergence, the post-mating changes in host selection may be attributed to changes in the central nervous system.

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Why must *Aphis gossypii* Glover change the host plant ?

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The cotton aphid, *Aphis gossypii* Glover (Hemiptera: Aphidadae) which selects *Hibiscus syriacus* L. as its primary host, explosively increases in population by parthenogenesis in early spring. Until end of spring or early summer they shift to secondary host plants including Solanaceous, Cucurbitae and Astraceae families with a morphological switch from the non-winged form to the winged form. In the fall, they return to the primary host, *H. syriacus* to lay eggs on it by syngenesis.

This study has tried to elucidate the reason why the cotton aphid must be forced out its primary host from the point view of the seasonal variations in the chemical constituents in the primary hosts.

The seasonal variations in the chemical constituents in the primary hosts by using HPLC were analyzed every two days. As a result, the non-winged form insects accompanying with increase of some peaks tended to increase and the winged form insects accompanying with increase of some peaks tend to increase. Of these components with seasonal variations during spring, concentration of uridine increased maximally (150 ppm in fresh leaf) before maximum number of the non-winged form insects and concentration of fumaric acid increased maximally (600 ppm in fresh leaf) before maximum number of the winged form insects. Number of non-winged form insects increased when uridine (150 ppm) was given to the aphids with artificial diet (organic acid, amino acid, vitamins in 35% sucrose solution) through parafilm membrane. On the other hand, number of the insects decreased when fumaric acid (600 ppm) was given to the aphids. Winged form insects significantly appeared in the test tube when fumaric acid was given. However, they decreased when both uridine(150 ppm) and fumaric (600 ppm) were given. From these results, we concluded that cotton aphid explosively increased by using uridine in early spring and that it was forced out from its primary host plant due to plant chemicals, that is fumaric acid.

S1-P-016

Evolution of induced response to herbivory and competition in invasive goldenrod

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Plants respond to biotic stresses, such as herbivory and competition, by inducing production of secondary metabolites that mediate ecological interactions. Such plastic responses may be adaptive, but whether and how plasticity evolves in a novel environment—where plants escape co-evolved herbivores while facing novel competitors—is not well understood. Competing hypotheses suggest that mean inducibility of an introduced population can either evolve to increase, decrease, or remain unchanged. Plasticity may be selected for when it allows individuals to achieve the optimal phenotype in the novel environment. Plasticity could also increase due to indirect selection against constitutive expression of phenotype that are in tradeoff with inducibility. In contrast, plasticity could be selected against if costs of maintaining machinery for induced response are high. Finally, plasticity may not evolve if there is no opportunity for selection to act on in the absence of biotic stresses that induces plant response (e.g. lack of herbivory).

Here, I tested if plants' plastic responses to 1) herbivory and 2) competition had evolved in introduced populations (Japanese and Australian) of tall goldenrod (*Solidago altissima*) compared to that in native North American populations. In a common garden, I first compared constitutive and induced expression of defense-related, leaf secondary metabolites (i.e. phenolics and diterpene acids) under control and simulated herbivory treatments (mechanical plus MeJA application). In the second experiment, plants were grown with or without a competitor (*Poa pratensis*), and expression of root secondary metabolites (i.e. polyacetylenes) that mediate interference competition was compared.

Constitutively, introduced populations expressed lower levels of defense metabolites than that of native North American plants, suggesting evolution of decreased defense upon introduction. While the damage treatment induced these metabolites, I found no difference in the levels of induction between the ranges. In the absence of a competitor, introduced populations expressed higher levels of allelopathic polyacetylenes than the native populations. Australian populations further induced polyacetylene production in response to competition, while Japanese and North American plants did not. Overall, while constitutive expression of secondary metabolites consistently diverged between native and introduced populations of goldenrod, the plastic response to herbivory and competition did not. The absence of evolution in inducibility may stem from lack of selection on plastic response, or lack of genetic variation for the plasticity.

S1-P-017

Synergistic Defensive Function of Raphides and chitinase/chitinase-like protein from Streptomyces griseus and yam (*Dioscorea japonica*) leaf extract through the Needle Effect

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Raphides, needle-shaped calcium oxalate crystals in tissues of many plants, have been thought to play defensive roles against herbivores. We have recently reported the synergistic defensive effects of raphides and plant protease [1]. In order to examine the synergistic defensive roles of raphides and chitinase, which often coincide with raphides in plant tissues, we performed bioassays giving the larvae of the Eri silkmoth, *Samia ricini* (Saturniidae), leaves of their host plant, the castor oil plant, *Ricinus communis* (Euphorbiaceae), painted with the raphides purified from kiwifruits, *Actinidia deliciosa* (Actinidiaceae), in presence or absence of *Streptomyces griseus* chitinase (sg-chi) and yam (*Dioscorea japonica*) leaf extracts (Dje) that are suggested to contain chitinase. Raphides alone or sg-chi / Dje alone showed only weak defensive activities around experimental concentrations. However, when raphides and sg-chi / Dje coexisted, they synergistically showed very strong growth-reducing activities, and the mortality of caterpillars was very high (i.e. the needle effect).

Dje solutions were separated into four fractions using three sizes Amicon® centrifugal filter units (i.e. 3kDa, 50kDa, 100kDa), and a bioassay was performed for each fraction. The needle effect was confirmed in the fraction of 100kDa or larger (Dje≧100kDa). Then, native PAGE were performed with Dje≧100kDa, and after the native PAGE, the gel sample was transferred to an agarose gel containing ethyleneglycol-chitin following the method for chitinase detection [2]. Two bands in the fraction of 95-135kDa showing the chitinase activity were found. Since these two bands seemed to be Chitinase-like proteins (CLPs), the fraction of 95-135kDa was further divided into three fractions by cutting the gel, and bioassays were performed on Eri silkworm. Only the fraction of 115-135kDa showed a strong synergistic defensive effect. The results suggest that this putative 115-135kDa CLP protein of yam functions as a defense factor against herbivorous insects through the synergistic effects with needles in the plant.

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Fluorescence from flower pollen and anther as a visual cue in plant-pollinator

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Pollination is predominantly achieved by insects. The factors determining the foraging behaviour of pollinating insects remain a major issue, although a number of studies demonstrated that flowering plants attract pollinators via various stimuli, such as odour, colour, and shape. Biocommunication between plants and insects through flower fluorescence has been proposed and discussed for decades. Pollen and anther of many plants show blue fluorescence under UV light. Anthers usually extend beyond the corolla, and so genes in pollen are exposed to the risk of damage from solar UV radiation. Primitive function of fluorescence may be protection of genes in pollen from UV-induced damage by the absorption and subsequent emission of its energy. Another function may be attraction of pollinators by highlighting pollens as foods for them. However, fluorescent compounds have not been identified, and their ecological function is still unclear. The objectives of this study were identification of the fluorescent compounds, and examination of their ecological function by behavioural experiments employing honeybees (*Apis mellifera*).

Plants with pollens and/or anthers showing intense blue fluorescence were screened from 598 species under 365 nm UV light (photographs available in the database, http://labo.kyoto-phu.ac.jp/mpgkpu/mushinome.html), and selected following five plants: *Plantago lanceolata*, *Dipsacus fullonum*, *Scabiosa atropurpurea* var. Snow Maiden, *Fatsia japonica*, and *Prunus persica*. The fluorescent compounds were isolated and identified as acteoside and its *cis*-isomer, 3,5-dicaffeoylquinic acid, chlorogenic acid, 1-*O*-feruloyl-β-D-glucose and its *cis*-isomer. The fluorophores of these compounds are caffeoyl and feruloyl moieties with emission maxima at about 460 nm and 420 nm, respectively. These wavelengths correspond to them of the anthers containing pollens, suggesting that these compounds are responsible for fluorescence from pollen and anther. These results indicated that hydroxycinnamoyl derivatives are widely distributed in flower pollen and anther as fluorescent compounds. These compounds are also known as antioxidants. Fluorescent compounds would protect pollen genes not only by absorbing UV harmful energy, but also scavenging UV-induced reactive oxygen species.

The potential of fluorescence as a visual cue for insects was examined by two-choice assays with honeybees under the sunlight. The assays were based on a choice between a filter paper containing chlorogenic acid and a plain filer paper. The concentration of chlorogenic acid ranging from 0 to 100 mg/g filter paper were tested. The results indicated that honeybees can perceive the fluorescence and be significantly attracted by it in correlation with the intensity of fluorescence from chlorogenic acid.

Fluorescent visual cues probably serve in mutualistic interactions between plants and pollinators in association with other cues. Pollens of gymnosperms also show blue fluorescence under UV light, although they do not need pollinators. This suggests that the fluorescent compounds may have been originally evolved to protect genes from UV light in the initial evolution process, and later insects might use them as a guide towards pollens.

Feeding Stimulants for a Larva of *Graphium doson*

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Ovipositional & feeding stimulants for Papilionini and Troidini insects in Papilioninae family have been studied on in the world. However, there is little research for Graphiini family insects, because artificial breeding and oviposition of Graphiini family insects are difficult due to their nervousness. Previously, our laboratory succeeded in isolation and identification ovipositional & feeding stimulants of *G. sarpedon* (Common Bluebottle) from its host plant: *Cinnamomum camphora*, but it is still insufficient to discuss what kind of compound is the key substance for host selection in the Papilioninae. Therefore, in order to elucidate the eating evolution of the swallowtail butterfly, this study have tried to isolate and identifiy the feeding stimulants for larva of *G. doson* (Common Jay) from its host plant: *Michelia compressa*.

The evaluation of feeding activity was measured by the average area of a styrofoam disks (45 mm diameter) with semicircular shape applied with 1 g fresh leaves equivalent of plant extract or fractions, eaten by the 5th instar larva of *G. doson*. All larvae were collected from field in Kochi City.

Bioassay guided fractionation led to isolate and identify the three active components, that is, Sucrose, Chlorogenic acid and Rhamnocitrin 3-*O*-neohesperidoside from water-soluble layer and also four active components, Echinacoside, Rhamnetin 3-*O*-neohesperidoside, 11,13-Dehydrolanuginolide and compound α (Unknown) from hexane-soluble layer. Though the activity of each compound is very weak, the activity was the same to that of the original extract only when they were combined. From these results, we concluded that these seven compounds were feeding stimulants for larva of *G. doson.*

According to previous study, it has been reported that *G.sarpedon* utilizes Sucrose, Chlorogenic acid , Quercetin 3-*O*-glucopyranoside and α-Linolenic acid as the feeding stimulants.In this study, it is elucidated that *G. doson* utilizes Sucrose & Chlorogenic acid as the feeding stimulants.Therefore, it was suggested that Sucrose & Chlorogenic acid are utilized as the common feeding stimulants in the Graphiini family. Furthermore, *G.sarpedon* also utilizes three of the feeding stimulants: Sucrose, Chlorogenic acid and Quercetin 3-*O*-glucopyranoside as the ovipositional stimulants.These facts may indicate that *G.doson* utilizes Sucrose, Chlorogenic acid, Rhamnocitrin 3-*O*-neohesperidoside and Rhamnetin 3-*O*-neohesperidoside or all seven feeding stimulants as the ovipositional stimulants.

S1-P-020

Olfactory cues and honeybee foraging in response to flower scent compounds

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Two wild subspecies of *Robinia pseudoacacia* L. differ in floral color and can be visually discriminated by insect pollinators. The extent to which olfactory cues derived from floral scents contribute to discrimination between *Robinia* subspecies is however unknown. We tested whether these two subspecies differ in floral scent and whether these olfactory differences are used by honeybees (*Apis mellifera*) to discriminate between them. In the present study, we investigated the volatile compounds from two types of flowers of *Robinia* species occurring in Korea. The volatile compounds analysis was performed by gas chromatography with mass selective detector combined with solid-phase microextraction (SPME). The floral scent of white *Robinia* flowers contained three volatile chemicals absent in the floral scent of red *Robinia* flowers. One of them, linalool contributed over 30% of the emissions of white flowers. We also performed behavioral choice tests to determine the innate response of honeybees to the main floral volatile chemicals emitted by our *Robinia* subspecies. Our findings indicate that honeybees are able to discriminate between the two *Robinia* subspecies.

Tissue specific fine tuning of toxin resistance and overall activity of the Na,K-ATPase in milkweed bugs

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Insects that feed on plants containing cardiac glycosides (cardenolides and bufadienolides) have repeatedly evolved amino acid substitutions in the Na,K-ATPase to prevent these toxins from binding to and blocking this essential enzyme. In lygaeid bugs, such as the large milkweed bug, *Oncopeltus fasciatus*, the Na,K-ATPase alpha gene in addition has undergone two rounds of duplications resulting in three gene copies (α1A, α1B and α1C). These copies have differing numbers and identities of amino acid substitutions in the target site for cardiac glycosides. To form a fully functional enzyme the α subunits must team up with a β subunit that acts as a chaperone and modulates the enzyme's kinetics. In this study, we identified three full length β subunits (β1, β2, and β3) from transcriptomes of *O. fasciatus* and expressed them in all possible combinations with the three α subunits using baculovirus infections in Sf9 cells. Functional assays of the harvested enzymes demonstrated vast differences in sensitivity to cardiac glycosides among the three α subunits. In addition, for the α1B and α1C copies the β subunit had a strong influence on the enzymes' overall activity. Tissue specific qPCR showed that enzyme forms highly resistant to cardiac glycosides, such as α1Aβ1 and α1Bβ2, are located in the Malpighian tubules, the insects' excretory organs. The Malpighian tubules are directly exposed to cardiac glycosides in the hemolymph, making higher resistance advantageous; however, this increased resistance comes at the cost of low overall activity of these subunit combinations. In contrast, the most active, but also most sensitive combination of subunits, α1Cβ3, predominated in the nervous tissue, where effective ion transport is essential for the restoration of resting potentials. As neural tissue is protected from cardiac glycosides by the blood-brain barrier, the Na,K-ATPase is exposed to lower concentrations of the toxins. Thus, the different combinations of α and β subunits enable a fine-tuning of enzyme function depending on the tissues in which the genes are expressed and the needs for highly effective ion transport.

Modification of interactions between a whitefly vector and host plants by a semipersistent plant virus

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It is known that plant viruses can change the phenotype and/or defense pathways of the host plants and the performance of their vectors by which they modify the interaction relationships between insects and host plants. *Cucurbit chlorotic yellows virus* (CCYV) is a newly reported virus occurring on cucurbit plants and many other plant species. CCYV is transmitted specifically by B and Q biotypes of tobacco whitefly, *Bemisia tabaci*, in a semipersistent manner. In this study, we investigated whether or how a semipersistent plant virus manipulates the feeding behaviors of its whitefly vectors and change the plant volatile profiles and thereby modify the orietation behaviors of whitefly vector to the infected plants. we used electrical penetration graph (EPG) technique to record feeding behaviors of *B. tabaci* carrying CCYV. We also observed orientation preference of whiteflies to CCYV-infected cucumber plants or heathy plants, and collected and analyzed volatile chemicals from these plants. The results showed that CCYV altered feeding behaviors of both biotypes and sexes of *B. tabaci* with different degrees. CCYV had stronger effects on feeding behaviors of Q biotype than those of B biotype, by increasing duration of phloem salivation and sap ingestion, and could differentially manipulate feeding behaviors of males and females in both biotype whiteflies, with more phloem ingestion in biotype Q males and more non-phloem probing in biotype B males than their respective females. Whiteflies basically were attracted to heathy cucumber plants rather than CCYV-infected plants, and the chemical profiles of CCYV-infected plants were different from those of heathy plants. From the present results we can conclude that CCYV can change the vector feeding behaviors and host plant volatile profiles and thereby the vector orietantion preference. Further research is needed to investigate whether or not the modification of plant-insect interactions by a semipersistent plant virus is different from those by non-persistent or persistent viruses.

The cytotoxicology of momordicins on *Spodoptera litura* ovary cell line SL-1 and *Ostrinina furnacalis* hemocytes Ofh cells

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Abstract: [Background] *Momordica charantia* L. (Cucurbitaceae), called bitter melon or ‘kugua’, is a tropical or subtropical vine widely distributed in Asian, African, and Caribbean. Momordicins are secondary metabolites from *M. charantia* stems and leaves that confer resistance to many phytophagous pests. They belong to cucurbitane-type triterpene glycosides and have only slight structural differences. Two tetracyclic triterpenoids, momordicin I and momordicin II have been demonstrated with novel structure and satisfactory insecticidal activity, including antifeedant, growth inhibition, and ovipositional defects against *Spodoptera litura* Fabricius, *Ostrinina furnacalis* Güenée, and *Plutella xylostella* Linnaeus. However, the mode of action and toxicity mechanism of momordicin I and II are poorly understood. **[Objectives]** In the present study, we deeply investigated the cytotoxicology of momordicin I and II on *Spodoptera litura* ovary cell line SL-1 and *Ostrinina furnacalis* hemocytes Ofh cells by combining usages of several technologies, including MTT assay, CCK-8 assay, flow cytometry technology, inverted phase contrast microscope and fluorescence microscopy observation methods. **[Findings]** The results indicated that both momordicin I and II significantly inhibited SL-1 and Ofh cellular proliferation, and showed in a time- and a concentration-dependent manner. Moreover, the toxicity of momordicin I exceeded than that of momordicin II and azadirachtin A. Using inverted phase contrast microscopy we found that after exposure to momordicin I and II, SL-1 amd Ofh cells shapes changed to circular, swelling increased, adherence ability declined and the cellular membrane bubble; vacuole deformation and cytoplasm leakage indicated that both momordicin I and II dramatically suppression of cytoskeletal function. Following treatment with momordicin I and II caused the karyotheca of SL-1 and Ofh cells dissolved, the chromatin condensed abnormally and the nucleoli were damaged, migrated, or disappeared, showing a typical characteristic of necrosis. The PI fluorescent value detected by FCM showed that the relative fluorescent intensity of SL-1 cells induced by momordicin I and II increased to 521.45±3.33 and 370.17±3.02, respectively, higher than 135.04±3.65 induced by control group treatment for 48 h. This result demonstrated that momordicin I and II caused greater damage to cell membrane integrity and significantly decreased the glucose absorption capacity of SL-1 cells. Correspondingly, the cellular total protein content and mitochondrial membrane potential were enriched after SL-1 cells were exposed to momordicin I and II as documented by FCM and FITC. Flow cytometry analysis suggested that momordicin I and II significantly inhibited the proliferation of SL-1 cells by arresting the cell cycle in G2/M phase. In addition, Ofh cells treated with momordicin I were dramatically induced to undergo necrosis, and the total cell necrosis rate was 74.92±2.02% after 36 h treatment with 8 μg /mL momordicin I. **[Conclusions]** To conclude, we believe that inhibition of cytoskeletal function, interference with mitosis and destruction of nuclear structure may be major modes of action of momordicin I and II in insect cells. Together, these factors could be responsible for disrupting normal insect growth and development. Since the chemical structure of momordicins differs from other botanical insecticides, our results suggest that cucurbitane-type triterpene glycosides have the potential for use as precursors for novel insecticides of value in the control of agricultural pests.

Key words: Momordicins, SL-1 cell, Ofh cell, cell proliferation, cytotoxicology

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Resistance/Susceptibility of 11 tea plant cultivars to the tea green leafhopper determined by EPG technique and its correlation with the defensive substances in the tea leaves

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In order to better understand the mechanisms of tea plant against pest, the feeding behaviors of the tea green leafhopper, *Empoasca vitis* (Göthe) on 11 cultivars were studied using EPG technique to measure tea plant resistance/susceptibility levels to the leafhopper; and to determine their potential correlations with the major defensive components in the tea leaves. The results showed that: 1) seven distinct EPG waveforms with possible biological significances during the leafhopper feeding, i.e. waveforms Np, A, S, C, E, F and R were recorded; and E, F and R might be resistance-related waveforms. Based on the number of probes, waveform E duration and waveform F duration, these eleven tea cultivars were classified into two groups using cluster analysis, i.e. cultivars Anjibaicha, Wuniuzao, Zimudan, Zhongcha 108, Huangmudan and Tieguanyin in group I, and cultivars Zijuan, Longjing43, Ningbohuangjinya, Huangguanyin and Jinguanyinanother in group II. The resistance levels of these cultivars in group II were stronger than those in the group I. From the susceptible cultivars to the resistant cultivars, the catechin contents in the tea leaves negatively correlated with the duration of waveform E ($P < 0.05$); however, caffeine, amino acids and soluble sugar contents did not show significant correlations with the resistance/susceptibility of tea cultivars. We conclude that waveform E duration, waveform F duration and probing number can be considered as the main EPG parameters useful for measuring the tea plant resistance/susceptibility to the leafhopper. Catechins seem to be the key defensive compounds in the tea leaves related to feeding resistance.

Key words: *Empoasca vitis* Göthe, EPG, Probing behavior, Resistance, Tea plant.

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Biotic and abiotic factors influencing the feeding behavior of *Cacopsylla pruni,* the vector of European Stone Fruit Yellows (ESFY)

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Many psyllid species are known to be vectors of phloem dwelling bacteria (phytoplasmas and liberibacter), which cause severe plant diseases and economically important crop damage. Some of those psyllid species have a particular life cycle, in which they alternate their host plants two times within one generation. For example, the plum psyllid (*Cacopsylla pruni*), the vector of one of the most serious pests in European fruit production, migrates to stone fruit orchards in early spring for reproduction. After mating and female oviposition on their reproduction host plant *Prunus* spp., the nymphs of the next generation hatch. Having passed five nymphal stages on stone fruit cultivars, the young adults emigrate to their overwintering hosts like spruce and other conifers. *C. pruni* transmits the phytoplasma '*Candidatus* Phytoplasma prunorum' by phloem-feeding to *Prunus* rootstocks and cultivars. '*Ca.* P. prunorum' induce the European Stone Fruit Yellows (ESFY). Because infected trees yield poorly and die fast, this plant disease causes high economic losses in European fruit production every year.

Still very little is known about the factors responsible for migration behavior and host alternation of migrating psyllid species and their behavior during their phase of life on conifers. Because insect feeding behavior and host acceptance is driven by different biotic factors like olfactory and gustatory cues as well as mechanical barriers, we carried out electrical penetration graph (EPG) studies with *C. pruni* on different conifer species as potential overwintering hosts. The presented study is the first to prove that psyllids do feed on spruce and other conifers. Further, we investigated the feeding behavior of *C. pruni* in dependence from ambient temperatures to figure out which cold tolerance strategy psyllids may pursue. Additionally, the acceptance of conifers as feeding hosts for *C. pruni* nymphs was investigated by EPG recordings.

Our studies show, that both adults and nymphs are able to ingest plant sap from conifers and adults feed at very low temperatures. We hypothesize, that no mechanical or chemical obstacle influence their feeding behavior, but that the content or composition of nutrients in conifer's phloem is too low for proper development of *C. pruni.* Phloem sap has been analyzed by GC-MS after derivatisation of the samples. The comparison of the chemical composition of phloem and xylem will give some indication about the possible nutrient supply of different hosts.

S1-P-026

Identification of oviposition stimulants of the leaf-mining moth *Acrocercops transecta*

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A leaf-mining moth, *Acrocercops transecta* (Gracillariidae), consists of two populations that specialize different host plants, as one is a *Juglans*-associated population and the other is a *Lyonia*-associated population. Whereas *Juglans* (Juglandaceae) is taxonomically far remote from *Lyonia* (Ericaceae), it is estimated that the *Lyonia*-associated population has derived from the *Juglans*-associated population via a host shift. During the process of the host shift, chemical factors contained in the host plants could play a decisive role. Therefore, we addressed to identify oviposition stimulants, focusing on adaptation of the *Lyonia*-associated population to its host plant. Because *Lyonia*-associated females lay eggs on a cover glass treated with an extract from leaves of *Lyonia ovalifolia*, we designed a bioassay in that an individual mated female was allowed to select cover glasses treated either with sample(s) or solvent (ethanol) for oviposition placed in a plastic container. After overnight, the number of eggs on and around the cover glasses were counted to evaluate an oviposition stimulant activity. We firstly tested several solvents including ethyl acetate, ethanol, ether and hexane efficiently to extract *Lyonia* leaf components. As a result, the ethyl acetate extract showed the highest activity. This extract was fractionated by silicagel- and then ODS column chromatographies. Of the separated fractions, a fraction containing terpenoids as main components showed low activity. Because it is known that lepidopteran species generally utilizes highly polar compounds including sugars and amino acid derivatives as oviposition stimulants, a mixture of a highly polar fraction prepared from an ethanol extract of *Lyonia* leaves and the terpenoid fraction was tested. This mixture showed significant activity. From the terpenoid fraction, three terpenoids, lyofolic acid, 24-oxo-lyofolic acid and ovalifolioside, were identified as main components. Of them, lyofolic acid and 24-oxo-lyofolic acid showed significant activities when mixed with the highly polar fraction. Because the *Lyonia*-associated population never oviposit on *Juglans* leaves, we assumed that *Juglans* leaves do not contain essential oviposition stimulant(s) for *Lyonia*-associated females. To confirm this, individual of *Lyonia*-associated females were exposed to fresh leaves of *Juglans regia* treated with 24-oxo-lyofolic acid and untreated leaves (solvent only). We found more eggs on *Juglans* leaves treated with 24-oxo-lyofolic acid than on untreated leaves. In conclusion, lyofolic acid and 24-oxo-lyofolic acid were characterized as oviposition stimulants for *Lyonia*-associated *A. transecta*. Moreover, it is suggested that *Lyonia*-associated females do not lay eggs on *Juglans* leaves due to a lack of specific oviposition stimulant(s) in *Lyonia*-associated females, but not likely due to the presence of oviposition deterrent or repellent.

How does an invasive plant affect a native plant and associated butterflies across altitude?

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Plant invasions are a global concern. However, less is known about how invasive plants directly and indirectly influence native plants across spatial gradients, and how this might subsequently affects associated arthropods. To answer these questions, we examined an invasive plant (*Bidens pilosa* var. *Radiata*; hereafter *Bidens*), a native plant (*Rorippa indica*; hereafter *Rorippa*), and associated *Pieris* butterflies at low (100m) and medium (1000m) altitudes in subtropical Taiwan. *Pieris* are pollinators of *Bidens* but herbivores of *Rorippa*. We conducted four experiments: 1)To examine the *Bidens-Rorippa* competition across altitude, we set up a laboratory experiment and measured plant performance in *Bidens-Bidens*, *Rorippa-Rorippa*, and *Bidens-Rorippa* pots. Plants, collected as seeds from low or medium altitude, were paired in a pot based on origin (altitude). 2)To identify the interspecific competition mechanisms, we performed laboratory factorial experiments examining above-ground (shading/non-shading) and below-ground (with/without/partial root contact) factors. 3)To investigate the indirect effect of *Bidens* on *Rorippa* via attracting *Pieris*, we manipulated *Bidens-Rorippa* distance in the field and examined the distance effect on *Pieris* oviposition on *Rorippa*. 4)To test whether the impact of *Bidens* on *Rorippa* generates a cascading effect on *Rorippa*'s herbivores, we raised and monitored *Pieirs canidia* on *Rorippa* with or without *Bidens* competition. We found that the invasive *Bidens* suppressed the native *Rorippa* at low altitude only (Exp.1), contributed by the below-ground effect of *Bidens* on *Rorippa* (e.g., soil chemicals) (Exp.2). The distance between *Bidens* and *Rorippa* didn't affect *Pieris* oviposition on *Rorippa* (Exp.3), suggesting no indirect effect of *Bidens* on *Rorippa* via pollinator and herbivore (*Pieris*) attraction. Furthermore, *Bidens* impact on *Rorippa* subsequently reduced *P. canidia* performance (Exp.4), indicating a cascading effect of plant invasion on a higher trophic level. In conclusion, the invasive *Bidens* suppressed the native *Rorippa* at low altitude, likely via below-ground mechanisms. This altitude-dependent, negative impact on *Rorippa* could subsequently cascade up to associated pollinators/herbivores (*Pieris* butterflies). As plant invasions are ubiquitous and occur across altitude or latitude, the spatial variation of their impact on different trophic levels deserves further investigation.

S1-P-028

Genetic variations of Kanzawa spider mites in their levels of detoxification and defense induction in plants

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Kanzawa spider mites (*Tetranychus kanzawai*) are polyphagous herbivores that leave different colors of scars (red or white) on leaves of host plants including *Phaseolus spp*. We identified the genetically distinct strains that left either red scars or white scars (hereafter called Red strain and White strain), in order to understand the intrinsic trait of those genotypes. We carried out RNAseq analysis for mRNA of Red and White strains on kidney bean plants (*Phaseolus vulgaris*). Then we assessed their acaricide resistance on *Phaseolus* leaves. As a result, genes involved in the detoxification system (cytochrome P450, carboxylesterase, glutathione *S*-transferase and ABC transporter) were expressed in Red strains for more than in White strains. The survival rate of Red strains was higher than that of White strains when the mites were exposed with 3 different acaricides.

Further, we obtained next generation of surviving females after they had been exposed to one of three acaricides in Red and White strains. We tested resistance ability againt the acaricide and detoxification enzyme activities in individuals of the next generation. We also measured levels of defense-related phytohormones (jasmonic acid and salicylic acid) that were induced by next generaton individuals in *P. vulgaris*. We found the differences of the detoxificaton system ability and levels of phytohormones among individual mites within White strain. Based on our findings, the implications of the significance of those genotypes are discussed.

Identification and investigation of compounds induced in apple by insect attack.

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The peach fruit moth, *Carposina sasakii*, is the most destructive insect pest for apples in Japan. Adults lay eggs on young apples, and hatched neonates burrow into the fruits. They spend their whole larval stage inside the fruits. Emerged fully grown larvae drop onto the ground to weave cocoons, where they pupate or enter diapause in order to overwinter.

In orchard under natural conditions, the larval survival rate was found to be greatly reduced, compared to that was observed in lab-rearing colonies. The larval survival rate differed significantly depending on whether the fruit was picked from the tree. More larvae survived in "picked" fruits than unpicked "on-tree" fruits.

In this study, we found many compounds induced in young apple fruits when attacked by the insects. Two of them were identified as chlorogenic acid (CGA) and *p*-coumaroylquinic acid (*p*-CoQA) by NMR and MS analyses. CGA and *p*-CoQA were more induced in "on-tree" fruits than in "picked" fruits, suggesting a defensive role against herbivory. Using artificial diet enriched with CGA or *p*-CoQA, the toxicity against lepidopteran larvae was examined. The results will be discussed in the context of their physiological mechanisms and ecological impacts.

The scent of male and female flowers in the *Glochidion–Epicephala* brood-site pollination mutualism in India

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Brood-site pollination mutualisms are characterised by specialised chemical communication between plant species and their usually species-specific pollinators. The best studied examples of such systems include the relationship between figs and pollinating fig wasps, yucca and yucca moths, and globeflowers and their fly pollinators. Another important brood-site pollination system with its centre of diversity in Asia is that involving *Glochidion* (Phyllanthaceae) and its pollinating *Epicephala* moths (Gracillariidae). We investigated the floral morphology and floral scent of male and female flowers of *Glochidion hohenackeri* in the Western Ghats of India in a seasonal cloud forest site in Maharashtra. This species is monoecious, male flowers are long-stalked while female flowers are sessile. We found differences in the scent of male and female flowers with the scents containing many terpenes, and lipidic compounds such as hexenyl acetates and hexenyl butyrates. A generic insect-repellent compound particularly in male flowers was noted and its significance for pollinator behaviour is discussed.

S1-P-031

Effect of inhibitory substances contained in calcium carbonate wettable powder on oviposition of peach fruit moth, *Carposina sasakii* (Lepidoptera: Carposinidae)

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The peach fruit moth, *Carposina sasakii* Matsumura, is a serious insect pest of apples and other roseceous fruits in Japan. Because newly hatched larvae burrow into the fruits, their infestation directly causes a big damage on the products‘ market value and also is regarded as international quarantine matters. Besides that, the ecological trait of being an internal fruit feeder make insecticides useless during the larval stage. Mating disruption by synthetic sex pheromone is currently practical but more effective management is urgently required.

It is known that the chemical solution containing lime has an effect of suppressing damage by this pest. Particularly, spraying the calcium carbonate wettable powder ″white coat″ (Shiraishi Calcium Co., Ltd.) significantly decreased the number of laying eggs. This is partly explained by a physical change of the fruit surface and a possible mechanical damage on the female moth antenae. In this study, we investigated another possibility of chemical deterrent mechanisms, by using GC-EAD (gas chromatography-electroantennographic detection), and laboratory bioassays.

Adult female antennae showed strong responses against nonanal and decanal in the volatile components derived from young apple fruits. Equivalent responses were obtained for two unknown components specific to the fruits treated with calcium carbonate hydrate. These two components were puritied by silica gel column and the structures were determined by NMR. One was 2,2,4-trimethyl-1,3-pentanediol diisobutyrate (Txib™), which is added as placticizer to the agents. And the other was its analogue. To estimate the effect of Txib™ to oviposition behavior, choice tests in a small glass cage were conducted using apples treated and untreated with Txib™.The result showed that significantly fewer eggs were laid on the treated fruits, suggesting Txib™ have a repellent activity against oviposition behavior of *C. sasakii* female moth.

S1-P-032

Bt rice provides ecological resistance to non-target planthoppers

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Genetically engineered (GE) rice lines expressing Lepidoptera-active insecticidal genes from the bacterium *Bacillus thuringiensis* (*Bt*) have been developed in China. Field surveys indicate that *Bt* rice harbours fewer rice planthoppers than non-*Bt* rice although planthoppers are not sensitive to the *Bt* proteins produced by *Bt* rice. The chemical mechanisms of this phenomenon remain unknown. Here we show that the low numbers of planthoppers on *Bt* rice is associated with reduced caterpillar damage. In laboratory and field-cage experiments, the rice planthopper*Nilapavatalugens*had no feeding preference for undamaged *Bt* or non-*Bt* plants but exhibited a strong preference for caterpillar-damaged plants whether *Bt* or non-*Bt*. Under open field conditions, rice planthoppers were more abundant on caterpillar-damaged non-*Bt* rice than on neighbouring healthy *Bt* rice. Furthermore, GC-MS and metabolome analyses expounded the potential chemical mechanisms of the phenomenon that rice planthoppers prefer caterpillar-damaged rice plants. Our results suggest that *Bt*rice provides ecological resistance to planthoppers. Non-*Bt* rice planted as a refuge to delay the evolution of *Bt* resistance is thus likely to divert planthoppers away from *Bt* rice and may therefore restrain planthopper outbreaks.

S2-P-033

Unusual mechanism of aliphatic formate formation closely related to hydrocarbon formation in the astigmatid mite, *Sancassania* sp. Sasagawa

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All hydrocarbons observed in the opisthonotal gland secretions of acarid mites are straight-chain aliphatic compounds, and the chains are characteristically shorter than those of the hydrocarbons found in insects. (*Z,Z*)-6,9-Heptadecadiene (6,9-C17), derived from linoleic acid (LA), and (*Z*)-8-heptadecene, derived from oleic acid (OA) are detected in a high proportion of acarid mites, yet there are almost no reports of their detection in insects. As a result of isotope-labeled compound uptake experiments using *Carpoglyphus lactis* (Acari: Carpoglyphidae), the biosynthetic conversion of LA to 6,9-C17 has been demonstrated. Aldehydes are known intermediates of hydrocarbons in insects, and our own studies make it increasingly clear that aldehydes are also intermediates of hydrocarbons in mites. Despite aliphatic formates are commonly detected in mite secretions together with hydrocarbons, details surrounding the biosynthetic pathway(s) remain unclear. Aliphatic formates such as (*Z*,*Z*)-8,11-heptadecadienyl formate and (*Z*)-8-heptadecenyl formate are rarely encountered natural products that are abundant in *Sancassania* sp. Sasagawa (Acari: Acaridae) mite secretions. LA and OA are predicted as key intermediates in the synthesis of the closely related aliphatic formates. To gain insights in this biosynthetic pathway, acarid mite feeding experiments were conducted using ^{13}C-labeled precursors to precisely track incorporation. Analyses using ^{13}C NMR spectroscopy demonstrated that the ^{13}C-labeling pattern of the precursors was detectable on formates in exocrine secretions and likewise on fatty acids in total lipid pools. Curiously, the results demonstrated that the formates were biosynthesized without the dehomologation of corresponding fatty acids. Consistent with a Baeyer–Villiger oxidation reaction, labeling studies support the insertion of an oxygen atom between the carbonyl group and carbon chain of aldehyde. Empirical data support the existence of a Baeyer–Villiger monooxygenase responsible for the catalyzation of the Baeyer–Villiger oxidation. Since the mite secrete hydrocarbons and formates at the same time, it is hypothesized that two different types of enzymes act on aldehydes as a common substrate in the secretory glands and generate hydrocarbons and formates separately.

Evaluation of male and female extracts upon *Opsiphanes invirae* Hubner (Lepidoptera: Nymphalidae) antennae.

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The caterpillar *Opsiphanes invirae* is a major pest of Arecaceae, such as coconut (*Cocos nucifer*a L.) and oil palm (*Elaeis guineensis* Jacq.), causing several damages to these crops of great socioeconomic interest. The use of sex pheromone has been considered an important tactic for the Integrated Pest Management. The objective of this study was to evaluate the bioactive effect of male and female extracts from headspace extraction upon *O. invirae* antennae. The research was carried out at the Laboratory of Natural Resources Research (LPqRN), Federal University of Alagoas (UFAL). The insects were collected at AGROPALMA farm, in Tailândia, Pará. The analysis of *O. invirae* male and female extracts in GC-FID and GC-MS revealed the presence of a specific compound in the male extracts. The compounds identified in the male insect extract of *O. invirae* including monoterpenes, sesquiterpene and alcenes. Electroantenographic (EAG) bioassays were carried out with male and female extracts to investigate biological activity upon *O. invirae* antennae. The stimuli air, hexane, male extract and female extract were used in antennae. The data were submitted to analysis of variance and the means compared by Scott-Knott test at 5% of probability. The results demonstrated that female antennae presented no difference in responses to female extracts and the hexane. However, there was a significant response from female antennae to the male extracts. Regarding the male antennae, there was no difference between the responses to the hexane and the used extracts. This way, the results indicate the presence of biologically active compounds in the male extract that produces significant responses in the female antennae of *O. invirae*.

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S2-P-035

Absolute configuration of pheromones of the bumblebee wax moth, *Aphomia sociella*

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Aphomia sociella L. is a parasite of bumblebees. The larvae destroy host nest, eat the brood comb and the offspring. Unlike most Lepidoptera, reproductive behavior of *A. sociella* (Pyralidae: Galleriinae) is initiated by males and includes both ultrasonic and pheromone signals. Females respond to the males' chemical and acoustic signals by approaching while releasing their own courtship pheromone.

The pheromone of both males and females contains 6,10,14-trimethylpentadecan-2-one (phytone) in sex-specific quantities [1,2]. Main component of the female courtship pheromone is the corresponding alcohol, 6,10,14-trimethylpentadecan-2-ol [2], which is absent in the male pheromone. Here we report on the absolute configuration of these two pheromonal components.

Standards of all stereoisomers of 6,10,14-trimethylpentadecan-2-one and 6,10,14-trimethylpentadecan-2-ol were prepared from phytol (1:1:1:1 mixture of stereoisomers) using a described procedure [3]. Ketone in the insect extract was reduced to alcohol and derivatized with (*S*)-acetoxypropionyl chloride. The obtained diastereoisomers were analyzed on GC/MS and compared with synthetic standards. The absolute configuration of phytone in the male pheromone has been proved as 6*R*,10*R*, while in females it could not be determined due to its low concentration. The configuration of 6,10,14-trimethylpentadecan-2-ol in the female pheromone was found to be 2*R*,6*R*,10*R*. Determination of the absolute configuration is important with respect to potential use of pheromones in monitoring or control of *A. sociella*.

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Multidimensional chromatographic techniques applied to chemical ecology

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The characterization of biological matrices is an unavoidable demand in chemical ecology. Notwithstanding the continuous development of gas chromatography (GC) and mass-spectrometry (MS) instrumentation techniques and analytical methodologies, the total separation of all compounds and their unequivocal full or partial identification, in complex biological samples, is generally impractical or unachievable. Classical one dimensional chromatographic (1D) approaches for characterization of biologica complex matrices in spite of achieving valid results, may not always give satisfactory results due to a potential considerable amount of information that remains unexploited or hidden, and thus demanding an alternative strategy to the use of a single column separation, if an increased resolution is needed.

The emergence of comprehensive two dimensional chromatography (GC×GC) in the last decade, and the resurgence, of a new generation of heart-cutting devices (MDGC) capable of delivering multidimensional gas chromatography (MDGC) with high accuracy (e.g. capillary flow Deans switch) opens a new door to allow the characterization of complex mixtures by enabling the separation of analytes in complex mixtures that cannot be otherwise achieved.

In this work, samples of extracts and volatiles emitted by trees and insects were analysed using one-dimensional gas chromatography (1D-GC), comprehensive two-dimensional gas chromatography (GCxGC), heart-cut MDGC and by GC-MS/EAD. The increased resolution and sensitivity achieved proved to be an advantage, which is beneficial for the needs of trace analysis and the complex sample characterization usually essential in chemical ecology research.

Antifungal effects of volatile organic compounds emitted during infection of barley roots by fungal pathogens

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With 123 million tons produced in the world in 2010, barley has high agronomical significance. It is threatened by various edaphic fungal diseases among which common root rot caused by *Cochliobolus sativus* and *Fusarium culmorum*, is one of the most damageable.

Today, since an important part of chemical fungicides used for crop protection are being forbidden, there is a growing need of sustainable ways to control plant diseases. Therefore, the aim of this work was to investigate, characterize and valorize the use of volatile organic compounds (VOCs) as a new and innovative agronomic tool to control plant pathogens.

In this work we have used solid phase microextraction (SPME) coupled with gas chromatography mass spectrometry to extract and identify VOCs emitted by the two fungi and by infected and non-infected barley roots. Interestingly, we have observed that VOCs, especially terpenes, are newly emitted during the infection.

We have afterwards assessed the effect of fungal volatiles on barley growth and the effect of barley root volatiles on fungal growth by cultivating both organisms in a shared atmosphere without any physical contact. In such conditions, we have observed that the blend of VOCs emitted by infected barley roots decrease *C. sativus* growth by 13 to 17% while have no significant effect on *F. culmorum*.

Testing the major compounds of the blend individually highlighted that methyl acrylate (MA) and methyl propionate (MP) significantly reduced the development of both fungi *in vitro*, in direct contact (in the culture medium) as well as in gaseous contact. An inhibition of the spores' germination was also observed in the presence of each ester.

The effect of the molecules on infected barley seeds was also tested. Plants coming from the seeds in contact with the VOCs did not develop symptoms of the disease.

The efficiency of these esters was tested on a large panel of pathogens and obtained complete growth inhibition for most of them. As our results were opening promising perspectives concerning the biological control of edaphic diseases, the mechanism of action of the two organic esters was investigated trough observations in transmission electron microscopy, fluorescence microscopy and biochemical reactions.

In conclusion, the effects of MA and MP observed *in vitro* are promising and the studied esters could be regarded as an interesting and innovative starting point in the development of sustainable way to control barley and plants ‘diseases.

Keywords: barley, volatile organic compounds, *C. sativus*, *F. culmorum*

S3-P-039

Biflavones of *Cycus revoluta* induce differentiation of filamentous *Nostoc* cyanobacteria into motile hormogonia

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Nitrogen-fixing cyanobacteria of genus *Nostoc* often establish symbioses with some selected plants, such as *Anthoceros* (hornworts), *Azolla* (fern), *Cycus* (gymnosperm), and *Gunnera* (angiospermae). For this symbiotic event, the host plants may release signaling substances to attract *Nostoc* cyanobacteria and induce morphological and functional differentiation of the symbiont. Hormogonium is the motile state of *Nostoc* cyanobacteria to acquire an ability of accessing to host plants at the primary stage of host-sybiont interaction. Such physiologically active substances in host plants have not yet been characterized. We have investigated such hormogonia-inducing factor (HIF) from *Cycus revoluta* coralloid root, and identified 1-palmitoyl-2-linoleoyl-*sn*-glycerol and some other diacylglycerols as HIFs. Diacylglycerols are, however, common primary metabolites as main components of biomembrane among most living creatures.

In this study, we hypothesized that *Cycus revoluta* release more species-specific signaling chemicals to induce hormogonium differentiation for soil-born cyanobacteria and activate them in the bed soil. We first focused on fresh leaf litters, swept the leaf surface with absorbent cotton balls wet with 70% ethanol. Leaf exudate thus recovered showed a clear HIF-like activity at 22 μg per a paper disc, but due to too small absolute amount of the leaf exudate, we have changed target material to the dead leaves of *C. revoluta*. Hormogonia-inducing factor was investigated in the methanolic extract of the completely brown and dry leaves of *C. revoluta.* The leaf materials (2.6 kg) that have passed a winter season were collected at Yaku Island, Kagoshima prefecture, Japan, and subjected to extraction with methanol. Bioassay to detect HIF-like activity was done, using a *Nostoc* sp. wild strain isolated from a coralloid root of *C. revoluta* grown in Yaku Island.

The filamentous *Nostoc* colonies exposed to the crude methanolic extract showed a potent HIF-like activity, and EtOAc and *n*-BuOH solubles from methanol extracts also showed a potent hormogonium-induction toward filamentous *Nostoc* colonies at 1.6 μg per a paper disc. Using hormogonia-inducing activity as an index, HIF was chased in the EtOAc solubles were further purified by several column chromatographies, and finally two active substances were obtained as single peaks in HPLC connected with a ODS-column (7.5 mm diameter × 250 mm in length) and monitored by UV 220 nm. The solvent system for separation was CH_3CN-H_2O (50:50) and the flow rate was 1.5 mL/min. These active substances detected at *tR* 11.8 min and 21.9 min were elucidated as biflavones of molecular formula $C_{30}H_{22}O_{10}$ and $C_{30}H_{20}O_{10}$ by FD-HR-MS and 2D-NMR analyses. Biflavones are known as secondary metabolites found only among gymnosperm plants. Some flavonoids have been characterized as regulatory factor for *hrmA* expression involved in hormogonium formation, suggesting that the biflavonoidal HIFs are specific signaling chemicals to function at the primary stage of *Nostoc* sp.-*C. revoluta* symbiosis.

S3-P-040

Diacylglycerols isolated from *Cycus revoluta* coralloid root and analogous anacardic acid derivatives induce morpho-differentiation of filamentous *Nostoc* cyanobacteria into motile hormogonia

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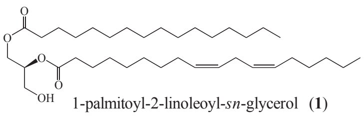
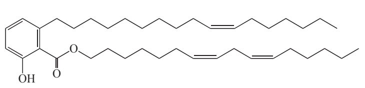
Nitrogen-fixing cyanobacteria of genus *Nostoc* in order Nostocales often establish symbiosis with some limited plants, such as *Anthoceros* (hornworts), *Azolla* (fern), *Cycus* (gymnosperm), and *Gunnera* (angiospermae). It is speculated that these selected host plants produce and release hormogonia-inducing factor (HIF) that leads to differentiation of cyanobacterial aggregated filaments to hormogonia, known as motile filaments. This hypothetical HIF that would assist *Nostoc* cyanobacteria in accessing to the host plants has long been searched, but signaling substances have not yet obtained from the host plants.

In searching for HIF, we used coralloid roots of *Cycus revoluta.* According to bioassay-guided fractionation using several chromatographic techniques, we chased HIF in the methanol extract, and eventually identified 1-palmitoyl-2-linoleoyl-*sn*-glycerol (**1**) as an HIF-like substance. Some homologs of **1** (1,2-diacylglycerols, DGs) tested were also active, but neither triacylglycerol nor 1-palmitoyl-2-linoleoyl-*sn*-phosphatidylglycerol showed any HIF-like activity. In addition, both (*S*)- and (*R*)-1-palmitoyl-2-linoleoylglycerols chemically synthesized were equally active as HIF. These facts implied that DGs not function as host-specific signal substances to recognize the host plant but act as root-sensing substances.

Since DG is known as a protein kinase C (PKC) activator, we also investigated several PKC activators, such as 12-*O*-tetradecanoylphorbol-13-acetate, urushiol ($15{:}3\Delta^{8,11,14}$), and $22{:}1\Delta^{8}$-anacardic acid (**2**), for our hormogonia-inducing assay. Neither 12-*O*-tetradecanoylphorbol-13-acetate nor urushiol (15:3) showed hormogonia induction. Although compound **2** had an HIF-like activity at lower concentration ranged from 0.1 to 10 nmol/disc (34.6 ng-3.46 µg charged on a ø 9 mm paper disc), this compound showed a lethal toxicity against the filamentous cyanobacteria at a level of 31.4 µg/disc (100 nmol/disc). Conversely, $22{:}1\Delta^{8}$-anacardic acid methyl ester (**2a**) derivatized from **2** showed no more toxicity even at 100 nmol/disc, but it maintained HIF-like activity. Instead of **2a**, esters with longer alkyl alcohol, such as *n*-hexanol (C_6) and *n*-octanol (C_8), were derivatized from **2** because they would be DG analogs. Among the $22{:}1\Delta^{8}$-anacardic acid alkyl esters (**2b**, **2c**, **2d**, and **2e** with C_6, C_8, C_{18}, and $C_{18:1}$ (Δ^{8}) alkoxy groups, respectively) showing no lethal toxicity against cyanobacteria, compounds **2b**, **2c**, and **2d** retained HIF-like activities at 100 nmol/disc. As the active anacardic acid alkyl esters structurally analogous to DG would be a fine chemical tool to investigate activated step in the intercellular signal transduction pathway for morpho- and functional differentiation of filamentous *Nostoc* cells into hormogonia, our results might suggest interaction between host plant and nitrogen-fixing cyanobacteria evolved.



1-palmitoyl-2-linoleoyl-*sn*-glycerol (**1**)



$22{:}1\Delta^{8}$-anacardic acid linoleyl ester (**2d**)

Responses of *Cnaphalocrocis medinalis* resistant varieties in rice.

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Rice leaffolder (*Cnaphalocrocis medinalis* Guenee) is one of major rice pests in Taiwan. Previous studies showed the migratory *C. medinalis* in Taiwan come from Mainland China every year. Thus, we collected *C. medinalis* throughout Taiwan to understand the genetic variation of *C. medinalis* in Taiwan. In addition, we have been selected several rice varieties which showed *C. medinalis* resistant trait in the field test. Thus, we further measured the several defensive enzyme responses (such as polyphenol oxidase (PPO), peroxidase (POD), phenyl ammonia lyase (PAL) and trypsin protease inhibitor) of *C. medinalis* resistant varieties against *C. medinalis*. Furthermore, the defensive responsive gene expression and phytohormone profiles in rice is also been examination. In addition, we measured the defensive responses of *C. medinalis* resistant varieties against *C. medinalis* exposed to elevated CO_2 and temperature conditions.

Discovering Fungal Pathogen-Responsive Terpene Synthase Genes in Australian Broadleaf Paperbark (*Melaleuca quinquenervia*)

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Increased human disturbance to forest ecosystems has exacerbated the spread of fungal pathogens to non-native environments. Rust pathogens (Pucciniales) can spread long distances by human activity and wind dispersal, and can cause severe disease outbreaks in cereal crops and in forest trees. The exotic fungus myrtle rust (*Austropuccinia psidii*) arrived in Australia in 2010. *Austropuccinia psidii*, which originated from Central and South America, is capable of infecting a broad range of species in the Myrtaceae family and so poses a serious threat to Australian ecosystems. The broadleaf paperbark (*Melaleuca quinquenervia*) is the foundation species of the east coast wetlands and appears to be variably susceptible to the rust. Finding the molecular basis of defence against rust in *M. quinquenervia* is therefore essential in facilitating resistant plant breeding for re-afforestation purposes.

Plants utilize a wide array of secondary metabolites such as terpenes to defend against herbivore and pathogen attack. We studied the chemical and molecular basis of resistance against myrtle rust in *Melaleuca quinquenervia*, with a focus on terpenes. We used GC-MS to identify constituent terpenes in resistant and susceptible plants. Subsequent transcriptome profiling of these plants showed that several terpene synthase genes (*TPS*) were strongly induced in response to myrtle rust infection. Functional characterization of these *TPS* showed them to be mono- and sesquiterpenes synthases producing compounds such as 1,8-cineole, nerolidol and viridiflorol. This study identified myrtle rust-responsive *TPS* genes in *M. quinquenervia* which may contribute to defence against the pathogen.

S4-P-043

Silencing of the gibberellin biosynthesis-related gene *OsGAS1* decreases the resistance of rice to the brown planthopper *Nilaparvata lugens*

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Gibberellins (GAs) are phytohormones that play an important role in plant growth and development. However, their roles in plant defenses in response to herbivore infestation are not fully understood. Here, we cloned a GA biosynthesis-related gene in rice (*Oryza sativa*) *OsGAS1*, which was remarkably up-regulated in response to infestation by the brown planthopper (BPH, *Nilaparvata lugens* (Stål)). Antisense expression of *OsGAS1* (as-*gas1*) resulted in a typical GA deficient phenotype. On the other hand, antisense expression of *OsGAS1* increased BPH-induced transcript levels of two defense-related mitogen-activated protein kinase (MPK) genes (*MPK3* and *MPK6*), two WRKY transcript factor genes (*WRKY30* and *WRKY53*) and an ethylene responsive factor (ERF) gene *ERF3*. Moreover, antisense expression of *OsGAS1* decreased elicited levels of ethylene and H_2O_2, but not affected JA and SA levels. Bioassays revealed that survival rates of BPH nymphs and eggs on as-*gas1* plants were significantly higher than those on wild type plants. The findings suggest that GA pathway plays an important role in resistance of rice to BPH by modulating MPK, ethylene and H_2O_2 signaling.

Mechanism of heavy-metal tolerance in shade plant *Aucuba japonica* and the effects of endophyte

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Aucuba japonica is an evergreen shrub and grows normally in forest floor of Hitachi mine, which has been contaminated with Pb, Cu, Zn and other heavy-metal elements. Recently, endophytes are reported to enhance the heavy-metal tolerance of plant hosts by producing siderophores, such as organic acid, which detoxify heavy metals by chelating. Our purpose is to clarify the mechanism of heavy-metal tolerance of *A. japonica*, considering the interaction with root endophytes.

Our study site is deciduous mixed forest in Hitachi mine and *A. japonica* trees are growing at the forest floor. In July 2016 (summer) and January 2017 (winter), we measured the heavy-metal concentrations of root-zone soil, leaves, branches, and roots with inductively coupled plasma optical emission spectrometry. Simultaneously, we isolated fungal endophytes from surface-sterilized root segments, and evaluated microbial ability to chelate heavy metals (zinc and cadmium) by the culture medium containing insoluble heavy metals. We also observed the distribution of heavy metals in roots with optical microscope by staining with diphenylthiocarbazone, which binds heavy metals, and analyzed the detoxicants (phenolics and organic acids) of heavy metals in roots by high performance liquid chromatography and gas chromatography and mass spectroscopy.

A. japonica highly accumulated zinc and cadmium in roots in summer and winter (zinc; 733.47 ± 92.67 mg/kg, cdminum; 29.78 mg ± 4.12 mg/kg in summer). Root-endophytic *Cryptosporiopsis* sp. was frequently isolated in summer and winter, and showed the highest Zn- and Cd-chelating abilities. Malic and citric acids, which are well-known to be detoxicants of heavy metals, were detected in roots. Aucubin, which is known to be a peculiar iridoid glycoside to *A. japonica,* was also detected in roots, but its chelating activity has not been clarified yet. Microscopic observation of heavy metals in roots indicates that zinc and cadmium were localized in cell walls as well as the vacuoles, in which heavy metals would be detoxified via chelation with organic acids. Furthermore, *A. japonica* might detoxify zinc and cadmium, interacting with *Cryptosporiopsis* sp., which can produce heavy-metal detoxicants. We are going to clarify the function of aucubin in heavy-metal tolerance. In the future, we are going to perform the inoculation test, to clarify the mechanism of heavy-metal tolerance and the effects of endophytes.

S4-P-045

Root endophytic *Chaetomium cupreum* enhanced tolerance of aluminum in *Miscanthus sinensis* and produced a siderophore

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Root endophytic fungi, which are miscellaneous group of root-inhibiting fungi, are known to be symbiotic fungi to plants. Root endophytes enhance plant growth via promotion of nutrient uptake and phytohormone production. Moreover, they can also improve the tolerance in plants against harmful metals via siderophores, which detoxify harmful metals by chelating. *Miscanthus sinensis* Andersson grows naturally in old mine sites, and is known as an Al tolerant plant under acidic soil conditions. Our previous research showed that the root-zone soil contained high concentration of Al and Cu, Pb, and Zn. On the other hand, *M. sinensis* contains high concentration of Al and Fe but not other heavy metals. The purpose of this study is to elucidate contribution of root endophytic fungi to the Al tolerance mechanism in *M. sinensis*, and to identify siderophores produced by root endophytic fungi.

We isolated *Chaetomium cupreum* at high frequency and their siderophores productions were high in chrome azurol S assay. *C. cupreum* was incubated in 1% malt extract liquid medium for 12 days and we confirmed that the obtained culture filtrate showed Al-chelating activity. Upon separation of siderophores in the culture filtrate, Al-chelating activity was detected in ethyl acetate layer. The ethyl acetate layer was evaporated and the precipitation was washed by small amount of cold ethanol. The obtained powder crystal showed Al-chelating activity as same as the culture filtrate. The crystal was analyzed by X-ray powder diffraction. The results were compared with already reported data, and the crystal was identified as oosporein. At present, we measure the formation constant for the complex between oosporein and Al. In inoculation tests of *C. cupreum*, we examined the growth, Al and nutrient absorption of *M. sinensis* seedlings growing in sterile study-site soil with or without root endophytic *C. cupreum*, and detected Al-detoxicants in the roots by HPLC-DAD and GC-MS. *C. cupreum* promoted *M. sinensis* seedling growth and increased Al uptake in the roots, though *C. cupreum* did not stimulate Al detoxicants production in the roots. We also observed the Al localization in the roots via lumagallion staining. *C. cupreum* compartmentalized Al into fungal mycelia surrounding the roots and it suggests that *C. cupreum* produced oosporein in their mycelia and detoxified Al. Furthermore, *C. cupreum* changed Al localization pattern into the more efficient pattern to detoxify Al, such as the epidermis, endodermis, casparian strip and stele of roots. Al accumulation in epidermis and casparian strip indicate that Al would be excluded via the cell turnover and Al transfer into the terrestrial parts, which are vulnerable to Al toxicity, would be inhibited, respectively. In conclusion, *C. cupreum* increased Al tolerance in *M. sinensis* via accumulating and detoxifying Al into their mycelia using oosporein, and via changing the Al localization pattern in the roots, which was more efficient to detoxify Al.

^{137}Cs absorption in *Pertya triloba* (Makino) Makino and the potential effect of root endophytes on ^{137}Cs desorption from soil.

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The accident of Fukushima Daiichi Nuclear Power Plant resulted in widespread contamination of the environment via the release of radionuclides. Among these radionuclides, radiocesium (^{137}Cs) is an especially serious environmental contaminant because of its long half-life (30.2 years) and the large amount of released. The released ^{137}Cs is reportedly mainly associated with clay minerals such as illite and vermiculite. Though adsorbed ^{137}C is considered to be non-bioavailable forms in the soil, several plant species could absorb ^{137}Cs from the soil. It has been known that root endophytes isolated from plants, which could accumulate ^{137}Cs, produce siderophores (Al-chelating compounds), suggesting that the siderophores from root endophytes may dissolve soil minerals to release ^{137}Cs from the soil. Our preliminary research indicates that the Japanese endemic species *Pertya triloba* accumulated high concentration of ^{137}Cs. In this study, we isolated root endophytes from *P. triloba* and examined the mechanisms of ^{137}Cs absorption by the root endophytes.

Five individuals of *P. triloba* were collected in September and October 2016 from the area (40 m×25 m) of deciduous broad-leaved forest at Fukusima prefecture, Japan. Simultaneously, root-zone soil (100 × 100 × 50 mm) was collected from each individual plant, air-dried and passed though a sieve (<2 mm). The collected plants were washed with tap water followed by deionized water, and were then separated into the shoot, the rhizome, and roots. The separated samples were dried at 80 ℃ for 48 h and ground. Concentrations of ^{137}Cs in the ground plant samples and soil were analyzed using a germanium semiconductor detector. Other elements (Al, K, etc.) concentrations in the plant samples were quantified by inductively coupled plasma optical emission spectrometry (ICP-OES) after acid-digestion of the ground plant materials. Some fresh roots collected in September 2016 were used for isolation of root endophytes, after the root-surface sterilization. The 250 root pieces (5 mm each) were put on 1% malt extract ager and incubated at 23 ℃ in the dark for 14 days.

We detected high concentrations of ^{137}Cs in *P. triloba* on each month (September; $3.40 \pm 1.65 \times 10^4$ Bq/kg dry weight, October; $2.10 \pm 0.634 \times 10^4$ Bq/kg dry weight). The concentrations of ^{137}Cs in the root-zone soil showed great differences ($1.70\text{-}16.1\times 10^4$ Bq/kg dry weight) at our study site. Pearson's correlation test revealed significant positive correlations between concentrations of ^{137}Cs in *P. triloba* and concentrations of ^{137}Cs in root-zone soils ($r = 0.799$, $P < 0.01$). Among elements analyzed by ICP-OES, high Al concentrations were detected in the roots of *P. triloba* on each month (September; 1,509 ± 194 mg/kg dry weight, October; 1,336 ± 168 mg/kg dry weight). The main fungal species isolated from *P. triloba* were *Cryptosporiopsis, Phialocephala, Acephala,* and *Colletotrichum.*

Our results suggest that high uptakes of ^{137}Cs and Al in the roots might be related with the desorption of ^{137}Cs from clay minerals by microbial siderophores, which can chelate with Al. Currently, we are under examination of the ability of those endophytes to produce siderophores, which will report in the presentation.

Insights into rice (*Oryza sativa* L.) root growth promotion mediated by β-1,3/1,6-glucans present in yeast (*Saccharomyces cerevisiae)* cell walls

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Bioactive oligosaccharides derived from plant and fungal cell wall polysaccharides mediate diverse responses in plants. For example, fungal β-glucans elicit defense responses in plants including the induced accumulation of low-molecular weight antimicrobial phytoalexins. Likewise alginate oligosaccharides present in brown seaweed cell walls can stimulate plant root growth. Given the growing societal need to further optimize crop yields and disease resistance, we are currently investing the interactions of Brewer's yeast cell walls (BCW, *Saccharomyces cerevisiae*), mainly composed of β-1,3/1,6-glucan, on rice (*Oryza sativa* L.) root growth promotion. Specifically, underlying physiological mechanisms mediated by auxin signaling and root-shoot interactions are further detailed. To demonstrate growth promotion, rice plants with fully expanded fourth leaves were transferred hydroponic solution containing BCW (50 ppm) for 7 days. Resulting root dry masses in plants from control and BCW containing hydroponic solutions were 21.6 ± 1.1 and 43.2 ± 0.8 mg, respectively. Thus, rice interactions with BCW meditated a significant 2-fold increase total root growth. To examine if key phytohormones associated with root growth underlay the response, levels of auxins (indole-3-acetic acid: IAA) and cytokinins (*trans*-zeatin: *t*-Z) were determined by LC-MS/MS analyses. It is well established that auxin and cytokinin ratios can govern the regulation of root meristems. We hypothesized that significant increases in IAA and/or significant decreases of *trans*-zeatin could contribute to BCW-mediated root growth promotion. Using a 7 day split-root experiment, the roots of individual plants were divided into two halves with one bundle submerged in control hydroponic solution and the other in BCW solution. Roots-halves treated with BCW grew longer than those in control solution despite signaling integration within each plants. We further demonstrated that higher levels of IAA were produced in the BCW-treated root halves. Surprisingly, no effects on the asymmetrical root elongation and IAA accumulation were detected in the treatment roots if leaf five was excised. Collectively, these results demonstrate physiological interactions between roots and shoots in the BCW-elicited root elongation and IAA accumulation.

Making sense of contact: the role of a single gustatory receptor in host plant identification in the specialised poplar leaf beetle (*Chrysomela populi*)

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For close range identification of plants, insect herbivores sense nonvolatile phytochemicals via direct contact with the receptive surface. Such contact chemosensation, or tasting, is an essential ability serving as final checkpoint for the acceptance or rejection of host plants. Gustatory receptors (GRs) play a central role in contact chemosensation, since they contribute to deciphering a host plant's metabolic code. Despite their importance, little is known about GRs, notably in specialised beetles (Coleoptera).

This project aims to identify those GRs that mediate specific host plant selection in the poplar leaf beetle (*Chrysomela populi*, Chrysomelidae), a specialist feeder on leaves of poplar (*Populus*, Salicaceae) being rich in phenolic glucosides. It is hypothesised that salicin and salicortin are major feeding stimulants for *C. populi*. However, it is unknown how these secondary metabolites or, alternatively, additional feeding stimulants such as primary metabolites, are detected by and interact with single or multiple GRs in *C. populi* to generate an adequate feeding response. Here we use RNA-sequencing and proteomics indicating that *C. populi* beetles possess 27 GRs. One GR candidate, the trehalose receptor CpTre1, was further investigated in detail. By using various molecular, physiological, chemical and bioassay methods, we show that CpTre1 is involved in compensatory host plant selection of *C. populi* beetles. These results show how a single GR may function as regulator for host plant identification in a specialised beetle.

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Metabolic changes in *Oryza sativa* accompanying acquisition of drought tolerance by acetic acid treatment

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When plants face abiotic stresses such as high temperature, freezing and drought, plants try to cope with the stresses by changing various gene expression. In recent years, genes responsible for the biosynthesis of acetic acid in response to drought stress was found in *Arabidopsis thaliana*. Besides, it was elucidated that rice (*Oryza sativa*) plants treated with acetic acid acquire drought tolerance. It is known that plants in drought stress accumulate gamma-aminobutyric acid (GABA) and the accumulated GABA plays the important role in drought stress acclimation. In this study, we investigated metabolic changes related to GABA induced by the acetic acid treatment in rice.

Rice seedlings (cultivar Nipponbare) grown for 2 weeks were soaked in tap water (control) or 30 mM acetic acid (treatment) for 4 days. The seedlings were given drought stress for 4 days and then were rehydrated. The survival rate of the treatment groups was higher than that of control groups. Similar drought tolerance was induced also by potassium acetate and ammonium acetate at the same concentration.

To examine changes of GABA quantity in the rice plants induced by the treatment, shoots and roots of treated seedlings were extracted with 80% methanol/water and GABA in the extracts was quantified by LC/MS. The result showed that the amount of GABA in the roots increased on the first day after the treatment but that in shoots increased only after the third day. In order to clarify how treated acetic acid was metabolized, we treated rice plants with ^{13}C-labeled acetic acid and examined ^{13}C labeled ratio of amino acids or organic acids related to tricarboxylic acid (TCA) cycle. As a result, the ^{13}C labeled ratio increased only in substances downstream of acetyl CoA. Furthermore, ^{13}C-NMR of the rice root extracts treated with ^{13}C-labeled acetic acid showed that GABA in the roots was synthesized from glutamic acid via glutamic acid decarboxylase (GAD). Collectively, these results demonstrate that treated acetic acid was absorbed into the rice plants, metabolized to GABA via GAD in rice plants.

Evolutionary changes in defensive secondary metabolism in the genus *Hordeum*

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Plants have developed defensive secondary metabolism during the course of evolution. Secondary metabolites, such as hordatines, benzoxazinones, and gramine, have been identified in the genus *Hordeum*, which includes the important cereal crop barley. *Hordeum* species are classified into four clades, H, Xu, Xa, and I. However, the correlation between phylogeny and the distribution of defensive secondary metabolites in *Hordeum* has not been clarified. In this study, we aimed to reveal the evolutionary changes in secondary metabolism in the genus *Hordeum*. The presence or absence of defensive secondary metabolites was analyzed in representative *Hordeum* species from all four clades. In addition, because *H. bulbosum* in H clade and *H. murinum* in Xu clade contained several unidentified compounds, we identified their chemical structures, and investigated their antimicrobial spectra.

In the H clade, *Hordeum vulgare* accumulated hordatines, but not benzoxazinones, while *H. bulbosum* did not accumulate either compound. Some accessions in the H clade accumulated gramine. Species in the I and Xa clades accumulated benzoxazinones, but not hordatines. In the Xu clade, neither hordatines nor benzoxazinones were detected in *H. murinum*.

H. bulbosum accumulated unidentified compounds **1** and **2**, while *H. murinum* accumulated **3** in addition to **1** and **2**. These compounds were isolated from *H. murinum* seedlings. On the basis of spectroscopic analyses, **1** and **2** were identified as dehydrodimers of feruloylagmatine and **3** was a compound composed of two feruloylagmatine units and a tyramine molecule. They are referred to as murinamides A, B, and C, respectively. Peroxidase reactions by with feruloylagmatine as a substrate resulted in the formation of murinamides A and B.

Hordatines are also dehydrodimers of hydroxycinnamic acid amides (HCAAs) of agmatine. Therefore, both the H and Xu clade species are considered to accumulate the same class of compounds. When H/Xu clades split from the I/Xa clades during evolution, the defensive metabolites shifted from benzoxazinones to dehydrodimers of agmatine HCAAs plus gramine in the H/Xu clades.

The antimicrobial spectra of hordatines, murinamides, benzoxazinones, and gramine were investigated in order to identify the functional differences among them. Dimers of HCAAs showed antifungal activity against the fungal pathogens *Bipolaris sorokiniana* and *Fusarium graminearum*. However, benzoxazinones did not show any activity against fungal pathogens, but exhibited strong inhibitory activity against the bacterial pathogen *Pseudomonas syringae* pv. *japonica* compared to that of the HCAA dimers. Gramine showed weak activity against pathogens at high concentrations. The differences in the antimicrobial spectra among the compounds might be a driving force that caused the metabolic shift from benzoxazinones to HCAA dimers in the genus *Hordeum*.

Mechanisms of gustatory receptor gene for host selection in swallowtail butterflies

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One of the keys factors to success in specialized phytophagous insects is the ability to efficiently find their host plant. Swallowtail butterflies belonging to the family of Papilionidae selectively utilize a limited number of plants from a single or a few families as their host. Female butterflies lay eggs on their host plants only if they detect specific chemicals through their foreleg chemosensilla while drumming reaction on the leaf surface.

We identified a gustatory receptor involved in the recognition of an oviposition stimulant, synephrine, from the butterfly *Papilio xuthus* using a combination of *in silico*, *in vitro* and *in vivo* approaches. The receptor, PxutGr1, specifically responded to synephrine and the sensitivities of tarsal taste sensilla to synephrine in electrophysiological approach and the oviposition behavior assay were strongly reduced by RNAi. These observations indicate that PxutGr1 represents a key factor in host specialization in *P. xuthus*.

We performe extended search other receptor genes involved in host selection by transcriptome analysis using next generation sequencer from several species of butterflies. We have elucidated the mechanism underlying the regulation of oviposition behavior by multiple oviposition stimulants using electrophysiological recordings. We are going to discuss the evolution of swallowtail butterflies that were driven by changing in the mechanism of host recognition.

Regulation of reproductive division of labor in bumble bees – a solved puzzle or an ongoing mystery?

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A hallmark of sociality is reproductive division of labor between females that specialize in reproduction vs. helping behavior. An important group of pheromones in social insects are chemical compounds that regulate reproduction in the worker caste. These pheromones are typically produced by the fertile individuals and function as queen pheromones or signals that manipulate worker reproduction against their interests, or advertise the queen fecundity, allowing workers to gain greater fitness by remaining sterile and helping the queen. Various chemical signals that correlate with reproductive status of females ("fertility signals") were identified in social groups, however, true queen pheromones that can be isolated and inhibit worker reproduction independently of the queen's presence were identified in only a handful of species.

Bumble bees provide a particularly interesting system to examine reproductive division of labor. Worker reproduction is inhibited at the presence of the queen, but also at the presence of fertile workers, and even then, for only a limited period of time. Despite extensive research in the field, there is no evidence, thus far, that queen-produced chemicals alone can significantly inhibit worker reproduction in bumble bees. Only a few queen-produced exocrine secretions have been shown to vary quantitatively in a timeframe that correlates with the initiation of worker reproduction in bumble bee colonies, and several attempts to pinpoint the glandular source or to show that these secretions act as a putative queen pheromone that reduce worker reproduction have been ambiguous.

In recent studies using caged queens in *Bombus impatiens* we demonstrated that contact with a live caged queen alone was not sufficient to inhibit worker reproduction and that active interaction of the queen with her workers is necessary. Here we further show that worker reproduction is inhibited by any egg laying queen, regardless of their mating status, but neither the queen's whole body extracts nor selected cuticular hydrocarbons, that are produced in higher quantaties by queens compared to workers, were able to inhibit reproduction in workers. Overall, we suggest that fertility signals produced by the queen in bumble bees, if exist, operate in a context dependent manner and only function in the presence of a freely behaving queen.

Identification and expression profile analysis of chemosensory protein genes in grasshopper, *Oedaleus asiaticus* (Orthoptera: Acridoidea)

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Oedaleus asiaticus B.-Bienko is one of the most dominant grasshoppers on steppes and agriculture-animal husbandry ecotones in northern China. Chemosensory proteins (CSPs) are a class of small soluble proteins in insects, and the olfactory functions of CSPs are involved in chemoreception, identification, transportation, chemical signal transduction, as well as participating in the regulation of insect development and circadian cycles. In the present study, we identified 18 CSP genes, named as OasiCSP1-18, from the antennal transcriptome of *O. asiaticus* adults using RNA-Seq. Phylogentic analysis indicated that the OasiCSPs had full-length open-reading frames (ORFs) ranging from 372 bp to 516 bp, and encoded the putative proteins of amino acids ranging from 124 to 172 with molecular weights from 13.38 KD to 19.08 KD, and their isoelectric points ranged from 5.37 to 9.56. The amino acid sequence homology was between 24.77% and 98.17%, indicating a high degree of differentiation in the OasiCSP1-18. The signal peptides were from 18 amino acids to 25 amino acids, and the multiple alignment of the amino acid sequences indicated that the OasiCSPs contained four conserved cysteine residues and shared the conserved characteristics of typical insect CSP families.

RT-PCR technology was employed to investigate the expression profiles of the OasiCSPs in the head, thorax, abdomen, wing, antenna, tarsus, genital, upper lip, lower lip of adult male and female, and labial palp and maxillary palp of adult female in *O. asiaticus.* The results showed that the OasiCSP1-6、OasiCSP9 and OasiCSP14-18 were expressed in all above tissues. Except for the lower lip, the OasiCSP7 was expressed in all other tissues. The OasiCSP8 was not expressed in the upper lip, slightly expressed in the lower lip, head , thorax and abdomen，and highly expressed in all other tissues. The CSP10 was only expressed in the antenna, ovipositor, lower lip of the female, and in the tarsus and wings of both male and female. The OasiCSP11 and OasiCSP13 were only expressed in the antenna, lower lip and tarsus， and much more in the antenna than in the tarsus and lower lip.

Expression profile by quantitative real-time PCR (qPCR) revealed that there were significant differences in the expression of different OasiCSPs in different tissues. Most of genes were more expressed in the antenna, tarsus, labial palp and maxillary palp than in other tissues, secondly in the upper lip and lower lip, and lowest in the wing and genitalia. These results provided an essential foundation for further studying the physiological functions of the OasiCSPs.

Keywords: *Oedaleus asiaticus*, chemosensory protein, tissue-specific expression

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S5-P-054

Bees eavesdrop upon informative and persistent signal compounds in alarm pheromones

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Pollinators such as bees provide a critical ecosystem service that can be impaired by information about predation. We provide the first evidence for olfactory eavesdropping and avoidance of heterospecific alarm signals, alarm pheromones, at food sources in bees. We predicted that foragers could eavesdrop upon heterospecific alarm pheromones, and would detect and avoid conspicuous individual pheromone compounds, defined by abundance and their ability to persist. We show that *Apis cerana* foragers avoid the distinctive alarm pheromones of *A. dorsata* and *A. mellifera*, species that share the same floral resources and predators. We next examined responses to individual alarm pheromone compounds. *Apis cerana* foragers avoided isopentyl acetate (IPA), which is found in all three species and is the most abundant and volatile of the tested compounds. Interestingly, *A. cerana* also avoided an odor component, gamma-octanoic lactone (GOL), which is >150-fold less volatile than IPA. Chemical analyses confirmed that GOL is only present in *A. dorsata*, not in *A. cerana*. Electroantennogram (EAG) recordings revealed that *A. cerana* antennae are 10-fold more sensitive to GOL than to other tested compounds. Thus, the eavesdropping strategy is shaped by signal conspicuousness (abundance and commonality) and signal persistence (volatility).

S5-P-055

Sex pheromone biosynthesis in the beet armyworm, *Spodoptera exigua*

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The beet armyworm, *Spodoptera exigua* uses (*Z*,*E*)-9,12-tetradecadienyl acetate and (*Z*)-9-tetradecenol as the attractive pheromone components. In the present study we investigated the biosynthesis of the sex pheromone in this species. Our *in vivo* labelling experiment showed that deuterium atoms were incorporated from labelled palmitic acid, monounsaturated (*Z*)-11-hexadecenoic acid and (*Z*)-9-tetradecenoic acid into (*Z*)-11-hexadecenoic acid, (*Z*)-9-tetradecenoic acid and (*Z*,*E*)-9,12-tetradecadienyl acid. These results proved that a Δ11 desaturation on hexadecanoic acid is involved in the biosynthetic pathway producing (*Z*)-11-hexadecenoic acid, which is then chain-shortened to (*Z*)-9-tetradecenoic acid, followed by an unusual Δ12 desaturation to produce the key precursor (*Z*,*E*)-9,12-tetradecenoic acid. These unsaturated acyl precursors are further reduced to alcohols, and then transformed to corresponding acetates. The relevant desaturase genes were functionally characterized via yeast heterologous expression system, and a pheromone gland specific desaturase, SexiDes5 was found to use palmitic acid and (*Z*)-9-tetradecenoic acid as precursors to produce (*Z*)-11-hexadecenoic acid and (*Z*,*E*)-9,12-tetradecadienoic acid, repectively. In addition, the homologous desaturase SlitDes5 from another *Spodoptera* moth, *S. litura* was found to have similar functions.

Key words. *Spodoptera exigua*; *Spodoptera litura*; pheromone biosynthesis; Δ11 desaturation; Δ12 desaturation

Morphology and physiology of antennal lobe projection neurons in the hawkmoth *Agrius convolvuli*

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The neuronal pathways involved in the processing of sex pheromone information were investigated in the hawkmoth *Agrius convolvuli*, which uses (*E,E*)-11,13-hexadecadienal (E11,E13-16:Ald) as the single sex pheromone component. The use of E11,E13-16:Ald in Sphingid species other than *A. convolvuli* has not been reported. Instead of E11,E13-16:Ald, (*E,Z*)-10,12-hexadecadienal (E10,Z12-16:Ald, bombykal) is widely used as a sex pheromone component in Sphingidae. In *A. convoluvli*, however, bombykal inhibits the attraction to the sex pheromone. The wide use of bombykal in Sphingidae and non-use in *A. convolvuli* suggest that bombykal was used as a sex pheromone component by the common ancestor of Sphingidae, and *A. convolvuli* may have evolved a novel sex pheromone communication system. Thus, studies on the sex pheromone communication system of *A. convolvuli* may provide insights into the evolution of sex pheromone communicating systems in moths.

We first clarified the anatomical organization of the antennal lobe, the primary olfactory center of the brain, of *A. convolvuli*. The male antennal lobe contains a group of enlarged glomeruli, macroglomerular complex (MGC). The MGC of *A. convolvuli* was composed of three glomeruli, cumulus, toroid, and internal glomerulus. The volume of the cumulus in *A. convolvuli* was significantly larger than that of the toroid, whereas the cumulus and toroid are similar in volume in a sphingid moth, *Manduca sexta*.

Subsequently, we investigated morphology and physiology of the projection neurons in the antennal lobe using intracellular recording technique. As a result, two groups of projection neurons, which responded to E11,E13-16:Ald and bombykal, respectively, were identified in the antennal lobe. In the projection neurons arborized in the cumulus, the response to E11,E13-16:Ald was significantly stronger than those to hexane and bombykal. In contrast, the projection neurons arborized in the toroid showed strong excitatory response to bombykal in comparison with hexane and E11,E13-16:Ald. These results indicate that, in *A. convolvuli*, cumulus processes the information of E11,E13-16:Ald, which works as a sex pheromone, and toroid processes the information of bombykal, which works as an inhibitor. In *M. sexta*, cumulus and toroid process the information of the two sex pheromone components, (*E,E,Z*)-10,12,14-hexadecatrienal and bombykal, respectively. Taken altogether, in both *A. convolvuli* and *M. sexta*, the cumulus processes the sex pheromone information albeit the pheromone compound is different. In contrast, the toroid processes the information of the same compound, bombykal, in both species, but the function is reversed (stimulatory in *M. sexta* whereas inhibitory in *A. convolvuli*).

Comparative analysis of the sex pheromone biosynthesis related genes between the *Spodoptera exigua* and *S. litura*

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Abstract

Species-specific sex pheromone is biosynthesized and released in most female moths as a chemical cue in mating communication. However, information on genes involved in this pathway is limited. The two *Spodoptera* species, *Spodoptera exigua* and *S. litura* are cosmopolitan agricultural pest that causes severe economic losses to many crops. In China, the female sex pheromones in sex pheromone glands (PGs) of *S. exigua* have been measured which comprises Z9,E12-14:OAc, Z9-14:OH, Z9-14:OAc, and Z9,E12-14:OH in a ratio of 47:18:18:17, but the sex pheromone of *S. litura* comprises Z9,E11-14:OAc, Z9,E12-14:OAc, Z9-14:OAc, and E11-14:OAc in a ratio of 100:27:20:27. However, the molecular mechanism of sex pheromones biosynthesis in the two *Spodoptera* species is unclear. By sequencing and analyzing the transcriptomic data of the PGs, we identified 59 and 55 genes related to sex pheromone biosynthesis in *S. exigua* and *S. litura*, respectively. Expression profiles revealed that 5 (*SexiDes5*, *SexiDes11*, *SexiFAR2*, SexiFAR*3*, and *SexiFAR9*) in *S. exigua* and 3 (*SlitDes5*, *SlitDes11*, and *SlitFAR3*) in *S. litura* genes had PG-specific expression and phylogenetic analysis demonstrated that they clustered with genes known to be involved in pheromone synthesis in other moth species. Our results provide crucial background information that could facilitate the elucidation of sex pheromone biosynthesis pathway and species differentiation of the two *Spodoptera* species, and also help identify potential targets for disrupting sexual communication in *S. exigua* and S. litura for developing novel environment-friendly pesticides.

Keywords *Spodoptera*; sex pheromone; transcriptome analysis; sex pheromone biosynthesis; tissue expression

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Understanding the past, planning the future: directed evolution of pheromone desaturases

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Moth sex pheromones are species-specific chemical signals responsible for mate recognition [1]. Enzymes involved in pheromone biosynthesis are able to modify saturated long chain fatty acid substrates by introducing double bonds at different locations in *E* or *Z* configurations. Many desaturases use the same substrate, but their products can have different stereochemistry, which can greatly impact species specificity. Identifying which residues confer substrate and chain length specificity, and how the desaturase enzyme introduces double bonds at various positions to create specific pheromones will help us to understand species specificity.

Several structurally important sites have previously been identified, such as the histidine rich motifs that play a key role in the catalytic activity of desaturases and the highly-conserved tyrosine residues in the substrate binding channel. We performed an alignment with homologous amino acid sequences of multiple desaturases that are known to be functionally different to pinpoint candidate sites that control substrate preference and double bond position or stereochemistry.

The multiple sequence alignment was analyzed to create a phylogeny in order to reconstruct the ancestral states and identify signatures of functional divergence along branches, which served as a guidance to design a variant library. CroΔ11 desaturase from *Choristoneura rosaceana* [2] and CpaE11 from *Choristoneura paralella* [3] used as models, and the libraries were experimentally constructed by using artificial gene (GeneArt) synthesis. With these models, the experiments focus on the understanding of the sequential differences responsible for different geometry introduction. Multiple variations are functionally tested in yeast cell assay in the presence of different substrates and the resulting fatty acid composition are analyzed by GC-MS. In addition, single site-directed mutagenesis is used to further investigate the important sites pointed out by the functional analyses of the GeneArt library.

Combining the ancestral protein state and its evolution toward different functions together with modelling of mutated versions of desaturases provides great potential to understand the enzymatic properties underlying species specific pheromone production. Moreover, this will give us a powerful tool for protein engineering to modify or improve protein function. These tailor-made desaturases may be used in cost-effective and environmentally friendly pheromone production in cell [4] and plant [5] factories and apply in integrated pest management (IPM) programs.

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S5-P-059

The story of a long range sex pheromone and how it affects the attractiveness of male burying beetles in the field

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Long range sex pheromones have been identified and studied in various groups of insects, primarily in moths, butterflies, bees and beetles. Fundamental to the research on sex attractants was the assumption that they serve solely as species recognition signals. However, the evolution of heightened condition dependence might be expected and long range sex pheromones might therefore also inform about a signaller's quality. Using a combination of chemical analyses, video observations and field experiments we provide a comprehensive study about the role of a male's long range pheromone in female mate choice by manipulating a males' condition. We show that the emission of the sex pheromone of the burying beetle, *Nicrophorus vespilloides*, has a high information content and reflects the actual nutritional state, age, body size, and parasite load – key determinants of an individual's condition. Both, the quantity as well as the ratio of the pheromone components were affected whereas the time invested in pheromone emission was largely unaffected by a male's condition. In a field study we found that this in turn affects the attractiveness of the males in the field. Males in better nutritional condition, of older age, larger body size and bearing less parasites were more attractive.

Furthermore, we analyzed the relationship between mating and parental effort in male *N. vespilloides*. Burying beetles reproduce on small vertebrate resources and upon hatching parents protect and feed their young. We found neither a positive nor a negative correlation between the intensity of the sexual signal and the amount of care provided. However, we discovered that males that were given the opportunity to breed and care for young went on to produce a higher amount of their sexual signal than control males that were not given the opportunity to breed. This in turn affects a male's attractiveness in the field; after the period of brood care males were three times as attractive for females as control males that had not raised a brood. Males might benefit from staying with their young as this enables them to feed from the carrion resource.

Our study provides good evidence that long range pheromones contain information beyond species and are reliable indicator of a male's quality. Moreover the heightened attractiveness after parental care is likely a factor that has facilitated the evolution of male assistance in parental care, as it selected males to remain on the carcass.

Production Rhythm, Isolation and Field Evaluation of Sex Pheromone from Calling Females in *Diaphanina angustalis* (Lepidoptera: Crambidae), A Medicinal Plant Moth

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Insect sex pheromones appear to play a crucial role in the mate finding and calling behavior of Lepidoptera pests. Currently, little was known about the chemical ecology of *Diaphanina angustalis* Snellen (Lepidoptera: Crambidae), a severe and important defoliator attacking the medical plant, *Alstonia scholaris*. In the present study, the pheromone components of *D. angustalis* females by males were investigated using electrophysiological and behavioral methods. The distilled hexane extracts of female pheromone glands were analyzed through electroantennogram (EAG) and gas chromatography-electroantennogram detector (GC-EAD) and the active compounds were identified through gas chromatography-mass spectrometry (GC-MS). Production peak of female sex pheromone was mainly 3-d-old at 5 h into the scotophase with the EAG test and the hexane extracts were attractive to males in the wind tunnel test. GC-EAD analysis of virgin males to gland extracts was subsequently evaluated showed two active compounds, (E,E)-10,12-hexadecadienal (E10E12-16:Ald) and (E,E)-10,12-hexadecadien-1-ol (E10E12-16:OH), based on the comparison the retention time and mass spectrum, with suitable synthetic compounds. Under laboratory conditions, the blend of E10E12-16:Ald and E10E12-16:OH in a ratio of 9:1 could elicit a stronger EAG response than other treatments or single component. In the field, more male moths were captured baited with the binary of E10E12-16:Ald and E10E12-16:OH in a ratio of 9:1, whereas, a binary of 8:1 and 10:1 also caught males. Accordingly, E10E12-16:Ald and E10E12-16:OH were regarded as the major sex pheromone components in the *D. angustalis* females.

Identification of female sex pheromones of rare moth species: perspective for conservation biology

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Assessing the conservation status of rare/threatened insect species is important for keeping species diversity. Pheromone-based monitoring is a useful method especially moth species which possesses a long-range communication system. We are interested in the studies of rare/threatened moth species and carring out now their potential application for assessments of biodiversity, conservation of the species in population declines, and environmental protection.

Prismosticta hyalinata (Bombycoidea: Prismostictidae) has been thought to be a rare species because female and male adults of this species are not phototactic. By a happy coincidence, we could collect a pair of newly mated adults, and the female oviposited fertilized eggs onto cuttings of *Symplocos sawafutagi* (Ericales: Symplocaceae, formally *S. chinensis*) in laboratory conditions. We successfully bred them using fresh leaves of *S. coreana*, and could perform the observations of circadian periodicity. In laboratory observation, adults flew only during the daytime and showed mating behavior mainly in the afternoon. GC-EAD analysis of the crude extract of female glands indicated only one EAG-active component, and GC/MS analysis assigned (*E*,*E*)-bombykal to a chemical structure of the pheromone candidate.

Rubber septa impregnated with the synthetic (*E*,*E*)-bombykal and/or related compounds were prepared as pheromone lures. Sticky traps baited with the lures were placed on 1.5 m above at coppices in Tottori Prefecture, Western region of Honshu, Japan. Surprisingly, many males flew around the traps baited with (*E*,*E*)-bombykal just after hunging on a twing in the afternoon. A large number of males could be captured specifically by (*E*,*E*)-bombykal suggesting that population level of *P. hyalinata* is not low.

The field test revealed three novel findings. First, *P. hyalinata* is a common species and may be distributed in various environments where *Symplocos* trees are distributed. Second, the adults are diurnal but their catching using a butterfly net seems to be difficult because of their unexpected quick flight. Third, this species is multivoltine; the males were captured by traps in late May, June, and early September in Tottori Prefecture.

Furthermore, we are conducting to identify female sex pheromones of some so-called rare moth species; e.g., a lappet moth *Pyrosis idiota* and a winter geometrid moth *Sebastosema bubonarium*. Various biological aspects of this species, e.g., distribution, habitat, rarity/risk of extinction, daily rhythms and seasonal prevalence, will be understand.

S5-P-064

Identification of inhibitory compounds against sex pheromone communication of light brown apple moth, *Epiphyas postvittana*

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Aliphatic compounds inhibiting male attraction of the female sex pheromone of light brown apple moth, *Epiphyas postvittana* (Lepidoptera: Tortricidae), were identified through electroantennogram (EAG) and field trapping tests. In EAG screening with 46 compounds that were structurally related to the female sex pheromone components of *E. postvittana*, several compounds exhibited significant male-specific EAG responses, in which 14-carbon acetates displayed stronger EAG activities than 12 or 16 carbon acetates. The EAG-active compounds were then evaluated in the fields for their behavioral activities to the male attraction of the binary blend (*E*11-14:Ac + *E*9*E*11-14:Ac) of sex pheromone of *E. postvittana*. In a series of field trapping experiments, each of nine compounds (E9-12:Ac, Z9-12:Ac, E5-14:Ac E9-14:Ac, E10-14:Ac, E12-14:Ac, Z12-14:Ac, Z9E11-14:Ac and Z9E12-14:Ac) displayed strong inhibition against male attraction of sex pheromone, indicating that these compounds interrupt the pheromone communication of *E. postvittana*. .

Key words: antagonist, electroantennogram, field trapping, pheromone attraction, sex pheromone

Female sex pheromone of Athetis lepigone (Lepidoptera: Noctuidae): identification and field evaluation

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Athetis lepigone has been recorded in many countries in Europe and Asia, but it had never been documented as an agricultural pest until 2005. For the purpose of using the sex pheromone to control this pest, we conducted a study to identify the sex pheromone of *A. lepigone* by gas chromatography with an electroantennographic detector (GC-EAD) and GC coupled with mass spectrometry (GC/MS) analyses. Three pheromone candidates were detected by GC-EAD analysis in the extracts of the female sex pheromone gland, and two candidates were identified as (Z)-7-dodecenyl acetate (Z7-12:OAc) and (*Z*)- 9-tetradecenyl acetate (Z9-14:OAc) in a ratio of 1:5 by mass spectral analysis of natural pheromone components and dimethyl disulfide adducts. In the field male trapping test, the traps baited with the binary blend captured high number of males, while traps with single component hardly caught males, indicating that the two components are essential for the male attractiveness. In addition, the optimum ratios of Z7-12:OAc and Z9-14:OAc were determined as 3:7-7:3, and the best doses for the binary blend (at ratio of 3:7 between Z7-12:OAc and Z9-14:OAc) were 0.25-0.5 mg/trap, based on the number of male catches. The identification of a highly attractive sex pheromone will help in developing efficient strategies for monitoring and control of *A. lepigone*.

S5-P-066

Pheromone components biosynthesized in tarsi of Heliothine moths

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The Noctuidae is one of the most specious moth families and contains the genera *Helicoverpa* and *Heliothis*. Their major sex pheromone component is (*Z*)-11-hexadecenal except for *Helicoverpa assulta* and *Helicoverpa gelotopoeon* both of which utilize (*Z*)-9-hexadecenal. The minor components of heliothine sex pheromones vary with species but hexadecanal has been found in the pheromone gland of almost all heliothine females so far investigated.

In this study we found a large amount (μg levels) of hexadecanal (16:Ald) and octadecanal (18:Ald) using by GC-MS in the male legs of four heliothine species, *Helicoverpa zea*, *Helicoverpa armigera*, *H. assulta*, and *Heliothis virescens*. The hexadecanal was found on and released from the tarsi, and was in much lower levels or not detected in the other leg parts including tibia, femur, trochanter and coxa. A relatively small amount of hexadecanal was found on female tarsi. This is the first time that a known sex pheromone component was identified from the legs of nocturnal moths. Decapitation did not reduce the levels of hexadecanal on the tarsi of *H. zea* males, indicating that hexadecanal production is not under the same neuroendocrine regulation system as the production of female sex pheromone.

The biological role of aldehydes (16:Ald and 18:Ald) produced from male's tarsi, especially a large amount (μg level), remains unknown. We propose that some or all of these compounds function as short-range male attractants in conjunction with the hair-pencil bouquet to facilitate courtship and female acceptance.

Unsaturated cuticle hydrocarbons contributed to sex pheromone attraction of the eggplant fruit borer, *Leucinodes orbonalis* Gueneé (Lepidoptera: Crambidae) inhabiting the Mekong delta of Vietnam

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Leucinodes orbonalis is one of the most serious pest insects of the eggplant in the Mekong delta of Vietnam. While (*E*)-11-hexadecenyl acetate (E11-16:OAc) and its alcohol (E11-16:OH, a minor component) have been identified as the sex pheromone, the activity of male attraction is weak in the Vietnamese fields. In order to utilize its pheromone as a monitoring and mass trapping tools for sustainable pest management programs, development of a lure comparable to the virgin female is necessary. We focused on extra pheromone components in another chemical group such as unsaturated hydrocarbons and conducted instrumental analyses of some extracts prepared newly from the *L. orbonolis* females. Then, roles of the identified hydrocarbons were investigated by field evaluation of the corresponding synthetic compounds.

GC-EAD and GC-MS analyses of the abdominal tip extracts revealed a trace of (3*Z*,6*Z*,9*Z*)-3,6,9-tricosatriene (Z3,Z6,Z9-23:H) as an EAG-active component in addition to E11-16:OAc. The ratio of E11-16:OAc and Z3,Z6,Z9-23:H is about 100:2. On the other hand, extracts of the whole body with a pheromone gland showed an abundant of Z3,Z6,Z9-23:H and one more component, (3*Z*,6*Z*,9*Z*)-3,6,9-docosatriene (Z3,Z6,Z9-22:H). The ratio of E11-16:OAc, Z3,Z6,Z9-22:H and Z3,Z6,Z9-23:H is about 100:1:45. Quantitative analysis showed that amounts of E11-16:OAc and Z3,Z6,Z9-23:H were ca. 33.7 and 18.5 ng/female, respectively. Z3,Z6,Z9-23:H was also detected in the wing extract. These results indicate that the two EAG-active trienes are mainly released from the body surface, not from the pheromone gland.

In the fields, traps baited with E11-16:OAc alone could captured significantly more males than those of control. However, its activity was weaker than the virgin female and addition of E11-16:OH to the lure as a minor component did not improve the attraction. The addition of E11-16:OH at a higher ratio than 10%, the attraction by E11-16:OAc was inhibited. On the contrary, addition of Z3,Z6,Z9-22:H or Z3,Z6,Z9-23:H significantly increased the number of captured males, confirming that these C_{22} and C_{23} trienes effectively contributed as the sex pheromone components to attract the males synergistically. Interestingly, in the field test of some other synthetic unsaturated hydrocarbons, unnatural C_{23} (3*Z*,6*Z*,9*Z*,12*Z*,15*Z*)-pentaene also showed a good synergistic effect, but C_{21} and C_{25} trienes and C_{25} pentaene didn't. The male moths seem to recognize more strictly chain length of the hydrocarbons than unsaturated degrees.

Keywords. *Leucinodes orbonalis*, Crambidae, sex pheromone, (*E*)-11-tetracenyl acetate, (3Z,6Z,9Z)-3,6,9-tricosatriene, type II pheromone, cuticle hydrocarbon.

Application of the sex pheromone and plant volatiles for mass trapping of the diamond back moth, *Plutella xylostella* L. (Lepidoptera: Plutellidae), in the Southern Vietnam

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The diamond back moth, *Plutella xylostella,* is one of the most serious pest insects of cabbage in the Southern Vietnam. Since effective control of *P. xylostella* by spraying insecticides is difficult due to its high resistance against the chemicals, mass trapping by its sex pheromone and other volatile compounds plant volatiles is expected to be an alternative effective method. As a first step, we examined effective synthetic lures for attraction of the moth. The pheromone lure composed of three components (Z11-16:Ald, Z11-16:OAc, and Z11-16:OH) at a ratio of 5:5:1 could attracted *P. xylostella* males as strong as the virgin female did in our field tests, while one- or two-component lures attracted significantly lower numbers of the males than those by the three-component lure. Another test of the three-component lures at some different doses from 0.01 to 1.0 mg/lure showed that 0.01 mg/lure was an optimal dose for attraction of the males (averagely 158 males/trap/week). On the other hand, lures prepared with (*Z*)-3-hexenyl acetate (0.01–1.0 mg/lure), allyl isothiocyanate (AITC, 0.01–1.0 mg/lure), or a brassica leaf grinded solution (1.0 ml) showed high attraction of the moths of both sexes. Interestingly, the males and females were not equally attracted and the ratio of the captured females was 5–10%. Furthermore, our field examination confirmed that AITC caused the number of captured moths to increase when it was added to the pheromone lure. Next, based on these results, a mass trapping experiment was conducted. The traps, which were bated with a lure composed of the sex pheromone and AITC and placed at a density of 120 traps/ha, showed the same effectiveness as application of conventional insecticides (5-time sprays) for the control of *P. xylostella* in a cabbage field.

Keywords. *Plutella xylostella*, sex pheromone, (*E*)-11-tetracenal, plant volatile compounds

Early-exposure to new sex pheromone blend alters mate preferences of female butterflies and those of their female offspring

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Insects communicate their identity to members of the same species using unique combinations and ratios of sex pheromones and correspondent antennal receptor systems. Deviations from the usual blend are known to make the emitter less attractive, so it is currently not well understood how populations start diverging in their odor perception systems. We hypothesized that insects develop preferences for new sex pheromone blends via the process of learning. In addition, we propose that these learned preferences may be transmitted to the next generation via epigenetic mechanisms. We tested these hypotheses by exposing newly emerged naïve butterfly females to modified (unattractive) male sex pheromone blends and by monitoring their subsequent mate choices. We also recorded naïve mate choice of female offspring of exposed mothers to test for the inheritance of learned preferences. Naïve females mated preferentially with wild type blend males, while pre-exposed females shifted their preference to the modified blend males; mating equally with both male types. Naïve daughters of females exposed to new blend showed similar mating patterns as their mothers. Our findings demonstrate that females are able to change their preference to a less attractive blend in response to a short social experience. This suggests that learning can be a key factor in the evolution of sex pheromone blend recognition, ultimately impacting chemosensory speciation.

Evolution of Resistance to Mating Disruption in the Pink Bollworm Moth

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The pink bollworm, *Pectinophora gossypiella,* (Lepidoptera: Gelechiidae) is a key pest of cotton worldwide. In Israel mating disruption (MD), using synthetic sex pheromone in all cotton fields resulted in a significant reduction in the use of toxic chemical applications. However, in recent years repeated outbreaks of pink bollworm populations have been reported in various cotton fields over Israel. We hypothesized that after a long-term of selective pressure from mating disruption, a change in the population's sex pheromone characteristics and/or changes in the males' abilities to detect females have occurred.

We will present evidence for developed resistance to the mating disruption method. Pink bollworms were collected from a cotton field in Israel where the mating disruption method had failed and reared in the laboratory. Sex pheromone ratios and amounts produced were determined from the resistant population and compared to a standard lab population. The amount of pheromone was not statistically different between the two populations but the ratio of major components was different. The resistant population had a higher ratio of the ZZ isomer (Z7,Z11-16:OAc) compared to the laboratory population. Pheromone glands from the two populations were extracted and mRNA purified and sequenced to obtain a transcriptomic profile. Sequence differences in some of the enzymes involved in the biosynthetic pathway will be presented. These results suggest that the enzymatic differences might result in an altered pheromone ratio. Behavioral results using an olfactometer and large cages tests suggest that although males are equally attracted to females of both populations, under pheromone saturated environment males are more attracted to females that were constantly exposed to pheromone and apparently have a slightly different ratio of the major components. Changes in the biosynthesis pathway of pheromone in the two populations and further population tests will reveal whether this change is due to individual variance in natural populations, a mutation, or plasticity in the pathway of pheromone production.

S5-P-071

Targeted mutagenesis of an odorant receptor co-receptor using TALEN in *Ostrinia furnacalis*

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Genome editing tools such as TALEN or CRISPR has been applied for various model organisms but not yet for agricultural pest insects. In this study, TALEN-mediated mutagenesis of the gene encoding odorant receptor co-receptor (Orco) of an important agricultural pest *Ostrinia furnacalis* (*OfurORco*) was carried out. Of the two pairs of TALEN constructs designed for *OfurORco*, one generated high somatic and germline mutation rates. Physiological and behavioral analyses using a gas chromatograph–electroantennographic detector system and a wind tunnel, respectively, revealed that antennal responses to sex pheromone components were decreased to trace levels, and behavioral responses were abolished in *OfurOrco* mutants. This study demonstrated that TALEN-mediated mutagenesis is applicable to pest insects, and these results will open the way for a better understanding of chemosensory systems in wild insects.

S5-P-072

Functional characterization of olfactory receptors responding to plant volatiles in the Oriental fruit fly, *Bactrocera dorsalis*

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The Oriental fruit fly, *Bactrocera dorsalis*, is a highly destructive global fruit pest in both tropical and temperature regions. Life cycle of this species is implicated with several major semiochemicals. One such example is the strong attraction of male flies to a specific phenylpropanoid, methyl eugenol, leading to voracious feeding of this compound so that male flies utilize it as a sex pheromone precursor. In addition, volatiles derived from host fruits are also known to influence the selection of suitable oviposition sites in females. Although chemoreception of plant semiochemicals is important for *B. dorsalis*, chemosensory mechanisms have not been well elucidated. To characterize chemosensory receptors required for chemoreception of semiochemicals, we conducted RNA sequencing analysis of chemosensory organs of *B. dorsalis* to identify genes coding for chemosensory receptors. From the transcriptome of male antennae and proboscises, 45 candidate olfactory receptors (ORs) including ORCO, 7 gustatory receptors and 10 ionotropic receptors were identified. We focused on ORs highly expressed in the antennae for functional analyses. To identify ligands for ORs, each of the eleven receptors were co-expressed with the obligatory co-receptor ORCO in *Xenopus* oocytes. We tested *ca*. 30 compounds including attractants for several *Bactrocera* species and volatiles in host fruits of *B. dorsalis*. Of them, we found responses of two ORs, BdorOR1 and BdorOR2, to several compounds of the ligands tested. BdorOR1 co-expressed with ORCO robustly responded to 1-octen-3-ol, but weakly responded to 1-pentyl acetate, 1-hexyl acetate and (*E*)-2-hexenal. BdorOR2 co-expressed with ORCO robustly responded to geranyl acetate, and also significantly responded to linalyl acetate and farnesene (a mixture of isomers). Thereafter, the attractiveness of 1-octen-3-ol, geranyl acetate, linalyl acetate and farnesene to *B. dorsalis* was further examined in caged assays. When filter papers treated with either one of the aforementioned compounds were exposed to flies, almost none of them were attracted to these compounds. However, when green colored balls were placed on those filter papers treated with ethanol as a control in the cage, several mated male and female flies were attracted to the balls, probably due to their attractive shape and color. Furthermore, when those green balls placed on filter papers treated with either 1-octen-3-ol, geranyl acetate, linalyl acetate and farnesene were exposed to those flies, significantly higher number of gravid females landed on those balls compared to control. These results suggest that female flies detect plant volatiles via highly expressed ORs, but require visual cue in exhibiting attraction.

CRISPR/Cas9 and its use in functional study of PBP genes in three moth species

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The pheromone-binding proteins (PBPs) are thought to play important roles in perception of sex pheromones in moths, by binding and transporting hydrophobic pheromone molecules across the aqueous sensilla lymph to the receptor proteins. The fact that multiple PBP genes in a single species strongly suggests functional differentiation among PBP genes, in terms of pheromone component preference and relative importance in the perception. However, this has not been verified, mostly due to the lack of proper in vivo technique for lepidopteran species. The newly developed CRISPR/Cas9 system, derived from a bacterial adaptive immune system, has been successfully used for genome editing in lepidopteran insects, allowing us to study the functional differentiation among PBPs.

To explore the functional differentiaiton among moth PBPs, we carried out experiments on the genome editing of PBP genes using CRISPR/Cas9 system, screening of homozygous mutants, and electrophysiological and behavioral assays in three important moth pests of *Spodoptera litura, Chilo suppressalis and Helicoverpa armigera*. The results showed that 1） high chimera mutation rates were induced in injected eggs, and the mutations were transmitted to insects of next generation; 2) moths with homozygous mutant PBP1-3 in *S. litura*, PBP1 and 3 in *C. suppressalis* and PBP1 in *H. armigera* were obtained respectively in three generations of screening; and 3) male moths with mutant PBPs resulted in highly impaired electrophysiological or/and behavioral response to sex pheromone components. Based on these reslutes, we concluded that multiple PBPs in a species differentiated in pheromone component selectivity and relative importance in the pheromone perception.

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Male moths prefer females of higher quality using their airborne sex pheromone as signal

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Females of the pink bollworm moth, *Pectinophora gossypiella* signal for mate searching males using a sex pheromone, as do most moths. *Pectinophora gossypiella* 3 day old females, reared in laboratory, were selected based on their size at the pupa stage (large vs. small), age as adults (3 day old vs. 7 day old) and fed and starved adults (sucrose solution vs. water). We also tested the males' preference of females of their own population vs. females of a different population. Using a y-tube olfactometer behavioral assay we tested the virgin males' preference of females by means of their pheromone signaling. We found that males were able to discriminate between females from different treatments, preferring females of better quality: large over small females, young over old, but did not prefer fed over starved females. Interestingly, males preferred females of their own populations over females of a different population. Pheromone glands were extracted from females of the different treatments and the pheromones quantified using a GC/MS. These results will be discussed in the context of how males are able to discriminate between females in the behavioral assay.

A male pheromone that predicts dominance in the bronze bug, *Thaumastocoris peregrinus*, basic and applied prospects

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Forest plantations in Uruguay cover about one million hectares, 75% of them planted with *Eucalyptus* species. The bronze bug, *Thaumastocoris peregrinus*, is a relevant pest in this crop, with unknown potential damage and no defined control measures. Semiochemicals are promisory tools for the management of *T. peregrinus* through attractants and/or repellents that act as natural signals or cues for this insect. We have previously described a male-emitted pheromone, 3-methyl-2-butenyl butyrate, that attracts males, both juveniles and adults, but not females. This compound, with yet undefined ecological function, is emitted with a diel pattern, which appears to be suppressed by the presence of females. Since this species occurs in aggregations, our current hypothesis is that this compound mediates interactions among males, possibly by determining dominance status. Using behavioral studies and GC-MS analysis of individual surface extracts, we here show that 3-methyl-2-butenyl butyrate is present in higher amounts in males that perform as dominant in behavioral interactions among two virgin males and a virgin female. Hence, the compound appears to either determine male-male competition outcomes, or correlate with other characters involved in such competition. We also show that the amount of 3-methyl-2-butenyl butyrate does not correlate with body weight, hence it is not a byproduct of male size, and that males in isolation produce similar amounts than dominant males, suggesting that submissive males decrease this potential signal for male dominant status. Finally, we have found that the presence of synthetic 3-methyl-2-butenyl butyrate in cages with males and females slightly but significantly decrease the number of eggs laid by the females, suggesting that the compound may have potential as a mating disruptant for the bronze bug in eucalypt crops.

S5-P-076

Identification of sex and aggregation pheromone-receptive sensilla in nymphal cockroaches.

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Pheromones are chemical agents that trigger specific behavioral responses in conspecific members and thus are important for mating and social behaviors in insects. Recently, periplanolide-1 (PLD-1) was identified as an aggregation pheromone of the cockroach *Periplaneta americana*. The molecule was purified as a main component of attractans extracted from feces of *P. americana* and had strong attractive effects to nymphal cockroaches(Y.Nishimura et al., (2015) WO2015/098681A1). On the other hand, sex pheromone, periplanone-B (PB), had already identified from the cockroach. PB secreted from female adult cockroach triggers mating behavior in male adult cockroaches. Previous electroantennogram have been reported that nymphal cockroach received both types of pheromones. However, it is still unclear peripheral pheromone processing in nymphal cockroaches.

In this study, SEM observation of male and female antennae in 4th instar nymphs revealed that the *single-walled* A (*sw*-A) sensilla could be classified into two sub-types based on differences of olfactory pores; slit-like aperture type (*sw*-A1) and circular aperture type (*sw*-A2). Next, we performed single sensillum recordings from *sw*-A1 and *sw*-A2 and successfully recorded impulse responses to PLD-1 and PB. These experiments showed that PLD-1 receptive neurons were selectively identified in *sw*-A1 sensilla, whereas PB receptive neurons in *sw*-A2 sensilla. Furthermore, we examined responses of the both types of sensilla to other general and pheromonal odor molecules, and obtained the response spectra of these sensilla types. We will report morphological and physiological features of peripheral sensory systems in the nymphal cockroach.

S5-P-077

Population fluctuation of Oriental fruit fly *Bactrocera dorsalis* Hendel influenced by abiotic factors in Yezin, Myanmar

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Oriental fruit fly is a very wide spread and important pest of mango, citrus, guava and cashew in Myanmar, (Morris and Waterhouse 1998). *Bactrocera dorsalis* species complex can be found in Myanmar as an economic pest (Drew and Hancock 1994). The abundance of larval hosts is one of the major factors regulating fruit fly populations (Kapatos and Fletcher 1984). Climate, particularly temperature and rainfall, is the main factor influencing the distribution of the fly. Local infestation patterns of *B. dorsalis* reportedly reflect local temperatures (Chen and Ye 2006). *Bactrocera dorsalis* completes more than five generations per year in most tropical regions (Shi et al. 2005). It is very difficult to control the pest simply through the application of chemical pesticides due to their peculiar biological features. The objectives of this study were to monitor the population fluctuation patterns of *Bactrocera dorsalis*, and to determine the major factors affecting the population incidence in Yezin area. Population fluctuation of oriental fruit fly, *Bactrocera dorsalis* (Hendel) (Diptera: Tephritidae), were monitored by using methyl eugenol traps during January, 2012 to December, 2012 and May, 2016 to December, 2016 in mango orchard farms of Department of Agricultural Research in Yezin, Myanmar. Traps were handmade and constructed based on steiner trap. Three steiner traps, more than 50 m apart, were placed on fruit trees at a height of 2 m above the ground at each site. The lure consisted of a small cotton ball soaked with 2 ml of methyl eugenol, which was replaced at one week interval during the study year. Male flies were attracted by the lures when they touched the cotton ball and were identified and counted at one week interval starting from January, 2011 to December, 2011 and May, 2016 to December, 2016. The meteorological data were recorded from Yezin meteorological station in Department of Agricultural Research. In 2012, the highest mean number of male oriental fruit flies/trap/day (108.24 ± 3.65) was recorded in June and the lowest (2.95 ± 0.20) in December. The highest mean number of male oriental fruit flies/trap/day (388.95 ± 8.56) was recorded in the month of June and the lowest (2.33 ± 0.20) in December, 2016. Population data were analyzed with meteorological data including temperature, duration of sunshine, and rainfall. Population fluctuation of male fruit flies were positively correlated with temperature and rainfall, and negatively correlated with the duration of sunshine.

Key words: Oriental fruit fly, *Bactrocera dorsalis*, population fluctuation, abiotic factor, mango orchard

S5-P-078

Mechanosensory and chemosensory cues mediate the sex pheromone-induced upwind orientation behavior in walking Indian meal moth

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In most moth species including Indian meal moth *Plodia interpunctella*, males flying in wind track a plume of sex pheromone to locate conspecific females. They steer themselves upwind via optomotor anemotaxis by visually detecting wind-induced drift. However, the fact that pheromone-stimulated moths on the ground also orient upwind while walking suggests that they possess sensory mechanisms to detect wind direction other than the visual cue.

To investigate sensory mechanisms underlying the upwind orientation of walking moths, we analysed the free- and tethered-walking Indian meal moth on a locomotion compensator and a trackball system, respectively. Males in the darkness readily oriented upwind as well as under the illumination of visible light, suggesting that their anemotactic ability is independent of visual cues. However, moths' orientation direction was completely randomised when basal segments of both antennae are immobilized with an adhesive to impair mechanosensory input. Then, either one of the antennae was amputated to see the involvement of chemotactic mechanisms. When these males were allowed to walk freely, their overall paths were still directed upwind, though slightly biased toward the side of the intact antenna. However, tethered-walking males kept turning toward the intact side during the stimulation of pheromone-laden airflow, indicating the existence of the chemotactic mechanisms.

We conclude that upwind anemotaxis in walking Indian meal moths is mediated by detecting wind direction with their antennal mechanosensory organs. In addition, the chemotaxis mechanism is suggested to coexist, though it may be overriden by anemotaxis in natural condition.

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Identification of olfactory-related genes in the brownwinged green bug, *Plausia stali*

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The brownwinged green bug, *Plausia stali*, is a fruit-spotting stinkbug that often causes extensive damage to various fruit trees. The feeding damage becomes serious by gathering of enormous number of adults, which are attracted by male-produced aggregation pheromone, methyl (*E, E, Z*)-2,3,6-decatrienoate.

A total of 37,385,798 RNA sequences of male and female adult antennae, we identified a conserved olfactory receptor co receptor (ORCO) gene, 140 olfactory receptor (OR)-like genes, 18 odorant binding protein (OBP) genes, 5 chemosensory protein (CSP) genes, and 5 Takeout (TO) genes.

The OBP genes were classified 14 classic type OBPs and 4 plus-C type OBPs. qRT-PCR amayses revealed that only 4 of the classic type OBPs and 1 plus-C type OBP were expressed in the antennae specifically. The other OBP genes were expressed in not only antenna but also the other organs including stylet, head, legs, wings, or whole body without those appendages. Expressions of the total of 5 antenna-specific OBPs were detected in 4th- and 5th-instar larval antennae as well as adult antennae both male and female. Although expression level of these OBPs was varied in sex, individuals, and age, most of the OBPs were expressed higher in adults than larvae.

S5-P-080

Functional characterization of a Niemann–Pick type C2 protein in the parasitoid wasp *Microplitis mediator*

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Niemann–Pick type C2 (NPC2) is a type of small soluble protein involved in lipid metabolism and triglyceride accumulation in vertebrates and arthropods. Recent studies have determined that NPC2 also participates in chemical communication of arthropods. In this work, two novel NPC2 proteins (MmedNPC2a and MmedNPC2b) in *Microplitis mediator* were identified. Real-time quantitative PCR (qPCR) analysis revealed that *MmedNPC2a* was expressed more higher in the antennae than in other tissues of adult wasps, compared with *MmedNPC2b*. Subsequent immunolocalization results demonstrated that NPC2a was located in the lymph cavities of sensilla placodea. To further explore the binding characterization of recombinant MmedNPC2a to 54 candidate odor molecules, a fluorescence binding assay was performed. It was found MmedNPC2a couldn't bind with the selected fatty acid such as linoleic acid, palmitic acid, stearic acid and octadecenoic acid. However, seven cotton volatiles, 4-ethylbenzaldehyde, 3,4-dimethylbenzaldehyde, β-ionone, linalool, m-xylene, benzaldehyde, and *trans*-2-hexen-1-al showed certain binding abilities with MmedNPC2a. Moreover, the predicted 3D model of MmedNPC2a was composed of seven β-sheets and three pairs of disulfide bridges. In this model, the key binding residues for oleic acid in CjapNPC2 of *Camponotus japonicus*, Lue68, Lys69, Lys70, Phe97, Thr103, and Phe127, are replaced with Phe85, Ser86, His87, Leu113, Tyr119, and Ile143 in MmedNPC2a, respectively. We proposed that MmedNPC2a in *M. mediator* may play roles in perception of plant volatiles.

Chemical ecology and pheromone evolution in *Leptopilina,* a parasitoid of *Drosophila*

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The origin and evolution of insect pheromones is a central topic in chemical ecology. The so-called precursor hypothesis predicts that any compound already produced by a species can evolve to adopt communicative functions. Recently it was shown, that females of the parasitoid wasp *Leptopilina heterotoma* (Hymenoptera: Figitidae) produce a defensive secretion consisting mainly of (-)-iridomyrmecin, which has evolved secondary functions as a chemical cue to avoid competition between females on host patches and as a major component of the female sex pheromone.

To better understand the mechanisms of pheromone evolution in the genus *Leptopilina* we are investigating the chemical ecology of up to 12 *Leptopilina* species. All nine species of *Leptopilina* investigated so far produce iridomyrmecin and other iridoid compounds. Our data show little variation in the use of the iridoid compounds for defence against predators and as chemical cue to avoid competition on host patches. However, we find large differences between species in the composition of the female sex pheromone and the response of males towards the iridoid compounds. *Leptopilina heterotoma* is the only species whose female sex pheromone consists of iridoid compounds. The sex pheromone of the other species consist either of a mixture of iridoid compounds and cuticular hydrocarbons (CHCs) or of CHCs only. The phylogenetic relationship of the species correlates with the production of (-)-iridomyrmecin and its enantiomer (+)-iridomyrmecin, but the phylogeny fails to explain the composition of the female sex pheromone. Alternatively, differences between species in the response of the males towards the iridoid compounds while searching for females might explain the strong divergence in the composition of the sex pheromone in the genus. We find support for this hypothesis in *L. clavipes,* whose female sex pheromone consists of cuticular hydrocarbons, which elicit courtship in males. However, males of *L. clavipes* did not react to female CHCs if presented in combination with the defensive secretion. This is in contrast to other species of *Leptopilina*, in which the defensive secretion has no inhibiting or even a stimulating effect on the males' courtship behaviour. On one hand, males benefit from being attracted towards the iridoid compounds by increasing the possibility of finding a female. Iridoid compounds are more volatile than most CHCs produced by insects and therefore the male would be able to locate a female from a greater distance using iridoid compounds compared to CHCs. On the other hand, males attracted by the defensive secretion experience a higher risk of predation, as the predator triggering the release of the defensive secretion might still be present. The ratio of these costs and benefits determines the selective pressure on the males and consequently on the route in the evolution of the female sex pheromone.

S5-P-082

Pheromone Diversity in Heliothine Moths

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Insects, and particularly moths, rely heavily on sex pheromones as a means of odor-based communication to draw together opposite sexes. To date, thousands of pheromones have been identified, with each species having its own unique blend of chemicals, which will attract a mate. There is incredible diversity in pheromone composition, but limited understanding of the evolution and shifts in production, detection and preference of these chemicals as new species evolve and diverge from one another. In other words, "why are there so many unique pheromone blends?" We are investigating the mechanisms by which pheromone composition and preferences shift during, or drive speciation. Understanding such shifts are paramount to our understanding of the olfactory system, and moreover, to adapting insect control strategies which use pheromones to monitor and control pests.

Heliothine moths represent an excellent model system for examining divergence of pheromone production, and mechanisms of detection and processing in closely related species. Divergence in olfactory communication is evident among heliothine species, based on shifts in the use of key components within each species' sex pheromone blend -blends that function to both attract members of the same species, and inhibit mating errors between closely related species. This talk will synthesize studies on pheromone composition, receptor identification and neuronal physiology, and behavioral blend preference in the lab and field. Through a better understanding pheromone evolution, build significantly upon the current knowledge of the evolution of pheromone-based species isolation, basic olfactory processing, and enable improved development of pesticide-reduced, pheromone-based insect management of many damaging species.

Mating time of *Maruca vitrata* and electroanntennographic responses of males to extracts and volatiles from its female body

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Maruca vitrata (Lepidoptera: Crambidae) is an insect pest against several leguminous crops. Its larvae prefer to feed on flowers and pods during the reproductive stage of plant, and cause severe reduction in seed yield. In order to develop a sex attractant as a monitoring tool for adults, emergence and mating and behaviors were observed under a red dim light in a 15/9h (light/dark) photoperiod, 25℃ and 50% RH condition. In addition, electroanntennographic (EAG) responses of males to known chemicals, and extracts and volatiles from female bodies, and were measured.

Adult emergence occurred mainly during the first five hours of scotophase. Mating occurred from the two days after emergence, and the mated females started to lay eggs from the next day. A maximal mating rate was observed in the night of five days after emergence. Mating occurred significantly more often during the time from 2 hours before to 3 hours after lights-off, but older females mated more frequently during the photophase.

The sex pheromone compounds, (*E*,*E*)-10,12-hexadecadienal (E10E12-16Ald), (*E*)-10-hexadecenal (E10-16Ald) and (*E*,*E*)-10,12-hexadecadien-1-ol (E10E12-16OH) previously-reported from the insect were identified from the hexane extract of female sex pheromone gland and air-collection of volatiles during night. EAG responses to the individual compounds were higher than hexane control, but any significant synergistic responses to mixtures of two or three components with equal amounts were not observed. A gland extract of 50 females showed a significant higher EAG response than its solvent control, but its individual fractions through a silica-gel column chromatography did not show active responses, respectively. An extract of 158 female whole bodies was fractionated on a silica-gel column chromatography, and a 97/3 fraction of hexane/ether showed the highest EAG response than other fractions. An air collection of volatiles from 50 females using a porapak Q column were fractionated on a silica-gel column, and its individual fractions did not show any significan EAG responses than control. When preliminary trapping tests using several compositions including the reported ones were conducted in a net-room and a field, respectively, only virgin female trap could catch males. From the result, it was concluded that virgin female has a sex pheromone and attracted males. For practical use in fields, however, sex pheromone compounds should be studied precisely for more efficient trapping.

S5-P-084

Antennal transcriptional analysis of seed beetles *(Coleoptera: Chrysomelidae: Bruchinae)*

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Seed beetles (*Coleoptera: Chrysomelidae: Bruchinae*) are major pests of stored legumes. Their mating behaviour and pheromones has been investigated in many years. Their antenna play an important role in searching and discriminating their foods and mates. However, the molecular basis of chemical perception are still unknown. Our goal is to identify chemosensory genes involved in mate recognition, especially pheromone reception. We conducted RNA-seq to identify differentially expressed genes (DEGs) in antenna between male and female of *Callosobruchus maculatus*. Sequencing library were prepared from mRNA in antenna of 3 replicates in each sex. We sequenced 240 million of 100 bp paired reads and 20 million of 300 bp paired reads in total. All reads after trim and filter were de novo assembled using the Trinity software. For expressional analysis, 100bp paired reads were mapped to assembled transcripts. DEGs were generated using statistical tests in gene expression analysis package such as edgeR and DESeq2. In DEGs, there are some chemosensory genes. In male, 1 odorant binding protein and 2 olfactory receptors were highly expressed, while 2 olfactory genes were highly expressed in female. These sex-specific chemosensory genes would be related to sexual behaviour in male, and oviposition in female. Furthermore, we also sequenced transcripts in antenna of other *Callosobruchus* species and annotated chemosensory genes for comparative study of pheromone reception in the closely-related species.

Attraction of herbivore enemies to *Mentha* volatiles

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Mentha plants constitutively emit volatiles, which may potentially affect the behaviour of surrounding organisms, including pests, predaceous arthropods, and plants. We assessed whether volatiles from *Mentha* species (candy mint (*Mentha* x *piperita*), spearmint (*Mentha spicata*) and apple mint (*Mentha suaveolens*)) attract predators using a Y-olfactometer. All the volatiles from the three mint species assessed were attractive for *Nesidiocoris tenuis* when compared with clean air. Moreover, candy mint and spearmint could even attract *N. tenuis* over eggplant, the host plant of the predator. However, none of mints assessed was attractive for *O. strigicollis* over eggplant. We also explored the effects of mint volatiles when blended with volatiles from eggplant, and found strong attractiveness of the blend for *N. tenuis* over eggplant volatiles alone. Together, these results suggest that candy mint and spearmint could be useful as a companion plant to attract the predators. The behavioural response of carnivore mites to mint volatiles was similarly assessed, and will be discussed.

Hexenal reductase modifies composition of green leaf volatiles in Arabidopsis

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Green leaf volatiles (GLVs) consist of six carbon (C6) aldehydes, alcohols, and their esters. GLVs are produced when leaves are injured by pathogen and herbivore attacks, and they exert direct and indirect defenses. In some plant species, such as tomatoes, lima beans, maize, or poplars, GLVs are involved in their airborne plant-plant interactions. Electrophysiological and behavioural response, for example, of a parasitoid, *Glyptapanteles liparidis*, is different depending on each component of GLVs, thus, the composition of GLVs emitted out from attacked plants could bear distinct information.

(*Z*)-3-Hexenal is the component of GLVs formed at first. A portion of the aldehyde would be isomerized to form (*E*)-2-hexenal, and another portion would be reduced to form (*Z*)-3-hexen-1-ol. In some plant species, (*E*)-2-hexenal would be also reduced to yield (*E*)-2-hexen-1-ol. A portion of these alcohols would be converted into their acetates. As such, at least six components are possible, and their composition would be determined by the efficiencies of reduction and acetylation. The enzyme accountable for acetylation has been determined; however, that for reduction has not been identified. In this study, we purified hexenal reductase from Arabidopsis, and confirmed its involvement in changing the GLV composition.

The enzyme purified from mature leaves of Arabidopsis required NADPH as a cofactor for its activity, and showed its optimal activity at pH 7.5-8.0. After cloning the gene encoding the enzyme, a T-DNA knock out mutant of Arabidopsis was isolated. After partial mechanical wounding on the leaves of mutant line, (*Z*)-3-hexen-1-ol was formed but its amount was approximately one-third of that found with the leaves of wild type plants. At the same time, the mutant line formed only one-tenth of (*Z*)-3-hexen-1-yl acetate. Based on these results, it was indicated that hexenal reductase we purified was involved in regulation of GLV composition even though the other components still exist to reduce hexenal.

Chemical components involved in the cross-stimuli modulation of olfactory response of a generalist parasitic wasp *Aphidius gifuensis*

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Experience modifies organisms' responses to environmental stimuli. It enables the organisms to perform adaptive behaviors in variable environments. In parasitic wasps, for example, the experience of oviposition on hosts by female wasps in a microhabitat reinforces the response of the wasps to the microhabitat odour associated with the experience. However, some parasitic wasps use broad ranges of aphid species in various habitat environments. Those habitat generalists need to cope with the novel stimuli in the new habitat environments in the life time of the individuals. There is a limitation for an individual parasitoid to have the ability to respond to the enormous variety of stimuli as an innate repertoire. Therefore, the use of information by the wasps are predicted to be modulated indirectly through experiences of other information (crossover modulation). We previously found that the generalist parasitic wasp *Aphidius gifuensis* Ashmead changed their olfactory responses to host-infestations after continuous exposure to novel habitat backgrounds (plants). In laboratory olfactometer tests, female wasps that had been exposed overnight to the non-natal broad bean plants showed olfactory responses to host-infestations not only on the non-natal bean plants but also on the natal wheat plants, whereas the wasps that had been exposed to only the natal wheat plants did not show the olfactory response to the host-infestation on the natal plants. These results could be partly explained by unrewarding experience of the non-natal habitat background but the change of response to the natal plants suggested more complex mechanisms. We here show the results of analysis of chemical components in the natal and non-natal plant odors and discuss their involvements in the cross-stimulus behavioural modulation.

S7-P-091

Brassica flower affects behavior of parasitoid, predator and herbivore

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It is known that many parasitoid females use volatile information released from foliage infested with host herbivores. Some herbivore-induced plant volatiles have been identified to attract parasitoids. Less is known about how floral scents affect parasitoids, their host herbivores, and predators of the herbivores. In this study, we examined effects of flower of *Brassica rapa* on behavior of a parasitoid, its host herbivore, and a predator of the herbivore.

A dual-choice test demonstrated that starved parasitoid females, *Cotesia vestalis*, preferred intact plants associated with flowers over the plants infested by host herbivores, *Plutella xylostella*, but not associated with flowers. In contrast, well-fed females preferred the host-infested plants. Chemical analysis revealed some volatile compounds released from flowers, but not from host-infested foliage. By an assay using an artificial blend of the flower specific compounds and a previously identified herbivore-induced foliar volatiles, it is revealed that starved females preferred the floral blend over the foliar blend, but that well-fed females preferred the foliar blend over the floral blend.

We further assayed effects of flowers on oviposition behavior of the host adults and on foraging behavior of a predatory stink bug, *Orius nagaii*, which can prey eggs and young larvae of *P. xylostella*. Multifunctional role of flowers is to be discussed.

S7-P-092

Roles of chemical and visual cues in host foraging behavior of some dipteran parasitoids

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In contrast to hymenopteran parasitoids, the cues important for host foraging behavior by dipteran parasitoids remain largely unknown. In this study, we investigated the roles of chemical and visual cues in host foraging behavior of dipteran parasitoids with different oviposition strategies of the family Tachinidae, the most dominant group of dipteran parasitoids. Maize plants infested by final instar larvae of the noctuid moth *Mythimna separata* release volatiles that are composed of a non-specific blend (mainly green leaf volatiles: GLVs) and a host-induced blend that includes the homoterpene (*E*)-4,8-dimethyl-1,3,7-nonatriene and indole. Females of the tachinid fly *Exorista japonica* that directly lay macrotype eggs on the host surface, showed a high level of response to a synthetic mixture of the non-specific and host-induced blends, but not to each separate blend in wind tunnel bioassays. This finding suggests the fly uses a combination of non-specific and host-induced blends as an olfactory cue for locating *M. separata*-infested plants. In addition to being attracted by the plant odor, females of *E. japonica* appear to employ plant color to locate host-infested plants: females showed a significantly higher landing rate on green paper plant models than three other color (yellow, blue, red) models when odors of the host-infested plants were present in the wind tunnel. *E. japonica* uses visual as well as olfactory cues to locate the host habitat. Microtype tachinids deposit numerous “micro-type” eggs on foliage rather than on the host insects. Parasitization is successful only when the hosts ingest these eggs. To increase the chance of hosts encountering the eggs, microtype tachinids must choose a suitable plant that harbors hosts and lay their eggs near the hosts. We found two microtype tachinid species can effectively search host habitat by using different semiochemicals derived from plants infested by their host caterpillars. *Pales pavida* uses *M. separata*-infested plant volatiles as cues to locate plants that contain potential hosts, whereas *Zenillia dolosa* uses GLVs as cues to pinpoint the location where their hosts were recently feeding. Our results suggest that each tachinid parasitoid employs a different host foraging strategy to exploit visual and chemical stimuli coming from plant–herbivore interaction as cues to increase their parasitization success.

Cuticular lipids, but not cuticular hydrocarbons mediate mate recognition in the subsocial burying beetle *Nicrophorus vespilloides*

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Insect cuticular hydrocarbons (CHCs) belong to the most intensively studied semiochemicals. Although primarily anti-desiccation agents, CHCs play an important role in inter- and intraspecific communication and are known or assumed to serve as contact sex pheromones in several taxa. Burying beetles perform extended biparental care that includes the protection and feeding of offspring. Even though the beetles are known to have evolved sophisticated recognition and communication processes, it is currently unknown how they recognize sex and which cues trigger male mating behavior.

In this study, we investigated whether males of the subsocial burying beetle *Nicrophorus vespilloides* use chemical cues to recognize females and to discriminate between sexes. Behavioral experiments with alive, anaesthetized and freshly killed beetles allowed us to exclude a role of the female's behavior for sex recognition. Further assays in which we applied extracts of male and female beetles to washed dummies confirmed the use of chemical compounds for sex recognition in *N. vespilloides*. Because previous study revealed sex differences in the CHC composition of the burying beetle *N. vespilloides*, we tested here whether males use CHCs to discriminate between the sexes. However, using a fractionated-guided bioassay we could show, that CHCs do not mediate sex recognition by males of N. vespilloides, although CHCs make up more than 98% of the total amount of compounds extracted from the cuticle of females. Chemical analyses of the behaviorally active fraction revealed 17 compounds, mainly aldehydes and fatty acid esters, with small quantitative but no qualitative differences between the sexes. One compound, hexadecanal contributed most to the statistical separation of chemical profiles of males and females. However supplementation of males with hexadecanal did not trigger copulation attempts by males. Therefore, a possible explanation is that the whole profile of polar lipids mediates sex recognition in *N. vespilloides*.

S8-P-094

Interspecies and intercaste comparisons of antennal lobe constitution in seven species of termites

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Termites are eusocial insects and their colony consists of neuter castes (worker and soldier) and a reproductive caste (male and female). The constituent members play the cast-specific roles and mutual chemical communication is crucial for maintaining their highly developed society. Within the colony, termites communicate using olfactory signals such as pheromones. In insects, pheromonal signals are generally processed in specific glomeruli in the primary olfactory center, the antennal lobe (AL). However, olfactory system relating to the pheromonal communication has been little studied in termites. In this study, we examined glomerular organizations of ALs of seven termite species, *Zootermopsis nevadensis, Incisitermes minor*, *Reticulitermes speratus*, *Coptotermes formosanus*, *Odontotermes formosanus*, *Pericapritermes nitobei* and *Nasutitermes takasagoensis*, by visualizing glomeruli with either anterograde staining of antennal afferents or immunohistochemistry using anti-synapsin antibody. Subsequently, glomeruli were reconstructed three-dimensionally to elucidate their numbers, loci and volumes. The number of glomeruli varied from 100 to 150 depending on species, whereas it was identical between sexes and castes in each species. Reproductive males and females were equipped with one to three extremely large glomeruli, termed tentatively macroglomeruli (MGs), among which one MG was conserved in locus across all species of both sexes and there were no sexual dimorphisms in volumes of all MGs. In addition, we also identified MGs in phylogenetically close termites. Next, we compared morphologies of MGs across castes in the three species; *Z. nevadensis, I. minor* and *N. takasagoensis*. In these species, workers and soldiers had glomeruli homologous to the MGs of reproductive castes, but the volumes of the glomeruli were significantly smaller than those in reproductive castes. It suggests involvement of reproductive caste-specific macroglomeruli in sexual behavior of these termites.

Effects of prey vital condition on recruitment behavior in a pavement ant *Tetramorium tsushimae*

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A pavement ant *Tetramorium tsushimae* sometimes captures large prey items, including injured small animals that could resist or escape against the forager ants, and their dead bodies that were immobile. There the scouter ants would recruit their nestmate workers by laying trail pheromone on the ground since they usually construct foraging queue. Such trail-recruitment systems might not always work well especially when the prey items were moved spontaneously even after the scouter ant went back to the nest. Hence, we hypothesized that recruited ants could arrange their searching behavior depending on information provided by the scouter ants. To investigate this hypothesis we compared the recruitment behavior of the scouter ants that had found either dead or alive prey, and also compared the foraging behaviors of the recruited workers in each case. When observing overall recruitment process on those preys separately, the scouter ants started recruitment more quick to a dead prey than alive one. The recruited workers approached a distance within ca. 1 cm from the dead prey. Concerning the alive prey, however, some workers approached it in the same way but the others kept staying surroundings of it. As a result, larger numbers of the workers were recruited surroundings away ca. 3 cm from the prey. Since those recruited workers exactly reached the site where the scouter left for the nest, it is most likely that the scouter informed the site by depositing the trail pheromone as well known. Different foraging behaviors after arrivals, however, would not be evoked by only the trail pheromone. It suggests that the scouter ants would convey additional information including vital condition of the preys.

What´s for dinner: olfactory food choice in soil mites (Oribatida)

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Animals need to consume food to maintain their metabolism and generate energy for growth and reproduction. Hence, various ways for the detection, foraging and processing of food evolved to exploit a wide range of resources. In above-ground systems, olfactory traits play an important role for many heterophic organisms to find and identify their food. In soil ecosystems, however, olfactory food selection has only been fragmentarily explored and it remains to be uncovered whether olfactory signals play a role in finding suitable food sources for mainly decomposing microarthropods (e.g., oribatid mites and springtails) in the highly structured soil microsphere. Hence, more data is needed for an understanding of the soil food web structure. We used two phylogenetically distinct oribatid mite species [*Archegozetes longisetosus* (parthenogenetic, pantropical, opportunistic feeder) and *Scheloribates sp.* (sexual, tropical, myco-/phytophagous feeder)] in 780 laboratory food-choice bioassays (= 7800 individuals) to access two basal questions. Do oribatid mites use olfactory cues for food selection and if so, can they discriminate between different food sources. We further asked whether oribatid mites are habituated to odors of food they know (= “learning”). Additionally we chemically characterized volatiles and nutrients of the food sources (litter, lichen, fungi, bacteria). We found that oribatid mites use olfactory signals to find food and also differentiate among resources. Mites did not prefer well known resources (no “learning”-effect) over their generally preferred food. While *A. longesitosus* preferred a fatty acid rich bacterial diet, *Scheloribates sp.* mainly fed on fungal-based food sources (fungi and lichen). We also presented synthetic amino/fatty acid mixtures and glucose; again *A. longesitosus* preferred fatty acids, while *Scheloribates* showed no preferences. However, when we added the fungal volatile 3-octen-1-ol to glucose, *Scheloribates sp.* subsequently started to favour the modified glucose.

S9-P-097

The (oxalato)aluminate complex as an antimicrobial substance protecting the "shiro" of *Tricholoma matsutake* from soil microorganisms

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Mycorrhiza is a symbiotic organ consisting of plant roots and fungi. The plant is supplied with nitrogen and mineral nutrients such as phosphorus, potassium, and water from fungi, and the fungi receive photosynthetic sugars such as glucose from the plant through the mycorrhiza. *Tricholoma matsutake* is an ectomycorrhizal fungus whose major host tree in Japan is the Japanese red pine, *Pinus densiflora*. Its fruiting body, "matsutake" in Japanese, is a commercially valuable mushroom in Japan. The artificial cultivation of *T. matsutake* has been attempted for a long time, but it has not yet succeeded.

Tricholoma matsutake forms a whitish mycelium-soil aggregated zone, called "shiro" in Japanese. The shiro front corresponds to an active mycorrhizal zone forming a fairy ring of the fruiting body. The horizontal width of the shiro front is approximately 15 cm, and its front annually advances outwards at 10-15 cm. Bacteria and actinomycetes reportedly disappeared, and only *Mortierella* sp. appeared in the shiro front, whereas they were abundant in the soil outside the shiro front and appeared inside the shiro front. Ohara demonstrated in 1966 that the active mycorrhizas and soil crusts from the shiro front inhibited the growth of bacteria from outside of the shiro, showing that an antimicrobial substance was present in the active mycorrhizas. However, the antimicrobial substance has remained unidentified for 50 years.

We purified the antimicrobial substance from the shiro front, and identified it by an X-ray analysis as the (oxalato)aluminate complex. The complex is known as a reaction product of oxalic acid and aluminum phosphate to release soluble phosphorus, but its antimicrobial activity has not been known so far. Aluminum adopts a regular octahedral complex where one to three molecules of oxalic acid can coordinate. The order of the antimicrobial activity was tris(oxalato)aluminate, bis(oxalato)aluminate, and (oxalato)aluminate from lowest to highest, suggesting that vacant coordination position of the (oxalato)aluminate complex was important for the antimicrobial activity. We examined the distribution of mycelia of *T. matsutake*, the (oxalato)aluminate complex, antimicrobial activity, microorganisms, and soluble phosphate in the shiro area in a season forming fruiting body. The results clearly showed a good correlation between the distribution of the (oxalato)aluminate complex and that of the other items. Bacteria were isolated from the shiro area, and their sensitivity to the complex was also examined. The bacteria from the shiro front were resistant to the complex, while bacteria sensitive to the complex were partially found inside and outside the shiro front. This suggested that the complex expels sensitive bacteria from the shiro front along with the extension of the shiro. Based on these results, we propose that the (oxalato)aluminate complex protects the shiro from microorganisms, and contributes to its development.

Mating Behavior Propelled Dynamic Changes of Chemosensory Genes during Whole Mating Progress in *Dendrolimus punctatus*

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Chemosensory system is among the most pivotal systems of insect, and olfactory is especially sensitive for moths. But few studies have linked plasticity of olfactory-guided behavior and electrophysiology to molecular level of insects. Pine caterpillar moth, *Dendrolimus Punctatus* walker is a dominant conifer defoliator in China, and mating is priority for adult *D. punctatus*, while sex pheromone recognition and oviposition site location were their main activities. These activities are all closely related to chemosensory genes. In this study, we expect to identify chemosensory related genes andmonitor thespectrum of their expression dynamic during the whole mating progress. The major gene families that encode olfactory-related proteins were annotated. Expression dynamics analysis of the olfactory related gene during mating behaviors indicating that considerable numbers of odorant binding proteins (OBP)， chemosensory proteins (CSP), and odorant receptors (OR) were closely correlated to mating behaviors. Subsequently comparing with ORs from other *Dendrolimus* and Lepidoptera species lead to the discovery of a *Dendrolimus*specific ORs group. Furthermore, we found several genes in OBPs and ORs upregulated after mating in females, which may be the genes response to host location related plant volatiles. This work not only will facilitate the follow-up function research of *D. Punctatus* chemosensory genes, but also give a spectrum relationship between chemosensory genes and important physiology and behavioral activities, and promote the research on the mechanism of insect olfactory recognition.

Key Words: Chemosensory gene, Mating, Expression, Pheromone receptor, Dynamic.

Dose and density effects on the use of aggregation pheromones by longhorn beetles (Coleoptera: Cerambycidae), and their possible role in optimal density strategy.

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Longhorn beetles (Coleoptera: Cerambycidae) are wood-boring insects that infest trees and woody plants. Many species utilize male-produced pheromones that attract both males and females. Based on other coleopteran species that utilize male-produced pheromones, the goal of this research was to test the hypotheses that 1) cerambycids respond to pheromones in a dose-dependent (=release-rate) manner and 2) pheromone emission is density-dependent. If true, these characteristics of pheromone use could be indicative that some cerambycid species utilize an optimal density strategy to limit competition for scarce and ephemeral hosts, i.e., the stressed or dying trees that are susceptible to infestation. Attraction to increasing release rates of two common pheromone components – 2-methylbutan-1-ol and 3-hydroxyhexan-2-one – was tested, and it was found that responses increased with release rates for four of the five test species, even at the highest rates tested (~1,450 µg/h and ~720 µg/h, respectively). However, responses of the fifth species, *Phymatodes obliquus,* plateaued with increasing release rates. The effect of density of conspecific males on per capita pheromone production was tested by collecting the volatiles produced by male *Phymatodes grandis* individuals, pairs, or groups of three or four beetles held in 500 ml aeration chambers. Emission rates of their pheromone (*R*)-2-methylbutan-1-ol were found to decrease with increasing density, suggesting that cerambycids may use optimal density strategies, as has been shown for other coleopteran species that utilize male-produced sex-aggregation pheromones.

S9-P-100

Antennal transcriptome analysis of the chemosensory gene families in the longhorned beetles, *Anoplophora glabripennis* and *A. chinensis* (Coleoptera: Cerambycidae)

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The Asian longhorned beetle (ALB) *Anoplophora glabripennis* (Motschulsky) and citrus longhorned beetle (CLB) *A. chinensis* (Forster) are native to Asia and East-Asia respectively. These two dangerous pests in forestry are polyphagous xylophage and have been introduced to North America and Europe, causing considerable economic and ecological losses. These affinis species share many semiochemicals, and we found both beetles ecological niche are same in some provinces of China, for example damaging willow and elm simultaneously. Therefore, based on olfactory accurate host tree and pheromone sense are of utmost importance for fitness and reproductive isolation. Through antenna transcriptome analysis of *A. glabripennis* and *A. chinensis*, we identified 42 odorant-binding proteins (OBPs), 12 chemosensory proteins (CSPs), 14 pheromone-degrading enzymes (PDEs), 1 odorant-degrading enzymes (ODE)，37 odorant receptors (ORs), 11 gustatory receptors (GRs), 2 sensory neuron membrane proteins (SNMPs), and 4 ionotropic receptor (IR) in *A. glabripennis;* and identified 46 OBPs, 16 CSPs, 44 ORs, 19 GRs, 23IRs and 3SNMPs in *A. Chinensis.* Expression profile of all studied CSPs and PBPs in both species showed all expressed in antenna. Vast majority of CSPs of both beetles were highly expressed in multiple chemosensory tissues, suggesting their participation in olfactory recognition in almost all olfactory tissues. Intriguingly, the *AglaPBP2* and *Achi*PBP2 was preferentially expressed in antenna, indicating that it is the main protein involved in efficient and sensitive pheromone recognition. This study establishes a foundation for determining the chemoreception molecular mechanisms of *A. glabripennis* and *A. chinensis*, which will also provide a new perspective for controlling longhorned beetle populations through olfactory interference.

S9-P-101

Seasonal variations and bioactivities of resin acid components in *Cryptomeria japonica* cones

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Cones are essential to the self-propagation of conifers. Their resin components mainly consisted of terpenoid have been suggested to act as the defensive substances against insects and fungi [1]. The aim of our study is to investigate the self-defensive roles of terpenoids in conifer cones. *Cryptomeria japonica* (Cupressaceae) is the most important conifer species in Japan for timber production and consequently important for the tree breeding. However, qualitative and quantitative analyses of cone components have not been fully demonstrated. In this study, we report the time-courses as well as identification and quantification of major components in the benzene extract of *C. japonica* cones, together with the evaluation of their bioactivities against pest insect.

Healthy cones were collected monthly from the same native variety of 20-year-old *C. japonica* trees (*n*=5) from June to January. Frozen cones were milled and then successively extracted with benzene, chloroform and methanol each three times. The benzene extracts were partitioned into acidic and neutral fractions, and the former was purified by silica gel column chromatography. Each isolate was identified by 1D and 2D NMR analyses, and their contents (%/dw, ±SD) in the cones were measured by quantitative NMR (q-NMR) using dimethyl sulfone as an internal standard. Then, bioactivities of the extracts and isolates on brown-winged green stink bug, *Plautia stali*, which hosts *C. japonica* cones were tested.

Yields of benzene extract gradually increased from 9.5±1.9 to 16.1±3.1%/dw from June to January. The composition ratio of acidic and neutral fraction was almost 1:1 in all samples. The GC analyses revealed that acidic fraction contained less than 10 diterpenoids, whereas neutral fraction contained more than 30 terpenoids. Interestingly, yields of acidic fraction had not changed until November (ca. 5.5%/dw) but then steeply increased to 7.7±1.6%/dw up to January, after the scales of mature cones had opened. Water contents dramatically decreased at this time, therefore, their resin production might be affected by some kind of drought stress. Five resin acids were isolated from the acidic fractions: they were identified as isopimaric, sandaracopimaric, *cis*-communic, imbricataloic and imbricatoloic acid. The q-NMR analyses revealed that these five compounds accounted for ca. 70% of the acidic fractions. Isopimaric acid, the major compound, consisted of ca. 30% of each acidic fraction. Contents of isolated resin acids increased according to the maturing stages of cones. Only imbricatoloic acid especially increased from June to January. These labdane-type resin acids may play important roles as the defensive substance because of the presence of their high reactive side chains. As the results of bioactive test, notable repellences were not observed against adult *P. stali*, even though high concentrations of each successive extract were tested. Details of seasonal variations and bioactivities of resin acids against *P. stali* larvae will be presented.

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S9-P-102

trans-4-Thujanol host tree volatile: Anti-attractant activity or not in European spruce bark beetle *Ips typographus?*

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The *trans*-4-thujanol (alias sabinene hydrate) is a trace oxygenated terpene compound in the Norway spruce host tree, decreasing with tree age. It has strong electrophysiological activity on antenna of *Ips typographus* and olfactometer data indicate a repellent effect [1]. We decided to test repellent (anti-attractant) or attractant activity of *trans*-4-thujanol in the field adding it at three steps of release rates to the traps for *Ips typographus* with and w/o pheromone. As a positive control was used pheromone traps with 1,8-cineole added, an already known anti-attractant. In all, 14 treatments were deployed. We choose two localities of Norway spruce forest with high population density of *Ips typographus* in lowland (Libava, Czech Republic) and mountains (Tatra Mountains, Slovakia). The tests are now running.

Our hypothesis is that the *trans*-4-thujanol will show a dose-dependent inhibition of attraction to pheromone, but may as well at low doses shows some attractant activity alone. We also check the enantiomeric composition of the tested *trans*-4-thujanol (4 possible isomers) and in local spruce trees.

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(-)-*α*-Pinene and Ethanol: Attractants for bark, ambrosia and Curculionidae beetles at *Pinus koraiensis* and *Pinus densiflora* Stands in Korea

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We evaluated the attractiveness of two kairmones, (-)-*α*-pinene and ethanol to develop efficient monitoring method for bark, ambrosia beetles and weevils at *Pinus densiflora* and *Pinus koraiensis* stands in Gangwon province, Republic of Korea. Total 15,657 species of bark, ambrosia beetles and weevils were captured at *Pinus koraiensis* and *Pinus densiflora* stands from March to November, respectively. Multiple funnel traps baited with (-)-*α*-pinene alone were attractive to bark beetles, *Tomicus piniperda*, *Hylastes plumbeus*, *Tomicus pilifer*, and ambrosia beetle, *Trypodendron lineatum*. Ethanol synergized attraction of following bark beetles, *Tomicus piniperda*, *Hylurgops interstitialis*, *Orthotomicus laricis*, and ambrosia beetles, *Tyrpodendron lineatum* and *Xylosandrus crassiusculus*, and weevils, *Shirahoshizo insidiosus*, *Shirahoshizo rufescens*, *Rhadinomerus maebaral*, *Niphades verrucosus*, and *Hylobius haroldi*. Multiple funnel traps baited with ethanol alone were attractive to following amborsia beetles, *Xylosandrus germanus, Cyclorhipidion pelliculosum, Cyclorhipidion bodoanum, Cnestus mutilates, Xyleborinus attenuates*, *Xyleborinus saxesenii, Scolytoplatypus tycoon,* and *Scolytoplatypus sinensis.*

S9-P-104

Inhibition activity of components in Japanese conifer against invasive plant species

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An ecological problem caused by invasive plant has become intensified in Japan. Especially, an herbaceous of *Solidago Canadensis* ver *scabra* and a woody plant of *Robinia pseudoacacia* is invasive plant species required strongly to be exclusion, because the plants have grown thickly in Japan. On the other hand, several conifer species such as *Cryptomeria japonica* (Japanese name: Sugi) and *Chamaecypress obtusa* (Hinoki) are Japanese native plant and large amounts of its resource are accumulated as plantation tree in Japanese forest. It is known that the components of the conifer trees have allelopathy activity against plant and insect. Therefore, we have investigated to inhibit the invasive plant by using the conifer extracts[1]. In this study, it was to examine the growth inhibition activity of extracts obtained from leaves of the Japanese conifers and identify the active components.

Leaves of *C. japonica* and *C. obtusa* were used as sample materials. Each extract was prepared by successive extraction of the leaves with hexane, ethyl acetate and methanol. The extract or isolation compound was impregnated at certain concentration to filter paper. Seeds of *S. Canadensis* ver *scabra* or *R. pseudoacacia* were put on the filter paper with water in petri dish. The activity of extracts or compounds were examined by measurement of seed germination, and growth (radicle and hypocotyl) according to previous report[2].

For *S. Canadensis* ver *scabra,* hexane extracts obtained from leaves of *C. japonica* and *C. obtusa* showed potent inhibition activity on seed germination, but no or weak activity on radicle and hypocotyl growth. Ethyl acetate and methanol extracts obtained from both leaves did not show any inhibition activities against *S. Canadensis* ver *scabra*. For woody plant of *R. pseudoacacia,* hexane and ethyl acetate extracts obtained from leaves of *C. japonica* and *C. obtusa* showed growth inhibition activities on radicle and hypocotyl growth but no or weak activity on seed germination. Thus, it was indicated that low polar components in the leaves had inhibition activities against *S. Canadensis* ver *scabra* and *R. pseudoacacia.* The hexane extract of *C. japonica* was fractionated by column chromatography to identify active compounds. We found that cryptomeridiol of sesquiterpene diol was as an active component against *R. pseudoacacia.*

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S9-P-105

Utilization and synthesis of host kairomones of the European Beech Weevil (*Orchestes fagi*), a non-native pest of beech (*Fagus grandifolia*) in Nova Scotia, Canada

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The beech leaf-mining weevil (*Orchestes fagi* L.) (Curculionidae) is a non-native invasive pest of American Beech (*Fagus grandifolia* Ehrh.) in Nova Scotia, Canada. Mating and oviposition by adults occurs in conjunction with beech budburst, during which time females will deposit eggs into the primary vein of the developing beech leaves. As part of the efforts to monitor this pest, we sought to identify possible kairomones produced by the host tree that may be utilized by *O. fagi* in host location. Headspace samples collected from bursting beech buds using solid-phase microextraction (SPME) and analyzed using gas chromatogaphy/mass spectrometry (GC/MS) identified several potential kairomones, including: the diterpene hydrocarbons, 9-geranyl-*p*-cymene, 9-geranyl-α-terpinene and the acyclic 9-geranyl myrcene; and the sesquiterpene (*E,E*)-α-farnesene. In our first year of field experiments, we observed that both mean total and mean male trap catches were significantly greater in yellow traps baited with 9-geranyl-*p*-cymene versus unbaited yellow sticky traps. These results provide evidence that 9-geranyl-*p*-cymene may be a kairomone for *O. fagi* in the location of beech buds. We also report the results of current year trapping experiments with this compound in combination with two possible *O. fagi* pheromone components, and in combination with (*E,E*)-α-farnesene, and acyclic 9-geranyl myrcene.

The importance of Chrysomelidae tarsal gustation in host selection

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Tarsal gustation has been described in Diptera and Lepidoptera. Many morphological, electrophysiological, and behavioral studies on tarsal gustation in these orders have been conducted. However, tarsal gustation in Coleoptera, the largest order of insects, is unclear. In previous studies, we showed that 15 of 17 Chrysomelidae subfamilies have tarsal gustatory sensilla and that the strawberry leaf beetle *Galerucella gricescens*, used as a model species of Chrysomelidae, can discriminate leaf surface wax of the host plant from that of non-host plants.

In this study, we attempted to demonstrate the presence of tarsal gustatory organs in Chrysomelidae by scanning electron microscope (SEM) and transmission electron microscope (TEM) examination and electrophysiological experiments. In morphological studies, we observed the tarsi of three Orsodacinae or Clytrinae species to determine whether these subfamilies have tarsal chemosensilla, as their tarsi are yet to be examined. In addition to the SEM observations, we examined the internal morphology of the tarsal chemosensillum of *G. grisescens* using TEM. In electrophysiological studies, using the tip-recording method, we recorded the responses of the tarsal gustatory sensilla of *G. grisescens* to KCl and sucrose to examine the acceptance of taste substances in the tarsus. Moreover, we investigated the role of tarsal gustation in Chrysomelidae host selection by examining the response of *G. grisescens* to host or non-host plant leaf surface waxes, considered to be the substance touched first by their tarsus.

The SEM examination showed tarsal chemosensilla in all three species observed in this study. From this result and the findings of our previous study, we concluded that all Chrysomelidae subfamilies have tarsal gustatory sensilla. It is suggested that the presence of tarsal chemosensilla is common in Chrysomelidae. The TEM examination revealed that the tarsal gustatory organ is innervated by four dendrites that extend from taste cells. In the electrophysiological experiments, these gustatory sensilla showed a spike response to KCl and sucrose. This indicates that Chrysomelidae can recognize taste, such as that of salts and sugars, through their tarsal sensilla. Finally, we investigated the role of tarsal gustation in host selection. *G. grisescens* showed a positive response to the leaf surface wax of *Rumex obtusifolius*, which is their most suitable host. However, they did not show a positive response to other plant waxes including that of strawberry, another of their hosts. Therefore, it is considered that *G. grisescens* can discriminate their host from non-host plants, and furthermore select the most suitable hosts, by their tarsi.

S11-P-107

Search for the fatty acyl reductases involved in the formation of bumblebee male pheromone

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The marking pheromone of bumblebees, which is used by males to attract conspecific females, is composed of a species-specific blend of various fatty acid- and isoprenoid-derived compounds such as fatty acid ethyl esters, fatty alcohols or terpene alcohols. In this project, we focus on the final step of fatty alcohol biosynthesis catalyzed by enzymes fatty acyl-CoA reductases (FARs, EC 1.2.1.84). We hypothesize that the diverse catalytic specificities of these enzymes could result in the species-specific composition of the marking pheromone.

We analyzed male labial gland and fat body transcriptomes of three European bumblebee species, *Bombus terrestris*, *B. lucorum* and *B. lapidarius*, and identified several putative FAR transcripts that are abundantly and specifically expressed in LG. The tissue specificity of selected FAR transcripts was further confirmed by qRT-PCR. The analysis of the translated protein sequences of these novel LG-specific FARs indicated that three groups, termed FAR1, FAR2 and FAR3, are present in both *B. terrestris* and *B. lucorum*. In *B. lapidarius*, FAR1 and FAR3 groups are accompanied by an additional group coined as FAR4/FAR5. The coding sequences of all candidate FARs were cloned into an expression vector and subsequent functional characterization of the reductases was performed in *Saccharomyces cerevisiae*. The fatty alcohols produced by the recombinant enzymes were determined in yeast extracts using GC-MS.

The recombinant FARs produce C14–C26 saturated and unsaturated fatty alcohols in yeast. While FAR1 from *B. lapidarius* produces monounsaturated C16/C18 fatty alcohols, FAR1 of *B. terrestris* and *B. lucorum* both produce only saturated C18−C26 fatty alcohols. The FAR3 group catalyzes the production of C22−C26 saturated fatty alcohols. The enzymes FAR4 and FAR5 from *B. lapidarius* produce C16 fatty alcohols, which are the same fatty alcohols as observed in male LG.

We have identified the enzymes, which are preferentially and abundantly expressed in LGs of male bumblebees and which produce fatty alcohols when expressed in yeast. Two of these FARs, FAR4 and FAR5, are presumably involved in the formation of major fatty alcohols, hexadecanol and (9*Z*)-hexadecenol, in LG of *B. lapidarius* males.

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In defence of flowers: different strategies in the deployment of a chemical defence, cyanogenesis, in floral tissues

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Floral chemical defence strategies remain little tested with respect to the large body of plant chemical defence theory, despite the significance of flowers to plant fitness, the sizeable investment of resources in floral structures, and the potential impact of florivory. Cyanogenic glycosides are nitrogen containing plant secondary metabolites that deter herbivores by releasing toxic hydrogen cyanide upon tissue damage. According to Optimal Defence Theory higher concentrations of defence metabolites will be allocated to defend the most vulnerable and valuable plant parts, including reproductive structures such as flowers [1]. Broadly consistent with this theory are high concentrations of cyanogenic glycosides in flowers in the Proteaceae, a family with a high frequency of floral cyanogenesis [2]. Little is known, however, about the distribution of defences within flowers; at what scale is there strategic allocation of defence metabolites in flowers, and how might this distribution vary with other floral traits?

Here, we use flowers from different cyanogenic genera from across the Proteaceae phylogeny to quantitatively compare the distribution of defence metabolites (1) in different floral tissues, (2) in species with different floral morphology and colour, and (3) across different stages in floral development. Flowers were dissected and cyanogenic glycoside concentrations were quantified as evolved cyanide. Further spatial localisation of specific cyanogenic glycosides was performed using imaging MALDI mass spectrometry imaging.

Four different patterns in the distribution of cyanogenic glycosides within floral tissues were identified across 11 species. In several species, the highest concentrations of cyanogenic glycosides and greatest allocation of these compounds was in pistillate tissues (ovary and style). By contrast, in other species there was a relatively even distribution of cyanogenic glycosides between floral tissues, including the pistil, perianth and pedicel. In a third group, cyanogenic glycoside concentrations were highest in the perianth, and in the fourth group the pedicel was most highly defended. In addition, there were significant changes in cyanogenic glycoside concentration and allocation with floral development, and metabolite mapping identified differences in the localisation of specific cyanogenic glycosides in floral tissues during development. These patterns were not correlated with differences in floral colour, which along with pollination syndrome was found to correlate with the presence/absence of floral cyanogenesis within in the genus *Hakea* [3]

Relationships between the different floral chemical defence strategies and other floral traits, including pollination syndrome and floral morphology will be explored, and discussed with respect to theories of optimal defence allocation.

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Evaluation of the repellent effects of Six Preservatives on *Solenopsis invicta*

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Solenopsis invicta Buren is a server pest all over the world and posed serious threats to the environment and humans. Baiting is the main methods for the control of *S. invicta*. In previous studies, we found that corrupt food negatively affected the foraging behaviors of *S. invicta*. Adding preservatives into the baits might extend the period of bait attractiveness; however, it is not known whether these preservatives would repel foraging ants. In the present study, we evaluated the effects of six commonly used preservatives on *S. invicta* foragers under laboratory (four-choice tests) and field (multiple-choice tests) conditions. Results showed that five preservatives (sodium benzoate, sodium diacetate, nisin, potassium sorbate, and calcium propionate) exhibited no repellent effects on the *S. invicta* foragers, whereas sodium dehydroacetate significantly decrease the number foraging ants compared to the controls. Based on these results, we suggested that the use of sodium dehydroacetate should be avoided to produce baits against *S. invicta*.

Stimulation of pheromone production via pheromone biosynthesis activating neuropeptide in the legume pod borer, *Maruca vitrata*

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Pheromone biosynthesis activating neuropeptide (PBAN) produced in the suboesophageal ganglion has been known to stimulate pheromone production in lepidopteran species, mediating sexual behaviors. PBAN was identified from the transcriptome of the head in the legume pod borer, *Maruca vitrata*. To examine its pheromonotropic activity in the legume pod borer, *Maruca vitrata*, PBAN was chemically synthesized. When female adults were injected with synthetic PBAN, pheromone production showed a maximal increase 2 h post-injection. PBAN was expressed in all examined tissues and developmental stages. In contrast, PBAN receptor (PBANr) was detected in the female tissues and all developmental stages except for adult male. In addition, two types of PBANr were identified from the transcriptome of the pheromone gland, suggesting that the molecular signal on the pheromone gland may transduce via PBANr.

S11-P-111

Escaping and repairing behaviors of *Odontotermes formosanus* (Blattodea: Termitidae) in response to the mud-tube damage

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Although some studies focused on the behaviors of disturbed termites under laboratory conditions, no study has been conducted in the field. In the present study, 41 videos (total length ~ 2700 min) were taken on 22 field groups of the black-winged termite, *Odontotermes formosanus* (Shiraki), after their mud tubes were partially damaged. In general, mud-tube repairing was observed shortly after damage, and new mud tubes were preferentially constructed from down to up. In almost all videos, we observed three stages of termite escaping, including probing (termites wondered near the broken mud tubes), individual escaping (individual termites escaped from up to down), followed by massive termite movement in the unidirectional escaping flows (groups of termites moved downward in queues). Interestingly, termites in the escaping flows moved significantly faster than that escaped individually. Downward moving and repairing were dominated behavioral repertoires for individual termites, which were significantly higher in frequency compared to upward moving, turning/backward moving, or wondering/probing. When predators (i.e. ants) appeared, termites stopped moving and sealed mud tubes. This is the first report to show that an animal (besides humans) carried out pathway repairing and emergency evacuation simultaneously and non-interferingly. The potential mechanism of chemical ecology of this process is discussed.

S11-P-112

Morphology and ultrastructure of olfactory sensilla in the antennae and maxillary palps of pumpkin fruit fly, *Bactrocera depressa*

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The morphology and ultrastructure of olfactory sensilla present in the antennae and maxillary palps of pumpkin fruit fly, *Bactrocera depressa*, an important pest of Curcurbitaceae crops such as pumpkin and zucchini in Korea, were investigated by scanning electron microscopy (SEM) and transmission electron microscopy (TEM). Male and female *B. depressa* displayed morphologically distinct types of olfactory sensilla in the antennae and maxillary palps, with predominant populations of basiconica, coeloconica and auricilica. High-resolution SEM observation showed that a number of nanoscale pores were present on their cuticular surface, and TEM observation exhibited that the pores were connected to the sensillum lymph cavity through electron-lucent material. In contrast, numerous longitudinal grooves were present along the surface of coeloconic sensilla. Basiconic sensilla, the most abundant type of olfactory sensilla in the antennae, could be further classified into subtypes, based on their size, shape and the distribution pattern and density of nanoscale pores. In contrast to the antennae bearing basiconic, auricillic and coeloconic sensilla, maxillary palps displayed predominant population of curved basiconic sensilla with some coeloconic sensilla, indicating that the olfactory function of the sensilla in maxillary palps would be different from that of antennal sensilla in *B. depressa*. Although the cuticular surface of long setae in the antennae displayed nanoscale pores around their basal areas, it was unclear if they were related to olfactory function. Our results will be useful in further studies to characterize the function of each type of olfactory sensilla in *B. depressa*.

Morphology and ultrastructure of olfactory sensilla in the antennae and maxillary palps of spotted wing drosophila, *Drosophila Suzukii* (Matsumura, 1931) (Diptera, Drosophilidae)

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The morphology and ultrastructure of olfactory sensilla present in the antennae and maxillary palps of male and female spotted-wing drosophila, *Drosophila suzukii*, a serious pest of berry fruits, were investigated by scanning electron microscopy (SEM) and transmission electron microscopy (TEM). Morphologically distinct types of olfactory sensilla, mostly comprised of trichodea, auricillica and basiconica, were identified in the antennae and maxillary palps. High-resolution SEM observation exhibited the presence of numerous nanoscale pores on the cuticular surface of these sensilla, and TEM observation displayed that the pores were connected to the sensillum lymph cavity through electron-lucent material. Each type of sensilla could be further classified into subtypes, according to their size and the distribution pattern of nanoscale pores. In contrast to the antennae bearing large numbers of trichoid, basiconic and auricillic sensilla, respectively, maxillary palps displayed predominant population of auricillic sensilla, indicating that the olfactory function would be different between antennae and maxillary palps in *D. suzukii*. No clear sexual dimorphism in the morphology of olfactory sensilla was found in this species. Our results will be useful in further studies to characterize the function of each type of olfactory sensilla.

Pea peptides metabolism in the gut of *Spodoptera litura* larvae

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PA1b (pea albumin 1, subunit b) is a plant peptide purified from pea (*Pisum sativum*) seeds, that is lethal to some insects, such as the cereal weevils (*Sitophilus oryzae*, *S. zeamais*, *S. granarius*) or the pea aphid (*Acyrthosiphon pisum*). PA1b consists of 37 amino acids (ASCNGVCSPFEMPPCGTSACRCIPVGLVIGYCRNPSG), cross-linked by three disulfide bonds. It has been demonstrated that PA1b inhibits the V-ATPase activity by binding with the c and e subunit of V-ATPase. In insects, the V-ATPase is present on the midgut brush border and essential for energizing nutrient absorption. Whilst lepidopteran larvae are insensitive to PA1b, its binding to the membrane extracts has been observed in all larvae tested. Thus, PA1b seems to be prevented from reaching V-ATPase in the gut of lepidopteran larvae.

In this study, we compared profiles of PA1b-related peptides in pea seeds, gut contents and frass of *Spodopetra litura* larvae reared on the pea seeds by LC/MS. The pea seed extracts contained peptides with molecular masses of 3741 (PA1b), 3757 and 3805 Da, in which PA1b was detected at the level more than twice as much as those with 3757 or 3805 Da. However, the ratio of PA1b to the other peptides decreased in the frass, and the peptide with 3757 Da was the major component among them. This result suggests that degradation or modification of PA1b could occur in the gut to confer PA1b resistance. The presentation will also highlight lipid composition change of the larvae reared on the pea seeds.

Dominance of *n*-alkanes in cuticular hydrocarbons of lycaenid larvae: chemical insignificance to ants

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Chemical mimicry and camouflage based on cuticular hydrocarbons are the adaptive strategies frequently observed in myrmecophilous insects. In butterflies, a few lycaenid larvae are found to employ chemical mimicry to establish obligate ant associations. However, little is known about the adaptive strategy of lycaenid larvae in the facultative associations with multiple ant species. Since chemical analyses revealed that three lycaenid larvae, two facultative-ant-associated (*Lycaeides argyrognomon* and *Zizeeria maha*) and one non-ant-associated (*Lycaena phlaeas*), commonly contain *n*-alkanes as the major cuticular hydrocarbons (CHCs), we examined potential effects of these CHC profiles on predation by *Formica japonica* ants. In field bioassays, the workers in Hiroshima and Yamanashi populations, which possessed different CHC profiles, showed 5–22% aggression responses to these intact larvae. The treatments of these larvae with 0.1 μg dose of 7- or 9-heptacosene activated ant aggression especially in Hiroshima; the aggression responses reached over 60% at the dose of 10 μg. Both alkenes also enhanced the ant aggression against *Pieris rapae* larvae, which had a CHC profile qualitatively similar to *L. argyrognomon* and *L. phlaeas* larvae. Compared with these alkenes, 13-methylheptacosane weakly activated the ant aggression, while *n*-heptacosane and *n*-octacosane were less effective than 13-methylheptacosane. Moreover, the Hiroshima workers intensively attacked *Papilio polytes* larvae, CHCs of which were dominated by a series of homologous 7-alkenes. These results indicate that the CHC profiles, consisting of *n*-alkanes and lacking alkenes and branched alkanes, make larvae chemical insignificant to ants, which leads to the reduction of predation risk.

Bursaphelenchus xylophilus is killed by homologues of monochamol, 2-(1-undecyloxy)-1-ethanol

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2-(1-Undecyloxy)-1-ethanol, monochamol, is a male-produced aggregation pheromone of the *Monochamus* species, which are efficient vectors of the pine wood nematode (PWN), *Bursaphelenchus xylophilus*, which cause devastating damage to pines worldwide. The nematicidal activity of synthetic monochamol and its homologues (ROEtOH: R=C_7-C_{13}) were investigated to find potential alternatives to the currently used PWN control agents abamectin and emamectin. Compounds with C_7-C_{13} chain length alkyl groups exhibited 100% nematicidal activity at a concentration of 1000 mg/L. At a concentration of 100 mg/L, 2-(1-nonyloxy)-1-ethanol (C_9OEtOH), 2-(1-decyloxy)-1-ethanol (C_{10}OEtOH), 2-(1-undecyloxy)-1-ethanol (C_{11}OEtOH), and 2-(1-dodecyloxy)-1-ethanol (C_{12}OEtOH) showed 100% nematicidal activity, but the others showed weaker activities. C_{11}OEtOH showed similar nematicidal activity to abamectin in terms of LD_{90} values, which were 13.30 and 12.53 mg/L, respectively. However, C_9OEtOH, C_{10}OEtOH, and C_{12}OEtOH (LC_{90} values: 53.63, 38.18, and 46.68 mg/L, respectively) were less effective than C_{11}OEtOH and abamectin. These results indicate that 2-(1-undecyloxy)-1-ethanol, monochamol, could be an effective alternative agent against PWN. The relationship of insecticidal and nematicidal activity to different carbon chain lengths in compounds is discussed.

Keywords: 2-(1-alkyloxy)-2-ethanol, 2-(1-undecyloxy)-1-ethanol, pine wilt disease, pine wood nematode, Monochamus, nematicide, monochamol

Different feeding responses of two herbivorous ladybirds *Henosepilachna vigintioctomaculata* and *Henosepilachna vigintioctopunctata* to a solanaceous plant *Physalis alkekengi*

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Two herbivorous ladybirds *Henosepilachna vigintioctomaculata* and *H. vigintioctopunctata* are known as pests of solanaceous crops. *Henosepilachna vigintioctomaculata* is distributed in northern part of Japan, whereas *H. vigintioctopunctata* is found in the southern part. While host plants are almost same for these two ladybirds, the feeding response of *H. vigintioctomaculata* to *Physalis alkekengi* differs from that of *H. vigintioctopunctata*; *H. vigintioctopunctata*, but not *H. vigintioctomaculata* can feed on *Physalis alkekengi*. Therefore, we studied why feeding responses of these two ladybirds are different. *Henosepilachna vigintioctomaculata* did not feed on host leaf (tomato) treated with the methanol extract of *P. alkekengi*. Therefore, the chemical factors of *P. alkekengi* may prevent *H. vigintioctomaculata* from feeding. It was reported in a previous study that a feeding stimulant for the two ladybirds is contained in *P. alkekengi*. These results indicated that strong feeding deterrents superior to the feeding stimulant are present in *P. alkekengi*. The methanol extract of *P. alkekengi* was fractionated with chloroform and water. *Henosepilachna vigintioctomaculata* was deterred from feeding on tomato leaf discs treated with both fractions. The chloroform soluble fraction was fractionated with hexane and methanol saturated with hexane. The methanol saturated with hexane fraction showed strong feeding deterrent activity. Then the fraction was fractionated into 5 fractions via silica-gel column chromatography. Fraction 4 showed the strongest feeding deterrent activity. Fraction 4 was then fractionated into 4 fractions by using silica-gel chromatography and fraction 4-2 showed the strongest feeding deterrent activity. In contrast, *H. vigintioctopunctata* was not deterred from feeding on leaf discs treated with any fractions. These results indicated that different feeding responses of the two ladybirds to the deterrents underlie their different feeding responses to *P. alkekengi*. Further separation of the extract led to reduce activity; therefore, the strong feeding deterrence in the case of *H. vigintioctomaculata* may have been due to combined effect of different feeding deterrents.

S11-P-118

Chlorophyll degradation in a Lepidopteran pest *Spodoptera littoralis* is mediated by a gut-specific Chlorophyllide binding protein

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Chlorophyll is a natural pigment from plants that is synthesized and conjointly degraded in an enormous number. Chlorophyll is well studied in plants whereas there are limited findings on other organisms. As insect herbivores are organisms that naturally consume leaves and thus chlorophyll, they provide a good start to learn the degradation mechanism of Chlorophyll in non-plant organisms. Our previous studies using the notorious lepidopteran pest species, the Egyptian cotton leaf worm (*Spodoptera littoralis*) have shown that i.) Regurgitate has catalytic activity to degrade chlorophyll and ii.) Catabolites were detected inside the gut and frass (Chlorophyllide, Pheophorbide, and Pyropheophorbide). In the present study, the suspected liable protein – Chlorophyllide binding protein (CHBP) – has been identified using transcriptome and proteome analysis from regurgitate of *S. littoralis*. A similar gene was also found in other lepidopterans, such as *Bombyx mori* and *Helicoverpa amiverga*. First experiments on the gene expression level in different developmental stages and tissues indicate that CHBP is mainly expressed in the digestive tract in the 5th instar. Furthermore, transcript silencing via RNAinterference was applied to larvae and indicating a metabolite change as detected by LC/MS. We also found a lower survival rate in larvae injected with gene specific dsRNA, where gene expression was decreased up to 80%. Heterologous expression of CHBP in insect cells will reveal the substrate of CHBP in vitro, whereas the protein purification from regurgitate will show comprehensively the enzyme activity in each fractions. This study may have an impact in herbivore-plant interactions considering CHBP's role in survival rate, prospectively it would be of importance in agriculture to fight against lepidopteran pests.

S11-P-119

Hydrogen peroxide as a new defensive compound among “benzoyl cyanide” producing Polydesmida

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Most of cyanogenic polydesmid millipedes (53 species among 58 species so-far examined worldwide, Makarov 2015; Shear 2015) produce mandelonitrile, which is then decomposed mainly by two enzymes [hydroxynitrile lyase (HNL, characterized by Dadashipour et al. 2015 and responsible for producing not only HCN and benzaldehyde but also benzyl alcohol and benzoic acid), and mandelonitrile oxidase (MOX, characterized by Ishida et al 2016, and responsible not only for production of benzoyl cyanide but also subsequent generation of mandelonitrile benzoate and HCN via the Schotten-Bauman reaction between mandelonitrile and benzoyl cyanide, together with hydrolytic generation of benzoic acid and HCN from benzoyl cyanide)]. MOX is a flavine enzyme, and H_2O_2 is known as a by-product of the MOX reaction (Ishida et al. 2016), but its presence as a component in millipede defensive secretions has been obscure.

The discharges of H_2O_2 upon disturbance of 11 Japanese Polydesmida and a “benzoyl cyanide” producing centipede were qualitatively examined using Trinder reagent (modified from Barham and Trinder1972). Simultaneous detection of H_2O_2 (by spray of Trinder reagent) and HCN (by alkaline picrate paper) were tried for smears of defensive secretions collected by a filter paper from three species *Parafontaria falcifera*, *Chamberlinius hualienensis, and Riukiaria semicircularis semicircularis.* Correlation ships between H_2O_2 (colorimetric determination by Trinder reagent at 555 nm) and benzoyl cyanide (by GC/MS) were confirmed using *P. laminata* and *P. falcifera*.

H_2O_2 was newly and simultaneously demonstrated with well-known HCN as a component of defensive secretions of “benzoyl cyanide” producing Polydesmida. A total of 11 Japanese polydesmida (cosmopolitan and endemic) studied so far can be classified into three types; A (HCN emitters, 2 species), B (HCN and H_2O_2 emitters, six species and one stadium 1 nymph), and C (non-cyanogenic, two species and one adult). 18 species worldwide are suggested to belong to A, 30 species including species possessing mandelonitrile benzoate (down-flow component, more stable than benzoyl cyanide) to B, and five species to C. The other five species are excluded, because only HCN is known. As a whole, Polydesmida can be classified with a type ratio (%) of A:B:C = 34:57:9. More than half of Polydesmida worldwide are elucidated to possess the two enzymes system (HNL and MOX) for allomone production, indicating they are not only HCN producers but also H_2O_2 emitters.

Three species of centipedes belonging to the order Geophilomorpha are known as cyanogenic (Vujisić et al. 2013), and supposed to be H_2O_2 emitters. The Japanese *Strigamia* sp. was actually identified as H_2O_2 emitter by Trinder reagent.

Attraction of West Indian fruit fly *Anastrepha obliqua* (Diptera: Tephritidae) to mango *Mangifera indica* cultivars.

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The selection process by herbivorous insects encompasses several steps as host location, recognition and acceptance. The location of suitable host for a female is of crucial importance for the development and survival of their offspring. The knowledge of the interaction of West Indian fruit fly *Anastrepha obliqua* with their hosts is scarce, but is known that females prefer to lay their eggs in Anacardiaceae fruits, especially mango, *Mangifera indica*. The aim of this study was to understand the chemical ecology of this fruit fly. To accomplish this goal we collect volatiles of three mangoes cultivars with different degrees of female host plant preference “Carlota” (preferred), "Keitt" (intermediate) and "Espada" (non preferred). We collect the fruit headspace volatiles at the third stage of fruit ripening in filters of Super-Q®, which was subsequently washed with hexane. The response of females of *A. obliqua* to these volatiles was evaluated in a “Y” olfactometer, oviposition and GC-EAD. Because these mango cultivars show different amounts of natural resin, we also test the survival of the embryos and larvae in fruits of those cultivars. To assess the viability of the embryo eggs were placed "Carlota" and "Espada" resin for periods of 1, 3, 9, 17 and 24 hours. Then, the eggs were fixed and dehydrated in aqueous ethanol for histological evaluation of the embryo. The first instar larvae were conditioned to different diets, containing skin, pulp, resin of "Carlota" or "Espada" for a period of 96 hours. The olfactometer results shows that the females prefer volatiles of the cultivars when contrasted with control (hexane). When the volatiles of cultivars were contrasted two-by-two there is a clear preference to “Carlota” and no preference to "Keitt" or "Espada". In the oviposition test, females showed the same preference found in the olfactometer. The GC profiles are distinct among cultivars, as well as the antennal response. Females respond to ten compounds from "Carlota" and five of "Espada" and "Keitt". All compounds that elicited antennal response were identified by GC-MS. In the survival test, embryos die when they were conditioned in resin of "Espada" showing total deterioration in 24 hours. However, when the eggs were exposed to resin of "Carlota" or the control (water) the embryos developed normally. Likewise, when larvae fed diets containing "Espada" resin the mortality was 100% contrasting with the normal development in "Carlota" and controls. The "Carlota" proved to be a good host for *A. obliqua* both in location, acceptance and development of offspring. The "Espada" seems to have a resistance by antixenosis and antibiosis to this fruit fly.

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Chemical analysis of bufadienolides in Japanese Lampyrine fireflies

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The majority of fireflies use bioluminescence to communicate and attract mates by sending optical signals. Interestingly, their larvae are also able to produce bioluminescence, and are known to contain toxic compounds. Thus, it has been considered that bioluminescence had originally evolved as a warning signal to predators, and then became to be used in mate selection. Actually, some species of the subfamily Lampyrinae in North America and Europe are known to contain cardiotonic steroids, called bufadienolides. Except for the Lampyrine species living in these areas, there are no reports of bufadienolides from fireflies.

We conducted chemical analysis on six Lampyrine species in Japan to examine whether these fireflies have bufadienolides. The analysis showed that all of them contain bufadienolides and the compositional patterns of bufadienolides were species-specific. Furthermore, through NMR spectroscopic and GCMS analyses, two novel bufadienolides were identified from *Pyrocoelia atripennis* larvae. One is a bufadienolide xyloside and the other is a bufadienolide glucoside. The xyloside conjugates have been found from North American fireflies. Although bufadienolide glucosides have been found from several plants, this is the first report of the identification of the bufadienolide glucoside from animals. These results suggest that the geographically separated fireflies sequester different kinds of bufadienolide glycosides.

Suppressive effects of egg mass shell on nymphal cannibalism in the gregarious assassin bug, *Agriosphodrus dohrni* (Signoret)

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The assassin bug *Agriosphodrus dohrni* (Signoret) has a strong gergariousness in the nymphal stages, of which cannibalism is often observed among the newborns in the natural population. Such nymphal cannibalism would be adaptive when it occurred against nymphs hatched from other egg masses and could available food were limited. To test this hypothesis, we confirmed the frequency of cannibalisms among newborn nymphs from the same egg mass under the existence of egg masses covered with sticky substances that the nymphs hatched from egg mass. All those egg masses were ovipoisted by adult female assassin bug that were collected in Kyoto City in 2012 and 2013. Ten nymphs from the same egg mass were reared together in container at 25°C, 12L12D under following conditions: (1) with a piece of the hatched egg mass shell, (2) with no egg mass shell, (3) with a piece of the another hatched egg mass shell, (4) with the extracted egg mass shell, (5) with the painted egg mass shell extraction for the extracted egg mass shell (N=10 each). Most nymphs survived until the 2nd-instar when presenting the hatched egg mass and the hexane extract. By contrast, 57-68% of the nymphs were cannibalized when presenting either the extracted egg mass shell or no egg mass shell, as well. These suggest that hatched egg mass shell, would involved hexane soluble substances that suppress have nymphal cannibalism especially in the newborns of this gregarious assassin bug.

RNA interference of odorant receptor 117 affects blood-feeding behavior in *Culex pipiens quinquefasciatus*

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Background: The OR plays a critical role for mosquitoes in the identification of the opposite sex and blood-feeding hosts and other physiologic process. In a previous unpublished study, high-throughput sequencing and real-time quantitative PCR were used to detect the olfactory genes of *Cx. pipiens quinquefasciatus* and *Cx. pipiens molestus*. The CquiOR117 that showed a high expression difference between the two subspecies was screened from the olfactory genes.

Aim/Objectives: To understand the function of CquiOR117 in the blood-feeding behavior of *Cx. pipiens quinquefasciatus*.

Methods: In this study, the expression of CquiOR117 in the different growth stages of *Culex pipiens quinquefasciatus* was detected by the amplification of CquiOR117 with real-time fluorescence quantitative polymerase chain reaction (PCR). RNAi technology was used to interfere with the expression of CquiOR117 in females to observe the blood-feeding behavior change.

Results: The results showed that the expression level of CquiOR117 in the egg-to-pupa stage was significantly lower than that in the adult stage and that the expression level of the female mosquitoes peaked on the third day after emergence. The expression of CquiOR117 was significantly decreased in the 2-4 days after the injection of dsRNA compared with the control groups. The analysis of the blood-feeding behavior showed a significant positive correlation between CquiOR117 expression and the engorgement rate of the mosquitoes.

Findings and conclusions: CquiOR117 is speculated to have a positive effect on the blood-feeding behavior of *Cx. pipiens quinquefasciatus*.

S11-P-124

A viral protease relocalizes in the presence of the vector to promote vector performance

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Vector-borne pathogens influence host characteristics relevant to host–vector contact, increasing pathogen transmission and survival. Previously, we demonstrated that infection with *Turnip mosaic virus*, a member of one of the largest families of plant-infecting viruses, increases vector attraction and reproduction on infected hosts. These changes were due to a single viral protein, NIa-Pro. Here we show that NIa-Pro responds to the presence of the aphid vector during infection by relocalizing to the vacuole. Remarkably, vacuolar localization is required for NIa-Pro's ability to enhance aphid reproduction on host plants, vacuole localization disappears when aphids are removed, and this phenomenon occurs for another potyvirus, *Potato virus Y*, suggesting a conserved role for the protein in vector–host interactions. Taken together, these results suggest that potyviruses dynamically respond to the presence of their vectors, promoting insect performance and transmission only when needed.

S11-P-125

Pea aphids (*Acyrthosiphon pisum*) reduce secretion of extrafloral nectar in broad bean (*Vicia faba*)

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Herbivores sometimes suppress plant defenses. We tested whether the presence of pea aphids (*Acyrthosiphon pisum*) on broad bean (*Vicia faba*) led to decreased secretion of extrafloral nectar (EFN), which functions as an indirect defense against herbivores. To determine effects of aphid-infestation on EFN secretion, we compared EFN levels in uninfested plants and plants infested by *A. pisum* and another aphid species (*Aphis craccivora*). When broad bean plants were infested by *A. pisum*, they secreted significantly smaller amounts of EFN than uninfested plants and *A. craccivora*-infested plants did. There was no significant difference in EFN secretion between uninfested plants and *A. craccivora*-infested plants. Furthermore, the number of extrafloral nectaries did not differ among the three treatments. These results suggest that *A. pisum* reduced production of EFN in broad bean plants.

Visualization of semiochemicals in action: Mass spectrometric imaging at ambient conditions

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To see distributions of semiochemicals on biological entity, or how cehemical signals are emitted into environment is of utmost importance for understanding of chemical communication. However, so far there a limited number of techniques available for fulfilling this needs exist and no one is universal. Currently, mass spectrometry-based visualization and imaging of small molecules becomes one of the modern methods in chemical ecology and our laboratory contributed to this area significantly [1-4].

However there are still numerous problems waiting for solution. 1) To guarantee optimal laser focus during laser-based ionization experiments, the sample is required to be as flat as possible. When analyzing surfaces of insects, bacterial colonies or plants, the requirement of flatness is rarely met. So far, the effects of surface topography on consistent laser ablation in mass spectrometry imaging are largely ignored. Profilometry offers the possibility guide laser ablation in the 3rd dimension by acquiring height profiles of surface topography prior to laser-based ionization experiments [5].

A telescope infrared-laser optic was employed in a custom-built laser ablation electrospray ionization source to focus an OPO-pumped pulse laser operating at 2940 nm wavelength. Height profiles of *Raphanus sativus* (radish, piccolo cultivar) leaves were acquired with a lateral resolution from 200 µm down to 10 µm using the confocal sensor. Acquired height profiles were applied to perform metabolic profiling/imaging on radish leaf surfaces with topographic features differing in height up to 1400 µm. Consistent laser ablation was performed with a laser-ablation-mark diameter of 70 µm in average. Without topographic guidance, ablation mark diameter doubled within 200 µm above and below the focal plane. All processes are integrated in LabVIEW software and the measurements are executed automatically. Using R scripts, the resulting data files were integrated into a data cube for visualization and analysis.

Novel Aspect

IR-Laser ablation can be guided in the 3rd dimension to overcome the influence of surface topography on laser focus for consistent laser ablation marks size in mass spectrometry imaging experiments.

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Thistle phylogeny determines volatile-mediated host plant recognition in the weed biocontrol agent *Cassida rubiginosa*

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The oligiphagous leaf beetle *Cassida rubiginosa* was introduced to New Zealand in 2007, mainly to limit the impact of the noxious weed *Cirsium arvense* (Californian thistle). The beetle prefers *C. arvense* as its primary host plant, but also feeds on other thistles of the Cardueae tribe. In contrast to what might be expected, this is not considered a non-target risk but a desirable trait since all thistle species in New Zealand are introduced and therefore (potentially) invasive. The aim of this study was to test the suitability of related thistle species as hosts, which will allow for predictions on the likelihood of positive spill-over effects. To assess the beetle's host preferences and the chemical traits that determine long range host finding, a series of phylogenetically controlled experiments were carried out. In cage experiments, sixteen Cardueae and three Asteraceae species from other tribes were tested for their acceptability as oviposition substrates for female beetles and as food plants for supporting larval development. Furthermore, no choice and dual choice olfactometer experiments were carried out to examine the role of thistle volatiles in selecting suitable hosts. Using generalized linear model analyses, our results from the no choice experiment show a strong preference towards plants that belong to the same genus as the primary host and to *Carduus tenuiflorus*, which belongs to a closely related clade. In the dual choice experiment, a significant negative correlation was found between phylogenetic distance to the tested thistle species and the olfactory attraction to thistle volatiles. That is, the more closely related the tested thistle species were to *C. arvense*, the more beetles were attracted. These findings indicate that thistle volatiles play a pivotal role in long-range host finding and that the composition of thistle volatiles is determined by phylogeny. Thistle volatiles were collected and will be analysed to confirm this hypothesis. Our results further suggest that *C. rubiginosa* could potentially extend its host range to other thistles in New Zealand.

Development of food-based baits for spotted wing drosophila, *Drosophila suzukii*

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The spotted wing drosophila, *Drosophila suzukii* is a highly invasive species attacking a wide range of ripening soft-skinned fruits, and has caused millions of dollors of crop loss. A series of laboratory choice tests were conducted to determine whether different levels of rice vinegar affect attraction of Merlot wine to *D. suzukii*, as well as to compare attractiveness of two common fermentation food baits: wine-vinegar and yeast-sugar water mixtures. The relative attraction of various combinations was used to develop a bait whose effectiveness was tested in the field. In laboratory choice experiments, wine-vinegar (80:20, v:v, hereafter referred to as wine) was more preferred over a yeast-sugar water mixture (hereafter referred to as yeast) by *D. suzukii.* Combination baits, either a mixture of wine and yeast or a mixture of wine and a supernatant from the yeast (comboS), were significantly more attractive than each product alone. The two combination baits were equally attractive to *D. suzukii*, so were the yeast and its supernatant, suggesting that yeast supernatant could be used as a replacement for the yeast sugar mixture currently used for trapping *D. suzukii*. The additive effect between wine and yeast supernatant in the field was not as profound as observed in the laboratory. In the field trial, numerically more male and female *D. suzukii* were captured in traps baited with comboS than those baited with the wine or yeast alone, however, significant differences were only found between the comboS and wine or between the comboS and yeast in some weeks over the period of the experiment.

Evidence for a contextual olfactory avoidance of the ubiquitous phytopathogen *Botrytis cinerea* by *Drosophila suzukii*

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Herbivorous insects may benefit from avoiding the smell of phytopathogens that can reduce insect fitness. However, phytopathogens are often ubiquitous and can colonize plant tissues that are either relevant or not relevant for insect fitness. We hypothesize that these insects may further benefit by focusing on the odor from pathogens on “relevant” plant tissue, as paying extraneous attention to “irrelevant” pathogen odor may cause neural trade-offs that incur fitness costs. In this study we showed that *Drosophila suzukii*, a serious invasive frugivore, avoided the odor of *Botrytis cinerea*, a ubiquitous phytopathogen, when cultured on a host tissue, raspberry fruit, and oviposited fewer eggs on the infected fruit. These results were associated with significantly fewer and smaller adults that emerged from *B. cinerea* infected raspberries. When the avoidance experiment was repeated comparing uninfected raspberry fruit plus a strawberry leaf, a tissue attractive to *D. suzukii* but irrelevant for its fitness, with raspberry plus a *B. cinerea* infected strawberry leaf, *D. suzukii* showed no differences in attraction. This highlights a novel role that context can play in mediating evolution of insect-plant-microbe interaction. We further discuss implications of our findings for developing a deterrent that can be useful for the *D. suzukii* management.

Using odour to understand the ecology of the small hive beetle (*Aethina* tumida), a pest of European honeybees

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European honeybees (*Apis mellifera*) are critical pollinators for crops around the world. Bees are currently under threat from many pests and diseases, including the small hive beetle (*Aethina tumida*). The larval beetles feed on hive products which they contaminate with a yeast, which is responsible for the fermentation and destruction of these products. The odours of these fermenting hive products are very attractive to adult beetles. Our GC-MS analyses of these odours have shown that they consist of typical fermentation volatiles, many of which are known to be attractive to insects. Final instar larvae of these beetles emerge from the hive and pupate in the soil. Pupation time varies, but the environmental cues used by the beetle to time emergence are poorly understood. Our laboratory studies suggest a link between soil moisture and beetle emergence. To understand the movement of adult beetles in the field and link these emergences to environmental cues such as temperature, relative humidity and rainfall we developed a simple trapping system. Our system is based on a fermenting honey solution and the commercially-available yeast *Saccharomyces cerevisiae*.

In parallel we are developing an economical, synthetic lure for an *A. tumida*-specific trapping system in apiaries. The ecological data gathered with the simple yeast traps will inform the development of such a system. Monitoring of beetle movement, and the correlation of beetle behaviour with environmental conditions allows targeted trapping to be deployed by commercial and hobbyist beekeepers, reducing the need for year-round monitoring and control. This research has the potential to inform further applied work in controlling this economically important pest.

S12-P-133

LAESI-MSI as a tool to differentiate the root metabolome of native and range expanding plant species

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Our understanding of chemical diversity in biological samples has greatly improved through recent advances in mass spectrometry (MS). MS-based-imaging (MSI) techniques have further enhanced this by providing spatial information on the distribution of metabolites and their relative abundance. This technique can be applied to study different biological samples ranging from single cells to intact tissue sections. However, some ionization approaches employed in MSI are operated under vacuum, need sample preparation and use a matrix. These can somehow alter the biochemical status of the sample under study.

We employed laser assisted electrospray ionization (LAESI), to perform MSI and study the metabolomic diversity and differences in intact root samples of range-expanding *Centaurea stoebe* and native plant species *Centaurea jacea.* Here, we tested the hypothesis that successful range-expanders have more unique chemistry than related native species.

To examine this, LAESI-MSI was performed in positive ion mode and data was acquired in a mass range of *m/z* 50-1200 with a spatial resolution of 100 µm. The acquired data was analyzed using in-house scripts written in R and localization patterns were studied for identified discriminatory mass features.

The results revealed clear differences in the metabolite profiles for the two species, in the form of distinct metabolic fingerprints. The results are in-line with previous study performed using gas chromatography (GC)-MS and direct analysis in real time (DART)-MS. The differences in root chemistry explain the observed differences in root-feeding nematodes preference and performance on the range-expanding *C. stoebe* and the native plant species *C. jacea.*

S12-P-134

Cultural control in Switzerland continues to be a sustainable strategy for *Diabrotica v. virgifera* containment - long term field research from 2001 to 2016

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Trapping data of the *Zea mays* pest *D. v. virgifera* LeConte (Chrysomelidae) (*Dvv*) were taken with highly sensitive Metcalf type sticky traps baited with 0.1 mg of synthetic sex pheromone 8-methyl-decane-2-ol propanoate. Over the course of a 15 year monitoring period between 2001 and 2016, basically the same spatial and temporal trapping protocol was followed. We wanted to establish a reliable long term population trend survey for *Dvv* which resulted from mandatory 1:1 year crop rotation in maize cultures employed since 2004. *Dvv* migrates from maize fields located in Lombardy/Northern Italy and touches on its flight path between calendar week 25 to 39 (with peak in week 30-31) a series of about 29 trapping points within the adjacent Ticino Canton. These trapping stations were established roughly along a line running southeast to northwest, following a route along 10 Ticino districts. Long term trapping data from the labs of ACW Cadenazzo and the Phytosanitary Service at Bellinzona were combined to yield a comprehensive data set. From it, the following conclusions could be drawn:

(1) Due to the mandatory crop rotation system, the beetle density remained fairly constantly and significantly below the economic threshold for *Dvv* in maize, as the beetle density averaged around 0.69 beetles/trap/day (from 2004-2016).

(2) For the entire Canton Ticino, *Dvv* beetles migrating from south to north first appear in the traps starting in calendar week 25 (in the south) and disappear in week 39 (again in the south), with a maximum around weeks 30-31. Whereas in the south beetles can be found in the traps in a high number over the whole observation period, in the northern traps the number of beetles is much lower and beetles only appear for a few weeks during the main observation peak.

(3) Monitoring program financially feasible, as overall yearly costs are very low: 600 highly sensitive METCALF-traps (own production): ~100 €; 2 field excursions per week for trap inspection: ~10.000 €; student research assistant: ~30.000 €.

We have now a fairly reliable predictive population model linking enforced crop rotation with efficient and sustainable cultural control of *Dvv*. It is eminently suitable for the special case of the Ticino, but might be also adapted to other landscapes. As a welcome consequence, the strategy can also curb the export of beetles to the Swiss cantons situated north of the main Alpine chain and points beyond.

Potential sexual attractants for control of *Philornis downsi* an avian parasite in the Galapagos Islands.

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Since its introduction to the Galapagos Islands, the parasitic fly *Philornis downsi* has caused high levels of mortality in several species of endemic birds like the Floreana mockingbird, the medium ground finch, and the mangrove finch which are critically endangered. Mortality is caused by blood loss in chicks, which often cause the death of up to 100% of the offspring. In some insects, sex pheromone production and release is often associated with the genital region of the abdomen and in some cases in lipids of the cuticle of hydrocarbon. On the base of this information is intended to identify a potential attractant produced by *P. downsi* that could be used as a tool in the monitoring and potential control of this parasitic fly in the future.

We used 1440 flies, to obtained cuticles of hydrocarbon and genital extracts, in both female and male flies, as well as flies raised in the laboratory and captured in the field. In each trap, we used a sample composed of 10 extracts of flies extracted in Hexane (45 sec for the cuticle and 24 hours for the genitals). Treatments included A, B, C, D, E, F, G and H: Cuticular and genital extracts of female and male flies, both wild and laboratory breeding. We combined the extracts with papaya juice, previously tested as a good food attractant for Philornis, and a control trap which only contained papaya juice.

The results obtained in the field, suggest that the control traps captured fewer flies than the treatments. Treatments with extracts of genitalia from females caught in the field and extracts of cuticular of hydrocarbons from females and males raised in the laboratory captured more flies. In all the traps, more females were captured, however this seems not to be due to the treatments, since at the time of the monitoring the number of females seems to be higher. The results seem to suggest that the cuticular and genital extracts probably contain a likely sexual attractiveness potential, which once identified could be used in conjunction with synthetic food odors to produce a potent attractant.

S12-P-136

Structural and biological characterization of antimicrobial peptides in the venom of the scorpion *Isometrus maculatus*

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Scorpion venom is a rich source of bioactive peptides, most of which are neurotoxic to mammals, insects or crustaceans. These peptides play important roles in the scorpion venom to capture preys or to defend against predators. In addition to these toxins, many antimicrobial peptides (AMPs) have been identified from the scorpion venom in recent years. Although biological roles of AMPs in the scorpion venom are not completely understood, it is reasonable to assume that they are necessary to protect the venom gland from microbial infection. Furthermore, it has been known that AMPs show insect toxicity and/or enhance the actions of other neurotoxins in the venom by disrupting the cellular membrane structure. Thus, scorpion AMPs attract increasing interest due to their diverse biological functions and potential therapeutic applications. The scorpion *Isometrus maculatus*, which belongs to the Buthidae scorpion family, is found in tropical regions throughout the world including Japan. In this study, we searched for novel AMPs in the *I. maculatus* venom based on the structural features that are commonly observed in scorpion AMPs. Their structures were determined by the mass spectrometry (MS)-based method, referred to as *de novo* sequencing.

Most of scorpion AMPs are non-disulfide-bridged peptides, and possess relatively hydrophobic nature. Based on this fact, we found novel AMPs from the *I. maculatus* venom using a following strategy: (1) collection of hydrophobic components by RP-HPLC, (2) selection of linear peptides by comparing molecular masses before and after reduction and alkylation of cysteine residues, (3) *de novo* sequencing of candidate peptides, including Leu/Ile discrimination, (4) chemical synthesis of identified peptides to confirm their structure and activity. *De novo* sequencing was performed using both MALDI-TOF/TOF and IT-TOF mass spectrometers. The combination of two MS analysis allows the confident determination of peptide sequences. As a result, three AMPs (Im-4, Im-5 and Im-6) were successfully identified. These peptides were synthesized, and their antimicrobial activity was evaluated. Im-4 and Im-6 exhibited the significant antimicrobial activity against *S. aureus* and *B. subtilis* (Gram-positive) but not against *E. coli* (Gram-negative). Unlike these peptides, Im-5 showed the activity against all bacterial species tested. Their insecticidal activity was then evaluated against crickets. Only Im-5 showed insecticidal activity among three peptides. To gain insight into the mechanisms of the insecticidal action of these peptide, insect cell lytic activity was examined. Im-5 induced cell death in about 50% of treated cells after incubation at 100 μM for 1 h, but Im-4 and Im-6 did not. This suggests that the main cause of insect toxicity of Im-5 is cell lysis of insect body tissues, such as muscles or neuronal systems.

Im-4: **FIGMIPGLIGGLISAIK**-$\mathrm{NH_2}$
Im-5: **FLGSLFSIGSKLLPGVIKLFQRKKQ**
Im-6: **FFFLPSLIGGLVSAIK**-$\mathrm{NH_2}$

Freeze-thaw sample preparation method improves the detection of volatile compounds in insects using headspace solid-phase microextraction (HS-SPME)

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Headspace solid-phase microextraction (HS-SPME) coupled with gas chromatography–mass spectrometry (GC-MS) is commonly used in analyzing insect volatiles. In order to improve the detection of volatiles in insects, a freeze-thaw method was applied to insect samples before the HS-SPME-GC-MS analysis. Insect samples were first frozen at -80 ºC for 10 min and then thawed at 25 ºC for 5 min before SPME extraction was performed. The freeze-thaw method clearly improved the detection of volatile compounds for all six tested insect species, including red imported fire ants, *Solenopsis invicta* Buren, black imported fire ants, *Solenopsis richteri* Forel, little black ants, *Monomorium minimum* (Buckley), pharaoh ants, *Monomorium pharaonis* (Linnaeus); eastern subterranean termites, *Reticulitermes flavipes* (Kollar); and spotted lady beetles, *Coleomegilla maculate* De Geer. This method helped identify various volatile compounds in the tested insects, which have never been reported previously. This improved method may facilitate the identification of insect derived volatiles, such as insect semiochemicals.

S12-P-138

Transcriptomic analysis of *Varroa* mite olfactory organs: Focus on chemosensory machinery

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Chemosensing is a primary sense in nature, however little is known about its mechanism in Chelicerata. We focused our study on, a specific parasitic mite of honeybees *Varroa destructor*, today's main problem in bee-keeping. To reveal the components of the chemosensory mechanism we used a transcriptomic analysis of the mite's main olfactory organ in the foreleg followed by a search for the presence of conserved domains of the known olfactory proteins. This approach revealed transcripts of chemosensory related proteins belonging to several groups: Sensory neuron membrane proteins (SNMPss), odorant binding proteins (OBPs), Niemann-Pick disease proteins, type C2 (NPC2), gustatory receptors (GRs), and ionotropic receptors (IRs). Trancripts of SNMPs and OBPs, were found for the first time in Chelicerates. As expected, no insects' odorant receptors (ORs) and odorant co-receptors (ORcos) were found. On the other hand, a phylogenetic analysis of IR-family revealed a new Acari IR-related group which is absent in *Drosophila melanogaster*. In addition, a homolog of the most ancient IR co-receptor, IR25a, was identified in Varroa as well as in other members of Acari. The high expression of this transcript in the mite's forelegs, while not detectable in the other pairs of legs, suggests a function for IR25a in Varroa chemosensation.

Electrophysiological responses of the male moth antenna to compounds found in the female sex pheromone of *Loxostege sticticalis*

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Abstract: The EAG (Electroantennography, a bioassay instruments, which are widely used in the experiment in entomology, it is mainly used for detecting the insects to perceive the world by antenna) responses of the male moth antenna to different combinations, proportions, and doses of the sex pheromone of *Loxostege sticticalis* Linnaeus were observed in the laboratory. The results revealed that component A (E11-14: AC), the binary blends of AB (E11-14: AC; E11-14: AL) and AD (E11-14: AC; 14: OH), and the trinary blends of ABE (E11-14: AC; E11-14: AL; E11-14: OH) produced an EAG response that was significantly higher than that of n-hexane and air control, while 10 female moth gland extracts of EAG values did not differ significantly. The trinary blends of ABC (E11-14: AC; E11-14: AL; 14: AC), when in ratios of 5:3:9 and 5:3:12, produced significantly higher levels of EAG response than those in other ratios of the trinary blends. A dose-response test for ABE blend in ratio of 5:3:12 showed that the EAG values climbed quickly after the dose reached 1 μg/μL and differed entirely from the EAG values of other doses.

S13-P-140

Neuro-modulatory components of behavioral state-dependency: antennal gene expression profiles as a function of larval age in a Chagas disease vector

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- Background. Triatomines experience a behavioral maturation process along the first week after their ecdysis. Immediately following ecdysis, these insects are refractory to host cues and do not blood-feed. Nevertheless, this situation is transient, as they gradually increase their orientation to CO_2 and heat, as well as their tendency to blood-feed or mate (the latter in the case of adults). Neuropeptides (NPs) and biogenic amines (BAs) can modulate sensory physiology, and consequently behavior, i.e., they convey messages to tissues expressing specific G-protein-coupled receptors (GPCRs) informing about nutritional, reproductive or developmental changes affecting the organism.

- Aim/Objectives. To characterize the antennal expression profiles of genes coding for: enzymes that mediate BA synthesis, NPs, and GPCRs detecting them, through the first week after the ecdysis of *Rhodnius prolixus* 5th instar larvae.

- Methods. A transcriptome of 5th instar larvae antennal samples (seven RNA samples per age point: 0, 2, 4, 6 and 8 days after ecdysis) was sequenced using Illumina HiSeq-2000. The corresponding results from the 35 libraries were analyzed with the CLC genomics software.

- Findings. The expression of neuropeptide genes such as tachykinin, allatostatin-CC, corazonin and allatostatin-B (isoform a) increased significantly during the first week after ecdysis, while others like eclosion hormone and ionic transport peptide showed a significant decrease in antennal expression with increasing age. Furthermore, several GPCRs showed significant changes in antennal expression with larval age.

- Conclusions. A broad repertoire of genes coding for signaling molecules (or their synthetic pathways) and their receptors has been shown to be expressed in the antennae of a model insect, opening an avenue to study the peripheral regulation of sensory processes. The relevance of these results will be discussed in the context of state dependent behavior.

S13-P-141

Identification and expression pattern analysis of chemosensory receptor genes in the *Anoplophora chinensis* (Foster)

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The *Anoplophora chinensis* (Forster) (Coleoptera: Cerambycidae) is a destructive native pest that infests a wide range of broadleaved plants. The species is native to East Asia, has recently spread to Europe causing severe damage to ornamental and forest trees. Olfaction plays a large part in host-locating, mating, foraging, and oviposition of *A. chinensis.* We use antennal transcriptome sequencing information for *A. chinensis* to identify chemosensory gene. We obtained 64787784 clean reads, and 44938 unigenes were assembled from 89331 transcripts within the analysis of transcriptome. We identified 54 putative odorant-binding protein (OBP) genes, 14 chemosensory protein (CSP) genes, 57 olfactory receptor (OR) genes, 5 ionotropic receptor (IR) genes, 20 gustatory receptors (GR) and 3 sensory neuron membrane protein (SNMP) genes. The expression profiles of 57 ORs, 20GRs, 5IRs were built by quantitative real-time PCR (RT-qPCR) for the antenna, abdomen, leg, Wing, maxillary palp, labipalp of male and female *A. chinensis.* These results may establish a solid foundation for exploring olfactory olfactory process and provide a new approach for pest control.

Keywords: *Anoplophora chinensis* (Forster); antennal transcriptome; chemosensory gene; expression pattern

A universal insecticide from an Indian medicinal plant

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Owing to environmental hazards and health concerns, as well as the evolution of resistance in insect pests, there have been constant efforts to discover new biopesticides from natural origins. We have recently isolated a plant-derived compound from an Indian medicinal plant *Artemisia pallens* that serves as a broad spectrum fumigant insecticide across several insect orders. Electroanntenogram recordings revealed that the chemosensory systems of diverse insect species, ranging from Archaeogantha to Dipterans can also detect this volatile.

With the aim of understanding mode of toxicity and conserved chemosensory detection of this compound across all insect orders, we used various approaches such as toxicology and behavioural bio-assays of several species; neurogenetics, and electrophysiological recordings from insect olfactory sensory neurons (OSNs). To confirm how this compound interacts with insect chemoreceptors, we examined the response of various chemosensory mutants and heterologously-expressed insect ORs in HEK293 cell lines using calcium imaging.

This plant derived compound is able to activate all OSNs expressing the ORx/ Orco complex in *Drosophila melanogaster* except for highly specialised olfactory receptors such as pheromone OSNs. IR and GR-expressing cells do not respond, nor do Orco-null mutants or receptor-specific knockouts. Behavioral assays also indicate that *Drosophila* is repelled by compound. The mode of toxicity, however, is different from olfactory detection, correlating to activation of Na^+/K^+ ATP-ase via fumigation and not via topical application or injection.

To conclude, we have identified a single chemical compound from an Indian traditional medicinal plant that acts both as a broad-spectrum fumigant as well as ligand for the chemosensory system of all known orders of flying insects. Further bioinformatic studies will help elucidate the conserved activity of this compound over millions of years of evolutionary divergence.

S14-P-144

Ivermectin modulation of the pH-sensitive chloride channel

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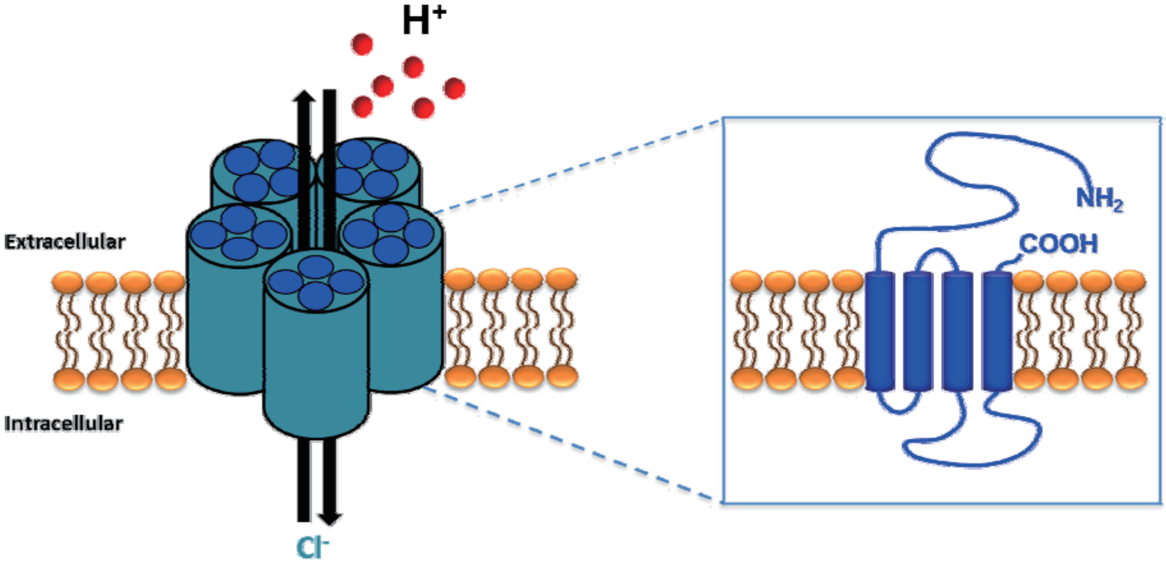
The insect pH-sensitive chloride channels (pHCls) are expressed in the brain and central nervous system etc, but little is known about their sensitivity to insecticides acting on chloride channels. Hence we have sequenced the pHCl-1 gene transcripts in the larvae of silkworm *Bombyx mori* to show that more than 50 variants were expressed with distinct splicing in the third thoracic ganglion as compared to the brain and midgut. Since variant 9 was expressed most abundantly in the larvae, it was expressed in *Xenopus laevis* oocytes for characterization. The variant 9 formed a functional pHCl, and was activated by ivermectin irrespective of the extracellular pH in contrast with variant 1, which was activated more profoundly at acidic pH. By site-directed mutagenesis, a key amino acid responsible for such differential ivermectin sensitivity was identified. Homology models of the *Bombyx* pHCls were constructed to explain the role for the pHCl amino acid determining ivermectin sensitivity.



Natural enemy attraction to herbivore induced asparagus volatiles

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Natural enemies are important regulators of pest pressure in managed and natural ecosystems, and often rely on volatile chemical cues to navigate complex landscapes in search of prey or hosts. Herbivore induced plant volatiles (HIPVs) have been a focal point in many studies that seek to increase efficacy of biological control programs by increasing recruitment and retention of natural enemies using volatile lures. Our research sought to explore the role HIPVs are playing in asparagus agroecosystems to determine: 1) are HIPVs being produced by asparagus ferns following feeding by common asparagus beetle larvae, 2) are convergent lady beetle or asparagus beetle adults attracted to asparagus volatiles, and 3) do field deployed lures baited with asparagus HIPVs attract natural enemies. Volatiles were field collected from the headspace of healthy asparagus ferns, mechanically damaged ferns, and ferns fed upon by asparagus beetle larvae. We found that asparagus beetle damaged asparagus had significantly higher concentrations of (*E*)-β-ocimene, (*E*,*E*)-α-farnesene, and (1)-tetradecanol than healthy or mechanically damaged ferns. In y-tube choice tests, we evaluated the attraction of adult convergent lady beetles and asparagus beetles to asparagus volatiles and odors from asparagus beetle larvae, and found evidence of chemotaxis by lady beetles to asparagus volatile isolates and asparagus beetle larvae. Lures using asparagus HIPVs were developed and deployed in commercial asparagus fields and significantly attracted more parasitoid wasps than controls, but did not attract to predators or key asparagus pests. Our results indicate that feeding by asparagus beetle larvae results in production of HIPVs in asparagus and that lures developed from isolates of asparagus HIPVs increase recruitment of parasitoids under field conditions.

Research on biology and attractant of *Rhagoletis batava obseuriosa* (Diptera: Tephritidae), a serious fruit fly of sea-buckthorn

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[**Background**] *Hippophae rhamnoides* (Elaeagnaceae), sea-buckthorn, distributes in many places in China and has functioned as a soil and water conservation tree species. Since its high economic values on Chinese medicine and health care, *H. rhamnoides* has also been planted in several provinces, including Xinjiang, Inner Mongolia, Heilungkiang Province. The fruit fly, *Rhagoletis batava obseuriosa* Kol. (RBO) (Diptera: Tephritidae), is a serious fruit borer of *H. rhamnoides*, and has caused heavy economic losses to local farmer.

[**Objectives**] In order to monitor and control RBO, we have been studied its biology and chemical ecology since 2013.

[**Methods**] The life cycle and biological characteristics of RBO, including eclosion, mating, oviposition, pupation and population dynamics were studied through laboratory and field observation in Dengkou county, Inner Mongolia, China. The attractants of RBO were screened from well-known male lures and food-based attractants of fruit flies.

[**Finds**] There was one generation of the RBO per year in Dengkou county, Inner Mongolia, but almost fifty percent of adults emerged in the third year. Adults occurred from the middle June to the early August. About 81.3% of adults emerged from the time 6:00 to 10:00, and the peak time was 8:00 to 9:00. The larvae came out of fruit and pupated mainly in the early morning from 0:00 to 6:00, especially from 3:00 to 6:00. The mating time mainly occurred in the daytime and there were two peaks from 12:00 to 13:00 and 16:00 to 17:00. Usually, the mating lasts 239 ± 11.86 minutes. The fruit fly preferred to lay one egg into one fruit. The mating and egg laying behavior of RBO were studied in details too. One larva can damage more than eight fruits by boring into fruits one by one since fruits of *H. rhamnoides* usually grow as the clusters. No male lure was found for RBO adults until now but ammonium acetate was proved to be one of effective components of attractant. Occurrence time and activity range of RBO adults, including their dispersal in the horizontal distance and height, were studied by using ammonium acetate as an attractant. Some other ammonium compounds were also tested for their attractive ability.

[**Conclusions**] Being a main fruit pest of *H. rhamnoides*, RBO can be monitored by ammonium acetate. Attract and kill techniques could be developed based on this ammonium compound. Attractive host plant volatiles and potential pheromone of RBO have also been exploring by our team members.

Key word: *Rhagoletis batava obseuriosa*; *Hippophae rhamnoides*; life cycle; attractant

Monitoring and mass-trapping methodologies using the lesser date moth *Batrachedra amydraula* pheromone

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The lesser date moth (LDM) *Batrachedra amydraula* is a significant pest of date palm fruits. Previously, detection and monitoring of the pest has been generally inaccurate due to high costs of sampling with lifting machines. We report dose–response experiments with the LDM pheromone indicating that a 1 mg lure appears optimal for monitoring and mass trapping. We also conducted experiments to improve trap performance and found delta traps with adhesive covering their entire inner surface gave the highest capture rates while trap color was unimportant. Sampling pheromone traps throughout the night indicated male flight begins at 1:00–2:00 and reaches a peak two hours before sunrise. Monitoring traps exposed from spring through summer in Israel revealed there were three generations with different abundance. Trapping transects in a date plantation indicated that interference from a monitoring trap became minimal at distances >27 m away. Inter–trap distances closer than this should lower the efficiency of monitoring and mass–trapping in control programs. Our estimate of the circular effective attraction radius (EARc) of a 1 mg delta trap for LDM (3.43 m) shows this bait is among the most attractive compared to baits for other insects. We developed encounter–rate equations with the pheromone trap EARc to model the interplay between population levels, trap density, and captures that are useful for detection of invasive LDM and its control by mass–trapping. The integrated methodologies are applicable to many pest species.

S15-P-150

Floral volatiles of cultivated and wild strawberries and their behaviour modifying impact on the strawberry blossom weevil

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The strawberry blossom weevil (SBW) *Anthonomus rubi* Herbst (Coleoptera: Curculionidae) is a major problem in strawberry fields throughout Europe. At present, traps baited with aggregation pheromone are applied to pest monitoring[1], however, in order to control the weevil populations, more effective lure formulation has to be developed. For number of pest species it was shown that the attractiveness of the pheromone can be enhanced with specific host plant volatiles. The goal of this study was to explore floral volatile bouquets of wild and cultivated strawberries aiming to determine compound(s) which would improve efficiency of existing lures for SBW.

Forty five, 50, 38, 42 and 41 floral volatiles were collected by aeration and SPME techniques from headspaces of cultivated strawberries *Fragaria x ananassa* Duchesne varieties Sonata, Beltran, Korona, Polka and wild strawberry *F. vesca* L., respectively. GC-MS analyses revealed that floral bouquets of both species were dominated by aromatic compounds and terpenoids. 4-methoxy-Benzaldehyde and α-muurolene were released att the largest quantities from the flowers of cultivated and wild strawberries, respectively.

Two choice behavioural tests revealed that SBW significantly preferred the odours of both flowering strawberry species compare to those of not flowering plants. Beetles did not discriminate between odours released from cultivated versus wild flowering strawberry plants. Addition of 6 synthetic odorants including 4-methoxy-benzaldehyde, benzaldehyde, benzyl alcohol, methyl salicylate, limonene and decanal to not flowering cultivated strawberry plants and addition of similar 6 component blend in which 4-methoxy-benzaldehyde was replaced by α-muurolene to not flowering wild strawberry plants significantly increased preference of those bouquets by SBW compare to the odours released by not flowering plants. None of the compounds constituting both six components blends increased preference of SBW when tested as a single compound revealing high synergistic effect between components. However, 1,4-dimetoxy benzene, structurally close compound to 4-methoxy-benzaldehyde was active tested as a single compound and significantly enhanced lure efficiency when added to the aggregation pheromone in the field tests.

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Plume structure of intercept traps for trapping *Monochamus* spp. (Coleoptera: Cerambycidae)

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Intercept traps are commonly used to survey and detect Cerambycid beetles in forests and plantations. A field trial showed that four intercept trap designs: the malaise slam, intercept panel, Lindgren funnel and modified Lindgren funnel traps captured significantly different numbers of *Monochamus scutellatus* and *M. notatus* beetles. These observed differences possibly originate from differences occurring in the pheromone plume associated with each design. Little is known about the structure of the volatile pheromone plume that is created downwind from intercept traps and even less is known about how intercept trap design impacts on pheromone plume shape. To investigate the effects of intercept trap design on plume structure we used CO_2 as a detectable semiochemical surrogate in a controlled greenhouse experiment. The plume from each trap was characterized by interpreting measurements made with a portable infrared spectroscopy device at different distances from the traps and CO_2 source. The plume shape of the four trap designs were compared by visualising the plume through contour plots and quantifying CO_2 filament frequency and amplitude at different distances from the traps. Contour heat maps show that CO_2 concentration rapidly declines with increases in distance from the traps. Differences in the amplitude and frequency of CO_2 filaments were also observed at different distances downwind of the traps and appear to be different among intercept trap types. These differences may be perceived by the *Monochamus* beetles and could contribute to the differences observed among trap designs in the number of individuals captured.

Utilization of cuticular hydrocarbon of the Japanese carpenter ant in pest management on the Argentine ant

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The Argentine ant, *Linepithema humile*, native to Northern Argentina is now a globally distributing invasive pest on six continents and pacific islands including New Zealand, Hawaii and Japan. Due to low levels of intraspecific aggression, high levels of cooperatively and rapid reproduction in a huge colony consisting of multiple nests with multiple queens, *L. humile* has rapidly been spreading and increasing damage not only to urban environments but also to natural and agricultural ecosystems. Ongoing researches into the implementation of chemical control programs using pesticides and poison baits limittedly decline the ant populations but not completely, hence strong aversion for this species has been awaited.

Here we found that the cuticle hydrocarbon (CHC) cocktail of the Japanese carpenter ant, *Camponotus japonicus* revealed an urgent aversive behavior in *L. humile*. Thus, the 18 components of the *Camponotus* CHC cocktail showed different strength of avrrsion effects as follows; normal alkanes < major unsaturated compounds, C_{27}:1(9), C_{27}:1(7), C_{29}:1(9) and C_{29}:1(7) < minor ones, C_{23}:1(9), C_{25}:1(9) and C_{26}:1(9). The Argentine ants, when touched highly aversive unsaturated CHC, immediately backed away at high speed and stay far from that. Then, they persistently repeated antennae gloomimg, suggesting that this compound may disturb their olfactory sensory system essential for their social communication. In a field experiment, we further succeeded to shut out the Argentina ants from the food souse area surrounded by the most effective compound. Based on these results, we advocate that appropriate usage of this compound could facilitate autonomous withdraw of the Argentine ants from their invasion area.

Rescue® spatial insect repellent devices for consumer markets

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Repellents provide excellent protection against vector-borne diseases, including malaria, zika, dengue fever, and leishmaniasis. In contrast to the well-known topical repellents (e.g. DEET), less attention has been paid toward the discovery of spatial repellents. In response to the U.S. DOD's calls for improving "personal protective measures" in military, a SBIR project (Phase I/II) entitled "Personal Insect Repellent Device" was proposed and funded by the U. S. Army. The key objective of this SBIR project was to develop a wearable portable device loaded with effective spatial insect repellents for personal protection against mosquito and sand fly bites in the military combat or other outdoor settings.

With the GC-EAD and GC-MS techniques, bioassay methods, efficient organic isolation/synthesis, a 3-D design and the latest prototyping technology, we finalized several essential oil-based (EO) spatial repellent mixture formulations; then we designed and prototyped two efficient release devices. Our cage-based lab and field evaluation tests showed significant repellency effects on various mosquito species under high biting pressure.

In 2016, two commerical products created from this SBIR project, Rescue$^{(R)}$ GoClip^{TM} (disposable)[1] and Rescue$^{(R)}$ DecoShield^{TM} (reusable)[2] spatial insect repellent devices, were launched in the U. S. consumer markets for outdoor use against common and dangerous vector insects, such as mosquitoes and other biting insects. They are available at all major retail stores in the United States, such as Wal-Mart, Home Depot, Lowes, ACE Hardware, and Costco, plus online sites like Amazon. With the same device designs loaded with a different EO-based spatial repellent formulation, the first ever GoClip^{TM} and DecoShield^{TM} repellent devices for pestiferous social wasps (yellowjackets, paper wasps and hornets)[3,4] were also developed and commericalized in North America.

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S15-P-155

Aversive olfactory learning in the oriental fruit fly, *Bactrocera dorsalis*

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The innate response to host may be shaped by learning in herbivorous insects, which could affect the control efficiency of attractant extracted from host plants. Methyl eugenol (ME), a male lure, is widely used to control populations of the oriental fruit fly, *Bactrocera dorsalis*, a serious pest of fruits and vegetables, through mass trapping. The effect of aversive olfactory training on the olfactory response to ME was investigated in restrained flies under laboratory conditions. Male flies were trained to associate ME odor with a food punishment, sodium chloride solution, and the conditioned suppression of the proboscis-extension response to ME was observed during training. Flies could learn to associate the ME odor with sodium chloride solution. We found that high ME concentration support a stronger association. With increasing concentration of sodium chloride solution, a steady decrease of proboscis-extension response during six training trials was observed. A high level of learning was achieved with an inter-trial interval of 1-10 min. However, extending the inter-trial interval to 15 min led to reduced learning. No effect of physiological status on learning performance was detected, nor was any non-associative learning effect induced by the repeat presentation of ME odor or sodium chloride solution alone. The memory formed after six training trials could be retained for at least 3h. Our results may provide more information on application of attractant for control.

Alarm signal parsimony in green lacewings (Neuroptera: Chrysopidae)

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Green lacewings (Chrysopidae) are important predators of several soft-bodied insect pests. Larvae of all species are predatory and several species are important in respect of biological control in agroecosystems. For this reason, knowledge of the chemical ecology of these beneficial predatory insects is of special importance.

The current study was undertaken to test the behavioural response of green lacewings to semiochemicals of potential interest identified from *Chrysopa formosa*. This species is one of the most important *Chrysopa* species in agroecosystems in Central Europe. *Chrysopa* species are of special importance, since unlike most green lacewing species, adults of these species are also predatory.

Extracts from the head and thorax of adult male *C. formosa* elicited electroantennographic responses (EAG) from antennae of conspecific lacewings, with an EAG-active compound being identified as (*Z*)-4-tridecene. In field experiments, the compound showed strong behavioural effects, decreasing the attraction of *C. formosa* and *Chrysoperla carnea* species complex lacewings to stimuli otherwise highly attractive to these species. To our knowledge, this is the first report of the behavioural activity of (*Z*)-4-tridecene in a *Chrysopa* species. Furthermore, although the presence of (*Z*)-4-tridecene has been reported from prothoracic gland extracts of different green lacewing species from different geographic regions, to our knowledge, only one previous study has been carried out on the behavioural response of a Nearctic *Chrysoperla* sp. to this compound.

The results suggest that (*Z*)-4-tridecene may serve as a common alarm signal among different green lacewing species. Potential practical applications are discussed.

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S15-P-157

Design of larval chemical attractants based on odorant response spectra of odorant receptors in the cotton bollworm

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Lepidopteran caterpillars rely on olfaction and gustation to discriminate among food sources. Compared to the larval gustation, the larval olfaction has been poorly investigated. To uncover the molecular basis of olfaction in *Helicoverpa armigera* larvae, we identified 17 odorant receptor (Or) genes in larval antennae and maxillae using transcriptome sequencing, and functionally identified the response spectra of seven Ors to ecologically relevant odorants. Innate behavioural responses of larvae to active odorants were evaluated in chemotaxis assays. Several odorant blends were formulated based on the Ors tuning spectra and caterpillar chemotaxis. A four-component blend strongly attracted *H. armigera* larvae, and cis-jasmone and 1-pentanol were identified as essential components. Localization analyses showed that the two Ors detecting these components (Or41 and Or52) were expressed in the same sensory neurons. This is the first evidence that Ors in a polyphagous caterpillar respond to odorants in a combinatorial manner. The design of attractants to target specific olfactory pathways may promote the development of new baits for pest management.

A new bisexual lure for the European corn borer (*Ostrinia nubilalis*) and comparison of its performance with that of synthetic pheromone

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There are conflicting reports on the performance of traps baited with the synthetic sex pheromone of the European corn borer *Ostrinia nubilalis*. In a European survey to assess the performance of traps baited with the synthetic pheromone, in 10 countries out of 13 the trap was reported to be not satisfactory [1]. The present research was undertaken in search of alternative lures which could be used for detection and monitoring of the pest.

In has been known that the floral compound phenylacetaldehyde – besides many other Lepidoptera – also attracts both sexes of *O. nubilalis* [2,3]. However, experience showed that the activity of this compound is too low and usually is not satisfactory for practical applications.

Through extensive screening of floral candidate compounds we discovered that 4-methoxyphenethyl alcohol, when added to phenylacetaldehyde, increased catches by 3 to 5-fold, and thus allowed the development of a practically usable trap lure for *O. nubilalis*. Highest catches were recorded at the 1:1 blend, and when formulated in cellulose dental rolls covered with polyethylene bag the bait dispenser started to loose its activity only after 3-4 weeks.

The performance of this new bisexual lure was compared with that of synthetic sex pheromone in 5 countries in Europe and the Middle East. In Hungary, Bulgaria ,Turkey (sites where the *Z* pheromone strain predominantly occurs) and Italy (*E* strain site), the catches of the bisexual lure highly exceeded those of the synthetic *Z* or *E* strain pheromone, which caught very little. A considerable percentage of moths caught by the bisexual lure was females.

In the Slovenian sites (where the *E* strain is present) there were also more moths caught in traps with the bisexual lure, but the mean catches were not significantly higher than those in traps baited with the synthetic *E* strain pheromone (which also caught sizeable numbers). It is noteworthy that at one Slovenian site the experiment was conducted in a hop field (while in all other places tests were performed in maize fields). In the future, a more detailed study on populations from Slovenia will be necessary.

In conclusion, conventional funnel traps baited with the new bisex lure give the first practical opportunity to farmers to conduct successful detection and monitoring trapping of the European corn borer in Europe and neighbouring areas.

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S15-P-159

A new possibility for LONG ROPE dispenser as hand-applied formulations for pheromone-mediated mating disruption

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The commercial successes of mating disruption have been achieved through the use of hand-applied pheromone formulations such as tubes, ampoules and envelopes. A greater adoption of these formulations will accompany the development of technologies that require less labor to apply.

The LONG ROPE dispenser, which has a long extended polymer tube with gelled pheromone(s) inside, is being developed for the control of the Codling moth (*Cydia pomonella* Linnaeus), the Oriental fruit moth (*Cydia molesta* Busck) and the European grapevine moth (*Lobesia botrana* Denis & Schiffermüller). This new type formulation releases the pheromone along the entire surface of the rope dispenser, and may act as a line source. It can be placed uniformly throughout the field, starting at the border row and applying at predetermined intervals. This formulation also has the following characteristics: 1) Leak free: the pheromone does not leak from the dispenser due to gelification, 2) Flexible application: can be cut and tied anywhere, 3) Easy deployment: no technique is necessary, and 4) Uniform concentration.

In 2016, LONG ROPE dispensers were applied for the Codling moth at the rate of 1,200 meters/hectare in southern France, for the Oriental fruit moth at the rate of 600 or 1,200 meters/hectare in California, USA and for the European grapevine moth at the rate of 1,200 meters/hectare in Tuscany, Italy. Trap capture and crop damage were assessed using the conventional tube dispensers as a reference.

The results revealed that LONG ROPE dispenser has a lot of potential and would be a promising tool for pheromone-mediated mating disruption.

Development of mating disruption against *Proeulia auraria* (Clarke) (Lepidoptera: Tortricidae) in fruit orchards in Chile

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Proeulia auraria (Clarke) is a native species, causing significant damage to foliage and fruits in several tree fruit species and vineyards in Chile, having also quarantine status in numerous markets as Japan, USA, and others [1]. Thus, *P. auraria* is frequently controlled by growers spraying conventional insecticides. Its sex pheromone consists of 14:OAc, E11-14:OAc, Z11-14:OAc, and E11-14:OH (11:100:4:37, relative ratio [2]). Looking for a more environmentally friendly control tactic, we conducted a government founded grant to develop mating disruption (MD) against *P. auraria*, using a dispenser material based on SPLAT ® technology loaded with the two main pheromonal components in a proportion 3:1, considering 10.5% of a.i. Field trials were set in apples, vineyards, and blueberries in central Chile at the beginning of the first flight (September, 2016), once we observed the first male captures in pheromone baited traps, and lasted until the end of the second and last flight (May, 2017). The equivalent to 250 point sources (3 g/point) per ha (apples and blueberries, 1.5 ha ea.) and 312/ha (vineyard, 1.2 ha) was homogeneously applied in a MD plot, not sprayed with insecticides (except apples). A control plot, not treated with MD, of similar size in every orchard, was also included. Two pheromone baited traps were set in every plot and served weekly the whole season. Cumulative control plot captures were 838 (vineyard), 343 (blueberries), and 192 (apples) *P. auraria* males during the season, whereas no males were caught in apples and blueberries, in the respective MD plots. Captures practically ceased immediately after applying the formulation in MD plots in all three orchards. Total disruption (100*[Control captures - MD plot captures]/Control captures) for at least 5-6 months (apple and blueberry orchards) was observed. 99.1% disruption was observed in the vineyard (with the highest pest pressure) during the 7 months evaluation period, mainly due to 7 males captured the30th week. These results demonstrated success in achieving mating disruption against *P. auraria* in orchards using the matrix material, pheromone load, and point source density described above. As far as we know, this is the first development for this technique in the region. However, the captures observed late in the season in the MD plot in the vineyard, and some damage observed in the MD plots in apples and the vineyard at harvest, indicate the strategy must be developed in a way to avoid both, late cease of male disruption and migration of mated females from nearby orchards in order to obtain satisfactory yields.

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Characterization of mosquitocidal activity of essentials oils derived from four herbal plants

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Mosquitoes act as vectors for most of life threatening diseases, such as malaria, dengue fever, and Zika virus infection. The management of mosquitoes mainly relies on synthetic insecticides which usually result in rapid development of resistance, therefore, it is urgent to develop alternative strategies for mosquito control. These studies focus on the characterization of larvicidal activity of essential oils (EOs). The EOs were extracted from four herbal plants by hydro-distillation and subjected to GC-MS analysis and larvicidal activity assay toward *Aedes aegypti* and *Culex quinquefasciatus*. Totally 14, 17, 12 and 15 compounds derived from the EOs of *Plectranthus amboinicus*, *Vitex rotundifolia*, *Crossostephium chinense* and *Mentha requienii* were identified, respectively. The EOs derived from the four herbal plants all exhibited remarkable larvicidal activity against both mosquitoes after 24 hours treatment. In particular, the EOs of *P. amboinicus* showed the highest larvicidal activity and the larva of *C. quinquefasciatus* were more sensitive to *P. amboinicus* EOs than that of *Ae. Aegypti* (*C. quinquefasciatus*: LT_{50} = 2.51 minutes; *Ae. Aegypti*: LT_{50} = 61.16 minutes at the concentration of 100 ppm). The predominant compositions of *P. amboinicus* EOs were carvacrol (61.53 %), β-caryophyllene (12.79%), γ-terpinene (8.5 %), *p*-cymene (9.4 %) and α-humulene (3.24%). The γ-terpinene and *p*-cymene were the precursors of carvacrol and exhibited extraordinary larvicidal activity toward *A. aegypti*. To facilitate its application in mosquito control, future studies will further delineate the mechanism of the differential toxicity of *P. amboinicus* EOs against *Culex* and *Aedes* mosquitoes. The information from these studies would benefit the incorporation of EOs in Integrate Vector Management.

Key words: Essential oil (EO), *Plectranthus amboinicus*, larvicidal activity

A system for quantitatively evaluating the chemo-orientation behavior of the two-spotted spider mite, *Tetranychus urticae*

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The two-spotted spider mite, *Tetranychus urticae* Koch (Acari: Tetranychidae), is a cosmopolitan pest species that feeds on >1100 plant species, including >100 important agricultural crops. As *T. urticae* shows resistance to at least 95 active pesticide ingredients, novel measures for the control of this species are required. The push-pull strategy is a pest-control measure based on behavioral manipulation of pests or beneficial organisms by using repellent (push) and attractive (pull) stimuli. It is well known that *T. urticae* shows a repellent response to synthetic pyrethroids such as fenpropathrin (FP). However, to date, behavioral assays have been conducted by visual observation, which limits the ability to continuously track the locomotion of a test mite for a quantitative evaluation of the chemo-orientation behavior. To address this problem, we developed of a digital camera-based tracking system to quantitatively evaluate the chemo-orientation behavior of *T. urticae*.

To conduct a concentration-series experiment, FP solution was prepared at concentrations of 0, 1×10^{-4}, 1×10^{-3}, 1×10^{-2}, 1×10^{-1}, and 1 mM in methanol with 0.01% Tween 20 (v/v). Next, half the area of a glass plate (9 mm × 9 mm) was coated with 3 μL of the prepared FP solution. In addition, glass plates without any coating were also prepared, to evaluate the effect of the surfactant. One adult *T. urticae* female was introduced onto each glass plate and the locomotion of the mite was recorded for 10 min using a digital camera, at a frame rate of 29.97 fps. Positional data of the mite at each frame were analyzed using a PC software. To evaluate the localization of the mite in coated and uncoated regions, an excess proportion index (*EPI*) was calculated as:

$$EPI = \frac{F_{\mathrm{C}} - F_{\mathrm{UC}}}{F_{\mathrm{C}} + F_{\mathrm{UC}}}$$

where F_{C} and F_{UC} are the frequencies of stay in the coated and uncoated regions, respectively. Thus, the *EPI* values can range from −1 (complete localization in the uncoated region) to 1 (complete localization in the coated region), and *EPI* = 0 indicates no preference for either region.

The median *EPI* at 0 mM FP was 0.065 (n = 12) and comparable to that (0.021) in the treatment without any coating (n = 20). This indicates that no effect of the solvent was observed on the chemo-orientation behavior of mites in the present study. The median *EPI* values at the FP concentrations of 1×10^{-4}, 1×10^{-3}, 1×10^{-2}, 1×10^{-1}, and 1 mM were 0.065 (n = 19), −0.083 (n = 19), −0.18 (n = 18), −0.54 (n = 18), −0.92 (n = 16), and −0.86 (n = 19), respectively. The median *EPI* values at FP concentrations $\geq 1 \times 10^{-3}$ mM were significantly lower than at 0 mM FP. From the dose-response relationship between FP and *EPI*, the median effective concentration (EC_{50}) was computed as 6.4×10^{-3} mM. This EC_{50} value was lower than that (1.1×10^{-1} mM) observed in a previous study based on visual observation of mite behavior on FP-treated leaves. This suggests that our system can evaluate the chemo-orientation behavior of *T. urticae* even at low concentrations of test chemicals. Our system is expected to be useful for performing a chemical screen to develop repellents/attractants for *T. urticae*.

Mating disruption effects on a parasitoid's ability to locate its host-pheromone

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Pest control through mating disruption (MD) is based on the saturation of agricultural plots with the pest's synthetic sex pheromone, thus interfering with its mate-finding and reproduction. The impact of MD on non-target arthropods is assumed to be low, because sex pheromones are highly species-specific. Nevertheless, the pheromone of the vine mealybug *Planococcus ficus* is known to attract females of its parasitoid, *Anagyrus pseudococci*. We therefore hypothesized that MD treatment may also interfere with the ability of this female wasps, but not necessarily of males, to orient to the host-pheromone source.

We compared captures of male and female wasps in pheromone baited traps in vineyards treated and untreated with MD, and in adjacent non-cultivated plots. Males were hardly captured and were not affected by the MD treatment. Females were captured in pheromone traps positioned in control plots but significantly fewer were captured in MD treated plots. In addition, in the control plots females were captured both in the vineyard and in the adjacent non-cultivated plots, whereas in the MD plots, most were captured in adjacent non-cultivated plots. Of the few captured males, most were captured inside the vineyards (whether treated or not).

These results suggest that male wasps do not use the host pheromone as a cue to locate females and that MD disrupts the ability of female wasps to locate their host by its pheromone signal. However, previous studies showed that MD enhances the wasp's parasitism rate. These conflicting results might be settled if multiple cues are involved in prey location by females.

The major sex pheromone Codlemone of Codling moth synergizes response to synthetic sex pheromone in Oriental fruit moth, *Grapholita molesta* in summer

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The Oriental fruit moth (OFM), *Grapholita molesta* (Busck) is a key pest of stone fruit throughout the world. Electroantennogram (EAG) studies and field trapping experiments were conducted in China and the USA to evaluate the use of lure combinations of sex pheromones to better capture the oriental fruit moth for monitoring as well as trapping of OFM in the field. OFM male antenna can detect the main sex pheromone component of *Cydia pomonella*, E8,E10-dodecadienol (Codlemone) with significantly increasing amplitudes from responses to over the dose of 1 μg. A mix of 1-10 ng OFM Pheromone and 1 μg Codlemone elicited stronger EAG responses in male OFM antennae than either OFM Pheromone or Codlemone alone. In the field trapping experiments in summer in China, 4–5 fold more OFM males were captured in traps baited with a combination of Codlemone and OFM pheromone, regardless of mixed or respective lure than in traps baited with Codlemone or OFM pheromone alone. For an apple orchard trapping experiments in summer in North Carolina State, USA, traps baited with a mix of Codlemone and OFM pheromone also caught significantly more OFM male moths than traps with either OFM Pheromone or Codlemone alone, but in the fall, no synergism was found between treatments. The more efficient lure could be used to control OFM by mass trapping and monitoring in orchards and the pheromone synergism mechanism merits futher research.

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S15-P-168

Trials of mating disruption for *Pseudococcus comstocki* (Kuwana) by using its sex pheromone in Japanese grape orchards under a greenhouse

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The Comstock mealybug, *Pseudococcus comstocki* (Kuwana), is distributed in 33 countries, including Japan, South Korea, China, United States, Brazil and France. The hosts of this mealybug are recorded from plants of 65 genera in 43 families. On crops, it has become a serious pest of several fruits, including grapes, pears, plums and apples. The sex pheromone of females was independently isolated and identified, as 2,6-dimethyl-3-acetoxy-1,5-heptadiene, by Negishi et al. (1980) and Bierl-Leonhardt et al. (1982).

We conducted five experiments for mating disruption of the mealybug by using a racemic mixture of the synthetic pheromone on small area (from $4m^2$ to $100m^2$) in grape orchards in a greenhouse from 2012 to 2015. The purpose of these tests had been to obtain information on the effective dose of sex pheromone for mating disruption. Here, we report the relationship of the dose of pheromone and the estimated efficacy of mating disruption. Treated dose of the sex pheromone per one are ($100m^2$) was 1 gram in 2012, 5 grams in 2013 and 2014, and 10 grams in 2015. The season that the sex pheromone was treated was from July to October. The treated period was 3-5 days in 2012 and 2013, 52 days in 2014, and 55 days in 2015. We used rubber septa (1F sleeve stopper 1888 grey; West Pharmaceutical Service) for despensers of the pheromone. Effect of mating disruption evaluated with two indices. One was the attraction inhibition rate by monitoring trap during the examination, and the other was the frequenceis of pregnant females in treated area compared to those in untreated area.

In these two evaluation indices, the attraction inhibition rates were from 0 to 0.946. On the other hand, the frequnecies of pregnant females in treated area, divided with those in untreated area, were 0.064 to 0.737. The observed indices of the attraction inhibition and the frequencies of pregnant females suggested that the mating disruption, at least partially, occurred. In addition, when using the rubber septum dispensers for the mating disruption, it was likely that 1 gram or more of sex pheromone per one are was necessary; even in the case of 10 grams per one are, mating disruption effects decreased after 16 days of the treatment. These results would be helpful for commercialization of the mating disruption.

Interaction between thrips and their host plants, and its application

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Herbivore attack is one the most important factors to decrease the agricultural production. Many types of insecticide were developed and utilized to control the insect pests in the world. Insecticides have been supporting the world agriculture. However recently, it has become a big problem that many herbivores with insecticide resistance were appeared. Especially, thrips developed highly resistance to many types of insecticide, and can cause serious problems in many crops, vegetables, fruits and flower plants. This tiny and polyphagous pest insect is an also vector of tospoviruses, Therefore, both the feeding damage and the tospovirus disease caused by thrips are serious problems. We have demonstrated that jasmonate (JA) dependent plant induced defense system restricts both thrips performance and preference. JA treatment to the plants increases the thrips avoidance and controls the thrips behavior. In this meeting, we also introduce our approach to use the JA analog, prohydrojasmon for thrips control.

Temporal variation in synergy effect of benzaldehyde to ethanol in *Xyleborinus saxesenii*

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Xyleborinus saxesenii Ratzeburg (Scolytinae, Coleoptera) is important pest of fruit trees such as apples and peaches in Korea. Ethanol acts as an attractant for many bark beetles, and traps baited with ethanol are commonly used to monitor flight activity. To optimize monitoring the fight activity of *X. saxesenii* in fruit orchards, our objective was to assess the effects of various green leaf volatiles (GLVs) emitted by damaged almonds on the attractiveness of traps baited with ethanol to this pest. We tested whether 23 GLVs (α-pinene, ethyl butyrate, ethyl 2-methylbutyrate, camphene, ethyl isovalerate, 3-pentanol, ethyl 2-butenoate, limonene, 3-methyl-1-butanol, 2-methyl-1-butanol, ethyl tiglate, ethyl hexanoate, 3-octanone, 1-octen-3-ol, benzaldehyde, aromadendrene, vanillin, 2-butanol, styrene, 3-hydroxy-2-butanone, 2-phenylethyl alcohol, 2-pentylfuran, ethyl 3.3-dimethylacrylate) act synergistically with ethanol in peach orchards. No beetles were captured in traps baited solely with GLVs. In spring, catch of *X. saxesenii* on ethanol-baited traps were not enhanced by the addition of GLVs. However, in summer, traps baited with the combination of benzaldehyde and ethanol captured more *X. saxesenii* than all other treatments, indicating that there is temporal variation in synergism between benzaldehyde and ethanol. These results suggest that differences in volatile compound production during growth and ripening of peaches may contribute to the effectiveness of attractants.

Analysis, screening and field evaluation of volatiles emitted from tea plants infested by *Ectropis obliqua* larvae

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Background: Herbivore-induced plant volatiles (HIPVs) are known to play critical roles in the efforts of many herbivorous insects to locate hosts and mates, which may have a repellent or attractive role for conspecific moths. *Ectropis obliqua* Prout (Lepideptera: Geometride) is a serious leaf-feeding pest that infests tea *Camellia sinensis* in East Asia, seriously reducing tea yield and quality. Attractants for *E. obliqua* moths are of particular interest as they might be used as a way to reduce pest levels.

Objectives: To elucidate the role of tea volatiles induced by the infestation of *E. obliqua* larvae in the host-plant-searching behavior of conspecific adults, and evaluate the attractiveness of different combination of volatile compounds in the lab or their attractive activity in the field.

Methods: Gas chromatograph-mass spectrometer (GC-MS) and gas chromatograph-electroantennographic (GC-EAD) were used to identify tea volatiles or distinguish the active components among the volatiles separately. Y-tube olfactometer was used to test responses of *E. obliqua* moth to odor stimuli. Larry Hull designs traps baited with synthetic volatile blend were conducted to evaluate the attractiveness in the field.

Findings and conclusions: We found that tea plants infested by *E. obliqua* caterpillars for 24 h were more attractive to both virgin male and female *E. obliqua* adults than were intact, uninfested tea plants; moreover, mated female moths were more attracted by infested tea plants and preferentially oviposited on these plants, whereas mated male moths were repelled. Among caterpillars-induced volatiles, 17 compounds elicited antennal responses from both male and female virginal moths. Using a Y-tube olfactometer, 3 of the 17 chemicals, benzyl alcohol, (Z)-3-hexenyl hexanoate, and (Z)-3-hexenal, were attractive to virgin male and female moths. One chemical, (Z)-3-hexenyl acetate, was attractive only to virgin males. Mated females were attracted by three compounds, (Z)-3-hexenyl hexanoate, (Z)-3-hexenyl acetate, and (Z)-3-hexenal. Successively, we found that a blend containing a ternary mixture containing (*Z*)-3-hexenal, (*Z*)-3-hexenyl hexanoate and benzyl alcohol clearly attracted to *E. obliqua* moths of both sex and that (*Z*)-3-hexenyl acetate could enhance the attractiveness of the ternary blend. Our study raises the tantalizing possibility that synthetic blends could be deployed as attractants for pests in the field.

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S15-P-175

A review and recent developments in research of chemical communication in *Sitona* spp. (Coleoptera: Curculionidae): pheromones and plant volatiles

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Weevils (Coleoptera: Curculionidae) in the genus *Sitona* are pests of many leguminous crops worldwide, and often more than one species causes significant damage to mostly young plants with a relatively small leaf area. *Sitona* spp. overwinter at the last feeding site, usually in perennial crops or in the stubble of annuals. In the spring, they may stay at the overwintering site or migrate to young annual crops by crawling or, at higher temperatures, by flying.

Timely detection of migrating adult weevils would indicate whether control measures are necessary. For this, synthetic pheromone- and/or plant volatile-baited traps provide a feasible approach. Depending upon the volatiles involved in the chemical communication within the genus, then either monitoring of the most abundant or the most damaging species selectively, or monitoring of a species complex with a single trap/bait combination, might be realistic practical approaches.

This presentation will summarize research conducted since the first semiochemical identifications up to recent findings, including results of extensive field experiments with some *Sitona* species.

We will discuss our latest findings on a newly identified plant volatile attractant of *S. humeralis,* including details of species-specificity and the period of year for successful trapping. We will also elaborate on the feasibility of a synergistic combination of the plant volatile attractant with 4-methyl-3,5-heptanedione-based pheromone lures. Our results will be compared to recent findings on *S. lineatus*.

S15-P-176

Field response of two cetoniin chafers, *Cetonia aurata aurata* and *Potosia cuprea* (Coleoptera, Scarabaeidae) to floral compounds in ternary and binary combinations

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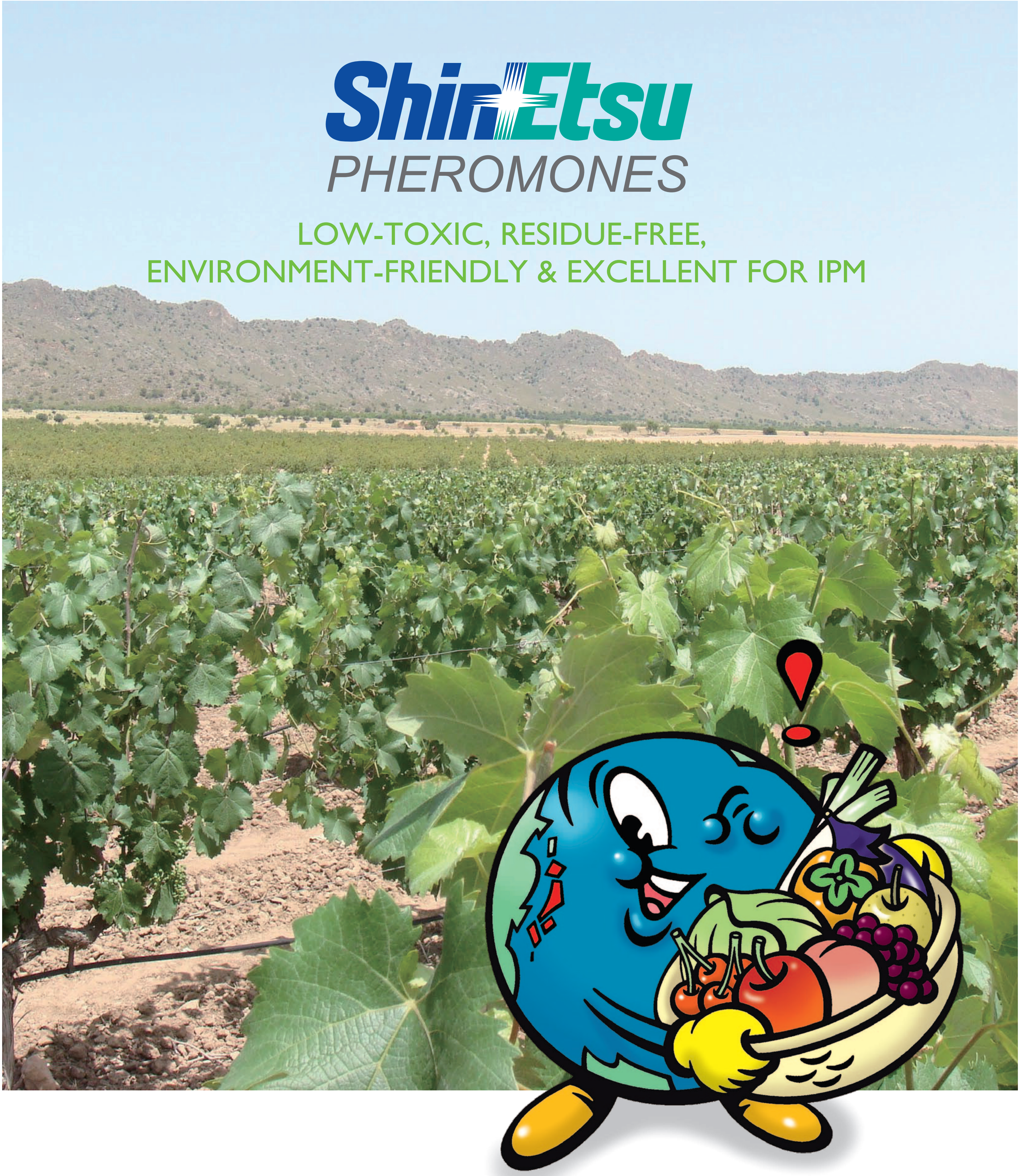
There are substantial differences in bouquets of flower volatiles of different flowers attractive to cetoniin chafers, with quantitative differences within plant species depending on environmental conditions and the phenotype of the plant. Cetoniin scarabs, including *Cetonia aurata aurata* L. and *Potosia cuprea* Fabr. (Coleoptera, Scarabaeidae) have a wide range of preference to flower volatiles and several synthetic floral lures have already been described.

The first objective of the present research was to test whether isoeugenol and eugenol, floral compounds with only a small difference in their molecular structure as compared to 3-methyl eugenol can substitute 3-methyl eugenol in its ternary mixture with 1-phenylethanol and (*E*)-anethol. This ternary mixture has previously been reported as a powerful synthetic floral attractant for both *C. a. aurata* and *P. cuprea*. All traps with baits caught significantly more of both chafers as compared to the unbaited control traps, indicating some level of attraction. At the same time baits containing 3-methyl eugenol caught more than the other two ternary blends indicating a fine tuning in behavioural response to 3-methyl eugenol.

The objective of a second test was originally the optimisation of floral lures for the closely related *Oxythyrea funesta* Poda, but resulted in catching significant numbers of *C. a. aurata* and *P. cuprea* also. A strong attraction to the combination of 2-phenylethanol and 4-methoxyphenethyl alcohol was found in both species, while the combination of 2-phenylethanol and 1,2,4-trimethoxybenzene resulted in significant increase in the catches of *P. cuprea* resulting in a certain level of selectivity towards *C. a. aurata*. This finding may lead to the development of more selective lures for *C. a. aurata* on the one hand and *P. cuprea* on the other, which gives more details to our knowledge in the food related olfactory preference of the two chafer species.

Author’s list



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