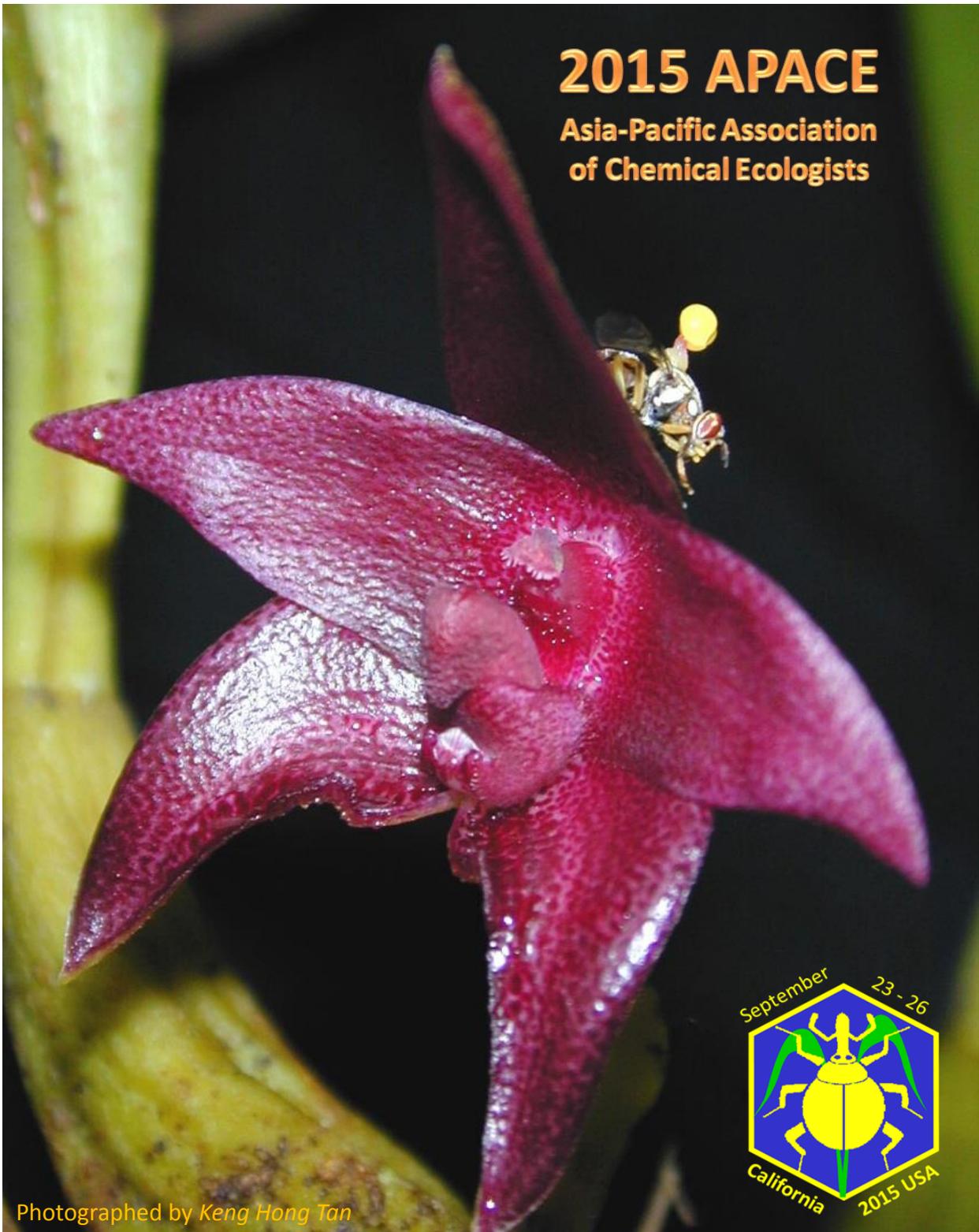


**2015 APACE**  
Asia-Pacific Association  
of Chemical Ecologists



Photographed by Keng Hong Tan



**APACE** Signaling in the 21<sup>st</sup> Century

# 8<sup>th</sup> Asia-Pacific Chemical Ecology Conference



APACE  
2015



Chemical Ecology: Signaling in the 21<sup>st</sup> Century

California, USA  
September 23 – 26 2015



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## Organizing Committee of 2015 APACE Conference

**Chair: Junwei Jerry Zhu (USDA-ARS, Lincoln, Nebraska, USA)**

**Gabrielle Nevitt** (University of California, Davis, California, USA)

**Agenor Mafra-Neto** (ISCA Technologies, Inc., Riverside, California, USA)

**Dangsheng Liang** (Apex Bait Technologies, Inc., Santa Clara, California, USA)

**Eric Jang** (USDA-ARS, Hilo, Hawaii, USA)

## Scientific Committee of 2015 APACE Conference

**Alexandre IL'ichev** (Primary Industry Research Victoria, Australia)

**Tilman Harder** (University of Bremen, Germany)

**Aijung Zhang** (USDA-ARS, Beltsville, Maryland, USA)

**Tom Baker** (Pennsylvania State University, University Park, Pennsylvania, USA)

**Kenneth Haynes** (University of Kentucky, Lexington, Kentucky, USA)

**Qinghe Zhang** (Sterling International, Inc., Spokane, Washington, USA)

**Coby Schal** (North Carolina State University, Raleigh, North Carolina, USA)

**Jocelyn Millar** (University of California, Riverside, California, USA)

**Ring Cardé** (University of California, Riverside, California, USA)

**Christer Löfstedt** (Lund University, Lund, Sweden)

**Yukio Ishikawa** (The University of Tokyo, Tokyo, Japan)

**Naoki Mori** (Kyoto University, Kyoto, Japan)

**Alvin Hee** (University of Putra Malaysia, Selangor, Malaysia)

**Kye-chung Park** (The New Zealand Institute for Plant & Food Research, Christchurch, New Zealand)

**Max Suckling** (The New Zealand Institute for Plant & Food Research, Christchurch, New Zealand)

**Yongping Huang** (Shanghai Institute for Biological Sciences, Shanghai, P.R. China)

**Rensen Zeng** (Fujian Agriculture and Forestry University, Fuzhou, P.R. China)

**Fengming Yan** (Henan Agricultural University, Zhenzhou, P.R. China)

## Registrar of 2015 APACE Conference

**Lee-Ann Choy** (Pacific Rim Concepts LLC)

## Sponsors

We appreciate our sponsors for their generous support for 2015 APACE Conference

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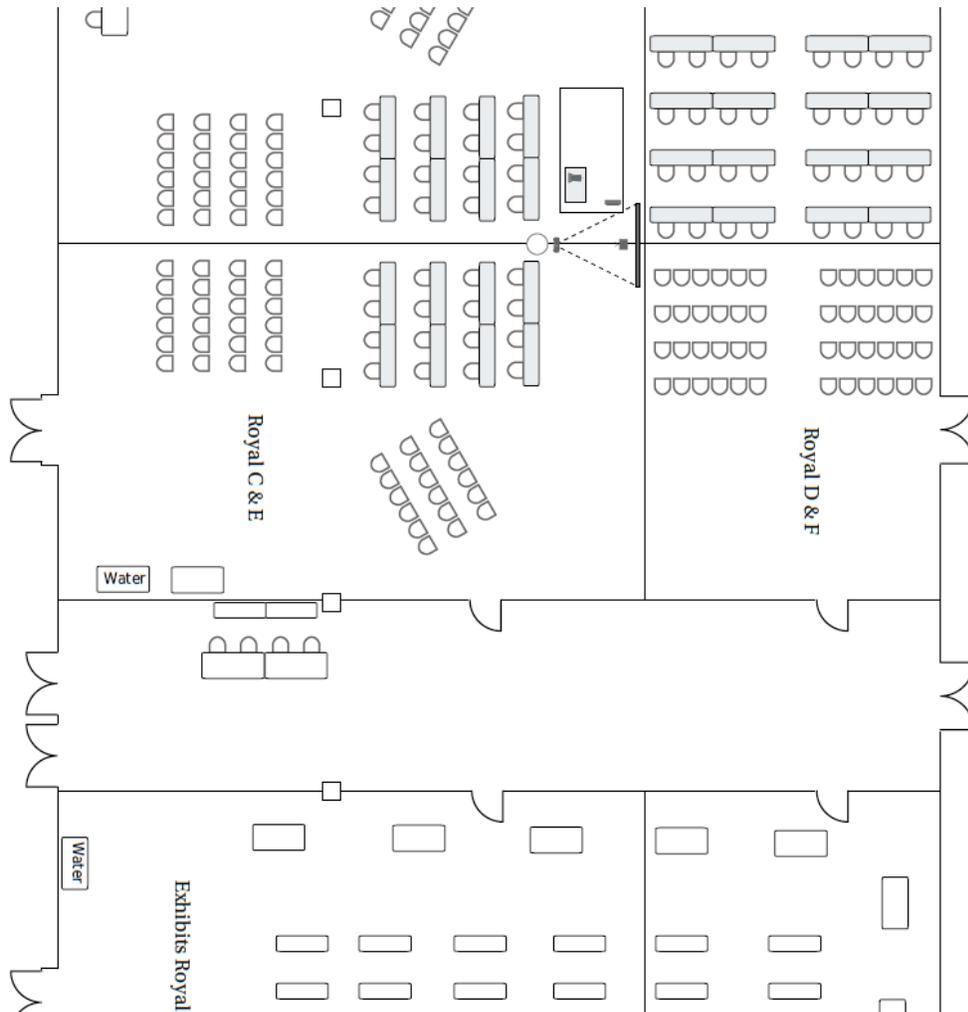


# Conference Room Arrangements

Symposium Rooms (Royal Ballroom D&F) and (Royal Ballroom C&E)

Poster Display Room and Exhibitors' Room (Royal Ballroom A&B)

Registration Room (Royal Hallway)





### 2015 APACE Conference Schedule

Wednesday September 23, 2015 RB D/F		Thursday September 24, 2015 Royal Ballroom C/E Royal Ballroom D/F		Friday September 25, 2015 Royal Ballroom C/E Royal Ballroom D/F		Saturday September 26, 2015 Royal Ballroom C/E Royal Ballroom D/F	
		Conference Opening (8:00 - 8:20)		Life-time Achievement Award (8:00 - 8:20)		JCE Briefing & 2016 ISCE Introduction (8:00 - 8:20)	
		8:20 - 8:55 Christer Löfstedt 8:55 - 9:30 Joanne Yee		8:20 - 8:55 Riag Cardé 8:55 - 9:30 Rebecca Zaag		8:20 - 8:55 Tristram Wyatt 8:55 - 9:30 Keiji Matsura	
		3:30 - 10:00am Morning Coffee and Tea Break					
Poster Session	Chemical Ecology of Biting Insects	Fruit Fly Chemical Ecology	Challenges for Invasive Species Pest management via Semiochemicals	Chemical Ecology of Forest Insects	Going from the Back to the Marketplace for Semiochemical products	Chemical Ecology in General	
	K Haynes, R Cardé & J Zhu	A Haa & S Olfsson	A Zhang & Asaf Levi-Zada	Z Zhang & S Seybold	J Zhu, T Baker & J Miller	G Wang	
	10:00 - 10:25 Aanandasaar Ray	10:00 - 10:25 Paul Cessingham	10:00 - 10:20 Cesar Rodriguez-Saona	10:00 - 10:04 Steve Seybold	10:00 - 10:25 Max Seckling	10:00 - 10:20 Paolo Zerbis	
	10:25 - 10:50 Keanth Haysae	10:25 - 10:50 Eric Jaag	10:04 - 10:25 Xiangbo Kong	10:04 - 10:25 Xiangbo Kong	10:25 - 10:50 Cam Oebischlager	10:20 - 10:40 Feigang Yao	
	10:50 - 11:05 Gerhard Gries	10:50 - 11:05 Alvia Hee	10:20 - 10:40 Doang Ho Cha	10:25 - 10:44 Zhen Zhang	10:50 - 11:05 Alex Hickey	10:40 - 11:00 Gabrielle Nevitt	
	11:05 - 11:35 Takuma Iwamoto	11:05 - 11:35 Amanda Ramsey	10:40 - 11:00 Aijun Zhang	10:44 - 11:03 Tom Coleman	11:05 - 11:40 David Lacey	11:00 - 11:20 Yongqiang Luo	
	11:35 - 11:55 Emerson Lacey	11:35 - 11:55 Shaosha Olfsson	11:00 - 11:20 Asraf El-Sayed	11:03 - 11:32 Yiguo Chen	11:15 - 11:40 Michael Rostas	11:20 - 11:40 Michael Rostas	
			11:20 - 11:40 Gregory Wheeler	11:32 - 11:41 Gezhang Cai	11:40 - 12:00 Gezhang Cai	11:40 - 12:00 Sub-Liang Yee	
			11:40 - 12:00 Asaf Levi-Zada	11:41 - 12:00 Maxwell Collingeon			
		12:00 - 13:30 Lunch break					
Poster Session	Molecular Chemical Ecology	Plant Allelopathy	Application of Electrophysiology in Chemical Ecology	New Physiological Aspects of Chemical Ecology	Going from the Back to the Marketplace for Semiochemical products	Chemical Ecology in General	
	T. Ishikawa & T. Hwang	R. Zaag & Z. Pan	K. Park & A. Wada-Katsumata	H. Mori & G. Falton	J. Zhu, T. Baker & J. Miller		
	13:30 - 14:00 May Borebaom	13:30 - 13:55 Zhiqiang Pan	13:30 - 13:55 Doang Ho Cha	13:30 - 13:55 Aanandasaar Ray	13:30 - 13:55 Satoshi Nojima	13:30 - 13:55 Zhaojun Xia	
	14:00 - 14:25 Min-Yoon Choi	13:55 - 14:20 Yessyua Soag	13:55 - 14:20 Ayako Wada-Katsumata	13:55 - 14:20 Joe Lewis	13:55 - 14:20 Qinghe Zhang	13:50 - 14:10 Baoyu Hao	
	14:25 - 14:50 Takeaki Sakurai	14:20 - 14:45 Jeff Weidenhamer	14:20 - 14:45 Hyun-Woo Oh	14:20 - 14:45 Gary Felton	14:20 - 14:45 Agosor Mafru-Meto	14:10 - 14:30 Zhiqian Li	
	14:50 - 15:10 Tsutomu Tsuchida	14:45 - 15:10 Zhongqi Zhang	14:45 - 15:10 Mickiya Kamio	14:45 - 15:10 Koji Noge	14:45 - 15:10 Larry Gett		
15:10 - 15:30 Yongqiang Luo	15:10 - 15:30 Muhammad Azim Khan	15:10 - 15:30	15:10 - 15:30 Shin Tejima	15:10 - 15:30 Matkew Boksart			
		15:30 - 16:00 Afternoon Coffee and Tea Break					
Poster Session	Molecular Chemical Ecology	Aquatic Chemical Ecology	Application of Electrophysiology in Chemical Ecology	New Physiological Aspects of Chemical Ecology	Going from the Back to the Marketplace for Semiochemical products		
	T. Ishikawa & T. Hwang	Tilman Harder	K. Park & A. Wada-Katsumata	H. Mori & G. Falton	J. Zhu, T. Baker & J. Miller		
	16:00 - 16:15 Shenglin Dong	16:00 - 16:20 Tristram Wyatt	16:00 - 16:25 Kee-Chang Park	16:00 - 16:25 Naoki Mori	16:00 - 16:20 Kiyoshi Nakamura		
	16:15 - 16:20 Yongqiang Luo	16:20 - 16:40 Tilman Harder	16:20 - 16:50 Takashi Inoue	16:20 - 16:45 Jared Gregory Ali	16:20 - 16:40 Peter Gregg		
	16:20 - 16:45 Gezhang Cai	16:40 - 17:00 Zongming Ren					
16:45 - 17:00 Yaag Liu							
		18:00 - 20:00 Welcome Reception (Terrace Garden)		Conference Dinner		Closing & Student Awards	

Registration & Pre-conference Mixer

Executive and Council Meeting (15:00 - 17:00 at Royal Ballroom CE)

## Program – Wednesday September 23

9:00 – 17:00	Registration (Registration Desk)
9:30 – 17:00	Industry Exhibitors (Set-up in Royal Ballroom A/B)
15:00 – 17:00	Executive & Councilor Meeting (Royal Ballroom C/E)
18:00 – 20:00	Welcome Reception (Terrace Garden)

## Program – Thursday September 24

7:00 – 16:00 Registration / Exhibits / Posters (Royal Hallway & Ballroom A/B)

8:00 – 9:30 Conference Opening & Plenary Talks (Royal Ballroom C/E)

### **From gene discovery to metabolic engineering: production of moth pheromones in plant and cell factories**

Christer Löfstedt (Lund University, Sweden)

8:20 – 8:55

### **A tale of two enzymes: unexpected insights into pheromone evolution and oenocyte survival**

Joanne Yew (University of Hawaii at Mānoa, USA)

8:55 – 9:30

### **Coffee/Tea Break (9:30 – 10:00)**

### **Chemical Ecology of Biting Insects (Royal Ballroom C/E, 10:00 – 12:00)**

*Chairs: Kenneth Haynes, Ring Cardé & Jerry Zhu*

10:00 – 10:25

Receptors, neurons and mechanisms for insect repellents and lures

*Anandasankar Ray (University of California-Riverside, USA)*

10:25 – 10:50

Chemical communication in bed bugs

*Ken Haynes (University of Kentucky, USA)*

10:50 – 11:15

180,000 bed bug bites later.... the bed bug aggregation pheromone is finally identified

*Gerhard Gries (Simon Fraser University, Canada)*

11:15 – 11:35

Search for odorants inducing olfactory behavior on the body louse, *Pediculus humanus corporis*, based on the response of olfactory receptor.

*Takuma Iwamatsu <sup>s</sup> (The University of Tokyo, Japan)*

11:35 – 11:55

Location of a carbon dioxide source by *Anopheles coluzzii* under conditions of laminar air flow and still air

*Emerson Lacey (University of California-Riverside, USA)*

## Program – Thursday September 24

### **Fruit Fly Chemical Ecology (Royal Ballroom D/F, 10:00 – 12:00)**

*Chairs: Alvin Hee & Shannon Olsson*

10:00 – 10:25

Fruit ripening volatiles act synergistically as host cues in a pest tephritid fruit fly  
*Paul Cunningham (Queensland University of Technology, Australia)*

10:25 – 10:50

Challenges and Constraints in Fruit Fly Chemical Ecology  
*Eric Jang (USDA-ARS, PBARC, Hilo, USA)*

10:50 – 11:15

Fruit Fly Chemical Ecology- An Update  
*Alvin Kah-Wei Hee (University of Putra Malaysia, Malaysia)*

11:15 – 11:35

Evaluation of gel commercial lure for spotted wing Drosophila, *Drosophila suzukii*  
*Amanda Ramsey (Scentry Biologicals, Inc. Billings, Montana, USA)*

11:35 – 11:55

Towards a Mechanistic Basis for Sympatric Speciation in *Rhagoletis* Fruit Flies  
*Shannon Olsson (National Centre for Biological Sciences, Bangalore, India)*

## Program – Thursday September 24

### Molecular Chemical Ecology (Royal Ballroom C/E, 13:30 – 17:00)

Chairs: Yukio Ishikawa & Yongping Huang

13:30 – 14:00

Pollinator chemical ecology and CYPome "blooms"

May Berenbaum (University of Illinois, Urbana-Champaign, Illinois, USA)

14:00 – 14:25

Insect PBANs: Structure, Function and Evolution

Man-Yeon Choi (USDA-ARS, Corvallis, Oregon, USA)

14:25 – 14:50

A single sex pheromone receptor mediates bombykol responses in the silkworm

Takeshi Sakurai (The University of Tokyo, Tokyo, Japan)

14:50 – 15:10

Symbiont-mediated body color change in the pea aphid

Tsutomu Tsuchida (University of Toyama, Toyama, Japan)

15:10 – 15:30

The edited doublesex gene (dsx) of silkworm alters mating behaviour

YongPing Huang (Institute of Plant Physiology and Ecology, Chinese Academy of Sciences, Shanghai, China)

### Coffee/Tea Break (15:30 – 16:00)

16:00 – 16:15

Functional characterization of carboxylesterases for degradation of ester odorants in adult antennae of *Spodoptera exigua*

ShuangLin Dong (Nanjing Agricultural University, Nanjing, China)

16:15 – 16:30

Binding specificity analysis of odorant binding protein AlucOBP8 of the *Apolygus lucorum* (Meyer-Dür)

YongJun Zhang (Institute of Plant Protection, Chinese Academy of Agricultural Sciences, Beijing, China)

16:30 – 16:45

High rate and heritable mutagenesis of SlitPBP1 in *Spodoptera litura* by CRISPR/Cas9 mediated genome editing

GuanHeng Zhu <sup>s</sup> (Nanjing Agricultural University, Nanjing, China)

16:45 – 17:00

Sensillar expression and responses of olfactory receptors reveal different peripheral coding in two *Helicoverpa* species using the same pheromone components

Yang Liu (Institute of Plant Protection, Chinese Academy of Agricultural Sciences, Beijing, China)

## Program – Thursday September 24

### Plant Allelopathy: Direct and indirect effects on plant interactions and community (Royal Ballroom D/F, 13:30 – 15:30)

*Chairs: Rensen Zeng & Zhiqiang Pan*

13:30 – 13:55

Investigating the phytotoxic activity of sorgoleone by heterologous reconstitution of the entire biosynthetic pathway

*Zhiqiang Pan (USDA-ARS-NPURU, Mississippi, USA)*

13:55 – 14:20

Underground common mycorrhizal networks mediate interplant defense communication

*Yuanyuan Song (Fujian Agriculture and Forestry University, Fuzhou, Fuzhou, China)*

14:20 – 14:45

Metabolomics in soil rhizospheres: A key to understanding plant-plant interactions

*Jeff Weidenhamer (Ashland University, Ohio, USA)*

14:45 – 15:10

A Research on autotoxins' temporal and spatial distribution and microbial communities within the soil of *Rehmannia glutinosa* root-zone

*Zhongyi Zhang (Fujian Agricultural and Forestry University, Fuzhou, China)*

15:10 – 15:30

Use of allelopathins with reduced dose of herbicides suppress weeds and microbial activities in wheat

*Muhammad Azim Khan (The University of Agriculture Peshawar, Peshawar, Pakistan)*

### Coffee/Tea Break (15:30 – 16:00)

### Aquatic Chemical Ecology (Royal Ballroom C/E, 16:00 – 17:00)

*Chairs: Tilmann Harder*

16:00 – 16:20

Chemical communication underwater: contrasts and similarities with terrestrial semiochemicals

*Tristram Wyatt (University of Oxford, Oxford, UK)*

16:20 – 16:40

Chemical mediation of coral larval settlement by crustose coralline algae

*Tilmann Harder (University of Bremen, Bremen, Germany)*

16:40 – 17:00

The role of AChE in swimming behavior of *Daphnia magna*: Correlation analysis of both parameters in the exposure of DM and MT

*Zongming Ren (Shandong Normal University, Jinan, China)*

## Program – Friday September 25

8:00 – 8:20 Lifetime Achievement Award (Royal Ballroom C/E)  
Emeritus Professor Jiawei Du (*Chinese Academy of Sciences, China*)

8:20 – 9:30 Plenary Talks

### **Moth pheromones: what are the known knowns, the known unknowns, and the unknown unknowns?**

Ring Cardé (University of California, Riverside, California, USA) 8:20 – 8:55

### **Plant induced defense and insect counter defense**

Rensen Zeng (Fujian Agriculture and Forestry University, Fuzhou, China) 8:55 – 9:30

### Coffee/Tea Break (9:30 – 10:00)

### Challenges for Invasive Species Pest Management via Semiochemicals (Royal Ballroom C/E, 10:00 – 12:00)

Chairs: Aijun Zhang & Anat Levi-Zada

10:00 – 10:20

Managing the invasive spotted wing drosophila using behavior-based strategies: Challenges and successes  
Cesar Rodriguez-Saona (*Rutgers University, New Brunswick, New Jersey, USA*)

10:20 – 10:40

Developing more sensitive and selective attractant for the management of *Drosophila suzukii*  
Dong H Cha (*Cornell University, Geneva, New York, USA*)

10:40 – 11:00

Invasive species brown marmorated stink bug semiochemical production and behavior response  
Aijun Zhang (*USDA-ARS, Beltsville, Maryland, USA*)

11:00 – 11:20

Managing invasive social wasps with semiochemicals  
Ashraf El-Sayed (*The New Zealand Institute for Plant & Food Research Limited, Lincoln, New Zealand*)

11:20 – 11:40

Pheromone based monitoring of *Neomusotima conspurcatalis* a biological control agent of the invasive weed *Lygodium microphyllum*  
Greg Wheeler (*USDA-ARS, Ft. Lauderdale, Florida, USA*)

11:40 – 12:00

Sequential SPME/GCMS Analysis (SSGA) - a new facile methodology for the identification of pheromones of fruit flies and other invasive species  
Anat Levi-Zada (*ARO, Volcani Center, Bet-Dagan, Israel*)

## Program – Friday September 25

### Chemical Ecology of Forest Insects (Royal Ballroom D/F, 10:00 – 12:00)

Chairs: Zhen Zhang & Steven Seybold

10:00 – 10:06

Introduction to APACE Symposium on Chemical Ecology of Forest Insects

Steve Seybold (USDA Forest Service, PSW Station, Davis, California, USA)

10:06 – 10:25

Complex semiochemical relationships in *Ips subelongatus* and its associated fungi

Xiang Bo Kong (Chinese Academy of Forestry, Beijing, China)

10:25 – 10:44

Semiochemicals regulating intraspecific and interspecific relationships of three *Tomicus* species in *Pinus yunnanensis* Franch

Zhen Zhang (Chinese Academy of Forestry, Beijing, China)

10:44 – 11:03

Developing monitoring techniques for the invasive goldspotted oak borer, *Agrilus auroguttatus*, in California

Tom Coleman (USDA Forest Health Protection, San Bernardino, California, USA)

11:03 – 11:22

Flight response of two invasive ambrosia beetles to ethanol and other semiochemicals in southern California

Yigen Chen (University of California, Davis, California, USA)

11:22 – 11:41

Insect pheromone-based management of forest pests in China: Current status and future development

Genzhong Cui (Pherobio Technology Co. Ltd., Beijing, China)

11:41 – 12:00

The role of host volatiles in the chemical ecology of a community of longhorn beetles

Maxwell Collignon <sup>s</sup> (University of California, Riverside, California, USA)

## Program – Friday September 25

### Application of Electrophysiology in Chemical Ecology (Royal Ballroom A/B, 13:30 – 17:00)

Chairs: Kye-Chung Park & Ayako Wada-Katsumata

13:30 – 13:55

GC-EAD as a tool for elucidating microbe-mediated attraction and avoidance in *Drosophila suzukii*  
Dong H Cha (Cornell University, Geneva, New York, USA)

13:55 – 14:20

Rapid change in gustatory sensilla drive behavioral resistance to baits  
Ayako Wada-Katsumata (North Carolina State University, Raleigh, North Carolina, USA)

14:20 – 14:45

Electron microscopy for electrophysiological recordings from chemosensory neurons  
Hyun-Woo Oh (Industrial Bio-materials Research Center, Daejeon, Republic of Korea)

14:45 – 15:10

Studies on sex pheromones in helmet crabs and blue crabs using a combination of approaches  
Michiya Kamio (Tokyo University of Marine and Technology, Tokyo, Japan)

15:10 – 15:30

*withdrew*

### Coffee/Tea Break (15:30 – 16:00)

16:00 – 16:25

A coupled HPLC-single sensillum recording system for identifying gustatory active compounds in insects  
Kye-Chung Park (New Zealand Institute for Plant and Food Research, Christchurch, New Zealand)

16:25 – 16:55

The purpose of puddling behavior of male *Papilio* butterflies (Video Presentation)  
Takashi Inoue (NIAS, Tsukuba, Ibaraki, Ibaraki, Japan)

## Program – Friday September 25

### **New Physiology Aspects of Chemical Ecology (Royal Ballroom C/E, 13:30 – 17:00)**

*Chairs: Naoki Mori & Gary Felton*

13:30 – 13:55

Targeted chemical ecology using chemical informatics and neurophysiology  
*Anandasankar Ray (University of California-Riverside, California, USA)*

13:55 – 14:20

Maize-corn leaf aphid interactions: Involvement of ethylene pathway  
*Joe Louis (University of Nebraska, Lincoln, Nebraska, USA)*

14:20 – 14:45

Microbial mediation of herbivore HAMPs and effectors  
*Gary Felton (Penn State University, USA)*

14:45 – 15:10

Handling foul-smelling stink bug: from heteropteran chemical ecology to a challenge toward insecticide development  
*Koji Noge (Akita Prefectural University, Akita, Japan)*

15:10 – 15:30

Multisensory modalities mediate the sex pheromone-induced upwind orientation behaviour in walking Indian meal moth  
*Shin Tejima <sup>s</sup> (The Kyoto University, Kyoto, Japan)*

### **Coffee/Tea Break (15:30 – 16:00)**

16:00 – 16:25

The tyrosine aminomutase TAM1 is required for  $\beta$ -tyrosine biosynthesis in rice  
*Naoki Mori (Kyoto University, Kyoto, Japan)*

16:25 – 16:50

Chemical ecology, behavior, and mutualistic interactions of beneficial nematodes as a belowground indirect plant defense  
*Jared Gregory Ali (Michigan State University, East Lansing, Michigan, USA)*

## Program – Saturday September 26

- 8:00 – 8:10 Briefing from JCE Editor, John Romeo (Royal Ballroom C/E)  
8:10 – 8:20 Introduction of ISCE Annual Meeting in Brazil, Paulo Zarbin (Royal Ballroom C/E)
- 8:20 – 9:30 Plenary Talks

### **Human pheromones: Where did we go wrong? What should we do next?**

Tristram Wyatt (University of Oxford, Oxford, UK) 8:20 – 8:55

### **Evolution of reproductive systems and pheromone communication in *Reticulitermes termites***

Kenji Matsuura (Kyoto University, Kyoto, Japan) 8:55 – 9:30

### **Coffee/Tea Break (9:30 – 10:00)**

### **Going from the Benchmark to the Marketplace for Semiochemical Products (Royal Ballroom C/E, 10:00 – 12:00)**

*Chairs: Jerry Zhu, Tom Baker & Jocelyn Millar*

10:00 – 10:25

Regulatory innovation, new mating disruption products in New Zealand, and the role of odor in 4-Play™  
*David Maxwell Suckling (The New Zealand Institute for Plant and Food Research Ltd., Christchurch and University of Auckland, New Zealand)*

10:25 – 10:50

Commercial Semiochemical Synthesis – Scaling and getting green  
*Cam Oehlschlager (ChemTica Internacional, S.A., Costa Rica)*

10:50 – 11:15

Retrospective of the 30-years long commercial application of mating disruption in Australian orchards and foreseeable challenges  
*Alex Ilichev (Primary Industry Research Victoria, Tatura, Victoria, Australia)*

11:15 – 11:40

Semiochemical-based suppression tactics for regulated plant pests  
*David Lance (USDA-APHIS-PPQ, Massachusetts, USA)*

11:40 – 12:00

The application of semiochemical-based products in China  
*Genzhong Cui (Pherobio Technology Co. Ltd., Beijing, China)*

## Program – Saturday September 26

### Going from the Benchmark to the Marketplace for Semiochemical Products (Royal Ballroom C/E, 13:30 – 17:00)

Chairs: Jerry Zhu, Tom Baker & Jocelyn Millar

13:30 – 13:55

Turning scientific results into business results: some key points to develop successful semiochemical products

*Satoshi Nojima (Shin-Etsu Chemical Co., Ltd., Davis, USA)*

13:55 – 14:20

Semiochemical-based pest control products for consumer markets: Opportunities and challenges

*Qing-He Zhang (Sterling International, Inc., Spokane, Washington, USA)*

14:20 – 14:45

Bringing Novel Semiochemical Formulations to the Market

*Agenor Mafra-Neto (ISCA Technologies, Inc., Riverside, California, USA)*

14:45 – 15:10

Optimizing pheromone aerosol emitters for codling moth mating disruption

*Larry Gut (Michigan State University, East Lansing, USA)*

15:10 – 15:30

Challenges on scaling up the use of semiochemicals from the experimentation settings to the pest control industry worldwide: A private company's point of view

*Matthew Bohnert (Suterra LLC, Bend, Oregon, USA)*

### Coffee/Tea Break (15:30 – 16:00)

16:00 – 16:25

Mating disruptant for the carpenter moth, *Cossus insularis* (Lepidoptera: Cossidae)

*Kiyoshi Nakamuta (Chiba University, Matsudo, Chiba, Japan)*

16:25 – 16:50

Registration and commercial development of a plant volatile based attract and kill system for *Helicoverpa* spp.

*Peter Gregg (University of New England, Armidale, NSW, Australia)*

## Program – Saturday September 26

### Chemical Ecology in General (Royal Ballroom D/F, 10:00 – 12:00)

Chairs: Guirong Wang

10:00 – 10:20

Recent Advances in the Pheromone Chemistry of Stink Bugs

*Paulo Zarbin (Federal University of Parana, Curitiba - PR, Brazil)*

10:20 – 10:40

Virus-mediated interactions of plants and insects

*Fengming Yan (Henan Agricultural University, Zhengzhou, China)*

10:40 – 11:00

MHC variation in Leach's storm-petrels (*Oceanodroma leucorhoa*); Implications for mate choice and selection via MHC-related odors

*Gabrielle Nevitt (University of California Davis, California, USA)*

11:00 – 11:20

Two salivary proteins of the rice brown planthopper *Nilaparvata lugens* function as effectors for defense responses in rice

*Yonggen Lou (Zhejiang University, Hangzhou, China)*

11:20 – 11:40

The effects of salt stress on herbivore defense in *Zea mays*

*Michael Rostas (Bio-Protection Research Centre, Lincoln, New Zealand)*

11:40 – 12:00

Exploitation of fruit fly innate behaviour: methyl eugenol response and ingestion - an insight into the synonymization of four invasive species of *Bactrocera dorsalis* complex

*Suk-Ling Wee (Universiti Kebangsaan Malaysia, Bangi, Selangor Darul Ehsan, Malaysia)*

### Chemical Ecology in General (Royal Ballroom D/F, 13:30 – 17:00)

Chairs: Guirong Wang

13:30 – 13:50

Application of chemical regulators modulate direct and indirect plant defenses against tea geometrid *Ectropis oblique*

*Zhaojun Xin (Tea Research Institute, Chinese Academy of Agricultural Sciences, Hangzhou, China)*

13:50 – 14:10

Current status of tea pest management via semiochemical-based approaches in China

*Baoyu Han (Zhejiang Provincial Key Laboratory of Biometrology and Inspection & Quarantine, China Jiliang University, Hangzhou, China)*

13:50 – 14:10

Enhancement of larval RNAi efficiency by overexpressing Argonaute2 gene in the silkworm, *Bombyx mori*

*Zhiqian Li <sup>s</sup> (Institute of Plant Physiology and Ecology, Chinese Academy of Sciences, Shanghai, China)*

<sup>s</sup> Student Presenter

# Oral Presentation Abstracts

## PLENARY

September 24-26, 2015

0001

## From gene discovery to metabolic engineering: Production of moth pheromones in plant and cell factories

Christer Löfstedt

*Department of Biology, Lund University, Sweden*

The use of pheromones for control of pest insects has many advantages over the use of traditional pesticides. The global market for pheromone-based control products is currently estimated to approximately \$200 millions and tons of synthetic pheromones are produced commercially for this purpose. We currently explore two “green chemistry” alternatives to conventional synthetic production of pheromones. One option is the pheromone brewery, a yeast cell factory for pheromone production. Another option is a plant factory, using genetically modified plants for production of pheromones or pheromone precursors. As a proof of the pheromone brewery concept, we co-expressed a  $\Delta 11$  desaturase and a FAR in the Brewer’s yeast *Saccharomyces cerevisiae* and produced (*Z*)-11-hexadecenol. Metabolic engineering of *S. cerevisiae* and optimized fermentation conditions has the potential to increase production titres dramatically. Using *Nicotiana benthamiana* as a plant factory, we produced several typical 14C and 16C moth sex pheromone components by transient expression of up to four genes coding for consecutive biosynthetic steps. The fatty alcohol fractions from the genetically modified plants were acetylated and mixed to mimic the respective sex pheromones of the small ermine moths *Yponomeuta evonymella* and *Y. padella*. These mixtures were very efficient and specific for trapping of male moths and matched the activity of conventionally produced synthetic pheromones. Semi-synthetic preparation of sex pheromones may be a novel and cost-effective way of producing moderate to large quantities of pheromones with high purity and a minimum of hazardous waste. One such possibility involves the use of genetically modified oil crops for production of pheromone components and precursors.

Hagström Å. K., Wang, H.-L., Liénard, M.A., Lassance, J.M., Johansson, T. and Löfstedt, C. 2013. A moth pheromone brewery: production of (*Z*)-11-hexadecenol by heterologous co-expression of two biosynthetic genes from a noctuid moth in a yeast cell factory. *Microbial Cell Factories* 12:125:1-11.

Ding, B.-J., Hofvander, P., Wang, H.-L., Durrett, T.P., Stymne, S., and Löfstedt, C. 2014. A plant factory for moth pheromone production. *Nature commun.* 5:3353 (<http://dx.doi.org/10.1038/ncomms4353>)

0002

## A Tale of Two Enzymes: Unexpected Insights into Pheromone Evolution and Oenocyte Survival

Joanne Yew

*University of Hawaii at Mānoa, Honolulu, HI, USA*

Insect behaviours are often guided by chemical signals, but little is known about how pheromone diversity evolves. To identify novel regulators of pheromones and other cuticular lipid components in *Drosophila*, we performed a RNA interference screen coupled with mass spectrometry. I will describe the characterization of two previously uncharacterized pheromone enzymes and their contribution to chemosensory diversity.

Elimination of an elongase, named *bond*, resulted in the loss of a male sex pheromone and unexpectedly, a reduction in male fertility as well as that of their conspecific rivals. Comparative analysis of *bond* expression in other drosophilids reveals that the gain of a cis-regulatory transcription site enabled expression of *bond* in the male ejaculatory bulb, a specialized site of pheromone production.

I will discuss also the role of a steroid dehydrogenase, which we name *spidey*. Silencing *spidey* expression during early adulthood results in oenocyte loss and a concomitant reduction of cuticular hydrocarbons, desiccation resistance, and life span. Changes in ecdysteroid levels during development and adulthood may be one way by which sustained alterations in cuticular lipid profiles are induced by environmental conditions, potentially contributing to reproductive isolation.

0003

## **Moth Pheromones: What are the Known Knowns, the Known Unknowns, and the Unknown Unknowns?**

Ring Cardé

*Department of Entomology, University of California, Riverside, USA*

How do male moths find a pheromone-emitting female? This issue is a “known known” in that, following Kennedy’s 1940 study demonstrating optomotor anemotaxis in the yellow fever mosquito, it has been accepted that moths use this mechanism to track along a pheromone plume. But a “known unknown” is: what are the optimal strategies for finding the plume? When wind direction is relatively constant, crosswind flights could be favored. When wind direction shifts over 60°, upwind and downwind paths could be optimal. Alternatively, the path may be random as in either Lévy or Random Walks. Simulations using the flight characteristics of gypsy moths suggest that strategies similar to Lévy Walks are most apt to result in plume contact. In two available field observations, moths adopted a random orientation with respect to concurrent wind direction.

How do pheromone channels change? We are only privy to their current state and we must speculate on mechanisms that may operate over 100s of generations to foster divergence—the “known unknowns.” the most commonly cited selective force is stabilizing selection, but even here direct proof is elusive. Change by a sudden “mutational leap” requires concomitant change in the signaler and receiver. One mechanism is reproductive character displacement wherein competition in the communication channel by two already differentiated “species” fosters divergence. There are, however, few candidate cases. The rapid characterization of genes and gene products that determine the structure of these channels define the factors that underlie divergence, but the processes driving change remains a “known unknown.”

0004

### Plant induced defense and insect counter defense

Rensen Zeng<sup>1,2</sup>, Yuanyuan Song<sup>1,2</sup>, Shiming Luo<sup>2</sup>, Ruilong Wang<sup>2</sup>, Wenxiong Lin<sup>1</sup>

<sup>1</sup>College of Life Sciences, Fujian Agriculture and Forestry University, Fuzhou, China, <sup>2</sup>Institute of Tropical & Subtropical Ecology, South China Agricultural University, Guangzhou, China

During long coevolution between plants and insects, plants have evolved an array of defensive strategies against insect herbivores, and meantime insects have developed multiple counter-defense strategies to respond to plant defense. Upon insect attack, plants initiate defense responses aided by the recognition of herbivore-specific molecular patterns, followed by the activation of a complex regulatory network involving jasmonate and other signalings, the expression of defense-related genes and the production of defense compounds which can enhance plant resistance against subsequent attack. We found that arbuscular mycorrhizal fungi (AMF), Plant growth-promoting rhizobacteria (PGPR) and silicon (Si) induced plant defense against insect herbivores. We used RNA interference (RNAi) and CRISPR-Cas system to determine the role of particular defense signaling pathway in rice defense against insect herbivores. Interactions between jasmonate (JA) signaling pathway and Si in response to insect attack were examined. The role of JA signaling in AMF induced defense was determined. Polyphagous herbivore insects encounter numerous allelochemicals in their different host plants. Certain plant allelochemicals reduced toxicity of mycotoxins and insecticides to insects by inducing detoxification systems, including cytochrome P450 monooxygenases (P450s), which can detoxify a broad range of substances. Ecological significances of xenobiotic resistance induced by plant allelochemicals will be discussed. Our results suggest that plant chemical defense can be induced by multiple agents, but insects can employ plant defensive compounds as antidotes to up-regulate their detoxification enzymes. Appropriate manipulation of plant induced defense and insect counter-defense is an important approach to optimize chemically based pest management programs.

0005

**Human pheromones: Where did we go wrong? What should we do next?**

Tristram Wyatt

*Department of Zoology, University of Oxford, UK*

As humans are mammals, it is possible, perhaps even probable, that we have pheromones. However, there is no robust bioassay-led evidence for the widely published claims, started by a corporation interested in patenting them, that the molecules androstadienone and estratetraenol are human pheromones. Positive results in studies of these molecules need to be treated with scepticism as these are likely to be false positives.

Instead, if we are to find human pheromones, we need to treat ourselves as if we were a newly discovered mammal, and use the rigorous methods already proven successful in pheromone research on other species. Establishing a pheromone relies on demonstration of an odour-mediated behavioural or physiological response, identification and synthesis of the bioactive molecule(s), followed by bioassay confirmation of activity. Comparison of secretions from adult and pre-pubertal humans may highlight potential molecules involved in sexual behaviour. One of the most promising human pheromone leads is a nipple secretion from the areola glands produced by all lactating mothers, which stimulates suckling by any baby not just their own. [www.zoo.ox.ac.uk/group/pheromones/](http://www.zoo.ox.ac.uk/group/pheromones/)

0006

## Evolution of reproductive systems and pheromone communication in *Reticulitermes termites*

Kenji Matsuura

*Laboratory of Insect Ecology, Graduate School of Agriculture, Kyoto University, Japan*

The evolution and maintenance of sexual reproduction is believed to involve important tradeoffs. The queens of social insects are faced with a dilemma over the costs and benefits of sexual and asexual reproduction. Asexual reproduction by a queen doubles her contribution to the gene pool. However, overuse of asexual reproduction reduces the genetic diversity of the offspring and thus the ability of the colony to adapt to environmental stress. Recent research suggests that queens of some termite species can solve this trade-off by the conditional use of sexual and asexual reproduction, whereby queens produce the next generation of queens by parthenogenesis but use sexual reproduction to produce other colony members. This reproductive system, so called AQS (Asexual Queen Succession), has been found in subterranean termites *Reticulitermes speratus*, *R. virginicus*, *R. lucifugus* and in a Neotropical higher termite *Embiratermes neotenicus*. It is known that AQS evolved multiple times independently in termites. In the AQS species, queens produce parthenogenetic offspring under the presence of kings by producing eggs without micropyles (sperm-gates, i.e., tiny openings for sperm entry). We also identified the active components of the queen pheromone, which regulate the differentiation of new neotenic queens, in *R. speratus*. I discuss how queen pheromone and genomic imprinting are involved in the evolution of asexual queen succession system.

# Oral Presentation Abstracts

## SYMPOSIUM

Chemical Ecology of Biting Insects

September 24, 2015

**0007**

**Receptors, Neurons and Mechanisms for Insect Repellents and Lures**

Anandasankar Ray

*University of California Riverside, Riverside, CA, USA*

Biting insects find human hosts using olfactory cues present in breath and skin. Their olfactory systems have evolved to detect odorants emitted from hosts which they use efficiently to navigate. A better understanding of the odorant receptors and olfactory neurons in mosquitoes that detect the human odorants has enabled us to target specific strategies to disrupt attraction. These strategies include the identification of odorants that are agonists or antagonists, as well as odorants that show prolonged tonic responses in the neurons. Behavioral analyses of the different classes of odorants identify several opportunities to prevent attraction of mosquitoes to humans. These affordable and safe odor-based behavioral control strategies are expected to have a significant impact in slowing the spread of deadly diseases worldwide.

0008

### **Chemical communication in bed bugs**

Kenneth Haynes, Sydney Crawley  
*University of Kentucky, Lexington, KY, USA*

Here we review what is known about the chemical chemical communication in bed bugs, and present results of ongoing studies that suggest novel ways that chemical signals may be involved. Our observations of bed bug behavior suggest new dimensions to bed bug interactions that may indicate additional benefits of aggregation. We hypothesize that adult females release chemical signals that improve foraging behavior by their offspring. Females also appear to mark their own eggs, suggesting the possibility that a signal is used to maintain contact between mother and offspring (amongst other possibilities). Also we find that presence of an adult female reduces predation on early stage nymphs. If the hypotheses implicit in these observations are supported, the results would suggest that bed bugs go beyond aggregation to include behaviors that are typically considered subsocial. We present these observations and results at this early stage to generate discussion and hopefully new ideas for experimental directions.

0009

**180,000 bites later.....the aggregation pheromone of the common bed bug is finally identified**

Regine Gries<sup>1</sup>, Robert Britton<sup>2</sup>, Michael Holmes<sup>2</sup> and Gerhard Gries<sup>1</sup>

<sup>1</sup>*Department of Biological Sciences, Simon Fraser University, Burnaby, British Columbia V5A1S6, Canada;*

<sup>2</sup>*Chemistry Department, Simon Fraser University, British Columbia V5A1S6, Canada*

Drawing on our 2014-publication in *Angewandte Chemie (International Edition)*, this presentation will describe our approach to accumulate sufficient pheromone source for identifying the aggregation pheromone of the common bed bug (*Cimex lectularius*; Hemiptera: Cimicidae), the analytical steps we have taken to identify the pheromone blend, the pheromone components that mediate attraction and arrestment of bed bugs, and the experiments we have run in the laboratory and in bed bug-infested apartments to test the effect of synthetic pheromone as a trap lure. The presentation will also highlight future objectives including the development of a commercial lure and trap.

**Search for odorants inducing olfactory behavior on the body louse, *Pediculus humanus corporis*, based on the response of olfactory receptor.**

Takuma Iwamatsu<sup>1,2,5</sup>, Daisuke Miyamoto<sup>1</sup>, Hidefumi Mitsuno<sup>1</sup>, Yoshiaki Yoshioka<sup>3</sup>, Takeshi Sakurai<sup>1</sup>, Ryohei Kanzaki<sup>1</sup>

<sup>1</sup>The University of Tokyo, Tokyo, Japan, <sup>2</sup>JSPS Research Fellow, Tokyo, Japan, <sup>3</sup>Osaka Pharmaceutical Co.,Ltd., Osaka, Japan

The pest control of body lice is medical importance because they mediate three pathogenic bacteria. Body lice show olfactory behaviors to odorants and they reportedly possess an olfactory organ called sensillar pegs on their antenna, suggesting that they detect odorants with their antennae. In addition, 9 olfactory receptor (OR) candidate genes named PhORs and one olfactory co-receptor candidate gene named PhOrco were discovered in genome of body lice through whole-genome analysis. However, it remains unknown whether PhORs possess ability to detect odorants and mediate olfactory behaviors in body lice. Thus, identification of the odorant inducing olfactory behavior based on the response of ORs would enable us to develop repellent efficiently. Here, we report the functional characterization of a PhOR and behavioral responses of body lice towards odorants selected based on the responsiveness of the PhOR. First, RT-PCR analysis indicated that 5 PhORs and PhOrco genes were confirmed to be expressed in the head with antenna of adult body lice. Then, we performed functional analysis of these 5 PhORs using *Xenopus* oocytes expression system with the electrophysiological method to screen odorants that activate PhORs from among 11 groups of the odorant cocktails (total 66 odorants). As a result, we found that oocytes co-expressing PhOR2 and PhOrco responded to some odorants tested. Behavioral analysis to these odorants using two-choice assay revealed that a vanilloid and a terpene elicit olfactory behaviors in body lice. These results suggest that the odorants selected based on the responses of PhOR induce olfactory behaviors of body lice.

0011

**Location of a carbon dioxide source by *Anopheles coluzzii* under conditions of laminar air flow and still air**

Emerson Lacey, Ring Cardé  
University of California Riverside, Riverside, CA, USA

Recent research suggests that detection of carbon dioxide (CO<sub>2</sub>) "gates" other modalities employed host-location (e.g. sequentially sensitizes mosquitoes to skin odor and temperature in the vicinity of a host). However, CO<sub>2</sub> on its own has long been recognized as a strong attractant for many mosquito species and is often employed in traps. Orientation and navigation upwind along a plume to a source of CO<sub>2</sub> is presumed to be the method used to locate the source however, mosquitoes are challenged with environments where air movement does not provide a reliable directional cue (i.e. inside a human dwelling). In this study we test the hypothesis that female *Anopheles coluzzii* (formerly *An. gambiae* s.s., M-form) can locate a source of CO<sub>2</sub> in still air. Mosquitoes were flown in a 1.5 m long x 0.5 m wide x 0.5 m tall wind tunnel with either laminar air flow of 20 cm / sec or still air. The stimulus was 300 mL / min of air enriched to 4% with CO<sub>2</sub>. Numbers locating the source in still air (~40%) was significantly greater than numbers locating a clean air control (0%) and significantly lower than in a laminar air flow (~85%). We conducted another experiment to test the hypothesis that additional semiochemicals in human breath would increase the numbers locating the source in still air vs. 4% CO<sub>2</sub>. There was no difference in numbers locating 4% CO<sub>2</sub> in moving air, or breath in still or moving air (>80%).

# Oral Presentation Abstracts

**SYMPOSIUM**

**Fruit Fly Chemical Ecology**

**September 24, 2015**

0012

**Fruit ripening volatiles act synergistically as host cues in a pest tephritid fruit fly.**

Paul L. Cunningham

*Queensland University of Technology. Brisbane. Australia*

Many economically damaging tephritid fruit fly pests are highly polyphagous, attacking a broad range of cultivated and wild fruit species. How these insects are able to recognise the odours of so many different host species is still poorly understood. Here, we studied behavioural responses of female Queensland fruit fly, *Bactrocera tryoni*, to odours of whole guava fruits (*Psidium guajava*) during different stages of ripening. Flies showed the strongest attraction to odours of fully mature fruit, compared to three other ripening stages and pureed guava juice. Volatile analysis of guava odour identified three esters that increased significantly in the most attractive stage, and were low in pureed fruit juice. Behavioural experiments demonstrated that a synthetic blend of the three esters was significantly more attractive to flies than an eight volatile guava-based synthetic blend without these esters, and also more attractive than guava juice. Further experiments demonstrated that when each ester was presented individually, flies showed no significant difference in attraction compared to an odourless control, demonstrating behavioural synergism in responses towards these compounds. Moreover, injecting poorly attractive hosts (squash and cucumber) with the ester blend increased their attractiveness to equal that of a highly attractive host (peach). Though these fruit flies are classed as generalist insects, they could also be regarded as fruit specialists, with groups of volatiles forming predictable host signals. Viewing olfaction in this way may improve our understanding of the evolution of host choice in polyphagous insects, and the selection of volatiles to be used as lure-and-kill attractants in insect pest management.

0013

### **Challenges and constraints in fruit fly chemical ecology**

Eric Jang

*USDA-ARS- Pacific Basin Agricultural Research Center (PBARC), 64 Nowelo Street, Hilo, HI, USA*

The chemical ecology of tephritid fruit flies represents a broad field that deals with many aspects of the life history of the species. A major focus for the economic species of importance has been the development of semiochemical lures and attractants for tephritids. Researchers have a better understanding of the chemical ecology of these tephritids. Central to an improved understanding is our increased knowledge of the physiology and ecology of tephritids, specifically the influence of physiological state and how kairomones influence behavior. Similarly, improvements in our understanding of fruit fly chemoreception and chemical organic synthesis is allowing us to develop new and improved lures for detection, delimitation and control. Yet despite these advances there are clearly major challenges ahead in the field. Questions remain as to why certain families respond to semiochemicals while others show little or no response. What paradigms might we challenge to gain further insight into the chemical ecology of these species. What has worked and not worked in the past and what lies ahead as a new generation of fruit fly chemical ecologists embark on their careers.

0014

### Fruit fly chemical ecology- an update

Alvin Kah-Wei Hee<sup>1</sup>, Suk-Ling Wee<sup>2</sup>, Ritsuo Nishida<sup>3</sup>, Keng-Hong Tan<sup>4</sup>

<sup>1</sup>Universiti Putra Malaysia, Serdang, Selangor Darul Ehsan, Malaysia, <sup>2</sup>Universiti Kebangsaan Malaysia, Bangi, Selangor Darul Ehsan, Malaysia, <sup>3</sup>Kyoto University, Kyoto, Japan, <sup>4</sup>Tan Hak Heng Co., Penang, Malaysia, Malaysia

The use of potent tephritid male attractants such as methyl eugenol (ME) and cue lure (CL) has contributed immensely to the successful control and eradication of pest fruit flies. Since the discovery of male fly attraction to ME over a century ago, the role of ME constituted "a great tephritid mystery" in the unique attraction of certain male *Bactrocera* species to this very potent chemical. As a natural compound, ME is found in over 450 plant species. However, in the last two decades, the relationship between certain male flies and ME has been finally elucidated. Male Oriental fruit fly, *B. dorsalis*, biotransforms ingested ME to sex pheromone components, 2-allyl-4,5-dimethoxyphenol (DMP) and (*E*)-coniferyl alcohol (CF) that are sequestered into the rectal gland for emission during dusk to attract females for copulation. These chemicals are similarly utilized by the flies as allomone to deter predation. In male melon fly, *Zeugodacus cucurbitae*, consumption of CL by male flies has also boosted its mating competitiveness. Furthermore, several putative sibling species, *B. invadens*, *B. papayae* and *B. philippinensis* in the *B. dorsalis* complex also release CF and DMP as sex pheromone components following ME consumption. The realization of this fact eventually contributed to the recent success in confirming that those species are in fact *B. dorsalis*. In the search for new attractants, an understanding of the fruit fly-plant (e.g. *Bulbophyllum* orchid) relationship has yielded novel compounds as attractants. These findings continue to highlight the fascinating discoveries in unravelling the *Bactrocera* fruit flies-host plant relationship.

0015

**Evaluation of gel commercial lure for spotted wing drosophila, *Drosophila suzukii***

Amanda Ramsey<sup>1</sup>, Gerry Bohmfalk<sup>1</sup>, Elizabeth Beers<sup>2</sup>

<sup>1</sup>*Scentry Biologicals, Inc., Billings, MT, USA,* <sup>2</sup>*TFREC- Univ. of WA, Wenatchee, WA, USA*

Scentry Biologicals, Inc. has been researching and developing devices for insect monitoring and control for over 30 years. Invasive pest detection, delimitation, and control using semiochemical-based attractants form the foundation of pest management programs. We report a summary and an update of recent research conducted to monitor and control Spotted Wing Drosophila (*Drosophila suzukii*).

The Spotted Wing Drosophila (SWD) is a vinegar fly of East Asian origin that can cause damage to many fruit crops of economic importance. SWD has been in the United States in Hawaii since the 1980s, and was detected in California in 2008 and has spread through-out many fruit crop growing regions. Recently, volatiles have been identified from apple cider vinegar and red wine (Landolt et. al.), and combining the identified chemistries with acetic acid and ethanol provide favorable field results compared to wet traps baited with vinegar. These compounds of varying solubility were formulated into a gel pouch dispenser and field tested to optimize attraction and field life. This improved detection method provides a more convenient and reliable method for detection of SWD. The lure design has been marketed with a trap and thoroughly field tested both domestically and internationally. Future work includes a complimentary dry-trap and improved specificity.

0016

**Towards a mechanistic basis for sympatric speciation in *Rhagoletis* fruit flies**

Cheyenne Tait<sup>1</sup>, Jeff Feder<sup>1</sup>, Shannon Olsson<sup>2</sup>

<sup>1</sup>University of Notre Dame, South Bend, IN, USA, <sup>2</sup>National Centre for Biological Sciences, Bangalore, India

The *Rhagoletis* species complex has long served as a paradigm for sympatric speciation in action. These Tephritid fruit flies are believed to be undergoing incipient sympatric speciation via shifts from one host plant to the other. In field and laboratory experiments, sympatric populations infesting apple (*Malus pumila*) and hawthorn (*Crataegus* spp.) not only preferentially orient to host volatiles emitted from their respective host fruits, but are antagonized by the addition of non-host volatiles to their blend. Flies mate and oviposit directly on host fruit. Thus, differences in host volatile choice generate prezygotic reproductive isolation between flies infesting different host plants. A comparison of F<sub>1</sub> and F<sub>2</sub> crosses and backcrosses also indicate that this behavioral preference is controlled by only a few genetic loci, implying that the mechanistic basis for host preference involves very few neurological changes in the olfactory system.

Our previous studies suggest that genetic changes in *Rhagoletis* fruit odor preference mainly target the brain. The stereotyped, innate, and heritable nature of fruit search also indicates a dedicated sensory pathway consistent across populations. Here, we discuss the various mechanisms by which changes in olfactory preference could occur, and present a series of histochemical, biochemical, and physiological experiments to address these hypotheses. We also compare our results in the context of other Tephritid and Dipteran neuroecologies.

# Oral Presentation Abstracts

**SYMPOSIUM**

**Molecular Chemical Ecology**

**September 24, 2015**

0017

**Pollinator chemical ecology and CYPome "blooms"**

May R. Berenbaum

*Department of Entomology, University of Illinois, Urbana-Champaign, Illinois, USA*

When the genome of *Apis mellifera*, the western honeybee, was sequenced, among noteworthy findings was the reduced inventory of detoxification genes relative to other insect genomes. With only 46 cytochrome P450 genes, the honey bee genome encodes half the number of P450s in the hymenopteran parasitoid *Nasonia vitripennis* genome. This reduction seems paradoxical in view of the fact that the polylectic honeybee encounters a tremendous diversity of phytochemicals in the nectar and pollen utilized for food production. Of the 46 P450s, 28 genes belong to the CYP3 clan, a lineage that includes the CYP6 family, many of which serve detoxificative functions in other insects. The reduced repertoire of P450 genes may relate to the ability of honeybees to reduce toxin exposure by behavioral means. Foragers can detect and avoid potential toxins, reducing inputs, and processing of nectar into honey and pollen into beebread may reduce toxin exposure even further, as does storage at hive temperatures. However, expansion within the CYP6AS subfamily, relative to *N. vitripennis*, suggests that there may be detoxificative specialization associated with consumption of nectar and pollen. The recent sequencing of ten bee genomes provides a wealth of tools for investigating the evolution of xenobiotic detoxification in a pollinator lineage, a critical component for understanding how mutualists cope with phytochemicals in their natural diets and how they process synthetic pesticides they are encountering with increasing frequency in contemporary agroecosystems.

0018

## **Insect PBANs: Structure, Function and Evolution**

Man-Yeon Choi

USDA-ARS, Corvallis, OR, USA

In moths, sex pheromone biosynthesis is stimulated and/or induced by a hormonal signal produced from the subesophageal ganglia to be released in the hemolymph activating their receptor in the pheromone gland. The hormone is called pheromone biosynthesis activating neuropeptide (PBAN) belonged in PBAN/pyrokinin family peptides that are defined by a common FXPRLamide or similar sequences at the C-terminal end. To date, about 200 PBAN family peptides have been reported from over 40 species, is the largest family of insect neuropeptides found in various insect groups, and implicated in regulating various physiological functions. The most well studied physiological function is regulation of moth sex pheromone biosynthesis through the PBAN, although several developmental functions have also been reported. These peptides, found in all insects studied thus far, have been conserved with the core structure and activity throughout evolutionary process. The peptide sequences are also widely diverse with gene expressions, and peptide processing and functionality. Extended knowledge of the PBAN/pyrokinin family of peptides to different insect groups, and mapped the insect PBAN gene structures and determined the tissue expression level in the central nervous system. The architecture of insect PBAN/pyrokinin indicates *Drosophila* is simpler than other insects elucidated to date, that also well match with the insect evolution. Summarize here our research to date on the molecular structure and evolutionary diversity of insect PBAN/pyrokinin genes and peptides in preparation for determining the function of the product neuropeptides.

0019

### **A single sex pheromone receptor mediates bombykol responses in the silkmoth**

Takeshi Sakurai, Ryohei Kanzaki

*Research Center for Advanced Science and Technology, The University of Tokyo, Tokyo, Japan*

Male moths locate their mates using species-specific sex pheromones emitted by conspecific females. One striking feature of sex pheromone recognition in male moths is the high degree of specificity at all levels from primary sensory processes to behavior. In this talk, recent work that addresses mechanisms underlying behavioral response specificity to conspecific pheromones using a genetically tractable moth species the silkmoth, *Bombyx mori*, will be presented.

In the silkmoth a single pheromone component, (*E,Z*)-10,12-hexadecadienol (bombykol), is sufficient to elicit full sexual behavior in males. A sex pheromone receptor BmOR1 is specifically tuned to bombykol and is expressed in specialized olfactory receptor neurons (ORNs) in the pheromone sensitive long sensilla trichodea of male antennae. To examine causal relationship between BmOR1 function and pheromone response *in vivo*, we generated BmOR1-knockout moths using transcription activator-like effector nucleases. The disruption of BmOR1 gene completely removes sensitivity of ORNs to bombykol and corresponding sexual behavior in males, demonstrating that BmOR1 is necessary for the recognition of bombykol. Using transgenic moths expressing a sex pheromone receptor PxOR1 of the diamondback moth *Plutella xylostella* in BmOR1-expressing ORNs, we found that artificial activation of BmOR1-expressing ORNs alone is sufficient to trigger sexual behavior. Together, our results show that the activation of BmOR1-expressing ORNs evoked by the interaction between BmOR1 and bombykol is necessary and sufficient for triggering sexual behavior in male silkmoths. Thus, BmOR1 is the sole receptor that mediates the bombykol response and its ligand selectivity defines behavioral specificity in the silkmoth.

0020

### Symbiont-mediated body color change in the pea aphid

Tsutomu Tsuchida

*University of Toyama, Toyama, Japan*

In the pea aphid *Acyrtosiphon pisum*, red and green color morphs are found in the same populations. Early ecological studies showed that the color variations are important traits, often involved in the prey-predation relationships. Red morph of the aphid is more attractive to a predator ladybird beetle, while green morph is more cryptic. In the previous study, we found an aphid endosymbiont of the genus *Rickettsiella* that modifies the aphid body color from red to green. Therefore, the symbiont-induced body color change seems to have an effect not only on infected aphid but also on prey-predation relationships and food web in ecosystem. We examined phylogenetic relationship, microbiological natures, and phenotypic traits of *Rickettsiella* symbiont, and revealed that the symbiont is distinctly different from previously reported *Rickettsiella* species. Therefore, we proposed the designation “*Candidatus Rickettsiella viridis*” for the symbiont clade.

We chemically characterized the main green pigments that are increased by *Rickettsiella* infection. Quantification using LC-MS confirmed that both of *Rickettsiella*-infected and uninfected aphids contain same green pigments, although the amounts of the pigments were remarkably different between them. From those results, *Rickettsiella* probably does not synthesize the green pigments by itself but somehow interferes with the aphid metabolism to activate the green pigment production. In this conference, I will also present our recent outcomes regarding molecular mechanisms underlying *Rickettsiella*-induced body color change.

0021

### The edited doublesex gene (dsx) of silkworm alters mating behaviour

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Chemical ecology is developing from behavioural observation, semiochemical identification, perception, behavioral response and chemical application to the understanding of the mechanisms at the physiological and molecular level. The further exploitation not only offers the profound knowledge to this Subject, but also serves to the new methods for insect behavioural and population regulation. *Drosophila*, the model insect, has been applied for this purpose and does provide valuable knowledge. But the diversity of insect species needs more targets and tools. Thanks to the progresses of genome research and the genome editing tools, the mechanism exploitation could be extended to many other insects.

Armed with genome information, transgenesis, and genome editing techniques, silkworm is considered as an ideal model for various researches of lepidopteran insects. In addition, with increasing demand for environmentally-friendly pest control, species specific gene targeting and genetic regulation are widely considered to be the future choice. Recently, the new technique of genome editing, Talents and CRISPR/CAS9, offers us convenient alternatives for gene knock out and knock in. Recently, we edited the doublesex gene of silkworm using genome editing tools. Doublesex gene has five exons, female has completely five exons, while male only has three. Because the site specific operation properties, we could edit male and female specific exons. The results showed that mating behaviour has seriously changed after the exons were knock out. Males could find females but the copulation attempt was failure. From this research, we could find that the editing not only affect the female egg formation, but also male copulation behaviour.

0022

**Functional characterization of carboxylesterases for degradation of ester odorants in adult antennae of *Spodoptera exigua***

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To characterize carboxylesterases (CXEs) that may act as Odorant Degrading Enzymes (ODEs) in *Spodoptera exigua*, a homologue search in transcriptome was performed and 57 putative CXE cDNA fragments were obtained. Then, qPCR measurements indicated that 3 CXE genes (SexiCXE4, 10 and 14) were antenna predominantly expressed. Phylogenetic tree showed that SexiCXE4 and 14 were distributed into group A (Mitochondrial, cytosolic and secreted esterases) and SexiCXE10 was into group B&C (Higher and lower dipteran microsomal  $\alpha$ -esterases), suggesting that the 3 CXEs prefer different substrates. In vivo functional study determined that SexiCXE4 and 14 had high degrading activity ( $V_{max}$ ) to host plant volatiles; and to the sex pheromones, the  $V_{max}$  and affinity parameter ( $K_m$ ) were comparable to those of reported pheromone degrading enzymes (PDEs). In contrast, SexiCXE10 had high activity specifically for some ester plant volatiles. Analysis with SexiCXE14 demonstrated that carbon chain length is a major influential factor, while the number of double bonds also affects the enzymatic activity of a substrate. In addition, SexiCXE14 displayed a “ $\wedge$ ” curve and peaked at pH 6.5 in the activity, with the pH increasing from 5 to 9; while SexiCXE10 displayed a “S” curve, with much lower activity at acidic pH (pH 5.0) than at neutral and alkaline conditions (pH 6.5-9.0). In conclusion, our study revealed two types of antennal SexiCXEs regarding the specificity in degradation of ester odorants. SexiCXE4 and 14 could degrade both sex pheromone components and plant volatiles, while SexiCXE10 could degrade only plant volatiles.

0023

**Binding specificity analysis of odorant binding protein AlucOBP8 of the *Apolygus lucorum* (Meyer-Dür)**

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Odorant binding proteins (OBPs), an important class of proteins, are involved in the process of insect olfactory perception. One of OBP's functions is to bind and transport odor molecules through the lymph to reach neuronal dendrites. In the present study, we cloned an OBP gene of *Apolygus lucorum* (Meyer-Dür) (GenBank No.JQ675725) and named *AlucOBP8*. The quantitative real-time quantitative PCR (qPCR) analysis demonstrated that the *AlucOBP8* was mainly expressed in the antennae of both male and female adult bugs. The fluorescence competitive binding assays were conducted to measure the binding abilities of recombinant *AlucOBP8* to seven sex pheromone analogs and thirty-five cotton volatiles. The results revealed that *AlucOBP8* could not bind to sex pheromone analogs. Only two cotton volatiles,  $\alpha$ -caryophyllene and dodecyl aldehyde showed strong binding affinities and the dissociation constant were 8.74  $\mu\text{mol/L}$  and 9.99  $\mu\text{mol/L}$ , respectively. We measured the taxis selection of *A.lucorum* to  $\alpha$ -caryophyllene and dodecyl aldehyde by using Y tube olfactometer. The results showed that dodecyl aldehyde had significantly repellent action on *A.lucorum* adult males. We suspected that the *AlucOBP8* might be a general odorant binding protein to play roles in odor recognition of host plant volatiles.

0024

**High rate and heritable mutagenesis of SlitPBP1 in *Spodoptera litura* by CRISPR/Cas9 mediated genome editing**

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The success in gene knockout by genome editing techniques is limited in some model insects. Here, we reported a highly efficient and heritable gene mutagenesis method in *Spodoptera litura* using a CRISPR/Cas9 system. The target gene in our study is the pheromone-binding protein 1 (SlitPBP1) gene, a key gene involved in the perception of the female sex pheromones. By co-injection of Cas9 mRNA and sgRNA into *S. litura* eggs, high rate (about 60%) mutations in specific sites of the genomic DNA of SlitPBP1 were detected both in the pooled sample of injected eggs and in the resulted moth individuals. We used the chimeric moths as parents to obtain the G1 offspring. In the G1 offspring from 9 single-pair lines, the percentage of heterozygous mutant individuals were averaged 60%, checked by HpyCH4 III genotyping and TA-clone sequencing. The establishment of several homozygous lines with SlitPBP1 gene knocked out was ongoing, which will be crucial for elucidating the function of SlitPBP1 in the sex pheromone perception in *S. litura*. Meanwhile, our study with CRISPR/Cas9 system provides a powerful means in gene functional study, particular for lepidopteran species in which the RNAi does not work well.

0025

**Sensillar expression and responses of olfactory receptors reveal different peripheral coding in two *Helicoverpa* species using the same pheromone components**

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Male moths can accurately perceive the sex pheromone emitted from conspecific females by their highly accurate and specific olfactory sensory system. The periphery pheromone encoding is attributed to the response strength of individual olfactory sensory neurons (OSNs) and the population of responding OSNs. The *Heliothis/Helicoverpa* species are regarded as good models for studying the perception of sex pheromones. In this study, we performed a series of experiments to investigate the peripheral mechanisms of pheromone coding in two-closely related species, *Helicoverpa armigera* and *H. assulta*. The morphology and distribution patterns of sensilla trichoidea are similar between the two species when observed at the scanning electron microscope, but their performances are different. In *H. armigera*, three functional types of sensilla trichoidea (A, B and C) were found to respond to different pheromone components, while in *H. assulta* only two types of such sensilla (A and C) could be detected. We also identified all the PRs and characterised them in terms of responses to pheromone components, using two electrode voltage clamp recordings in *H. armigera* and *H. assulta*. The response profiles of all types of sensilla trichoidea in the two species well matched the specificities of the pheromone receptors (PRs) expressed in the same sensilla, as measured in patch-clamp experiments. The expressions of PRs in neighboring OSNs within the same trichoid sensillum were further confirmed by *in situ* hybridization. Our results show how the same pheromone components can code for different messages at the periphery of two *Helicoverpa* species.

# Oral Presentation Abstracts

**SYMPOSIUM**

Plant Allelopathy

**September 24, 2015**

0026

**Investigating the phytotoxic activity of sorgoleone by heterologous reconstitution of the biosynthetic pathway**

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Allelopathy is a phenomenon of growth suppression of one plant species by another through the release of chemical compounds. Sorghum (*Sorghum bicolor*) had been shown to have inhibitory effects on growth of surrounding weeds. The major constituent of sorghum root exudates that causes this allelopathic activity is sorgoleone. The biosynthetic pathway of sorgoleone had been recently elucidated and the enzymes involved in the synthesis of this compound had also been identified and functionally characterized. To investigate the mechanism and in planta phytotoxic activity of sorgoleone, a series of constructs for expressing the genes for the entire pathway was made. *Agrobacterium*-mediated transient expression in *Nicotiana benthamiana* led to the accumulation of sorgoleone in agroinfiltrated leaves and resulted in formation of necrotic lesions in the infiltrated areas, indicating that in vivo synthesized compound causes phytotoxicity in plant cells. This approach holds great potential in characterizing gene function and provides a tool for uncovering the mode of action of allelochemicals.

0027

### Underground common mycorrhizal networks mediate interplant defense communication

Yuanyuan Song<sup>1,2</sup>, Shiming Luo<sup>2</sup>, Wenxiong Lin<sup>1</sup>, Rensen Zeng<sup>1,2</sup>

<sup>1</sup>College of Life Sciences, Fujian Agriculture and Forestry University, Fuzhou, China, <sup>2</sup>Institute of Tropical & Subtropical Ecology, South China Agricultural University, Guangzhou, China

Mycorrhizas are ubiquitous plant-fungus symbiotic association in terrestrial ecosystems. They play a key role in plant nutrition acquisition and stress resistance. Common mycorrhizal networks (CMNs) link multiple plants together in ecosystems. Our study shows that CMNs mediate plant-plant communication between healthy plants and enemy-challenged tomato plants. After establishment of CMNs with the arbuscular mycorrhizal fungus *Glomus mosseae* between tomato plants, inoculation of 'donor' plants with pathogen or herbivore insectled to increases in resistance and activities of the putative defensive enzymes, as well as induction of defense-related genes in healthy neighboring 'receiver' plants, suggesting that CMNs may function as a plant-plant underground talking conduit for systemic defense. However, use of a JA biosynthesis defective mutant *spr2* as 'donor' plants resulted in no induction of defence responses and no change in insect resistance in 'receiver' plants, suggesting that JA signaling is required for CMNs-mediated interplant communication. CMNs of both arbuscular mycorrhiza and ectomycorrhiza can serve as interplant communication conduits. Our results indicate that plants are able to hijack CMNs for herbivore-induced defence signal transfer and interplant defence communication. The significance of CMNs-mediated interplant communication will be discussed.

0028

## Metabolomics in soil rhizospheres: A key to understanding plant-plant interactions

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Metabolomics has great potential to provide insights to plant-plant interactions. Plants and microbes release many bioactive metabolites into the rhizosphere. These compounds generally occur in low concentrations but can serve important roles in communication and defense. Measurement of their dynamics in the soil environment is challenging but crucial to understanding rhizosphere interactions. We are applying new methods to measure allelochemical fluxes in the rhizosphere to investigate plant-plant interactions in Australian agroecosystems.

Paterson's curse (*Echium plantagineum*) is invasive in Australia. Echium roots produce an array of naphtho- and anthraquinones. Under certain environmental conditions, young roots produce large quantities of naphthoquinones in outer root periderm layers, and seedling root hairs exude dark red droplets of naphthoquinones. These naphthoquinones show potent antimicrobial, fungitoxic and phytotoxic activity through impacts on electron transport and cellular respiration. Field sampling of rhizosphere and bulk soils, followed by LC-MS QToF (HPLC coupled to time of flight mass spectrometry) can establish a more complete metabolic profile of the soil. Polydimethylsiloxane (PDMS) microtubing is useful for sorption of lipophilic compounds with minimal soil disturbance, and has been used to probe the potential dynamics of these compounds over time upon release from Echium roots.

Similar techniques are being applied to profile rhizospheres of competitive and less competitive wheat, barley and canola cultivars to gain insights into mechanisms of weed suppression by competitive cultivars. For many plants, polar allelochemicals are important in rhizosphere interactions and we are working to chemically modify PDMS probes to more effectively trap polar and moderately polar compounds.

0029

**A Research on autotoxins' temporal and spatial distribution and microbial communities within the soil of *Rehmannia glutinosa* root-zone**

Bao Zhang<sup>1</sup>, Xuanzheng Li<sup>2</sup>, Mingjie Li<sup>1</sup>, Li Gu<sup>1</sup>, Zhongyi Zhang<sup>1</sup>

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*Rehmannia glutinosa* L. (*R. glutinosa*), belongs to the *Scrophulariaceae* family, possess precisely clinic effect. However, the consecutive monoculture problems (replanting problems) resulted in tremendous decrease of its yield and quality. To reveal the factors in the soil that induced this issue, we performed four level experiments as followed. Firstly, based on assessment of autotoxicity effects on root exudates from different development stages, and from different root-zone soils, we found the sensitive stage when *R. glutinosa* perceive the autotoxin effect was at early stage of tuberous root expanding, and the farthest range of this effect could reach to 20 cm from the plant. Secondly, the root exudates of *R. glutinosa* at different development stages were in detail analysis using HPLC technology. The results strong suggested that syringic acid might be the dominated autotoxin secreted by *R. glutinosa*. Thirdly, the compounds in root-zone soils of *R. glutinosa* were further extracted by methanol and n-pentane before analyzing; the results showed that besides phenolic acids, other potential autotoxins might play important roles in autotoxicity. Finally, the microbial communities in soils of different root-zone were analyzed by PCR-DGGE technology and results showed that the microbial communities were remarkably changed and some new pathogenic microbes were induced by *R. glutinosa* cultivation. In addition, we identified a series of the candidate compounds and pathogenic microorganisms that might cause replanting disease. These results give insight into understanding formation mechanism of the consecutive monoculture problems of *R. glutinosa*.

0030

**Use of allelopathins with reduced dose of herbicides suppress weeds and microbial activities in wheat**

Muhammad Azim Khan

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The use of allelopathic plants and their potential as bioherbicide in combination with low rates of herbicides in wheat has become an attractive option for researchers. Allelopathic effects of crops and weeds were studied during 2011 and was repeated in 2012 by utilizing water extracts of allelopathic plants viz., *Oryza sativa* L., *Parthenium hysterophorus* L., *Phragmites australis* Cav. and *Datura alba* L. with reduced rates of herbicides for controlling weeds. *P. australis* and *P. hysterophorus* with lower rates of fenoxaprop-p-ethyl and bromoxinil+MCPA showed promising results by controlling weeds and improving wheat grain yield. In addition, these treatments were economical by giving higher values of cost benefit ratios. The study of microbial activity showed higher amount of mineralizable carbon in *D. alba* + ½ fenoxaprop-p-ethyl treated plots, while the least of mineralizable carbon was observed in control plots. Overall, the data showed that 50% reduced herbicide rates in combination with *P. australis* or *P. hysterophorus* extracts suppressed weeds and increased grain yield of wheat. The presence of allelopathic plants in field crops and subsequent mixing in soil by ploughing may create problems in crop production, as allelochemicals will be accumulated in the soil. Adverse effects of allelochemicals and herbicides also increased mortality of soil biota that is vital for sustainable crop production. Hence extensive and meaningful studies are suggested to fully explore all the possible interactions among allelochemicals and herbicides. It seems that plant derived compounds provide more opportunities as appreciated.

# Oral Presentation Abstracts

**SYMPOSIUM**

Aquatic Chemical Ecology

**September 24, 2015**

0031

**Chemical communication underwater: contrasts and similarities with terrestrial semiochemicals**

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Pheromones and other semiochemicals are essential for mediating aquatic species' behaviors, including those associated with mating, foraging, recruitment, and alarm. The ways that pheromones evolve in aquatic and terrestrial environments are similar but the kinds of molecules which become selected for use as pheromones are different. Longer distance aquatic pheromones can be large molecules so long as they are soluble. For example, proteins and peptides are used by many aquatic organisms from sea slugs to newts. One way that pheromones evolve is via espionage, for example with selection on males responding to cues such as hormones leaking from gravid females while their eggs develop. This appears to explain the evolution of goldfish sex pheromones. Multicomponent pheromones allow related sympatric fish species to share components but avoid cross-species attraction. In copepods, glycoproteins may take the place of cuticular hydrocarbons of insects in contact species-recognition. However, our understanding of aquatic pheromones seems limited by the greater challenges involved in isolating and identifying aquatic pheromones compared with those of terrestrial animals. Most pheromones are detected and processed by olfaction (in vertebrates) or, in crustaceans, analogous chemosensory systems with a glomerular design. There are few applied uses of aquatic pheromones but lamprey pheromones show promise for control of this vertebrate pest. A particular and dramatic threat to marine life comes from global disruption of the perception of crucial chemical cues and signals by falling aquatic pH due to rising atmospheric carbon dioxide levels.

0032

### **Chemical mediation of coral larval settlement by crustose coralline algae**

Tilmann Harder<sup>1,2</sup>, Jan Tebben<sup>1,3</sup>, Andrew Negri<sup>2</sup>, Cherie Motti<sup>2</sup>, Peter Steinberg<sup>3</sup>, Peter Schupp<sup>4</sup>

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<sup>3</sup>*University of New South Wales, Sydney, Australia,* <sup>4</sup>*University of Oldenburg, Oldenburg, Germany*

The majority of marine invertebrates produce dispersive larvae which, in order to complete their life cycles, must attach and metamorphose into benthic forms. This process, collectively referred to as settlement, is often guided by habitat-specific cues. While the sources of such cues are well known, the links between their biological activity, chemical identity, presence and quantification in situ are largely missing. Previous work on coral larval settlement in vitro has shown widespread induction by crustose coralline algae (CCA) and in particular their associated bacteria. However, we found that bacterial biofilms on CCA did not initiate ecologically realistic settlement responses in larvae of 11 hard coral species from Australia, Guam, Singapore and Japan. We instead found that algal chemical cues induce identical behavioral responses of larvae as per live CCA. We identified two classes of CCA cell wall-associated compounds - glycolipids and polysaccharides - as the main constituents of settlement inducing fractions. These algae-derived fractions induce settlement and metamorphosis at equivalent concentrations as present in CCA, both in small scale laboratory assays and under flow through conditions, suggesting their ability to act in an ecologically relevant fashion to steer larval settlement of corals. Both compound classes were readily detected in natural samples.

0033

**The role of AChE in swimming behavior of *Daphnia magna*: Correlation analysis of both parameters in the exposure of DM and MT**

Qing Ren<sup>1</sup>, Tingting Zhang<sup>1</sup>, Luhuzi Qi<sup>1</sup>, Shuaishuai Liu<sup>1</sup>, Na Xing<sup>1</sup>, Xun Wang<sup>2</sup>, Baixiang Ren<sup>1</sup>, Zongming Ren

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The 24 hours continuous effects of both deltamethrin (DM) and methomyl (MT) on swimming behavior and Acetylcholinesterase (AChE) inhibition of *D. magna* were investigated in this research. The half lethal concentrations of 24h (LC50-24h) of DM and MT to *D. magna* were 9.83 µg/L and 6.68µg/L respectively. The behavioral responses in different treatments of DM (13.36µg/L and 33.40µg/L) and MT (19.66µg/L and 49.15µg/L) suggested that recovery behavior strength in adjustment was important, and behavior homeostasis could provide *D. magna* a perfect way to have a wide tolerance ability of environmental limiting factors. During the experiment, some positive effects on AChE activity at the beginning of exposures and some evident recovery of AChE activity occurred in different treatments. Though the de novo synthesis of AChE in *D. magna* might help AChE activity recover, the trends during the 24 h exposure in different treatments were downward. The correlation analysis of the relationship between swimming behavior and AChE activity showed that both behavior strength and correspondent AChE activity inhibition of *D. magna* in most treatments stayed in a same field in the correlation circle. A 50% activity decrease of AChE may cause the toxic effect of swimming behavior, and fluctuating environmental stress may induce an induction effect on AChE activity sometimes. These results illustrated that the environmental stress caused by both DM and MT could inhibit AChE activity and then induce a stepwise behavioral response, though they act their toxic effects as indirect and direct inhibitors of AChE separately.

# Life-Time Achievement Award

*Emeritus Professor Jiawei Du*



The APACE Life-time Achievement Award recognizes long-term career achievement and significant contribution to Chemical Ecology research in Asia-Pacific region. APACE Life-Time Achievement Award 2015 will be presented to one of APACE co-founders, Emeritus Professor Jiawei Du. APACE Life-Time Achievement Award 2015 presentation is kindly supported by Chemtica Internacional, S.A., Costa Rica.

**September 25, 2015**

# Oral Presentation Abstracts

## SYMPOSIUM

Challenges for Invasive Species  
Pest Management via Semiochemicals

September 25, 2015

0034

### **Managing the invasive spotted wing drosophila using behavior-based strategies: Challenges and successes**

Cesar Rodriguez-Saona<sup>1</sup>, Aijun Zhang<sup>3</sup>, Tracy Leskey<sup>2</sup>, Anne Nielsen<sup>1</sup>

<sup>1</sup>Rutgers University, New Brunswick, NJ, USA, <sup>2</sup>USDA-ARS, Kearneysville, WV, USA, <sup>3</sup>USDA-ARS, Beltsville, MD, USA

We conducted studies to develop an attract-and-kill approach based on a combination of olfactory (fruit-based) and visual cues and a killing agent for managing the invasive pest spotted wing drosophila (SWD) (*Drosophila suzukii*) in small fruit crops. Specific objectives were to identify fruit-based attractants for SWD, identify visual cues important in host location, and develop an attract-and-kill approach. Coupled gas chromatography electro-antennographic detection (GC-EAD) experiments revealed 11 antennally-active volatiles from raspberries. In choice tests, SWD flies were attracted to a blend containing the 11 antennally-active raspberry compounds. In laboratory, semi-field, and field trials, spheres painted black or red had greater SWD captures statistically and/or numerically compared with other colors. Based on these results, we evaluated SWD fly response to a number of insecticides formulated into red attracticidal spheres (originally designed for apple maggot) which included sugar as a feeding stimulant and wax. Based on 24h mortality, spinosad, spinetoram, lambda-cyhalothrin, bifenthrin, acephate, permethrin, and dinotefuran resulted in mortality greater than 90%. In field trials, we compared attracticidal spheres formulated with spinetoram with weekly insecticide treatments, weekly insecticide treatments combined with attracticidal spheres, and an untreated control. There was a significant difference among treatments, with attracticidal spheres alone and weekly sprays reducing SWD infestation by over 50% compared with the untreated control. When combined, attracticidal spheres and weekly insecticide applications reduced SWD infestation by ~66%. In summary, these results indicate that attracticidal spheres offer promise in terms of a behaviorally-based management strategy to protect small fruit from SWD and reduce insecticide applications.

0035

**Developing more sensitive and selective attractant for the management of *Drosophila suzukii***

Dong H. Cha<sup>1</sup>, Peter Landolt<sup>2</sup>, Gregory Loeb<sup>1</sup>

<sup>1</sup>Cornell University, Geneva, NY, USA, <sup>2</sup>USDA-ARS, Wapato, WA, USA

The spotted wing drosophila (SWD), *Drosophila suzukii*, is a new invasive pest that challenges the production of soft-skinned fresh fruits in US and Europe. The presence of SWD has been traditionally monitored using fermented baits, such as yeast-sugar solution, wines and vinegars. However, such fermentation baits are often messy, effective over a relatively short period of time, and attract many non-target insects that adds difficulty in monitoring. We have identified a 4-component chemical attractant from a mixture of wine and vinegar. Among 13-antennaly active wine and vinegar volatile compounds, we have found that only 4 chemicals (acetic acid, ethanol, acetoin and methionol) are necessary to elicit a similar level of attraction as the wine plus vinegar. We have found that this synthetic attractant is more selective than its original material, wine plus vinegar, in trapping non-target drosophilids, muscid flies, moths and yellow jacket wasps. We have been able to improve the attractiveness of this 4-component lure by optimizing the chemical release rates of different components. The improved formulation is two-times more attractive than the original formulation and our finding suggests that the synergistic interaction between the increased amount of acetic acid and acetoin is responsible for the increase. The improved formulation is now commercially available from Scentry and Trecé with a recommendation of monthly lure replacement. We have been comparing the commercial and the original formulations with fermentation baits to evaluate their differences in detecting the presence of adult SWD in relation to timing of infestation in raspberry and blueberry plantings.

0036

**Invasive species brown marmorated stink bug semiochemical production and behavioural response**

Aijun Zhang<sup>1</sup>, Christina Harris<sup>1,2</sup>, Mengmeng Yu<sup>3</sup>

<sup>1</sup>USDA-ARS, Beltsville, MD, USA, <sup>2</sup>Entomology Dept, Virginia Tech., Blacksburg, VA, USA, <sup>3</sup>China Agriculture University, Beijing, China

The brown marmorated stinkbug, *Halyomorpha halys* (Hemiptera: Pentatomidae), is an invasive insect pest to the U.S. that has been causing substantial damage to fruit crops in the Mid-Atlantic region since its introduction in 2001. A male-produced pheromone blend has been previously identified from *H. halys* adults held under low densities. In this study, we report the temporal patterns of release of this pheromone blend and other semiochemicals from adult males, as well as the subsequent attractiveness to conspecifics. Y-tube olfactometer bioassays indicate that pheromone-emitting adult males were highly attractive to other adult males, but only weakly attractive to nymphs and adult females. In addition to the pheromone components, tridecane (C<sub>13</sub>) and E-2-decenal (an alarm compound) were observed in headspace collections of males, as well as in females and nymphs. Exposure of pheromone-emitting adult males to synthetic C<sub>13</sub> greatly reduced pheromone emission. However, C<sub>13</sub> and E-2-decenal combined do not reduce pheromone emission.

0037

### **Managing invasive social wasps with semiochemicals**

Ashraf M. El-Sayed

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Social wasps are major pests in New Zealand, having large effects on ecosystems and native fauna. *Vespula* wasps stand out as the worst intractable pest problem in New Zealand, with massive impacts on indigenous biodiversity and parts of the productive sector, and being a public health and nuisance issue. A major program was initiated at Plant and Food Research to develop semiochemicals based system for managing invasive social wasps. The research program focus on three main aspects; 1) developing a potent kairomones for invasive social wasps, 2) identification of pheromones that regulate chemical communication in social wasps, 3) the application of semiochemicals to manage invasive wasps in New Zealand beech forest. The talk will present the recent progress in developing semiochemicals based system to manage invasive social wasps with highlight on opportunities and challenges.

0038

**Pheromone based monitoring of *Neomusotima conspurcatalis* a biological control agent of the invasive weed *Lygodium microphyllum***

Greg Wheeler<sup>1</sup>, Aijun Zhang<sup>2</sup>

<sup>1</sup>USDA/ARS, Ft Lauderdale, FL, USA, <sup>2</sup>USDA/ARS, Beltsville, MD, USA

The Everglades is a unique ecosystem of slow flowing fresh waters and minute changes in topography coupled with a convergence of species at the limits of their ranges. Invasive plants overrun extensive swaths of this bastion of North American biodiversity - a consequence of climate and cultivation. Among the worst of these threats is the weed *Lygodium microphyllum*. This fern is native to tropical Asia and covers substantial portions of multiple habitat types in the Florida Everglades. Classical biological control offers a potentially cost effective tool to reduce the competitive ability of *L. microphyllum*. The moth *Neomusotima conspurcatalis* (Lepidoptera: Crambidae), released in Florida in 2009, is spreading and exerting control against the weed. Pheromone based field monitoring of moth populations would be a useful tool to track this species. Volatile collections, subsequent field trapping and EAD analysis indicate that a number of volatile constituents are produced by females that attract males. Using this monitoring technique we will be able to monitor dispersal, distribution, and seasonal densities of adults.

0039

**Sequential SPME/GCMS Analysis (SSGA) - a new facile methodology for the identification of pheromones of fruit flies and other invasive species**

Anat Levi-Zada

*ARO, Volcani Center, Bet-Dagan, Israel*

The classical methods for identification of sex pheromones are based either on solvent extraction of sex pheromone glands or whole insects, or on airborne collection of the released pheromones on appropriate adsorbents. Both methods have the disadvantage of collecting non-relevant compounds and loss of pheromone components during solvent extraction and subsequent concentration of the solutions for GC (Gas Chromatography), GCMS (GC Mass Spectrometry) and GC-EAD (GC Electroantennographic Detection) analyses. These procedures are time consuming and require relatively large numbers of insects. Recently we developed a system of sequential SPME (Solid Phase MicroExtraction)/GCMS analysis; the system combines automatic sampling of the released pheromone components and on-line GCMS. The method enables repeated collections of volatiles released by only a few living insects in a predetermined temporal pattern throughout day and night. Compounds that are released in a circadian rhythm are usually potential pheromone components. The method has been already successfully applied to a number of representative cases. The wide potential of using the sequential SPME/GCMS analysis method for pheromone identification of various invasive insects, including fruit flies, will be discussed.

# Oral Presentation Abstracts

## SYMPOSIUM

Chemical Ecology of Forest Insects

September 25, 2015

0040

## Introduction to APACE Symposium on Chemical Ecology of Forest Insects

Steven J. Seybold (USDA Forest Service, PSW Station, Davis, CA, USA)

Zhen Zhang (Chinese Academy of Forestry, Beijing, China)

Issues in forest entomology related to chemical ecology include the isolation, identification, and application of semiochemicals for: 1) individual and areawide protection of trees (generally repellents); 2) improvement of lures for native insects (based on tree- or fungal-derived kairomones or pheromone “co-attractants” from trophic levels besides the herbivore); and 3) detection of invasive forest insect species. The role of behavioral chemicals in structuring communities of herbivore complexes is another area of relatively intense research.

This session will focus on chemical ecological aspects of several wood- and phloem-boring species that have invaded North America from Asia (e.g., the polyphagous shot hole borer, *Euwallacea* sp., and the fruit-tree pinhole borer, *Xyleborinus saxeseni*). It will also focus on the chemical ecology of several Asian species (the bark beetles, *Ips subelongatus* in *Abies*, *Larix*, and *Picea*, and *Tomicus* spp. in *Pinus yunnanensis*) that could invade North America. Furthermore, the symposium will cover attempts to develop a lure for a North American indigenous exotic flatheaded borer pest of oaks, the goldspotted oak borer, *Agrilus auroguttatus*. This borer has invaded California from its native range in southeastern Arizona. The role of host compounds in the chemical ecology of other woodborers (Cerambycidae) will also be addressed in the symposium.

Forest entomology has been dominated in recent years by a focus on invasive species. For example, survey of the invasive bark and ambrosia beetle fauna of California identified 22 species that had established populations in the state; about half of these invasions have occurred in the last 15 years. Many of the new species were first detected in heavily urbanized southern California where many exotic tree species are planted and trees are often under stress from exposure to conditions marked by drought and poor air quality. The original detections were nearly always made with traps baited with generic lures or through discovery of infested hosts. Subsequently, some of the invasive populations were more accurately delimited with species-specific lures.

**Complex semiochemical relationships in *Ips subelongatus* and its associated fungi**

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Two strains of *Ceratocystis fujensis*, which were the main associated fungi with *Ips subelongatus* in China, were inoculated in mature Prince Rupprecht larch (*Larix principis-rupprechtii* Mayr.), and then the host volatiles were sampled by dynamic headspace collection method on the 2nd and 90th day, respectively. The contents of main host monoterpenes (for example, S- $\alpha$ -pinene, S- $\beta$ -pinene, and R- $\alpha$ -pinene, etc) increased rapidly on the 2th day, which were significantly different from the contents sampled on the 90th day. Behavioral bioassay showed that S- $\beta$ -pinene, myrcene, and  $\alpha$ -phellandrene had very significant promoting effect on the attraction of aggregation pheromone of *I. subelongatus*, while R- $\alpha$ -pinene had very significant inhibiting effect. For non-host volatiles, Z-3-hexen-1-ol had significant inhibiting effect on the attraction of aggregation pheromone of *I. subelongatus*, while other non-host components tested had no effect on the attraction. Fungi culture experiments indicated that four pheromone candidates [R-(+)-ipsenol, S-(-)-ipsenol, S-(+)-ipsdienol, and R-(-)-ipsdienol] had weak promoting effect on the growth rates of fungi *C. fujensis* on malt extract agar (MEA) medium. But, different concentrations of four pheromone candidates had no significant difference in the fungi growth rate. Furthermore, the main host monoterpenes can inhibit the growth of the associated fungi. Especially, terpinolene, S- $\beta$ -pinene, S- $\alpha$ -pinene, and R- $\alpha$ -pinene had significant inhibitory effect on the growth rate of fungi on MEA. Non-host volatiles had no effects when the tested concentrations were very low, but the higher of the concentrations, the stronger of the inhibition, and finally the associated fungi stop growth on MEA when the concentrations reached a certain dose

**Semiochemicals regulating intraspecific and interspecific relationships of three *Tomicus* species in *Pinus yunnanensis* Franch**

Zhen Zhang, Junhui Wang, Pingyan Wang, Xiangbo Kong, Sufang Zhang, Hongbin Wang  
Key Laboratory of Forest Protection of State Forest Administration, Research Institute of Forest Ecology,  
Environment and Protection, Chinese Academy of Forestry, Beijing, China

The shoot beetles *Tomicus minor*, *Tomicus yunnanensis*, and *Tomicus brevipilosus* have been decimating *Pinus yunnanensis* trees for more than 30 years in southwest China. To understand the chemical ecological relationship between pines and *Tomicus*, and among the three beetle species, we compared the attraction of the damaged and undamaged Yunnan pine bolt bundles and shoots. We found that during shoot-feeding stage, *T. minor* and *T. yunnanensis* had stronger attraction to volatiles which collected from shoots damaged by the same beetle species and sex than to volatiles collected from other damaged shoots. *T. brevipilosus* not only had strong attraction to volatiles collected from shoots damaged by its own species and sex, but also had attraction to volatiles collected from shoots damaged by *T. minor* and *T. yunnanensis*. Meanwhile, the three beetle species showed strong attraction to volatiles collected from its own hindguts, so did the situation of volatiles from each other hindguts. During trunk breeding phase, *T. minor* and *T. yunnanensis* had strong attraction to volatiles which collected from trunks damaged by the three beetle species, but *T. brevipilosus* mainly had strong attraction to volatiles collected from trunks damaged by its own. In addition, the three beetle species had strong attraction to volatiles collected from hindguts of their own and each other. The main semiochemicals regulating the aggregation behaviours and interspecific interactions were identified by GC, GC-MS, EAG, walking bioassay and field bioassay.

**Developing monitoring techniques for the invasive goldspotted oak borer, *Agrilus auroguttatus*, in California**

Tom Coleman<sup>1</sup>, Damon Crook<sup>2</sup>, Yigen Chen<sup>3</sup>, Steven Seybold<sup>4</sup>

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The goldspotted oak borer, *Agrilus auroguttatus* Schaeffer (Coleoptera: Buprestidae), is an invasive species that has colonized oak woodlands in southern California. From 2002 to 2015, *A. auroguttatus* has caused elevated levels of tree mortality, killing >25,000 oaks within an area of 212,784 ha in San Diego Co. Introduced populations of *A. auroguttatus* in California are believed to have originated from southeastern Arizona. To better define its seasonal flight activity, assist with forest and integrated pest management activities, and define the current distribution in California, an effective monitoring technique for *A. auroguttatus* is necessary. From 2009 to 2013, three approaches were assessed to improve GSOB trapping methods: 1) scan host foliage wavelengths and match trap color to them; 2) use electrophysiological techniques (electro-retinogram) to measure what wavelengths of light *A. auroguttatus* is most sensitive to, then test on traps; and 3) bark volatiles from coast live oak, *Quercus agrifolia*, were tested for electrophysiological activity by *A. auroguttatus* using gas chromatographic-electroantennographic detection (GC-EAD) and behavioral activity purple prism flight-intercept traps. Bark volatiles were sampled from uninfested and *A. auroguttatus*-infested *Q. agrifolia*. *Agrilus auroguttatus* responded to numerous wavelengths in laboratory bioassays, but responded best to purple prism flight-intercept trap developed for the emerald ash borer, *A. planipennis* Fairmaire. Several monoterpenes and sesquiterpenes were identified in the GC-EAD bioassays, but individual compounds and blends of compounds did not significantly enhance *A. auroguttatus* trap catch in southern California.

## Flight response of two invasive ambrosia beetles to ethanol and other semiochemicals in southern California

Yigen Chen<sup>1</sup>, Tom Coleman<sup>2</sup>, Deguang Liu<sup>3</sup>, Christopher Ranger<sup>2</sup>, Steven Seybold<sup>2</sup>  
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Two studies were conducted to test the effectiveness of ethanol, (-)- $\alpha$ -pinene, and box elder bolts in attracting females of the polyphagous shot hole borer (PSHB), *Euwallacea* sp., and the fruit-tree pinhole borer, *Xyleborinus saxesenii*. The first study examined the flight response of both species to ethanol and box elder bolts in San Diego County, California. Treatment did not affect PSHB catches significantly. Ethanol by itself is not an attractant for PSHB. The response of *X. saxesenii* was much higher and showed a clear pattern with ethanol: box elder + ethanol attracted more beetles than ethanol alone, which was in turn more attractive than box elder + water. Unbaited traps were the least attractive. This indicated that ethanol and potentially certain kairomones from box elder may have interacted to increase the attraction of *X. saxesenii*.

The second experiment was designed to examine the flight response of *X. saxesenii* to ethanol and (-)- $\alpha$ -pinene at four urban landscapes in LA and Riverside Counties. The responses to ethanol and ethanol plus (-)- $\alpha$ -pinene-baited traps were significantly greater than unbaited trap and (-)- $\alpha$ -pinene-baited trap. The addition of (-)- $\alpha$ -pinene significantly decreased the attraction of this species to ethanol.

Differential responses of the two ambrosia beetles to ethanol might be related to their invasive host colonization behavior. PSHB attacks healthy trees that likely lack detectable amounts of ethanol, whereas *X. saxesenii* usually bores into the wood of dying or recently dead trees that likely contain high amounts of ethanol caused by fermentation.

0045

## **Insect pheromone-based management of forest pests in China: Current status and future development**

Genzhong Cui

*Pherobio Technology Co. Ltd.*

In the past 10 years, research on applications of using insect pheromones for forest pest control has made significant progress in China, with over 70 species of insect pheromones have been applied for forestry pest management.

This report provides a very brief summary about the current status of using pheromones in forestry and fruit tree pest controls in China.

By the end of 2013, the total forestry areas reached 208 million hectares in China, with additional 12 million hectares of fruit trees. This fast development ensures the massive deployment of insect pheromone technique has gained significant footsteps in forest pest control, with a bright future in years ahead.

0046

**The role of host volatiles in the chemical ecology of a community of longhorn beetles.**

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Research on longhorn beetle (Coleoptera: Cerambycidae) chemical ecology has shown that for some species, host plant volatiles strongly synergize attraction to pheromones. Typically, the species for which this effect is most pronounced are those infesting conifers. To explore the extent of this phenomenon and the factors that influence it, two blends of synthetic host plant volatiles were developed based on volatiles collected from four conifer and three oak species native to southern California. The blends were tested at various release rates, with and without cerambycid pheromones. Only a high release rate of the synthetic conifer blend with pheromones was effective in attracting conifer-infesting cerambycids, whereas the synthetic oak blend did not increase attraction for oak-infesting species to their pheromones at any release rate tested. The synthetic conifer blend was then tested versus alpha-pinene alone for conifer-infesting species, and ethanol was tested as a possible HPV used by oak-infesting species. We report here the results of these various bioassays.

# Oral Presentation Abstracts

## SYMPOSIUM

Application of Electrophysiology  
In Chemical Ecology

September 25, 2015

**GC-EAD as a tool for elucidating microbe-mediated attraction and avoidance in *Drosophila suzukii***

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GC-EAD (gas chromatography-electroantennographic detection) and behavioral analyses are two essential tools in the identification of insect behavior modifying chemicals. The spotted wing drosophila (SWD), *Drosophila suzukii* Matsumura (Diptera: Drosophilidae), is a new wide-spread invasive pest, threatening the yield and quality of soft-skinned fresh fruit production in US and Europe. We have been using GC-EAD and behavioral assays to identify potential attractant and antagonistic chemicals that can be used in a push-pull management system for SWD. Adult SWD can be attracted with a mixture of wine and vinegar in the laboratory and field. Based on our assessment of wine and vinegar volatiles, we identified a 4-component microbial fermentation chemical blend (acetic acid, ethanol, acetoin and methionol) that is as sensitive as and more specific than its original material of wine plus vinegar for SWD. To develop a SWD deterrent, we have been evaluating a plant pathogen, gray mold, *Botrytis cinerea*, as a potential antagonist for SWD. Our findings show that more female SWD avoid traps baited with an attractive source (raspberry infused agar media) plus a small piece (1 cm<sup>2</sup>) of the raspberry agar with *Botrytis* compared to traps baited with the same attractive source plus 1 cm<sup>2</sup> piece of sterile raspberry agar. We have been collecting headspace volatiles from the raspberry fruit agar with or without *Botrytis*, analyzing the adsorbent extracts using GC-EAD, and evaluating the effect of EAD-active *Botrytis* compounds on female SWD behaviour.

**Rapid changes in gustatory sensilla drive behavioral resistance to baits**

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In response to persistent selection with insecticidal baits containing glucose as a phagostimulant, populations of the German cockroach have rapidly evolved a novel behavior: aversion of glucose, which is highly adaptive because it lets cockroaches avoid the bait. To understand the mechanisms underlying this behavior, we compared the electrophysiological responses of gustatory receptor neurons (GRNs) of the mouthparts to glucose, fructose and caffeine between wild-type and glucose-averse cockroaches. In both strains, the phagostimulant fructose stimulated a sugar-GRN, whereas a bitter deterrent compound caffeine stimulated a bitter-GRN. Glucose, like fructose, also stimulated the sugar-GRN in wild-type cockroaches, but in glucose-averse cockroaches it stimulated both sugar- and bitter-GRNs. The results suggested that the acquisition of sensitivity for glucose in bitter-GRNs is responsible for glucose-aversion behaviour. Moreover, chemical structure-GRN activity experiments with glucose and related compounds indicated that the glucose-GRs of the bitter-GRN in glucose-averse cockroaches recognize glucose-like molecules differently from the native glucose-GRs of the sugar-GRN. Results suggest that in glucose-averse cockroaches the expression of a broadly tuned receptor, or multiple narrowly tuned receptors, may contribute to the broad acceptance of glucose and related compounds by the bitter-GRN, driving aversive behaviour. Recently we found other sugar-averse cockroaches (aversion for fructose and other sugars) in field populations. We discuss how gustatory polymorphisms contribute to survivorship of the German cockroach under the strong selection pressure of sugar-containing insecticide baits.

## Electron microscopy for electrophysiological recordings from chemosensory neurons

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Electron microscopy provides useful information for electrophysiological monitoring of the activities of chemosensory neurons in insects. Most common use of electron microscopy for insect chemoreception studies would be the identification of morphological types of sensilla by scanning electron microscopy (SEM). High-resolution SEM observation makes it possible to predict the chemosensory function of each type of sensilla. For example, the presence of numerous pores or grooves along the sensillum surface is a good indication of olfactory function. Sensilla with different morphology generally have different function; therefore the classification of morphological types of sensilla is useful in deciding target sensilla and sample size in single sensillum recording. Sex-specific or developmental stage-specific sensilla can be a good indication of sex-specific or stage-specific function of the sensilla, as shown in male specific trichoid sensilla in moths and form-specific secondary rhinaria in aphids for their specialized roles in detecting sex pheromones. Transmission electron microscopy can provide further information on sensilla, such as the presence of pore tubules, dendritic branches of sensory neurons and cell bodies. The number of co-compartmentalized neurons in a sensillum is often an important question in single sensillum recordings, and SEM observation of sectioned samples can be an efficient way to investigate the number of co-compartmentalized neurons. Low-vacuum SEM observation of specimens with no metal coating can be a high-throughput tool for the morphometrics of chemosensilla.

0050

## Studies on sex pheromones in helmet crabs and blue crabs using a combination of approaches

Michiya Kamio

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Identification of sex pheromone molecules requires purification of the molecules guided by behavioral bioassays of courtship or mating behavior. However, data collection may be slow due to a number of issues. First, the short mating season of some crabs can limit the number of assays that can be run. The pheromones may be mixtures of molecules that lose activity in the process of purification because each component molecule alone may have no or very limited pheromonal activity. Testing the activity of various combinations of purified molecules requires running many bioassays, which is time-consuming. To cope with these problems, we have utilized metabolomics approaches and electrophysiological methods on antennules in two species of crabs, helmet crab *Telmessus cheiragonus* and blue crab *Callinectes sapidus*. NMR based metabolomics discovered a premolt biomarker metabolite together with unidentified molecules in urine of blue crab and helmet crab as a candidate sex pheromone molecule. Multiunit electrophysiological recording from olfactory receptors on outer flagellum of the antennule showed that the male's antennule can detect differences between male and female urine. Calcium imaging of olfactory receptor cells in antennule of blue crab showed that the antennule can detect a candidate sex pheromone molecule. The combination of metabolomics, electrophysiological assays, and behavioral assays with separation of candidate molecules from urine may elucidate the sex pheromone molecules of the pheromones.

0051

### **A coupled HPLC-single sensillum recording system for identifying gustatory active compounds in insects**

Kye Chung Park, Lee-Anne Manning

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A coupled HPLC-gustatory sensory neuron (GSN) monitoring system was developed for identifying gustatory active compounds in insects. To achieve this, new techniques were developed to enable continuous monitoring of electrophysiological responses of individual GSNs while maintaining the stimulation of target neurons with a continuous water flow containing various stimuli from the effluent of HPLC column. A tungsten electrode connected to the basal area of a sensillum could be used to monitor the responses of GSNs while exposing the tip of the target sensilla for continuous stimulation. However, the application of this technique appears to be limited to certain types of sensilla since the basal areas of gustatory sensilla are often inaccessible with tungsten electrodes because of dense scales and hairs surrounding them. To overcome this, we have developed another recording technique using a microglass recording electrode. The responses of GSNs to continuous stimulation with water flow containing various stimuli could be successfully monitored by placing the microglass electrode close to the tip of a gustatory sensillum and sending a stimulus flow through the gap. A new stimulus delivery system was also developed to stimulate the target gustatory sensilla with continuous water flow containing effluents from HPLC column or other test stimuli while maintaining low level of electromagnetic noise so that action potentials from target GSNs can be continuously monitored. The electromagnetic noise picked up by the water column for continuous stimulus delivery could be effectively eliminated by having a series of discontinuous glass capillaries for stimulus delivery.

0052

### The purpose of puddling behaviour of male *Papilio* butterflies (Video Presentation)

Takashi INOUE

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It is well known in the field a large number of butterflies come to damp ground together to sip water. This behaviour is called puddling. *Papilio* butterflies mostly male also do. ARMS et al (1974) is the first persons who showed an answer to this question. They found the most preferred solution is that contains 10mM Na<sup>+</sup>. Some other researchers followed their experiments, however questions like “how do the butterflies find the proper site for puddling? Is there any special organ or tissue for detecting Na<sup>+</sup> taste? Do butterflies really absorb Na<sup>+</sup> in their alimentary tract?” So we started the research of puddling morphologically, ecologically, and neurophysiologically. In addition, we also measured concentrations of Na<sup>+</sup>, K<sup>+</sup>, Ca<sup>2+</sup> and Mg<sup>2+</sup> in the water which was puddled by butterflies and butterfly urine excreted during butterfly puddled by HPLC.

We can show that 1) The contact chemosensilla in trompe of *Papilio* butterflies responded to the Na<sup>+</sup> taste; 2) The most preferred concentration of Na<sup>+</sup> is 10mM; 3) *Papilio* butterflies trying to find the proper site for puddling were attracted NH<sub>3</sub> vapour and H<sub>2</sub>O vapour; 4) Alimentary tract of *Papilio* butterflies basically absorb Na<sup>+</sup> and excrete K<sup>+</sup>, however this function goes wrong by more than 10mM-Na<sup>+</sup> water solution.

In conclusion, the purpose of puddling behaviour of male *Papilio* butterflies are mainly absorb Na<sup>+</sup>, and they excrete over ingested K<sup>+</sup> from leaves during their larval stages. For this purpose, the *Papilio* butterfly uses Na<sup>+</sup> as a taste signal and H<sub>3</sub>N and H<sub>2</sub>O vapour as odour signals.

# Oral Presentation Abstracts

## SYMPOSIUM

New Physiological Aspects  
In Chemical Ecology

September 25, 2015

0053

### **Targeted Chemical Ecology using Chemical Informatics and Neurophysiology**

Anandasankar Ray

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Recent advances in chemical informatics and electrophysiology have created new opportunities to understand and manipulate the insect olfactory system. These have led to new classes of volatile attractants and repellents, species-specific as well as broadly effective against multiple species. Additional advantages of this form of targeted chemical ecology is the ability to continuously improve the properties of the active compounds, as well as the ability to select affordable, safe and pleasant smelling attractants and repellents for practical use.

0054

### Maize-corn leaf aphid interactions: Involvement of ethylene pathway

Joe Louis

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Maize (*Zea mays* L.) is arguably the world's significant cereal crop, but also suffers severe yield losses due to insect infestation. Corn leaf aphid [CLA; *Rhopalosiphum maidis* (Fitch)] is an economically important pest of several monocot crops, including maize. In addition to extensive crop damage, CLA also acts as a vector for viruses that cause devastating diseases in maize. Feeding by CLA triggers the rapid accumulation of the *maize insect resistance1* (*mir1*), which encodes a cysteine protease, in the resistant maize Mp708 genotype. In addition, CLA feeding rapidly accumulates *mir1* both locally and systemically, suggesting that mRNA transcripts encoding Mir1-CP contribute to intraplant defense signaling in Mp708 genotype. Resistance to CLA in Mp708 involves both antibiotic and antixenotic factors, compared to B73 and Tx601 maize susceptible inbred lines. Furthermore, feeding behavior studies using Electrical Penetration Graph (EPG) technique revealed that CLA spent less time in the sieve elements of Mp708, thus confirming that Mp708's resistance to CLA is phloem-localized. Previously, it was reported that the combined actions of ethylene (ET) and jasmonic acid (JA) was required for providing *mir1*-dependent defense against chewing group of insect pests in maize. However, *mir1*-mediated defense against CLA is independent of JA, but dependent on ET pathway.

## Microbial Mediation of Herbivore HAMPs and Effectors

Gary W. Felton

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Chewing herbivores such as beetles and caterpillars cause massive tissue damage during feeding and release not only damage associated molecular patterns (=DAMPs) and herbivore-associated molecular patterns (=HAMPs) but also microbial associated molecular patterns (=MAMPs) associated with their gut symbionts. Plants possess receptors that can recognize these cues and then initiate signalling pathways that mobilize the appropriate defences.

Using an experimental system comprised of tomato and maize and caterpillar and beetle herbivores, we have shown that gut bacteria play an exceedingly important role in the herbivores' ability to circumvent plant defences. Gut bacteria and their MAMPs may be directly applied to the plant during herbivore feeding and regurgitation. Plants may then perceive the MAMPs and upregulate the salicylic acid pathway, with a concomitant suppression of jasmonate signalling. This negative cross-talk may be relatively common among chewing herbivores such as beetles that freely regurgitate during feeding.

Alternatively, we have shown that the gut bacteria in some herbivores may indirectly regulate the expression or synthesis of herbivore effectors and HAMPs thus affecting plant signalling. This latter finding has likely been overlooked because many studies with herbivores such as caterpillars have been conducted with lab colonies of insects, which have lost a significant segment of their bacterial community during rearing in the lab. In summary, we contend that the study of plant-herbivore interactions needs to be considered in the context of the microbiomes of both the plant and the herbivore.

0056

## Handling foul-smelling stink bug: from heteropteran chemical ecology to a challenge toward insecticide development

Koji Noge

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Terrestrial heteropteran bugs produce foul-smelling odors commonly composed of short-chain aliphatic aldehydes, alcohols and esters, alkanes, terpenes and phenolics. Each compound or compound blends secreted from the scent glands play important roles in chemical communication and defense of this insect group. As the defense substances, for example, (*E*)-2-hexenal, one of the well-known heteropteran compounds, functions as a repellent against insect predators and as an antibacterial agent. In contrast, 4-oxo-(*E*)-2-hexenal (OHE), frequently found from coreid, mirid and pentatomid bugs, shows deterrent and toxic effects, but not repellent against predators. However, in spite of advances in heteropteran chemistry, it is still unclear how these compounds affect insect physiology. Unlike 2-alkenals, OHE induces permanent locomotive impairment and death in crickets, dragonflies, and other insect species. In OHE-treated crickets, the amounts of free thiols are significantly decreased compared to healthy crickets. OHE reacts with thiol compounds *in vitro*, suggesting that covalent binding of OHE to biologically active molecules correlates with locomotive impairment in insects. Our group has revealed that OHE interferes with energy metabolism of insects. OHE inhibits proline metabolism that is involved in ATP production. OHE itself is toxic to human, but if there is the insect-specific target molecule(s) of OHE, it may be useful to develop a new insecticide. Recent progress in identification of the target molecules of OHE will be discussed.

0057

## **Multisensory modalities mediate the sex pheromone-induced upwind orientation behaviour in walking Indian meal moth**

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When stimulated by sex pheromone, male moths often fly upwind to locate conspecific females. Flying moths are known to direct themselves upwind by detecting visual drift caused by wind, termed optomotor anemotaxis. However, the fact that pheromone-stimulated moths on the ground also orient upwind while walking suggests they could 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 walking behaviour of wing-amputated Indian meal moth *Plodia interpunctella* on a locomotion compensator. Males under the illumination of visible light and in the darkness both readily oriented upwind when stimulated by pheromone-laden airflow, suggesting that their anemotactic ability is independent of visual cues. When one of moths' antennae was amputated, their overall paths were still directed upwind, though they were biased toward the side of the intact antenna. However, moths' orientation direction was completely randomised when basal segments of antennae are immobilized with an adhesive to impair mechanosensory function. In addition, their anemotactic ability was restored when the water soluble adhesive was removed. Thus we conclude that walking Indian meal moths orient upwind mainly by detecting wind direction with their mechanosensory organs on the antennae, but the bilateral stimulation of olfactory organs may also contribute to adjust the orientation.

0058

### The tyrosine aminomutase TAM1 is required for $\beta$ -tyrosine biosynthesis in rice

Jian Yan<sup>1</sup>, Takako Aboshi<sup>2</sup>, Takayuki Yokoo<sup>2</sup>, Naoki Mori<sup>2</sup>, Gerog Jander<sup>1</sup>

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A targeted search for defense-related metabolites in cultivated rice (*Oryza sativa*) identified (R)- $\beta$ -tyrosine, an isomer of the common (S)- $\alpha$ -tyrosine in the seeds, leaves, roots, and root exudates of the Nipponbare cultivar. Measurement of  $\beta$ -tyrosine in 119 diverse rice cultivars showed a distinct presence/absence polymorphism, with the compound being most prevalent in Japanese rice cultivars. Genetic mapping using chromosome segment substitution lines derived from Nipponbare x Kasalath, showed that a region of chromosome 12 is associated with  $\beta$ -tyrosine production. A candidate gene in the mapping interval was confirmed to be a tyrosine aminomutase (*OsTAM1*) by transient expression in *Nicotiana benthamiana* and *in vitro* enzyme assays. Although  $\beta$ -tyrosine accumulation in Nipponbare was induced by treatment with the defense signaling molecule jasmonic acid, bioassays with lepidopteran herbivores showed no negative effects at physiologically relevant  $\beta$ -tyrosine concentrations. In contrast, root growth of *Arabidopsis thaliana* was inhibited by  $\beta$ -tyrosine concentrations as low as 1 micromolar. As  $\beta$ -tyrosine is exuded into hydroponic medium at higher concentrations than this, it may contribute to the allelopathic potential of rice seedlings.

0059

**Chemical ecology, behavior, and multitrophic interactions of beneficial nematodes as a belowground indirect plant defense.**

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Plant signals play diverse roles to the many organisms that surround them. One facet of this is their ability to manipulate organisms in a manner which protects them or harms herbivores that feed on them. This relationship has more recently been recognized to occur belowground. Here we discuss these belowground interactions, techniques, and findings, focusing on entomopathogenic nematodes and soil nematode chemotaxis in response to plant root cues and potential implications for agroecosystems and fundamental concepts in ecological trophic cascades.

# Oral Presentation Abstracts

## SYMPOSIUM

Going from the Bench to the Marketplace  
For Semiochemical Products

September 26, 2015

0060

## **Regulatory Innovation, New Mating Disruption Products in New Zealand, and The Role Of Odor in 4-Play™**

David Maxwell Suckling<sup>1</sup>, James TS Walter<sup>2</sup>

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Straight-Chained Lepidopteran Pheromones (SCLPs) are now regulated under a Group Standard in New Zealand, based on products of similar low risk, under the Hazardous Substances and New Organisms Act (1996). This means that compliant new pheromone products can be developed and commercialized with low regulatory requirements, encouraging innovation and supporting export fruit industries interested in meeting export phytosanitary standards while targetting low or nil residues of pesticides. We have chosen a four species mating disruption product commercialized in 2012 by Etec Crop Solutions Ltd, to illustrate how this system now operates. Changes to the complex blend for reasons such as technical improvements or variations in pest species composition in different crops can now be made with minimal regulatory involvement. The odors involved in 4-Play™ consist of a range of components used by codling moth, lightbrown apple moth, green-headed leafroller, and brown-headed leafroller. This presentation will discuss the story of 4-Play™ and how mating disruption of insects can support industry goals.

0061

## **Commercial Semiochemical Synthesis – Scaling and getting green**

Cam Oehlschlager

*ChemTica Intercisional, S.A., Costa Rica*

The semiochemical industry is comprised of two large companies (Shin-Etsu and BASF) and over 30 smaller companies. Because of the “founder effect” each company strives to rapidly identify targets, develop products and market. While large companies are positioned to achieve dominant market positions in major crops, smaller companies attempt to develop products for niche markets that have the potential to become significant. A key element of a company’s ability to compete in any market is its ability to synthesize and formulate semiochemicals of the target insects. Increasingly, green techniques of synthesis are chosen. At most companies there have been concerted efforts to use green techniques in synthesis such as the use of metal ions that have low environmental impact, solvent recycling and multiple step processes where the same solvent is used for several steps. Examples of the application of the principles of green chemistry to the synthesis of codlemone (large scale) and to a recently identified attractant for an ambrosia beetle (small scale) will be discussed.

0062

## **Retrospective of the 30-years long commercial application of mating disruption in Australian orchards and foreseeable challenges**

Alex Ilichev

*Biosciences Research Division of DEDJTR, Tatura, Victoria, Australia*

Adoption of mating disruption (MD) in Australian orchards started 30 years ago by treatment of individual orchard blocks and only known host-plants against individual pest species. The ability of oriental fruit moth (OFM) and codling moth (CM) to migrate between orchards, quickly invade new host-plants and together damage the same variety stimulated grower's need to control both pests in an area-wide scale. From an initial OFM area-wide MD program (1,100 ha of 40 contiguous orchards with all fruit varieties under MD) to multispecies selective treatments, MD progressed to become the key element of cost effective strategies for pest control while protecting the environment by reducing pesticide pressure in orchards. In some stone fruit orchards continuously treated with MD for over 30 years in northern Victoria, small OFM populations had survived and damaged stone fruits. Our study indicated that OFM females are capable of detecting their own sex pheromone and that prolonged exposure to sex pheromone changed their behaviour. Also, high concentration of the artificial sex pheromone for MD could repel OFM females and they could avoid the effect of MD by moving outside the treated area to seek mates. After successful mating, fertile females could return to preferred host-plant areas to lay eggs on the food plants for future larvae. Such type of behaviour could help in developing a behavioural avoidance mechanism that may support the survival of OFM under long-term MD treatments and possibly trigger the development of resistance to MD. Problems of multispecies MD will also be discussed.

0063

## **Semiochemical-based suppression tactics for regulated plant pests**

David R. Lance

*USDA APHIS PPQ, Center for plant HITH SCI & TECH, Massachusetts, USA*

Programs to mitigate effects of introductions of regulated invasive plant pests are often large, area-wide projects run by public agencies. Environmental and human health concerns generate pressures to use species-specific, non-toxic tactics as control measures for such efforts, and semiochemical-based suppression tactics seem a natural fit. This presentation will discuss various aspects of incorporating semiochemical-based suppression into programs for regulatory pests, with emphasis on mating disruption of Lepidoptera. Mating disruption has long been used in management and containment (slow-the-spread) programs, but is increasingly being considered as either a supplementary or primary suppression tactic in eradication. The theoretical basis for this is sound, being rooted in mode of action and Allee effect theory, but this is not always well understood by either the public or the scientific community. Use of semiochemical-based control methods in regulatory pest programs is not without challenges, including a need for rigorous development and evaluation of formulations and application methods, reduced ability to detect/monitor the target population due to effects of treatments on trap effectiveness, and ensuring acceptance of the strategy among the public and program cooperators. These factors, and resulting successes and pitfalls, will be illustrated using case studies on at least three major invasive pests: gypsy moth, light-brown apple moth, and European grapevine moth.

## The application of semiochemical-based products in China

Genzhong Cui

*Pherobio Technology Co. Ltd.*

The rapid increases in number of insect pests on agricultural crops and animals (including invasive species) have led to devastating economic damages in Chinese agriculture that have ultimately accompanied with the massive pesticide application for control. This has also brought many major environmental issues and the development of insecticide resistance. Scientists and pest control specialists have explored the complex semiochemical communication in pest insects and their ecosystems with discovering well-classified assorted behavioral-modifying semiochemicals (pheromones and kairomones) for use to develop associated practical products for their commercial use. These novel environmental-friendly approaches not only provide opportunities for Chinese semiochemical industries, but also stimulate the food productivity and ensure the food safety. Here, I will present a brief introduction of practical approaches in control of various pests, as well as the current status of Chinese semiochemical market, with encountered constrains.

To date, semiochemical research and product development in China play important roles in sustainable pest management. Annual production of lures for surveillance and mass trapping of pests has reached over 170 species, covering at least 4 million hectares of crop fields. The demand has been increasing every year. The dynamics of major pests are well profiled according to comprehensive database integrated by long-term collection. Enterprises in China have developed many semiochemical-based products used for alternative controls, which successfully reduced application costs of pesticide in several regions. Research on behavioral-modifying semiochemicals and promotion as alternative tools are greatly advocated by central and local government authorities. Some state-of-the-art innovations, i.e., collaborative or compounded signals, have been applied in commercial development with demonstrated huge advantages in practical control.

After a decade of market-driven semiochemical product trials in Northwest of China, we have experienced pros and cons, with some valuable lessons learned: 1) Massive efforts in agricultural and forestry products, but lack of development for pest control in horticulture, sanitary and store-product pests. 2) A lack of local manufacturers for producing cost-effective technical solutions (syntheses) to compete with those of oversea counterparts. 3) More cutting-edge technologies are much needed to facilitate world-class recognition of Chinese brands. At present, Chinese companies are technically committed to screening active minor or secondary components of sex pheromones, optimizing dispensers on consistency and longevity, modifying trap designs to maximize trapping efficacy and exploiting multifunctional products for multi-species control. Manifold and accessible ways in mating disruption are continuously being experimented. In addition, both domestic and international collaborations in both basic and applied research among universities, research institutes and industries need to be strengthening. All these efforts will enable us to develop better products and bring us more profits.

0065

## **Turning scientific results into business results: some key points to develop successful semiochemical products**

Satoshi Nojima

*Shin-Etsu Chemical Co., Ltd., Davis, USA*

In order to develop successful semiochemical products in markets, one of the most important keys is "benefit and profit". Good products have to provide cost-effective pest control for growers and also have to bring companies enough profit to keep their business running. From a business point of view, it is the matter of a balance between biological efficacy and costs of products. For example, a product works to control a pest greatly, but if it is very expensive, it won't succeed in markets.

Cost of a product is mainly companies' business, while biological efficacy is mainly researchers' business. Designing an effective formulation by companies greatly depends on the results of researches. However, in some cases, there are still missing factors to develop effective formulations. In addition, figuring out these factors is beyond companies' capabilities in some cases. Greater supports from researchers are very important for the semiochemical industry in this respect. In this presentation, some key points to solve the missing factors will be discussed.

0066

## **Semiochemical-based pest control products for consumer markets: Opportunities and challenges**

Qing-He Zhang

*Sterling International, Inc., Spokane, WA, USA*

Sterling International, Inc., is a semiochemical-based company manufacturing Rescue® brand traps and attractants for pestiferous social wasps (yellowjackets, paper wasps and hornets), filth flies, Japanese/Oriental beetles, stink bugs, vinegar fruit flies, and spiders; plus, natural essential oil-based insect repellents for the consumer retail market. Unlike agricultural, commercial and professional pest control operator (PCO) markets, the consumer retail market consists primarily of household consumers who buy semiochemical-based pest control products as a "*Do it yourself*" (DIY) approach for individual or family consumption. The retail stores for our products include many well-known giant retailers such as Wal-Mart, Home Depot, Lowes, ACE Hardware, Costco, and online stores such as Amazon.com. The massive scale of these retailers provides tremendous opportunities for sale revenues and profits; but this marketplace is also extremely competitive for companies such as ours competing for limited shelf space. For any product, especially consumer products, performance (efficacy; R&D core) is most crucial for end users. However, other factors such as pricing, packaging, design (appearance/look), TV commercials, floor display, instructions, child resistant features, and customer service (among many other things) are also keys to success. In this presentation, I will share my joys and burdens as a chemical ecologist and director of research in an industry that develops and sells semiochemical-based pest control products for the gigantic consumer market.

**Bringing Novel Semiochemical Formulations to the Market**

Agenor Mafra-Neto<sup>1,4</sup>, Christopher Fettig<sup>2</sup>, Steve Munson<sup>3</sup>, Romeno Faleiro<sup>5</sup>, Abdallah ben Abdallah<sup>5</sup>, Cesar Rodriguez-Saona<sup>7</sup>, Márcio Fernandes Peixoto<sup>6</sup>, Robert Holdcraft<sup>7</sup>, Carmem Bernardi<sup>1</sup>, William Urrutia<sup>1</sup>, Rodrigo Silva<sup>1,4</sup>, Rafael Borges<sup>4,1</sup>, Shawn Steffan<sup>8</sup>, Jonathan Rico<sup>1</sup>, Kavita Sharma<sup>1</sup>, Robert Progar<sup>9</sup>

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SPLAT<sup>®</sup> (Specialized Pheromone and Lure Application Technology) matrix is a unique controlled-release technology that can be adapted to dispense and protect a wide variety of compounds from degradation, including semiochemicals, pesticides, and phagostimulants, in diverse environments. ISCA Technologies in collaboration with colleagues in academia, government, and industry, has been developing SPLAT<sup>®</sup>-based insect control products for over a decade. We will provide an overview of SPLAT<sup>®</sup> technology and existing commercial formulations and describe ongoing efforts to develop new SPLAT<sup>®</sup> mating disruption, attract-and-kill, and repellent products for pest control in agricultural and forest environments.

0068

## Optimizing pheromone aerosol emitters for codling moth mating disruption

Larry Gut, Peter Mcghee, Michael Haas, James Miller  
*Michigan State University, East Lansing, Michigan, USA*

Oriental disruption of codling moth (CM) has been achieved with deployment of as few as two aerosol-emitting devices per hectare. Although encouraging, making the approach robust or consistently effective will arise through sorting out the mechanism by which disruption is achieved and improving the economics. Aerosol devices emitting CM pheromone were deployed at various densities in commercial orchards to generate dosage-response profiles in order to elucidate the behavioral mechanism of disruption. The resulting set of profiles matched the predictions for competitive rather than non-competitive disruption. Aerosol emitters disrupt CM principally by inducing false-plume following. Males move toward the aerosol emitters, bypassing females and traps along the way. Growers are limited to deploying only one or two aerosol emitters per acre because of the high cost of the unit and especially the pheromone. In an effort to refine this technology and increase its cost-competitiveness, we conducted a series of studies evaluating the effectiveness of emitters designed to release smaller than standard amounts of pheromone active ingredient. Field experiments demonstrated that excellent CM suppression could be achieved using aerosol dispensers filled with half the current amount of pheromone. Additional trials revealed that further reductions are possible by limiting emissions to the first few hours of peak evening flight activity and reducing the number of emissions per hour to one or two compared to the current four per hour. Combined, these reductions in the pheromone requirement could significantly reduce the cost of aerosol emitters. Furthermore, the control achieved using aerosol emitters may be improved if less costly units that release lower rates of pheromone were deployed at densities of 3-6 per acre.

0069

**Challenges on scaling up the use of semiochemicals from the experimentation settings to the pest control industry worldwide: A private company's point of view**

Matthew Bohnert

*Suterra LLC, Bend OR 97701, USA*

One characteristic of semio-chemical markets in agriculture, and of pheromones in particular, has been the historical lack of professional investment activity to commercialize products at large scale. This is due to the unattractiveness of the industry measured by many classic investment factors such as market size, adoption rates, and technological barriers. In particular, the factors of (1) acceptable return thresholds and (2) time-to-liquidation are significant challenges that have restricted professional investment activity. Those specific factors will be discussed, as well as how they have driven much of the industry structure as it exists today. As certain factors change over time, the industry structure may change as new opportunities arise for professional investment.

0070

**Mating disruptant for the carpenter moth, *Cossus insularis* (Lepidoptera: Cossidae).**

Kiyoshi Nakamuta

*Chiba University, Matsudo, Chiba, Japan*

Recently the larvae of *Cossus insularis* (Lepidoptera: Cossidae) were found to infest the main branches and trunks of Japanese pear (sand pear) trees as a new host plant in 2001. Afterwards, damage by the species has been found on apple and Japanese pear orchards in many prefectures in central and northern Japan. Larvae bore into the branches or trunks of the trees, and develop there until adult emergence. Thus, all life stages except the adult are hidden in the bark or wood, and it is thought that insecticide spraying is not effective against eggs and larvae.

Sex pheromone of this species has been identified as a 95:5 mixture of (*E*)-3-tetradecenyl acetate and (*Z*)-3-tetradecenyl acetate. Mating disruption, therefore, was tested in apple and pear orchard in three locations in Japan from 2011 to 2013, and it showed the reduction of damages by the species. Based on these results, the mating disruptant (Bokutoukon-H<sup>®</sup>) has been registered as a control agent against *C. insularis* in Japan and now has been used practically in Japan.

To complete the life cycle from egg hatching to adult emergence, the species may require a few years. Thus mating disruption should be continued for more than three years with an expectation of cumulative effect.

0071

**Registration and commercial development of a plant volatile based attract and kill system for *Helicoverpa* spp.**

Peter Gregg<sup>1</sup>, Alice Del Socorro<sup>1</sup>, Anthony Hawes<sup>2</sup>

<sup>1</sup>University of New England, Armidale, NSW, Australia, <sup>2</sup>AgBiTech Pty Ltd, Toowoomba, Queensland, Australia

In 2009 we registered an attract-and-kill product for *Helicoverpa* spp. in cotton and corn, under the trade name Magnet. This was the first such product, using synthetic plant volatiles, including insecticides and intended for direct application to crops, to be registered in Australia and possibly the world. We will describe the processes and difficulties encountered during registration, and lessons for commercialisation of semiochemical products. The product was originally intended for use in IPM schemes for *Helicoverpa* spp. in cotton, but as with many conventional insecticides this role has been substantially reduced due to the widespread adoption of transgenic (Bt) cotton. We are now investigating other potential roles in management of resistance to Bt, and in other crops.

# Oral Presentation Abstracts

**SYMPOSIUM**

Chemical Ecology in General

**September 26, 2015**

0072

## Recent Advances in the Pheromone Chemistry of Stink Bugs

Paulo Zarbin

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Stink bugs are among the main agricultural pests in the world, and they are increasingly important with the advent of genetically modified crops. The piercing-sucking mode of feeding exhibited by stink bugs is particularly damaging to maturing fruit and seeds, and stink bugs often migrate undetected into maturing crops from wild hosts plants or other crops.

New methods are needed to minimize or eliminate application of environmentally harmful insecticides used to control this stink bugs, as well as other pest species. Pheromones are potentially useful for monitoring and otherwise managing pest species.

In this presentation, recent results of our research group related to the structural identification and synthesis of pheromones of several species of stink bugs will be discussed, including: *Edessa meditabunda*, *Agroecus griseus*, *Pallantia macunaima* and *Pellaea sticta*.

**Virus-mediated interactions of plants and insects**

Jingjing Li, Shaohua Lu, Xiangzhi Liang, Xueli Wang, Fengming Yan  
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*Cucurbit chlorotic yellows virus* (CCYV) is a newly reported virus occurring on melon plants and many other plant species. CCYV is transmitted specifically by B and Q biotypes of tobacco whitefly, *Bemisia tabaci*, in a semipersistent manner. A DC-EPG technique was applied to record feeding behaviors of viruliferous *B. tabaci* B and Q biotype adults on healthy cotton plants in order to understand influence of the virus on its vector performance. Both sexes of viruliferous (21 females, 20 males) and non-viruliferous adults (30 female, 20 males) were used in 6 h EPG recordings on cotton plant which is not a host of CCYV. Viruliferous and non-viruliferous whiteflies (mixed sexes) performed very differently based on analysis of above EPG variables. Viruliferous whiteflies had significantly longer durations of each phloem waveform, E1, E2 and E2>10min, than control insect group, but behaved similarly in pathway waveforms in both groups. These results indicated that viruliferous whiteflies fed better on cotton plants than non-viruliferous did, implying that CCYV could manipulate vector feeding behaviors to facilitate virus transmission.

Virus-infested plants are different from healthy plants in many aspects, including color, volatile chemicals, nutrition, and so on. Changes of these plant features affect host searching, selection and feeding behaviors of vector insects.

New strategies for control of plant virus diseases and vectors can be applied based on further understanding of virus-mediated plant-insect interactions.

0074

## MHC variation in Leach's storm-petrels (*Oceanodroma leucorhoa*); Implications for mate choice and selection via MHC-related odors

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The Major Histocompatibility Complex (MHC) is a multi-gene complex associated with immune function, and is known to be highly variable between individuals of the same species. Variation in MHC genes has also been shown to influence individual body odor, and may impact recognition and mate preference in some species. MHC-based mate selection in wild populations has not been conclusively proven, however, likely due to insufficient sample sizes. Procellariiformes are an ideal model for investigating the role of MHC in a natural population, as they are highly olfactory, long-lived, and form lifelong pair bonds, and our research group has previously shown that some species can differentiate between individuals using only odor. Using a robust five-year dataset (n=1350 genotyped individuals) of monitored Leach's storm-petrels (*Oceanodroma leucorhoa*) in Nova Scotia, Canada, we genotyped partial genomic fragments of two MHC Class IIB gene duplicates (Ocle-DAB1 and Ocle-DAB2) to characterize the MHC variability in a natural population and test for evidence of MHC-based disassortative mating. We used randomization tests to compare observed and bootstrapped medians of three MHC similarity metrics (pairwise heterozygosity differences, band-sharing coefficients, amino-acid substitutions) in 294 established pairs and found no significant evidence of disassortative mating (HZ:  $p=0.32$ ; band-sharing:  $p=0.47$ ; AA:  $p=0.27$ ). This sample size is unprecedented in any MHC study of a wild population and the probability of detecting a Type 1 error is  $<0.07$ , providing the first definitive characterization of the role of MHC in a natural population. Furthermore, this study highlights the need to use appropriate sample size to allow for the meaningful interpretation of results.

0075

**Two salivary proteins of the rice brown planthopper *Nilaparvata lugens* function as effectors for defense responses in rice**

Yonggen Lou

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The rice brown planthopper (BPH) *Nilaparvata lugens* (Stål) is one of the most destructive insect pests on rice in Asia. BPH is able to successfully puncture sieve tubes in rice with its piercing stylet and then to ingest phloem sap and can rapidly adapt to new rice resistance factors. However, how BPH manages to continuously feed on rice and how BPH quickly overcome rice resistance remain unclear. We discovered two BPH salivary proteins, an endo- $\beta$ -1,4-glucanase (NIEG1) and an EF-hand  $\text{Ca}^{2+}$ -binding protein (NISEF1), both of which are secreted into rice plants when BPH feed. Both *NIEG1* and NISEF1 are highly expressed in the salivary glands of BPH. By combining reverse genetics, molecular biology and bioassay, we found that NIEG1 is crucial for BPH to penetrate the plant cell wall and feeding. Moreover, NIEG1 acts as an effector that can come under positive selection and thereby enable BPH to rapidly adapt to a resistant rice variety Mudgo (carrying the *Bph1* resistance gene). NISEF1 can suppress the production of  $\text{Ca}^{2+}$ , salicylic acid and  $\text{H}_2\text{O}_2$  in rice, which enhanced the survival rate and feeding capacity of BPHs. In addition, compared with control BPH, *NISEF1*-knockdown BPH induced rice to produce higher amounts of volatiles, especially 2-heptanol and linalool, which subsequently increased the attractiveness of rice to *Anagrus nilaparvatae*, an egg parasitoid of BPH. These results indicate that both of the two salivary proteins, NIEG1 and NISEF1, function as effectors and plays important roles in interactions between BPH and rice.

### The effects of salt stress on herbivore defense in *Zea mays*

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In nature, plants are often exposed to multiple stress factors at the same time. The effects of single biotic or abiotic stresses on plant metabolism are well documented but how plants respond to a combination of these is little researched. Here we studied the effects of high salinity and herbivory on levels of secondary compounds and gene expression associated with defences against insects. Hydroponically grown maize plants were subjected to NaCl (1, 50, 100 mM) and/or damage by caterpillars of *Spodoptera exigua*. Salt-stressed plants showed stunted growth, reduced chlorophyll fluorescence and enhanced levels of reactive oxygen species and 1,4-benzoxazin-3-one aglycones (aBX). Herbivory induced higher transcript levels of the *Zm-Bx1* gene involved in aBX biosynthesis and of the *Zm-SerPIN* gene coding for a serine proteinase inhibitor which might affect plant feeding insects. Herbivory also triggered the emission of volatile organic compounds (VOCs) that are attractive signals for parasitoids and predators and thus regarded as an indirect defence. Herbivore-induced metabolites were differentially affected in salt-stressed plants. High salinity resulted in transient priming of jasmonic acid while aBX levels were reduced in double-stressed plants. Salt stress led to lower herbivore-induced VOC emission per plant but not per unit biomass. However, quantitative shifts in individual compounds were found in both cases. Our study confirms the notion that combined stresses produce a unique phenotype that cannot be derived from single-stress effects. The ecological implications of these changes for organisms from different trophic levels and for plant fitness remain to be tested.

0077

**Exploitation of fruit fly innate behaviour: methyl eugenol response and ingestion - an insight into the synonymization of four invasive species of *Bactrocera dorsalis* complex**

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Males of certain putative sibling species belonging to the *Bactrocera dorsalis* complex possess high affinity to and feed voraciously on methyl eugenol (ME), a potent male lure found in over 450 plant species worldwide. Amongst these species are the highly destructive Oriental fruit fly *B. dorsalis* s.s. and its sibling species- the highly invasive *B. papayae*, *B. philippinensis* and *B. invadens*. Those four taxa are very similar morphologically, possess identical rectal/pheromonal components; and close genetic relationships with high mating compatibility. Here, we further demonstrate that the fly innate behaviour of ME responsiveness may be used as a behavioural parameter for species delimitation by quantifying the quantum response of the four siblings to ME via behavioural bioassays to estimate the median effective dose of each species population using the Probit analysis. Furthermore, quantitative chemical analysis of two ME-derived sex pheromonal components, 2-allyl-4,5-dimethoxyphenol (DMP) and (*E*)-coniferyl alcohol (CF), and their ratios in male rectal glands and volatile emissions after ME consumption revealed two interesting facts: i) prior to release as sex pheromone, ratios of DMP: CF in the rectal gland were similar across the four species; and ii) male volatile emission during courtship revealed similar ratios of DMP: CF across the four species but differ from that of the rectal gland. These revealed the dynamics of sex pheromone components in intraspecific sexual communication and provide further supportive evidence in complementing previous data which confirmed that the four taxa in fact, belong to one and the same biological species - *B. dorsalis*.

**Application of chemical regulators modulate direct and indirect plant defenses against tea geometrid *Ectropis obliqua***

Xin Zhaojun, Li Xiwang, Li Jiancai, Sun Xiaoling

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Plants are sessile organisms that are continuously subjected to numerous insect pests. As a result, plants have developed a massive reprogramming of metabolic pathways and formed induced defense mechanisms to protect themselves. Plant defense regulators (elicitors / inhibitors) play vital roles in regulating the plant defense response against herbivores. In our study, we investigated the effect of (Z)-3-hexenol (z3HOL) and salicylhydroxamic acid (SHAM) on the induced defense against a tea geometrid (TG) *Ectropis obliqua* Prout in tea plants. We found that treatment with z3HOL elicits increased levels of jasmonic acid (JA) and ethylene (ET) as well as of expression of JA- and ET-related genes. Such reactions resulted in a marked increase in polyphenol oxidase (PPO) activity and herbivore-associate plant volatiles (HAPVs) production. The induced tea plants reduced the performance of TG and became highly attractive to the main parasitoid wasp of TG larvae, *Apanteles* sp. However, treatment of tea leaves with SHAM enhanced the performance of TG and TG-elicited level of JA-related genes. The negative effect of SHAM dramatically reduced the total HAPVs and the attractiveness to *A. sp.* These results indicated that SHAM may negatively mediate tea defense response against TG by modulating the expression of genes involved in oxylipin pathway, and the emission of HAPV compounds that mediate direct and indirect defenses. The success of the attraction of parasitoids to HAPVs has been observed in many plant species. We can speculate that plant regulators would be employed in biological tea pests control in the near future.

0079

## Current status of tea pest management via semiochemical-based approaches in China

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The area of tea plantations is 2,580,000 hm<sup>2</sup>, and in the tea plant growing area in the mainland over 800 species insect pests were recorded, in which 5 % of the total species richness needed to be controlled sometimes, and 2.5% of the total species richness were occurred usually. During current status, the important pests include tea green leafhopper, *Empoasca vitis* Göthe, tea geometrid, *Ectropis obliqua* (Prout), citrus spiny whitefly, *Aleurocanthus spiniferus* (Quaintance), tea aphid, *Toxoptera aurantii* Boyer, tea caterpillar, *Euproctis pseudoconspersa* Strand, *Adoxophyes orana* Fischer von Roslerstamm, *Acaphylla theae* Watt, *Brevipalpus obovatus* Donnadied, *Polyphagotarsonemus latus* (Banks), *Caloptilia theivora* (Walsingham), *Scirtothrips dorsalis* Hood, etc. The adopted main control methods include biological control, chemical control, physical and mechanical control, agricultural control, etc. During the past several years, the pheromone preparations were used as one of the main control agents, which were usually combined with colored sticky boards to compose the traps for trapping the adults of pests. The aim pests which can be trapped were tea green leafhopper, *Empoasca vitis* Göthe, tea geometrid, *Ectropis obliqua* (Prout), citrus spiny whitefly, *Aleurocanthus spiniferus* (Quaintance), tea aphid, *Toxoptera aurantii* Boyer, tea caterpillar, *Euproctis pseudoconspersa* Strand, *Adoxophyes orana* Fischer von Roslerstamm. Colored sticky boards were used to control the adults of *Scirtothrips dorsalis* Hood adults. The color sticky boards baited with sex pheromones could use to control tea aphid, *Toxoptera aurantii* Boyer, tea caterpillar, *Euproctis pseudoconspersa* Strand, and *Adoxophyes orana* Fischer von Roslerstamm. The color sticky boards baited with the botanic attractants were applied for the trap of tea green leafhopper, *Empoasca vitis* Göthe, tea geometrid, *Ectropis obliqua* (Prout), citrus spiny whitefly, *Aleurocanthus spiniferus* (Quaintance). Within the proper time, the natural enemy attractants, which composed of the plant synomones, were attached on the colored non-sticky boards to make up the attracting device. Then the device was used to assemble many natural enemies to suppress the aim pests. For example, Assemble *Stethynium empoascae* Subba Rao and *Schizophrag maparvula* Ogloblin to parasitize the eggs of tea green leafhopper, gather *Apanteles* spp. together to parasitize the larva of the tea geometrid, as well as assemble *Aphidius* sp. to parasitize the nymphs of tea aphid. The extension area of the pheromonal preparations and colored sticky boards is being increased rapidly. The application area of per year exceeded 200000 hm<sup>2</sup>.

**Enhancement of larval RNAi efficiency by overexpressing Argonaute2 gene in the silkworm, *Bombyx mori***

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RNA interference (RNAi) has been proved to be a powerful reverse-genetic tool for insect gene functional analysis and a promising approach for pest management. However, RNAi efficiency varies significantly among different insect species due to distinct RNAi machineries. Lepidopteran insects represent large amount of pests as well as the model insects including the silkworm, *Bombyx mori*. Limited success of in vivo RNAi has been reported in Lepidoptera, especially during larval stages. In the present study, we focus on the conserved RNAi core factor, Argonaute2 (Ago2), to investigate BmAgo2 function in mediating *B. mori* RNAi efficiency. We proved that introducing BmAgo2 dsRNA can inhibit RNAi efficiency of dsRNA-mediated RNAi in both BmN cells and embryos. To further assess the roles of BmAgo2 in larval RNAi, we established transgenic silkworm lines which ubiquitously expressed BmAgo2 under the control of OpIE2 promoter (OpIE2-BmAgo2) and the control lines which expressed red fluorescence protein dsRed under the control of IE1 promoter (IE1-dsRed), as well as transgenic lines which expressed shRNA targeting BmBlos2 under the control of U6 promoter (U6-BmBlos2 shRNA). As the result, overexpression of BmAgo2 significantly facilitated both dsRNA- and shRNA-mediated RNAi during larval stage when targeting dsRed or BmBlos2 respectively. Our results prove that BmAgo2 is one of the rate-limiting steps of RNAi in *B. mori*, and provide a promising approach for improving larval RNAi efficiency in *B. mori* and lepidopteran insects as well.

# Poster Presentation Abstracts

September 24-26, 2015

P001

**Oxalic acid as a larval feeding stimulant for the Pale Grass Blue butterfly, *Zizeeria maha***

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Over 80% of the herbivorous insect species are specialized feeders (Schoonhoven et al., 1998). Their behavioral decisions to accept plants as foods or oviposition substrates are predominantly based on sensory information. Especially for Lepidoptera, host plant recognition, selection and oviposition are crucial, because they determine the survival of their progeny (Kostantopoulou et al., 2002; Renwick, 1989). Larvae of the Pale Grass Blue butterfly (*Zizeeria maha*, Lycaenidae) feed exclusively on *Oxalis corniculata* (Oxalidales: Oxalidaceae) whose plant family is characterized by the accumulation of oxalic acid. Larvae were strongly stimulated to feed on artificial diets containing crude methanol extract of host plant leaves. Fractionations and bioassays revealed that the strongest feeding stimulating activity retained in the water layer from which oxalic acid was detected as a major compound. Oxalic acid consisted as much as 15wt% of the methanolic extract, and more than 90% of extracted oxalic acid was found in the water layer. Removal of oxalic acid as calcium oxalate precipitates by addition of calcium chloride into the water layer resulted in the significant decrease of feeding activity in the filtrate. Re-addition of oxalic acid in the filtrate recovered the feeding activity. The addition of oxalic acid in the range of 3.15 to 6.30 mmol, which corresponds to 0.5 to 1.0 g fresh leaves of *Oxalis*, and to one gram of artificial diet, significantly stimulated feeding compared to the intact artificial diet. Therefore, oxalic acid was concluded as a major feeding stimulant for *Zizeeria maha* larvae.

P002

**Electrophysiological and behavioural responses of *Isoceras sibirica* Alpheraky (Lepidoptera, Cossidae) to volatiles from its host plant asparagus (*Asparagus officinalis* L.)**

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Plant volatiles are important cues for the orientation of herbivorous insects. It is possible that these compounds indicate whether the plant is suitable for feeding and larval development, or for mating aggregation. The aim of this study was to identify the bioactive components within asparagus by gas chromatography–electroantennographic detection (GC-EAD) and gas chromatography–mass spectrometry (GC-MS), and to test the antennal and behavioural responses of *Isoceras sibirica* to these chemicals. GC-EAD experiments indicated that *Isoceras sibirica* antennae responded to the following volatile compounds from asparagus: tetramethylurea; 2-ethyl-2-hexenal; 3-heptanone; 2,4-dimethylheptane; heptanal;  $\beta$ -pinene;  $\alpha$ -pinene; limonene; p-cymene; and menthone. In further electroantennographic (EAG) assays with synthetic compounds, high responses by the antennae of both males and females were recorded to these compounds identified: 2-ethyl-2-hexenal, hexanal, p-cymene, heptanal and limonene. Female showed a response equal to or higher than males to all compounds. Subsequently the behavioural response of *Isoceras sibirica* moths to EAD-active compounds presented singly and in mixtures was recorded in a wind tunnel. *Isoceras sibirica* moths engaged in upwind flights to the three compounds: hexanal, p-cymene and 2-ethyl-2-hexenal. Our findings suggest that host volatiles play a role in *Isoceras sibirica* host recognition. The results are discussed in relation to mechanisms behind host odor recognition and the evolution of insect–plant associations.

**P003**

### **Brushtail possum urinary attractants as lures**

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Australian brushtail possums (*Trichosurus vulpecula*) are the leading mammalian agricultural and environmental pest in New Zealand. They have traditionally been controlled using food-based lures that work well for population suppression with toxic baits but probably less well at low and invading densities. Possum pheromones might be adapted as lures to improve detection and kill rates, especially when eradication is the objective. Possums communicate using olfactory cues, but no possum pheromones have been identified to date. We used solid phase micro-extraction gas chromatography mass spectrometry (GC/MS) to characterize inter-individual variation (n=6) in possum urine volatiles and investigated the responses of wild conspecifics to the same urine samples in two field trials (151 trap nights over 30 trap sites). Conspecific trap visitation, and investigation and contact behaviours, of urine-baited traps were at least double and twice as long as of control traps. Partial Least Squares Regression Analysis (PLS) was used to determine which urinary volatiles explain the increase. The list of volatiles responsible for possum attraction and engagement with traps will be tested in further field trials to determine which compounds function as attractants and might be developed as a pheromone lure for possums. Results from GC/MS analyses of male and female possum urinary volatiles and from field trials will be presented along with PLS analysis outcomes.

P004

### Feeding-stimulant activities of rice-ear compounds for rice-ear bugs

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Occurrence of "pecky rice" due to feeding by stink bugs is one of the most serious problems in rice cultivation. "Rice-ear bug" is the name given to the species of stink bug that causes pecky rice, and thereby cause enormous economic losses. The chemical factors that stimulate feeding behaviors of the bugs are still unknown. In this study, we investigated the activities of feeding stimulants for the rice leaf bug, *Trigonotylus caelestialium* (Kirkaldy), which is a common rice-ear bug in Japan. Feeding-stimulant activities of four different substances - rice starch, soluble starch, ethanol extract of rice ear, and water extract of rice ear - for the bugs were analyzed by using an electrical penetration graph (EPG). From the EPG waveforms, feeding behaviors were categorized into four distinct processes: insertion, salivation, test probe, and ingestion. Subsequently, the frequency and total duration of each behavior were analyzed. Rice starch increased the total duration of insertion at a concentration of 50% (in distilled water). Soluble starch increased the total duration of ingestion, the frequency of insertion, and frequency of test probe, at concentrations of 30%, 40%, and 30-50%, respectively. Ethanol extract increased the frequency and total duration of ingestion. Water extract increased the frequency of insertion, salivation, and test probe, and prolonged total duration of insertion and salivation. These findings suggest that rice ear contains several feeding stimulants for *T. caelestialium*, and that starch is one of main feeding stimulants present in rice ear.

P005

### The role of tarsi in host selection by Chrysomelidae

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In previous studies, we have shown that some coleopteran insects use their tarsi as a gustatory organ. In morphological studies using scanning electron microscopy, it was shown that almost all chrysomelid beetles observed had gustatory sensilla on their tarsi. In the present study, we revealed that two subfamilies, Chlamisinae and Sagrinae, which had not been examined in the previous studies, also have tarsal gustatory sensilla. Consequently, we could confirm the presence of tarsal gustatory sensilla in 15 of the 17 subfamilies that constitute Chrysomelidae. In our previous behavioral studies using *Galerucella griseescens* Joannis, we revealed that chrysomelid beetles could recognize gustatory substances, such as sucrose and bitter compounds, by only their tarsi. However, the discrimination between host and nonhost plant chemicals by using tarsi had not yet been investigated. In the present study, we examined the behavioral response of *G. griseescens* to the leaf surface waxes of the host plant *Rumex obtusifolius* and the nonhost plant *Solanum melongena*. *G. griseescens* showed behavioral response to the leaf surface wax of the host plant by only their tarsi. In contrast, the beetles showed no response to the leaf surface wax of the nonhost plant. These results indicate that *G. griseescens* use their tarsi in host selection, and that they can discriminate between host and nonhost plant by using their tarsi. Thus, using their tarsi, the beetles can decide whether to accept plants as their hosts before they bite the leaves.

P006

**A synthetic small molecular compound WJ72 increases rice resistance to the white-backed planthopper *Sogatella furcifera***

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Exogenous application of chemical elicitors is an effective way to help plants against pathogens and herbivores. In this study, the effect of exogenous application of a synthetic small molecular weight compound WJ72 on resistance of rice to the white-backed planthopper (WBPH) *Sogatella furcifera* and its regulating mechanism were explored. Bioassays showed that plants treated with WJ72, ranging from 0.5mg/L to 5mg/L, obviously reduced the survival rate of WBPH nymphs, whereas WJ72 itself had no direct effects on the survival of WBPH nymphs. Exogenous application of WJ72 had a significant effect on plant root and stem growth. Interestingly, three analogues of WJ72 had no effect on survival rate of WBPH nymphs although they also suppressed plant growth when the same concentration as WJ72 was applied. The mechanism study revealed that WJ72 had little influence on JA, ET and H<sub>2</sub>O<sub>2</sub> biosynthesis but slightly suppressed SA production. These findings demonstrate that WJ72 functions as a chemical elicitor and could be exploited as a plant protection agent.

P007

**Expressing *OsHI-MAPK* enhances resistance in rice to the brown planthopper *Nilaparvata lugens***

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MAPKs have been reported to play an important role in plant defense responses against pathogens and insects in dicotyledons. Yet, their role in monocots, including rice remains largely unknown. We here cloned a rice MAPK gene *OsHI-MAPK* whose expression was induced by mechanical wound and feeding of the striped stem borer (SSB) *Chilo suppressalis* but not by the infestation of the brown plant hopper (BPH) *Nilaparvata lugens*. Over-expressing *OsHI-MAPK* (oe-MAPK) reduced levels of ethylene and H<sub>2</sub>O<sub>2</sub> but enhanced JA and SA levels in rice infested by the BPH. BPH preferred to feed and oviposit on WT plants, on which they consumed more and survived better than oe-MAPK lines. Moreover, although no difference was observed in the survival rate of the nymphs and the number of eggs laid by one female adult between BPHs that fed on oe-MAPK lines and those that fed on WT plants, the hatching rate of BPH eggs on oe-MAPK lines was lower than that on WT plants, most possibly because of a lower ethylene level. The results demonstrate that *OsHI-MAPK* plays a pivotal role in modulating herbivore-induced defense-related signaling and defense responses in rice.

**Sex pheromones and reproductive isolation in five mirid species**

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Mate location in many mirid bugs (Heteroptera: Miridae) is mediated by female-released sex pheromones. To elucidate the potential role of the pheromones in prezygotic reproductive isolation between sympatric species, we investigated differences in the pheromone systems of five mirid species, *Apolygus lucorum*, *Apolygus spinolae*, *Orthops campestris*, *Stenotus rubrovittatus* and *Taylorilygus apicalis*. GC/MS analyses of metathoracic scent gland extracts of virgin females showed that all five species produced mixtures of hexyl butyrate, (*E*)-2-hexenyl butyrate and (*E*)-4-oxo-2-hexenal, but in quite different ratios. (*E*)-2-hexenyl butyrate was the major component of *A. spinolae*, while hexyl butyrate was the most abundant component in the pheromone blends of the other four species. In addition to the three compounds, a fourth component, (*E*)-2-octenyl butyrate, was present in the gland extracts of *A. lucorum* and *T. apicalis* females. Field tests suggest that the ternary blends of hexyl butyrate, (*E*)-2-hexenyl butyrate and (*E*)-4-oxo-2-hexenal as found in the extracts of the females of each species do not inhibit attraction of conspecific males but ensure species-specificity of attraction between *A. lucorum*, *O. campestris* and *T. apicalis*. Furthermore, (*E*)-2-octenyl butyrate was essential for attraction of *A. lucorum* and *T. apicalis* males, but strongly inhibited attraction of male *A. spinolae*, *O. campestris* and *S. rubrovittatus*. The combined results from this study and previous studies suggest that the minor component and pheromone dose in addition to the relative ratios of the major components play an important role in reproductive isolation between mirid species.

P009

**The seasonal prevalence of occurrence of the Matsumoto mealybug, *Crisicoccus matsumotoi*, monitored by its sex pheromone-baited trap in a grape orchard**

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The Matsumoto mealybug, *Crisicoccus matsumotoi*, is distributed in India, Philippines, Japan and South Korea. This mealybug is a pest in deciduous fruit, including grapes, pears, figs and persimmons. Tabata et al. (2012) identified a sex pheromone of the pest. We have monitored the seasonal prevalence of the occurrence on the mealybug by a sticky trap baited with the pheromone along with counting the pest on trees in a grape orchard (cultivation style: unheated plastic greenhouse, variety: Sekirei, house area: 2000m<sup>2</sup>, research field: 35 degrees 19.9 minutes north latitude, 132 degrees 43.8 minutes east longitude, elevation 20m). Here, we report the occurrence of the pest in a grape orchard in Japan from 2010 to 2013. Males were attracted to a pheromone trap from the middle of April to the end of October. Three peaks were clearly found in May, July, and late August to the middle of September. Pregnant females and egg sacs were also observed on trees after each peak in every year examined. We therefore concluded that the seasonal prevalence of occurrence of males attracted to the pheromone trap could reflect the occurrence of the pest in a grape orchard. Our monitoring data demonstrated that the mealybug increases three times in a year. The pheromone trap is provided as an efficient and reliable tool for monitoring this pest.

**P010**

**Oviposition stimulants for *Lasioderma serricorne* in roasted coffee beans**

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The cigarette beetle, *Lasioderma serricorne*, is a serious global pest of stored products. We studied oviposition stimulants for the cigarette beetle to control their oviposition. Roasted coffee beans, in which cigarette beetles lay many eggs, were extracted by hexane, chloroform, 1-butanol, 100% methanol, and 20% methanol in water. The beetles exhibited higher oviposition response to the extract of chloroform, 1-butanol, 100% methanol, and 20% methanol in water than to the control. From chloroform extract, we identified catechol as an oviposition stimulant for the beetles (Nagasawa et al. 2014). Methanol extract (100%) was separated into five fractions by using octadecylsilane (ODS) column chromatography. Each of these five fractions showed no significant activity alone, whereas their mixture showed oviposition stimulant activity. Methanol extract (20%) was separated by using ODS column chromatography. A fraction that was eluted with water (Fr.1-1) showed significant activity. The beetles exhibited weak oviposition response to each fraction that was separated from Fr.1-1 by high performance liquid chromatography. Mixture of the fractions of Fr.1-1 from 20% methanol extract was needed to obtain oviposition stimulant response in the beetles to the fractions. Therefore, multiple chemical components act synergistically as an oviposition stimulant for the cigarette beetle.

P011

### Development of a cell-based odorant sensor for mold odors based on insect odorant receptors

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Insects use numerous olfactory sensory cells, which express different odorant receptors (ORs), to sensitively detect various types of environmental odorants. Although odorant sensors based on metal oxide semiconductors have been put to practical use for detecting various odorants, the performances of these sensors are much inferior to those of insects in terms of sensitivity and selectivity. To overcome these problems, we attempt to develop cell-based odorant sensors using insects' ORs. Until now, we have demonstrated that Sf21 cells co-expressing silkworm's pheromone receptors and co-receptor, Orco, along with fluorescent calcium indicator proteins are available as odorant sensor elements with high degree of sensitivity and selectivity. However, we have not yet led this to the development of a practical odorant sensor for the detection of target odorants. Here we report the development of a cell-based odorant sensor that enable us to sensitively and selectively detect mold odorants. We used two *Drosophila melanogaster* ORs (Or56a; Geosmin, Or13a; 1-octen-3-ol) to establish the Sf21 cell lines. Or56a and Or13a cell lines selectively detected Geosmin and 1-octen-3-ol by increasing their fluorescent intensities. Both cell lines dose-dependently detected odorants and their lower detection limits were 300 nM, which corresponds to several tens of ppb. In addition, by integrating these cell lines into microfluidic channels, we constructed a compact odorant sensor chip that can detect two mold odorants as a pattern of increased fluorescence intensity. These results represent a first step towards practical cell-based odorant sensors that detect target odorants with high degree of sensitivity and selectivity.

P012

### A mungbean cultivar that gives high mortality to the bean bug

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It has been known that pods and seeds of some *Vigna* species plants give high mortality to weevils and stink bugs. We observed several behavioral responses of the bean bug, *Riptortus pedestris*, in feeding experiments to seeds of a mungbean (*Vigna radiata*) cultivar, and from the seed extract, isolated some active fractions that show high mortalities to the insect. The insect couldn't survive when reared with seeds of the cultivar. Feeding time and contact frequency of the insect on the seed was not significantly different from those on seeds of a susceptible variety. However, the feeding amount on the resistant cultivar was significantly less than that on the susceptible variety. Through a rearing experiment with various nutrients-feeding condition and survivorship tests with artificial seeds, it was presumed that the high mortality on the seeds of resistant cultivar is related with chemical factors and starvation. The seed flour was extracted with methanol and ethanol solutions, and the extract was fractionated with several parts with solvent/solvent partition and chromatography. In addition, it was confirmed that several peaks of an active fraction are related with the high mortality of insect through HPLC analyses and bioassay. Finally a partial chemical structure in one of those peaks was observed with a chemical reaction and NMR spectroscopy.

**P013**

**Identification and functional characterization of TRPA1 in *Apolygus lucorum***

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Sensing and responding to changes in the external environment is important for insect survival. Transient receptor potential (TRP) channels are crucial for various sensory modalities including olfaction, vision, hearing, thermosensation and mechanosensation. Here a TRPA1 gene was identified and cloned from the green plant bug, *Apolygus lucorum* (Meyer-Dür). The AlucTRPA1 transcripts are abundantly expressed in antennae. In *Xenopus*-based functional assays, AlucTRPA1 was activated by increasing temperatures from 20°C to 40°C with no significant adaptation observed after repeated temperature stimulus. High concentrations of allyl isothiocyanate, cinnamaldehyde, and citronellal directly activated only AlucTRPA1. These results indicate that AlucTRPA1 might function in vivo as both thermal and chemical sensors. This study not only enriches our understanding of TRPA1 function in Hemiptera (Miridae), but also offers a foundation for developing new pest control strategies.

P014

**Identification of olfactory and gustatory perception genes of a primitive hemimetabolous insect, the German cockroach (*Blattella germanica*)**

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The German cockroach, *Blattella germanica*, is an important household pest and has long served as a model system for studies of insect biology, physiology and ecology.

We subjected the *B. germanica* transcriptome to massively parallel pyrosequencing and identified gene families that have been implicated in chemosensory reception including odorant binding proteins (OBPs), chemosensory proteins (CSPs), odorant receptors (ORs) and gustatory receptors (GRs). Fourteen putative OBP transcripts were identified in this study, far less than other model insect. We identified a total of 12 CSP-coding gene fragments in the present study, with 8 CSPs being homologous to CSP7 and CSP18 from *Tribolium castaneum*, which contained the typical four-cysteine signature and a common cysteine sequence motif of C1-X6-8-C2-X16-21-C3-X2-C4 of insect CSPs. Only 2 OR unigenes were annotated in this transcriptome, far fewer than those from other insects. We identified 4 transcripts encoding homologous gustatory receptor in the cockroach, unigene c5971 showed homology to Gr64f from *Drosophila melanogaster*. Gr64f is required broadly as a co-receptor for the detection of sugars. We propose that c5971 gene identified in this study may also be involved in sugar perception of German cockroach. Unigene rep c30027 similarly showed close evolutionary distances with Gr63a and Gr21a, which are co-expressed in CO<sub>2</sub>-responsive neurons and play an important role in the fruit fly food-seeking, suggesting the involvement of unigene rep c30027 in food seeking in the cockroach. The information presented here will be useful to improve our understanding about the molecular mechanism of cockroach chemoreception and gene regulation.

P015

**Volatiles from the coffee berry blossom end and their potential applications in pest management of Coffee Berry Borer *Hypothenemus hampei* (Coleoptera: Curculionidae)**

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The Coffee Berry Borer (CBB), *Hypothenemus hampei* Ferrari (Coleoptera: Scolytidae) is the most destructive pest of commercial coffee worldwide. We proposed two strategies to control CBB: push-pull and push-kill, using repellents and attractants. Push-pull works by pushing CBB out of and away from coffee berries using repellents and then attracting these CBB toward the pull traps baited with attractants. Push-kill works by mixing repellents with pesticides so that CBB are pushed out of the bored tunnel of the coffee berry and exposed to pesticides resulting in enhanced pesticide efficacy. As observed, the blossom end of a green coffee berry is the only entrance CBB bore into, so we proposed that this section of the berry contains more attractants and fewer repellents than the epicarp. Therefore, we carried out a comparison study of the volatile profiles between the blossom end and epicarp of green coffee berries. Using a Headspace-GC-MS system we found that three compounds were significantly higher in the blossom end of non-infested green berries, and two compounds were significantly higher in the blossom end of infested green berries. We further demonstrated through field trials that some of these compounds showed repellent activity to CBB infesting Hawaii coffee farms. Initial field tests demonstrated that both strategies of push-pull and push-kill can potentially be applied for CBB control in an integrated pest management system.

P016

**Cedar Wood Oil and Cedrol as Repellents against Little Fire Ant (*Wasmannia auropunctata*)**

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Originally from South America, little fire ant (*Wasmannia auropunctata*) ranks amongst the top 100 worst invasive species due the threats it poses to human health and agriculture through painful venomous stings and tending homopterans, which directly damage crops (Lowe et al. 2000; Conant 2000); additionally, little fire ant negatively impacts biodiversity by displacing and out competing native species. Eller et al, 2014 conducted an outdoor bioassay and determined that red imported fire ants (*Solenopsis invicta*) were significantly repelled by the presence of cedar wood oil on a pole leading to a water-sugar solution. We are in the process on conducting similar field bioassays using chopsticks bated with peanut butter to test the effectiveness of cedar wood oil as a repellent against little fire ant (*Wasmannia auropunctata*). Our preliminary data conducted in a macadamia nut orchard on Hawaii Island suggests that cedar wood oil, which contains cedrol as an active ingredient is capable of repelling little fire ant. We hope to demonstrate the potential use for cedar wood oil as a pest control agent and mitigate the negative consequences of little fire ant.

P017

### Differential Attraction of Horn Flies, *Haematobia irritans*, to European and Zebu Breeds of Cattle

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Brazil is the largest exporter of beef and fifth highest producer of milk worldwide. The horn fly, *Haematobia irritans* (Diptera: Muscidae) is a major bovine ectoparasite, causing significant economic losses to agribusiness in Brazil. Control of *H. irritans* is mainly delivered through the application of insecticides, other control strategies need to be developed, including the use of volatile semiochemicals that can be deployed to modify host location behaviour. The aim of this current study was to investigate the semiochemical basis of location, by *H. irritans*, of different cattle breeds based in Brazil. Volatile compounds were obtained from six different cattle breeds and used for behavioral assays and electrophysiological studies. Active compounds were identified using gas chromatography coupled mass spectrometry and comparison with standard compounds. Results showed that three cattle breeds were not significantly attractive to the *H. irritans*. The volatile compound profile of the unattractive breeds presented three compounds that were not present in the other breed's volatile compound profile. When testing these compounds in behavioral assays there was a clear repellence from the *H. irritans*. This work provides preliminary evidence of a semiochemical basis for differences in preference of cattle breeds for *H. irritans* in Brazil.

P018

## **Toxicity and Efficacy of Two Emulsifiable Concentrates of 2-Tridecanone against Red Imported Fire Ants**

Jian Chen

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2-Tridecanone is a major constituent of defensive secretion in tawny crazy ants, *Nylanderia fulva* that is an ant species reported to be able to displace red imported fire ants, *Solenopsis invicta* in the field. 2-tridecanone was proven to be toxic to *S. invicta*. Although 2-tridecanone is commercially available, utilization of this naturally occurring compound in fire ant management has received little attention. In this study, toxicity and efficacy of two 2-tridecanone formulations were assessed against *S. invicta*. Two emulsifiable concentrates were prepared using 2-tridecanone as an active ingredient, vegetable oil as a solvent and polyoxyethylene tridecyl ether as a surfactant. Piperonyl butoxide (PBO) was used as a synergist in one formulation. Both formulations showed significant toxicity in laboratory bioassays. In a field trial, at application rate of 5.28 mL/L and 14 days after mound drench treatment, 100% control was achieved for formulation with PBO and 90% control for the formulation without PBO. In conclusion, both formulations had significant efficacy against *S. invicta*. Due to the low mammalian toxicity of 2-tridecanone, no involvement of hazardous organic solvents, no phytotoxicity at applied concentrations, and relatively low cost, both formulations are promising alternatives to commercial insecticide products for fire ant mound drench. The outstanding efficacy of both formulations observed in this study warrants further research on their efficacy against other pest insects.

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