

## Abstract Book





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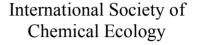












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#### 34<sup>th</sup> Annual Meeting of the International Society of Chemical Ecology 12-18 August 2018, Budapest, Hungary

## **ABSTRACT BOOK**

**Cover photo:** The insect on the ISCE logo is a moth, a young female *Erannis bajaria* (Geometridae), in calling posture, emitting her pheromone [(3Z,6Z,9Z)-3,6,9-octadecatriene and (3Z,6Z,9Z)-3,6,9-nonadecatriene, G. Szőcs, W. Francke et al., unpublished] (photo L.Z. Nagy)

The ISCE 2018 Conference was organized jointly by the Plant Protection Institute, Centre for Agricultural Research, Hungarian Academy of Sciences and the Hungarian Plant Protection Society

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Zoltán Imrei

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## Session committees Session 1. New Chemical Structures:

Jan Bermann Ené Leppik Gábor Szőcs

#### **Session 2. Interspecific relationships:**

Mike Domingue Gerhard Gries Júlia K. Jósvai

#### **Session 3. Intraspecific relationships:**

Claus Tittiger Emmanuelle Jacquin-Joly Sándor Koczor

#### **Session 4. Practical applications:**

Max Suckling Anne-Marie Cortesero Béla Molnár

#### **Specific symposia:**

#### **Session New Chemical Structures:**

- Symposium "Omics in chemical ecology" by Aleš Svatoš and Emmanuel Gaquerel.
- Symposium "Invasions under the bark: chemical ecology of woodboring beetles" by Steven Seybold and Gábor Szőcs

#### **Session Interspecific Relationships:**

- Symposium "Tibor Jermy's Legacy in insect-plant evolution" by Anurag Agrawal
- Symposium "Semiochemistry of aphidophagous insects" by Gunda Thöming and Sándor Koczor
- Symposium "Volatile-mediated microbe-plant interactions" by Birgit Piechulla
- Symposium "Ecology and evolution of toxins in vertebrate animals" by Veronika Bókony and Bálint Üveges
- Symposium "Insect-microbe interactions" by Almuth Hammerbacher
- Symposium "Arthropod Chemoreceptors" by Rob Mitchell and Martin N.
   Andersson

 Symposium "Metabolomics Approaches in the Brassicaceae" by Torsten Meiners and Caroline Müller

#### **Session Intraspecific Relationships:**

- Symposium "Complementary or predominant role of non-chemical based communication and orientation of insects" by Zoltán Imrei
- Symposium: "Chemical ecology of click beetles (Elateridae): practical applications and advances in the field" by Jacqueline Serrano and József Vuts
- Symposium "Importance of multicomponent mixtures for chemical cues" by Brian Smith

#### **Session Practical Applications:**

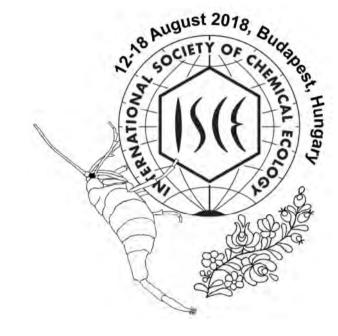
- Symposium "Natural products for integrated pest management" by Jerry
   Zhu
- Symposium "Comparison of semiochemical control methods of pest insects" by Anat Zada-Byers
- Symposium "Semiochemical application for invasive species" by Aijun Zhang and Jian Chen

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### **PROGRAM**

(minor last-minute changes are possible)



#### Sunday, August 12

16:00	Executive meeting of ISCE (Lehár hall)	Registration starting
19:00	Welcome mixer (restaurant of hotel)	
21:00	End of service	

Monday, August 13

wionuay	, August 13	
8:30	Registration continued	
9:30	Pátria hall	
	Opening ceremony	
	Chair: Anne-Geneviève Bagnères	
10:00	Chair: COBY SCHAL	
	Silverstein-Simeone Award lecture	
	ANURAG AGRAWAL: Evolution of two systems of plant defense: milkweed's latex and cardenolides	
11:00	Plenary lecture	
	CONSUELO DE MORAES: Olfactory	
	information in ecology and disease	
	diagnosis	
12:00	Lunch	

	Bartók hall	Liszt hall	Lehár hall
13:00	Session Interspecific Relationships,	Session Practical Applications,	Session New Chemical Structures,
	Symposium ''Tibor Jermy's Legacy	Symposium "Natural products for	Symposium "Omics in chemical
	in insect-plant evolution" by	integrated pest management" by	ecology" by Aleš Svatoš and
	Anurag Agrawal	Jerry Zhu	Emmanuel Gaquerel.
	Chair: Anurag Agrawal	Chair: JERRY ZHU	Chair: ALEŠ SVATOŠ
	SZENTESI Á.: A local perspective on	STEPHEN O. DUKE: Novel modes of	ALEŠ SVATOŠ: Omics in chemical
	Tibor Jermy's contributions to insect-	action of natural phytotoxins can meet	ecology: a gateway to synthetic
	plant evolutionary interactions	a growing herbicide resistance	ecology?
		management needs	
13:15	continued	continued	continued
13:30	Keynote: MICHAEL C. SINGER.: Tibor	QING-HE ZHANG: Food-based fruit fly	MARINE VALLET, TIM BAUMEISTER,
	Jermy and host shifts: are those shifts	trap for the consumer market	FILIP KAFTAN, ALEŠ SVATOŠ, GEORG
	easy or hard?		POHNERT: Investigation of cellular
			heterogeneity in phytoplankton
			communities with comparative
			metabolomics and high resolution
			mass spectrometry imaging
13:45	continued	NICOLETTA FARAONE*, SAMANTHA	continued
		MACPHERSON, VETT K. LLOYD, NEIL	
		KIRK HILLIER: Olfactory basis of tick	
		behavior and novel repellent	
		development	

Monday	, August 13 (continued)		
14:00	JOHN L. MARON: Impact of herbivores	da Silva M. Fátima G.F.*, Soares	VICTORIA MORIS*, THOMAS SCHMITT,
	on plant fitness and populations	MÁRCIO S., SILVA DANIELLE F.,	OLIVER NIEHUIS: Enzymes involved
		Amaral Jéssica C., Silva Michelli	in cuticular hydrocarbon (CHC)
		M., FORIM MOACIR R., FERNANDES	diversity in insects: insights from an
		João B., Souza Alessandra A.,	extraordinary case of intrasexual CHC
		MACHADO ANA M., LOPES ADRIANA	profile dimorphism in a mason wasp
		A.: The citrus defense strategy in	
		response to phytopathogen Xylella	
		fastidiosa and Candidatus	
		Liberibacter asiaticus: quorum	
		sensing signals used by X. fastidiosa	
		and increased biosynthesis of	
		coumarins	
14:15	María-José Endara*, James A.	PICKETT J.A.: Integrated management	ALEXANDER WEINHOLD*, AINHOA
	NICHOLLS, PHYLLIS D. COLEY, DALE	of pest and beneficial insects by	MARTINEZ MEDINA, AND NICOLE M.
	L. FORRISTER, GORDON C. YOUNKIN,	manipulating natural products using	VAN DAM: Shoot herbivory on tomato
	KYLE G. DEXTER, CATHERINE A.	companion crops, GM and synthetic	affects the root metabolome and
	KIDNER, R. TOBY PENNINGTON,	biology	resistance to root knot nematode
	GRAHAM N. STONE, THOMAS A.		
	KURSAR: Tracking of host defences		
	vs. tracking of host phylogeny during		
	the radiation of neotropical Inga		
	insect herbivores		

	, August 15 (continueu)		T
14:30	Volf M*, Segar ST, Miller SE,	FANGZHOU LIU, BIN YANG*, AIHONG	Emmanuel Gacquerel, Aura
	ISUA B, SISOL M, AUBONA G, ŠIMEK	ZHANG, DERONG DING, GUIRONG	Navarro Quezada, Thomas
	P, Moos M, Laitila J, Kim J, Rota J,	WANG: Plant-mediated RNAi in	NAAKE, MONIKA KUPKE, DAPENG LI:
	WEIBLEN GD, WOSSA S, SALMINEN	controlling Apolygus lucorum	Joining forces: – an integrative
	JP, NOVOTNY V: Phylogenetic		approach to decipher anti-herbivore
	escalation and divergence of plant		defense innovations in Nicotiana
	defense and their effects on insect		allopolyploids
	herbivores		
14:45	NIKLAS JANZ: A race or a chase?	JUNWEI JERRY ZHU: Discovery and	continued
	Revisiting Ehrlich and Raven in light	development of natural products for	
	of Tibor Jermy	managing blood-sucking insect	
		vectors	
15:00	Emmanuelle Jousselin:		JAMES M. MWENDWA, PAUL A.
	Phylogenetic approaches to study		Weston, Inge Fomsgaard,
	host-plant driven speciation in		WILLIAM B. BROWN, GREG
	phytophagous insects: insights from		REBETZKE, JEFFREY D.
	fig wasps and aphids		WEIDENHAMER, LESLIE A. WESTON*:
			Metabolic profiling of benzoxazinoids
			in weed suppressive and early vigour
			wheat genotypes.
15:15	FORISTER, M. L.: Evolution of insect		SAJID LATIF*, PAUL A. WESTON,
	host range		SALIYA GURUSINGHE, JANE C. QUINN,
			LESLIE A. WESTON: Metabolic
			profiling of bioactive flavonoids in
			selected annual pasture legumes

15:30	, August 13 (continued)		
15.50	coffee break	coffee break	coffee break
16:00	Session Intespecific Relationships,	Session Practical Applications,	Session New Chemical Structures,
	independent lectures	Independent lectures	independent lectures
	Chair: TED TURLINGS	Chair: JÜRGEN GROSS	Chair: STEFAN SCHULZ
	Ortiz, A.*, Pérez-Andueza, G. and	MEINERS, T.: Chemical diversity in	<b>Keynote:</b> WITTKO FRANCKE:
	SAUCEDO, C.: The curious case of	plant/beneficial arthropod	Identification and synthesis of some
	black truffle beetle (Leiodes	interactions: Tool or obstacle?	new insect volatiles
	cinnamomeus) attractant based on		
	host volatiles		
16:15	ARCE C.*, MACHADO R., MAMIN M.,	HAYES, R. ANDREW*; AMOS,	continued
	RÖDER G, GLAUSER G., ERB M.,	Brogan; Rice, Steven;	
	ROBERT C., TURLINGS T.: Diabrotica	McGlashan, Kate; Baker,	
	virgifera females use maize plant	DALTON; LEEMON, DIANA: An	
	benzoxazinoids to protect their eggs	external attractant trap for the small	
	against predators	hive beetle (Aethina tumida), a pest of	
		European honeybees	
16:30	FÜRSTENAU B.*, AWATER S. D.,	Eraldo Lima*, Fernanda F. Sousa,	STEFAN SCHULZ*, JAN BELLO, WITALI
	HILKER M.: What is the key to host	Nathaly de La Pava, Laura M.	SCHMIDT, MICHEL STEINBISS,
	recognition by the larval	MACHUCA-MESA, NATÁLIA RIBAS,	CHRISTIAN SCHLAWIS, MORITZ
	ectoparasitoid Holepyris sylvanidis?	Eliseu J. G. Pereira, Jeremy	GERBAULET, HANS PETTER LEINAAS:
		McNeil: Are refuge areas effective as	The unique chemistry of springtails
		a Bt resistance management strategy	from a chemical ecology perspective
		when using transgenic plants?	
		Pheromone ecology of Spodoptera	
		frugiperda in Brazil.	

Monday	, August 13 (continued)		
16:45	HERTAEG C.*, VORBURGER C.,	JÜRGEN GROSS*, MARGIT RID, ANNA	STEPHAN H. VON REUSS*, CÉLIA
	MESCHER M. C., DE MORAES C. M.:	MARKHEISER, CHRISTOPH	BERGAME, ROCÍO RIVERA SÁNCHEZ,
	Identifying cuticular hydrocarbon	HOFFMANN: For development of an	CHUANFU DONG, FRANZISKA DOLKE:
	signatures that mediate chemical	oviposition monitoring tool (M-	Exploring the molecular basis for
	mimicry of host aphids by	Ovicard): unravelling the key factors	species-specific ascaroside signaling
	Lysiphlebus parasitoids	for oviposition of grape berry moths	in Nematodes
17:00	DEANNA S BEATTY, JINU MATHEW	CRAIG D. STEWART, RUSSELL A.	BJÖRN BOHMAN*, GAVIN R
	VALAYIL, CODY S CLEMENTS, FRANK	BARROW*, SAJID LATIF, LESLIE A.	FLEMATTI, RUSSELL A BARROW,
	J STEWART, KIM B RITCHIE, MARK E	WESTON: An allelopathic compound	RYAN D PHILLIPS, ALYSSA M
	HAY*: Coral anti-pathogen chemical	from Festuca spp. as a lead molecule	WEINSTEIN AND ROD PEAKALL: 3,6-
	defense: Does seaweed dominance	for development of pre-emergent	Dialkyldihydro-2H-pyran-2,4(3H)-
	suppress coral resistance?	herbicides for weed management	diones: new pollinator attractants in
			Australian orchids
17:15	MICHEREFF MFF, GRYNBERG P,	DANIEL MUTYAMBAI*, ZEYAUR	Session Intraspecific Relationships,
	TOGAWA R, LAUMANN RA, JING-	KHAN, ANDRE KESSLER: Soil	independent lectures
	JIANG Z, SCHIMMELPFENG PHC,	conditioning by a novel maize	ABITH VATTEKKATTE, WILHELM
	BORGES M, PICKETT JA, BIRKETT	cropping system affects constitutive	BOLAND*: Enhancing structural
	MA, BLASSIOLI-MORAES MC*: Maize	plant defences	diversity in terpenoid biosynthesis:
	herbivore-induced plant volatiles		enzymes, substrates and cofactors
	(HIPVs) elicited by Spodoptera		
	frugiperda larvae prime neighboring		
	plants for enhanced indirect defence		

Monday	, August 13 (continueu)		
17:30	SIMON SEGAR*, MARTIN VOLF,	KIRK, W. D. J.: Thrips aggregation	ABDULLAHI YUSUF*, FABIEN
	DANIEL SOUTO, JAN MICHALEK, BRUS	pheromones and their use in pest	DEMARES, CHRISTIAN PIRK: Effect of
	ISUA, MENTAP SISOL, THOMAS	management	brood pheromone on survival and
	Kuyaiva, George Weiblen, Juha-		nutrient intake of African honey bees
	PEKKA SALMINEN, MAGALI PROFFIT,		(Apis mellifera scutellata) under
	CLIVE DARWELL, VOJTECH		controlled conditions
	NOVOTNY: Friends and foes,		
	elevational trends in plant defences		
	and pollinator attractants mirror insect		
	community structure and gene flow		
	along a mountain gradient.		

Tuesday, August 14

	Bartók hall	Liszt hall	Lehár hall
9:00	Silver Medal Lecture		
	Chair: WITTKO FRANCKE		
	TETSU ANDO: Lepidopteran sex		
	pheromones: wonderland for a natural		
	product chemist		
10:00	coffee break	coffee break	coffee break
10:30	Session Interspecific Relationships,	Session Practical Applications,	Session New Chemical Structures,
	Symposium "Semiochemistry of	independent lectures.	Symposium "Invasions under the
	aphidophagous insects" by Gunda	Chair: TOM BAKER	bark: chemical ecology of
	Thöming and Sándor Koczor Chair: GUNDA THÖMING	Keynote: BAŽOK, R.*, TOTH, M.,	woodboring beetles" by Steven
	Keynote: PICKETT J.A.: The	Lemić, D., Čačija, M., Virić	Seybold and Gábor Szőcs
	influence of aphid chemical ecology	Gašparić, H., Rešić, I., Drmić, Z.:	Chair: STEVEN SEYBOLD
	on the aphidophagous insects	First area-wide pest control program	Keynote: CHRISTOPHER M. RANGER*,
	on the upmoophagous miseets	carried out in Europe by the use of	PETER H. W. BIEDERMANN, VIPAPORN
		aggregation pheromones: lessons	Phuntumart, Gayathri U.
		learnt	BELIGALA, SATYAKI GHOSH, ROBERT
			MUELLER, PETER B. SCHULTZ,
			MICHAEL E. REDING, AND J. PHILIPP
			BENZ: A new perspective on the
			affinity of fungus farming ambrosia
			beetles for ethanol

Tuesday	, August 14 (continued)		
10:45	continued	Keynote: IVAN SIVČEV, LAZAR	continued
		SIVČEV: Further results of research on	
		Bothynoderes punctiventris	
		aggregation attractant	
11:00	THÖMING G.*, KNUDSEN G. K.: A	Björn Eriksson*, Alexander	JOCELYN G. MILLAR*, WELITON D.
	semiochemical attractant for common	NILSSON, MATTIAS LARSSON:	SILVA, YUNFAN ZOU, JOSE MAURICIO
	green lacewings combined with floral	Pheromone-based sampling for	S. BENTO, LAWRENCE M. HANKS:
	buffer stripes to enhance biological	conservation: determining the habitat	Chemical parsimony and chemical
	control of aphids in barley	requirements of Tragosoma	diversity in the pheromones of
		depsarium	cerambycid beetles
11:15	BRUCE T.J.A.: The chemical world of	Mikael A. Molander*, Björn	ŽUNIČ KOSI A.*, STRITIH PELJHAN N.,
	aphids, their host plants and their	Eriksson, Marcus Vestlund,	ZOU Y., McElfresh S. and Millar
	enemies	YUNFAN ZOU, JOCELYN G. MILLAR,	J.: A male-produced pheromone of
		MATTIAS C. LARSSON: Quantifying	Arhopalus rusticus may be useful in
		the short-term effects of logging in	managing this invasive longhorn
		oak dominated forests on threatened	beetle
		longhorn beetles (Cerambycidae) with	
		a pheromone-based trapping system	
11:30	HERMANN S.*, AND LANDIS D.: Flying	THOMAS C. BAKER* AND ANDREW J.	FREDRIK SCHLYTER: Host selection in
	into the face of Fear: Predator cues	MYRICK: Feeding, mating, and flight	<i>Ips typographus</i> : have we been
	influence aphid development and	dispersal behavior of the invasive pest	barking up the wrong tree?
	behavior	species, Lycorma delicatula	
		(Hemiptera: Fulgoridae)	

19:00

Editorial Board Meeting of JCE (Janet

Slobodian)

Tuesday	, August 14 (continued)		
11:45	JOHN J SLOGGETT*, ZOWI	PAUDEL S., LIN P., RAJOTTE EG, AND	C X Wu1, Fu Liu1, X B Kong, S F
	OUDENDIJK: Intraspecific variation in	FELTON GW: Facing the heat: how	ZHANG, Z ZHANG*: Semiochemical
	ladybird beetle chemical defences:	global warming might affect tomatoes	regulation of interactions between
	implications for theories of honest	and their interactions with insect	Tomicus minor and Tomicus
	signalling	pests?	yunnanensis during the shoot-feeding
12:00	JEFFREY R. ALDRICH*, ELDA		
	VITANOVIC, SHAUN L. WINTERTON,		
	FRANK G. ZALOM: Green lacewing		
	(Neuroptera: Chrysopidae:		
	Chrysoperla) attraction to yeast		
12:15	Lunch	Lunch	Lunch
13:00	Poster session (uneven numbers):		
	Poster Exhibition Rooms		
14:30	coffee break		
15:15	Poster session (even numbers):		
	Poster Exhibition Rooms		

#### Wednesday, August 15

	Bartók hall	Liszt hall	Lehár hall
9:00	Chair: CHRISTER LÖFSTEDT		
	Plenary lecture		
	MONIKA HILKER: Early herbivore alert: plant chemical and molecular responses to insect eggs		
10:00	coffee break	coffee break	coffee break
10:30	Session Intraspecific Relationships, Symposium "Complementary or predominant role of non-chemical based communication and orientation of insects" by Zoltán Imrei Chair: ZOLTÁN IMREI Keynote: HORVÁTH G. Polarization- based (mis)orientation of insects: water detection, host finding, polarized photopollution and ecological traps	Session Interspecific Relationships, Symposium "Volatile-mediated microbe-plant interactions" by Birgit Piechulla Chair: BIRGIT PIECHULLA Keynote: CHOONG-MIN RYU: Sniffing bacterial volatiles for healthier plants	Session Interspecific Relationships, Symposium "Ecology and evolution of toxins in vertebrate animals" by Veronika Bókony and Bálint Üveges Chair: VERONIKA BÓKONY Keynote: ROBERT J CAPON: Cane toad toxin chemical ecology: the untold story
10:45	continued	continued	continued

#### Wednesday, August 15 (continued)

	day, Magast 15 (continued)		
11:00	continued	MARIE CHANTAL LEMFACK*, NANCY	BÁLINT ÜVEGES*, ANNIE BASSON,
		Magnus, Dajana Domik, Teresa	ÁGNES M. MÓRICZ, VERONIKA
		WEISE, STEPHAN VON REUSS, BIRGIT	BÓKONY, ATTILA HETTYEY: Changes
		PIECHULLA: Sodorifen - a key volatile	in the chemical defence of common
		organic compound (VOC) of Serratia	toad (Bufo bufo) tadpoles
		species	experiencing complex environmental
			challenges
11:15	Keynote: ANDREJ ČOKL: Talking	OSSOWICKI A.*, JAFRA S., GARBEVA	ZOLTÁN TÓTH*, ANIKÓ KURALI,
	through plants as part of multimodal	P: Plant protection from a distance	ÁGNES M. MÓRICZ, ATTILA HETTYEY:
	communication in insects		Toxin replenishment following
			experimental depletion of toxin
			reserves in Bufo bufo tadpoles
11:30	continued	MOUT DE VRIEZE, AURÉLIE GFELLER,	VERONIKA BÓKONY*, BÁLINT
		DELPHINE CHINCHILLA, AURÉLIEN	ÜVEGES, ÁGNES M. MÓRICZ: Toxin
		Bailly, Floriane L'Haridon,	composition of adult common toads
		LAURE WEISSKOPF*: News from the	(Bufo bufo) in natural, agricultural and
		volatile warfare between potato-	urban habitats
		associated <i>Pseudomonas</i> and the late	
		blight causing agent Phytophthora	
		infestans	

#### Wednesday, August 15 (continued)

***************************************	uay, August 15 (continucu)		
11:45	STRITIH PELJHAN N.* AND ŽUNIČ KOSI	DINESHKUMAR KANDASAMY,	MARKUS MENKE*, KRISTINA MELNIK,
	A.: Olfactory signals of the cave	JONATHAN GERSHENZON, ALMUTH	Pardha S. Peram, Iris
	cricket Troglophilus neglectus	HAMMERBACHER*: The sweet smell	Starnberger, Walter Hödl,
	(Rhaphidophoridae; Orthoptera): a	of decay: bark beetles are attracted to	MIGUEL VENCES, STEFAN SCHULZ:
	rare example of temporally modulated	volatile by-products produced during	Identification, synthesis and
	chemical threats	detoxification of host defenses by	determination of the absolute
		their associated fungi	configuration of new compounds in
			scent glands of African frogs
12:00	SÁNDOR KOCZOR*, FERENC SZENTKIRÁLYI, MIKLÓS TÓTH: Combination of chemical and tactile stimuli results in locally increased oviposition in <i>Chrysoperla carnea</i> complex lacewings (Chrysopidae)	BAROJA-FERNÁNDEZ E, ALMAGRO G, BAHAJI A, SÁNCHEZ-LÓPEZ Á, GARCÍA-GÓMEZ P, DE DIEGO N, DOLEZAL K, MUÑOZ FJ, AMEZTOY K, RUBIO L, FERNÁNDEZ JA, POZUETA- ROMERO: Soil application of filtrates and distilled extracts from cultures of fungal phytopathogens enhance yield of pepper (Capsicum annuum L.) plants	OLABIMPE Y. OLAIDE*, DAVID P. TCHOUASSI, ABDULLAHI A. YUSUF, CHRISTIAN W. PIRK, DANIEL K. MASIGA, BALDWYN TORTO, RAJINDER K. SAINI: Equids as potential sources of repellents for Savannah tsetse fly control

#### Wednesday, August 15 (continued)

19:00	cultural event	cultural event	cultural event
14:00	excursion	excursion	excursion
12:30	Lunch	Lunch	Lunch
		phase microextraction	
		citricarpa using headspace solid-	
		after inoculation of <i>Phyllosticta</i>	
		compounds of leaves citrus before and	
		Chemical profiling of volatile	
		RENATO LAJARIN CARNEIRO:	
	•	FERNANDES, MOACIR ROSSI FORIM,	
	Drosophila suzukii	SILVA JUNIOR, JOÃO BATISTA	
	blueberries to infestation by	GONÇALVES PEREIRA, GERALDO JOSÉ	
	colour on susceptibility of highbush	FERNANDES DA SILVA, ROSANA	
	HILLIER N. K.: Role of fruit and leaf	Maria Fátima das Graças	
12:15	LITTLE C. M.*, CHAPMAN T. W.,	HOCELAYNE PAULINO FERNANDES*,	

#### Thursday, August 16

	Bartók hall	Liszt hall	Lehár hall
9:00	Chair: RICHARD NEWCOMB		
	Early Career Award Lecture		
	MARTIN N. ANDERSSON: Insect olfaction: receptors, neurons and behavior		
10:00	coffee break	coffee break	coffee break
10:30	Session Interspecific Relationships, Symposium "Arthropod Chemoreceptors" by Rob Mitchell and Martin N. Andersson Chair: ROB MITCHELL Keynote: RICHARD NEWCOMB*, MELISSA JORDAN, MICHAEL THOMA, AMALI THRIMAWITHANA, SRIDEVI BHAMIDIPATI, CHRISTINE MISSBACH, EWALD GROSSE-WILDE, BILL HANSSON, THOMAS BUCKLEY: The origin of the odorant receptor gene family within the Hexapoda	Session Interspecific Realtionships, Symposium "Metabolomics Approaches in the Brassicaceae" by Torsten Meiners and Caroline Müller Chair: TORSTEN MEINERS NADINE AUSTEL*, CHRISTOPH BÖTTCHER, TORSTEN MEINERS: Non- targeted metabolome profiling of green flower buds in oilseed rape: Screening for resistance against the pollen beetle	Chair: ALMUTH HAMERBACHER Keynote: JON CLARDY: Fungus- growimng ants, molecular diversity, and

Indibat	ay, August 10 (continueu)		
10:45	continued	NICOLE M. VAN DAM*, GALINI	continued
		PAPADOPOULOU, BOB T. RAIJMAKERS,	
		AXEL TOUW, KATHARINA GROSSER,	
		Ainhoa Martinez-Medina ,	
		TOMONORI TSUNODA: Defending a	
		fortress under siege: Optimizing	
		glucosinolate allocation upon root and	
		shoot herbivory in <i>Brassica</i> species	
11:00	JACQUIN-JOLY E.*, MESLIN C., DE	RUO SUN*, RIETA GOLS, JEFF HARVEY,	DINESHKUMAR KANDASAMY*,
	FOUCHIER A., MAINET P., WALKER III	MICHAEL REICHELT, DANIEL VASSÃO,	JONATHAN GERSHENZON, MARTIN N
	W., HANSSON B., LARSSON M.,	JONATHAN GERSHENZON, SAGAR	ANDERSSON, ALMUTH
	MONTAGNÉ N.: Diversity of odorant	PANDIT: Metabolomics of Brassicaceae	HAMMERBACHER: The attraction of the
	and gustatory receptors in the moth	plants and their herbivores help explain	Eurasian spruce bark beetle (Ips
	Spodoptera littoralis: towards	the costs and benefits of glucosinolate	typographus) to its fungal associates is
	understanding what makes a	detoxification	mediated by their unique volatile
	polyphagous species a pest.		bouquets
11:15	DAN-DAN ZHANG*, HONG-LEI WANG,	CAROLINE MÜLLER*, RABEA	C. RIKARD UNELIUS, TAO ZHAO*,
	XIAOQING HOU, ASTRID GROOT,	SCHWEIGER, ANJA BONTE, JULIA	SURESH GANJI, CHRISTIAN SCHIEBE,
	JÜRGEN KRIEGER, CHRISTER LÖFSTEDT:	VOELSEN, CAROLINE PONS, LUDGER	BJÖRN BOHMAN, PAAL KROKENE,
	Male hairpencil polyenes activate	BRÜHL, BERTRAND MATTHÄUS: Moist	PHILIP WEINSTEIN, AND ANNA-KARIN
	receptors located in sensilla housing	storage of <i>Brassica napus</i> seeds induces	BORG-KARLSON: Bark beetle
	receptors for female-produced sex	rapid changes in the metabolic profiles	pheromones are produced by their
	pheromones in noctuid moths	of stored material and the resulting oils	associated symbiotic fungi

12:15	Lunch	Lunch	Lunch
		trophic plant interactions	
		to decipher the role of metabolites in	
		ROBERT D. HALL: Metabolomics as tool	
12:00		ROLAND MUMM, RIC C.H. DE VOS,	
		abiotic stress scenario	
		mustard under a multiple biotic and	
		plant-insect interactions in black	
	0	Untangle complexity: Metabolomics of	
	female <i>Rhagoletis</i> fruit flies.	AND BENEDICTE R. ALBRECTSEN:	
	receptors in the antennae of male and	THOMAS MORITZ, JAMES D. BLANDE,	spread by aphid vectors.
	WEE YEE: Expression of odorant	LASSUEUR, PHILIPPE REYMOND,	Ethylene signaling mediates Potyvirus
	WITZGALL, STEPHEN F. GARCZYNSKI,	KHALING, CHRISTELLE BONNET, STEVE	HENAO L.M.P., CASTEEL C.L.*:
11:45	WILLIAM B. WALKER III*, PETER	Stefano Papazian*, Eliezer	BAK A., PATTON M.F., MURIKI M.,
		interaction	growing termites
	lineages across the Coleoptera	_	communication associdated with fungus
	persistence) of odorant receptor	plant resistance to insects: an example	chemical treasure of microbial
	gain and loss (and occasional	biochemical biomarkers for screening	CHRISTINE BEEMELMANNS*: The
	MCKENNA: Ten genomes reveal the	AM.: Field identification of	MARTIN DAHSE, MICHAEL POULSEN,
	MARTIN N. ANDERSSON, DUANE D.	OLLIVIER J., FAURE S., CORTESERO	BEER, CHRISTIANE WEIGEL, HANS-
11.50	SCHNEIDER, ADAM M. SCHWARTZ,	ESCANDE L., HECKY T., LARIEPE A.,	ALEXANDER SCHMIDT, WILHELM DE
11:30	ROBERT F. MITCHELL*, TROY M.	SEIMANDI CORDA G.*, RENAUD D.,	HUIJUAN GUO, RENÉ BENNDORF,

I iiui sua	ay, August 10 (continueu)		
13:00	Chair: MARTIN N. ANDERSSON	Session Intraspecific Relationships,	Laura V. Flórez*, Kirstin
	SEONG-IL EYUN, HO YOUNG SOH,	Symposium: "Chemical ecology of	SCHERLACH, PAUL GAUBE, JÜRGEN
	MARIJAN POSAVI, STEPHEN RICHARDS,	click beetles (Elateridae): practical	Wierz, Christian Hertweck, Martin
	CAROL EUNMI LEE*: Evolutionary	applications and advances in the	KALTENPOTH: From hitchhiker to
	history of chemosensory-related gene	field" by Jacqueline Serrano and	bodyguard: the evolution of defensive
	families across the Arthropoda	József Vuts	symbionts of beetles from plant-
	•	Chair: JACQUELINE SERRANO Keynote: TOLASCH, T.: Click beetle	associated bacteria
			associated bacteria
		pheromones - more than pest control	
13:15	ROBERTS RE*, POWELL D, WANG T,	continued	ROHLFS M.: Volatiles from mutualist
	HALL MH, MOTTI CA, CUMMINS SF:		yeast protect saprophagous insects
	Putative chemosensory receptors are		against invasion by chemically
	differentially expressed in the sensory		defended mould fungi
	organs of male and female Crown-of-		
	thorns starfish, Acanthaster planci.		
13:30	CHRISTER LÖFSTEDT*, MARTIN N	JACQUELINE M. SERRANO*, R.	CHING-WEN TAN*, MICHELLE PEIFFER,
	ANDERSSON, JACOB CORCORAN, JOTHI	MAXWELL COLLIGNON, YUNFAN ZOU,	GARY FELTON: Symbiotic polydnavirus
	K Yuvaraj, Dan-Dan Zhang,	JOCELYN G. MILLAR: Sex pheromones	of a parasitoid manipulates caterpillar
	XIAOQING HOU, OLLE ANDERBRANT,	and sex attractants of North American	and plant immunity
	RICHARD D NEWCOMB: Comparative	click beetles in the genus Cardiophorus	
	studies of moth pheromone receptors		
	using two complementary platforms		

	iy, August 10 (continueu)		
13:45	BING WANG, WEI-CHAN CUI, MENG-BO	LARSSON MATTIAS C., SVENSSON,	TILOTTAMA MAZUMDAR*, BENG SOON
	Guo, Yang Liu, Emmanuelle	GLENN P., BURMAN JOE, WINDE INIS,	TEH, AISHWARIYA MURALI, WILHELM
	JACQUIN-JOLY, SHAN-CHUN YAN, GUI-	Nyabuga Franklin, Milberg Per,	BOLAND: Stress survival strategies of
	RONG WANG*: A receptor-neuron	BERGMAN KARL-OLOF, JANSSON	the dominating bacteria Enterococcus
	correlate for the detection of attractive	NICKLAS, WESTERBERG LARS, PALTTO	mundtii in the gut of Spodoptera
	plant volatiles in Helicoverpa assulta	Heidi, Oleksa, Andrzej, Harvey	littoralis
	(Lepidoptera: Noctuidae)	DEBORAH, KADEJ MARCIN, MOLANDER	
		Mikael, Eriksson Björn, Andersson	
		Fredrik, Hedenström Erik:	
		Pheromone monitoring turn cryptic	
		click beetles into regional conservation	
		indicators	
14:00	HILL S.R.*, ZASPEL J., IGNELL R.: What	VUTS J.*, FURLAN L., BIRKETT M. A.,	FENGMING YAN*, JINGJING LI,
	makes a blood sucker? Vampire moth,	То́тн М.: Enhancement of click beetle	Danyang Song, Xuefei Tang,
	Calyptra thalictri, chemosensory	surveillance using female attractants	SHAOHUA LU: Chemical and behavioral
	receptor gene expression reflects blood-		ecology of virus-vector-plant
	feeding phenotype		interactions
14:15	ZAINULABEUDDIN SYED: Molecular and	VAN HERK W., VERNON B.:	EBERL F., HAMMERBACHER A.,
	physiological correlates of peripheral	Development of a pheromone-based	GERSHENZON J., UNSICKER S.B.: Small
	olfactory modulation	IPM program to manage Agriotes spp.	size, big effects: how a plant pathogen
		click beetles in Canada	influences tree-insect interactions

	T (Continued)		T
14:30	AMIR D., DAVID R., YAKIR E., BOHBOT	<b>Keynote:</b> LORENZO FURLAN*,	Session Interspecific Relationships,
	J.D.*: The puzzling role of mosquito	Francesca Chiarini, Barbara	independent lectures
	host attractants: The case of sulcatone	CONTIERO: How to use pheromone	Chair: JEREMY MCNEIL
		traps to implement effective IPM of	JEAN-MARC LASSANCE, GLENN P.
		wireworms (Agriotes spp.)	SVENSSON, MIKHAIL V. KOZLOV,
			WITTKO FRANCKE, CHRISTER
			LÖFSTEDT*: Pheromones and barcoding
			delimit boundaries between cryptic
			species in the primitive moth genus
			Eriocrania (Lepidoptera: Eriocraniidae)
14:45	BINU ANTONY*, JIBIN JOHNY AND	continued	JEREMY N. MCNEIL*, ZOE LINDO AND
	MOHAMMAD ALI ALSALEH: The		M. ISABEL RAMERIZ: The impact of
	narrowly tuned odorant receptor		monarch chemical defences on soil
	RferOR5519 and antenna-specific		invertebrates.
	odorant binding protein RferOBP1768		
	are highly selective for ferrugineol, the		
	major aggregation pheromone		
	compound of red palm weevil		
15:00	coffee break	coffee break	coffee break

	iy, August 16 (continuea)		
15:45	Session Intraspecific Relationships,	Chair: József Vuts	
	independent lectures	TEODORA B. TOSHOVA*, MIKLÓS TÓTH,	LENSCHOW M., CORDEL M., POKORNY
	Chair: PATRICIA NAGNAN-LE	Lorenzo Furlan, József Vuts,	T., MAIR M.M., HOFFERBERTH J.,
	MEILLOUR Voymeter SANDRA STEIGER, The	DIMITAR I. VELCHEV, MITKO SUBCHEV:	RUTHER J.*: The biochemical and
	<b>Keynote:</b> SANDRA STEIGER: The chemistry of mating: how pheromones	Detection and seasonal monitoring of	neuromodulatory mechanism
	help to spread and receive sperms	Agriotes species (Coleoptera:	underlying the plastic sex pheromone
	neip to spread and receive sperms	Elateridae) in different vegetation	response in Nasonia vitripennis
		habitats in Bulgaria by pheromone traps	
16:00	continued	LIVY WILLIAMS, III*, JACQUELINE M.	KEVIN FARNIER, DAVID MADGE, PAUL
		SERRANO, JOCELYN G. MILLAR, PAUL J.	CUNNINGHAM*:
		JOHNSON: 13-tetradecenyl acetate, a	Two stones, one bird: Are two distinct
		female-produced sex pheromone	female-specific lures necessary to
		component of Melanotus communis	efficiently monitor and control
		(Gyllenhal) (Coleoptera: Elateridae)	Queensland fruit fly (Bactrocera tryoni)
			populations in orchards?
16:15	PATRICIA NAGNAN-LE MEILLOUR*,	VAN HERK W.*, VERNON B. Trap-based	NANNA HJORT VIDKJAER*, PER
	Amandine Descamps, Peruth	estimates of click beetle populations	Kryger, Inge S. Fomsgaard: Can
	IHIMBAZWE, CHRYSTELLE LE DANVIC,	can vary depending on duration of trap	nature's pharmacy influence the health
	KEVIN POISSENOT, KELLER MATTHIEU:	placement	of honey bees?.
	Chemical ecology of the water vole		
	Arvicola terrestris (Cricetidae) for		
	sustainable population control in		
	Europe		

	iy, August 10 (continueu)		T
16:30	NJIHIA TERESIAH*, TORTO BALDWYN,	Session Intraspecific Relationships,	HILLARY K. KIRWA, LUCY K.
	Murungi Lucy, Irungu Janet,	independent lectures	Murungi, Danny Coyne, John J.
	MWENDA DICKSON, BABIN RÉGIS:	Chair: ALVIN KAH-WEI HEE	BECK, AND BALDWYN TORTO:
	Methyl-2,4,6-decatrienoate identified as	ALI, J.: Who's smelling who? Bi-	Responses of the root knot nematode
	a potential male- produced aggregation	directional signaling and implications	Meloidogyne incognita to specific
	pheromone of Antestia bug	for mutli-trophic chemical ecology.	metabolites identified in the root
	(Heteroptera: Pentatomidae:		exudate of Solanum lycopersicum
	Antestiopsis thunbergii): a coffee pest		
	native to Africa		
16:45	PATRICK GARVEY*, ROGER PECH,	JULIA BING*, DANNY KESSLER, IAN T.	ARNAUD AMELINE*, QUENTIN
	ALISTAIR GLEN, MICHAEL JACKSON,	BALDWIN: Uncoupling pollinator	CHESNAIS, GABRIELA CABALLERO
	WAYNE LINKLATER, GRANT NORBURY:	attraction and post-pollination mate	VIDAL, FLORENT BOGAERT, PATRICIA
	Fatal attraction: exploiting olfactory	selection in Nicotiana attenuata	CLAUDEL, ANAS CHERQUI A, AUDE
	eavesdropping for wildlife conservation		COUTY, PHILIPPE HUGUENEY,
			ALESSANDRA MAIA-GRONDARD,
			Véronique Ziegler-Graff,
			VÉRONIQUE BRAULT: Chemical ecology
			of plant- aphid vector-virus interactions:
			a case of manipulation?
17:00	CHRYSTELLE LE DANVIC*, MARINA	STEITZ I., AYASSE M.*: Macrocyclic	ALYSSA M WEINSTEIN*, BJÖRN
	DILLENBOURG, MATTHIEU KELLER,	lactones act as a queen pheromone in	BOHMAN, RYAN D PHILLIPS, GAVIN R
	LAURENT SCHIBLER, PATRICIA	the primitively eusocial sweat bee	FLEMATTI, ROD PEAKALL: Chemical
	NAGNAN-LE MEILLOUR: The male	Lasioglossum malachurum	clues help resolve the Warty Hammer
	effect: on the way to elucidating the male fragrance inducing ovulation in	(Hymenoptera: Halictidae)	Orchid complex
	ewe and goat	- -	_
	T T		

17:15	Session Interspecific Relationships,	ANNA CHUI-TING CHIENG, SUK-LING	MAGALI PROFEIT* RENOIT I ADEVDE
17:13	independent lectures GEOFFREY JAFFUEL*, SRIBALA DURAIRAJ, RAQUEL CAMPOS-HERRERA AND TED TURLINGS: Identification of ant deterrents emitted by insect cadavers infected with entomopathogenic nematodes	WEE, ALVIN KAH-WEI HEE*:  Involvement of maxillary palp in detection of methyl eugenol by male oriental fruit fly, <i>Bactrocera dorsalis</i> (Diptera: Tephritidae)	MAGALI PROFFIT*, BENOIT LAPEYRE, BRUNO BUATOIS, PIERRE ARNAL, PAUL AUBIER, FLORA GOUZERH, MANON OPSOMMER, MICHAEL STAUDT, MARTINE HOSSAERT-MCKEY: Ozone pollution alters the olfaction and behavior of a specialized pollinator
17:30	SATYAJEET GUPTA*, ANUSHA L K KUMBLE, SATHISH DESIREDDY, JEAN- MARIE BESSIÈRE, RENEE M. BORGES: The scent of life: phoretic nematodes choose live over dead wasps as vehicles	MALKA O*, FELDMESSER E, SANTOS D, VAN BRUNSCHOT S, REICHELT M., EASSON M, GERSHENZON J, VASSÃO DG, SEAL S, COLVIN J MORIN S: Metabolic responses to variable host plants play a key role in different degrees of polyphagy in the <i>Bemisia</i> tabaci species complex	FELIPE BORRERO-ECHEVERRY*, MARIE BENGTSSON, PETER WITZGALL: Host plant odour is an integral part of sex pheromone communication in an insect herbivore

#### Friday, August 17

	Bartók hall	Liszt hall	Lehár hall
9:00	Session Practical Applications, Symposium "Comparison of semiochemical control methods of pest insects" by Anat Zada-Byers Chair: ANAT ZADA-BYERS Keynote: JAMES R. MILLER: Principles of pest management guiding determinations of appropriateness for semiochemical control tactics	Session Intraspecific Relationships, Symposium "Importance of multicomponent mixtures for chemical cues" by Brian Smith Chair: BRIAN SMITH DANIEL L. KLINE: Attraction of Ceratopogonids to Plant and Animal Volatiles	Session Practical Applications. Symposium "Semiochemical application for invasive species" by Aijun Zhang and Jian Chen Chair: AIJUN ZHANG Keynote: YIN ZHONG CUI: Semiochemical application for invasive species in China
9:15	continued	PAUL SZYSZKA*, ALEXANDER EGEA- WEISS, AARTI SEHDEV, YUNUSA MOHAMMED, ALPHA RENNER: Odor source separation and rapid odor coding in insects	DONG H. CHA*, PETER J. LANDOLT, AND GREGORY M. LOEB: Discovery of attractants and repellents for managing <i>Drosophila suzukii</i>
9:30	JOHN A. BYERS: Modeling detection, mating disruption, mass trapping, and push-pull management strategies using semiochemicals	HETAN CHANG, YANG LIU, DONG AI, XINGCHUAN JIANG, SHUANGLIN DONG, GUIRONG WANG*: The antagonist Z11-16:OH emitted by females regulates optimal mating time in Helicoverpa armigera	AMALIN, D.M.*, TAVERA, M.A.A., ORMENITA, L.A.C., CARANDANG J.S.R., FLORES, M.J.C., LORENZANA, L.DJ., JANAIRO, G.C., AND JANAIRO, J.I.B.: Understanding Semiochemicals for Detection and Control of Major Insect Pests of Cacao in the Philippines

#### Friday, August 17 (continued)

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9:45	PICKETT J.A.*, KHAN Z.R. AND	ALLY HARARI*, VICTORIA SOROKER,	Man-Qun Wang: Structural
	MIDEGA C.A.O.: The Push-Pull	Adrea Gonzalez-Karlsson,	Transformation Detection Contributes
	system for controlling lepidopterous	YFTACH GOLOV, RUSSELL JURENKA:	to Screening of Behaviorally Active
	pests in cereals: new developments in	Evidence for resistance to mating	Compounds: Dynamic Binding
	exploiting GM in delivering the Push-	disruption technique in the pink	Process Analysis of DhelOBP21 from
	Pull strategy	bollworm a pest of cotton	Dastarcus helophoroides
10:00	coffee break	coffee break	coffee break
10:30	CLARE SAMPSON: Mass trapping with	CHAN H. K., HERSPERGER F.,	DONG-HWAN CHOE: Chemical
	pheromone incorporated sticky traps	MARACHLIAN E., SMITH B. H.,	ecology of Argentine ant and its
	makes integrated thrips management	LOCATELLI F., SZYSZKA P., NOWOTNY	implication for practical pest
	programmes more robust	T.*: Mixtures are more robust stimuli	management
		in olfaction	
10:45	LARRY GUT* AND JAMES MILLER:	APHRODITE KANTSA*, ROBERT A.	FLORE MAS*, OLIVIA REYNOLDS,
	Accelerating the adoption of mating	RAGUSO, THEODORA PETANIDOU:	RACHAEL HORNER, AIMEE HARPER,
	disruption: matching technology with	Natural olfactory landscapes and	LEE-ANNE M. MANNING, TERRY
	mechanism	pollination networks	OSBORNE, ANDREW KRALICEK, D.M.
			SUCKLING: Odorant-based detection
			of invasive species in fresh fruit
11:00	KSENIA S. ONUFRIEVA*, ALEXEY	PENGFEI LU*, MIN BAO: Mating	MICHAEL J. DOMINGUE*, SCOTT W.
	Onufriev, Andrea D. Hickman,	behavior and attractiveness of male	MYERS, THOMAS W. PHILLIPS: Larvae
	Donna S. Leonard, Patrick C.	cuticle extracts based on	of Trogoderma respond behaviorally
	TOBIN: Finding maximum gypsy	electroantennogram and behavioral	to whole body extracts
	moth population density controlled by	assay in Sirex noctilio Fabricius	
	mating disruption		

#### Friday, August 17 (continued)

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11:15	DAVID MAXWELL SUCKLING:	VANDER MEER R, JONES T., CHINTA	ZHANG, AIJUN*, FENG, YAN: Activity
	Behavioural disruption for	S.: Chemicals produced by males and	evaluation of attractants for spotted
	suppression presents different	transferred to females during mating	wing drosophila Drosophila suzukii in
	challenges in different insect orders	prevent multiple matings	the field
11:30	PETER S. McGHEE*, LARRY J. GUT, JAMES R. MILLER, DONALD THOMSON: Optimizing pheromone dispensers for codling moth mating disruption	ALEŠ SVATOŠ*, RICHARD A. FANDINO, SYBILLE LORENZ, YVONNE HUPFER, ALEŠ BUČEK, IVA PICHOVÁ:  Manduca sexta sex pheromone biosynthesis: desaturase point	DONALD C. WEBER*, ANNA K. WALLINGFORD, WILLIAM R. MORRISON III, TRACY C. LESKEY, ASHOT KHRIMIAN: Invasive stink bugs: semiochemical patterns of field- based attraction, cross-attraction,
		mutations and CRISPR/Cas8 genes editing	synergy, and application
11:45	ANAT LEVI-ZADA*, AVRAHAM	RUI-TING LI, CHAO NING, LING-QIAO	CHEN, J.*, FENG, Y., ZHANG, A.,
	SADOWSKY, SVETLANA DOBRININ,	HUANG, JUN-FENG DONG, XIANCHUN	GRODOWITZ, M.J.: Utilization of
	TAMIR TICUCHINSKI, MAAYAN	LI, CHEN-ZHU WANG*: The molecular	naturally occurring compounds in
	David, Daniela Fefer, Ezra	bases of producing sex pheromones	controlling fire ants
	DUNKELBLUM, JOHN A. BYERS:	with the opposite component ratios in	
	Control of lesser date moth	two Helicoverpa moth species	
	Batrachedra amydraula by mass- trapping with pheromone		
12:00	Session Interspecific Relationships,	STOWERS L.: Circuit coding of innate	RAY A. M.*, FRANCESE J. A., ZOU Y.,
	independent lectures Chair: MIKLÓS TÓTH ADRIENNE L. GODSCHALX*, MARK A. SZENTECZKI, NADIR ALVAREZ, AND SERGIO RASMANN: Floral scent variation in smelling like poop	olfactory behavior	WATSON K., CROOK D. C., MILLAR J. G.: Detection of velvet longhorned beetle populations using attractant baited traps

#### Friday, August 17 (continued)

r i iuay,	rugust 17 (continucu)		
12:15	OMER NEVO*, DIARY	S. Noushin Emami*, Lisa C.	B.J. Almarinez*, M. Arcelo, R.
	RAZAFIMANDIMBY, JUAN ANTONIO	RANFORD-CARTWRIGHT, HEATHER M.	CENA, R. CASTILLO, J.I. JANAIRO, A.
	JAMES JEFFREY, STEFAN SCHULZ,	FERGUSON: Do transmission potential	ZHANG, AND D. AMALIN: Field
	Manfred Ayasse: Is fruit scent an	of infected mosquitoes alter by the	bioassay of the synthetic sex
	adaptation to primate seed dispersal?	type of vertebrate blood?	pheromone of the cacao pod borer,
			Conopomorpha cramerella, at
			different height
12:30	Costa A.*, Moos M.,	L.E. ROSCOE*, P. SILK, E.S.	CESAR RODRIGUEZ-SAONA,
	Zahradníčková H., Šimek P.,	EVELEIGH, M. BROPHY, K. BURGESS:	FERNANDO SANCHEZ, MONICA
	ZEMEK R.: The effect of wounding	Identification of male hairpencil	Guisti, Yucheng Zhou, Betty
	and Spodoptera littoralis herbivory on	pheromone components in	BENREY: Effects of domestication of
	plant VOCs emissions in caraway	Choristoneura fumiferana Clemens	blueberries on the invasive vinegar fly
	Carum carvi	(Lepidoptera: Tortricidae): chemistry	Drosophila suzukii
		and function	
12:45	Lunch	Lunch	Lunch
14:00	Business Meeting and Closing Remarks		
	Obituary of Jerrold Meinwald		
	Other matters		
15:15	coffee break		
19:00	I .		

# Plenary and Award Lectures

# Silverstein-Simeone Award Lecture

Anurag Agrawal

Evolution of two systems of plant defense: milkweed's latex and cardenolides

Cornell University

Corresponding author's e-mail address: aa337@cornell.edu

Studies of evolution of plant defense have a long and venerable history, with major advances coming through the age of quantitative genetics, phylogenetics, and genomics. In this talk I will summarize what we know about the micro- and macroevolution of defense in a genus of herbaceous plants, the milkweeds (*Asclepias* species). In particular, I will focus on allocation trade-offs between constitutive and induced production of latex (a physical and chemical defense) and cardenolides (steroidal toxins). These two defense systems show different patterns of evolution within and between species, which sheds light on constraints and trade-offs during adaptive evolution across scales. The lecture will be rich with natural history and interesting biology of the plants and their herbivores, including the monarch butterfly. This group will serve as a model for understanding general principles in the chemically mediated interactions between species.

#### **Silver Medal Award Lecture**

Tetsu Ando

#### Lepidopteran sex pheromones: wonderland for a natural product chemist

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

Corresponding author's e-mail address: antetsu@cc.tuat.ac.jp

Lepidopteran sex pheromones have been identified from more than 680 species by chemical analysis, and the sex attractants of 1280 other species have been found in field tests of synthetic pheromones and related compounds. Our laboratory studies based on organic synthesis contribute to the research of the pheromones of 64 species in 16 moth families and the attractants of 250 species. While our information is still rudimentary considering the species diversity, other natural products have not been investigated for as many different species as the lepidopteran sex pheromone.

By analyzing many mass spectra of Type I and II pheromones, we clarified the diagnostic fragment ions reflecting the positions of double bonds and an epoxy ring in order to utilize them for studies on new natural pheromones. In the case of the epoxy pheromones, their stereochemistry was successfully determined by applying enantioselective HPLC analysis. Furthermore, we found novel methyl-branched pheromones and developed a widely applicable synthetic route via the SN2 reaction of a secondary tosylate derived from optically active propylene oxide.

Our laboratory is also interested in insect physiology and has investigated pheromone biosynthesis. Experiments with handmade 14C- or D-labeled precursors proposed the biosynthetic pathways of some pheromones, and molecular biology experiments successfully cloned key enzymes of the biosynthesis, such as a desaturase for Type I pheromones and an epoxidase for Type II pheromones. In addition to the pheromones, pheromone-biosynthesis-activating neuropeptides were identified, and biosynthetic steps regulated by the hormone were revealed. Our understanding increased rapidly, but the diversity of lepidopteran insects still provides many interesting research topics, such as evolution.

# **Early Career Award Lecture**

Martin N. Andersson

Insect olfaction: receptors, neurons and behavior

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Insects detect odors primarily using odorant receptors (ORs) that are expressed in olfactory sensory neurons (OSNs) housed in the olfactory appendages. The coding of odors by these key players of olfaction drives behaviors crucial to insect fitness. The large and divergent family of insect ORs evolve according to a "birth-and-death" model involving both neutral and adaptive events. This evolutionary model is thought to be important for the coexistence of both narrowly and broadly tuned receptors, and the combinatorial mode of odor coding that is crucial to insect ecology. My research on insect odor recognition primarily involves non-model organisms. These species have proven useful for addressing questions of the evolution and function of insect ORs, and peripheral odor coding mechanisms that underlie behavioral responses. In this presentation, I will summarize the major findings that have resulted from my studies of the olfactory systems of bark beetles, gall midges, and moths. These findings include novel insight into the effects of OSN colocalization in sensilla on neurophysiological and behavioral responses in insects. Specifically, OSN co-localization allows for signal modulation in the periphery, and it also improves the insects' ability to detect spatially separated odor sources in the field. Our functional work on insect ORs using heterologous systems has provided insight into the origin of sex pheromone receptors in Lepidoptera, suggesting that these receptors have evolved from ORs detecting plant volatiles. Our studies have also resulted in the identification of a sex pheromone receptor and a deviant olfactory co-receptor (Orco) in gall midges.

# **Plenary Lecture**

Consuelo M. De Moraes

Olfactory information in ecology and disease diagnosis.

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Information transferred via olfactory cues and signals plays a central role in mediating inter-specific ecological interactions. We have been exploring the communicative functions of volatiles in a wide range of systems, including multi-trophic interactions among plants and other organisms and the transmission of plant and animal pathogens by insect vectors. In addition to exploring the ecological functions of volatiles and their roles in structuring natural and agricultural ecosystems, we are also interested in the potential use of volatiles as biomarkers in the contexts of agriculture and medicine. Some of our recent work has focused on exploring how malaria induced changes in human odours influence vector attraction, as well as the potential exploitation of odour cues for disease diagnosis. There is a pressing need for improved diagnostic methods for malaria, and particularly for the reliable detection of asymptomatic infections that play an important role in perpetuating the disease within human populations but often go undetected and untreated. Some of our recent findings from work on human populations in Kenya suggests that screening skin volatile profiles can identify both symptomatic and asymptomatic malaria infections with high sensitivity. In this talk, I will discuss these studies, as well as other recent highlights of our work on volatile signalling.

# **Plenary Lecture**

Monika Hilker

# Early herbivore alert: Plant chemical and molecular responses to insect eggs

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Plants respond to egg depositions of herbivorous insects on their leaves by various defenses harming the attacker. How do plants "recognize" the egg depositions on their leaves? Several compounds of amphiphilic character have been identified that are released by the insect female with the eggs and elicit defensive plant responses which reduce survival of the eggs. However, highly specialized insects show counter-adaptations to plant defense responses against their eggs. For example, pine needles with eggs of the pine sawfly accumulate hydrogen peroxide which might be involved in defense signaling and/or directly harm the eggs. However, the pine sawfly can obviously limit accumulation of hydrogen peroxide by releasing an egg-associated secretion which shows catalase activity, thus degrading hydrogen peroxide. Interestingly, plant responses to eggs do not only target the eggs. Several plant species have been shown to intensify their defense against hatching larvae when having received insect eggs prior to larval feeding. Previously egg-deposited plants reduce performance of herbivorous larvae more effectively than egg-free ones. Our data indicate that this effect is due to enhanced levels of phenylpropanoid plant compounds in previously egg-deposited leaves. Furthermore, a recent study revealed that egg depositions preceding larval hatching shape the transcriptomic dynamics of plant responses to feeding damage. We found that previously egg-deposited plants show faster/earlier differential regulation of defense genes in response to feeding damage than egg-free plants. Hence, plant responses to the initial step of insect attack, the egg deposition, impact also on subsequent responses to the feeding insect stages.

Hilker M., Fatouros N.E. (2016) Curr. Opin. Plant Biol. 32: 9-16.

# Abstracts of oral and poster presentations

The writings, concepts and opinions expressed in the abstracts of this publication are the responsibility of their authors. The Organizing Committee doesn't assume responsibility for data and conclusions in the abstracts

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# Interaction between thrips and their host plants, and its application

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Herbivore attack is one the most important factors to decrease the agricultural production. Many types of insecticide were developed and utilized to control the insect pests, and have been supporting the agriculture. However recently, it has become a big problem that many herbivores with insecticide resistance were appeared. Especially, thrips developed highly resistance to many types of insecticide, and can cause serious problems in many crops, vegetables, fruits and flower plants. This tiny and polyphagous pest insect is an also vector of tospoviruses, therefore, both the feeding damage and the tospovirus disease caused by thrips are serious problems in the world.

In these surroundings, we elucidated the molecular mechanism of the plant response against thrips (*Frankliniella occidentalis*) feeding to develop the new methods of thrips control. We demonstrated that jasmonate (JA) dependent plant induced defense system restricts both thrips performance and preference. JA treatment to the plants increased the thrips avoidance and controls the thrips behavior in the experimental laboratory. On the other hand, salicylic acid (SA) treatment to the plants increased the thrips attraction. Moreover, we indicated that tospovirus utilizes these antagonistic plant defense systems to attract its vector thrips to tospovirus infected plants. These results imply the possible practical effects of plant defense system to control thrips behavior.

In this meeting, we show our recent approach to develop the thrips repellent. The experiments for verification of our candidate to activate plant defense system successfully control thrips population in agricultural greenhouse.

Abe H et al. (2008) Plant and Cell Physiology 49: 68-80.

Abe H et al. (2009) BMC Plant Biology 9: 97.

Abe H et al. (2012) Plant and Cell Physiology 53: 204-212.

Session/symposium: Practical applications/independent presentation

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# Male thrips discriminate females by age when mating

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In insect species where the male is sperm-limited, males would be expected to discriminate between females when mating so as to maximise their total number of offspring. Males should favour: (1) virgin females; (2) young females, which will live longer and so have higher future reproductive potential; and (3) females that resist less by abdominal flipping, because that reduces the risk of male injury. We tested the hypothesis that female age influences the likelihood that a male will copulate in the bean flower thrips, Megalurothrips sjostedti (Thysanoptera: Thripidae). Individual known-age males were observed with individual virgin females that were 0-1 days, 1-3 days and 7-9 days old postemergence. The proportion of males that copulated decreased markedly with female age. To test whether this was an effect of chemical cues rather than female behaviour, such as abdominal flipping, the experiment was repeated with females (0-1 days and 7-9 days old post emergence) that were killed by being frozen and defrosted. Males were still able to copulate with dead females and there was a similar difference in copulation proportion with female age. This is evidence that the males were using chemical cues from the female, such as cuticular hydrocarbons. This is the first report of male assessment of female age before copulation in the order Thysanoptera.

Session/symposium: Intraspecific relationships/independent presentation

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Field bioassay of the synthetic sex pheromone of the cacao pod borer, *Conopomorpha cramerella*, at different height

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The cacao pod borer (CPB), Conopomorpha cramerella, is one of the major insect pests of cacao in Southeast Asia. In the Philippines, this pest can cause up to 50 per cent annual loss or even higher if no proper management is employed. Management of CPB heavily relies on chemical control but very expensive and not safe to human and environment. Several efforts are being done to develop Integrated Pest Management (IPM) program for CPB with the inclusion of the use of sex pheromone for monitoring and control. A new blend of the synthetic sex pheromone of CBP showed potential in monitoring and mass trapping activities. Initial trial in one the cacao farms in Cotabato, southern Philippines showed an average CPB trap catches of 50 individuals per trap from traps installed 1.0m above the canopy. A follow-up study is being conducted to evaluate the effect of trap height on the insect trap catches. Determination of the correct height of the CPB trap catches is valuable in maximizing the efficiency of the sex pheromone traps for inclusion in the IPM program of CPB in the Philippines.

Session/symposium: *Practical applications/Semiochemical application for invasive species*ORAL PRESENTATION

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Green lacewing (Neuroptera: Chrysopidae: Chrysoperla) attraction to yeast

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Green lacewings (Chrysopidae; ~1200 species), especially Chrysopa and Chrysoperla species whose larvae are predators of aphids, are invaluable biological control agents. In the green lacewing genus, Chrysopa, adults are also predacious, and these are the only lacewings known to produce aggregation pheromones. However, adults of most other green lacewings, including Chrysoperla species, are not predacious; instead they feed on nectar and pollen (termed glyco-pollenophagy). Attractant pheromones are unknown for glyco-pollenophagous lacewings; the only attractant currently available for Chrysoperla lacewings is a blend of floral compound. During research supervised by Dr. Frank Zalom, University of California, Davis, on the attractiveness of various yeasts to olive fruit flies, large numbers of green lacewings were incidentally attracted (Vitanovic, unpublished data). These lacewings were predominantly Chrysoperla comanche (Winterton, unpublished), one of the most common green lacewings in California. Earlier research by Suh et al. (2004) reported identification of the main gut yeasts of C. comanche as Metschnikowia chrysoperlae and Candida picachoensis. These yeasts are closely related to those found attractive to C. comanche in the olive fruit fly tests. Taken together, these data indicate that adults of glyco-pollenophagous green lacewings, such as Chrysoperla species, are attracted to certain yeasts in nature in order to inoculate themselves with these essential symbionts.

Suh S.-O. et al. (2004) Int. J. Syst. Evol. Microbiol. 54: 1883-1890.

Session/symposium: Interspecific relationships/Semiochemistry of aphidophagous insects
ORAL PRESENTATION

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Fruit flies *Rhagoletis batava* perceive odours emitted by yeasts from berries of their host plant

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Economic demand of sea buckthorn (*Hippophae rhamnoides* Hering) berries is growing. It is because of their wide use in cosmetics, food and medicine industry. sea buckthorn fly *Rhagoletis batava* L. is the most serious pest of the berries. Fruit flies lay their eggs into the berries and after maggot feeding the berries loses their nutritional and economical value. *R. batava* can destroy up to 100% of harvest in organic farms (Shamanskaya et al., 2009; Shamanskaya, 2014; Shalkevich et al., 2014).

Fruit flies, as well as many other insects, locate and recognise host plant by perceiving its volatile organic compounds (VOCs). However, there are microorganisms on plant surface, and those also emit their VOCs. The odours can play a role as extra signals for the insects, indicating plant condition and its suitability for egg laying.

Three yeast species from sea buckthorn berries surface were isolated and cultivated. Their VOCs were collected by SPME. GC-EAD revealed 10 VOCs which evoked electroantennographic responses in *R. batava* males and females. These compounds were identified by GC-MS. Further analysis of *R. batava* behavioural responses to the VOCs is in progress.

Session/symposium: Interspecific relationships/independent presentation

Ali J.

Who's smelling who? Bi-directional signaling and implications for multi-trophic chemical ecology.

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Plants, along with their friends and foes are inherently linked by signals that are dynamic, variable and impartial to perception by the surrounding community. Using examples from our lab studies we will examine instances demonstrating how these signals can affect plants, herbivore or natural enemies in new and interesting ways. These topics will be discussed in the contexts of harnessing them for better understanding of multi-trophic interaction in both fundamental and agro- ecological contexts.

Session/symposium: Intraspecific relationships/independent presentation ORAL PRESENTATION

Al-Jalely B.H.<sup>1</sup>\*, Xu W.<sup>2</sup>, Ren Y.<sup>2</sup>, Agarwal M.<sup>2</sup>

Electrophysiology response of the parasitoid wasp *Diadegma semiclausum* (Hymenoptera: Ichneumonidae) to plant volatile compounds

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Brassicaceae family crops are of a significant importance on direct consumption crops such as Cauliflower, Cabbage, Broccoli etc. and indirect consumption crops such as Canola. The family is a target for a number of destructive insects including the Diamondback moth (DBM) *Plutella xylostella* (Linnaeus), which can rapidly develop resistance to chemical pesticides.

Thus, biological control agents are safe alternatives for being eco-friendly sustainable elements. The parasitoid wasp, *Diadegma semiclausum* (Hellen), stands out as a very efficient agent in controlling the DBM populations. Using its sensory organ (the antenna), the female has the ability to recognize the favourite larval stage to oviposit its egg, not to mention the ability to find the plant that hosts the DBM larva. The antenna works as a receptor for the Volatile Organic Compounds (VOCs) that accompanies the plants and DBM, in addition to other elements present in the ecosystem.

In this study, antennae of male and female *D. semiclausum* adults were exposed to a number of volatile organic compounds and the response was measured and recorded using the Electroantennography (EAG). Results showed significant differences in the response of male's antennae compared to female's antennae. Age of tested insects was tested too. The improved understanding of the chemical detection of *D. semiclausum* will help develop more efficient and environmentally friendly DBM control strategies.

Session/symposium: Intraspecific relationships/independent presentation

Allan S.A.\*, Cromartie R.

# Potential chemical attractants for corn silk flies (Diptera: Uliidae)

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Corn silk flies are primary pests of fresh market sweet corn, particularly in south Florida where over 60% of the US fresh market sweet corn in produced. Surveillance methods consist of sweeping, collection of infested corn ears or visual inspection and are unreliable, insensitive and time consumptive. Food-associated materials attractive to several corn silk fly species were identified in the field and laboratory. Attraction to various volatiles from these materials were evaluated in the laboratory.

Session/symposium: Practical applications/independent presentation

Allison J.D.<sup>1</sup>, Andrade S.<sup>2</sup>, Millar J.G.<sup>3</sup>

 $\label{lem:complex} Pheromones\ and\ reproductive\ isolation\ in\ pine\ sawyers\ (Coleoptera:\ Cerambycidae)\ in\ North\ America$ 

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Chemical specificity in specific mate recognition systems is thought to be a product of qualitative (i.e., presence/ absence of components and differences in component double bond configuration or position, enantiomer configurations, chain length, functional moieties) and quantitative (component ratios) differences in pheromone blends. Although there is little debate about whether these differences can confer reproductive isolation, additional mechanisms capable of creating reproductive isolation exist (e.g., daily and seasonal activity patterns, signals in other modalities, host volatile effects on pheromone response) potentially obviating the need for chemical specificity in the pheromone blend. Further, there is considerable debate concerning the role of pheromone divergence in the speciation process. Until recently, little was known about the pheromone biology of longhorned beetles (Coleoptera: Cerambycidae); however, in recent years a large number of pheromone and pheromone attractants have been identified for these beetles. Interestingly, patterns or pheromone parsimony with closely related species (i.e., congeneric) and sometimes more distantly related species often using the same pheromone components have begun to emerge. For example, in North America 2-(undecyloxy)ethanol (monochamol) has been identified as a pheromone or pheromone attractant for several sympatric groups of *Monochamus* spp. The objective of this study was to explore the role of volatile pheromones in the maintenance of reproductive isolation of the sympatric species Monochamus scutellatus, Monochamus mutator and Monochamus notatus.

Session/symposium: New chemical structures/Invasions under the bark: chemical ecology of woodboring beetles

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# Understanding semiochemicals for detection and control of major insect pests of cacao in the Philippines

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With the global concern of projected high deficit of raw materials for chocolate making, the Philippines is considered as one of the key player suppliers because of its location in the equatorial plate and suitable environment in growing cacao. However, before the Philippines can take this market opportunity, production constraints should be addressed. One of the major constraints in cacao production is damages from insect pests and diseases. Two of the most important insect pests of cacao in the Philippines are the cacao pod borer (CPB) and cacao mirid bug (CMB). Farmers usually resort to the use of insecticides to control these pests, but becoming unpopular because of high cost and health risk to people and environment. Alternatively, sleeving of pods is being use but very labor intensive. Thus, effective but farmers and environment - friendly pest management system is needed. The potential of semiochemicals to manage the population of CPB and CMB is currently being evaluated in the Philippines as component of pest management system. The volatile chemical profiles of the cacao and other hosts of CMB were obtained using SPME coupled with GC-MS. From the profiles, a common bicyclic sesquiterpene compound was observed. Olfactometry analysis using lab-reared CMB confirmed positive attraction towards this natural compound suggesting potential for a kairomone - based pest control strategy for CMB as feeding attractant, which can be tested also as oviposition attractant for CPB. Additionally, mating behavior study of CMB showed that the female CMB is the mating caller and therefore possibly releasing the sex pheromone. Using GC-MS, alpha-pinene was extracted from the abdomen of female CMBs, which is a known sex pheromone in insect. Bioassay of alpha-pinene will be done to assess the attraction of the male CMB. This will be the basis for the development of pheromone impregnated lures for CMB monitoring and control.

Session/symposium: Practical applications/Semiochemical application for invasive species
ORAL PRESENTATION

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Chemical ecology of plant- aphid vector-virus interactions: a case of manipulation?

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Viruses induce changes in plant metabolism that can further affect behaviour and physiology of plant pathogen-transmitting aphids. In this project, we compared the secondary metabolite profiles of infected Brassicaceae with those from uninfected plants. We then analysed the effects of these modifications on insect vectors by studying aphid physiology, feeding behaviour, and aphid's colonization of a plant. The present study was conducted using the aphid-transmitted polerovirus Turnip yellows virus (TuYV), two Brassicaceae (Arabidopsis thaliana and Camelina sativa) and the polyphagous aphid Myzus persicae. TuYV infection induced a different composition of headspace volatiles of C. sativa but not of A. thaliana plants. In contrast, secondary metabolites in leaves clearly differed between infected and uninfected plants of both species. Interestingly, phloem sap ingestion and development of M. persicae were negatively affected on infected A. thaliana, whereas these characteristics were enhanced on infected C. sativa plants. Not surprisingly then, aphid colonization was less persistent on infected A. thaliana than on infected C. sativa plants. We hypothesize that these contrasting findings strongly affect virus dissemination by aphid vectors on different host plants. In conclusion, within the Brassicaceae family, virus effects on plant biochemistry can affect aphids differentially. Viral manipulation of vector behaviour could therefore be dependent not only on the virus mode of transmission, which is commonly assumed, but also on the plant species; indeed, even phylogenetically closely-related plant species differed in their chemical response to the same virus infection.

Session/symposium: Interspecific relationships/independent presentation ORAL PRESENTATION

Amir D., David R., Yakir E., Bohbot J.D.\*

# The puzzling role of mosquito host attractants: the case of sulcatone

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Almost a century after the identification of the first mosquito attractants, the equivalent of a moth pheromone lure is still wanting. One of the reasons for this situation is due to our poor understanding of the ecological context of known mosquito attractants. The few volatile organic compounds identified so far are present in most ecological niches visited by mosquitoes, yet they have been mainly understood in the context of female host-seeking. We illustrate the ubiquity of mosquito attractants by exploring the pharmacological relationship between the animal-host attractant sulcatone and the odorant receptor 4 from blood and nectar feeding mosquito species. Our findings suggest that mosquito odorant receptors operate in contexts other than animal-host-seeking. From this analysis, emerges a confounding yet exciting perspective regarding mosquito chemical ecology, which is congruent with the idea that known mosquito attractants are pleiotropic signals.

Session/symposium: Interspecific relationships/Arthropod chemoreceptors
ORAL PRESENTATION

Ammagarahalli B.\*, Layne J.E., Rollmann S.M.

Changes to the peripheral olfactory system of *Drosophila mojavensis* with shifts in host plant use

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Heterogeneous host plant distribution, and adaptations to these can present reproductive barriers to phytophagous insects. In such conditions differences in host plant use can lead to population divergence. The desert dwelling fly, *Drosophila mojavensis* is a model in which the proximate mechanisms underlying population divergence can be examined. Four populations each utilizing a specific host cactus are found across SW United States and NW Mexico. Host finding is guided by peripheral olfactory detection of distinct volatile profiles emitted during the fermentation of each cactus host. Here, we show how differences in odorants emit from cactus host plants are detected by two of the populations, each using a distinct cactus host. Specifically, response profiles of antennal olfactory receptor neurons to a suite of odorants were characterized using single sensillum recordings. Spike counts were found to be different between populations and sexes. Our findings suggest changes in the peripheral olfactory system responses can accompany shifts in host plant use.

Session/symposium: Interspecific relationships/independent presentation

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Small hive beetle, Aethina tumida: potential chemical mimicry of a honeybee pest

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The small hive beetle, Aethina tumida Murray (Coleoptera: Nitidulidae), is a pest of the European honeybee, Apis mellifera L. (Hymenoptera: Apidae). Aethina tumida distribution among A. mellifera colonies has been reported to be non-random with colony phenotype not influencing infestation levels. Aethina tumida is reported as demonstrating aggregative behaviour within host colonies and as part of mating behaviour. We found that adult A. tumida tend to cluster in groups even after bilateral antennectomy and hypothesise that CHCs may have a role in aggregation in this species. We collected hexane wash extracts of beetles reared in a laboratory culture and from A. mellifera colonies in three apiaries in Queensland and New South Wales, Australia. Through Gas Chromatography Mass Spectrometry (GCMS) we found that these extracts differed significantly, even between apiaries. We hypothesise that because adults spend most of their life inside A. mellifera hives they acquire a more complex and hive odour-derived cuticular profile. This may have a role in allowing them to remain undetected by their hosts and in the trophollaxis that occurs in these species which has been principally attributed to behavioural mimicry. The intraspecific interactions in A. tumida and the compounds which mediate them may help develop more targeted control strategies for this pest. This work also more broadly contributes to our understanding of social insect parasites and adaptive chemical mimicry.

Session/symposium: Interspecific relationships/independent presentation

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Northward expansion of the European corn borer *Ostrinia nubilalis* in Scandinavia

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Climate change causes range shifts in many plants and animals. During the last decades, cultivation of some crops has rapidly expanded northward. With the crops follow associated diseases and insects causing damage, if they can adapt to new environments. The agricultural sector calls for more research to be able to monitor, predict, and manage these new situations. Maize production has increased rapidly in Sweden, and in 2010 the European corn borer (ECB), Ostrinia nubilalis, maize host-race (Z pheromone strain) was first documented in this country [1]. To determine the occurrence and phenology of the two host races (or pheromone strains E and Z) of ECB in Sweden and Denmark we used pheromone traps and genetic markers to verify strain identity of catches and collected larvae. During 2013-2017 traps baited with E11- and Z11-tetradecenyl acetate ratios mimicking the sex pheromone blend of the two host-races and hybrids were placed in maize fields in Denmark and southern Sweden. The host-race identity of trapped males and larvae collected from maize stalks was determined by using Restriction Fragment Length Polymorphism [2]. Genetic analyses confirmed the strain specificity of the lures used. We caught similar numbers of Z- and Estrain males in traps. Catches were low and did not increase during the study period, indicating an early stage of population establishment in these areas. We conclude that the maize-ECB system in Scandinavia offers an excellent opportunity to study the spread of recently established populations of a pest in relation to climate change and host abundance. Some remaining questions: Is the E-strain also expanding? What are the E-strain individuals doing around maize fields? What is the pressure from natural enemies compared to more southern locations?

- [1] Lehmhus et al. (2012) J Kulturpfl. 64: 163-167.
- [2] Coates B.S. et al. (2013) Ecol. Evol. 3: 249-2470.

Session/symposium: Practical applications/Semiochemica application for invasive species

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The narrowly tuned odorant receptor *RferOR5519* and antenna-specific odorant binding protein *RferOBP1768* are highly selective for ferrugineol, the major aggregation pheromone compound of red palm weevil

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The Red Palm Weevil (RPW) Rhynchophorus ferrugineus (Olivier), is the most devastating quarantine pest of palm trees worldwide, invaded the Middle Eastern countries in the mid-1980s and has caused havoc with date plantations ever since. Palm weevil infestation is mediated primarily by the male aggregation pheromone (4RS,5RS)-4methylnonan-5-ol (ferrugineol), other weevil within the vicinity are attracted to the signal, which often leads to a coordinated mass attack. Because of the global threat and economic impacts of this pest, we selected it for study to obtain more extensive knowledge regarding its olfactory communication. We focus on the identification of ferrugineol-specific odorant receptors (ORs) and odorant binding proteins (OBPs). We constructed libraries of all ORs and OBPs and selected antenna-specific and highly expressing candidates for silencing through RNA interference. Aggregation pheromone compounds, ferrugineol and 4-methyl-5nonanone (ferruginone), and a kairomone, ethyl acetate, were sequentially presented to individual RPWs. The results showed that antenna-specific RferOBP1768 aids in the capture and transport of ferrugineol to ORs. Using behavioural trials and EAG recordings, we screened entire set of ORs and results revealed that ferrugineol activated only 1 receptor, RferOR5519, suggesting a unique role of RferOR5519 for the most sensitive detection of the major aggregation pheromone compound. It is worth mentioning that RferOR5519 highly sensitive to ferrugineol, not responded to ferruginone and ethyl acetate, indicating that RferOR5519 is a narrowly tuned receptor. It has also been shown that silencing of the RferOBP1768 and RferOR5519 expression as a result of dsRNA treatment alters RPW behavior. Our findings on R. ferrugineus ferrugineol specific OBP and OR holds great promise for the development of olfactory biosensors.

Session/symposium: Interspecific relationships/Arthropod chemoreceptors Oral Presentation

Antwi-Agyakwa A.K.<sup>1,2</sup>, Fombong A.T.<sup>1</sup>, Yusuf A.A.<sup>2</sup>, Pirk C.<sup>2</sup>, Deletre E.<sup>1,3</sup>, Torto B.<sup>1,2</sup> **Behavioural responses of African Citrus Triozid** *Trioza erytreae* **to odours from its common host plant** *Citrus jambhiri* – an evidence of host finding using olfaction

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Trioza erytreae Del Guercio (Hemiptera: Triozidae) is one of the primary vectors of Candidatus Liberibacter spp. the bacteria that causes citrus greening, a disease of economic importance in global citrus production. Management of T. erytreae using conventional insecticides has not been effective prompting the need for alternative management strategies. Using behavioural assays, we tested the behaviour of T. erytreae at different ages to odours from the headspace of intact host plant (Citrus jambhiri, rough lemon) and of different leaf textures and eluates. Attraction to host plant odours were influenced by age, are sex specific and dose dependent. Insects were more responsive at day 14, more females attracted than males and 80 Leaf Hour Equivalent (LHE) eliciting better response in T. erytreae than 40 LHE and 160 LHE respectively. Both flushing and mature leaves though unique in compositional quantity and quality elicited similar responses in males and females. This result suggests that olfactory cues may be responsible for host plant location in T. erytreae. The implications of these findings in the development of monitoring and management tools for this pest are discussed.

Session/symposium: Interspecific relationships/independent presentation

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# Diabrotica virgifera females use maize plant benzoxazinoids to protect their eggs against predators

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Various herbivorous insects sequester defensive secondary metabolites from their host plant and use them against their natural enemies. While these effects are well documented for insect larvae and adults, it is still rarely shown that sequestered metabolites can be transferred to eggs as a defensive mechanism. We studied this for benzoxazinoids (BX), the main chemical defense compounds of maize plant, which are known to be sequestered by larvae of the western corn rootworm, Diabrotica virgifera. Chemical analyses revealed that maize-fed D. virgifera beetles also transfer BX to the eggs, which was not the case for non-BX-sequestering Diabrotica balteata beetles. The BX profiles of the eggs were mainly composed of DIMBOA-Glc and HMBOA-Glc, followed by HDMBOA-Glc and MBOA-Glc. To test whether these toxins protect eggs against predators, we fed D. virgifera beetles with mutant maize plants impaired in BX production or their BX-producing isogenic counterparts, in order to obtain BX-free and BX-containing eggs. These eggs were offered to the rove beetle Atheta coriaria and the minute pirate bug Orius laevigatus. In choice and no-choice experiments, both predators consumed 2 to 3-times more BX-free eggs than BX-containing eggs. Moreover, in survival assays we confirmed the deleterious effects of BX-containing eggs on the predators. When fed on BX-free eggs instead of BX-containing eggs A. coriaria showed 30% increased survivorship and its mortality started 7 days later. Our results provide new insights into how beetles like D. virgifera use plant defensive chemicals against higher trophic levels, which may in part explain the extraordinary success of this invasive pest.

Session/symposium: Interspecific relationships/independent presentation ORAL PRESENTATION

Austel N.1\*, Böttcher C.2, Meiners T.2

Non-targeted metabolome profiling of green flower buds in oilseed rape: screening for resistance against the pollen beetle

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The pollen beetle (*Meligethes aeneus* F.; Coleoptera: Nitidulidae) is one of the major insect pests of oilseed rape (*Brassica napus* L.; Brassicaceae), with the potential of causing significant reductions in seed yield. Since pollen beetles become increasingly resistant to pyrethroids, alternative control strategies within the framework of integrated pest management are needed to reduce the use of insecticides and the undesirable selection of beetles for insecticide resistance. One strategy is to use the natural variation in brassicaceous plants species to identify potential chemical resistance parameters that enable plant breeders to enhance the resistance of oilseed rape against adult pollen beetles.

In this project we have i) screened *B. napus* cultivars and related brassicaceous plant species for their effect on the feeding behaviour of adult *M. aeneus* and ii) analysed the metabolome profiles of green flower buds by liquid chromatography electrospray ionisation time-of-flight mass spectrometry.

The feeding response of the pollen beetle was dependent on sex and plant species. Males discriminated stronger between plant cultivars and species than females. The beetles preferred plants closely related to *B. napus* over distantly related ones like *Sinapis alba*, *Eruca sativa* or *Barabarea vulgaris*. To identify candidate compounds as potential chemical resistance parameters, we correlated metabolome profiles and beetle feeding behaviour. Positive and negative correlations of plant compounds with the beetles feeding behaviour have been detected. The metabolome profiles were plant species and variety-specific. Oilseed rape varieties could also be clearly separated statistically. Statistical models resulted in a candidate list for metabolite identification. Non-targeted metabolome profiling can be a first step to unravel plant resistance traits via a chemical ecology approach.

Session/symposium: Interspecific relationships/Metabolomics approaches in the

Brassicaceae

**ORAL PRESENTATION** 

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# Effect of climate change on plant fatty acid and phenolic compounds

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Environmental changes influenced by climate change can directly and indirectly affect the biochemical constituents in different woody plants because the plants might recognize environmental changes as a stressor factor. In order to demonstrate possible interactions between climate change factors and physiochemical substance changes, this study focused on identifying the key bioactive components and demonstrating the endogenous compoundsclimate change interactions. Trachelospermum asiaticum, Hedera rhombea and Ilex cornutathree major species in southern ecosystems that can exert important effects on the fluctuations of different phenolic compounds and fatty acid volatile profiles under environmental conditions-were examined. A comparative analysis of phenolic compounds and fatty acids in the mature leaves and stems was performed using gas chromatography, a flame ionization detector, and Fourier-transform infrared spectrometry. Saturated fatty acids were abundant in foliar than in stems, although the amounts of fatty acids was low, while similar phenol content values were found in the three species. Regarding the relationship between fatty acids and phenolic compounds with climate change, finding suggest that the plant species' resistance to changeable environmental conditions depends not only on the fatty acid and phenolic compound contents but also on the individual phenol or fatty acid profile and ratio.

Session/symposium: Interspecific relationships/independent presentation

Bak A., Patton M.F., Muriki M., Henao L.M.P., Casteel C.L.\* Ethylene signaling mediates Potyvirus spread by aphid vectors.

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Studies have demonstrated insect vectors have higher fecundity and preferentially settle on potyvirus-infected plants compared to controls. Previously, we demonstrated that potyvirus infection increases plant ethylene production suggesting a potential role of ethylene in plant-virus-insect interactions. We report here the effect of ethylene and virus-induced changes in ethylene signaling on vector attraction to infected plants. We (1) surveyed aphid and virus incidence in the field and (2) measured aphid settlement and ethylene production in the lab using ethylene signaling inducers (Ethaphon), ethylene inhibitors (aminoethoxyvinylglycine (AVG) and 1-Methylcyclopropene (1-MCP)) and Arabidopsis mutants to study the role of ethylene signaling in these interactions. Potyvirus-infected plants were colonized by greater numbers of aphid vectors in the field and lab and ethylene induction mediated increased aphid attraction to host plants. Furthermore, aphid attraction to virus-infected plants and virus spread depends on a functioning ethylene signaling pathway. Decreased aphid attraction to virus-infected plants and decreased virus spread upon ethylene inhibition suggests ethylene biosynthesis is important for transmission dynamics and may represent an alternative control target in the future.

Session/symposium: Interspecific relationships/Insect-microbe interactions
ORAL PRESENTATION

Baker T.C.\*, Myrick A.J.

Feeding, mating, and flight dispersal behavior of the invasive pest species, *Lycorma delicatula* (Hemiptera: Fulgoridae)

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The spotted lanternfly, Lycorma delicatula, is an invasive fulgorid bug species that was recently accidentally introduced into North America, likely from indigenous populations in China. The first record of this species in the United States occurred in September, 2014 in Berks County, Pennsylvania. This incipient pest of vineyards, fruit orchards, and hardwood forests of the U.S. has now spread from a quarantine zone in a small portion of one county in eastern Pennsylvania in 2015 to now comprise in 2018 over 6,900 square miles of infestation, with recent detections reported also in two neighboring states - Delaware and New York. There is great concern of this species' certain continued spread over a broader geographic area in North America if it cannot be controlled. In 2017 we saw a progression of adult behaviors that culminated in a mass flight dispersal of thousands of adults followed by mating and egg laying. After one to two weeks of intense early-season feeding, short flights occurred indiscriminately from the foliage of one tree to another or between bushes, trees and vines. At the peak of adult population abundance in 2017 these flights became more prevalent and lengthier, with adults of both sexes observed to launch themselves into the wind from all types of host and non-host trees or from porches, posts and other human-made structures. The fairly straight-line level or gradually descending flight trajectories allowed the flying adults to traverse only usually 30 to 80 meters of ground in one episode, showing no preference for landing on any particular species of plant or inanimate object. They would then crawl up a nearby vertical object and launch themselves into flight again. Thousands of adult L. delicatula undertook such flights together on any day, creating massive swarms in local areas. We developed a novel technique to study the in-flight wind-orientation of adults during their flight dispersal bouts.

Session/symposium: *Practical applications/independent presentation*ORAL PRESENTATION

Barcellos Franca P.H.<sup>1\*</sup>, Triana M.F.<sup>1</sup>, Goulart H.F.<sup>2</sup>, Sant'Ana A.E.G.<sup>2</sup> **Identification of major components from the sex pheromone of a sugarcane borer,** *Hyponeuma taltula* 

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Hyponeuma taltula (Schaus) (Lep. Herminiinae) is a pest that attacks sugarcane and it is widely distributed in all Brazilian regions where the sugarcane culture is prevalent. This species has been long known for its damage to sugarcane, whose evident symptom is referred to as dead heart. Some growing concern about H. taltula has arisen over the past couple of decades due to the recent increase in relative importance of the economic losses in some locations. Because environmental issues led to the adoption of mechanical procedures for harvesting and to the prohibition on the burning of sugarcane straw, greater population densities of several soil-dwelling pests have been observed. In order to contribute to the integrated pest management of H. taltula, our group has been pursuing the identification of the components from the sex pheromone of this species. In this work, the abdominal glands of H. taltula were excised from virgin females in calling behavior and were extracted with hexane. The hexane extract was then analyzed by gas chromatography and identification of compounds was conducted by individual analyses of the mass spectra and compared with KI's from reported compounds and reference standards. The electroantennographic response of male antennae to compounds in pheromone gland extracts showed three significant active signals. Two compounds, Z3,Z6,Z9-19:Hy and one epoxide from Z3,Z6,Z9-21:Hy were identified by the aforementioned methods and their structures were confirmed by synthesizing the corresponding molecules. The absolute configuration of the active epoxide was established by using a standard. With these results, we aim to assess the bioactivity of both components in field tests as well as to identify the third minor component of the sex pheromone.

Session/symposium: New chemical structures/independent presentation

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Soil application of filtrates and distilled extracts from cultures of fungal phytopathogens enhance yield of pepper (*Capsicum annuum* L.) plants

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Microbes synthesize a multitude of substances including carbohydrates, proteins, lipids, amino acids, hormones, volatiles, etc., which regulate plant growth and morphogenesis. Irrigation with culture filtrates (CFs) of some plant beneficial microorganisms promotes plant growth and enhances yield. However, nothing is known about the response of plants to CFs of microbial phytopathogens. In this work we explored the response of two pepper (Capsicum annuum L.) cultivars (i.e. Sweet Italian and Piquillo) to CFs of the beneficial fungus (i.e. Trichoderma harzianum) and two fungal phytopathogens (i.e. Alternaria alternata and Penicillium aurantiogriseum). We found that irrigation with CFs of the three fungal species promotes root growth and increased yield under greenhouse and open field conditions. This response was associated with changes in the membrane potential and intracellular pH of root cells. Compositional analyses revealed the presence of hormones and volatile organic compounds (VOCs) in the fungal CFs. To investigate the contribution of VOCs to the response of pepper plants to fungal CFs, we characterized plants irrigated with extracts obtained by CFs distillation. Notably, the response of plants to irrigation with CFs distillates was comparable to that of plants irrigated with complete CFs. Our findings provide strong evidence that application to soil of volatile compounds emitted by microbial phytopathogens represents a potentially very useful strategy for increasing yield of horticultural crops in a sustainable and environmentally friendly way.

Keywords: Yield, hormone, culture filtrate, plant pathogens, root membrane potential, biostimulants, microbial volatiles, fruit quality

Session/symposium: Interspecific relationships/Volatile-mediated microbe-plant interactions ORAL PRESENTATION

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Bazok R. 1\*, Tóth M. 2, Lemic D. 1, Cacija M. 1, Viric Gasparic H. 1, Resic I. 1, Drmic Z. 1 First area-wide pest control program carried out in Europe by the use of aggregation pheromones: lessons learnt

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Area-Wide Pest Management (AWPM) is a form of Integrated Pest Management (IPM) that aims to reduce pests in a particular area underneath those numbers that can cause damage. Among many tools available to be used in AWPM, pheromones are very efficient. In four year field study (2012-2015) carried out in east Croatia on an area of 6 and 14.8 km2 we attempted to reduce the population of sugar beet weevil (SBW) (Bothynoderes punctiventris Germar) on overwintering sites (i.e. "donor" fields) thus reducing the need for insecticide treatment on the newly sown sugar beet fields. In 2017 and 2018 mass trapping has been performed only on the "donor" sugar beet fields neighboring the new sugar beet fields chosen to be a part of the study. The CSALOMON® TAL traps (modified pitfall traps) baited with aggregation pheromones for sugar beet weevil were placed at a density of 15 pheromone traps/ha. Additionally to mass trapping, in the mass trapping area (MTA) insecticides were applied if pest population reached the thresholds. Depending on the year, the mass trapping of SBW on the "donor" sugar beet fields reduced the population from 0.73 to 11.59%. Comparing to the fields outside the MTA, the number of insecticide applications and the amount of active ingredient per ha were 3.5-4 times greater on the fields outside the MTA compared to the fields inside the MTA. The study confirmed that AWPM programs are feasible to carry out in larger area. It may not be possible to state that trapping alone will reduce the SBW population below the economic threshold level. However, the data do suggest that trapping can play an important role in the reduction of insecticide applications and in creating an IPM plan for dealing with SBW under similar circumstances. AWPM are connected with specific problems related to the organizational issues, participation of the farmers and to handling the traps after their use.

Session/symposium: Practical applications/independent presentation

KEYNOTE LECTURE

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Coral anti-pathogen chemical defense: does seaweed dominance suppress coral resistance?

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Coral reefs are in precipitous global decline with seaweeds replacing corals. Seaweeds have been hypothesized to suppress reef recovery by shifting coral microbiomes from mutualistic to pathogenic microbes. Seaweeds, or reef degradation in general, could also reduce coral chemical defense against pathogens via altering coral physiology or microbiomes. We assessed this hypothesis by evaluating the potency of extracts from coral mucus and surficial layers against the coral pathogen Vibrio coralliilyticus using three corals (Porites cylindrica, Pocillopora damicornis, and Acropora millepora) collected from three pairs of coral-dominated no-take marine protected areas (MPAs) and adjacent seaweeddominated fished reefs in Fiji. P. cylindrical extracts suppressed the pathogen by 35-90+%, depending on temperature and density of initial pathogen inoculum, but activity did not differ between corals from MPA and fished areas. P. damicornis extracts had minimal effects on the pathogen. A. millepora extracts suppressed the pathogen by 20-85% with potency being significantly greater if corals were from healthy reefs in the MPAs. Microbiome community composition differed strongly among coral species but did not differ within species as a function of collection from the MPAs versus fished areas; however, microbiome dispersion (variance) was greater for A. millepora from the seaweed-dominated areas than from the MPAs. Reciprocally transplanting A. millepora between MPAs and fished areas demonstrated that chemical defense was upregulated within 28 days of moving corals into MPAs. Thus, some corals harbor chemical defenses against a common coral pathogen and, for some species, these defenses are compromised in areas of seaweed dominance. Bioassay-guided separations are being conducted to isolate and identify the compounds involved.

Session/symposium: Interspecific relationships/independent presentation ORAL PRESENTATION

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Uncoupling pollinator attraction and post-pollination mate selection in *Nicotiana* attenuata

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Many studies have shown that a large number of plant species prefer pollen of certain genotypes over others, i.e. mate selection. So far, most of these studies focused on the mechanisms of mate selection after pollen deposition (post-pollination selection), thereby neglecting that the attraction of pollinators could be already an important component of this process. It's known that different floral visitors vary in their response to certain floral traits the plant uses to allure its pollinators. Due to huge variations in floral traits between different *Nicotiana attenuata* genotypes within a population, plants attract pollinators differently and thus influence what kind of conspecific pollen they receive (pre-pollination selection). As a result, this affects which pollen mates are available for selection to set seeds.

In this study we combine both pre- and post-pollination selection, to investigate the importance of pollinator attraction in the process of mate choice in plants. Pairs of selecting (EV, empty vector control) and non-selecting plants (ACO, silenced in post-pollination ethylene burst) were planted in a population of 4 native accessions that vary in floral traits important for pollinator attraction. Seeds produced after pollinator visitation and hand-pollination were used for genotyping to uncouple which pollen was brought by pollinators (ACO) and which pollen was selected by the plant (EV). While capsules visited by day pollinators didn't show outcrossing, capsules pollinated by the hawkmoth *Manduca sexta* contained seeds set by pollen of all 4 accessions but varied in their proportions. Preliminary data show that capsules from ACO plants sired most seeds from pollen of the native accession with the highest scent emission, which suggests that adapting floral traits to favor a certain pollinator could entail a higher diversity of conspecific pollen that is available for the plant's post-pollination mate selection.

Session/symposium: Intraspecific relationships/independent presentation ORAL PRESENTATION

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The male-produced sex pheromone of *Tibraca limbativentris* revisited: absolute configurations of zingiberenol stereoisomers

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The rice stalk stink bug, *Tibraca limbativentris*, is an important rice pest in Brazil with a high invasive potential in Mexico and USA. Its control in Brazil is based heavily on insecticide applications; however, there is no efficient monitoring method. The sexpheromone of this species was identified previously as a combination of two stereoisomers of 1,10-bisaboladien-3-ol (a.k.a. zingiberenol), but the absolute configuration of these sesquiterpenoids were not determined. Therefore, the aim of this study was two-fold: to identify the absolute configurations of the two isomers of 1,10-bisaboladien-3-ol produced by males, and to evaluate the influence of these isomers on conspecific females behaviour in laboratory. The GC analyses of air-entrainment samples from males, and all possible stereoisomers of 1,10-bisaboladien-3-ol on two chiral columns, proved that males produce (3S,6S,7R)-1,10-bisaboladien-3-ol and (3R,6S,7R)-1,10-bisaboladien-3-ol in a 7:3 ratio. The Y-tube olfactomenter bioassays showed that the major isomer (3S,6S,7R) was essential in the attraction of females, but the minor component (3R,6S,7R) was not different from control, nor did it show synergistic/antagonistic effects in females attraction. The other isomers were evaluated as binary mixtures and did not attract females.

Session/symposium: Practical applications/Natural products for integrated pest management

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Blažytė-Čereškienė L. 1\*, Apšegaitė V. 1, Mozūraitis R. 1,2, Būda V. 1 New compound in chemical interaction: *Ips typhographus* and *Picea abies* 

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Bark beetle *Ips typographus* is one of the most economically important pests of Norway spruce (*Picea abies*). It is now well known, that the olfactory system of the beetle responds to several host-tree odours. However, the role of these compounds for host attraction is not completely understood. Our objective was to identify host-plant volatiles that were EAD-active and to evaluate the behavioural activity of the beetles to these compounds under laboratory conditions.

GC-EAD analysis of spruce tree bark extract revealed the presence of several EAD-active compounds. Among these the new compound with consistent activity was revealed. The compound was identified by GC-MS.

The analysis of dose-EAG response to identified compound demonstrated that both males and females of *I. typographus* responded to the lowest dose (0.01  $\mu$ L) tested. EAG responses increased with increasing doses (0.01  $\mu$ L – 100  $\mu$ L) and did not differ between males and females. Behavioural assay in Y-tube olfactometer revealed that this compound was repellent to females at lower dose (0.1  $\mu$ L) comparing to males (1  $\mu$ L).

Results of this study suggest that the compound, which affects spruce bark beetle behaviour under laboratory conditions, might have a role in spruce tree defence and could be suitable for choosing host-tree by the beetle pest.

Session/symposium: Interspecific relationships/independent presentation

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Spatiotemporal heterogeneity of phenolics within a population of black poplar trees

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The distribution of secondary metabolites in trees is heterogeneous in space and time and often strongly dependent on the season. Due to the importance of some secondary metabolites in anti-herbivore and antimicrobial tree defense, their spatiotemporal patterns may also strongly affect higher trophic levels and *vice versa*. We investigated the annual trajectories of secondary metabolites in different tissues of 20 mature black poplar (*Populus nigra*) trees growing in a natural floodplain population. Major phenolic classes in black poplar, salicinoids and condensed tannins, increase in the course of the vegetation period, while phenolic acids and flavonol glycosides and declined. Salicinoid concentrations were higher in the lower canopy and generally increased along the shoot axis. Correlation analyses suggest that the interaction of black poplar trees with insect herbivores and fungal pathogens is strongly affected by the spatiotemporal distribution patterns of phenolic compounds.

Session/symposium: Interspecific relationships/independent presentation

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# 3,6-Dialkyldihydro-2H-pyran-2,4(3H)-diones: new pollinator attractants in Australian orchids

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Sexual deception is becoming one of the most well biologically characterised pollination systems. Yet, relatively few studies have combined chemical investigations with behavioural bioassays to directly link specific chemicals with a biological function. Commonly, it is assumed that electrophysiologically active compounds, or compounds known to be active in other pollination systems, are the key components. However, when detailed studies have been executed, combining organic- and analytical chemistry with field bioassays, novel natural products have often been discovered.

Here we present a new semiochemical class, active as attractants for thynnine wasp pollinators of Western Australian hammer- and spider orchids. In previous work, we identified several hydroxymethyl-pyrazines as EAD-active compounds in floral extracts of *Drakaea livida* and *D. micrantha*. Field bioassays, however, failed to demonstrate any pollinator attraction to these pyrazine compounds on their own.

Subsequently, we conducted a field-based bioassay-guided fractionation with semi-preparative GC. This methodology ultimately provided fractions containing single compounds that were active with the previously identified hydroxymethyl-pyrazines. Lacking sufficient quantities for NMR-analysis, GC-MS was used as the sole identification method for the isolated compounds.

After synthesis of a set of candidates, we identified the two new attractive compounds as 3-ethyl-6-propyldihydro-2H-pyran-2,4(3H)-dione and 3-methyl-6-(2-methylbutyl)dihydro-2H-pyran-2,4(3H)-dione. Determination of the absolute configurations of both compounds is ongoing.

Session/symposium: New chemical structures/independent presentation

**ORAL PRESENTATION** 

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Toxin composition of adult common toads  $(Bufo\ bufo)$  in natural, agricultural and urban habitats

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Human-induced environmental change, including habitat destruction and chemical pollution, is a ubiquitous threat to wildlife, especially to amphibians, the globally most threatened group of vertebrates. Many amphibians use defensive chemicals for protection from predators and diseases, yet we know little about the effects of human-induced habitat alterations on this important fitness component. We studied the defensive toxins (bufadienolides) of common toads in natural, agricultural and urban habitats in the spawning season. We took samples from the parotoid glands of 168 adult toads from 9 ponds (3 ponds per habitat type) and quantified their bufadienolides by HPLC-DAD-ESI-MS using C18 column and water-acetonitrile gradient. We detected 31 bufadienolide compounds, 20 of which were bufotoxins (m/z>700) and 11 were bufagenins (m/z<500). Toxin composition varied with sex and habitat type in a complex way. The amount of most compounds differed between sexes, males having larger amounts of compounds with the shortest (3-4 min) and longest (10-14 min) retention times and females having larger amounts of compounds with medium retention times (4-10 min) in HPLC. Toads in urban habitats had higher amounts of several bufagenins but lower amounts of several bufotoxins than toads in natural habitats. The relative amounts of some bufotoxins also differed between agricultural and natural habitats. Some of the habitat differences were sex-specific, urban males having higher amounts of some bufagenins and urban females having higher amounts of some bufotoxins. These results suggest that agricultural and urban land use influences the chemical defence of toads, possibly via adaptive phenotypic plasticity. Increased production of bufagenins in urban habitats, especially by males, may be beneficial for deterring non-specialized predators like dogs, while females may produce bufotoxins with relatively high polarity to protect their eggs especially in polluted habitats.

Session/symposium: Interspecific relationships/Ecology and evolution of toxins in vertebrate animals

**ORAL PRESENTATION** 

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Borrero-Echeverry F.<sup>1</sup>\*, Bengtsson M.<sup>2</sup>, Witzgall P.<sup>2</sup>

Host plant odour is an integral part of sex pheromone communication in an insect herbivore

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Specific mate recognition (SMR) serves to maintain the integrity of species and contributes towards the formation of premating isolation between diverging populations. In many animals SMR strongly relies on chemical signals. Moths, in particular, due to their nocturnal habits, strongly rely on odours to find host plants, using host plant volatiles (HPVs), and mates, using female produced pheromones. Pheromones have traditionally been considered to be the key component of SMR in moths; however, under natural conditions pheromones are immersed in a background of environmental odours. They are, accordingly, perceived simultaneously by males and the integration of these two distinct signals may play a role in the development of specific mate recognition systems and premating isolation barriers. Using authentic and synthetic stimuli, we show that cotton leafworm males (Spodoptera littoralis) perceive and encode female-produced sex pheromone and host plant volatiles as a unit, rather than as separate messages. Deviations from either the optimal pheromone or plant volatile blend composition deteriorates male response. Attraction towards the incomplete synthetic pheromone was diminished when blended with synthetic and plant produced host volatiles. Similarly, attraction towards female produced sex pheromone of a sibling species was also reduced when blended with host odours. Male attraction towards the complete synthetic pheromone, or conspecific female produced pheromone was not reduced or increased by HPVs from healthy plants, but was reduced by HPVs from plants with damage cause by herbivory. Our results suggest that specific mate recognition in moths is made up of sex pheromones and host plant volatiles, rather than just sex pheromones. As such, changes in female host preference, or pheromone biosynthesis could more easily break SMR and lead to the reinforcement of premating barriers between diverging populations.

Session/symposium: Interspecific relationships/independent presentation ORAL PRESENTATION

Bouwer M.C.<sup>1,2</sup>\*, Slippers B.<sup>3</sup>, Wingfield M.J.<sup>1</sup>, Rohwer E.R.<sup>2</sup>

Mass trapping of the Eucalyptus cossid moth (*Coryphodema tristis*) in South Africa

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The Eucalyptus cossid moth (*Coryphodema tristis*) is a native insect in South Africa and it has been a pest in non-native *Eucalyptus nitens* plantations for more than 10 years. The larvae cause damage by feeding gregariously in the wood and they weaken trees to such an extent that they snap at the site of infestation during windy periods. Research conducted at the Forestry and Agricultural Biotechnology Institute (FABI) and the Department of Chemistry at the University of Pretoria has resulted in the identification of a pheromone formulation for this pest. The pheromone was subsequently used in several trials to test various variables with the aim of optimizing the success rates of field traps. The South African Pulp and Paper Industry (SAPPI) has initiated a pilot mass trapping trial with the aim of reducing cossid moth numbers in the field. The trial was conducted over a period of two years and included the deployment of 6425 traps in various compartments in the Lothair plantations in Mpumalanga. In this talk the pheromone identification process will be presented together with the preliminary results of some of the field trials.

Session/symposium: Practical applications/independent presentation

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Bozsik G. 1\*, Szőcs G. 1, Seybold S.J. 2, Tröger A. 3, Francke W. 3

Electroantennographic responses of the cypress bark beetle, *Phloeosinus aubei*, to plant volatile components of *Thuja occidentalis* Smaragd

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The invasive cypress bark beetle, Phloeosinus aubei (Coleoptera: Curculionidae, Scolytinae) has become a major pest of ornamental scale-leafed conifer trees (Cupressaceae) in tree nurseries, as well as in urban landscapes in the temperate zone of Europe. One of its most preferred cultivars is the Emerald variety of American arborvitae, Thuja occidentalis Smaragd. Earlier 21 antennally active components had been identified among green leaf / twig volatiles of T. occidentalis Smaragd, and relative antennal responses of P. aubei were recorded to 100 µg doses of synthetic samples. To better understand the sensitivity of males and females to these compounds, dose-response curves were established, using electroantennography (EAG). Emphasis was placed on certain chiral compounds, where responses were compared to the paired enantiomers. While all plant volatiles in the tests elicited significant responses from both male and female antennae at a 100 µg, 12 components (the same in males and females) evoked responses at 10 µg. Seven (from males) and 11 components (from females) elicited responses at a 1 µg dose. (-)-1-Octen-3-ol and (+)-fenchone evoked responses from both sexes at the lowest dose level in the test (100 ng), whereas Z3-hexen-1-ol elicited a response at this dose only from females. (R)-1-Octen-3-ol and (S)-sulcatol elicited higher responses than their enantiomers, whereas responses to the enantiomers of terpinen-4-ol did not differ significantly. Antennal responses to plant volatile collections from bark of healthy as well as of decaying T. occidentalis Smaragd and T. plicata Atrovirens were also compared by GC-EAD. Flight behavioral tests to a reduced number of possible key kairomone components are underway.

Session/symposium: New chemical structures/Invasions under the bark: chemical ecology of woodboring beetles

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Böttinger L.<sup>1</sup>\*, Pfeiffer L.<sup>2</sup>, Hofferberth J.<sup>3</sup>, Ruther J.<sup>2</sup>, Stökl J.<sup>4</sup>

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

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Recently, it was shown that females of the parasitic wasp Leptopilina heterotoma produce a defensive secretion consisting of iridoid compounds, which have also evolved a communicative function as chemical cue to avoid competition between females on host patches and as major component of the female sex pheromone. This supports the so-called precursor hypothesis, which predicts that pheromones can evolve from previously used noncommunicative chemical compounds. To reconstruct the evolution of pheromones in the genus Leptopilina, chemical, behavioral and ecological data of several species are compared in this study. We show that L. ryukyuensis, L. japonica, L. tsushimaensis and L. pacifica also produce iridoids and use them for defense against predators. In L. ryukyuensis these iridoids are also used by females to avoid host patches exploited by conspecific females, while females of L. japonica do not avoid other conspecific females during host search. We further show that the sex pheromone of female L. ryukyuensis consists of cuticular hydrocarbons (CHCs), as males show strong courtship behavior towards these compounds. In contrast, males of L. japonica prefer their females' iridoids but might need the combination of CHCs and iridoids to elicit the full courtship behavior. Our current knowledge suggests that the usage of iridoid compounds as defensive allomones seems to be common in the genus Leptopilina, while the use for competition avoidance and as a sex pheromone only evolved in few species. The phylogenetic relationships of the species correlate with the production of certain iridoid compounds, but fail to explain the strong divergence in the composition of the female sex pheromone in the genus. Therefore, the dispersal behavior of males and females as well as the search strategies for mating partners are currently under investigation in order to gain further insights into the evolutionary forces shaping the composition of the sex pheromones in Leptopilina.

Session/symposium: Interspecific relationships/independent presentation

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#### Bruce T.J.A.

## The chemical world of aphids, their host plants and their enemies

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Interactions between aphids, their host plants and their predators and parasitoids are influenced by chemical cues and signals. Aphids use olfaction to recognise their host plants and avoid non-hosts. Their natural enemies also perceive and respond to blends of plant volatiles but are tuned in to odours induced by their prey. Interestingly, insect responses to a whole blend of plant volatiles (as a mixture) is often stronger than responses to single compounds. Sometimes there can even be the opposite behavioural response to mixtures and single compounds. Thus, the insect nervous system integrates inputs and uses a combinatorial coding system to make sense of scents. There are some taxonomically characteristic plant volatiles that specialist aphids may use to recognise their host plants. For example, isothiocyantes characteristic of brassica plants. However, host recognition through blends of commonly occurring volatiles is more prevalent. Even subtle changes in the ratio of certain key compounds, often those associated with stress or induction of plant defence, such as methyl salicylate, can change responses. We often find the volatile blends that repel herbivores attract their natural enemies. This makes ecological sense because aphids are avoiding plants induced by aphids that arrived earlier whereas the natural enemies are purposefully seeking plants with aphids. Furthermore, insects can change their responses over time. This was clearly shown with transgenic plants engineered to constitutively emit the aphid alarm pheromone, (E)- $\beta$ -farnesene. With short term exposure, aphids were repelled and their parasitoids attracted. With prolonged exposure, aphids were no longer repelled and there was no difference in parasitism levels. The olfactory system of aphids and their enemies is adaptive because it allows them to integrate the spatio-temporal patterns of volatiles and associate them with presence or absence of suitable food resources.

Session/symposium: Interspecific relationships/Semiochemistry of aphidophagous insects Oral presentation

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Impact of benzoxazinoid sequestration by the Western Corn Rootworm on the biocontrol potential of entomopathogenic nematode isolates from Mexico

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The Western Corn Rootworm (WCR) Diabrotica virgifera virgifera LeConte (Coleoptera: Chrysomelidae), is a root-feeding specialist on maize (Zea mays L.). Entomopathogenic nematodes (EPNs) are used as biological control agents against this important pest. Robert et al. (2017, eLife, https://doi.org/10.7554/eLife.29307.001) showed that WCR sequesters benzoxazinoid (BX) chemical defenses and that this increases WCR resistance against a commercial strain of EPN, thereby possibly limiting their efficacy as biological control agents. However, to date, little is known about the impact of BX sequestration on different EPNs, including naturally occurring strains. In the context of a collaborative project, we want to test the biological control potential of EPNs from Mexico, from where WCR originated approx. 1000 years ago, and how they may be affected by WCR BX sequestration. In a first step, we surveyed and screened EPNs from Mexican maize fields and evaluated their virulence against WCR larvae and against the non-BX sequestering D. balteata. Of the five EPN species that we found, Heterorhabditis bacteriophora was by far the most common and different isolates showed substantial variability in causing mortality in WCR larvae, in contrast to the overall very high mortality caused in D. balteata larvae. Currently, we use mutant maize plants impaired in benzoxazinoid production (Maag et al., 2016, The Plant Journal, https://doi.org/10.1111/tpj.13308) to evaluate to what extent BX sequestration by WCR is affecting the ability of the different EPN isolates to kill WCR larvae. These results will increase our understanding of the impact of WCR self-defense on the efficacy of EPNs as biological control agents against WCR.

Session/symposium: Practical applications/independent presentation

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Byers J.A.

Modeling detection, mating disruption, mass trapping, and push-pull management strategies using semiochemicals

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Models of monitoring/detection and mass trapping can be done with individual insects moving in a correlated random walk (CRW) based on only three variables, step size, turning angle, and number of steps. The simulated insects either encounter pheromone traps, opposite sex, or mating aggregations on hosts. The catch on an attractive trap is modelled not as orientation to a source within a plume but equivalently as an effective attraction radius (EAR) which in principle does not change in size regardless of population density. The EAR depends on the trap, pheromone blend, release rate and the species considered. All individuals that encounter the spherical EAR in three dimensions are captured, and for models in two dimensions the EAR is converted to a circular EARc by using the standard deviation of the vertical flight distribution. The EARc can be used in simulations and in encounter rate equations. Mating disruption with competitive attraction and camouflage can be modelled by using the same EAR or EARc and causing an insect encountering an EARc to "wait" a period of time that is equivalent to the time the insect spends "false-trailfollowing". However, this wait period is poorly known. In models of push-pull systems, the mass trapping model (pull) is combined with an inhibition of attraction and landing after encountering an inhibitory radius (push). The inhibition can be imposed for several movement steps after the encounter with the inhibitor. The inhibition part of the push-pull model is also not well understood. The simulation models of these control strategies give approximate estimates of mortality and mating outcomes but the main purpose is a better understanding of the effects of important parameters and processes to provide insight and help in the design of the control methods. Another goal of the models is to suggest an appropriate experimental density of traps (and inhibitors) for a desired level of control.

Session/symposium: Practical applications/Comparison of semiochemical control methods of pest insects

ORAL PRESENTATION

## Capon R.J.

## Cane toad toxin chemical ecology: the untold story

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The cane toad *Rhinella marina*, indigenous to south/middle America, was introduced to major sugar growing regions of the world last century as a biological control for cane beetles. A biocontrol failure, the cane toad went on to become a devastating invasive pest species, particularly in Australia. With an invasion front extending across millions of km2, from Queensland to Western Australia, and south to New South Wales, cane toads are poisoning and killing iconic Australian native predator species, including lizards, snakes, crocodiles and marsupials, as well as domestic pets. Despite a clear and urgent need for action, significant and lasting cane toad control has proved elusive.

In an effort to discover new, ecologically-inspired and sustainable control strategies, we turned our attention to cane toad toxin chemical ecology, to reveal a far more sophisticated and complex system than previously imagined. Through these investigations we came to understand the role of toxin storage and delivery, the existence of pro-toxins and a cosecreted enzyme that mediates their activation, the complementary impact of bacterial biodegradation and biotransformation on cane toad toxins, and perhaps most important of all, the nature of toxins as pheromones. This presentation provides an account of these discoveries, culminating in the development of a tadpole trapping technology that employs a cane toad toxin-derived pheromone to selectively attract cane toad tadpoles into traps for the purpose of eradication. This technology has been patented and licensed for commercial development, and is currently being rolled out under the Cane Toad Challenge, a University of Queensland community engagement and citizen science initiative (canetoadchallenge.org.au).

Session/symposium: Interspecific relationships/Ecology and evolution of toxins in vertebrate animals

KEYNOTE LECTURE

Cardé R.T.\*, Hughes G., Zhang J., Lacey E.

## Pheromone antagonists of Helicoverpa armigera and Helicoverpa zea

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The female-produced pheromones of *Helicoverpa zea*, the corn earworm moth, and *Helicoverpa armigera*, the Old World bollworm moth, are comprised of the same 97:3 mixture of (*Z*)-11- and (*Z*)-9-hexadecenals. For *Helicoverpa armigera* in China, (*Z*)-9-tetradecenal was previously reported to add to the attractivity of the pheromone. Our collaborative field tests in Australia with *H. armigera* confirmed that (*Z*)-9-tetradecenal augments attractivity about 2.5 fold over the two-component pheromone alone. Several related compounds, including (*Z*)-11-hexadecenol, antagonize attraction to pheromone in both species. It has been proposed that the antagonist (*Z*)-11-hexadecenol regulates "optimal" mating in *H. armigera* by delaying male attraction until females are mature. To explore this hypothesis, we have used wind-tunnel studies to establish male sensitivity to antagonists, particularly (*Z*)-11-hexadecenol. Documentation of possible emission of this compound included behavioral observations of calling periodicities in immature and mature females, pheromone gland extractions and SPME wipes, and airborne collections.

Our studies were supported by grants from the USDA-APHIS. Patricia Balzer and Myron Zalucki conducted the field trials in Australia.

Session/symposium: Practical applications/independent presentation

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Efficacy of several repellent molecules on pyrethroid resistant (kdr) and non-resistant *Aedes aegypti* mosquitoes

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The extensive use of pyrethroid insecticides has favored the appearance of widespread insecticide-resistant mosquito populations, thus the reduction on the efficacy of insecticidetreated nets. This fact highlights the need of deploying alternative methods for vectorcontrol, and the use of repellents in combination with insecticides has been proposed. The most common repellent compounds used nowadays are DEET, IR3535, icaridin, PMD and citronellal among others, but their efficacy against mosquitoes is variable, remaining DEET as the gold standard. Knock down resistant (kdr) genotypes have associated one or several mutations on the sodium channels present on neuron membranes. Such amino acid replacements limit the quantity of pyrethroid insecticide that can bind to the cell target, thus conferring mutant individuals the ability to stand longer time without being affected by the insecticide (i.e. kdr). However, besides the presence of higher fitness costs in kdr mutants compared to non-resistant mosquitoes in several life history traits, there is no information on whether there is a difference of the repellent efficacy between the strains. In this study, we investigate the efficacy and exposure costs of the above mention repellents on two strains, sensitive and pyrethroid resistant (kdr), of Aedes aegypti mosquitoes having the same genetic background. Using a dose-response approach on a membrane feeding assay, we determine feeding rates at different concentrations and the potential fitness related costs (i.e. egg laying and survival) after exposure. Preliminary results on feeding rates indicate a repellent specific higher sensitivity of kdr mutants compared to non-resistant individuals. The presence of a differential sensitivity depending on the repellent molecule may bring new insights for the development of alternative control methods as well as on the mode of action of such molecules.

Session/symposium: Practical applications/independent presentation

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Čepulyte R.<sup>1,2</sup>\*, Danquah W.B.<sup>1</sup>, Bruening G.E.<sup>1</sup>, Williamson V.M.<sup>1</sup>
Assessing the response of root-knot nematodes to plant semiochemicals

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Root-knot nematodes (*Meloidogyne* spp.) can parasitize over 2,000 plant species and are generally considered the most damaging and economically important group of plant-parasitic nematodes worldwide. Infective juveniles (J2) are non-feeding and must locate and invade a host before their reserves are depleted, but what attracts them to appropriate entry sites is not known. We show that exudates collected from tomato seedling root tips are highly attractive to *M. javanica* J2. We developed a pluronic gel-based microassay to assess activity of root exudate fractions. Size fractionation chromatography suggests that major active component has a mass of ~400. Root exudates from the legume *Medicago truncatula* were similarly attractive to J2 and fractionated in a similar way to tomato suggesting that a metabolite of the same or similar structure from root tips of both species may be a potent attractant.

Session/symposium: Interspecific relationships/independent presentation

Cha D.H.<sup>1</sup>\*, Landolt P.J.<sup>2</sup>, Loeb G.M.<sup>3</sup> **Discovery of attractants and repellents for managing** *Drosophila suzukii* 

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The spotted wing drosophila (SWD), introduced from Asia, is now a serious pest of numerous soft fruits throughout North America and Europe. Efforts continue to improve monitoring and control methods. A four-component lure comprised of ethanol, acetic acid, acetoin, and methionol was developed and marketed by several companies for trapping SWD. We describe here the multidisciplinary processes used to isolate, identify and develop that lure, and provide specifications for its use as a trap lure. We also discuss the recent progress on the development of specific lure and novel deterrent for managing SWD.

Session/symposium: *Practical applications/Semiochemical application for invasive species*ORAL PRESENTATION

Cha D.H. 1\*, Skabeikis D. 1, Choi M.Y. 2, Vander Meer R.K. 3

Behavioral response of Wasmannia auropunctata to chemical trails laid on epiphytic moss

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The little fire ant (LFA), *Wasmannia auropunctata* Roger (Hymenoptera: Formicidae), is native to the neotropics, but has become one of the world's most widespread and destructive invasive ants. In Hawaii, LFA was first discovered in 1999 on the Big Island and since then it has rapidly spread to neighboring islands, causing ecological and economic damage. LFA can develop fully functional nests on the ground and arboreally. Foraging and retrieval of food resources is facilitated by a well-developed recruitment system. LFA were found to form recruitment trails on epiphytic moss growing on Macadamia nut trees. As a first step to identify LFA recruitment pheromone components, we tested the LFA worker trail-following response to naturally marked epiphytic moss trails and to experimental epiphytic moss trails made with a hexane extract of epiphytic moss trails. LFA workers followed the artificial hexane/moss trail, as they did on the natural trail. In laboratory choice assays, LFA workers preferred to follow a trail drawn with a hexane extract of moss only. Our results confirm that LFA workers readily follow a trail marking substance(s) laid down on epiphytic moss.

Session/symposium: Practical applications/Semiochemical application for invasive species

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## Mixtures are more robust stimuli in olfaction

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In a natural environment, insects typically encounter complex mixtures of odorants, often with numerous super-threshold components. It is an important open question whether and how neuronal encoding of odorant mixtures differs from that of single odorants.

We have built a statistical model of the full receptor repertoire and of the antennal lobe of honey bees. Our model was developed to reproduce a variety of experimentally observed statistics of olfactory response patterns and taking into account biophysical processes, such as receptor binding and activation, and spike generation and transmission. It can predict responses to both, single components and mixtures, and reproduces temporal and spatial features of neuronal odour representations that were not used when building it.

Using simulations and mathematical analysis, we found that the processes of receptor binding and activation alone already lead to significant differences between the responses to mixtures and to single components, long before any neural processing takes place. The model predicts that i) the response latency of olfactory receptor neurons decreases and ii) response patterns become less variable across concentrations with increasing number of chemical components in a mixture. These effects are preserved in the projection neurons. We confirm our predictions for response latencies by single sensillum recordings in *Drosophila* and our predictions on the robustness of odour response patterns across concentrations using calcium imaging in the antennal lobe.

Our results suggest that the olfactory system in insects encodes mixtures more efficiently than single odorants, which resonates well with the observation that chemical signaling in nature predominantly utilizes mixtures.

Acknowledgements

This work was supported by HFSP, RGP0053/2015 and EPSRC, EP/P006094/1.

Session/symposium: Intraspecific relationships/Importance of multicomponent mixtures for chemical cues

**ORAL PRESENTATION** 

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## Chang C.L.

## Responses of adult fruit flies after feeding on a diet with male lure-lufenuron mixture

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Irradiation has been used to sterilize fruit fly males to support Sterile Insect Technique (SIT) for fruit fly control. If one can develop a diet that can attract insects, provide needed nutrients and energy for strong survival, and induce 100% sterility would replace the costly irradiation process. Lufenuron (LFN) has been demonstrated as a fruit fly chemosterilant through a 7 days ingestion. However, it needs an attractant that is competitive to natural fruit odors to attract flies to feed on diets. We incorporated LFN and fruit fly lures to an agar diet to assess the daily response of adults after feeding from day 1. The results showed that egg production was significantly lower than those of control or lures alone diets and egg hatchability was reduced with increasing LFN does. The sterility is almost 100% at optimal does. Fruit flies are extremely active and better survival after 30 d feeding on a diet with LFN and lure mixture although delayed maturity was observed through the fully packed abdomen of both male and female at 8 d old control flies, but not in LFN and lures treated ones. These response after LFN and lures feeding were indications for a successful mass rearing of fruit fly sterile strain without irradiation.

Session/symposium: Practical applications/independent presentation

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The antagonist Z11-16:OH emitted by females regulates optimal mating time in *Helicoverpa armigera* 

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One of the major unanswered questions in sex pheromone communication of moths is why females produce antagonists as components of their pheromone blend. Here we show that the emission of the pheromone antagonist Z11-16:OH by *H. armigera* females regulates the optimum mating time corresponding to the most efficient development of the offspring. At the same time, we identify OR16 as the odorant receptor responsible for detecting Z11-16:OH and thus assessing the best female reproductive status.

The discrepancy between the maximum of calling activity and that of mating success in *H. armigera* suggests the action of sex pheromone antagonists. We first monitored the concentration of pheromone components from female sex glands and found that the concentration of Z11-16:OH follows a trend opposite to that of mating success. We further knocked out the only pheromone receptor (OR16) tuned to Z11-16:OH using genome-editing method CRISPR/Cas9. Electrophysiological recordings indicated that OR16 neurons depleted of OR16 receptor did not respond to Z11-16:OH nor to Z9-14:Ald at any of the tested concentrations, while the same neurons in wild type moths responded to both Z11-16:OH and Z9-14:Ald. Behavioral assays revealed that mutant males were not inhibited to mate with immature females, with the result of producing a limited number of hatching eggs. Taken together, our results show that the emission of Z11-16:OH by females regulates optimum mating time with the advantage of offspring development. We have elucidated the molecular mechanisms used by males to distinguish between immature and mature females, thus preventing them from courting females not ready for mating.

Session/symposium: Intraspecific relationships/Importance of multicomponent mixtures for chemical cues

**ORAL PRESENTATION** 

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## Utilization of naturally occurring compounds in controlling fire ants

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Due to their aggressiveness and poisonous sting, the red imported fire ants, *Solenopsis invicta* Buren, are a significant threat to humans, wildlife, crops, and livestock. Like many other pest insects, management of *S. invicta* heavily depends on the application of synthetic insecticides. There is an increasing desire to use toxicologically and environmentally benign chemicals in pest insect management. Toxins that occur in the nature are believed to be more desirable insecticides than conventional synthetic insecticides, due to their rapid environmental biodegradation and potential lower toxicity to natural enemies, humans and other mammals. In our effort in searching for naturally occurring fire ant toxins, it was found 2-tridecanone and hexyl benzoate are two potent contact toxins against red imported fire ants. Mound treatment formulations were developed using these two compounds. Reported here are results of laboratory toxicity bioassays and field trials. The outstanding efficacy of these formulations against fire ants my warrant further research on their efficacy against other pest in-sects.

Session/symposium: *Practical applications/Semiochemical application for invasive species*ORAL PRESENTATION

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Involvement of maxillary palp in detection of methyl eugenol by male Oriental fruit fly, *Bactrocera dorsalis* (Diptera: Tephritidae)

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The discovery of methyl eugenol (ME) as a potent male attractant for the Oriental fruit fly, Bactrocera dorsalis (Diptera: Tephritidae) has led to its successful use in area-wide fruit fly monitoring and control programmes such as male annihilation. Whilst it is generally acknowledged that the antenna is the primary olfactory organ directly responsible for the male flies' detection of ME, little is known of the maxillary palp involvement although olfactory sensilla are also present in the palp. We seek to ascertain, using behavioural assays if the maxillary palp is also involved in the male fly detection of ME. In assuming the involvement of the male antenna in detecting ME, it was expected that antenna-ablated male and maxillary palp-ablated male will demonstrate none or trace, and strong attraction of those males to ME respectively. Interestingly, we observed that a significant proportion of antenna-ablated males was still attracted to ME and that the sum of total males from that of antenna-ablated and maxillary palp-ablated groups when exposed to ME independently, was similar to that of control males (with intact antennae and palp) attracted to ME. Further, wind tunnel tests showed that maxillary palp-ablated male took significantly longer duration to detect and feed on ME following initial flight and landing on the source containing the lure. These results suggest the possible function of the maxillary palp of the male fly in detecting ME at close range.

Session/symposium: Intraspecific relationships/independent presentation ORAL PRESENTATION

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#### Choe D.H.

## Chemical ecology of Argentine ant and its implication for practical pest management

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Social insects such as ants, honeybees, and termites use a diverse array of pheromones for organization and coordination of all aspects of their colony development and maintenance. In particular, the trail pheromones of ants are known to play critical roles in their foraging and nest relocation activities. The trail pheromone of the Argentine ant has been the focus of numerous studies because of its significance in the species' mass recruitment behavior. Since the original isolation and characterization of (Z)-9-hexadecenal from Argentine ants, the compound has been studied as a trail pheromone component of the Argentine ant. My research group has been exploring the use of (Z)-9-hexadecenal to attract Argentine workers to an area treated with insecticides. This "lure-and-kill" approach would be advantageous over conventional, stand-alone application of insecticides because the insecticide-pheromone mixture will attract foraging ants from nearby trails and even from the nest entrances, maximizing the number of ants exposed to the treatments. The pheromone also facilitates the initial discovery of the insecticidal bait by foraging ants, significantly increasing the overall efficacy of the bait. A recent study with two iridoids produced by Argentine ants during interspecific aggression will be presented and discussed in the context of potential application of the allomone for practical pest management of this invasive ant.

Session/symposium: *Practical applications/Semiochemical application for invasive species*ORAL PRESENTATION

Chrzanowski G.\*, Matok H., Klewek A., Czerniewicz P.

## The aphicidal activity of alkaloid fraction from four medicinal plants

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Secondary metabolites play an important role in plant-insect interactions. Among them, alkaloids are the large group of nitrogen-containing natural compounds. Many of alkaloids with regard to their structure are toxic, and have been proposed as antibiotic/anixenotic compounds or botanical insecticides. The purpose of the present studies was to determine the effect of alkaloid fraction on behavior and development of two aphid species, bird cherry-oat aphid (Rhopalosiphum padi L.) and green peach aphid (Myzus persicae Sulz.). Alkaloids were extracted from Nicotiana tabacum (L.), Juglans regia (L.), Hypericum perforatum (L.) and Sambucus nigra (L.) using 20 % (v/v) methanol acidified with H2SO4 to pH 2.5-3.0. After centrifugation aqueous phase was adjusted to pH 10 with ammonia (25 %) and reextracted with organic phase consisting of diethyl ether and chloroform (4:1, v/v). Alkaloids were evaporated under reduced pressure, dissolved in methanol and subjected to GC-MS chromatography and entomological tests. EPG and choice tests were used for evaluation of feeding behavior and deterrent activity. Moreover, the effect of alkaloids treatment on development of R. padi and M. persicae was examined. Obtained results showed that analyzed group of natural compounds inhibited phloem ingestion by R. padi, and the strongest effects were observed after N. tabacum treatment. Moreover, N. tabacum and J. regia alkaloids showed deterrent activity against R. padi and M. persicae whereas alkaloids from S. nigra and H. perforatum were very weak attractants. Among analyzed plants, the highest toxicity and reduction of aphid development were found after application tobacco and walnut alkaloids. The oligophagous bird cherry-oat aphid was more sensitive to the applied alcaloid extracts than polyphagous green peach aphid.

Session/symposium: Practical applications/Natural products for integrated pest

management
POSTER
number: 128

## Clardy J.

## Fungus-growing ants, molecular diversity, and drug discovery

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Chemical ecology has largely focused on describing the many ways in which organisms exchange information, modulate behaviors, and alter life history trajectories through the exchange of (small) molecules. Aggregated and systematic studies can address broader questions about how the current suite of ecologically relevant molecules evolved or how ecology can aid in drug discovery. For the past decade we have tried to address these more general questions as part of a multidisciplinary team studying the fungus-growing ants of the Neotropics. This lecture will focus on the genetic organization driving a remarkably high level of molecular diversity along with an example in which ecological context led to the discovery of a small molecule with therapeutic potential.

Session/symposium: Interspecific relationships/Insect-microbe interactions
KEYNOTE LECTURE

Cloonan K.\*, Rodriguez-Saona C.

Testing novel attractants for *Drosophila suzukii* 

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In an effort to investigate the lure specificity of the invasive spotted wing drosophila, Drosophila suzukii, we investigated different lure combinations in the laboratory and field. We first tested the following lures: fermentation (acetic acid, acetoin, methionol, and ethanol), yeast (isoamyl acetate and isobutyl acetate), and leaf (β-cyclocitral) for adult attraction in two-choice laboratory assays. We then tested if lures synergize SWD attraction in the lab by combining lures in the following treatments: fermentation lure alone, fermentation lure plus yeast lure, fermentation lure plus leaf lure, and finally fermentation lure plus leaf plus yeast lures. These lures were then tested under field conditions in New Jersey, New York, North Carolina, Michigan, and Oregon in several crop types including blueberry, raspberry, blackberry, and cherry. At each field site the following treatments were deployed using Yellow Jacket and Flying Insect Traps to monitor adult capture: blank water as a control, a mixture of yeast and sugar, fermentation lure alone, fermentation lure plus yeast lure, fermentation lure plus leaf lure, and finally fermentation lure plus leaf plus yeast lures. We found that each individual compound is more attractive to adults than water controls in laboratory assays. However, only the fermentation lure was attractive under laboratory and field settings during the growing season. Adding the yeast, leaf, and yeast + leaf lures to the fermentation lure decreased adult attraction compared to fermentation lures alone in laboratory and field settings. Our results indicate that although the individual yeast and leaf compounds are attractive in a laboratory setting, when combined together they attract very few adults in the field compared to our fermentation lure. Further, the addition of these yeast and leaf lures to the fermentation lure reduces its attractiveness to flies in both a laboratory and field setting.

Session/symposium: Practical applications/independent presentation

#### Čokl A.

## Talking through plants as part of multimodal communication in insects

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Communication enables solitary insects to meet in the field and holds insect societies together. Different environments demand tuning of signal characteristics with transmission properties of media. Insects developed mechanisms to communicate by signals of different modalities. Plants represent the common substrate that gives insects shelter, food and medium where they exchange information during mating. Dense vegetation as environment and substrate with specific characteristics forces communication with vibratory signals tuned with its mechanical properties. Predominant communication with plant-borne vibratory signals does not exclude the use of chemical, airborne vibratory, contact mechanical and visual signals. Mating behaviour of stinkbugs (Pentatomidae) represents an instructive example of multimodal communication that different phases run with signals transmitted through different media. The general model includes air transmitted chemical signals that in the field attract at the longer-range scale mates to meet on the same plant. The male pheromone triggers females on a plant to emit vibratory signals that transmitted through it stimulate males for copulation. Recognition of species-specific female calling song signals prevents hybridisation, activates males to respond with calling and courtship song and to approach the signal source by vibrational directionality mediated by female calls, Vibratory signals, produced by different mechanisms have frequency and temporal characteristics tuned with mechanical properties of plants. The relevant vibration sensitive mechanoreceptors are situated in the legs and on the body surface. Substrate-borne vibratory communication is upgraded on the plant by information exchange with airborne component of high amplitude buzzing and tremulatory signals in near-field and closer-range conditions as well as by contact mechanical signals and visual cues that both terminate courtship and precede copulation.

Session/symposium: Intraspecific relationships/Complementary or predominant role of nonchemical based communication and orientation of insects

KEYNOTE LECTURE Conchou L.\*, Lucas P., Renou M.

Perception of sex pheromones in complex odour landscapes – towards a better reconstitution of ecologically relevant olfactory scenes.

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For male moths to be able to follow a sex pheromone plume is crucial for reproductive success. In natural conditions the signal is picked out from a complex and variable olfactory background, mostly built from plant emissions of volatile compounds (VPC). Since it has been shown that a puff with a single VPC may alter the responses of receptor neurons to the pheromone, we aimed to analyze the effects of more complex and intermittent natural backgrounds. One of the major challenges we face is to reproduce ecologically relevant olfactory scenes made of numerous VPC in terms of stimulus intensity and dynamics using a multichannel delivery system (MDS). Starting from a panel of 8 VPC from different chemical families, ecologically relevant to noctuid moth Agrotis ipsilon, we calibrated the MDS, measured the dynamics and tested the effects of single vs binary blends. Each VPCs was diluted in mineral oil (MO) and injected through a separate channel into a carrying air flow. After measuring air/MO partition coefficients we could correct for differences in volatility and compare dose-response curves and effect types of single VPCs. When tested at the same concentration, 3 of our 8 volatiles significantly affected firing activity of pheromone-sensitive olfactory neurons. Their presence in the background reduced the contrast of pheromone responses in a dose-dependent way. The effects of binary blends on pheromone response were mostly hypo-additive compared to single compounds. The timecourse of short pulses was followed with a mini photo-ionization detector showing differences in hysteresis between compounds and according to the geometry of the main and odorized flows. Further developments will consist in modeling volatile diffusion in our stimulator to be able to adjust MO concentrations to achieve known aerial concentrations. We will also improve the dynamics of our stimulus delivering system to produce fast fluctuating backgrounds.

Session/symposium: Intraspecific relationships/Importance of multicomponent mixtures for chemical cues

Costa A.<sup>1</sup>\*, Moos M.<sup>2</sup>, Zahradnícková H.<sup>2</sup>, Simek P.<sup>2</sup>, Zemek R.<sup>3</sup>

The effect of wounding and *Spodoptera littoralis* herbivory on plant VOCs emissions in caraway *Carum carvi* 

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Caraway (Carum carvi) is a biennial plant grown for its seeds in Central Europe and in Scandinavia. In this work, we aimed to determine how biotic and abiotic factors could affect the Volatile Organic Compounds (VOCs) caraway emissions, both quantitatively (absolute amounts) and qualitatively (relative amounts). We measured how wounding (W) or herbivory (H) by the polyphagous pest Spodoptera littoralis modified the host plant Carum carvi VOCs profiles. Volatiles from live plants were sampled in the laboratory at repeated times (0-4 hrs, 4-28 hrs after wounding and 0-4 hrs, 48-72 hrs after herbivory) and identified using GC-MS. Undamaged plants were characterised by a high proportion of two monoterpenes, (+)-limonene (from 33 to 45%,  $11.96 \pm 2.13$  ng h<sup>-1</sup> g<sup>-1</sup> plant dry weight DW) and cis-ocimene (from 12 to 22%,  $8.03 \pm 1.77$  ng h<sup>-1</sup> g<sup>-1</sup> plant DW). In addition, two sesquiterpenes, Germacrene D (10.9%, 5.05  $\pm$  0.86 ng h<sup>-1</sup> g<sup>-1</sup> plant DW) and  $\beta$ caryophyllene (3.4%, 1.51  $\pm$  0.51 ng h<sup>-1</sup> g<sup>-1</sup> plant DW) were also found in safe plants but in lower levels. In the first 4 hours following treatment, Germacrene D emissions were increased by 130 times (W=×127; H=×132), while β-caryophyllene increased by 60 times (W=×56; H=×62). Interestingly, the increase of these two VOCs were very similar after (W) or (H). Germacrene D became highly dominant with more than 59% of the total VOCs emitted. Whereas (W) and (H) produced similar effects in VOCs emissions on a short term (0-4 hrs), analyses showed that in S. littoralis treated plants, a higher and longer lasting increase of (+)-limonene (45.03  $\pm$  2.13 ng  $^{-1}$  g $^{-1}$ ) and four other monoterpenes (sabinene, myrcene, β-pinene, trans-ocimene) was observed (48-72 hrs). We discussed how these changes of VOCs amounts and ratio can further influence the behaviour of conspecific adults or larvae.

Session/symposium: Interspecific relationships/independent presentation ORAL PRESENTATION

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Use of semiochemicals in Integrated Pest Management strategies in South East Asia: current status and future challenges

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Current practices in farming systems are rapidly changing in South East Asia due to a demand for higher food standards and due to concerns with heavy pesticide use. Worldwide, semiochemicals have been extensively used in various cropping systems to reduce the use of insecticides. Recent outbreaks of beet armyworm *Spodoptera exigua* in Philippines have reduced by 74% the production of onion, with an estimated loss of 34.9 million (\$). In Vietnam, the occurrence of major pests can considerably hinder the yield in Mekong orchards due to citrus pests such as the citrus leaf miner *Phyllocnistis citrella* or the citrus pock caterpillar *Prays endocarpa*. To tackle large outbreaks that often are driven by unsuitable pest management strategies followed by insect resistance to pesticides, it is essential that the use of semiochemicals is optimised and adapted to farmers' needs in order to be readily adopted.

Among the most common techniques currently used in Integrated Pest Management are the trapping of pests using attractants to monitor insect adult flight and determine the best timing for insecticide spray, the mass-trapping of pests, or the disruption of their mating. Additional techniques include their contamination by entomopathogenic fungi and the recording of establishment from new pests. In the sweet potato weevil *Cylas formicarius*, several uses of pheromone traps can reduce the pest occurrence, either by mass trapping to reduce the population, by monitoring the peak of male flight, or by disrupting the mating with high concentrations of pheromone. In this review, we focus on four major pests: the coffee berry borer *Hypothenemus hampei*, the sweet potato weevil *Cylas formicarius*, fruit flies *Bractocera* spp. and Lepidopteran pests in citrus trees. Further, we discuss why in some cases these methods are not widely used and what can be done to widespread their use by farmers.

Session/symposium: Practical applications/independent presentation

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Presence of C10 fatty acids in the cephalic secretions of bees.

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The fatty acids produced in the mandibular glands of honey bees are unique in having four important functions: the first is to act as an acidification agent for royal jelly that allows for queen rearing (10 hydroxy- 2-decenioic acid - 10HDA), the second is to act as a sex pheromone for the attraction of drones (9-keto-2-decenoic acid – 9ODA), the third is to act as a social regulator in caste determination through the inhibition of ovary activation in workers (QMP -queen mandibular gland pheromone) and the fourth is to act as an antibiotic in larval food (10 HDA). The multiple functions of the C10 fatty acids in honey bee social behavior raises questions about the origin and evolution of this communication system both in honey bees and bees in general.

The presence of C10 fatty acids in a variety of bee species will be explored in order to determine the extent to which C10 fatty acids are present in the cephalic secretions of a variety of bee species and to provide an insight into their evolutionary origin in the honey bees. The question to be answered will be the extent to which these fatty acids are unique to honey bees and whether their functions in honey bees represent a novel use of these compounds in the Apid bees. The use of these C10 fatty acids as social regulators in honey bees appears to a novel characteristic of Apid bees and suggests that social regulatory systems can arise from chemical origins not confined to cuticular hydrocarbons.

Session/symposium: Interspecific relationships/independent presentation

## Cui Y.

## Semiochemical application for invasive species in China

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Semiochemical-based pest management technology has been widely used to monitor and control insect pests in agricultural, forestry, and public health sectors in China. We will present the early history of pheromone research and current practical applications in China, particularly pheromone-based invasive species monitoring and management. We also provide information regarding the pheromone product manufacturing, marketing, and regulatory issues related to local semiochemical industries.

Keywords: Sex pheromone, Mass trapping, Mating disruption, Marketing, Regulation, Invasive Pest management

Session/symposium: *Practical applications/Semiochemical application for invasive species* KEYNOTE LECTURE

Cunningham J.P.\*, Baig F., Farnier K., Hossain M.

Yeast-beetle interactions and the control of Carpophilus beetles in stone fruits and almonds

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Carpophilus beetles are important pests of fruit and nut orchards. We investigated yeastinsect interactions in Carpophilus behavioural ecology, with the aim of applying this knowledge to the development of new attractants for mass-trapping strategies. This is particularly important for Australian almonds, where Carpophilus has become a major problem and current mass trapping is proving ineffective. We isolated and identified several species of yeast from the gut of wild collected Carpophilus adults of C. hemipterus (which prefers rotting fruit), C. davidsoni (which prefers ripe fruit on trees), and a newly identified species (C. nr dimidiatus), the major pest of Australian almonds. In fruit attacking species, Carpophilus beetles were closely associated with two yeasts, Pichia kluyveri and Hanseniaspora guilliermondii. In cage trials on attraction and oviposition, adult C. davidsoni and C. hemipterus beetles exhibited significantly stronger preferences for fruit substrates inoculated with H. guilliermondii, compared to P. kluyveri. Interestingly, medium inoculated with P. kluyveri, (the less preferred yeast) yielded greater larval survival in both beetle species. GC-MS analysis revealed differences in volatile emissions between the two yeasts, identifying volatiles that may play an important role in Carpophilus behaviour. A yeast species in the genus Wickerhamomyces was most commonly isolated from C. nr dimidiatus (almond-attacking species), and had a different odour profile to that of H. guilliermondii. Substrates inoculated with this yeast trapped significantly higher numbers of beetles than H. guilliermondii during trials in almond and stone fruit orchards. Our next steps are to design and test new attractant blends based on these findings.

Session/symposium: Practical applications/Comparison of semiochemical control methods of pest insects

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Feeding behaviour of the bird cherry-oat aphid on seedlings of selected maize (Zea mays L.) cultivars

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Aphids are serious group of insect pests of many cultivated plants, including maize. One of the predominant aphid species that may reduce maize crop is the bird cherry-oat aphid (*Rhopalosiphum padi* L.). This pest damage crops both directly, through phloem feeding, and indirectly, by the transmission of plant viruses. In the protection of plants against aphids, especially according to integrated pest management strategies, it is quite important to understand the ecological and biochemical relations between insects and their host plants.

Feeding behaviour of the bird cherry-oat aphid on seedlings of two cultivars of maize with different level of resistance (Waza – relative resistant, Złota Karłowa – susceptible) was monitored using the electrical penetration graph (EPG) technique. The relative degree of resistance of the tested plants was determined based on our previous studies on the aphid development on fifteen maize cultivars.

Analyses of the EPG recordings showed significant differences between aphid probing on susceptible and resistant maize plants. On the susceptible cultivar, after short period of non-probing, aphid females started penetration of the epidermis and the mesophyll, and relatively quickly reached phloem vessels. In contrary to susceptible plants, probing of the peripheral tissues on the resistant cultivar started later, and this activity comprised more than half of the total penetration time. Additionally, phloem phase on the resistant seedlings was two times shorter than on susceptible one. The relatively long time of phloem feeding indicates that maize Złota Karłowa cv. creates more favourable feeding conditions for the bird cherry-oat aphid.

Acknowledgements. The research was financially supported by the National Science Centre (NSC; Poland) under the grant no. 2016/21/B/NZ9/00612.

Session/symposium: Practical applications/independent presentation

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The citrus defense strategy in response to phytopathogen *Xylella fastidiosa* and Candidatus *Liberibacter asiaticus*: quorum sensing signals used by *X. fastidiosa* and increased biosynthesis of coumarins

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The major biotic diseases in Citrus include citrus variegated chlorosis (CVC), and more recently, huanglongbing. To establish a representative chromatographic fingerprint for the leaves, stems, rootstock stems and roots of C. sinensis grafted on C. limonia cv. Pêra with and without diseases symptoms, respective samples have been individually analyzed using the established LC-UV-MS/MS methods, and the peaks indicated the highest content among others with good resolution, and they were identified. Then, LC-UV-MS/MS methods were developed for quantifying these compounds. These methods have become powerful tools for detecting diseases in citrus before the symptoms appear, thereby informing in advance when the plant should be removed from the orchard. These methods have been demonstrated which constituents varied in concentration in response to the presence of bacteria, such as the flavonoids hesperidin in the presence of X. fastidiosa, dydimine in plants with HLB symptoms, the coumarins xanthyletin and seselin in CVC and HLB symptomatic citrus. Increased biosynthesis of this metabolite represents a plant defense strategy in response to pathogen attack, since these compounds showed antimicrobial activity on the respective bacteria. Thus, studies on biosynthesis of flavonoids and coumarins with isotopic labeling were development. Possibilities for engineering enzymes to increase levels of some coumarins to induce resistance in citrus are in development. In addition, a simple defined medium (XFM) containing a single carbon source and no preformed amino acids was developed as a method with a particularly high potential for the grown of X. fastidiosa and to produce genuine natural products, as a range of diketopiperazines. This class is a novel signal molecule of Gram-negative bacteria or interacts with AHL-based quorum sensing.

The authors thank: CNPq 465357/2014-8, FAPESP, 2014/509187; 2012/25299-6.

Session/symposium: Practical applications/Natural products for integrated pest management

**ORAL PRESENTATION** 

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Neighbour- specific plant-plant communication influences herbivore suppression in cultivar mixtures

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Increased plant genotypic diversity in crop fields can promote ecosystem services including pest control, but understanding of mechanisms behind herbivore population responses to cultivar mixtures is limited.

We studied aphid settling on barley plants exposed to volatiles from different cultivars, aphid population development in monocultures and two-cultivar mixtures, and differences in volatile composition between studied cultivars.

Aphid responses to one cultivar in a mixture were neighbour-specific, and this was more important for pest suppression than the overall mixture effect, aphid colonization patterns or natural enemy abundance. Aphid populations decreased most in a mixture where both cultivars showed a reduced aphid plant acceptance after reciprocal volatile exposure in the laboratory, and reduced population growth compared to monocultures in the field.

Our findings suggest that herbivore population responses to crop genotypic diversity can depend on plant-plant volatile interactions, which can lead to changes in herbivore response to individual cultivars in a mixture resulting in slower population growth. The impact of plant-plant interaction through volatiles on associated herbivore species is rarely considered, but improved understanding of these mechanisms would advance our understanding of the ecological consequences of biodiversity and guide development of sustainable agricultural practices. Combining cultivars in mixtures based on how they interact with each other is a promising strategy for sustainable pest management.

Session/symposium: Practical applications/Natural products for integrated pest

management

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Davidson-Lowe E.\*, Ali J.

# Arbuscular mycorrhizal associations impact plant resistance to a belowground feeding herbivore

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Plants interact with multiple organisms throughout their lifetime and respond dynamically to changes in their environment and within their community. Arbuscular mycorrhizal fungi (AMF) form mutualistic associations with the roots of most land plants. Plants provide AMF with carbohydrates in exchange for mineral nutrients, such as phosphorous. However, AMF colonization can also facilitate changes in phytochemistry, including phytohormones and secondary metabolites. The majority of studies that focus on AMF-related changes in plant defenses focus on responses to aboveground damage. In this study, we investigated how AMF colonization in *Zea mays* affects jasmonic (JA) and salicylic (SA) signaling and other plant responses to belowground damage by the root-feeding larvae of western corn rootworm (*Diabrotica virgifera virgifera*). Larvae were added to plants with and without AMF colonization. Above and belowground plant tissue was collected for plant defense analyses and larval survival was used as a measure of herbivore performance. This study demonstrates that mycorrhizal associations can impact herbivore performance by altering plant defenses, which has significant implications in agricultural practices for improved pest management.

Session/symposium: Interspecific relationships/independent presentation

De Vrieze M.<sup>1</sup>, Gfeller A.<sup>1,2</sup>, Chinchilla D.<sup>3</sup>, Bailly A.<sup>1</sup>, L'Haridon F.<sup>3</sup>, Weisskopf L.<sup>1,2,3</sup>\* News from the volatile warfare between potato-associated *Pseudomonas* and the late blight causing agent *Phytophthora infestans* 

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During the last decade, the importance of bacterial volatiles in cross-kingdom interactions has become evident. In addition to promoting plant growth and root development, bacterial volatiles have been repeatedly shown to inhibit the growth of phytopathogenic fungi, although the molecules responsible for this effect are still largely unknown, with the notable exception of hydrogen cyanide. Our recent work has shown that oomycete pathogens such as Phytophthora infestans, causing late blight in potato, are particularly sensitive to the volatiles of potato-associated Pseudomonas strains, when compared to other potato disease-causing agents such as Rhizoctonia solani or Helminthosporium solani. In a screen aimed at identifying the chemical composition of the volatile blends from those efficient antioomycete Pseudomonas, Sulphur-based compounds were identified as potent inhibitors of all life stages of the pathogen, including mycelial growth, sporangia production and germination, as well as zoospore motility. Some of these Sulphur-containing volatiles were able to prevent disease establishment on infected plant material. One important and so-far unresolved question concerns the ability of bacteria to emit those bioactive volatiles when living in natural conditions, e.g. on leaf surfaces. We are currently investigating this question using sterile potato plantlets inoculated with bacterial strains of known volatile blend emission. First results indicate that typical bacterial smells such as the long-chained alkene 1-Undecene previously shown to inhibit growth and sporulation of P. infestans can be detected on inoculated plants. This highlights the so-far underexplored potential of bacterial volatile compounds for sustainable crop protection.

Session/symposium: Interspecific relationships/Volatile-mediated microbe-plant interactions ORAL PRESENTATION

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Moth sex pheromone precursors produced as constituents of wax ester in the oil crop *Camelina sativa* 

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Application of synthetic sex pheromones for mating disruption is an environmentally friendly method for pest control and one of the foremost alternatives to the use of conventional pesticides. The high cost and the environmental concerns associated with the synthetic pheromone production, however, prevents the use of this technique in many crops and against many pest insects. By expressing the necessary and sufficient biosynthetic genes of insect and plant origin, we have previously made plants produce monounsaturated alcohols, which after acetylation proved to be efficient for trapping of target insects. We aim to produce moth pheromone intermediates esterified as wax esters in oil crop seeds, which can be extracted and saponification to fatty alcohols that are either used directly, or oxidized to the corresponding aldehyde or acetylated, and then used to control pest insects by mating disruption. We tested 10 wax synthases from various sources in the tobacco and selected the best candidates for the esterification of short-chain monounsaturated fatty alcohols (14C-16C). Eventually, we made transgenic Camelina lines expressing thioesterase, fatty acyl desaturase, fatty acyl reductase and wax synthase which are capable of producing pheromones in the form of wax esters in the seed oil. The fatty alcohol components were isolated from the oil by saponification and solvent extraction and converted by acetylation to the corresponding acetate or by oxidation to the corresponding fatty aldehyde and then used to trap pest insects or disrupt their mating. Moreover, the resulting acyl and alcohol moieties in the wax esters were shifted heavily to the 14C and 16C level that confers the desirable properties of spermaceti oil (high thermal stability and low melting point). Such semisynthetic preparation of sex pheromones is a novel and cost-effective way of producing large quantities of pheromones with high purity and a minimum of hazardous waste.

Session/symposium: Interspecific relationships/Metabolomics approaches in the

Brassicaceae POSTER number: 134

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Dolejšová K.<sup>1</sup>, Křivánek J.<sup>1</sup>, Kalinová B.<sup>2</sup>, Hadravová R.<sup>1</sup>, Kyjaková P.<sup>1</sup>, Hanus R.<sup>1\*</sup> Sex-pairing pheromones in three sympatric neotropical termite species, *Embiratermes neotenicus, Silvestritermes heyeri*, and *Silvestritermes minutus* (Isoptera: Termitidae: Syntermitinae)

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Termite colonies are almost always founded by a pair of winged dispersers. The dispersal flights of imagoes from natal colonies are followed by mate search, mediated by sex-pairing pheromones. Here, we studied the chemistry of sex-pairing pheromones and the related aspects of mate search in winged imagoes of three species from the subfamily Syntermitinae, Embiratermes neotenicus, Silvestritermes minutus, and Silvestritermes heyeri. All three species are widespread in the Neotropics, including the rainforests of French Guiana. After the dispersal flight and spontaneous loss of wings, females expose their hypertrophied tergal glands situated under abdominal tergites VIII – X. The females are attractive to males and, upon direct contact, the two sexes form characteristic tandems. Chemical analyses indicated that the females secrete species-specific combinations of unbranched, unsaturated C12 primary alcohols from the tergal glands, (3Z,6Z,8E)-dodeca-3,6,8-trien-1-ol (approx. 200 pg per female) and (3Z)-dodec-3-enol (185 pg) in E. neotenicus, (3Z,6Z)-dodeca-3,6-dien-1-ol (3500 pg) in S. heyeri, and (3Z,6Z)-dodeca-3,6-dien-1-ol (300 pg) and (3Z)-dodec-3-enol (50 pg) in S. minutus. (3Z,6Z,8E)-Dodeca-3,6,8-trien-1-ol and (3Z,6Z)-dodeca-3,6-dien-1-ol act as major pheromone components in the respective species and mimic the function of female tergal gland extracts in electrophysiological and behavioral experiments. Biologically relevant amounts of the third compound, (3Z)-dodec-3-enol, elicited non-significant reactions in males of E. neotenicus and S. minutus, and slight synergistic effects in males of S. minutus when tested in combination with the major component.

Session/symposium: Intraspecific relationships/independent presentation

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### Multiple patterns of human scent signature recognition by trained dogs

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Human scent signature is a set of volatile organic molecules forming individual odor print that enables unequivocally identify people by trained dogs. In order to understand which molecules are responsible for human scent individuality, we collected and separated human scent samples into three fractions based on the volatility of the analytes using preparative gas chromatography. Subsequently, each fraction was subjected to the scent identification protocol with specially trained canines.

The least volatile of the three fractions has been the most often correctly attributed by the dogs (in 78% of cases). Nevertheless, also the two remaining fractions have been positively discriminated, suggesting that the scent trace contains multiple signature patterns utilized by canines for identification of individual persons.

Using GC×GC-TOFMS, we identified 137 individual compounds in the human scent samples, belonging to a wide range of chemical classes, among them fatty acids, ketones, aldehydes, alcohols and fatty acid esters.

This work was supported by the Ministry of the Interior of the Czech Republic (www.mvcr.cz), projects #Vf20142016036 and VI20172020075

Session/symposium: Interspecific relationships/independent presentation

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Domingue M.J.<sup>1,2</sup>\*, Myers S.W.<sup>1</sup>, Phillips T.W.<sup>2</sup>
Larvae of *Trogoderma* respond behaviorally to whole body extracts

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Behavioral responses to semiochemicals by Trogoderma (Coleoptera: Dermestidae) stored product pests were assayed in a small arena. Hexane extracts were obtained from Khapra beetle, Trogoderma granarium, warehouse beetle, Trogoderma variable, and the larger cabinet beetle Trogoderma inclusum that were killed by being frozen for 48 hours at -20° degrees C. These extracts were analyzed using gas chromatography coupled with mass spectrometry (GC/MS), and it was confirmed that they contain several cuticular hydrocarbons, fatty acids and sterols. Two choice experiments were performed inside petri dish arenas, with filter paper fully covering the bottom surfaces. Two smaller 3cm filter papers were placed on opposite ends within each arena. Each of the smaller papers were folded three times in parallel to present a corrugated surface that the insects could move underneath if they chose. In each case, one paper had a 100µl aliquot of one of the extracts, and the other 100µl of hexane as a control. 10 large larvae of the same species as the treatment extract were placed in the arena and allowed to acclimate overnight in a dark room. For all three species, it was found that larvae were more likely to be found on the side of the petri dish with the hexane control rather than the conspecific larval extract. They were also more likely to be on or near the smaller corrugated filter paper treated with the control as opposed to the filter paper treated with the larval extract. Thus repellency of the conspecific extract was demonstrated at that particular dose. Further assays using different doses of the raw extracts and their individual chemical components are planned. The use of these semiochemicals in novel management strategies will be considered.

Session/symposium: *Practical applications/Semiochemical application for invasive species*ORAL PRESENTATION

Duhin A.\*, Turlings T., Röder G.

#### Primitive plants: plentiful but neglected resources for herbivores?

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Plants and their herbivores are engaged in a coevolutionary arms race, whereby plants evolve strategies to cope with herbivores and the herbivores evolve traits to overcome these obstacles. Some plants may also be avoided because they lack suitable nutrients for development. Interestingly, primitive plants like mosses and ferns do not appear to exhibit advanced defenses, but they are rarely eaten by herbivores. It has been hypothesized that this is because their tissues are of poor nutrient quality, but this awaits scientific confirmation. In this study, the nutritive suitability of 15 moss (Bryophyta s. l.) and fern (pteridophytes) species for herbivores was investigated using feeding assays combined with analytical quantification of nutrients. Growth and survival of the two generalist herbivores Spodoptera exigua (Insecta, Lepidoptera) and Cochlicella barbara (Gasteropoda, Pulmonata) on primitive plants were assessed and compared to performance on maize plants (Zea mays), a well-studied angiosperm. In parallel, levels of proteins, amino acids and sugars were quantified. Overall, our results suggest that the poor performance of herbivores on primitive plants is not correlated with nutritional quality. Although development and survival were considerably better on maize, this could not be explained by differences in nutrient contents. Interestingly, taxonomically related species showed similar protein levels and amino acids profiles, but not sugars. These results have prompted further investigations into the reasons for the avoidance of primitive plants by herbivores. These include the evaluations of constitutive and induced defences, including volatiles organic compounds (VOCs) emitted by mosses and ferns. Ultimately, our findings may assess an entirely neglected domain of plant-herbivore interactions. A better understanding of such interactions in primitive plants should shed new light on the evolutionary history of these interactions.

Session/symposium: Interspecific relationships/independent presentation

Duke S.O.

Novel modes of action of natural phytotoxins can meet a growing herbicide resistance management needs

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Synthetic herbicides have dominated weed management for the past 70 years in developed countries. The many commercial herbicides represent only about twenty modes of action, and a new mode of action has not been introduced for about 30 years. Resistance to these herbicides is rapidly evolving, with fewer options available for alternating and/or stacking modes of action to delay or mitigate evolved herbicide resistance. There is renewed interest in discovery and development of new herbicide modes of action for improved resistance management. Natural phytotoxins often have novel modes of action that have not been used in commercial herbicides. Both phytochemical (e.g., *m*-tyrosine) and microbial phytotoxins (e.g., AAL-toxin) with novel modes of action will be discussed. Some phytochemical phytotoxins, but not all, have been proven to be allelochemicals involved in plant-plant interactions. Some of the most active microbial phytotoxins are produced by plant pathogens. Thus, chemical ecology provides clues for discovery of phytochemicals of interest. The use of omics methods in determination of modes of action of natural phytotoxins will be discussed, as will discovery strategies.

Session/symposium: Practical applications/Natural products for integrated pest management

KEYNOTE LECTURE

Eberl F. 1\*, Hammerbacher A. 1,2, Gershenzon J. 1, Unsicker S.B. 1 Small size, big effects: how a plant pathogen influences tree-insect interactions

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The world's terrestrial ecosystems are dominated by woody plant species harboring an enormous diversity of insect and microbial species. As most chemical ecology studies were carried out on short-lived herbaceous plants using only a single attacker species, our knowledge on the interaction between insects and pathogens in trees is poor. We therefore used black poplar (Populus nigra) as a model organism to study complex pathogen-treeinsect interactions. Black poplar trees were infected with rust (Melampsora larici-populina), one of the most devastating diseases threatening cultivated and natural popular populations worldwide. A subsequent attack by insect herbivores (Lymantria dispar caterpillars) evoked a less intense defense response in rust-infected trees compared to uninfected controls. This diminution of defense parameters could be explained by antagonistic phytohormone crosstalk that influenced downstream molecular mechanisms. Moreover, L. dispar caterpillars preferred rust-infected over uninfected leaves and were attracted by the smell of rust spores. Our results show that different plant attackers influence each other in a direct and indirect (plant-mediated) manner. They further provide knowledge on the complex web of interactions in natural ecosystems as well as information for increasing yield in silvicultural plantations.

Session/symposium: Interspecific relationships/Insect-microbe interactions
ORAL PRESENTATION

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### Chemosensing of Varroa mite: proteomic and transcriptomic study

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The tight synchronization between the life cycle of the parasitic mite Varroa destructor and its host, the honeybee is mediated by chemical stimuli. These are mainly perceived by a pit organ located at the distal part of the mite's foreleg. The mites' reproduction is known to be regulated by cues from the honeybee brood; while at the phoretic stage, host orientation and selection are mediated by adult bee volatiles. Despite the significance of chemosensing in the parasite lifecycle, information on the molecular structure and function of its peripheral mechanisms in Varroa is mostly lacking. Recently we identified in the Varroa foreleg, transcripts of chemosensory related genes belonging to several groups. These included Niemann-Pick disease protein type 2 (NPC2), gustatory receptors (GRs), ionotropic receptors (IRs), sensory neuron membrane proteins (SNMPs) and odorant binding proteins (OBPs). However, no insect odorant receptors (ORs) and odorant co-receptors (ORcos) were found.

For further identification of specific chemosensory proteins we used a transcriptomics and proteomic approach. In particularly we compared forelegs relative to the rear legs (as control). We also compared the expression profile of mites' forelegs from the two main physiological stages of the mite, phoretic and reproductive, those differ in their host selection behaviour. Since the Varroa genome is not yet annotated, a transcriptome was de novo assembled using Trinity and served as a base for proteomics. The analysis revealed transcripts and proteins unique to the mite's foreleg. As expected, most of the transcripts were uncharacterized. We focused on transcripts of putative chemosensory gene families, odorant carrier proteins and membrane bound chemoreceptors. Using a conserved domains approach, we found significant higher expression of 14 of the chemosensory putative transcripts. The phylogeny of those transcripts will be discussed.

Session/symposium: Interspecific relationships/independent presentation

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## Do transmission potential of infected mosquitoes alter by the type of vertebrate blood?

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The efficiency of malaria parasite development within mosquito vectors is a critical determinant of transmission. Sporogony is thought to be controlled by environmental conditions and mosquito / parasite genetic factors. We tested this assumption by investigating whether successful sporogony of Plasmodium falciparum parasites through to human-infectious transmission stages is influenced by the host species upon which infected mosquitoes feed. Studies were conducted on two major African vector species that generally are found to differ in their innate host preferences: Anopheles arabiensis and An. gambiae sensu stricto. We show that the proportion of vectors developing transmissible infections (sporozoites) was influenced by the source of host blood consumed during sporogony. The direction of this effect was associated with the innate host preference of vectors: higher sporozoite prevalences were generated in the usually human-specialist An. gambiae s.s. feeding on human compared to cow blood, whereas the more zoophilic An. arabiensishad significantly higher prevalences after feeding on cow blood. Our results tentatively suggest that the benefits of eliminating highly anthropophilic species may be less than expected, if the more zoophilic feeding behaviour in the less permissive vectors that remain endow them with an equal probability of developing sporozoite stage infections.

Session/symposium: Intraspecific relationships/Importance of multicomponent mixtures for chemical cues

ORAL PRESENTATION

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Tracking of host defences vs. tracking of host phylogeny during the radiation of neotropical *Inga* insect herbivores

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The arms race between plants and insect herbivores has been invoked as one of the main mechanisms driving trait diversification and coevolution for both groups. Although explicit tests are few, the evidence suggests that patterns of herbivore-host codiversification are rare. We currently are examining the evolution of herbivore-host relationships in detail and determining the role of plant defensive traits in the evolution of associations for leaf-feeding herbivores and their *Inga* (Leguminosae) hosts in the Neotropics. We have developed multilocus phylogenies for both plants and insects and quantified host associations and plant defenses. We find that the sawfly herbivores (Hymenoptera, Symphyta, Tenthredinidae) show strong associations with specific species of *Inga*. Our initial results indicate a pattern of herbivore-host codiversification that is closely (but not strictly) tied to host chemistry. In contrast, for Lepidoptera, we found low topological congruence between *Inga* and herbivore phylogenies, suggesting taxon-specific histories of herbivore -host plant interactions.

Session/symposium: Interspecific relationships/Tibor Jermy's Legacy in insect-plant evolution

ORAL PRESENTATION

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Pheromone-based sampling for conservation: determining the habitat requirements of *Tragosoma depsarium* 

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Changes in forest management regimes have during the last centuries turned Swedish forests increasingly more homogeneous, mostly dominated by dense and young spruce stands. This has led to the disappearance of many previously common habitats with complex effects on biodiversity. Insects, which as a group constitutes a large proportion of forest biodiversity have often been underrepresented in habitat surveys, often due to difficult and expensive survey methods. During recent years pheromone based survey methods has started to ameliorate these problems by allowing for large scale studies of threatened insects.

Tragosoma depsarium is a saproxylic beetle spending its larval stage inside logs of old *Pinus sylvestris* trees. The species was previously common in large parts of Sweden but has declined during the last century and is today considered Near Threatened on the Swedish Red List. Although a large characteristic beetle, not much is known about its habitat requirements and dispersal capacity. To investigate this a study of *T. depsarium* occurrence was performed on a regional scale with 162 investigated potential habitats in the Swedish counties of Kalmar and Östergötland. A mark and recapture study was simultaneously conducted in a hotspot area in Kalmar county.

By combining, at the sites locally measured, habitat characteristics and satellite based forest volume GIS data, models explaining species presence were created. The most important explanatory variables seemed to be the volume of *P. sylvestris* in a three km radius, the density of trees around the traps and the number of logs within 100 m of the traps. The mark and recapture study showed movements of up to almost 4 km and indicated large beneficial effects of targeted conservation efforts on the population abundance of *T. depsarium*.

Session/symposium: *Practical applications/independent presentation*ORAL PRESENTATION

Eyun S.<sup>1</sup>, Soh H.Y.<sup>2</sup>, Posavi M.<sup>3</sup>, Richards S.<sup>4</sup>, Lee C.E.<sup>3</sup>\*

# Evolutionary history of chemosensory-related gene families across the Arthropoda

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Chemosensory-related gene (CRG) families have been studied extensively in insects, but their evolutionary history across the Arthropoda had remained relatively unexplored. Here, we address current hypotheses and prior conclusions on CRG family evolution using a more comprehensive dataset. In particular, odorant receptors (ORs) were hypothesized to have proliferated during terrestrial colonization by insects (hexapods), but their association with other pancrustacean clades and with independent terrestrial colonizations in other arthropod subphyla have been unclear. We also examine hypotheses on which arthropod CRG family is most ancient. Thus, we reconstructed phylogenies of CRGs, including those from new arthropod genomes and transcriptomes, and mapped CRG gains and losses across arthropod lineages. Our analysis was strengthened by including crustaceans, especially copepods, which reside outside the hexapod/branchiopod clade within the subphylum Pancrustacea. We found ORs and odorant binding proteins (OBPs) present only in hexapods (insects) and absent from all other arthropod lineages, indicating that they are not universal adaptations to land. Gustatory receptors (GRs) likely represent the oldest chemosensory receptors among CRGs, dating back to the Placozoa. We also clarified and confirmed the evolutionary history of antennal ionotropic receptors (IRs) across the Arthropoda. All antennal IRs in E. affinis were expressed more highly in males than in females, suggesting an association with male mate-recognition behavior. This study is the most comprehensive comparative analysis to date of CRG family evolution across the largest and most speciose metazoan phylum Arthropoda.

Session/symposium: Interspecific relationships/Arthropod chemoreceptors
ORAL PRESENTATION

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Cashew volatile extracts reduce attractiveness of host plant volatiles to *Diaphorina citri* Kuwayama (Hemiptera: Liviidae)

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Huanglongbing (HLB) is the most severe disease of citrus. Diaphorina citri is a vector of the bacterial causative agent of HLB. Management practices for reducing HLB impacts include eradication of symptomatic trees, use of healthy plants and vector control. Manipulating insect behaviour through deployment of semiochemicals offers a promising prospect for protecting citrus crops. The aim of this research was to evaluate the behavioural responses of D. citri to plant volatile extracts. Volatiles were collected from host plants Murraya paniculata, Citrus sinensis, C. reshni, C. limettioides, Poncirus trifoliata, and from non-host plants Psidium guajava, Mangifera indica, Anacardium occidentale. In behavioural assays (four-arm olfactometry), D. citri spent more time in arms containing volatiles from either M. paniculata or C. sinensis compared to the control arms. In response to volatiles from A. occidentale, they preferred the control arm. Volatiles from the other plants did not influence the insect behaviour. Chemical analyses of volatiles from C. sinensis, M. paniculata, and A. occidentale revealed the presence of (E)-4,8-dimethylnona-1,3,7-triene (DMNT) and (E,E)-4,8,12-trimethyltrideca-1,3,7,11-tetraene (TMTT) in higher amounts in A. occidentale. In another series of behavioural bioassays, D. citri spent less time in arms containing a synthetic blend of DMNT and TMTT and in arms containing the synthetic blend in combination with volatiles from either M. paniculata or C. sinensis compared to the control arms. A blend of DMNT and TMTT, combined with the VOCs from host plants, inhibited the foraging behaviour of D. citri, indicating that these chemicals could be involved in the lack of attraction of *D. citri* to cashew volatiles.

Session/symposium: Interspecific relationships/independent presentation

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Olfactory basis of tick behavior and novel repellent development

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The blacklegged tick (Ixodes scapularis) is the main vector of Borrelia burgdorferi, the etiological agent of Lyme disease. In North America, the spread of *I. scapularis* ticks and the high incidence of Lyme disease cases reported in recent years have raised concerns for public health [1]. The threat of disease transmission has resulted in an increasing demand for effective, safe and environmentally-friendly repellent products. Plant-derived essential oils are natural products that exhibit insecticidal and repellant activities and represent a promising alternative to synthetic repellants [2]. Preliminary bioassays suggest that certain essential oils (i.e. lemongrass) have potential in repelling I. scapularis. However, the mechanisms by which ticks detect odor stimuli and how these stimuli are perceived as repellents is not well described. An enhanced understanding of tick olfaction is critical for better selecting the appropriate chemicals and developing suitable formulations for protecting people against tick bites [3]. Moreover, it is not clear if the presence of bacterial infection(s) may alter the response of ticks to odor stimuli4. In this project, we investigate behavioral and electrophysiological responses by ticks to stimulation with selected essential oils and their main components, compare the responses to those of infected ticks, and use this information to develop new repellent products for personal protection.

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- 4Arsnoe et al. (2015) PlosONE doi:10.1371/journal.pone.0127450.

Session/symposium: Practical applications/Natural products for integrated pest management

ORAL PRESENTATION

Farnier K.<sup>1</sup>, Madge D.<sup>2</sup>, Cunningham P.<sup>1</sup>\*

Two stones, one bird: are two distinct female-specific lures necessary to efficiently monitor and control Queensland fruit fly (*Bactrocera tryoni*) populations in orchards?

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The Queensland fruit fly (Bactrocera tryoni) is a major horticultural pest in Australia, attacking a wide variety of fruits (stone fruit, pome fruit and citrus) and vegetable crops and posing a serious threat to domestic fruit production and export market. The recent withdrawal of the only effective insecticides has exhorted the need for a viable alternative to control fruit fly populations. To date, semio-chemicals used in 'lure and kill' device have consisted of either male-specific attractants (cuelure) or protein-based baits that mostly attract immature/virgin females. The absence of a lure targeting sexually mature and/or gravid females that are directly responsible for fruit damage has, for many years, been identified as a major hurdle to the success of lure and kill strategies. Previous laboratory-based studies have shown that mature female fruit flies are strongly attracted to volatiles abundantly produced in ripe guavas. Here, we conducted field studies in which we sought to implement these compounds, alone and/or in combination with fruit- associated microbial volatiles. Various prototype lures placed inside red spheres of visual "LADD" traps were tested in orchards and their efficacy compared with that of the current best protein-based commercial traps.

Our prototype fruit odour-based lures caught significantly more flies than commercial protein traps in some of our trials, whilst the opposite effect was observed in others. Greatest fly catches were obtained from prototype lures composed of microbial compounds applied concomitantly with fruit odours, suggesting a synergistic effect. Variation in trap efficacy may be linked to the mating status (or sexual maturity) of resident fly populations, and a mixed trap strategy using distinct lures to catch mated and virgin female flies could be more advantageous for controlling this important horticultural pest.

Session/symposium: Interspecific relationships/independent presentation ORAL PRESENTATION

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Chemical profiling of volatile compounds of leaves citrus before and after inoculation of *Phyllosticta citricarpa* using headspace solid-phase microesxtraction

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The citrus black spot caused by the fungus *Phyllosticta citricarpa* is an important citrus disease, which affects most citric varieties, causing relevant economic damages. Several systems have been adapted to analyze, identify, and quantify airborne metabolites released by plants. For example, MS based platforms are widely used in studies of the metabolome of plants. This study presents the application of fingerprinting profiling by GC-MS using the solid phase microextraction technique of the Headspace-SPME in studies of two citrus species, Citrus lemon and Citrus latifolia inoculated by fungus P. citricarpa, susceptible and resistant species, respectively. Both samples were inoculated with fungus and the control samples (non-inoculated) were obtained in the same conditions. The inoculated and control leaves were obtained in different time periods after the inoculation. After the factorial planning to choose the best conditions for analysis, the leaves were weighed and placed in a headspace viel, sealed and were taken for GC-MS analysis. The chemometric study showed a tendency of distinction between susceptible and resistant species. The susceptible specie showed strong distinction of inoculated and control samples. The comparison of the chromatograms obtained from analyzes showed that only the susceptible sample presented an accumulation of several terpenes in leaves inoculated. This may be related to the action of the phytopathogen in the plant, in which itself can be increasing the production of this compound such as defense responses. It is important to note that C. latifolia is a variety with high resistance to the fungus in the field, and through analyses by headspace it was not observed increase in concentration of these terpenes. This may be related with the resistance of this plant to *P. citricarpa*, inversely observed in the susceptible plant.

Session/symposium: Interspecific relationships/Volatile-mediated microbe-plant interactions ORAL PRESENTATION

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Fingu Mabola J.C., Lognoul M., Bosquee E., Serteyn L., Sarles L., Verheggen F., Francis F  $\ast$ 

### Impact of entomopathogenic fungi on multitrophic insect-plant interactions

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Entomopathogen microorganisms such as fungi are biological control agents that are able to disturb host plant - herbivore relations due to their interactions with some target insects. Particularly, these fungi grow on insect hosts, secrete secondary metabolites and finally kill them. Here, we investigated the influence of diverse entomopathogenic fungi (Beauveria, Metarhizium, Aspergillus) on different insect hosts but also on non target beneficials. Aphids and bugs for herbivore guild while aphidophagous predators for tritrophic interactions were selected respectively. Multitrophic approaches were developed to determine potential effects of these fungi on the behaviour of intra- and interguild protagonists. Beside the observation of insect developmental parameters, the orientation preferences were tested by taking into consideration the insect and/or plant fungal infection status. Olfactometry devices were used, complemented by choice tests in Petri dishes and analysis of emitted volatile organic compounds. Also, electropenetrography (EPG) technique was developed to assess the changes of aphid and bug sucking feeding behaviour related to fungal infestation status. Our results are discussed in relation to the complexity of interactions at different trophic levels, with a particular focus on behavioural more than only developmental aspects of the impact of entomopathogenic fungi in plant - insect interactions.

Keywords: entomopathogen, aphid, bug, predator, behaviour

Session/symposium: Interspecific relationships/independent presentation

Florencio-Ortiz V.<sup>1</sup>, Novak O.<sup>2</sup>, Casas J.L.<sup>1</sup>\*

Jasmonate and salicylate accumulation in pepper (*Capsicum annuum* L.) leaves induced by aphid (*Myzus persicae* Sulzer) infestation. A local or systemic response?

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Aphids are among the most economically important insect pests in the world agriculture even though, as phloem-feeders, they produce little injury to plant foliage during feeding in comparison with chewing insects like caterpillars or grasshoppers. Their negative impact can thus be considered as indirect, related to the high population densities they achieve leading to significant nutrient withdrawal from the phloem sap, the vectoring of plant viruses, and the huge deposition of honeydew, which impairs the proper leaf functioning and favors fungal infection. Plant defenses against herbivores are vast and efficient, but aphids frequently circumvent these defenses and succeed in feeding and reproducing on the host plant. In this context, the present study was aimed to describe the content of defense-involved molecules in leaves of pepper (Capsicum annuum L.) plants in response to green peach aphid (Myzus persicae Sulzer) infestation. We focused on jasmonates (JA) and salicylate (SA) because they are both major compounds involved in plant responses to pathogens and herbivores. We conducted a detailed time-course experiment and the content of defensive compounds was evaluated at both local and systemic level, by preventing aphids to freely move in the host plant with the aid of clip cages. Aphid infestation did not alter the content of cis-12-oxophytodienoic acid, the JA precursor, even though endogenous levels of JA and its bioactive isoleucine-conjugated form (JA-Ile) significantly increased from 8 to 96 hours in local infested leaves. Systemic effects in JA content were only showed at 48 hours in the case of JA, and 8 and 48 hours in the case of JA-Ile. An antagonistic interaction between JA and SA pathways may be deduced, given that SA accumulated only in local infested leaves after 96 hours of infestation, when the level of JA-Ile decreased.

Session/symposium: Interspecific relationships/independent presentation

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From hitchhiker to bodyguard: the evolution of defensive symbionts of beetles from plant-associated bacteria

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While the study of many animal- and plant-microbe relationships has traditionally focused on one-on-one interactions, there is much to learn about these systems by considering the multiple interacting partners on a common scenario. Several Lagriinae beetle species engage in a symbiosis with Burkholderia gladioli bacteria that belong to a clade of plant pathogens. The beetles consistently carry coinfecting symbiont strains in specific organs within their body and can transmit these from mother to offspring as well as acquire them from the environment. We are interested in understanding how and why such long-term associations are established, and whether a plant host can play an important role for the symbiosis. Manipulative bioassays showed that the symbiotic bacteria inhibit antagonistic fungi on the beetle eggs, and a combination of chemical and genomic analyses on the symbiont strains revealed the production of at least four different bioactive compounds that might explain the protective effect. Interestingly, the bioactive potential varies for the different coinfecting strains, as well as their occurrence, relative abundance and degree of host dependence. At least some of the symbiont strains can also infect and survive within a host plant, and can be transmitted to and from the insect to a plant, suggesting that a dynamic tripartite interplay is likely taking place. We shed light on the evolutionary ecology of symbiont-mediated defense in Lagriinae beetles and propose the insect-plant-microbe interaction as a key aspect influencing the emergence and maintenance of a protective symbiosis.

Session/symposium: Interspecific relationships/Insect-microbe interactions ORAL PRESENTATION

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Fonseca M.M.<sup>1</sup>\*, Lima E.<sup>1</sup>, Pallini A.<sup>1</sup>, Alecrim I.<sup>1</sup>, Nascimento P.H.<sup>1</sup>, Janssen A.<sup>2</sup> Volatile-mediated responses of predatory mites to heterospecific competitors

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Arthropod predators are known to use a wide diversity of volatile cues when foraging. The response to these volatiles determines habitat selection and distribution of species, as well as the occurrence and strength of interactions within food webs. For example, chemical cues are known to mediate avoidance of competitors within trophic levels. It is therefore of paramount importance to explore how the response of predators to volatiles associated with potentially interacting species affects their distribution. Predatory mites are commonly used as biological control agents and the species Phytoseiulus macropilis and Neoseiulus californicus are considered to be released together to control an important pest, the twospotted spider mite Tetranychus urticae. It is not known how these predators respond to volatiles emanating from prey patches invaded by heterospecifics, which is important to determine their interactions and, ultimately, their combined effects on the pest. Using a Ytube olfactometer, we therefore investigated whether these two predatory mites avoid the presence of the other species using the volatiles emanating from plants with prey and heterospecifics. Whereas both P. macropilis and N. californicus were significantly attracted to volatiles of plants infested with spider mites, neither of the predators avoided volatiles from prey patches occupied by the heterospecifics. The increased joint use of several species of natural enemies for biological control highlights the importance of exploring the mechanisms and functions of volatiles mediating interactions within the third trophic level. Chemical cues have important implications for ecological interactions and evolutionary processes, however, studies of volatile-mediated avoidance of competitors are limited to a small number of species, yet deserve more attention.

Session/symposium: Interspecific relationships/independent presentation

Forim M.R.<sup>1</sup>\*, Aquino F.A.<sup>1</sup>, Carneiro R.L.<sup>1</sup>, Silva M.F.G.F.<sup>1</sup>, Fernandes J.B.<sup>1</sup>, Freitas C.A.<sup>2</sup>, Boica-Junior A.L.<sup>2</sup>

Metabolomic evaluation of *Gossypium hirsutum* volatile organic compounds against *Spodoptera cosmioide*: resistant and susceptible genotypes

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Cotton (Gossypium hirsutum, Malvaceae) is an important crop from Brazilian agribusiness. Therefore, it is important to develop sustainable mechanisms of protection against insect as Spodoptera spp., improving its production and quality. As alternative, we may use resistant genotypes in crops. However, not only is important to find resistant genotypes, but also to understand the associated mechanisms. An interspecific defense mechanism usually present in plants it is the biosynthesis of volatile organic compounds (VOCs) in induced, or constitutive systems. Plants employ VOCs as communication channels to manipulate their environment. The knowledge how VOC are involved to the communication between cotton and other organisms, like plant-herbivore interactions, it can help in the development of new pest control strategies. Then, the main aim of this study was to evaluate the metabolomic interaction between G. hirsutum genotypes, resistant and susceptible, and S. coismioide through the VOCs. Initially, twelve different cotton genotypes were submitted to antibiosis bioassays against S. coismioide. Genotypes with high capacity to avoid this biotic stress, and other susceptive were chosen to metabolomic studies of VOCs. Qualitative and quantitative analyses by Headspace Gas Chromatography hyphenated to Tandem Mass Spectrometer were carried out investigating the variation in the chemical profiles. Chemical and biological data were together processed by MATLAB software package providing the identification of volatile compounds correlated of resistance. In order to improve the chemical model, we also performed some treatments to these genotypes under stress situations such as herbivory, hydric stress, and in the presence of methyl jasmonate. In these treatments, we were able to identify chemical modifications in the VOCs profile, as well as to identify secondary metabolites likely linked to plant resistance mechanism.

Session/symposium: Interspecific relationships/independent presentation

Forim M.R. <sup>1</sup>\*, Madera K.S.M. <sup>2</sup>, Durango L.G.C. <sup>1</sup>, Lima E.M. <sup>1</sup>, Martínez O.C. <sup>2</sup>, Gorecki T. <sup>3</sup> Use of gastrointestinal microorganisms from *Diabrotica speciosa* in biodegradation of xenobiotic compounds to the environment

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Antibiotics are one of the most important medicinal compounds, widely applied in treatment of human diseases. At the same time, insecticides are largely used to the control of crops. Both classes of those compounds are xenobiotic to the environment. As a result, their inappropriate use has been showing negative consequences such as antimicrobial and insecticidal resistance, and contamination of the environment. In your turn, insects have developed several resistance mechanisms using enzyme(s) that break down xenobiotic compounds to non-toxic structures. Some resistance mechanism found in insects are results of symbiotic associations with microorganisms. Thus, insects have harbored some microbial strains to protect themselves against toxic compounds. Therefore, the gastrointestinal tract of the insects may be a rich source of microorganism with biotechnology potential. Hence, the main aim of this project was evaluate if bacteria of the gut from Diabrotica speciosa were able to biodegrade antibiotics and insecticides such as doxycycline, carbofuran, chlorpyrifos, and cypermethrin. In this work, we isolated 73 bacterial strains, which were characterized by 16S rDNA, and MALDI-TOF MS analyses. The main isolated genera were Enterobacter (21%), Pseudomonas (18%), and Acinetobacter (16%). The species Enterobacter cloacae and Stenotrophomonas maltophilia were growth in broths previously doped with the chosen analytes. Gas Chromatography-Tandem Mass Spectrometry and Liquid Chromatography-Tandem Mass Spectrometry were applied to quantify the biodegradation processes. E. cloacae and S. maltophilia showed similar grow between controls and treatments. At the same time, S. maltophilia showed the biodegradation of 52, 44, 31% to the cypermethrin, chlorpyrifos and carbofuran, respectively, and E. cloacae degraded 42% of the doxycycline, at 96 h. This model may be used in the development of new bioremediation programs.

Session/symposium: Practical applications/Natural products for integrated pest

management POSTER number: 135

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Forister M.L. **Evolution of insect host range** 

Univeristy of Nevada, Reno

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Understanding the evolution of insect host range has implications for the study of specialization and speciation, as well as the emergence of new crop pests and diseases. Alfalfa throughout the Great Basin of western North America has been a useful model for studying the process by which novel plant-insect interactions are formed. I will discuss the colonization of alfalfa from three perspectives: (1) proximate barriers or controls on use of the novel host by a focal butterfly; (2) the genetic architecture of novel host use, also by the focal butterfly; (3) finally, from the plant's perspective, I will ask how plants manage complex communities of parasites and other associates (incorporating metabolomic and microbiome data). Results are discussed in the context of a model for the evolution of insect host range that prioritizes ecological contingency and genetic drift, but not antagonistic pleiotropy of associated genetic architecture.

Session/symposium: Interspecific relationships/Tibor Jermy's Legacy in insect-plant evolution

ORAL PRESENTATION

Francke W.

#### Identification and synthesis of some new insect volatiles

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Structure elucidation of trace amounts of new natural products in multicomponent mixtures is largely restricted to the application of GC/MS and HR-GC/MS. Derivatization of the natural products (hydrogenation, acetylation etc.) may be helpful. Indirect proof of postulated structures is carried out by comparison of analytical data of the target compounds and synthetic reference samples. Structures of some new compounds will be presented.

Compound 1 is the first spiroacetal showing a branched carbon skeleton with methylene groups at both a-positions to the oxygens. The compound co-occurs with several terpenoids such as 2 in the cephalic secretion of females of the cleptoparasitic bee, *Nomada goodeniana*. The same and similar terpenoids showing oxygenation at the same carbon along the chain are present in other *Nomada* species, too. *Nomada* also contain 5-oxofarnesol and some of its esters. Interestingly, 5-hydroxyfarnesol 3 is the main volatile in skin extracts of the grashopper *Phymataeus leprosus*. The terpenoid 4 and closely related structures are characteristic volatiles in several *Monomorium* species.

Session/symposium: *New chemical structures/independent presentation* KEYNOTE LECTURE

Fu N.\*, Becker T., Kunert M., Burse A., Boland W.

A cytochrome P450 responsible for 3-nitropropionic acid biosynthesis in leaf beetles *Phaedon cochleariae* 

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3-nitropropionic acid (3-NPA) is a neurotoxin and causes neurodegeneration with symptoms similar to that triggered by Huntington's disease. Presence of this compound and its derivatives has been reported in a board range of living organisms like plants, insects, bacteria and fungi. In leaf beetles, Phaedon cochleariae, 3-NPA is stored as pre-toxic compound, esterified to isoxazolin-5-one glucoside, which is presented in all life stages for protective purpose. It's known that 3-NPA is derived from β-alanine, however, which enzyme is recruited to catalyze this reaction remains obscure. Here we report a P. cochleariae cytochrome P450 CYP347W1 that is involved in the production of 3-NPA. Transcriptional analysis in various tissues and life stages suggested that the presence of the isoxazolin-5-one glucoside in eggs is due to maternal transfer. Knock down of CYP347W1 via RNAi in larvae at beginning of the 2nd instar led to a dramatic decrease of the stored pretoxic defensive compound in the hemolymph. This unique β-alanine and P450-dependend synthetic tactic is different from the aspartic acid and flavin-dependent oxidoreductase based mechanism in prokaryotic organisms. Our results indicated that different organisms choose their favorable amino acids and enzymes to produce the same products, which contributes to the widespread occurrence of 3-NPA in eukaryotic and prokaryotic kingdom.

Session/symposium: Interspecific relationships/independent presentation

Furlan L.<sup>1</sup>\*, Chiarini F.<sup>1</sup>, Contiero B.<sup>2</sup>

### How to use pheromone traps to implement effective IPM of wireworms (Agriotes spp.)

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Non-saturable (YATLORf) sex-pheromone traps have been set up to monitor the click beetles of all the most important European Agriotes species (A. brevis, A. lineatus, A. litigiosus, A. obscurus, A. sputator, A. rufipalpis/sordidus and A. ustulatus). Experiments carried out in several European countries have shown that the same trap can be baited with different lures, making it possible to monitor all of the key species at one site at very low costs. In order to exploit the potential of this IPM tool, experiments have been carried with baited YATLORf traps to spot fields at risk of economic wireworm populations. This can be achieved if reliable correlations between adult populations and consequent larval density/plant damage are established. With reference to some harmful Agriotes species (A. brevis, A. sordidus and A. ustulatus), multi-baited trap capture levels gave an estimate of the risk posed to maize by each pest species. When local population levels were low (<200 click beetles/trap/season), the risk of economic plant damage was negligible, regardless of the species; there have been no seriously damaged fields in more than 30 years of observations. For A. brevis, a significant correlation between YATLORf beetle captures and wireworm density/maize-plant damage the following year were identified and a practical threshold has been made available for farmers. The evaluation of pheromone-trap captures and risk factors enabled risk assessment of wireworm damage to maize - and potentially to other crops - to be made accurately, thus making it feasible to implement IPM of wireworms affordably.

Session/symposium: Intraspecific relationships/Chemical ecology of click beetles (Elateridae): practical applications and advances in the field.

KEYNOTE LECTURE

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### What is the key to host recognition by the larval ectoparasitoid Holepyris sylvanidis?

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The larval ectoparasitoid Holepyris sylvanidis (Hymenoptera, Bethylidae) has been described as natural enemy of several beetle species infesting stored grain products. Our previous studies revealed that H. sylvanidis is attracted to larvae of Tribolium confusum, a major stored-product pest, by a blend of specifically host-associated compounds released from host larval feces and ubiquitous volatiles from the host's feeding substrate. Moreover, cuticular hydrocarbons (CHC) of T. confusum larvae serve H. sylvanidis as contact kairomones. The parasitoids follow host larval CHC trails and show host recognition behavior toward host larval CHC extracts [1,2]. Here, we studied the question whether the various host species of H. sylvanidis share common CHC patterns that mediate host recognition. In contact bioassays, we exposed parasitoid females (i) to alive larvae of four species of stored-product beetles, (ii) to dead larvae whose CHCs were removed by hexane extraction, (iii) to dead larvae with re-applied CHC extracts, and (iv) to extracted dead larvae treated with fractions of those CHC extracts which elicited host recognition. While H. sylvanidis showed host recognition behavior toward three tested Tribolium species and extracted larvae treated with their CHC extracts (T. confusum, T. castaneum and T. destructor), larvae of Oryzaephilus surinamensis and the corresponding CHC extracts elicited no response. Comparative GC-MS analysis of CHC extracts of the recognized and non-recognized species as well as bioassays with CHC extract fractions showed that methylbranched alkanes (chain length C25 to C30) present on the cuticle of host larvae are key compounds necessary for host recognition by H. sylvanidis.

- [1] Fürstenau B. et al. (2016) Chem. Sens. 41: 611-621.
- [2] Fürstenau B., Hilker M. (2017) J. Chem. Ecol. 43: 858-868.

Session/symposium: Interspecific relationships/independent presentation ORAL PRESENTATION

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Galis I.\*, Fukumoto K., Hojo Y., Nakatani H., Shinya T.

A hormone with multiple roles: jasmonate-related functions in defense and development of rice plants

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While jasmonates accumulate in plants after wounding and herbivore attack, they also appear in flower tissues when plants reach reproduction. Previously, we reported jasmonate profiles in rice leaves treated with simulated herbivory, showing that OsJAR1 gene was essential for the synthesis of most of the active hormone, JA-Ile, in these tissues. At the same time OsJAR1 mutant plants (Osjar1) were sterile. When we now analyzed the levels of jasmonic acid (JA), JA-Ile, salicylic acid (SA), and abscisic acid (ABA) at four different stages of rice panicle (flower) development in the wild-type plants, highest levels of JA and JA-Ile were found at anthesis, followed by a sharp decline at later stages. As expected, Osjar1 mutants lacked JA-Ile but accumulated much higher levels of free JA in the flowers. Levels of SA gradually increased and culminated during seed filling, whereas ABA appeared mostly constant. From the sharp peak accumulation of JA-Ile at anthesis, we assumed that JA-Ile should play its major role at this stage to promote fertility. We then examined several flower-specific MADS-box gene transcripts as potential JA-Ile targets in flower development but none of them was strongly affected by the deficiency of JA-Ile. Interestingly, transcripts of several other genes expressed in flowers peaked during anthesis in wild-type but remained much higher in Osjar1 plants after this point, consistent with the idea of flowers being arrested at this stage of development without acquiring a stimulus of JA-Ile. Currently, we continue with characterization of several other transcriptional regulators whose action might be impaired in Osjar1 flowers and thus lead us to the key contributions of a canonical defense signal JA-Ile and its downstream signaling in the reproductive fitness of monocot plants.

Session/symposium: Interspecific relationships/independent presentation

Ganji S.\*, Zhao T., Schiebe C., Unelius C.R.

The influence of fatty acids on the production of bark beetle pheromones by their symbiotic fungi

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Bark beetles bring symbiotic fungi species into their host trees during mass attacks that overwhelm the trees defense system. Together with effective aggregation pheromones, the symbiotic relationship with bluestain fungi is thought to be the key to the beetles' ability to overpower and kill healthy trees. Our previous studies have showed that fungal symbionts associated with the spruce bark beetle, Ips typographus produce an aggregation pheromone component for this beetle; 2-methyl-3-buten-2-ol. In this study, seven fungal species of the Ophistomoid family Grosmannia europhioides, G. clavigera, Endoconidiophora rufipennis, Ophiostoma piceae, O. pseudotsugae, O. montium and Leptographium abietinum were individually incubated in agar and the volatile emission were analyzed by SPME-GC-MS for 30 days. Numerous fungal compounds were detected and confirmed and a number of them are electrophysiologically active in the noxious bark beetle, *Ips typographus*. The virulent Ophistomoid fungi carried by spruce bark beetle produced 2-methyl-3-buten-2-ol and other known semiochemicals when growing on the agar medium. Interestingly, the production of electrophysiologically active compounds were significantly enhanced and the proportions of compounds changed when the fungi were grown in agar medium with certain fatty acids added.

The possible role of fatty acids in host selection of bark beetles is discussed.

Session/symposium: Intraspecific relationships/Chemical ecology of click beetles (Elateridae): practical applications and advances in the field.

Gaquerel E. 1\*, Navarro Quezada A. 1, Naake T. 2, Kupke M. 1, Li D. 3

Joining forces – an integrative approach to decipher anti-hervivore defense innovations in *Nicotiana allopolyploids* 

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Plants adapt to their environments by diversifying their phenotypes in various ways. This diversification is intimately linked and reflected in plants' fascinating capacity to evolve novel specialized metabolites. It is now clearly recognized that all modern flowering plant genomes derive from processes set in motion by a history of repeated and episodic auto- or allopolyploidy events and that these polyploidy events act as important drivers of phenotypic innovations. However, the contribution of polyploidy events to the diversification of plant specialized metabolism defenses against insects has only rarely been examined.

Using a multidisciplinary approach combining transcriptomics, metabolomics and chemical ecology methods, we are currently dissecting biochemical pathways leading to "transgressive" defense-related metabolic characters for *Nicotiana* allopolyploids species of the section *Repandae*. Species from this section derive from a single allopolyploidy event, for which *N. sylvestris* and *N. obtusifolia* are the closest paternal and maternal genomes, respectively. We detected that *Repandae* species are unique among all *Nicotiana* species with respect to their capacity to synthesize long chain fatty acid-based N-acyl-nornicotine compounds (NANNs) in their trichomes where they act as superior toxins against insects. During this seminar, I will present results of our recent investigations on the biochemistry, molecular evolution as well as on the defensive mode of action of NANNs. Critical to this research has also been the development of novel computational approaches applied to mass spectrometry metabolomics profiles. Also, I will report on a novel "BLAST-like" pipeline (R package MetCirc) through which metabolite trees can be constructed. The use of this bioinformatics method to mine intra- and inter-specific metabolic variations will be exemplified.

Session/symposium: New chemical structures/Omics in chemical ecology

KEYNOTE LECTURE

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Fatal attraction: exploiting olfactory eavesdropping for wildlife conservation

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Interspecific competition among predators imposes selective pressures and influence animal behaviour. Natural selection will encourage mechanisms that allow mesopredators to recognise and respond to dominant predators, if this reduces the risk of a confrontation. The aim of our research was to explore the role of kairomones (chemical cues that benefit the detecting species) in interspecific interactions and investigate whether behavioural responses can be exploited for conservation.

In a series of pen and field experiments, we discovered that the body odour of an invasive apex predator (ferret *Mustela furo*) attracted mesopredators (stoats *Mustela erminea*, ship rats *Rattus rattus*, hedgehogs *Erinaceus europaeus*) to treatment sites. We initiated a mesopredator response-guided analysis of ferrets' olfactory profiles to identify the kairomones responsible for attraction. The profiles of 12 ferrets were described using solid phase microextraction (SPME) coupled with gas chromatography-mass spectrometry. Stoat responses to individual ferret profiles were assessed using a rapid behavioural bioassay in a balanced, randomised pen trial. Partial least-squares regression was used to identify profile compounds that were significantly and positively associated with stoat attraction. Eight candidate compounds were identified that are now the subject of confirmation trials towards the development of a synthetic lure.

Reducing the olfactory signal to its minimum chemical components, rather than the primary biological material, will extend the longevity, availability, and effectiveness of mesopredator lures. Such lures are effective when mesopredators invade a new system, when food is plentiful, or where food lures were previously deployed for wildlife management. Our research has applications for pest management in New Zealand and the technique of using kairomones to attract predators could have applications for conservation efforts worldwide.

Session/symposium: Intraspecific relationships/independent presentation ORAL PRESENTATION

Geisler S.\*, Schweiger R., Müller C.

#### Glucosinolate metabolism in Phaedon cochleariae

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The interaction between plants and herbivorous insects has led to the evolution of various strategies to defend against and attack the respective counterpart. Phaedon cochleariae is an oligophagous beetle that is specialized on plants of the Brassicaceae family, which uses the glucosinolate-myrosinase system as its primary defense against antagonists. When the plant tissue is damaged by a chewing herbivore, glucosinolates come into contact with myrosinases and are converted into a diverse spectrum of biologically active products. Here, we investigated how these compounds are metabolized by the specialist P. cochleariae. Therefore, feeding experiments were carried out by offering larvae host plant tissue without or with the addition of specific glucosinolates. Larvae and faeces were analyzed using LC-QTOF-MS. None of the samples could provide evidence for detoxification pathways known from other herbivorous insect species feeding on Brassicaceae, such as a conversion to desulfo glucosinolates, a sequestration of intact glucosinolates, or a production of isothiocyanates or cyanides. However, for the tested glucosinolates with a benzyl side chain, a common loss of the sulfate group and thioglucose moiety, conversion as well as conjugation, were found to occur in the larvae. These results show that the specialist P. cochleariae has evolved yet another way to cope with glucosinolates.

Session/symposium: Interspecific relationships/independent presentation

Ghaninia M.<sup>1</sup>\*, Knauer A.C.<sup>2</sup>, Schiestl F.P.<sup>2</sup>, Sharpee T.O.<sup>3</sup>, Smith B.H.<sup>1</sup> The neurophysiology of brassica odor detection by bees

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Olfactory signals play a vital role in plant-pollinator interplay. Pollinator insects, such as bees, can distinguish between rewarding and non-rewarding flowers by sniffing out floral scent and are subsequently attracted to the host plant only if the honesty of floral scent is validated. Floral scent, however, consists of a variety of different volatile compounds. Which of these compounds are detected by the sensitive olfactory system of bees and how it announces the honesty of a rewarding flower has been poorly explored. Here, by employing electoantennography (EAG), we investigated the detection of *Brassica rapa* odors by honey bees (Apis mellifera) and bumble bees (Bombus terristris). We used 12 odorants previously found in the headspace volatiles of Brassica rapa and first established a dose-response curve using individual compounds and a blend. Although the concentration of individual components in the mixture was one-twelfth of what they were presented with individually, the mixture elicited the most robust response. We then compared the intensity of the responses obtained when the compounds were presented individually at 1013 pg/L with that in the complete mixture. Results show that in Apis mellifera response to acetophenone, nonanal, and decanal was not significantly different from the mixture. In Bombus terristris, however, the response to acetophenone and phenyl acetaldehyde was similar to the mixture. We then analyzed mixtures from florets in relation to the nectar and pollen resources that bees seek. Specific submixtures were independently correlated to the presence/absence of pollen and nectar. Our data are consistent with a model in which components of submixtures together amplify an important message to bees about whether or not florets contain pollen and nectar, and the submixture is therefore more reliable than any individual component.

Session/symposium: Interspecific relationships/Arthropod chemoreceptors

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Girling R.D.<sup>1</sup>\*, Hayden P.<sup>2</sup>, Langford B.<sup>3</sup>, Mullinger N.<sup>3</sup>, Nemitz E.<sup>3</sup>, Pfrang C.<sup>4</sup>, Robins A.<sup>2</sup>, Touhami D.<sup>4</sup>

Elevated ozone alters the plume structure of an important floral volatile organic compound ( $\alpha$ -terpinene): a detailed mapping at temporal and spatial scales relevant to insects

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There is growing evidence to suggest that common air pollutants, such as ozone  $(O_3)$  and diesel exhaust, can chemically alter a range of Volatile Organic Compounds (VOCs) that are used by insects to navigate towards floral resources, hosts, mates etc. To-date, most of the chemical analyses in these studies have been confined to reaction chambers and flow tubes, which do not account for the physical structure of an odour plume. However, it is known that plume structure can significantly influence the success of an insect in locating an odour source.

Here, for the first time, we present measurements of the physicochemical degradation of an odour plume at temporal and spatial scales relevant to insects. We used a large open circuit "suck-down" model wind-tunnel with a working area of 20 (L) x 3.5 (W) x 1.5 metres (H). Odour plumes measurements were made at a rate approximately equivalent to the response time of an insect's antennae (ca. 10hz), using a proton transfer reaction time-of-flight mass spectrometer with quadrupole ion guide.

We investigated how  $O_3$  interacts with the plume of a model VOC, the monoterpene  $\alpha$ -terpinene. This VOC was selected because it has a relatively high documented rate constant for its reaction with  $O_3$  and in our previous behavioural studies was shown to be an important fVOC in honey bees' recognition of an odour plume.  $\alpha$ -terpinene was released from the upwind end of the tunnel and its plume mapped throughout the tunnel using a sampling line mounted on an X, Y, Z traverse. Plumes were mapped either at ambient  $O_3$  or at one of two atmospherically relevant concentrations (50 & 150 ppb).

 $O_3$  not only reduced the concentration of  $\alpha$ -terpinene but also affected the magnitude and frequency of odour filaments, significantly altering the plume structure, which suggests potentially wide-ranging consequences for insect orientation behaviour.

Session/symposium: Interspecific relationships/independent presentation

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Godschalx A.L.<sup>1</sup>\*, Szenteczki M.A.<sup>1</sup>, Alvarez N.<sup>2</sup>, Rasmann S.<sup>1</sup> Floral scent variation in smelling like poop

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Plants produce a staggering lexicon of scent compounds to maintain both specific and diverse channels of communication. Deciphering which plant biology-determining forces from the bottom-up, i.e. climate and phylogenetic constraints, versus top-down, biotic responses to plant chemistry, dictate volatile blends and drive variation in plant chemistry remains an important challenge to interpret the diverse suite of bewildering interactions in nature. Here we measure variation in the fetid aroma of Arum maculatum, a bewildering pollination system that mainly traps two species of Psychoda moth flies by putatively chemically mimicking the flies' oviposition site: cow dung. We collected A. maculatum volatiles across Europe, from France, Switzerland, Italy, Croatia, Serbia, and Bulgaria. To test biogeography, plant genetic background, and climatic conditions as bottom-up forces, and the dominant pollinator species in each region as a top-down force, we used a series of distance matrices to explain variation in "poop-scented" volatile production. We found population-level variation across Europe, with plant genetic cluster, and dominant pollinator species as significant factors explaining this variation. Climatic conditions did not explain floral scent variation. These data reinforce the potential for plant-insect interactions to contribute to the bewildering biochemical diversity and resulting scents emitted across the landscape.

Session/symposium: Interspecific relationships/independent presentation ORAL PRESENTATION

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Goncalves E. 1\*, Figueiredo A.C. 1, Barroso J.G. 1, Henriques J. 2, Sousa E. 2, Bonifácio L. 2 Volatiles released by two species of *Pinus* after feeding by *Monochamus* galloprovincialis insect-vector

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Pine wilt disease (PWD) is the conifers pathology with highest ecological and economic negative implications. PWD results from a complex interaction involving the infection agent, the pinewood nematode (PWN) Bursaphelenchus xylophilus, the transmission vector, beetles of the genus Monochamus, and the host, Pinus trees. In 1999 PWD was first described in Europe, in Portugal, in *Pinus pinaster* and associated to the longhorn beetle, *Monochamus* galloprovincialis. Using the most relevant species of the genus in Portugal, P. pinaster and P. pinea half-sib genetic contrasting families, the aim of this study was to determine the influence of M. galloprovincialis feeding on volatiles emission. One week after acclimatization under open air conditions, seven years old pine trees were tested individually, sheltered from direct sun incidence. Each Pinus canopy was covered by a metallic net, within which a male-female couple of newly formed starved M. galloprovincialis was placed. This setup was covered with a plastic bag closed at the openend in the Pinus trunk and the insects were left to feed during 24 h. The volatiles were collected, during 24h, by solid phase micro extraction (SPME) and analyzed by Gas Chromatography-Mass Spectrometry (GC-MS) and GC for component identification and quantification, respectively. Of the emitted volatiles detected in *P. pinaster*, α-pinene, βpinene, phenethyl 2-methyl isovalerate, germacrene D and bornyl acetate were among those that showed the highest fold increase after insect feeding. Eight components were detected in P. pinea volatiles, of which limonene and n-hexadecanol showed the highest fold increase after insect feeding.

Acknowledgment: Work partially funded by UID/AMB/50017-POCI-01-0145-FEDER-007638-PT2020-Compete 2020 and research contract PTDC/AGR-FOR/4391/2014.

Session/symposium: Interspecific relationships/independent presentation

Gongora C.\*, Tapias J., Castro A., Jaramillo J., Casanova H., González S., Benavides P. Sesquiterpene volatiles effect on Coffee Berry Borer *Hypothenemus hampei* 

Cenicafe- Federacafe. National Center of Coffee Research. Entomology Department. University of Antioquia-Colombia. Colloids group.

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Coffee Berry Borer CBB is the most important insect pest of coffee crops worlwide. In Colombia, the impact of CBB is agravated since there are coffee fruits all year long in the field, so survival and reproduction inside are guaranted. The IPM strategy to control CBB is based on cultural control and the use of chemical insecticides and entomopathogens as a complement. So for, the use of atractan and repellent volatiles arrived as a new chemical ecological strategy to be added to the IPM program. This research was carried out in order to evaluate the preference of CBB to five sesquiterpenes, previously identified in a plant, Lantana camara that repelled CBB. The volatiles were evaluated in concentrations between 25 to 200 ppm using a Y-olfactometer containing ripe coffee berries in one branch and the addition of the volatile in the opposite. Each volatile and concentration was tested using 50 CBB independent females and then replicated four times. The number of insects reaching each branch of the Y-olphactometer was registered, so the repellent effect was recorded as the proportion of females entering the branch containing only the ripe coffee berries. As a result, a-pinene at 25 ppm produced 38% repellency. Limonene attracted CBB females at 25 ppm, but repelled at concentrations surpassing 100 ppm. Terpinene did not induced any response between 25 and 100 ppm, attracted at 200 ppm and repelled at 1000 ppm. Farnesene did not induced any response at all. A new terpene volatile was repellent at 25 ppm and higher showing up to 78% repellency at 50 ppm. We will present ongoing field results using volatiles repelling CBB.

Session/symposium: Practical applications/independent presentation

Grandi L.<sup>1</sup>\*, Ye W.<sup>1</sup>, Vallat A.<sup>2</sup>, Glauser G.<sup>2</sup>, Benrey B.<sup>3</sup>, Turlings T.<sup>1</sup> Communication among cotton plants

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Cotton (*Gossypium* spp) is involved in several mutualistic and antagonistic interactions and it has evolved various direct and indirect defense strategies to ward off antagonists and to facilitate beneficial interactions. Studies have shown that volatile organic compounds (VOCs) that are emitted in response to insect herbivory can repel herbivores and are attractive to the natural enemies of the herbivores. These herbivore-induced volatiles also serve as signals between leaves and neighboring plants, priming them for enhanced defense induction. Recent studies suggest that the priming of neighbors has potential for application in pest resistance, but the underlying mechanisms are still poorly understood. In order to better understand communication among cotton plants, we exposed intact receiver plants to VOCs from cotton plants that were either infested by *Spodoptera* caterpillars or from control plants that were left unharmed. In plants that had been exposed to VOCs from infested plants we found a general upregulation of defense genes, increases in direct defense metabolites (i.e. gossypol) and reduced caterpillar feeding as compared to control plants.

Our results provide evidence of beneficial VOCs-mediated communication among cotton plants. Further experiments aim to identify the VOCs that trigger the enhanced defenses in neighboring plants. Eventually, these specific VOCs might be applied on cotton cultivars, as a novel strategy to enhance the resistance of cotton plants to pests.

Session/symposium: Interspecific relationships/independent presentation

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Black mustard plants counter butterfly eggs: genotypic variation of this ability differently affects butterfly and parasitoid preferences

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Plants perceive and respond to insect eggs in several ways. Some plants are capable of killing eggs before they hatch by either directly killing them via e.g. hypersensitive responses (HR-like) or indirectly by the recruitment of egg-killing parasitic wasps. Insect oviposition onto leaves can alter the profile of leaf volatiles, and such oviposition-induced plant volatiles (OIPVs) can affect different trophic levels, such as host preferences of herbivores, as well as the attraction of egg- and larval parasitoids.

To understand whether egg-killing through HR-like defence or attraction of parasitoids through the emission of OIPVs is beneficial for plants under complex natural conditions and whether this is correlated with natural variation, we used self-fertilised accessions of the annual black mustard (*Brassica nigra*). Those *B. nigra* accessions (SF48) that strongly expressed HR-like defence induced by eggs of the large cabbage white butterfly (*Pieris brassicae*) were more attractive to egg parasitoids when compared to clean control plants than accessions that rarely expressed HR-like necrosis (SF19). In contrast, OIPVs emitted by SF48 plants deterred female *P. brassicae* butterflies from oviposition. Differences in the emission of five terpenoids and one non-terpenoid alcohol compound might partly explain the observed behavioural patterns. SF19 plants showed a slightly higher fitness than SF48 but egg depositions had no effect. Both egg-killing defences vary with plant genotype but because survival of clustered *P. brassicae* eggs was not affected by HR-like necrosis and parasitism rates were low, we did not find effects on plant fitness.

Session/symposium: Interspecific relationships/independent presentation

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Gross J.<sup>1</sup>\*, Rid M.<sup>1</sup>, Markheiser A.<sup>2</sup>, Hoffmann C.<sup>2</sup>

For development of an oviposition monitoring tool (M-Ovicard): unravelling the key factors for oviposition of grape berry moths

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The European grape berry moth *Eupoecilia ambiguella* and the European grapevine moth *Lobesia botrana* are the most serious pests in vineyards. Moths locate their host plant from distance by olfactory cues whereas contact-chemosensory and visual cues are important for oviposition.

Although different grape varieties show a variable susceptibility to moth infestation, less is known about the signals emitted by different grape varieties during the growing season. Besides physical factors (color, shape and texture), volatile and non-volatile compounds produced by four different grape varieties at different phenological stages were analyzed and identified by GC-MS. The perception of potential attractive compounds by *L. botrana* and *E. ambiguella* has been verified via electroantennography (EAG). Further, the biological activity and variety preferences for both volatile and non-volatile components were tested in behavioral two-choice bioassays.

The pattern of volatile compounds differed significantly between the varieties, but by considering the perceived compounds only this effect disappeared. In contrast, non-volatile compounds on the waxy surface substrate were responsible for oviposition. Oleanolic acid, the major compound on the waxy surface of all cultivars, was found to highly stimulate oviposition acceptance in both moths species.

These findings will contribute for the development of an innovative tool (M-OVICARD) for monitoring the oviposition of both species in vineyards for determining the exact spraying time of insecticides.

Session/symposium: *Practical applications/independent presentation*ORAL PRESENTATION

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Volatile organic compounds emitted by *Brassica* seedlings as tools of *Bagrada hilaris* chemical orientation

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Bagrada hilaris Burmeister, also known as the painted bug, is a heteropterean pest native to Asia and Africa and invasive in the United States, Mexico, and more recently, South America. This species can cause heavy damage to several vegetable crops in the genus Brassica, in particular to young plants at seedling stage. Objective of this study was to evaluate the role of Volatile Organic Compounds (VOCs) emitted by seedlings of three Brassica species on the host location of B. hilaris individuals. Preliminary host preference experiments in dual choice arena and olfactometer, indicated that adult painted bugs preferred B. oleracea var. botrytis and B. napus over B. carinata. Brassica oleracea var. botrytis seedlings VOCs then were collected and tested with B. hilaris adults and late stage nymphs, with electroantennographic (EAG) and behavioral (olfactometer) techniques. The results indicated that B. hilaris adults and nymphs were attracted to the crude extract of B. oleracea var. botrytis seedlings, and to aliquid chromatography nonpolar fraction containing hydrocarbons, whereas there were no responses to the more polar fractions. Chemical analysis (GC-MS) showed that the main constituent of the nonpolar fraction was an as yet unidentified diterpene hydrocarbon, which occurred in both of the preferred hosts B. oleracea and B. napus, but not in B. carinata. This compound might act as the key mediator in this insect-plant interaction, and could be a good candidate for use in lures for monitoring B. hilaris in the field.

Session/symposium: Interspecific relationships/independent presentation

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# The chemical treasure of microbial communication associdated with fungus growing termites

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The rapid development of OMICs has enabled a renaissance of natural product research. In particular, the detailed chemical analysis of complex symbiotic systems has attracted attention amongst natural product chemists. As a result, many different microbial derived metabolites with diverse structural features were identified and found to be important regulators of symbiotic interactions and essential for microbial communication [Beemelmanns C. et al. (2016) Beilstein J. Org. Chem. 12: 314].

Here, I will present our recent results of the chemical analysis of symbiotic microbes associated with fungus-growing termites *Macrotermes natalensis*. Firstly, new highly substituted tropolone alkaloids rubterolones were identified from *Actinomadura* sp. 5-2, isolated from the termite gut, by using fungus-bacteria challenge assays, a HRMS-based dereplication, and were characterized by NMR and HRMS and X-ray crystallography. Genome sequencing revealed their putative biosynthetic pathway, and supported by isolation of precursors and feeding experiments. [Guo H. et al. (2017) Chem. Eur J. 23: 9338]

Secondly pseudoxylallemycins were identified from the fungus *Pseudoxylaria* sp. X802, an antagonist of the termite cultivar. Based on dereplication strategy and directed feeding of synthetic precursors, several new derivatives have been isolated. Derivatives B–D were particularly intriguing as they possess a rare allene moiety amenable for synthetic modifications and showed antimicrobial activity against human-pathogenic *Pseudomonas aeruginosa*. Genomic studies to analyse the biosynthetic pathway are currently under way. (

[Guo H. et al. (2016) Org Lett. 18: 3338) Both studies highlight that microbes present a prolific source of secondary metabolites and that we can use the chemical and genomic information to increase compound diversity.

Session/symposium: Interspecific relationships/Insect-microbe interactions
ORAL PRESENTATION

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Gupta S.<sup>1</sup>\*, Kumble A.L.K.<sup>1</sup>, Desireddy S.<sup>1</sup>, Bessière J.M.<sup>2</sup>, Borges R.M.<sup>1</sup> The scent of life: phoretic nematodes choose live over dead wasps as vehicles

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Phoresy is a phenomenon where one organism (traveler) attaches to another organism belonging to a different species (vehicle) for dispersal to suitable habitats. Organisms that show such interactions can become tightly associated with their vehicles if they occupy specialized habitats which show ephemerality, i.e. appear stochastically in time and space. In phoretic systems that need to cope with the constraint of habitat ephemerality, getting on to the correct vehicle forms a crucial step. Phoretic organisms are known to use various cues ranging from visual to chemical to locate their vehicles but so far no studies have investigated if they can differentiate between the physiological status of their vehicles, i.e. whether they are dead or alive. The brood site pollination mutualism involving figs and fig wasps provides an excellent opportunity to conduct such studies. This represents an ancient co-evolved, species-specific, obligate mutualism which show associations with phoretic nematode communities and that utilize pollinating wasps as vehicles for dispersing from one fig syconium (closed urn-shaped inflorescence) to another. We study the fig wasp species (one pollinator and six non-pollinator species) and the nematode community (three species with putative plant- and animal-parasitic lifestyles) associated with the Ficus racemosa, a wide-spread, common tropical fig species. We use behavioural assays to demonstrate that nematodes choose live over dead vehicles and ignore non-pollinating wasps that are not vehicles. Since earlier studies have shown that seven species of wasps differ in their metabolic rates, we investigate the role of wasp volatiles especially carbon dioxide concentrations in nematode differentiation between different species of wasp vehicles as well as between their physiological status. We use chemical (GC-MS analysis of volatiles) and metabolic (estimation of carbon dioxide released) analysis for thus determining the scent of life.

Session/symposium: Interspecific relationships/independent presentation
ORAL PRESENTATION

Gut L.\*, Miller J.

# Accelerating the adoption of mating disruption: matching technology with mechanism

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Although substantial inroads into commercial markets have been made, mating disruption products continue to hold a rather small share of the pest control market. What can be done to accelerate the successful use of mating disruption and minimize failures? We propose that improvements in the efficacy and economics of mating disruption will be catalyzed by: i) progress toward differentiating among mating disruption mechanisms and identification of the causal actions of given products and ii) understanding how to deliver the optimal dose of pheromone to affect the targeted pest while minimizing costs of disruption products and labor for application. Mechanisms of mating disruption fall into two major categories competitive and non-competitive. Mating disruption products of the 21st century must be characterized as to which of the two mechanisms is operational. The best way to do so is via dosage-response profiles. Key traits of purely competitive disruption are smoothly concave profiles when untransformed capture data are plotted against density of dispensers and a straight line with negative slope when catch is plotted against dispenser density x catch. Key traits of purely non-competitive disruption profiles include an initial linear disruption profile on untransformed axes and a re-curving complex plot. Hybrid profiles are possible when some males begin the activity period already incapacitated, while those not pre-exposed are able to respond to traps or pheromone dispensers. Examples within the two categories and suggested tactics for differentiating among them are offered through analysis of the disruption of important pests using various formulations. Once the principal and contributory causes of disruption are understood, different paths present themselves with respect to optimizing: i) loads of pheromone in the formulation and release rates, ii) dispenser spacing in the field, and iii) costs of the product and its application.

Session/symposium: Practical applications/Comparison of semiochemical control methods of pest insects

ORAL PRESENTATION

Hagenbucher S., Birgersson G., Chattington S.R.\*, Anderson P.

Domestication of cotton influences choice behaviour and performance of the generalist herbivore, *Spodoptera littoralis* 

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Wild ancestral plants have been drastically altered through domestication processes in order to see today's crop plants producing high yields and being well adapted to agricultural practices. However, domestication also causes an increase in susceptibility to herbivores and pathogens, bringing new challenges to agricultural practitioners. In this study we investigated the effects of domestication processes on the volatile profiles of a group of cotton accessions from three different species (*Gossypium hirsutum*, *Gossypium herbaceum*, and *Gossypium raimondii*), as well as the effects on the larval and adult host choices, and larval development, of the invasive moth, *Spodoptera littoralis*. Domesticated *G. hirsutum* plants were found to support better performance of *S. littoralis* than the wild accessions of cotton, but although qualitative and quantitative differences were found between the volatile bouquet of the different species and accessions, *S. littoralis* did not discriminate for better host plants in this way. However, one species in particular, *G. raimondii*, was found to be rejected by means of volatiles, with a preference for domesticated accessions. *G. raimondii* was also found to provide associational resistance to neighbouring susceptible plants. Our findings indicate that domestication in cotton affects host plant choice in S. littoralis.

Session/symposium: Interspecific relationships/independent presentation

Hammerbacher A.\*, Wingfield B.

Chemical defence responses of Australian Acacia trees to infection by Ceratocystis species

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Australian Acacia mearnsii and A. manginum are considered highly productive timber and tannin-producing plantation trees in the southern hemisphere. However, these trees are extremely susceptible to infections by Ceratocystis albifundus and C. manginecans respectively, which both cause high losses in tannin, wood and pulp production. The aim of this study was to determine how Acacia trees defend themselves against infections by these pathogens and the basis for their susceptibility. Artificial inoculation of young saplings from both tree species was conducted. Analysis of the defence hormones produced in response to fungal colonization revealed that both tree species produce adequate levels of the plant defense hormone jasmonic acid-isoleucine, which is an early signal for initiating defence mechanisms against infection by necotrophic pathogens. However, tannin levels in both tree species were declined after infection. On the other hand an increase in flavonoid concentrations in infected saplings was observed. In an in vitro assay both fungi grew significantly faster on media amended with extracts from infected trees compared to growth on extracts from uninfected trees. The fungi are clearly modulating their hosts' defense responses to increase their susceptibility and to decrease their tannin levels. Further research will elucidate the mechanism by which C. albifundus and C. manginecans manipulate their hosts to down-regulate tannin biosynthesis.

Session/symposium: Interspecific relationships/independent presentation

Harari A.<sup>1\*</sup>, Soroker V.<sup>1</sup>, Gonzalez-Karlsson A.<sup>1</sup>, Golov Y.<sup>1</sup>, Jurenka R.<sup>2</sup> Evidence for resistance to mating disruption technique in the pink bollworm a pest of cotton

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The pink bollworm, Pectinophora gossypiella (Gelechiidae) is a key pest of cotton worldwide. In Israel mating disruption (MD) is used successfully to control the pest in all cotton fields since the early 1990s. However, in recent years a significant increase in the pest population was observed. We studied the hypotheses that: (a) a change in the male's response to the ratio of sex specific pheromone has evolved, and (b) a change in the population mean in the pheromone amount and/or in its ratio of components have occurred. We tested both hypotheses using an old established population that was never exposed to MD and a newly field collected population that experienced pheromone (MD) environment. We compared the male response to each of the two pheromone components and both components combined. We analyzed females' glands of the two populations for their amount of pheromone and ratio of its components. In an olfactometer, we tested the male preference to females of their own population vs females of the other population and also tested male attactance to females of both populations under clean air and MD environment. We found a significant deviation from the pheromone population mean in the glands of MD experienced females. We also found that although males of the two populations were similarly attracted to the pheromone components, in clean air males preferred females of their own populations. Under MD environment males of both populations were attracted significantly more to MD experienced females. We believe that our results are first to demonstrate the evolution of "resistance" to mating disruption technique due to a deviation of the female produced sex pheromone away of the population mean, as is represented in the synthetic pheromone that is used for MD.

Session/symposium: Intraspecific relationships/Importance of multicomponent mixtures for chemical cues

**ORAL PRESENTATION** 

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<sup>&</sup>lt;sup>2</sup>Department of Entomology, Iowa state University, Ames, Iowa.

Haverkamp A.<sup>1</sup>, Bing J.<sup>2</sup>, Li X.<sup>2</sup>, Baldwin I.T.<sup>2</sup>, Hansson B.S.<sup>1</sup>, Knaden M.<sup>1</sup>, Yon F.<sup>1,3</sup>\* Floral trait variations adapt to a different range of pollinators or the lack of them

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Many plants adapt their flowers (form, scent and reward) to attract pollinators and some at a particular time of the day. Even so, in some areas it is hard for a plant to get successfully pollinated during consecutive days due to environmental conditions. In its natural range the wild tobacco Nicotiana attenuata developed various floral phenotypes possibly towards different pollinator communities and abundances. N. attenuata has a broad range in the southwest USA across altitudes but similar arid environments. In the lowlands many studies shown that this plant is outcrossed by the hawkmoth Manduca sexta, and its local floral display is synchronized with the hawkmoth's night activity such as flower opening and strong floral scent emission. In this study, floral traits from 4 populations (2 pollinated by hawkmoth) were characterized and tested for its attractiveness and pollination by hawkmoths. Despite flower size and scent emission differences, these flowers were approached equally, but differed in the time the hawkmoth spent on the flower. This difference is associated with the amount of the volatile benzyl acetone, which can function as a probing enhancer. The nectar reward was similar in caloric content but not in volume, nor in their sucrose proportion, thus not yielding a differential incentive. Flowers at higher altitudes had a stigma deeper into the corolla tube which would facilitate the selfing by pollen dropping. Also, these flowers presented a trend towards higher seed output by selfing and by hawkmoth pollination unlike lower altitude plants. The evidence suggests that in the absence of pollinators the plants will easily produce self-capsules, and likewise be more receptive to opportunistic outcrossing by a hawkmoth, unlike the lower altitude which could be considered as more selective with the received pollen. No matter chemical or form adaptations, plants can always go back to readapt to the pollinators absence.

Session/symposium: Interspecific relationships/independent presentation

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An external attractant trap for the small hive beetle (*Aethina tumida*), a pest of European honeybees

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The Australian honeybee industry contributes over \$101 million annually to the Australian economy through honey products, and an estimated \$1.7 billion through pollination services to crops and horticulture. European honeybees (Apis mellifera) (Hymenoptera: Apidae) are threatened by a variety of pests and diseases, including the exotic small hive beetle Aethina tumida Murray (Coleoptera: Nitidulidae). This beetle is a significant pest in Australia and the USA. The larvae are the destructive life-stage, causing damage to hives when they feed on bee brood and pollen stores. Small hive beetles carry a yeast (Kodamaea ohmeri) (Ascomycota: Saccharomycotina) which is primarily responsible for the fermentation of hive products associated with beetle larval development. Our GC-MS analyses of these fermenting hive products found that they consist of typical fermentation volatiles, many of which are known insect attractants. A range of in-hive control options are used by beekeepers, but there is additional need for out of hive control measures such as an external attractant trap that can stop the beetles entering hives, using a lure based on these fermentation odours. We used GC-MS and behavioural bioassays to identify the attractive components of fermenting hive products, which were then tested in the laboratory and field to develop an attractant lure. A simple yeast fermentate was used to study the impact of environmental conditions on beetle hive-finding behaviour in the field to develop targeted trapping for beekeepers, reducing the need for year-round monitoring and control. An external attractant trap will also be useful for biosecurity as a surveillance tool for monitoring small hive beetle in areas of uncertain infestation or to demonstrate area freedom in areas where they are not yet known to occur.

Session/symposium: *Practical applications/independent presentation*ORAL PRESENTATION

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Hermann S.\*, Landis D.

# Flying into the face of Fear: predator cues influence aphid development and behavior

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Understanding how insect predators alter prey abundance through direct consumption is critical to their population dynamics. However, prey can also adjust their behavior and physiology to avoid predation. For example, some prey 'eavesdrop' on predator cues and disperse to avoid attack before an encounter occurs. Impacts of predators on prey biology that do not involve direct consumption are termed non-consumptive effects (hereafter, NCEs). A fuller understanding of how NCEs alter predator-prey dynamics is particularly needed in agriculture where managers seek to manipulate these interactions for enhanced pest suppression (Hermann and Landis 2017). In our study we evaluate the influence of predator direct and indirect cues on aphid behavior, performance and development. We find that aphids significantly influenced by predator NCEs and that this carries significant implications in fundamental biology and agroecological pest management strategies.

Session/symposium: *Interspecific relationships/Semiochemistry of aphidophagous insects*ORAL PRESENTATION

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Identifying cuticular hydrocarbon signatures that mediate chemical mimicry of host aphids by Lysiphlebus parasitoids

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Parasitoids in the genus *Lysiphlebus* mimic the cuticular hydrocarbon (CHC) profiles of their aphid hosts to avoid detection by aphid-tending ants. The need to efficiently mimic hosts raises questions about how these wasps adapt to novel hosts in ecological or evolutionary time. Yet, the mechanisms underlying mimicry in this system remain largely unexplored.

There is evidence of genetic differentiation among *Lysiphlebus* wasps (*L. fabarum* group) collected from different hosts; however, no previous work has explored variation in the CHC profiles of wasps parasitizing different aphid species. We collected *Lysiphlebus* wasps from different aphids in the field and identified key features of their CHC profiles, including many methyl-branched alkanes, that were consistently more similar to the profile of the host aphid than to that of other aphid species.

To explore how genetic and host-derived factors influence wasp CHC profiles, we reared asexual wasp lines on different aphid species than those from which they were collected. This experiment revealed that the relative proportions of linear alkanes are influenced by genetic factors, while some methyl-branched alkanes—which are less abundant but may play an important role in signaling—exhibit patterns matching those of the host aphid.

All in all, these results suggest that methyl-branched alkanes acquired from host aphids play a crucial role in mimicry by *Lysiphlebus* wasps. Interestingly, however, the field and laboratory experiments identified different subsets of these compounds as important predictors of the aphid host from which a given wasp emerged, suggesting that somewhat different processes influence wasp CHC profiles in these settings. Ongoing work on this system will provide further insight into the mechanisms by which *Lysiphlebus* wasps mimic their aphid hosts and the implications of the mimicry system for host specificity.

Session/symposium: Interspecific relationships/independent presentation ORAL PRESENTATION

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Hettiarachchi D.K.<sup>1</sup>\*, Jackman S.D.<sup>2</sup>, van Koten C.<sup>2</sup>, Sullivan J.<sup>3</sup>, Cripps M.G.<sup>2</sup>, Rostás M.<sup>1</sup> Tripartite interaction between phytophagous *Cassida rubiginosa* and two fungal biocontrol agents on *Cirsium arvense* (Californian thistle): a plant volatile perspective

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In biological control, several herbivore and pathogen species are often released against the same target without knowing how these biological control agents interact. In New Zealand, two fungal pathogens (Puccinia punctiformis, Sclerotenia sclerotiorum) and the chrysomelid beetle Cassida rubiginosa are used to control Cirsium arvense. We investigated the compatibility of these biocontrol agents by studying the olfactory behaviour of adult C.rubiginosa towards fungus-infected thistles. A series of olfactometer experiments and dual-choice feeding experiments were conducted. Adult beetles were given the choice between healthy thistle leaves and leaves infected by one of the pathogens. Volatiles were collected and analyzed using GCMS. The beetles' attraction towards *C. arevense* was reduced when infected with the biotrophic P.punctiformis (P = 0.0001) while the necrotroph S. sclerotiorum had no effect (P = 0.232). Both pathogens however reduced leaf consumption. Feeding on the infected leaves was confined to infection-free areas. Volatile profiles showed a unique blend of compounds where the P.punctiformis infected thistles were characterized by the emission of benzenoids and indole, S. sclerotiorum infected plants by green leaves volatiles and healthy *C. arvense* by terpenoids. Kluth et.al. (2002, 2001) reported detrimental effects of *P. punctiformis* infection on *C. rubiginosa* larval development and host choice. Our study complements these findings by showing that adult beetles recognize rust infection by its typical scent and consequently avoid such low-quality plants. We speculate that the beetles were deterred by benzenoids and indole. The study highlights the complexity of the interactions between biocontrol agents. Knowing the underlying mechanisms may help to improve the success of weed biocontrol.

Session/symposium: Interspecific relationships/independent presentation

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Hill S.R.<sup>1</sup>\*, Zaspel J.<sup>2,3</sup>, Ignell R.<sup>1</sup>

What makes a blood sucker? Vampire moth, *Calyptra thalictri*, chemosensory receptor gene expression reflects blood-feeding phenotype

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The mechanisms by which blood feeding in insects has evolved are unclear, primarily because there has been no access to species in which there is a mixture of same-sex blood feeding and non-blood feeding individuals. The discovery of a subset of male *Calyptra thalictri* (Lepidoptera: Noctuidae: Calpini) that blood feed, while the majority of these males do not, provides a unique opportunity to investigate members of the same species for potential root mechanisms leading to the ability to blood feed. *Calyptra thalictripopulations* revealed no morphological differences in the classical structures used for species identification in individuals that took a blood meal compared with those that did not. However, a dimorphism exists in the number of antennal sensilla coeloconica, which are sensitive to vertebrate-related compounds, between individuals of male *C. thalictrithat* took a blood meal and those that did not. Here, we describe the antennal transcriptomes of wild caught adult male *C. thalictri*, as well as annotating and investigating the differential expression of chemosensory-related genes among males, including those demonstrating the vertebrate host-seeking phenotype and those which do not, in a Y-tube olfactometer.

Session/symposium: Interspecific relationships/Arthropod chemoreceptors
ORAL PRESENTATION

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Hillier N.K.

# **Evolution of Heliothine moth pheromone diversity**

Biology, Acadia University, Wolfville, NS, Canada

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

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

Session/symposium: Interspecific relationships/independent presentation

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Optimisation of *Hermetia illuscens* reproduction according to appropriate organic compounds

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Insects are frequently thought to be an important source of proteins for human food but also to feed animals such as poultry and fish. Many advantages are cited to rear insects: limited needs in space, efficient conversion rates from organic wastes, short developmental duration and high reproductive performances. One species, namely Hermetia illucens also called the Black Soldier Fly (BSF) is a model due to its ability to feed on a broad range of organic materials. To be able to produce very large amounts of fly larvae continuously, it is important to ensure optimal development and reproduction. For the latter, not only physical aspects of the strucrure is important for the female to lay their eggs. Indeed, the kind and abundance of organic materials and relative organic compounds are important. Here, the opimisation of BSF reproductive parameters was developped in order to maximise the number of eggs and the development of first instar larvae. Different plant but also house cooking residues were used as oviposition stimulant in BSF adult cages to assess the egg production of adult flies. After assessing the egg abundance, firts instar larvae behaviour was followed to assess the attractivity of the different organic wastes. Volatile analysis by gas chromatrography coupled with mass spectrometry were performed to identify the most attractive patterns related to the most efficient organic material to induce reproduction and larval searching efficiency.

Keywords: organic waste, ovipostimulant, VOCs, black soldier fly

Session/symposium: Practical applications/independent presentation

## Horváth G.

Polarization-based (mis)orientation of insects: water detection, host finding, polarized photopollution and ecological traps

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Many insect species perceives not only the intensity and colour (spectrum) of light, but also the direction of oscillation of the electric field vector of light (polarization), if its degree of polarization is high enough. Insects use their polarization sensitivity for orientation on the basis of sky polarization, to detect water and host animals, for example. In the talk I present natural and artificial polarization patterns measured by imaging polarimetry in the optical environment, and show how insects can use these patterns for orientation, as well as water and host detection. Artificial polarizers, such as asphalt roads, shiny black plastic sheets, glass panes and solar panels, for instance, can reflect highly and horizontally polarized light which can deceive, misorient and attract polarotactic, water-seeking, flying aquatic insects. I demonstrate with some case studies that such man-made polarizing surfaces are strong sources of polarized light pollution. This new kind of ecological photopollution can induce polarization ecological traps for polarotactic insects. I demonstrate how polarized-light-polluting black objects can be the basis of new polarization traps for insects, especially horseflies (tabanids).

Session/symposium: Intraspecific relationships/Complementary or predominant role of nonchemical based communication and orientation of insects

KEYNOTE LECTURE Hou X.<sup>1</sup>, Zhang D.D.<sup>1</sup>, Yuvaraj J.K.<sup>1</sup>, Corcoran J.<sup>1,2</sup>, Löfstedt C.<sup>1</sup>

Deorphanization of olfactory receptors in the moth *Eriocrania semipurpurella*: comparative studies of the *Xenopus* oocyte and HEK293 cell systems

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The Xenopus oocyte and the Human Embryonic Kidney (HEK) 293 cell expression systems have been used for deorphanization of pheromone receptor (PR) genes from a number of moth species. However, their inherent characteristics differ in several aspects, which raises the question of whether the different systems provide comparable results. Yuvaraj et al (2017) identified five candidate PR genes in the antennal transcriptome of the primitive moth Eriocrania semipurpurella and tested the response profiles of the receptors in HEK cells. In the current study, we re-examine the response profiles of candidate PR genes in the Xenopus oocyte system. In both systems, EsemOR3 responded stronger to the pheromone component (S,Z)-6-nonen-2-ol than to its enantiomer (R,Z)-6-nonen-2-ol, a second pheromone component. Conversely, EsemOR4 responded stronger to (R,Z)-6-nonen-2-ol than to (S,Z)-6-nonen-2-ol in the oocytes. EsemOR4 didn't respond to any tested compound in HEK cell system although it is highly expressed in the antennae1. EsemOR3 was also sensitive to β-caryophyllene whereas EsemOR4 was not. In both systems, EsemOR1 specifically responded to  $\beta$ -caryophyllene and EsemOR5 was broadly tuned to (S,Z)-6-nonen-2-ol, (R,Z)-6-nonen-2-ol and (Z)-6-nonen-2-one. EsemOR6 showed no response to any tested compound in either system. We compare the results obtained in the two different heterologous systems with activity recorded in vivo as well as expression patterns based on transcriptome data and in situ hybridization. In spite of overall similar results, conclusions about the function of individual receptor candidates may differ depending on the system used for deorphanization.

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Session/symposium: Interspecific relationships/Arthropod chemoreceptors

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Attraction of Drosophila suzukii to its symbiotic yeast strains

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Drosophila flies rely on microorganisms such as natural yeast symbionts for food and development. This close interaction with symbionts provide an opportunity for developing novel species-specific pest management strategies such as improved monitoring and detection. Here, we compared attractiveness of 6 yeast strains against Drosophila suzukii in the laboratory and field. Hanseniaspora uvarum and Pichia terricola are symbiotically associated with D. suzukii; Candida zemplinina and C. californica are associated with Drosophila melanogaster; Pichia kluyveri is hosted by both species; and Saccharomyces cerevisiae is the baker's yeast, a main component of yeast-sugar bait but rarely found with natural populations of Drosophila species in the field.

In 2-choice tests where attraction of each yeast strain grown on a PDA media was tested against the PDA without the yeast, *D. suzukii* was highly attracted to *H. uvarum*, the most abundant yeast strain found in its yeast flora. Surprisingly, *D. suzukii* was also attracted to *C. zemplinina*, a yeast strain associated with *D, melanogaster*, but not attracted to *S. cerevisiae*, *P. kluyveri*, *P. terricola*, and *C. californica*. When 6 yeast strains along with a PDA media were given to *D. suzukii* simultaneously, *H. uvarum* and *C. zemplinina* were the most attractive, followed by *S. cerevisiae* and *P. terricola*. *P. kluyveri* and *C. Californica* were the least preferred. In the cherry orchard, traps baited with *H. uvarum* captured the highest numbers of males and females among all the yeasts tested, followed by *C. zemplinina* and *S. cerevisiae*. These results indicate *D. suzukii* is capable of discriminating yeast species.

Session/symposium: Interspecific relationships/independent presentation

Hussain S., Wan Y., Wu Q.\*

Tomato spotted wilt virus infection mediates mating behavior in its western flower thrips vector, *Frankliniella occidentalis* (Pergande)

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Plant viruses can modulate the behavior of their insect vectors to enhance viral transmission and spread. Previous studies have shown that *Tomato spotted wilt virus* (TSWV) can influence feeding and preferential behavior of its vector *Frankliniella occidentalis* (Pergande). In the present study, four cross treatments were designed that consisted of paired TSWV-infected and uninfected male and female thrips. We found that TSWV infection caused the males to be more active than the females, indicated by a higher male harassment rate. Although copulation duration was significantly higher in the TSWV-infected treatments, virus infection significantly decreased the female re-mating frequency in viruliferous *F. occidentalis*. It is possible that TSWV infection may affect the ability of males to produce aggregated pheromones, which decreases mating.

Session/symposium: Intraspecific relationships/independent presentation

Jaccard C.1\*, Arce C.M.2, Benrey B.1

The consequences of squash domestication on chemical defenses and insect performance

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Plant domestication is assumed to result in reduced levels of defensive compounds in crops. A reduction in plant defensive traits is often expected to result in increased insect performance. However, recent studies have shown that this pattern is not ubiquitous.

The reason for this may lie in the purpose or the organ targeted by domestication. Such that, a positive relationship between reduction in plant defense and increased insect performance will be only, or more evident for cultivars and plant organs that have been selected for consumption. We tested this hypothesis by investigating the relationship between the chemical defense and insect performance for two squash varieties selected for two different purposes, for fruit consumption and as ornamental. The roots of these two varieties were analyzed for their content in cucurbitacins, known to be toxic to generalist herbivores. In parallel, we conducted laboratory assays to determine the preference and performance of larvae of the generalist herbivore *Diabrotica balteata* on these plants.

We found partial support for our hypothesis. No cucurbitacins were found in the roots of the variety selected for fruit consumption, while a high content was detected in the ornamental variety. Larval performance was not negatively affected by the concentration of cucurbitacins. Moreover, choice experiments revealed that larvae selected roots with high cucurbitacin content. These results suggest that *D. balteata* is adapted to the cucurbitacins present in the roots.

We conclude that although domestication has reduced the cucurbitacin content in the variety selected for consumption, this reduction does not result in increased insect performance of this generalist herbivore. Purpose-specific effects of plant domestication on plant defenses can be expected for other squash varieties and other crops as well.

Session/symposium: Interspecific relationships/independent presentation

Jacquin-Joly E.<sup>1</sup>\*, Meslin C.<sup>1</sup>, De Fouchier A.<sup>1,2</sup>, Mainet P.<sup>1</sup>, Walker III W.<sup>3</sup>, Hansson B.<sup>4</sup>, Larsson M.<sup>3</sup>, Montagné N.<sup>2</sup>.

Diversity of odorant and gustatory receptors in the moth *Spodoptera littoralis*: towards understanding what makes a polyphagous species a pest.

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In the recent years, the development of high-throughput sequencing technologies has highlighted the diversity of insect chemosensory receptors (odorant receptors: ORs, and gustatory receptors: GRs). In the cotton leafworm *Spodoptera littoralis*, a polyphagous crop pest found in all the Mediterranean Basin, Africa and West Asia, transcriptomic and genomic data led us to the identification of the whole repertoires of such ORs and GRs.

As expected for a Lepidoptera, the OR repertoire consisted of ~70 ORs, a number close to what has been found in other species. Part of this repertoire has been functionally characterized in *S. littoralis* using heterologous expression in *Drosophila* antennae coupled to electrophysiology, approaching for the first time the odor space of a Lepidoptera. Noteworthy, we found basic conservation of function within the receptor repertoire of Lepidoptera, across the different major OR clades.

Unexpectedly, the GR repertoire consisted of more than 250 GRs, a number much higher than those previously published in monophagous and oligophagous lepidopteran species (~70). Together with recent GR annotation in polyphagous noctuids revealing as well expansions of GR numbers, we propose that polyphagy is linked to bitter compound receptor duplications. Further GR functional studies are now needed to verify this hypothesis.

Session/symposium: Interspecific relationships/Arthropod chemoreceptors
ORAL PRESENTATION

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Identification of ant deterrents emitted by insect cadavers infected with entomopathogenic nematodes

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Among the vast variety of parasitic nematodes, some have evolved an association with pathogenic bacteria that together parasitize insects. The parasitic stages of these so-called "entomopathogenic nematodes" (EPNs) cannot survive outside of the cadavers and it is therefore essential that the cadavers remain intact throughout EPN lifecycles. The nematode/bacterium complex appears to protect the cadavers by producing deterrents, which prevent that the cadavers are eaten by scavengers. We extracted the surface compounds from larvae of the wax moth, *Galleria mellonella*, that were either healthy, freeze-killed, or infected by EPN. The repellency of these extracts to the ant *Lasius niger* was tested in laboratory assays. The extracts of EPN-infected larvae were found to be highly deterrent to the ants. Using gas chromatography coupled to a mass spectrometer (GC/MS) we identified two of the dominant compounds that were unique to the extracts of EPN-infected larvae. These were found to indeed be repellent and to show an additive effect when tested in combination. Whether these deterrent compounds are produced by the nematodes or by the bacteria remains to be determined.

Session/symposium: Interspecific relationships/independent presentation ORAL PRESENTATION

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## Janz N.

# A race or a chase? Revisiting Ehrlich and Raven in light of Tibor Jermy

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Ehrlich and Raven's paper on coevolution influenced work of plant-insect interactions for decades to come. Especially in the decades following its publication, its impact was pervasive. Tibor Jermy challenged this established view, starting a debate that hasn't quite settled yet. In retrospect, I believe that there were two main accomplishments of Jermy's paper. It gave us an alternative perspective to the escape-and-radiate scenario – suggesting a much more asymmetrical view of the interaction – but it also forced scholars in the field to focus on the mechanisms that shape the interaction. I will show how Jermy's "devil's advocate approach" has influenced my own view of how the insect-plant interaction evolves. I argue that there are indeed some fundamental ways in which this interaction is asymmetrical, and interestingly, some of this asymmetry is embedded also in the original coevolutionary scenario that Ehrlich and Raven proposed. I will show why I think the issues at the heart of this argument are still relevant today, and how some of them may be resolved.

Session/symposium: Interspecific relationships/Tibor Jermy's Legacy in insect-plant evolution

ORAL PRESENTATION

Jiang H.<sup>1,2</sup>, Xu L.<sup>1,2</sup>, Wang J.<sup>1,2</sup>\*

Screening of the odorant receptors responsible for methyl eugenol perception in the oriental fruit fly, *Bactrocera dorsalis* 

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The oriental fruit fly, Bactrocera dorsalis (Hendel), is one of the most devastating pests causing substantial economic damage to the fresh vegetables and fruits worldwide. The most effective way to control the fruit fly is luring and killing. The most effective commercial component of attractant is methyl eugenol (ME), but the molecular mechanism of the olfactory perception remains unknown. Here, we therefore attempted to identify the odorant receptors (ORs) responsible for ME in Bactrocera dorsalis. We annotated the 44 OR-like genes of the Bactrocera dorsalis based on its genome information. Furthermore, we investigated their temporal and spatial expression profiles. In addition, we determined their expression profiles of the male flies exposed to 5% ME for 5 hours to screen out 14 candidate OR-like genes responsible for ME perception in terms of the down-regulated expression. In order to further confirm the binding capability of the candidate ORs with ME, the live calcium imaging using HEK cell expression system as well as and voltage clamp recording in Xenopus oocytes. Besides, genome editing tool such as CRISPR/Cas9 is going to be employed to knock out the essential ORs to measure the olfaction guided behaviors in vivo. We expect to identify odorant receptors responsible for the ME perception in this notorious fly. Our results will not only reveal the molecular basis of ME perception in B. dorsalis, but also lay a solid foundation for the development of novel effective attractant targeting key ORs in this fly.

Session/symposium: Interspecific relationships/Arthropod chemoreceptors

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Genesis of *Ips typographus* aggregation pheromone production: early phases, sensitivity to JH III

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European spruce bark beetle (*Ips typographus*), (Coleoptera, Scolytinae) is the main bark beetle pest on Norway spruce in Europe, where it cause a large calamity in spruce monocultures. Male aggregation pheromone (a synergistic mixture of methyl-3-buten-2-ol (MB) and cis-verbenol (cV)) is produced in bark beetle hindgut. Experiments made in 90's showed the partial evidence that MB is synthetized *de novo* (Lanne 1989), while cV is created by conversion of host monoterpene α-pinene (Lindström 1989). While the biosynthesis and its regulation of the terpenic pheromone components of another bark beetles (*Ips, Dendroctonus*) were extensively studied (Tittiger&Blomquist 2016), the overall physiological knowledge of that process in *I. typographus* is still missing. Even though there are expected many principles to be similar, we decided to fill gaps in knowledge for this economically important specie. Later we plan to extend study with the molecular biology aspects of the pheromone biosynthetic pathway and its regulation cascade. Though massive pheromone production in *Ips typographus* males is associated with the early phases of host tree colonization before mating, there exist an indirect evidence that beetles can synthetize pheromone already before entering host bark (Birgersson 1984).

In the pilot study we focus on physiological factors that influence the first phases of pheromone production in teneral beetles, and the regulation role of juvenile hormone III in this process.

The EU project ""EXTEMIT - K"", No. CZ.02.1.01/0.0/0.0/15\_003/0000433 financed by OP RDE.

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Session/symposium: New chemical structures/Invasions under the bark: chemical ecology of woodboring beetles

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Jousselin E.

Phylogenetic approaches to study host-plant driven speciation in phytophagous insects: insights from fig wasps and aphids

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During the last two decades, ecological speciation has been a major research theme in evolutionary biology. Ecological speciation occurs when reproductive isolation between populations evolves as a result of divergent selection, arising from niche differences (Rundle & Nosil 2005; Schluter 2009). Phytophagous insects represent model systems for the study of this evolutionary process. The host-plants on which these insects feed constitute ideal agents of divergent selection for these organisms. Adaptation to feeding on different hostplant species can potentially lead to ecological specialization of insect populations and subsequent speciation (Nosil et al. 2002). This process is often thought to have given birth to the astonishing diversity of phytophagous insects. It plays a central role in the macroevolutionary scenarios of insect diversification that have been put forward in the literature (i.e. "Escape and Radiate", "Cospeciation", "Oscillation hypothesis", "Musical Chairs"). Numerous phylogenetic studies on herbivorous insects aim at testing the predictions of these scenarios and deciphering whether speciation driven by host-plant adaptation is the main process behind the diversification of the groups under investigation. I will present here the results of my own phylogenetic investigations on two model systems: fig wasps (Hymenoptera: Agaonidae) and aphids (Hemipera: Aphididae). I will discuss whether the results fit the predictions of the different macroevolutionary scenarios and present perspectives that aim at integrating diverse sources of data in a phylogenetic context to investigate radiation driven by interaction with hostplants.

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Session/symposium: Interspecific relationships/Tibor Jermy's Legacy in insect-plant evolution

**ORAL PRESENTATION** 

Junker R.R.

Integrating information on biosynthetic pathways into the statistical analysis of secondary metabolite profiles

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Plants synthesize a plethora of metabolites that have various functions in plant physiology and in interactions with other organisms. Chemical analytical techniques identifying secondary metabolites produced by plants and other organisms compile huge data sets that require meaningful statistical analyses to extract relevant information. The shared biosynthesis of many compounds within compositions of secondary metabolites, however, violates the assumption of independence between the compounds, which is not acknowledged in common statistical approaches. To account for the autocorrelative nature of plant metabolites as a consequence of biosynthetic constraints, I recently integrated information on the biosynthesis of compounds into statistical analyses of metabolite profiles (Junker 2018; Junker et al. 2018). I will demonstrate that the integration of biosynthetic pathways into the statistical treatment of secondary metabolite profiles provides novel information about the structure of and similarity between secondary metabolite profiles. The examples presented show that the acknowledgement of biosynthetic pathways and constraints represents an conceptual advancement for studies in chemical ecology.

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Session/symposium: Practical applications/independent presentation

Kaan Alkan M.<sup>1,2</sup>\*, Pfrang C.<sup>1</sup>, Girling R.<sup>2</sup>

Detailing the chemical dynamics of elevated ozone on floral VOCs using a *Brassica* napus model system

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Several studies have shown that tropospheric pollutants such as nitrous oxides (NOX) and ozone (O3) have the potential to disrupt Volatile Organic Compounds (VOCs) used in chemical communication. To date, whilst several studies have investigated the ecological aspects of communication disruption, few studies have investigated in detail the underlying chemistry of the interactions between VOCs and tropospheric pollutants.

A selection of model 'floral' VOCs (Sabinene, cis-3-hexenol, cis-3-hexenyl acetate, 1-dodecene, 1-undecene,  $\Delta$ -3-carene) derived from a model plant system (*Brassica napus*) were used in combination with ozone to investigate the underlying chemistry that may result in the disruption of chemo-communication. Relevant chemical vectors were recorded, such as rates of reaction using a gas chromatograph-flame ionisation detector (GC-FID), the identity of reaction products using gas chromatography-mass spectrometry (GC-MS) and rates of Secondary Organic Aerosol (SOA) formation using a scanning mobility particle sizer (SMPS).

At atmospherically relevant stoichiometries, our results indicate increased SOA yields and decreased atmospheric lifetimes in the presence of increased ozone levels. Several gaseous reaction products have also been identified for all of the model VOCs.

These findings support literature evidence that the atmospheric concentration of certain VOCs may decrease significantly as a result of atmospherically relevant increases in ozone concentration. As a consequence, less odour information will be available to pollinators to utilise in their foraging for floral resources, with potential negative effects for both pollinators and the plants they pollinate.

Session/symposium: Interspecific relationships/independent presentation

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The attraction of the Eurasian spruce bark beetle (*Ips typographus*) to its fungal associates is mediated by their unique volatile bouquets

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Many insects exhibit symbioses with other organisms through signaling compounds which drive the evolutionary maintenance of mutualistic associations. Signaling is well studied in plant-insect and plant-microbe mutualisms but little is known about the role of chemical signals in insect-microbe interactions. The invasive European bark beetle Ips typographus is the most serious killer of Norway spruce (Picea abies) in Europe and has a symbiotic relationship with ophiostomatoid fungi such as Endoconidiophora polonica, Ophiostoma bicolor, Grosmannia penicillata and Grosmannia europhioides. The basis of this symbiosis is not known, but it is thought that the fungi detoxify host defense compounds, supply vital nutrients to increase bark beetle fitness and substantially contribute to host tree mortality. The beetle, in return, facilitates fungal transmission from tree to tree. Here we tested the hypothesis that volatile organic compounds emitted from different fungal symbionts could act as signaling cues for bark beetles to recognize and distinguish its associated microbial community. Behavioral experiments with different fungi showed that adult I. typographus is highly attracted to food sources colonized by E. polonica, G. penicillata and G. europhioides, but not to O. bicolor and O. piceae. Through detailed GC-MS analysis, we identified several de novo synthesized volatile organic compounds from ophiostomatoid fungi. Testing these fungal compounds on beetle antennae using single sensillum recording revealed new olfactory sensory neuron classes specialized for detecting these de novo fungal volatiles. These findings collectively indicate that volatile compounds produced by fungi may act as recognition signals for bark beetles to maintain specific microbial communities with high accuracy which might impact on bark beetle fitness.

Session/symposium: Interspecific relationships/Insect-microbe interactions ORAL PRESENTATION

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The sweet smell of decay: bark beetles are attracted to volatile by-products produced during detoxification of host defenses by their associated fungi

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The European bark beetle *Ips typographus* is the most serious killer of Norway spruce trees (Picea abies) in Europe. The ophiostomatoid fungi Endoconidiophora polonica, Ophiostoma bicolor, Grosmannia penicillata and Grosmannia europhioides are frequently isolated from the beetle and attacked trees and thus are thought to have a close association with this insect. However, the basis of this association is yet not known. Norway spruce produces a formidable array of chemical defenses to ward off attacks by beetles and fungi, including terpenoid oleoresins and phenolic compounds. In the absence of their fungal associates, these chemical defenses were deterrent to bark beetles and altered their feeding behavior. However, when fungi were introduced, beetles chose high levels of defense metabolites in their diet. Through chemical analysis, we observed that the volatile profile emitted by spruce bark changed dramatically after infection by ophiostomatoid fungi. Targeted analysis revealed that the fungi transformed host-derived metabolites to small aromatic compounds as well as to oxygenated monoterpenoids. Using single sensillum recordings, we identified several new types of olfactory sensory neurons (OSNs) in bark beetles which were specific for fungal biotransformation products. Olfactometer assays with adult beetles revealed that the insects preferred spruce bark diet colonized by four of their five most frequent fungal associates. Taken together, this study shows that bark beetles may use fungal catabolites of host defense metabolites to accurately select their microbial symbionts, which detoxify host chemical defenses.

Session/symposium: Interspecific relationships/Volatile-mediated microbe-plant interactions ORAL PRESENTATION

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# Natural olfactory landscapes and pollination networks

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The angiosperm flower represents an excellent communication device enabling plants to interact with other organisms via visual and olfactory channels. The fundamental relationship between the diverse airborne chemical cues of plants and the behavior of arthropods has a prominent position in ecological research and is the basis for a wide range of applications that sustain human population. However, attempts to unravel the complex association patterns between plant semiochemicals and arthropod behavior in entire natural communities are currently lacking, as are community-wide datasets of plant volatilomic profiles. To address this gap, we collected and analyzed the spring floral volatile profiles of 41 insectpollinated plants in a Mediterranean scrubland and investigated their role in shaping the plant–pollinator network of the community. By using a multidisciplinary statistical approach, we unraveled the role of floral sensory stimuli in visitation patterns; our results indicate those chemical properties of floral scent that are most influential in shaping plant behavior in the community. Moreover, we compiled and present a unique VOC-insect network in order to explore association patterns between pollinator species and individual compounds. Our approach reveals the ecological functionality of a natural olfactory landscape and offers a predictive framework for future investigations that will decipher the communication strategies of plants and their interacting partners.

Session/symposium: Intraspecific relationships/Importance of multicomponent mixtures for chemical cues

ORAL PRESENTATION

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Environmental gradients and the evolution of chemically-mediated tri-trophic interactions

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Long standing theory has predicted that plants associated with herbivores and carnivores should experience selection for increased plant defenses, such as the production of volatile organic compounds, which attract enemies of herbivores. Along elevation gradients a general pattern is that herbivores and carnivores are abundant at low elevation and progressively drop off at higher elevations. To determine whether plant adaptation along such a gradient can shape species interactions and ultimately trophic cascades, we manipulated carnivores such as soil-dwelling entomopathogenic nematodes (EPNs), herbivore pressure, and plant genotypes in a field common garden. Trophic cascade strength was significantly higher for low elevation plants, and this was mediated by changes in volatile emissions between low and high elevation plants, which resulted in differential EPN attraction. This study not only provides a first assessment of how ecological gradients modulate the strength of trophic cascades, but also demonstrates how habitat variation imposes selection on indirect plant defenses.

Session/symposium: Interspecific relationships/independent presentation

Khrimian A.<sup>1</sup>\*, Lancaster J.<sup>2</sup>, Tholl D.<sup>2</sup> Sesquipiperitol as key precursor in harlequin bug pheromone biosynthesis

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The harlequin bug,  $Murgantia\ histrionica$ , is a specialist pest of crucifers that uses two stereoisomers of 10,11-epoxy-1-bisabolen-3-ol, SSRS and SSRR, as a male-produced aggregation pheromone. It has recently been shown that an enzyme unrelated to plant and microbial terpene synthases (MhTPS) catalyzed conversion of (E,E)-farnesyl diphosphate to (1S,6S,7R)-1,10-bisaboladien-1-ol, or sesquipiperitol. RNAi-mediated knockdown of MhTPS mRNA confirmed the role of MhTPS in the biosynthesis of M. histronica pheromone. The presentation will focus on the identification of sesquipiperitol and determination of its absolute configuration, which was confirmed by synthesis.

Session/symposium: New chemical structures/independent presentation

Kihika R.M.\*, Torto B.

Volatile chemical cues modulating root herbivory in root knot nematode-pepper interactions

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Root-knot nematodes (RKNs; Meloidogyne spp.) are polyphagous endoparasitic plant nematodes whose infestation results in poor crop yields as they form galls that inhibit uptake of nutrients and water by the host plants. Their damage symptoms are non-specific and include chlorosis, wilting, stunted growth and distorted shoots. In Africa, crop production losses of 40-100% can be experienced in vegetable crops and smallholder farmers are often unaware of root knot nematodes or the damage they cause but recognize reducing yields. Root-produced chemical signals mediate host finding in the second-stage infective juvenile (J2). This presentation will highlight identification of the volatile organic compounds (VOCs) produced by the roots of pepper, Capsicum annum, that influence the host seeking behavior of Meloidogyne incognita J2. Using behavioral assays, we found that the J2s showed preference for the roots of three RKN-susceptible pepper cultivars (70-82%) than an accession (47%) over sand controls. Further chemical analysis followed by laboratory bioassays showed that a blend of five compounds – methyl salicylate (MeSA), α-pinene, limonene, tridecane, and 2-methoxy- 3-(1-methylpropyl)-pyrazine) common to the four varieties elicited positive behavioral responses. Of the five compounds, MeSA elicited the highest response while the rest of the compounds had intermediate to low effects. On the other hand, thymol present only in the accession induced negative responses. Addition of thymol to the 5-component blend and to an attractive pepper plant further demonstrated its repellent effect. We will discuss the implication of these findings and their potential application in the control of RKNs.

Session/symposium: Interspecific relationships/independent presentation

Kinsho T.<sup>1\*</sup>, Baba A.<sup>1</sup>, Miyake Y.<sup>1</sup>, Kutsuwada Y.<sup>1</sup>, Fujii T.<sup>2</sup> Synthesis of San Jose scale sex pheromone and its application in field mating disruption

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The San Jose scale (SJS), *Quadraspidiotus perniciosus* (Comstock), is a cosmopolitan and polyphagous pest, and its infestation makes crops unmarketable, and severe infestations can kill plants. Although the insecticide application against the crawlers in early spring is a well-known conventional control method, it is sometimes insufficient to control the scale, and the later generations are difficult to manage by insecticide spraying. Pheromone-based mating disruption (MD) has been considered as an alternative eco-friendly pest control method of this serious pest.

Female produced sex pheromone of SJS consists of three isomeric components, namely, 7-methyl-3-methylene-7-octenyl propionate, (Z)-3,7-dimethyl-2,7-octadienyl propionate and (E)-3,7-dimethyl-2,7-octadienyl propionate. Synthesis employing coupling reaction of the two C-5 building blocks enables the facile construction of the molecule and serves as an industrially scalable method in order to supply sufficient amounts of the active ingredients for actual application in the field.

Field mating disruption trials using tube-type dispensers have been conducted in Chile, Argentina and Japan since 2015. MD efficacy was demonstrated through suppression of the male trap capture and reduction of the crawler density.

Session/symposium: Practical applications/independent presentation

#### Kirk W.D.J.

## Thrips aggregation pheromones and their use in pest management

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Thrips (Insecta: Thysanoptera) are major worldwide pests that damage many crops, mainly by feeding and transmitting tospoviruses. They are very difficult to control because they are resistant to many insecticides and they hide away in inaccessible spaces. The use of aggregation pheromones offers several possible ways of managing them. In 2001, we identified the first thrips aggregation pheromone, which was in the western flower thrips (Frankliniella occidentalis). The male-produced headspace volatiles were identified as neryl (S)-2-methylbutanoate and (R)-lavandulyl acetate, but many questions remain about the functions of these two compounds. Commercial products are available that use the aggregation pheromone for monitoring and mass trapping. Similar male-produced volatiles have been identified in the flower thrips (Frankliniella intonsa) and the melon thrips (Thrips palmi). A project ('SAFARI'), involving researchers from the UK and Kenya, is under way to investigate the function of the aggregation pheromone of the bean flower thrips (Megalurothrips sjostedti). This will allow the development of ways to manage the pest and reduce the need for insecticides, including the development of mass trapping and lure-andinfect devices with the entomopathogenic fungus Metarhizium. Progress on this project will be reported.

Session/symposium: Practical applications/independent presentation

ORAL PRESENTATION

Kirkman W.<sup>1</sup>\*, Moore S.<sup>1,2</sup>, Peyper M.<sup>2</sup>, Marsberg T.<sup>2</sup>, Albertyn S.<sup>2</sup>, Burger B.<sup>3</sup>, Goddard M.<sup>2</sup>, Love C.<sup>1</sup>, Thackeray S.<sup>4</sup>

A novel method for inhibiting mating of the false codling moth on citrus in South Africa: 7-vinyl-decyl acetate

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Several years ago 7-vinyldecyl acetate 1 (7-VDA) was identified as a contaminant in a faulty batch of synthesised false codling moth (FCM), Thaumatotibia leucotreta, pheromone (E- and Z-8 dodecenyl acetate). Deeper investigation proved that 7-VDA was capable of preventing adult FCM males from locating virgin females. A novel polyethylene dispenser was developed, which allowed a consistent release rate of 7-VDA, comparable that of the female FCM pheromone from a commercial dispenser. Laboratory mating inhibition trials were conducted with virgin pairs of moths in plastic containers, with. Both FCM pheromone and 7-VDA showed the ability to reduce fecundity, an indication of reduced mating. Results were variable, but on average FCM pheromone appeared to be more effective. However, a subsequently tested combination of 7-VDA (5-10%) and FCM pheromone (90-95%) proved to be the most effective treatment, reducing fecundity by 69%. Trials were repeated using a modified protocol, designed to reduce variability, where females were removed from the plastic containers after 48 h and placed into petri dishes to record fecundity and fertility. Four replicates were completed, showing a similar reduction in fecundity (by 69%) with FCM pheromone, 7-VDA and 10% 7-VDA in FCM pheromone. However, the last two treatments completely eliminated fertility of eggs, whereas there was a small percentage of fertilized eggs with the FCM pheromone treatment. In field trials, four treatments were used: untreated control, FCM pheromone, 7-VDA (10%) + FCM pheromone (90%) and Splat-FCM. A total of 300 moths were caught in the untreated control, 17 in the FCM pheromone treatment, 10 in the 7-VDA + FCM pheromone treatment and 20 in the Splat treatment. Infestation was monitored during January and February. The field trials will be repeated as infestation data did not concur with trap data.

Session/symposium: Practical applications/independent presentation

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Kirwa H.K.<sup>1,2</sup>, Murungi L.K.<sup>2</sup>, Coyne D.<sup>3</sup>, Beck J.J.<sup>4</sup>, Torto B.<sup>1</sup>\*

Responses of the root knot nematode *Meloidogyne incognita* to specific metabolites identified in the root exudate of *Solanum lycopersicum* 

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Plant roots release chemicals into the rhizosphere, which may interact with a wide range of organisms including root knot nematodes (RKNs). RKN species of the genus Meloidogyne are known to utilize some of these volatile and non-volatile chemicals in host location. In Africa, the most damaging RKN is Meloidogyne incognita, and its natural host plants include the Solanaceae species tomato Solanum lycopersicum and pepper Capsicum annum. Currently, the tomato root exudate compounds 2, 6-di-tert-butyl-p-cresol, L-ascorbyl 2, 6dipalmitate, dibutyl phthalate and dimethyl phthalate have been reported to influence the behavior of J2s of M. incognita. In the present study, the root exudate of the tomato cultivar Cal J, widely grown in Kenya, and specific components identified in bioactive fractions were investigated for host seeking activity of the motile stage J2 of M. incognita. Stylet thrusting and chemotaxis were used to screen for bioactivity of extracts and metabolites against J2s. Distilled water and 2% dimethyl sulphoxide served as negative controls, with methyl salicylate as the positive control. Chemical analysis by liquid chromatography- time of flight- mass spectrometry (LC-QTOF-MS) of bioactive fractions obtained from the root exudate, identified a high diversity of compounds including a phytohormone, flavonoids, alkaloids and several unidentified components. In all the assays, J2s showed dose-dependent differential responses to the different treatments relative to the controls. These results provide further evidence that non-volatile chemicals play a role in the host seeking behavior of *M. incognita*, which may be useful in the potential management of RKNs.

Session/symposium: Interspecific relationships/independent presentation ORAL PRESENTATION

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## Kline D.L.

## Attraction of ceratopogonids to plant and animal volatiles

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Ceratopogonidae, or biting midges, are a family of small flies (1-4 mm long) belonging to the order Diptera. Members of this family include many species which suck blood from humans and livestock, and many species which are important pollinators and never blood feed. The blood-sucking species require a blood meal to produce viable eggs. The blood-sucking species may be vectors of disease causing pathogens (e.g. viruses, protozoa, and/or filarial worms). All ceratopogonid species utilize nectar throughout their life cycle. This presentation will focus on the blood-sucking species and the volatiles they use to locate human, animal and plant hosts.

Session/symposium: Intraspecific relationships/Importance of multicomponent mixtures for chemical cues

ORAL PRESENTATION

Koczor S.\*, Szentkirályi F., Tóth M.

Combination of chemical and tactile stimuli results in locally increased oviposition in *Chrysoperla carnea* complex lacewings (Chrysopidae)

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Green lacewings (Chrysopidae) are important predators of many soft-bodied insect pests, such as aphids. Several studies have reported behavioural responses of green lacewings to olfactory stimuli, in some cases with highly remarkable effects. For instance a ternary blend of plant volatiles was reported to be highly attractive both for males and females of the *Chrysoperla carnea* species-complex, furthermore, females were found to lay their eggs in the vicinity of the baits. Beside chemical stimuli, previous studies reported colour preference of green lacewings and preferred oviposition on protruding parts of plants.

In our experiments we aimed to test the ternary floral bait in combination of either visual or tactile stimuli to see whether these have any effect on the attraction/oviposition of green lacewings.

Colours did not show significant effect on attraction of green lacewings to the ternary bait. Nevertheless, surface characteristics had a significant and remarkable effect, as females laid more eggs on baited spiny test plates, than on smooth ones. Test plates without baits had negligible numbers of eggs.

Thus, with the combination of appropriate chemical and tactile stimuli, oviposition of green lacewings can be concentrated locally. Potential practical applications will be discussed.

Acknowledgements: The current research was partially supported by the PD115938 NKFIH grant and the János Bolyai Research Scholarship of the Hungarian Academy of Sciences.

Session/symposium: Intraspecific relationships/Complementary or predominant role of nonchemical based communication and orientation of insects

ORAL PRESENTATION Kong C.\*, Ni H.

## The role of allelopathic crop cultivars in weed management in cropping systems

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The heavy use of herbicides for weed control is of great concern in environment and ecological problems. The over-reliance on herbicides can lead to the evolution of herbicide-resistant weeds. In this regard, there is a need to find a non-herbicidal alternative that permits ecological weed management in cropping systems. One promising weed control option is the potential for exploiting the weed-suppressing ability of crop cultivars themselves. In fact, a few crop cultivars are very effective against weeds in fields, resulting in a natural interference of crop plants with weeds. These allelopathic crop cultivars can produce and release their own 'herbicides', i.e. allelochemicals to interfere with the growth and establishment of associated weeds while reducing the use of herbicides. Therefore, much effort has gone into exploiting allelopathic crop cultivars as an efficient component of ecological weed management in cropping systems in the last decades. This report outlines recent research regarding the interference of allelopathic crop cultivars with weeds, and the mechanisms underlying such interactions. In particular, commercially acceptable allelopathic crop cultivars have been released, incorporating them into reducing herbicides for weed management in field practices in China.

Session/symposium: Practical applications/Natural products for integrated pest

management
POSTER
number: 142

Kong H.G.<sup>1</sup>, Shin T.S.<sup>2</sup>, Kim T.H.<sup>2</sup>, Ryu C.M.<sup>1</sup>\*

Stereoisomers of the bacterial volatile compound 2,3-butanediol differently elicit systemic defense responses of pepper against multiple viruses in the field

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The volatile compound 2,3-butanediol, which is produced by certain strains of rootassociated bacteria, consists of three stereoisomers, namely, two enantiomers (2R,3Rand 2S,3S-butanediol) and one meso compound (2R,3S-butanediol). The ability of 2,3-butanediol to induce plant resistance against pathogenic fungi and bacteria has been investigated; however, little is known about its effects on induced resistance against viruses in plants. To investigate the effects of 2,3-butanediol on plant systemic defense against viruses, we evaluated the disease control capacity of each of its three stereoisomers in pepper. Specifically, we investigated the optimal concentration of 2,3- butanediol to use for disease control against Cucumber mosaic virus and Tobacco mosaic virus in the greenhouse and examined the effects of drench application of these compounds in the field. In the field trial, treatment with 2R,3R-butanediol and 2R,3Sbutanediol significantly reduced the incidence of naturally occurring viruses compared with 2S,3S-butanediol and control treatments. In addition, 2R,3R-butanediol treatment induced the expression of plant defense marker genes in the salicylic acid, jasmonic acid, and ethylene signaling pathways to levels similar to those of the benzothiadiazoletreated positive control. This study reports the first field trial showing that specific stereoisomers of 2,3-butanediol trigger plant immunity against multiple viruses.

Session/symposium: Interspecific relationships/independent presentation

Kong X.\*, Zhang Z.

Mate signaling specificity and redundancy in closely related sympatric species Dendrolimus kikuchii and Dendrolimus houi (Lepidoptera: Lasiocampidae)

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Sympatric species Dendrolimus houi and D. kikuchii in Simao prefecture, Yunnan province generate unique pheromone blend to avoid interspecific attraction. Dendrolimus houi uses unique E5,Z7-stereoisomers of acetate/alcohol/aldehyde as components of its chemical communication signals. In contrast, all known sex pheromones and attractants in the sex pheromone reported species in Dendrolimus genus are of the Z5,E7acetate/alcohol/aldehyde type. Thus, there appears to be a distinct chemotaxonomic division between D. houi and the other Dendrolimus spp. Furthermore, real-time release of volatile pheromone gland components from D. kikuchii and D. houi were extensively studied by SPME-GC-MS. These data clearly demonstrated that, apart from (Z,E)-5,7-isomers of acetate and alcohol pheromone components, not any trace amount of (E,Z)-5,7-isomers of acetate and alcohol pheromone analogs were released from the pheromone glands of D. kikuchii with CW/DVB fiber coating headspace extraction method. In contrast, some (Z)-5and (Z,E)-5,7-isomers of acetate and alcohol pheromone analogs were also observed from pheromone glands of D. houi. Olfactory reception of conspecific and heterospecific pheromone components by male antennae of D. kikuchii and D. houi was also assessed by response differences to stimuli using SPME-GC-EAD techniques and field behavioral assays. Our results revealed that male response specificity is based on distinctive pheromone blends in these congeners, even though the mutual influence of individual pheromone components of sibling species was observed electrophysilogical and behaviorally. Traps baited with synthetic pheromone of both species captured significantly fewer male D. houi than traps baited solely with synthetic D. houi pheromone components. Dendrolimus kikuchii compete with D. houi for a distinctive channel with communication interference as the principal selective force causing the communication channel to diverge.

Session/symposium: Interspecific relationships/independent presentation

Koschier E.H.\*, Egger B.

## Potential for habituation to a feeding deterrent in Frankliniella occidentalis Pergande

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Frankliniella occidentalis Pergande (Thysanoptera: Thripidae), the western flower thrips, is a major pest in agricultural and horticultural crops worldwide. The feeding deterrence of the secondary plant compound cis-jasmone when directly applied to bean leaf discs to female thrips was investigated. In choice assays the concentration required to reduce the feeding damage by 50 % relative to the control treatment (FDC50) was determined. To test for habituation effects, thrips females were repeatedly exposed for a 6-hour period to the deterrent at the FDC15, the FDC50 and the FDC95 over 4 consecutive days in no-choice bioassays. Cis-jasmone at a low concentration showed to induce habituation in F. occidentalis on the third day, whereas no habituation occurred at a high concentration over 4 days. In choice assays we tested the influence of a continuous exposure of early-adult females to cis-jasmone at the FDC50 for varied time periods and compared their responses to this compound at the FDC50 to the responses of females that had no previous experience with cis-jasmone. When continuously exposed to cis-jasmone for 48 or 96 hours during their pre-oviposition period, F. occidentalis females did not habituate to the deterrent. Our study is the first to investigate the habituation potential in Thysanoptera, a generalist, cell sap-feeding insect with piercing-sucking mouthparts, to secondary plant compounds. The results may contribute to the development of integrated crop protection strategies with the implementation of allelochemicals as pest behaviour-modifying agents.

Session/symposium: Practical applications/Natural products for integrated pest

management

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Functional characterization of multi-drug resistance transporters in phytophagous insects

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The rapid evolution of toxin resistance in phytophagous insects creates pronounced problems on a global scale. Numerous herbivores are simultaneously resistant to several related or even unrelated phytochemicals and pesticides. This evolutionary flexibility seems to be owed to a large extent to newly adjusted transport processes. Our goal is to shed light on the molecular mechanisms underlying toxin resistance in highly specialized herbivores. The dogbane leaf beetle, Chrysochus auratus, incorporates host plant derived cardenolides in its own anti-predator defensive secretions. These potent toxins are specific inhibitors of the sodium-potassium pump (Na, K-ATPase). We suggest that integral membrane proteins in the hemolymph-brain barrier shield the sensitive nervous tissue which is especially rich in Na,K-ATPase against intruding cardenolides. Promising candidates for cardenolide carriers are members of the ABCB (ATP-binding cassette subfamily B) proteins. These transmembrane proteins mediate the translocation of xenobiotics across cellular membranes fueled by ATP hydrolysis. We chose a Baculovirus-Sf9 insect cells expression system to generate high levels of recombinantly expressed C. auratus ABCB transporters (Ca\_ABCB) and developed transporter ATPase assays to characterize the function and substrate specificity. Our results show that cardenolides consistently stimulate Ca\_ABCB ATPase activity to a high degree whereas specific inhibitors block the ATPase activity. We conclude that the evolution of ABCB transporters substrate specificity plays a key role in the adaptation of the beetles to their poisonous host plants and that Ca ABCB transporters differentiated over the course of evolution to take over specialized tasks in cardenolides transport and sequestration.

Session/symposium: Interspecific relationships/independent presentation

Kpongbe H.<sup>1,2,3</sup>, Khamis F.<sup>1</sup>, van den Berg J.<sup>2</sup>, Tamò M.<sup>3</sup>, Torto B.<sup>1</sup>\*
Semiochemical cues mediate aggregation behavior in *Clavigralla* species

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Grain legumes, especially cowpea (Vigna unguiculata Walp. L.) and common bean (Phaseolus vulgaris L.) are staple foods in Africa. Cultivation of these crops is constrained by Clavigralla spp., which can cause estimated yield losses of between 44% and 100%. Clavigralla tomentosicollis Stål (Hemiptera: Coreidae) is the most damaging when individuals of this species aggregate on the host crop. Previous studies demonstrated that many Coreidae species release volatiles that attract Scelionidae parasitoid wasps, which can be exploited for control of Clavigralla spp. For instance, volatiles released by C. tomentosicollis males is known to attract and aggregate both sexes, and the egg parasitoid, Gryon fulviventris Crawford (Hymenoptera: Scelionidae). However, the composition of this male-produced aggregation pheromone is unknown. The aim of this study was to determine which compounds make up the male-produced aggregation pheromone, and evaluate their effect on G. fulviventris behavior. Using behavioral and electrophysiological assays and chemical analysis, of headspace volatiles, pentane extracts of the thorax and abdomen of males, eight EAD-active components were isolated. GC/MS identified all these compounds, and in bioassays, three of these compounds elicited attraction in both sexes of C. tomentosicollis. Bioassays also indicated that the source of the aggregation pheromone was the male thorax. These results improve our understanding of the chemical ecology of Clavigralla spp. and potential use of male-produced aggregation pheromones in the management of the pest.

Keywords Clavigralla tomentosicollis, Gryon fulviventris, aggregation pheromone, semiochemical cues.

Session/symposium: Intraspecific relationships/independent presentation

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Kugimiya S.\*, Shimoda T.

## Effects of floral scent on response of a parasitoid wasp to different color models

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Females of a solitary parasitoid, *Cotesia vestalis*, need to search for food as well as hosts to maximize their reproductive success. They can utilize flowers of field mustard, *Brassica rapa*, as a food source and can use visual and olfactory cues to find floral food sources. However, it is not clear what colors of flowers they prefer and how floral scent affects their preference.

In a choice test using paper flower models in four colours (blue, green, yellow, and red), non-starved females visited green and yellow models equally, but seldom visited the blue or red models. When starved, they visited yellow models more frequently, suggesting that dietary status influences their color preference for yellow.

We sampled the headspace of *B. rapa* inflorescences and prepared solutions of their floral scents. The presence of the floral scent solutions increased total visits to green and yellow flower models, though the solution did not change the trends of color preferences shown by starved and non-starved females, respectively. These results indicate that both floral color and scent can provide more opportunity for parasitoids to find out food sources efficiently.

Session/symposium: Interspecific relationships/independent presentation

Kulkarni Y.<sup>1\*</sup>, Olsson S.<sup>2</sup>, Kunte K.<sup>2</sup>, Pragadheesh V.S.<sup>2</sup>, Indap M.<sup>1</sup> Sexual communication in the tropical swallowtail butterfly, *Graphium agamemnon* (Linnaeus, 1758), by chemical and visual cues

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Multimodal cues are often used in sexual communication in the animal kingdom. Within Lepidoptera, while chemical cues play a crucial role in sexual communication in moths, a combination of visual and chemical cues would be presumed to be used in diurnal butterflies. We studied the relative importance of chemical and visual cues in the courtship of the swallowtail butterfly *Graphium agamemnon* (Linnaeus, 1758).

We mapped and compared the volatile compounds present across male and female wings including the scent producing wing pouch of the male through gas chromatography coupled with mass spectrometry. Along with an array of straight-chain hydrocarbons, the wings of both the males and females contained a complex mixture of volatiles, including alphapinene, alpha terpinene and (E,E)-alpha-farnesene. (E,E)-alpha-farnesene was the only identified compound unique to the scent pouch of the males and was detected by the female antenna as revealed by GC coupled with electroantennographic detection (EAD). We then studied the relative importance of visual and chemical cues displayed by the male in the courtship ritual through a behaviour assay. Male models made from real male wings were used as the visual cue along with either scent pouch extracts or synthetic (E,E)-alpha-farnesene as the chemical cue. Responses were elicited from the females only in the presence of both visual and chemical cues. This suggests that both sets of cues work in tandem in the courtship of this species, and that (E,E)-alpha-farnesene could play a role in sexual communication.

Session/symposium: Intraspecific relationships/independent presentation

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Lackner S.\*, Lackus N., Köllner T., Unsicker S.B. Below- and aboveground defenses of black poplar

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Upon insect herbivore attack plants induce a series of indirect and direct defenses like phenolic glycosides, volatile organic compounds or protease inhibitors. When simultaneously challenged by a root feeding insect these well-known patterns become more complex. Most of the work trying to explain the dynamics of combined below- and aboveground attack was done in herbaceous plants focusing only on aboveground tissues. We investigated the effects of below- and aboveground herbivory on the black poplar (*Populus nigra*) comparing both above- and belowground tissues.

After 4 days of belowground herbivory by larvae of the cockchafer (*Melolontha melolontha*) young black poplar trees were infested with gypsy moth (*Lymantria dispar*) caterpillars for 40 hours; during this time volatiles were collected. Afterwards roots and leaves were harvested; defense hormones, phenolic compounds and protease inhibitors were measured; followed up by a caterpillar food choice assay.

Belowground herbivory caused drought stress indicated by increased levels of abscisic acid, free sugars and free amino acids in the leaves. In a separate food choice experiment caterpillars chose to fed on leaves that were influenced by belowground herbivory rather than on control leaves. Root volatiles induced after belowground herbivory lead to reduced growth of a generalist pathogen fungus (*Phytophtera cactorum*).

The results of our study give a first insight into the constitutive and induced defenses of black poplar roots and furthermore show that belowground herbivory can heavily influence aboveground defense mechanisms.

Session/symposium: Interspecific relationships/independent presentation

Lagoa A.C.G.<sup>1</sup>, Campos G.<sup>3</sup>, Blassioli Moraes M.C.<sup>1,2</sup>, Borges M.<sup>1,2</sup>, Laumann R.A.<sup>1,2</sup>\* Host searching behavior of an egg parasitoid in a multimodal stimuli environment.

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Little is known about the possible interaction between multimodal stimuli during host searching behavior of Platygastridae egg parasitoids. The aim of these study was to evaluate the searching behavior of Telenomus podisi in response to association of visual stimuli (color) with kairomones from their preferred host, the Neotropical brown stink bug Euschisthus heros (Hemiptera: Pentatomidae). In a first experiment, it was evaluated how the substrate color influence the as host searching behavior of the parastioid. For this 15 E. heros eggs were glued on cards with different colors (yellow (preferential color), green, red, white and black). Colored cards were arranged at the vertices of a square arena. In the second experiment, the choice of the parasitoid was evaluated to four different treatments: yellow substrate, white substrate and to yellow or white substrate combined with the principal component of the E. heros sex-pheromone (5 ng of methyl-2,6,10 timethyltridecanoate, placed in a filter paper). The third experiment was performed as the second, but the chemical stimuli were the natural footprints of E. heros females, obtained from filter paper previously exposed to walking females. In all experiments, a parasitoid female was released in the center of the arena and their choice for one of the treatments was recorded. The females were maintained for another 2 hours to allow the parasitism in egg masses. In the first experiment, the parasitoid preferred the yellow substrate compared to the substrates of other colors. There was no preference for the yellow color when in the presence of pheromone or footprints of E. heros (2nd and 3rd experiments). The results suggest that in presence of chemical stimuli the high attractiveness of visual stimuli, in this case the yellow substrate, is altered but no synergy between chemical and visual stimuli was observed.

Session/symposium: Interspecific relationships/independent presentation

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# The possible consequences of the suppression of plant volatiles by slugs

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In response to herbivory, plants emit blends of volatile organic compounds (VOCs) into their surroundings. Those volatiles have been shown to act as cues that mediate the interactions between the emitting plant, its herbivores and their natural enemies (predators and parasitoids), as well as the neighboring plants. While these compounds are solely plant-produced, they are neither under their exclusive control, nor do they always work to their advantage. Indeed, some herbivores are able to manipulate a plant's emission by inducing or suppressing selected compounds. Desurmont et al (2016) discovered that the slug *Arion vulgaris* suppresses the emission of green leaf volatiles and glucosinolate breakdown *products* in wild *Brassica rapa*. In this study, we examine the prevalence and specifics of this suppression among Brassicaceaes (*Brassica rapa*, *Isatis tinctoria*, *Cardamine pratensis*, *Sinapsis arvensis*) and an Asteracea (*Centaurea cyanus*). We also discuss the implications of this suppression for the efficiency of plant defenses against slugs and other herbivores.

Desurmont G. et at. (2016) J Chem, Ecol. 42(3): 183-192.

Session/symposium: Interspecific relationships/Tibor Jermy's Legacy in insect-plant

evolution
POSTER
number: 061

Larsson M.C.\*, Svensson G.P., Burman J., Winde I., Nyabuga F., Milberg P., Bergman K.O., Jansson N., Westerberg L., Paltto H., Oleksa A., Harvey D., Kadej M., Molander M., Eriksson B., Andersson F., Hedenström E.

#### Pheromone monitoring turn cryptic click beetles into regional conservation indicators

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Click beetles constitute important model species for evidence-based conservation of the saproxylic insect fauna associated with decaying wood and hollow trees. Larvae of saproxylic click beetles may be predatory or feed on decaying wood and typically occupy later successional decomposition stages of the wood than e.g. longhorn beetles. Many saproxylic click beetles likely recquire large, decaying or hollow trunks and branches for their persistence, potentially making them excellent indicators of old-growth forest continuity and saproxylic insect diversity. Their usefulness as indicators can be severely limited by the difficulty to systematically survey these elusive beetles, however. We have exploited the potent attractiveness of its sex pheromone for systematic monitoring of the rare and elusive click beetle Elater ferrugineus. Based on this pheromone we can now monitor its distribution and abundance with unprecedented spatial and temporal accuracy over large geographic regions. Within a few seasons we have been able to fast-forward decades of work corresponding to traditional methods and provide regional, accurate distribution maps highlighting the most important hotspots in the landscape. We have mapped its distributions and movements in relation to habitat and linked to the distribution of other saproxylic species, and studied its dispersal biology in fragmented landscapes. We have demonstrated that this species constitutes an excellent indicator for saproxylic insect biodiversity of largehollow trees and for identifying the most valuable and prioritized hotspots in the landscape. We have also provided habitat thresholds on different spatial scales, and indicated that female dispersal may constitute a likely limit for colonization of new habitats among patchily distributed habitat resources.

Session/symposium: Intraspecific relationships/Chemical ecology of click beetles (Elateridae): practical applications and advances in the field.

ORAL PRESENTATION

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Pheromones and barcoding delimit boundaries between cryptic species in the primitive moth genus *Eriocrania* (Lepidoptera: Eriocraniidae)

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Animal classification is primarily based on morphological characters, even though these are typically not the first to diverge during the speciation process. In many cases, however, closely related taxa are difficult to distinguish based on morphological characters alone, especially when there is no substantial niche differentiation. As a consequence, the diversity of certain groups is likely to be underestimated. Lepidoptera -moths and butterfliesrepresents the largest group of herbivorous insects, and the extensive diversification in the group is generally assumed to have its origin in the spectacular radiation of flowering plants. However, reproductive isolation between closely related species can be the result of divergence in mate preference and the associated pheromone communication system alone. Here we combine pheromone trapping and genetic analysis to elucidate the evolutionary relationships within a complex of sympatric primitive moths (Lepidoptera: Eriocraniidae). Mitochondrial and nuclear DNA markers provided evidence that *Eriocrania semipurpurella*, as currently defined by morphological characters, includes three cryptic species in Northern and Western Europe. Male moths of these cryptic species, as well as of the closely related E. sangii, exhibited a remarkable specificity in terms of their attraction to specific ratios of two major pheromone components, (2S,6Z)-nonen-2-ol and (2R,6Z)-nonen-2-ol). Our data suggest strong assortative mating in these species in the absence of niche separation, indicating that *Eriocrania* moths may represent an example of non-ecological speciation. Finally, our study argues in favour of combining pheromone trapping and DNA barcoding as powerful tools for identifying and delimitating species boundaries.

Session/symposium: Interspecific relationships/independent presentation ORAL PRESENTATION

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Latif S.<sup>1,2</sup>\*, Weston P.A.<sup>1,3</sup>, Gurusinghe S.<sup>1</sup>, Quinn J.C.<sup>1,2</sup>, Weston L.A.<sup>1,3</sup> **Metabolic profiling of bioactive flavonoids in selected annual pasture legumes** 

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Australian farmers and researchers have embraced the use of introduced annual pasture species originating from Mediterranean regions of Europe and nothern Africa and these have been continuously evaluated in the face of a changing climate to increase crop diversity and fill existing agro-ecological gaps. Recent efforts have focussed on less well-known annual legumes including Ornithopus spp. (French serradella and yellow serradella) and Biserrula pelecinus (biserrula). However there is a need to better understand the performance of each species as well as the bioactive plant secondary compounds associated with weed suppression, produced by these legumes under Australian climatic conditions. Legumes typically produce a diversity of secondary metabolites particularly flavonoids that are involved in nitrogen fixation, rhizobium signalling, and also possess antioxidant and allelopathic effects. Various plant tissue types including leaf, stem, flower and rhizoplane and bulk soil sampels were extracted with methanol using high temperature and pressure speed extractor. Metabolic profiling was performed using high pressure liquid chromatography coupled with quadrupole – time of flight mass spectrometry followed by chemometric analysis. Compounds were identified by comparing accurate mass, retention time and mass spectra with available libraries and known standards. Numerous bioactive flavonoids were profiled and these varied significantly with species and plant tissue types. Further data analyses revealed that field and laboratory experimental results were similar, with the highest abundance of key metabolites including bioactive flavonoids in plant tissues including rhizoplane and bulk soil. Stepwise linear regression showed strong correlation between phytotoxicity and presence of key flavonoids quercetin, kaempferol and their glycosides.

Session/symposium: *New chemical structures/Omics in chemical ecology*ORAL PRESENTATION

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The male effect: on the way to elucidating the male fragrance inducing ovulation in ewe and goat

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Small ungulates, like sheep and goat, have a seasonal breeding cycle. During the season of sexual rest, females are reproductively quiescent and have therefore no ovarian cycle. However, if during this period they are exposed to a sexually active male, they will undergo a complete reactivation of their gonadotropic axis leading to ovulation. This phenomenon, called the 'male effect', constitutes an attractive alternative to induce and synchronize ovulations in ungulates without exogenous hormones. However, the use of the male effect in farms is still limited because of a limited efficiency. The identification of the olfactory cues, predominant component in the setting up of this natural process, will allow for the development of techniques mimicking and/or potentializing the male effect in breeding farms in order to limit the use of hormones. If some potentially involved molecules have previously been identified (Cohen-Tanoudji et al., 1994; Murata et al., 2014), the complete signal (those inducing ovulation) remains to be clearly characterized. To achieve this, we adopted an original approach based on the comparison of olfactory profiles of males during nonreproductive and reproductive periods. The complete chemical cues characterization in the two species was undertaken by analyzing various biological samples (whool, anteorbital glands, ...) using two strategies. Chemical profiles were determined by GC/MS analysis after SPME or solvent extractions to analyze volatiles compounds and fatty acids respectively. Specific ram and bulk olfactory profiles could be observed during the period of maximal sexual activity with quantitative and qualitative variations. More than 150 molecules seasonspecific (with a fold-change >10) were characterized (ethyl esters, ketones, ...) in the two species. Assessment of their biological activity on female (ewe and goat) are on-going with the collaboration of our partners (INRA PRC, Nouzilly, France).

Session/symposium: Intraspecific relationships/independent presentation ORAL PRESENTATION

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Transient expression of whitefly effectors in *Nicotiana benthamiana* leaves activates systemic immunity against the leaf pathogen *Pseudomonas syringae* and soil-borne pathogen *Ralstonia solanacearum* 

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Infestation of plants with the phloem-feeding whitefly Bemisia tabaci modulates root microbiota and both local and systemic immunity against microbial pathogens. Specifically, aboveground whitefly infestation suppresses pathogen propagation and symptom development caused by the soil-borne pathogens Agrobacterium tumefaciens and Ralstonia solanacearum in the root system through systemic signal transduction. Therefore, we hypothesized that secreted protein(s)/non-protein factors from whitefly saliva (referred to as candidate effectors) might function as insect determinants that activate systemic acquired resistance (SAR) in the host plant. Here, we intensively screened a cDNA library constructed from mRNA from whitefly feeding on Nicotiana benthamiana leaves and selected three candidate effectors, 2G4, 2G5, and 6A10, that appear to reduce disease development caused by the aboveground pathogen Pseudomonas syringae pv. tabaci and the soil-borne pathogen R. solanacearum. Transient expression of the three candidate effector cDNAs in leaves primed the expression of SAR marker genes NbPR1a and NbPR2 in local and systemic leaves against P. syringae pv. tabaci, while leaf infiltration with 2G4 and 6A10 cDNA elicited strong defense priming of SAR markers following drench application of R. solanacearum on plant roots. In silico and qRT-PCR analyses revealed the presence of 2G5 and 6A10 transcripts in insect salivary glands. This is the first report of whitefly effectors that prime SAR against a root pathogen.

Session/symposium: Interspecific relationships/Insect-microbe interactions

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Lemfack M.C.<sup>1\*</sup>, Magnus N.<sup>1</sup>, Domik D.<sup>1,2</sup>, Weise T.<sup>1,3</sup>, von Reuss S.<sup>4</sup>, Piechulla B.<sup>1</sup> Sodorifen - a key volatile organic compound (VOC) of *Serratia* species

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Microorganisms populate almost all natural and artificial habitats, subsequently appear ubiquitously and have to be considered as distinct trophic levels in ecosystems. Very prominent and highly populated are e.g. animal gastrointestinal tracts and the rhizospheres of plants. The bacterium Serratia plymuthica 4Rx13 was isolated from the rhizosphere of Brassica napus, possessing a complex volatilome of at least 100 compounds. Among them, the dominant compound sodorifen was identified, which exhibits a very unusual structure. So far, it was only found to be produced in Serratia plymuthica and is an outstanding fingerprint VOC for this species, while the other 17 Serratia species do not emit this compound. Unique is also the 4 gene sodorifen cluster encoding a terpene cyclase, methyltransferase, DOXP synthase and IPP isomerase, present in the few S. plymuthica isolates sequenced so far. Due to its high abundance in the volatile bouquet of S. ply. 4Rx13, investigation of the biological function of sodorifen came into focus. Studies, using artificially synthesized sodorifen as a pure substance, neither revealed any phenotypic effect on the model plant Arabidopsis thaliana nor on Serratia marcescens. Several feeding experiments with different carbon sources were performed, especially interesting was the observation that sodorifen emission was repressed during presence of glucose as the sole carbon source. Using insertional deletion mutagenesis, the global regulatory mechanism of carbon catabolite repression was found to be responsible for transcriptional repression. Furthermore, specific promoter regions were identified which are specifically responsible for the expression of genes during stationary phase growth, allowing the speculation that sodorifen might enable its producers to cope with certain stress conditions and thus, provide a survival benefit to them.

Session/symposium: Interspecific relationships/Volatile-mediated microbe-plant interactions
ORAL PRESENTATION

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The biochemical and neuromodulatory mechanism underlying the plastic sex pheromone response in *Nasonia vitripennis* 

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The response of insects to chemical stimuli is plastic and depends on their physiological state and prior experience. In many insect species, the mating status influences the response to sex pheromones, but the underlying biochemical and neuromodulatory mechanisms are poorly understood. After mating, females of the parasitic wasp Nasonia vitripennis are no longer attracted to the male sex pheromone. This behavioral switch of the females is independent of sperm transfer and elicited by the males by applying an oral secretion to the females' antennae during courtship. The active compounds in the oral secretion are the three fatty acid esters ethyl oleate, ethyl linoleate and ethyl alpha-linolenate. By a pharmacological approach, we studied the neuromodulatory mechanism underlying the behavioral switch and found dopamine (DA) to be of crucial importance. Females fed a DA-receptor antagonist prior to mating maintained their attraction to the male pheromone after mating while virgin females injected with DA became unresponsive. However, the switch is reversible as mated females are able to regain their pheromone preference by appetitive learning. This demonstrates that the peripheral elements of the olfactory system underlying sex pheromone perception are still functional after mating. Feeding mated N. vitripennis females with antagonists of either octopamine- (OA) or DA-receptors prevented relearning of the pheromone suggesting that both receptors are involved in appetitive learning. Moreover, DA injection into mated females was sufficient to mimic the oviposition reward during odor conditioning with the male pheromone. Hence, our data indicate that DA plays a key role in the plastic pheromone response of N. vitripennis females and reveal some striking parallels between insects and mammals in the neuromodulatory mechanisms underlying olfactory plasticity.

Session/symposium: Interspecific relationships/independent presentation
ORAL PRESENTATION

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Unleash crop defences: silicon impact on corn volatile organic compounds

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Evidence has mounted in the last decade that silicon (Si) plays important roles in plant defence against biotic and abiotic stress. Silicon would enhance the ability of plants to defend themselves against herbivorous pests like folivores, borers or phloem and xylem feeders. Moreover, silicon impacts tritrophic interactions between plants, pests and natural enemies by modifying herbivore-induced plant volatiles. In this study, we aim at evaluating the cascade effect of silicon bioavailability in the rhizosphere on the emissions of volatile organic compounds (VOCs) and associated ability of natural enemies to locate their prey. We selected a tritrophic model made of Zea mays (Poaceae, var.Delprim), Spodoptera exigua (Lepidoptera: Noctuidae) and Cotesia marginiventris (Hymenoptera: Braconidae). We first developed a hydroponic medium allowing the culture of maize (Zea mays) under three silicon concentrations, provided by the addition of monosilicic acid (H4SiO4) in a nutrient solution. Silicon concentration was measured from maize leaves and was found to be linked with silicon concentration in the medium. Then, we collected the VOCs from healthy plants, as well as from plants exposed to caterpillars. VOCs were captured from plants placed in large glass chambers, and their VOCs were pulled from plant headspace and trapped on Tenax-TA adsorbent, before being separated, quantified and identified by gas chromatography. Qualitative and quantitative differences were highlighted among silicon concentrations. Finally, we used a wind tunnel to evaluate the ability of C. marginiventris to locate its host. We found that caterpillars-infested plants grown under elevated silicon concentrations were more attractive. How silicon impacts plant defence pathways remains to be elucidated.

Session/symposium: Interspecific relationships/independent presentation

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## Control of lesser date moth Batrachedra amydraula by mass-trapping with pheromone

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The lesser date moth (LDM) Batrachedra amydraula is a significant pest of date palm fruits in all date growing areas of the Middle East and North Africa, including Israel. Previously, detection and monitoring of the pest has often been less accurate and expensive due to sampling larvae with lifting machines. After 45 years of ongoing damage by the pest in Israel we finally succeeded to identify the pheromone as a mixture of three components: (Z4,Z7)-4,7-decadien-1-yl acetate, Z5-decen-1-yl acetate, and Z5-decen-1-ol in a ratio of 1:2:2. Monitoring traps with lures loaded with this mixture and exposed from spring through summer in Israel revealed there were three generations with different abundance and that there is a period of diapause in the winter. The pest is specific to date palms and the plantations located in desert areas. Therefore, mass-trapping appears to have good potential to control the pest. We will discuss the reasons why we chose mass-trapping and present the research steps that were taken to increase the efficiency of this method such as: dose response tests, trap height and type, inter-trap distances, circular effective attraction radius (EARc) calculations and computer models of mass-trapping that investigate the interplay between population levels, moth movement, trap density, and potency of lure/traps. The integrated methodologies are applicable to many pest species.

Session/symposium: Practical applications/Comparison of semiochemical control methods of pest insects

ORAL PRESENTATION

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The molecular bases of producing sex pheromones with the opposite component ratios in two *Helicoverpa* moth species

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The sympatric closely related species Helicoverpa armigera and Helicoverpa assulta use 97:3 and 7:93 of (Z)-11-hexadecenal and (Z)-9-hexadecenal, respectively, as their sex pheromone to find/locate correct sex mates. Moreover, (Z)-11-hexadecenyl alcohol and (Z)-9-hexadecenyl alcohol are more abundant in the pheromone gland of *H. assulta* than in that of H. armigera. To clarify the molecular basis of these differences, we sequenced the pheromone gland transcriptomes of the two species and compared the expression patterns of the candidate enzyme genes involved in the pheromone biosynthetic pathways by FPKM values and quantitative RT-PCR analysis. We found that the desaturase gene LPAQ expressed about 70 times higher in H. armigera than in H. assulta, whereas another desaturase gene NPVE expressed about 60 times higher in *H. assulta* than in *H. armigera*. We also observed significantly higher expression of the fatty acyl reductase (FAR) gene FAR1 and the aldehyde reductase (AR) gene AR3 in H. assulta than in H. armigera. Examination of the pheromone glands of the backcross offspring of their hybrids to H. assulta showed a positive linear correlation between the expression level of LPAQ and the amount of Z11-16:Ald and between the expression level of NPVE and the amount of Z9-16:Ald in the pheromone glands. Taken together, these data demonstrate that the expressional divergence of LPAQ and NPVE determine the opposite sex pheromone component ratios in the two species and the divergent expression of FAR1 and AR3 account for the greater accumulation of alcohols in the pheromone gland of *H. assulta*.

Session/symposium: Intraspecific relationships/Importance of multicomponent mixtures for chemical cues

ORAL PRESENTATION

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Species-specific aggregation pheromones contribute to coexistence in two closely related thrips species

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Pheromones play an important role in mediating interspecific interactions in insects. In an insect community, pheromones can reveal information about the senders, which could be used by other members of the food web (competitor, natural enemies, etc.) to their own advantage. The aggregation pheromones of two closely related thrips species, Frankliniella occidentalis and Frankliniella intonsa, have been identified with the same major compounds, (R)-lavandulyl acetate and neryl (S)-2-methylbutanoate, but in different ratios. However, the roles of the aggregation pheromones in the interspecific interactions between these two closely related species are unknown. Here, we investigated the roles of major aggregation pheromone compounds in interspecific interactions between F. occidentalis and F. intonsa for both long and short ranges. The results showed that, at tested doses, neither aggregation pheromone induced long range cross-attraction nor short range cross-mating was detected between F. occidentalis and F. intonsa. Field-trapping trials showed that the speciesspecificity in aggregation pheromones was regulated by the ratio of two major compounds. However, species-specific blends of the two major compounds had no effect on short range interactions between these two species. Our data from the thrips species provide support for the "aggregation model of coexistence", explaining the species-specific pheromone mediated coexistence of closely related species. Thus, species-specific pheromones could be one of the factors affecting population dynamics and community structure in closely related insects with similar niches.

Session/symposium: Interspecific relationships/independent presentation

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Are refuge areas effective as a Bt resistance management strategy when using transgenic plants? Pheromone ecology of *Spodoptera frugiperda* in Brazil.

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There are many studies demonstrating that the development of resistance to insecticides comes at a cost, reflected in traits such as extended larval developmental time, small adults and reduced fecundity. In contrast, while very few studies have examined the possibility of resistance might affect pheromone communication systems they have all reported some differences in the pheromone biology of resistant and susceptible individuals. In Brazil the fall armyworm has developed resistance to Bt so we tested the hypothesis that the development of resistance impacts female calling behavior, pheromone content and ovarian development and male responses to pheromones and spermatophore size, with the prediction that resistant virgin females and males would perform less than susceptible individuals. Contrary to our expectations resistant females spent significantly more time calling that susceptible ones suggesting that there is no cost reflected in calling duration. Interestingly, this would support the results of a parallel study that found no differences in various growth parameters of susceptible and resistant individuals. Experiments examining both pheromone emission and male responses shows that where are marked changes in the pheromone blend and/or the rate emission then with the extended calling window resistant females would have a higher probability of attracting mates. These finding will be discussed within the context of resistance management when using Bt corn as part of a pest management program.

Session/symposium: *Practical applications/independent presentation*ORAL PRESENTATION

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Little C.M.<sup>1,2</sup>\*, Chapman T.W.<sup>2</sup>, Hillier N.K.<sup>1</sup>

# Role of fruit and leaf colour on susceptibility of highbush blueberries to infestation by Drosophila suzukii

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Drosophila suzukii Matsumara (Diptera: Drosophilidae) has become a principal pest in soft-skin fruits and berries. Crop damage in highbush blueberry have been particularly severe, infesting both ripe and still ripening fruits. During blueberry harvest, fruits of various degrees of development and ripeness are present simultaneously. In addition, blueberry foliage senesces resulting in significant colour change as the season progresses. We investigated the influence of blueberry fruit and leaf colour on host-finding behavior in D. suzukii. Developmental changes in contrast between fruit colour and leaf colour may act as a visual contextual cue in finding suitable host fruits. Opposing shifts in reflectance spectra of ripening fruits and senescing leaves resulted in increased contrast between ripe fruit and senesced foliage. These opposing changes in colour may contribute to the attractiveness of blueberry fruit as a late season host for D. suzukii.

Session/symposium: Intraspecific relationships/Complementary or predominant role of nonchemical based communication and orientation of insects

ORAL PRESENTATION

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# Plant-mediated RNAi in controlling Apolygus lucorum

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Plant-mediated RNA interference (RNAi) methods are considered a new strategy for pest control. RNAi strategies are effective for decreasing disoperation of insecticides and are not limited to a few orders such as Lepidoptera. The small green plant bug, Apolygus lucorum (Hemiptera: Miridae), an important pest that damages multiple crops including maize, soybean and cotton, has already become a major pest that is hard to control using chemical pesticides. In this study, we screened and obtained eight target genes for plant-mediated RNAi to control A. lucorum. Among them, dsRNA of five target genes were successfully constructed into transgenic maize. In addition, two were successfully induced into transgenic soybean, with mortality rates of up to 69.21% (day 7) in the feeding bioassay. Plant-mediated RNAi for AlucV-ATPase-E significantly suppressed development and decreased the population of A. lucorum in both maize and soybean. However, even though the expression of dsRNA for AlucSnf7 was determined to be equal between the transgenic crops, the lethal effect of plant-mediated RNAi was not observed in transgenic soybean. Our results demonstrated a new strategy for controlling A. lucorum using plant-mediated RNAi. This study is the first time that RNAi effects for the same target genes have been compared between different transgenic crops. Moreover, we found that plant-mediated RNAi is not identical between crops and advanced several considerations for improving the application of plant-mediated RNAi in non-Bt pest control.

Session/symposium: Practical applications/Natural products for integrated pest management

ORAL PRESENTATION

Liu W.<sup>1</sup>, Jiang X.<sup>2</sup>, Cao S.<sup>1</sup>, Yang B.<sup>1</sup>\*, Wang G.<sup>1</sup>\*

# Functional studies of sex pheromone receptors in Asian corn borer Ostrinia furnacalis

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Lepidopteran insects use sex pheromones for sexual communication. Pheromone receptors expressed on peripheral olfactory receptor neurons (ORNs) are critical part to detect the sex pheromones. In genus Ostrinia, several pheromone receptors were functional analyzed in O. nubilalis and O. scapulalis but the knowledge in O. furnacalis was rare. In this study, seven pheromone receptors were functionalized by heterologous expression system of *Xenopus* oocytes. Functional types of sensilla trichoidea were classified by single sensillum recordings to interpret the response pattern of olfactory sensory neurons to Ostrinia pheromone components. OfurOR4 and OfurOR6 responded to the major sex pheromone Z/E12-14:OAc. OfurOR4 is the main receptor for both Z/E12-14:OAc and OfurOR6 mainly responded to E12-14:OAc. Functional differentiations of gene duplication were found between OfurOR5a and OfurOR5b. OfurOR5b showed a broad response to most of the pheromone components in O. furnacalis, whereas OfurOR5a was found with no ligands. OfurOR7 showed a specific response to Z9-14:OAc and OfurOR8 mainly responded to Z11-14:OAc and E11-14:OAc. OfurOR3 did not respond to any pheromone components. Our results improved the current knowledge of pheromone reception in Ostrinia species which may contribute to speciation.

Session/symposium: Interspecific relationships/Arthropod chemoreceptors

Löfstedt C.<sup>1</sup>\*, Andersson M.N.<sup>1</sup>, Corcoran J.<sup>1,2</sup>, Yuvaraj J.K.<sup>1</sup>, Zhang D.D.<sup>1</sup>, Hou X.<sup>1</sup>, Anderbrant O.<sup>1</sup>, Newcomb R.D.<sup>2,3</sup>

## Comparative studies of moth pheromone receptors using two complementary platforms

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Insect ORs are present in the olfactory sensory neurons, located mainly in the antenna. However, with few exceptions, we do not understand in which of the thousands of neurons in the antenna a specific OR is expressed. Thus, to be able to study the odour responses of a specific OR and furthermore to manipulate and study its specificity by mutagenesis, we must express the OR in a surrogate cell system where it naturally does not belong (so called heterologous system) and stimulate it with odour molecules. Several heterologous cell systems exist. However, their inherent characteristics differ in several aspects, such as intraand extracellular components, control of gene expression, and measurement technique. These differences raise questions regarding the comparison of results from these systems and how an OR responds in a heterologous system compared to the sensory neurons in the antenna (in vivo). We have assayed pheromone receptor candidates from three species of moths in the Xenopusoocyte system and the HEK293 cell system. Results obtained with the two in vitrosystems are compared to electrophysiological activity profiles of receptor cells recorded in vivo and expression patterns as determined by transcriptome analyses and in situhybridization. Prosand consof the two systems for deorphanization of olfactory receptors, false negatives and false positives, as well as explanations for differences in results in terms of response profiles will be discussed.

Zhang D.D., Löfstedt C. (2015) Front Ecol Evol 3: 105: 1. Zhang D.D., Löfstedt C. (2013) PLoS One 8: e77345: 1. Yuvaraj J.K. et al. (2017) Mol Biol Evol 34: 2733.

Session/symposium: Interspecific relationships/Arthropod chemoreceptors
ORAL PRESENTATION

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Lu P.\*, Bao M.

Mating behavior and attractiveness of male cuticle extracts based on electroantennogram and behavioral assay in *Sirex noctilio* Fabricius

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Sirex noctilio was a major forest invasive pest worldwide and has caused tremendous damages. After a careful observation of mating behavior and rhythm of Sirex noctilio, four types of male cuticle extracts were collected. Electroantennogram (EAG) and behavioral responses of both sexes to these extracts were investigated. According to sex ratio of 1:3 (female to male), wasps were put into the cages and then the number of mating couples was recorded throughout the day. Male cuticles were extracted by hexane (HPLC), and then EAG and olfactory responses of both sexes to the extracts were determined. The extracts were as follow, sample 1 (separately reared in plastic cage), sample 2 (males gathering without female), sample 3 (males attracted females, but no mating occurred), and sample 4 (after mating). The results showed that the mating process can be divided into five phases: searching, attracting, seizing, copulating and ending. The highest mating frequency occurred at 9:00 - 11:00 in a day. Both males and females have the highest selection to the sample 3 and sample 4. Male extracts can elicit much stronger EAG response from female wasps antennae than from male wasps antennae. Behavior test showed that only sample 3 was attractive to females, sample 1 - 4 were attractive to males, but with no significant difference. Males released pheromone which can attract both male and female wasps. We can hypothesis that males could release aggregative pheromone to attract males in the canopy after emergence, also can release likely sex pheromone to attract females once they were much closer to females.

Session/symposium: Intraspecific relationships/Importance of multicomponent mixtures for chemical cues

ORAL PRESENTATION

Lucas C.<sup>1</sup>, Brossette L.<sup>1</sup>, Lefloch L.<sup>1</sup>, Dupont S.<sup>1</sup>, Christides J.P.<sup>1</sup>, Bagneres A.G.<sup>1,2</sup>\* **Effects of predator odor on behavioral and chemical adaptations in two termite species** 

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Predation is a key process in evolution because it impacts directly individual survivorship. Being able to detect a predator before any direct contact is therefore primordial to improve prey's fitness. In insects, chemical communication is involved in several interactions like courtship behavior, circadian activity, parasitism and of course preypredator interactions. Among the active chemical signals, the cuticular hydrocarbons are known to rule the social organization of social insects including their caste and recognition systems.

In this study, we asked if the cuticular hydrocarbons produced by a predator could influence the social organization of its prey. Indeed, hydrocarbons are sticky and could be released in the environment giving indirect information about predators (or competitors). Preys could therefore adapt their behavior which could induce social-organization modifications of their colonies. To test this hypothesis, we studied interactions between two social insects, the ant predator and its termite prey. Two termite species among the most widespread species in Europe were chosen: Reticulitermes flavipes and *R. grassei*. Our results show that, under the influence of the predator odor, *R. grassei* presented a higher homogenous chemical profile and also increased its survivorship during competition tests. Interestingly, the predator-odor effects on the behavioral and chemical adaptations of the native species *R. grassei* are not effective in the invasive termite *R. flavipes*.

Session/symposium: Interspecific relationships/independent presentation

Machara A., Kyjaková P.\*, Hanus R.

## Queen-specific volatile of Silvestritermes minutus: (5Z,9S)-tetradec-5-en-9-olide

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Queens of the South American termite *Silvestritermes minutus* (Termitidae: Syntermitinae) produce large amounts of previously undescribed volatile, detected in their ovaries, haemolymph, but also at the body surface and in the headspace of the queens. To understand the biological significance of the compound, we studied its chemical identity using analytical chemistry combined with *de novo* synthesis. Isolation of the compound, followed by MS and GC-FTIR data analyses, led us to propose the structure of the unknown compound to be 10-pentyl-3,4,5,8,9,10-hexahydro-2*H*-oxecin-2-one. In order to unambiguously determine absolute and relative configurations of the 10-membered macrolide, we performed enantiodivergent syntheses starting from enantiopure (*S*)-glycidyl tosylate. The synthetic sequence proceeded from the preparation of one enantiomer to the synthesis of its stereochemical counterpart and involved macrolide-closing metathesis using ruthenium scavenging agent. The absolute and relative configuration of the natural compound was resolved to be (5*Z*,9*S*)-tetradec-5-en-9-olide based on the comparison of mass fragmentation pattern and chromatographic behaviour of the authentic sample and products of the *de novo* synthesis.

Session/symposium: New chemical structures/independent presentation

MacIsaac M., Faraone N.\*, Hillier N.K.

## Effects of granite dust treatment on herbivory and volatile emissions of lilies

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Plants produce a multitude of volatile compounds that have a role in reproduction, stress response, and offering olfactory cues for location by predatory pest insects. Current trends in agriculture seek to find safer, sustainable methods for crop protection against such pests. Recent work has determined a novel granite dust bio-product [1] as a natural alternative (similar to diatomaceous earth) which has been used as a pesticide with promising results in controlling *Lilioceris lilii* (lily beetles), an invasive pest [2] that causes significant damage to *Lilium* spp. It has been observed that dust-treated lily plants were rejected by lily beetles. This study will explore how this mineral dust may affect volatile emission and herbivory acceptance in lily plants. After application of granite dust on plants, volatiles were collected throughout developmental stages of lilies and analyzed via gas chromatography-mass spectrometry and compared to those volatiles from untreated plants. Electrophysiological analyses were performed in order to investigate the response of *L. lilii* adults towards volatiles from treated and control plants. Changes in the volatile emission in treated plants may provide novel approaches for managing insect pests, while improving the sustainability of conventional agricultural practices.

### References:

- [1] Faraone et al. (2018) J Pest Sci doi.org/10.1007/s10340-018-0986-9.
- [2] Salisbury et al. (2012) Physiol Entomol 37: 97-10.

Session/symposium: Practical applications/Natural products for integrated pest

management Poster

number: 144

MacPherson S., Faraone N., Hillier N.K.\*

Evaluation of repellent and insecticidal properties of a novel granite dust product in crop protection.

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Inert rock dusts, such as diatomaceous earth, represent effective products for insect pest management. We investigated the properties of a novel granite dust product in different formulations as a repellent and insecticidal agent against two important horticultural crop pests (*Plutella xylostella* and *Trichoplusia ni*). Foliar application of granite dust (dry and in aqueous solution) reduced larval feeding damage of *P. xylostella* and *T. ni* on cabbage leaves. In the case of *P. xylostella*, aqueous formulation of granite dust with surfactant had a significant insecticidal effect on larvae. In oviposition bioassays, adult *P. xylostella* female insects oviposited significantly fewer eggs on cabbage leaves treated with granite dust than control leaves. Our results demonstrate, for the first time, the potential use of granite dust as a pest management treatment providing repellent and insecticidal activities exceeding other mineral and dust-based pest management products.

Session/symposium: Practical applications/Natural products for integrated pest

management
POSTER
number: 145

Mailula D.\*, van der Nest M.A., Wingfield B.D., Misse A., Roux J., Hammerbacher A. Luring insects with enticing smells: fusel alcohol biosynthesis by fungi in the Ceratocystidaceae

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Many fungi rely on insects for their dispersal and employ different strategies to ensure a close association with their vectors. Fungi in the family Ceratocystidaceae attract insects by producing fusel alcohols and acetates which have fruity and floral odours. The characteristic scents of banana and roses produced by this family primarily attracts nitidulid beetles. Our aim was to analyse the volatiles produced by the different genera in the Ceratocystidaceae and to study their biosynthesis. All the strains included in our study produced isoamyl alcohol and isoamyl acetate from leucine. However, there was significant variation in production of either branched chain or aromatic fusel alcohols within the family. We discovered 3 genes encoding aromatic amino transferases and 3 genes encoding branched-chain amino transferases in the available genomes of the Ceratocystidaceae that might be involved in catalysing the first steps in the production of the fusel alcohols produced by these fungi. However, other fungi of the Sordariomycetes which do not produce fruity or floral smells also have similar amino transferase genes. Further functional analysis of these genes is therefore necessary to confirm their involvement in the biosynthesis of these volatiles.

Session/symposium: Interspecific relationships/independent presentation

Malka O.<sup>1</sup>\*, Feldmesser E.<sup>2</sup>, Santos D.<sup>1</sup>, Van Brunschot S.<sup>3</sup>, Reichelt M.<sup>4</sup>, Easson M.<sup>4</sup>, Gershenzon J.<sup>4</sup>, Vassao D.G.<sup>3</sup>, Seal S.<sup>3</sup>, Colvin J.<sup>3</sup>, Morin S.<sup>1</sup>

Metabolic responses to variable host plants play a key role in different degrees of polyphagy in the *Bemisia tabaci* species complex

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The cryptic polyphagous phloem-feeding whitefly Bemisia tabaci can feed on a broad range of host plants. However, the adaptation strategies utilized for handling the insect's diverse diet remain poorly understood. By using six species of B. tabaci and four host plants, we investigated how B. tabaci adults cope with the environmental unpredictability presented by the set of host plants. Behavioral studies indicated large differences in performance on the four hosts between the six species and also putative specialization of one of the species on cassava plants. Chemical analyses indicated that B. tabaci species are less efficient in their ability to excrete carbohydrates and plant secondary metabolites when compared to a reference specialist whitefly. Transcriptomic analyses of the six species responses to host switching revealed that large set of genes involved in the metabolism of carbohydrates, amino acids, xenobiotics and vitamins showed differences in their expression pattern between the six analyzed species. Within species, sets of genes associated with environmental information processing and various organismal systems, responded to host switching. Based on these results, we hypothesize that differences in the expression patterns of various metabolic pathways may hold the key for understanding the essence of host adaptation among closely related B. tabaci species and likely other generalist phloemfeeders. More precisely, our transcriptomics, behavioral and metabolomics data suggest that adaption to host plants in B. tabaci involves unique combinations and interactions of primary and secondary metabolic pathways which allow some of the species to efficiently metabolize both carbohydrates and plant secondary defense compounds.

Session/symposium: Intraspecific relationships/independent presentation ORAL PRESENTATION

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Species-complex evolution and host-plant associations in *Bemisia tabaci*: a plant-defense, detoxification perspective revealed by RNAseq analyses

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Bemisia tabaci is a complex of more than 35 cryptic species. The putative mechanisms driving this impressive diversification focused so far on allopatric forces that assume a common, broad, host-plant range. To ask if host-adaptation processes have played a role in B. tabaci diversification, we aimed, in this study, to identify macroevolution patterns in the relationship between B. tabaci species and their host plants that have the potential to promote diversification. At first, we conducted a literature survey, which indicated that many species in the complex have been documented to harbor a limited host-range, with only few showing a truly broad one. Next, we tested if differences in performance and in expression profiles of genes involved in host utilization (detoxification genes), are shaped more by the phylogenetic relationships amongst the species in the complex, or by the species ability to successfully utilize many plants as hosts. Six species, representing different geographical regions and documented host ranges were analyzed. Performance assays divided them into two groups, one showing higher performance on various host plants than the other. The same grouping pattern appeared when the species were clustered according to their expression profiles, despite the species in the higher-performance group being only distantly related phylogenetically. Finding that the ability to perform well on multiple hosts exists in various clades of the B. tabaci species complex and that these species share a "common" detoxification machinery, can support a species expansion model (known as the "oscillation hypothesis") in which an ancestral ability to perform well on multiple hosts sets the stage for subsequent local adaptation and specializations, leading to fragmentation and eventually speciation.

Session/symposium: Intraspecific relationships/independent presentation

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Markovic D.<sup>1,2</sup>\*, Ninkovic V.<sup>3</sup>

## Plants respond to touch by releasing volatiles that repels herbivore insects

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Touch is one of the most common signals to which plants has to respond in order to readjust growth of individual organs and thus prepare themselves for imminent competition with nearby neighbours. The possible ecological implications of a plant's response to touch on plant-insect interactions have not been explicitly investigated. In our recent studies we have used soft face brush to mimic contact between nearby plants and also to isolate the mechanical component of touch from leaf chemicals. One minute of brief and light mechanical contact to the leaf surface of tested plants significantly reduced all tested morphological parameters making treated plants more compact. Touched plants emitted in total the same amount of volatiles as untouched controls but they increased emission of specific compounds like (E)-caryophyllene, (E)-nerolidol, germacrene D-4-ol, bcaryophyllene and 6-methyl-5-hepten-2-one. Such changes in emission of specific volatile compounds has been detected by aphids (Rhopalosiphum padi L., Aphis fabae Scop., Macrosiphum euphorbiae (Thomas) and Myzus persica (Sulzer)) that resulted in reduced attraction. The volatiles from touched plants were also significantly less attracted for ladybirds (Coccinella septempunctata L.), aphid natural which indicates that predators prefer habitats where the probability of finding herbivores is increased. Taken together our recent findings demonstrated that plant response to mechanical contact may have broader ecological implications beyond the plant itself, affecting organisms at higher trophic levels.

Session/symposium: Interspecific relationships/Semiochemistry of aphidophagous insects

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#### Maron J.L.

## Impact of herbivores on plant fitness and populations

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One of the boldest, and most controversial, predications Tibor Jermy made in his seminal 1984 paper was that herbivores have limited impacts on plant fitness and abundance. While subsequent work has shown otherwise, predicting when and where herbivores have important effects on plant fitness and abundance continues to be a challenge. In this talk, I will discuss what is known about how herbivores influence the number of successful progeny plants produce (i.e. fitness/abundance). Jermy recognized that the percentage of seeds destroyed by consumers does not necessarily align with how strongly those consumers influence plant fitness or dynamics. I will present a framework for understanding these impacts. In particular, I will focus on how determining the underlying drivers of context-dependency could advance our ability to predict the ecological and evolutionary outcome of plant-herbivore interactions. I will also touch on our understanding of links between defense traits and how these traits influence seed loss and the number of progeny produced.

Session/symposium: Interspecific relationships/Tibor Jermy's Legacy in insect-plant evolution

**ORAL PRESENTATION** 

Martin-Sánchez L.\*, Garbeva P.

Communication in the rhizosphere: the role of bacterial terpenes in belowground interactions

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One of the most abundant and highly diverse groups of secondary metabolites are terpenes. While generally considered as plant and fungal metabolites, it has become clear in the last years that bacteria also have the potential to produce these metabolites since terpene synthase genes are widespread in bacteria. However, to date, the ecological roles of bacterial terpenes in microbe-microbe and plant-microbe communication remain largely unknown.

The main aim of our research is to understand the function of bacterial terpenes and their role in belowground interactions. We have detected and explored several terpene-mediated interactions involving bacteria, fungi, protists and plants. For example, the soil inhabiting β-proteobacteria *Collimonas pratensis* Ter91 can provoke activation of certain soil protists by means of terpenes; the fungal plant pathogen *Fusarium culmorum* induces motility via volatile organic compounds (most of which are terpenes) in the maize rhizosphere isolate *Serratia plymuthica* PRI2C. In response to the fungal volatiles *S. plymuthica* PRI2C upregulated a multiproduct terpene synthase gene and produced the terpene sodorifen together with a cluster of novel terpenes. Preliminary data indicates that sodorifen may play a role in promoting maize plant growth.

Here we present our recent findings about the role of bacterial terpenes in the belowground microbe-microbe and microbe-plant interactions.

Session/symposium: Interspecific relationships/independent presentation

Martorana L.<sup>1,2</sup>, Brodeur J.<sup>2</sup>, Foti M.C.<sup>1</sup>, Colazza S.<sup>1</sup>, Peri E.<sup>1</sup>\*

Role of volatile and contact semiochemicals in mediating response of local egg parasitoids to an invasive not associated host

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When foraging, egg parasitoids rely on chemical stimuli from the host/plant complex. In tritrophic systems consisting of cultivated plants, herbivorous stink bugs and their platygastrid egg parasitoids, previous studies demonstrated that female wasps are attracted by oviposition-induced plant volatiles (OIPVs). These compounds act as indirect plant defence mechanism allowing plants to recruit egg parasitoids, which are in general the most important natural enemies of stink bugs. Moreover, when wasps land on a plant, they can exploit chemical traces left by male and female stink bugs walking over the leaves as indirect host-related cues, since these chemicals can lead them in areas where newly laid host eggs are likely to be found. Finally, since host female traces are the most promising signals of host eggs, wasps evolved the ability to distinguish between footprints left by females and males of their associated hosts. These foraging behaviours are adopted by playgastrid egg parasitoids searching for native and associated hosts, but what if an alien herbivore invades these infochemical networks? Here we focus on infochemical networks including two platygastrid egg parasitoids, Trissolcus basalis and Telenomus podisi, the former from Europe and the latter from North America, and an invasive pest, the brown marmorated stink bug (BMSB), Halyomorpha halys, a species native to Asia, which was recently introduced in the Old and New World. Experiments conducted under laboratory conditions showed that both egg parasitoid species were not attracted by the OIPVs of the BMSB. Concerning the substrateborn cues, experiments conducted with T. basalis and H. halys demonstrated that the egg parasitoid is able to spy chemical footprints left by BMSB adults but not to discriminate the sex of the invasive and not-associated host by exploiting them.

Funded by the project Impact of invasive alien true bug species in native trophic webs-INVASIoN (N. 690952) - H2020-MSCA-RISE-2015

Session/symposium: Interspecific relationships/independent presentation

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## Odorant-based detection of invasive species in fresh fruit

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Invasive species often hitch-hike and hide in imported goods and thus are often difficult to detect before they establish and become noticeable in the environment. Also for most insects, the immature stages such as eggs, larvae/nymphs are less mobile therefore harder to detect and trap. Actual detection tools based on vision are limited in precision at small scale but odours, which are often a by-product of metabolism, can reflect the state of a host and thus leak information from potential threats. More and more research is being done to identify the early symptoms of plant pathogens or insects caused either by microorganism infection or pest infestation. Using volatile markers of infestation represents a non-invasive way to design new tools of detection of invasive species in fresh goods.

We present the results of two studies comparing the volatile signatures of: 1) one fruit (apple, *Malus domestica*) infested with three different insect pests, the codling moth (*Cydia pomonella*), the brown marmorated stink bug (*Halyomorpha halys*) and the Queensland fruit fly ("QFF", *Bactrocera tryoni*), as well as 2) five different fruits infested with the same pest (QFF). Multivariate analysis of the chemical profiles allowed us to determine the specificity of the chemical responses and the possibility of discriminating the pest origin depending on the volatile signatures.

We discuss the differences between feeding guild, specificity of the pest or the host influencing the herbivore-induced volatiles and implication for designing generic odorantbased sensor for detection.

Session/symposium: *Practical applications/Semiochemical application for invasive species*ORAL PRESENTATION

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Stress survival strategies of the dominating bacteria *Enterococcus mundtii* in the gut of *Spodoptera littoralis* 

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The complex interaction amongst a higher organism and its resident gut flora is a subject of immense interest in the field of symbiosis. Insects harbor a population of gut bacteria that play roles in their growth, development and immunity. The gut microbiota of Spodoptera littoralis varies spatially and temporally. The core community consists of Enterococci, Lactobacilli and Clostridia. The selection of one bacterial species over the other is quite evident throughout the lifecycle, so is the differing bacterial population and abundance among the fore, mid and hind gut of the larva. By the time the larva reaches fifth instar, Enterococcus mundtii persist and dominate. The gut environment dictates the persistence of its residents. There is a pH gradient from alkaline to neutral along fore to hind gut respectively, and a depleted iron condition as posed by the chelator 8-HQA (acid) produced by the insects. We ask the following: How does the E. mundtii dominate by surviving the gut stress? What kind of interaction goes on between them and their host? A GFP-tagged reporter E. mundtii has been constructed to answer our questions. They are fed to the insects at early instars, and sorted from the gut spatially and temporally using flow-cytometry. A simultaneous transcriptomic analysis of the retrieved bacteria and the host gut tissue must tell us how they interact. The fluorescent reporter confirmed the persistence of E. mundtii in the gut. RNA-sequencing of the sorted bacteria has given us preliminary answers to some questions. There are upregulated pathways for stress survival: alkaline stress, biofilm formation, two-component signaling systems, resistance towards oxidative stress. There is a differential regulation among various metabolic pathways too. Studying the host transcriptome will give us the full picture of their interaction.

Session/symposium: Interspecific relationships/Insect-microbe interactions
ORAL PRESENTATION

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## Optimizing pheromone dispensers for codling moth mating disruption

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The high cost of mating disruption is often cited as a major impediment to broader adoption of the tactic. The economics of pheromone releasing dispensers could be improved through more efficient use of the precious active ingredient (ai). Dosage response studies allow us to optimize the amount of ai needed by altering the: quantity/dispenser, number of dispensers/area, release rate, or period of release. Field studies conducted in Michigan, indicate very similar disruption profiles and impacts using hand-applied codling moth pheromone dispensers releasing at much lower rates (Isomate CM Flex) than the standard dispenser (Isomate C-Plus). Equivalent levels of disruption have been achieved using lowreleasing Flex dispensers and standard dispensers in both small-plot and on-farm experiments. Similarly, high levels of orientational disruption have been achieved with deployment of as few as two aerosol-emitting devices per hectare. From a practical standpoint, 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. The amount currently released per day was essentially chosen to equate the release rate from one emitter to that of 400 hand-applied dispensers. In an effort to refine this technology and increase its costcompetitiveness, 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 codling moth 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 pheromone emissions to the peak hours of flight activity and by reducing the number of emissions per hour from four to one or two. Combined, these reductions in the pheromone requirement could significantly reduce the cost of aerosol emitters.

Session/symposium: Practical applications/Comparison of semiochemical control methods of pest insects

ORAL PRESENTATION

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The impact of monarch chemical defences on soil invertebrates.

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A large number of adult monarchs die at the overwintering sites in Mexico and the defence compounds they sequestered from plants would be released as the butterflies decompose. We tested the hypothesis that the release of these compounds would impact soil fauna. We collected soil and duff samples within an overwintering colony and from an adjacent control area and extracted the soil invertebrates. Preliminary results indicate that there is a significant decline in soil invertebrates within the monarch colony, compared with the controls. The full results will be presented, examining the impact on both the density and diversity of the invertebrate communities.

Session/symposium: Interspecific relationships/independent presentation
ORAL PRESENTATION

#### Meiners T.

## Chemical diversity in plant/beneficial arthropod interactions: tool or obstacle?

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In many crop systems key parasitoids or predators of arthropod pests are sufficiently widespread and abundant for biocontrol of the pest organisms. However, their efficacy is often not high enough to warrant a substantial reduction of their hosts or prey - and of yield losses. Can plant chemical diversity be used as a tool to enhance the efficacy of beneficial arthropods, or is it mainly an obstacle? What kind of research is needed to answer this question? Plant - arthropod interactions have evolved in chemically complex environments, formed primarily by host and non-host plants. Natural enemies of crop pests have evolved physiological and behavioral mechanisms to cope with or even to utilize the chemical diversity of the crop environment for successful host finding. Plant constituents fulfill a multitude of biological functions and determine the relationships between crops, pests and beneficial arthropods from the level of molecules to their function in the ecosystem and the agricultural landscape. Strategies for conservation and enhancement of parasitoid or predator efficacy need to be developed or improved that involve a positive effect of chemical diversity on beneficial arthropods. One option to achieve this is to adjust the composition of field margins or flowering areas in a way that they are supporting functionally natural enemies of nearby crop pests. To achieve this the scent diversity of the a) entire flowering area and b) specific plants needs to be determined and compared with overall arthropod biodiversity and specific multitrophic interactions; c) the composition of primary and secondary plant compounds and their effects on arthropods in terms of plant quality, resistance and stress tolerance on the arable land and in the neighboring flowering areas needs to be investigated. This complex knowledge will allow deciding if and how chemical diversity in plant/beneficial arthropod interactions can be applied as biocontrol tool.

Session/symposium: *Practical applications/independent presentation*ORAL PRESENTATION

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Identification and synthesis of a novel macrolide of the mantellid frog *Gephyromantis luteus*, a putative signaling compound

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*Male mantellid* frogs, endemic to Madagascar, possess femoral glands on the back of their hind legs, disseminating volatiles. These volatiles comprise secondary alcohols but mainly macrolactones, such as 8-methyl-2-nonanol and phoracantholide J. [Angew. Chem. Int. Ed. (2012) 51: 2187-2190.] They are used for communication in addition to acoustic, visual and tactile signals.

The femoral gland extract from males of *Gephyromantis luteus* was analyzed by GC/MS, showing only two compounds. Gratifyingly, NMR analysis of the mixture was possible due to the high amount of material. A ten membered aliphatic macrolide 3,7-dimethyl-8-ethyldecanolide, was proposed for one of the compounds after NMR analysis. This structural motif, featuring three stereogenic centers and an ethyl side chain, is unique among frog macrolides.

A synthesis was developed, starting from enantiomerically pure (S)-citronellal, which was converted to (S)-4-methyl-6-heptenoic acid via Wittig reaction, epoxidation, oxidative cleavage and subsequent oxidation. The counterpart for a later esterification, the chiral alcohol (3S, 4S)-4-methyl-5-hexen-3-ol, was synthesized by asymmetric crotylation using allyldiisopinocampheylborane. After esterification and ring closing metathesis, the macrolide was hydrogenated with Rhodium on carbon. Comparison of the gas chromatographic retention indices MS and NMR data, of the natural sample and the synthetic proved their identity. Evaluation of the absolute configuration via synthesis of the enantiomer and chiral-phase gas chromatography is ongoing. The identity of the second major component of the secretion, also a macrolide, will also be reported.

Session/symposium: New chemical structures/independent presentation

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Menke M.<sup>1\*</sup>, Melnik K.<sup>1</sup>, Peram P.S.<sup>1</sup>, Starnberger I.<sup>2</sup>, Hödl W.<sup>2</sup>, Vences M.<sup>3</sup>, Schulz S.<sup>1</sup> **Identification, synthesis and determination of the absolute configuration of new compounds in scent glands of African frogs** 

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Anuran amphibians (frogs and toads) often use acoustic, visual and tactile signals for communication. Next to these traits, African frogs of the family Hyperoliidae and Mantellidae use the chemical communication channel to transmit information via volatile pheromones. Males of the family Mantellidae carry particular femoral glands on the ventral side of the shanks, which emit species-specific compounds. [Angew. Chem. Int. Ed. (2012) 51: 2187–2190]

In contrast to mantellides, males of the family Hyperoliidae carry colorful gular glands on their vocal sacs. These glands emit a complex mixture of aliphatic macrolides and sesquiterpenes, which are difficult to identify just by comparison with mass spectral databases. [Biol. J. Linn. Soc. (2013) 110: 828–838.]

Herein, we present the identification, characterization and synthesis of an unprecedented, novel sesquiterpenoid macrolide occuring in both hyperolides and mantellides. This compound named frogolide occurs naturally in nearly enantiomerically pure form and is the first macrolide of terpenoid origin occuring in frog gland extracts.

Furthermore, we present the distinct analysis of gland extracts of the African reed frog *Hyperolius cinnamomeoventris*. The macrolides Gephyromantolide A and (5*Z*, 13*S*)-tetradec-5-en-13-olide [Beilstein J. Org. Chem. (2016) 12: 2731–2738] were identified by synthesis and by comparison with authentic material. On top of that, a novel occurring sesquiterpenoid alcohol, bearing a cadinane backbone, will be presented.

The structure elucidation of these compounds via synthetic approaches and analysis of extracts via chiral gas chromatography is the first step of the recognition of their role in chemical communication.

Session/symposium: Interspecific relationships/Ecology and evolution of toxins in vertebrate animals

**ORAL PRESENTATION** 

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Chemical Warfare: The battle between termite-associated actinobacteria and *Trichoderma harzianum*, a fungal pathogen

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Termite-associated actinobacteria are prolific producers of biologically active small molecules that exhibit a range of potencies against pathogenic microbes, including Trichoderma harzianum, a common biocontrol agent. This constant exposure to antimicrobial agents pressures the pathogens to evolve defense mechanisms in order to counteract the antagonism. These mechanisms include detoxifying the agent, increasing the number of active efflux pumps to remove the toxin, or by switching 'on' a cryptic or silent gene cluster that encodes for an antibacterial agent. Our work elucidated a chemical conversation between the fungal pathogen, T. harzianum, and a termite-associated actinobacteria, revealing a likely co-evolution of defense metabolites. We have found that a bacterial antifungal agent - bafilomycin C1 - up-regulates the production of several metabolites in the fungal pathogen, including a metabolite that exhibits strong antibacterial activity. Three major up-regulated metabolites were identified, one with strong antibacterial activity and another with a novel carbon backbone. Structure elucidation of the latter metabolite was challenging due to its highly oxygenated nature and a low H/C ratio, 0.92. Traditional structure elucidation using 2D NMR was unsuccessful, however a combination of advanced NMR experiments and computer-assisted structure elucidation successfully elucidated the structure and revealed the presence of 15 quaternary carbons and 8 stereogenic centers. Overall, this project illuminated the diverse chemical warfare ongoing between termite-associated Streptomyces and a fungal pathogen, yielding a small glimpse of the complex interactions constantly occurring between microbes within the termite nests.

Session/symposium: New chemical structures/independent presentation

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Mfuti D.K.<sup>1</sup>\*, Subramanian S.<sup>1</sup>, Tamiru A.<sup>1</sup>, Akinyemi A.O.<sup>1,2</sup>, Drijfhout F.P.<sup>2</sup>, Pope T.W.<sup>3</sup>, Kirk W.D.J.<sup>2</sup>

Field efficacy of different concentrations of male bean flower thrips pheromone compounds in Kenya

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Bean flower thrips (BFT), *Megalurothrips sjostedti* (Thysanoptera: Thripidae), are a major pest of cowpea and other leguminous crops in Kenya, with high dependence of growers on pesticides for their management. Development of alternatives to insecticides for BFT management is critical to make food safe. Recently, we have identified two distinct pheromone compounds (major and minor) in the headspace of male BFT. These compounds were synthesized and tested in the field at doses of 3 µg, 30 µg and 300 µg. Hexane solvent was used as the control. The dose in 100 µl of solution was spread on rubber septum dispensers and field tested on commercial blue sticky traps in a cowpea (*Vigna unguiculata*) field. Results showed that attraction of female BFT varied significantly between the different concentrations for both major and minor BFT pheromone compounds. However, males were only attracted significantly to the major compound and the attraction differed with the concentration. Both sexes were more attracted to doses of 3 µg and 30 µg than to 300 µg or the control. Further studies are in progress to determine whether a blend of the compounds provides synergistic effect to maximize trap catches of both females and males *M. sjostedti*.

Session/symposium: Intraspecific relationships/independent presentation

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Michereff M.F.F.<sup>1</sup>, Grynberg P.<sup>2</sup>, Togawa R.<sup>2</sup>, Laumann R.A.<sup>1</sup>, Jing-Jiang Z.<sup>3</sup>, Schimmelpfeng P.H.C.<sup>1</sup>, Borges M.<sup>1</sup>, Pickett J.A.<sup>4</sup>, Birkett M.A.<sup>3</sup>, Blassioli-Moraes M.C.<sup>1\*</sup> Maize herbivore-induced plant volatiles (HIPVs) elicited by *Spodoptera frugiperda* larvae prime neighboring plants for enhanced indirect defence

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Priming is a defensive mechanism in plants whereby a faster and/or stronger response to herbivore challenge is elicited following previous exposure to herbivore-induced plant

volatiles (HIPVs). This study aimed to evaluate whether HIPVs emitted by *S. frugiperda*-damaged maize genotypes could trigger priming in neighboring undamaged maize plants. Maize genotypes with different levels of HIPV production were selected. Chemical analysis of volatiles emitted by primed and non-primed maize plants was conducted and the influence of volatiles emitted by these plants on the foraging behaviour of the egg parasitoid, *Telenomus remus*, a natural enemy of *S. frugiperda*, was assessed using olfactometer bioassays. The results showed that HIPVs emitted by maize genotype L3, which produces low levels of HIPVs, did not induce detectable priming of defence in neighbouring plants of the same genotype. However, HIPVs emitted by the genotype Sintetico Spodoptera, which produces high level of HIPVs, induced priming in neighbouring plants of the same genotype. In addition, volatiles from primed plants attracted *T. remus* at an earlier stage, compared to volatiles from maize plants that were directly damaged by *S. frugiperda*. In summary, our results show that HIPVs induced in maize by *S. frugiperda* can elicit priming in neighboring maize plants and enhance indirect maize defence, and also show that the level of HIPVs is important for elicitation of the priming effect.

Session/symposium: Interspecific relationships/independent presentation ORAL PRESENTATION

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## Chemical parsimony and chemical diversity in the pheromones of cerambycid beetles

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Pheromones or likely pheromones have now been identified from several hundred species worldwide, but the number of compounds that constitute these pheromones is <<100 because many compounds are widely shared among related species, genera, and tribes. Conversely, despite this widespread parsimony, there is increasing evidence that some species produce compounds which may only be shared among a very limited number of congeners, or may even be species specific. Thus, at one extreme, congeneric species on multiple different continents may have the same pheromone, whereas at the other extreme, sympatric congeners may have pheromones of widely differing structures, arising from entirely different biosynthetic pathways. Some examples of recently identified pheromones will be described, with a focus on invasive species from Asia, and species from South America.

Session/symposium: New chemical structures/Invasions under the bark: chemical ecology of woodboring beetles

ORAL PRESENTATION

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Miller J.R.

Principles of pest management guiding determinations of appropriateness for semiochemical control tactics

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This presentation will briefly review the pros and cons of semiochemical pest control tactics relative to other pest management approaches, including insecticides. Emphasis will be placed on whether or not efficacy is or is not dependent on pest density and why that is true. Also considered will be temporal differences over which a treatment must last. A very important factor influencing efficacy for semiochemical controls is the area of action per unit of chemical dispensed. For example, recent findings indicate that plume-reach for mating disruption dispensers are not as large as previously expected. Explanation will be offered for how such "reaches" can be measured experimentally and why they have great impact on behavioral controls of pests.

Session/symposium: Practical applications/Comparison of semiochemical control methods of pest insects
KEYNOTE LECTURE

Mitchell R.F. <sup>1</sup>\*, Schneider T.M. <sup>1</sup>, Schwartz A.M. <sup>1</sup>, Andersson M.N. <sup>2</sup>, McKenna D.D. <sup>3</sup>

Ten genomes reveal the gain and loss (and occasional persistence) of odorant receptor lineages across the Coleoptera

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The family of odorant receptors (ORs) is the primary means by which insects recognize volatile compounds, and their evolution across insect orders is characterized by extraordinary divergence and extensive gain and loss. However, the evolution of ORs is poorly recorded within many orders; for example, ORs from the largest order of insects – the Coleoptera – are fully annotated from the genomes of only three species in the polyphagan families Tenebrionidae, Chrysomelidae, and Cerambycidae. Here, we present the identification and analysis of ORs from the genomes of eight additional species that include a strepsipteran outgroup (family Mengenillidae), the three basal suborders of beetles (families Cupedidae, Hydroscaphidae, and Carabidae), and diverse members of the suborder Polyphaga (families Silphidae, Scarabaeidae, Buprestidae, and Curculionidae). Our phylogeny provides support for at least seven subfamilies of ORs; four of these appear to be specific to the Coleoptera, but none are present in all species of beetles. Genes within these subfamilies evolve in large, lineage-specific expansions, which frequently include the majority of a species' OR suite. However, some conserved orthologs persist throughout the beetle tree of life, suggesting a few candidate genes that may be foundational to the chemosensory biology of beetles.

Session/symposium: Interspecific relationships/Arthropod chemoreceptors
ORAL PRESENTATION

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Quantifying the short-term effects of logging in oak dominated forests on threatened longhorn beetles (Cerambycidae) with a pheromone-based trapping system

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Saproxylic longhorn beetles include a large number of threatened species and flagship species for insect conservation. However, many species are problematic to study quantitatively as they are dependent on ephemeral substrates such as fresh, recently dead wood, a habitat which is difficult to sample with conventional methods. Recently, we have identified the aggregation-sex pheromones of several longhorn beetles dependent on dead oak wood in order to construct an efficient tool for detection, quantification and monitoring of their local abundance. Using pheromone-based trapping, we conducted a two-year study measuring beetle abundance of seven species at 52 oak dominated forest stands and woodlands in Sweden. The sites were categorized into four types; treatment stands, controls, key biotopes and hotspots. Treatments were forests which had undergone recent logging activity with comparatively large amounts of fresh oak wood (primarily twigs and branches). Controls were similar, but untreated, stands which had much smaller amounts of dead oak wood. The key biotopes are small set aside forests intended to benefit biodiversity while hotspots are sites well known for their importance to threatened saproxylic beetles associated with oak. In general, treatment stands had a higher average number of beetles compared to the controls, but the strength of the effect varied depending on species. Surprisingly, key biotopes and hotspots did not perform better than the controls and had fewer beetles than the treatments. We conclude that thinning or logging in oak forests can benefit many species in this group of longhorn beetles, provided that some wood material is left on site, but ecologically demanding species, e.g. Poecilium pusillum and Plagionotus detritus, will require more specific conservation actions. In addition, pheromone-based monitoring appears to be a promising tool to study this group of elusive insects under field conditions.

Session/symposium: *Practical applications/independent presentation*ORAL PRESENTATION

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Large expansion of gustatory receptors in the genome of the noctuid moth Spodoptera littoralis

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The evolution of chemosensory receptors is expected to play an important role in the adaptation of insects to a diversity of ecological niches. Whereas the function and the evolution of olfactory receptors have been the focus of intense research in the last years, the gustatory receptors (GRs) have received less attention. GRs are expressed in taste organs and are believed to detect non-volatile molecules such as sugars and bitter compounds found on food sources and oviposition sites.

We annotated the repertoire of candidate GRs in the genome of *Spodoptera littoralis*, a polyphagous noctuid moth. We found a massive expansion of GRs, with more than 200 genes annotated. Most of these genes were found in large gene clusters, suggesting recent and repeated tandem duplications. Phylogenetic analyses revealed that this expansion occurred in candidate bitter receptor clades, and is not restricted to *S. littoralis* as a large number of candidate bitter receptor genes arranged in clusters could also be identified in closely related species. GR expansion could thus constitute a hallmark of Noctuidae, the most diversified family of Lepidoptera. In order to investigate expression patterns of GR genes, we also carried out a transcriptomic study on different taste organs of *S. littoralis*, and compared expression patterns with those observed in other lepidopteran species.

This work lays the foundation for future studies on the link between the evolution of GRs and the adaptation to polyphagy in Noctuidae.

Session/symposium: Interspecific relationships/Arthropod chemoreceptors

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<sup>&</sup>lt;sup>2</sup>Inra, iEES-Paris, Versailles, France

<sup>&</sup>lt;sup>3</sup>BioInformatics Platform for Agroecosystem Arthropods, Rennes, France

Moreira Dias A.<sup>1</sup>, Blassioli-Moraes M.C.<sup>2</sup>, Borges M.<sup>2</sup>, Čokl A.<sup>3</sup>, Laumann R.A.<sup>2</sup>\* Chemical footprints influence on vibratory communication and mating behaviour of the Neotropical brown stink bug, *Euschistus heros* (F) (Hemiptera: Pentatomidae).

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Interaction between signals from different channels could be decisive for satisfactory information interchange during insect communication. During the reproductive behavior stinkbug communicate by chemical and physical signals as pheromone, chemical traces (footprints) and substrate-bone vibrations. In this work we evaluated how chemical footprints of different gender can affect vibratory communication during the mating behaviour of Euschistus heros. Females, males or couples of virgin insects were exposed to footprints of females or males left on Petri dishes. To obtain the footprints 5 insects of the same sex were placed in a cleaned Petri dish (14 cm of diameter), which was positioned on a clean glass plate for two hours, after this the insects were taken off and the Petri dish with the stink bugs footprints was used for experiments. Clean Petri dish were used as control arenas. Experimental arenas were closed using another Petri dish with the same treatment. For all combination of treatments one insect or a couple (N = 30), were left inside the arena during a period of 15 minutes or until the couple mate. The behavior was monitored and the vibrational signals were registered from glass surface with a portable digital laser vibrometer. When insects were evaluated alone it was observed that male footprints trigger females to call emitting vibratory signals. On the other hand, males were note stimulated to sing in presence of both, female or male footprints. Couples in presence of female footprints showed higher copulation rate (83%) than couples in presence of male footprints (50%) or control (53%). Results suggest that male footprints stimulate calling behavior of females and females footprints stimulate courtship behaviour and copulation. Chemical analyses of footprints are being conducted to check for differences on chemical profile between the sexes that could explain their different stimulation of insect behaviour.

Session/symposium: Intraspecific relationships/Complementary or predominant role of non-chemical based communication and orientation of insects

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Enzymes involved in cuticular hydrocarbon (CHC) diversity in insects: insights from an extraordinary case of intrasexual CHC profile dimorphism in a mason wasp

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Cuticular hydrocarbons (CHC) diversity and behavioral effects have been intensively studied over the last decades. CHCs play two essential roles for insects: as anti-desiccation agents and for intra- and interspecific communication (e.g., as sex, territorial, and epideictic pheromones, and for caste and species recognition). Yet, only few genes involved in their biosynthesis have been identified. By studying an exceptionally case of intrasexual CHC profile dimorphism in the spiny mason wasp, Odynerus spinipes (Hymenopetera: Vespidae), we are able to shed light on the biosynthesis of CHCs. O. spinipes females express two CHC profiles (chemotypes) that differ in more than 70 CHCs qualitatively from each other. Females seem to differ exclusively in this specific trait and keep their chemotype during their entire lifespan. We searched for genes that are systematically differentially expressed in females of the two chemotypes. By doing so, we readily identified ca. 15 candidate genes likely involved in CHC biosynthesis. We currently seek to validate the function of these genes via RNAi-mediated knockdown experiments on O. spinipes and on the eusocial vespid wasp, Polistes dominula. The CHC dimorphism of O. spinipes females not only prove to be an intriguing gateway for shedding light in the genetics of CHC diversity, but also raises the question why and how this dimorphism evolved in the first place. The CHC dimorphism seems to be the result of intraspecific and interspecific kleptoparasitism. O. spinipes females steal weevil (Coleoptera: Curculionidae) prey from the nests of other O. spinipes females or they take the ownership of the nest of other O. spinipes females expressing the same chemotype. Furthermore, two cuckoo wasps (Hymenoptera: Chyrsididae) that each chemically mimic one of the two O. spinipes CHC profiles kleptoparasitize O. spinipes nests.

Session/symposium: New chemical structures/Omics in chemical ecology Oral presentation

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Mozuraitis R.<sup>1,2</sup>\*, Zurita J.<sup>3</sup>, Ilag L.<sup>3</sup>, Wiklund C.<sup>2</sup>, Borg Karlson A.K.<sup>4</sup> **Anti-aphrodisiac pheromone, a renewable signal in adult** *Pieris napi* butterflies

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The male butterfly *Pieris napi* produces the anti-aphrodisiac pheromone methyl salicylate (MeS) and transfers it to the female during mating. After mating female releases MeS, when courted by conspecific males, which decreases her attractiveness and the duration of male harassment, thus increases her time available for egg-laying. In previous studies we have shown that MeS is produced from the amino acid L-phenylalanine in males during larval development. Since P. napi is polyandrous, males would gain from being able to restore the titer of MeS after mating. In this study we show that adult males of P. napi can utilize phenylalanine and aromatic flower volatiles as building blocks for the production of their anti-aphrodisiac pheromone. We demonstrate this by feeding butterflies with stable isotope labelled molecules mixed in sugar solutions, and, to mimic the natural situation, we feed male butterflies with nectar rich host plants fertilized with labelled phenylalanine. The volatiles from butterflies and plants were collected and identified by solid phase micro extraction, gas chromatography and mass spectrometry techniques. Our results clearly showed that the L-phenylalanine and aromatic floral volatiles including benzaldehyde, benzyl alcohol, benzoic acid and methyl benzoate present in floral nectar are incorporated in the anti-aphrodisiac pheromone in adult butterflies. Hence, males are able to a certain extent restore the level of anti-aphrodisiac titer prior to their next mating. In addition to MeS two additional compounds, namely benzyl cyanide and 2-methoxyphenol, containing labelled atoms, were identified in males, transferred to females at mating in the spermatophore and together with MeS, were detected in effluvia from females when adopting the mate-refusal posture. The behavioural function of benzyl cyanide and 2-methoxyphenol is still unknown.

Session/symposium: Interspecific relationships/independent presentation

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Jasmonates ...but who are "the others" in rice defense against herbivores?

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While jasmonates are major players in the induced plant defense against herbivores, their function in plants is obviously much more complex. For example, apart from defense, jasmonates are needed for fertility in plants, which raises pragmatic questions of how defense and reproductive roles of jasmonates are coordinated in plant development. Our recent findings suggest that function of jasmonates could be even more complex in monocots. For example, we observed contrasting impacts of jasmonates on two woundinduced defense responses in rice, volatile organic compound (VOC) emissions and leafassociated accumulation of secondary metabolites. In brief, jasmonate-signaling-deficient mutants, Osjar1 and hebiba, emitted substantially less indirect defense VOC signals, in particular, the monoterpene linalool, but the levels of wound-induced direct defenseassociated phenolamides in the leaves remained intact, even if rice plants completely lacked jasmonates, or their major bio-active form, JA-Ile. This striking difference suggests that another wound-induced signaling pathway, independent of JA, must be involved in the regulation of phenolamide defense against insects in rice. As a closest natural candidate, ethylene signaling is also known to be induced by herbivory in plants. To examine possible involvement of ethylene, and its crosstalk with jasmonate signaling pathway in rice, we employed a well-characterized ethylene response blocker, 1-methylcyclopropene (1-MCP) and examined its impact on both VOC release and phenolamide accumulations in rice plants treated with simulated herbivory, i.e. leaves with wounds and application of oral secretions from the chewing rice herbivore Mythimna lorevi. Interestingly, 1-MCP showed differential effects on both metabolic pathways, suggesting direct involvement of ethylene, which is further examined in the context of significant changes in defense strategies found during natural aging of rice plants.

Session/symposium: Interspecific relationships/independent presentation

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Mushroom with host-associated cues enhance the olfactory response of the parasitoid wasp of fungivorous larvae

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Some parasitoids can locate the host using reliable and predictable cues such as smells derived from host plants or from the hosts themselves. Several studies have been conducted on the functions of plant infochemicals in the tritrophic interactions between plants, herbivores, and parasites. However, till date, there is limited information about the functions of fungi or other olfactory cues in the tritrophic interactions between fungi, fungivorous insects, and parasites. Recently, we found that female Orthocentrus sp. (Hymenoptera: Ichneumonidae) exhibit parasitic behavior for the larvae of the fungivorous fly Neoempheria spp. (Diptera: Mycetophilidae). Orthocentrus sp. is a solitary, internal parasitoid of the fungivorous larvae. In our study, we established the breeding system of the flies and parasitoids using the commercial mycelial block of the shiitake Lentinula edodes (Agaricales: Omphalotaceae) and investigated in detail the parasitic and the host-locating behavior of parasitoids in the laboratory. Volatile preference experiments using a Y-tube olfactometer demonstrated that the parasitoid females exhibit a response to the volatile of a piece of the mycelial block of the shiitake. Parasitoid females exhibited an even stronger preference for a piece of the mycelial block that was infested by the larvae of the flies. In addition, GC-MC Analyses revealed that the volatile of the fungivorous larva-infested mycelial block was different from that of the noninfested mycelial block. We also discuss about the roles of some volatiles released by fungivores-infested mycelial block in the parasitoid's orientation to host-specific volatile cues.

Session/symposium: Interspecific relationships/independent presentation

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## Metabolomics as tool to decipher the role of metabolites in trophic plant interactions

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Plants are continually challenged by competitiors, pests and pathogens and, being sessile, must be able to fight their ground. Most plants have developed a very sophisticated 'chemical arsenal' which enables them to respond adequately to these different biotic stresses. To elucidate and define this chemical arsenal we apply (un-)targeted metabolomics as deep-phytochemical profiling tool. Enabling technologies, such as metabolomics and proteomics, broaden our knowledge of how plants are molecularly organized and how genetic varitation is translated into phenotyes. Metabolomics in particular is gaining much attention mainly due both to the comprehensiveness of the technology and also the potentially close relationship between biochemical composition and phenotype. Here, we will give examples of how metabolomics technologies can be exploited to study the natural variation of the metabolome of brassicaceaous plants and to understand beter how this variation shapes the interaction with organisms of other trophic levels and vice versa. Members from the family Brassicaceae represent a diverse and very interesting group of plants. Their close relationship with the model plant, Arabidopsis thaliana, makes combined research on these species both scientifically valuable and of considerable commercial importance.

 $Session/symposium: \begin{tabular}{l} Interspecific & relationships/Metabolomics & approaches & in & the \\ Brassicaceae & & & & \\ \end{tabular}$ 

ORAL PRESENTATION

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## Soil conditioning by a novel maize cropping system affects constitutive plant defences

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Soil properties have been shown to affect plant phytochemistry. In this study we investigated the effect of emergent soil properties as a result of Push-Pull cropping technology on plant defences. Push-Pull technology is a pest management strategy that involves attracting cereal stemborers with trap plants; Napier grass (Pull) whilst driving them away from the main crop using a repellent intercrop; Desmodium (Push). The system has been shown to have ecological and socio-economic emergent properties that go far beyond the effects of efficient insect pest control. Chemicals released by intercrop roots induce abortive germination of the parasitic Striga weed, providing very effective control of this noxious weed. Soil fertility is improved through nitrogen fixation and increased soil organic matter by the intercrop and soil degradation is prevented as the intercrop acts as a cover crop. We hypothesised that the emergent improved soil quality affected secondary plant metabolites. Maize plants were grown in soil collected from push-pull and non-push pull fields in the screen house. Secondary plant metabolites were collected from 3-week old plants via headspace sampling and harvesting of leaf and root tissue. Coupled Gas Chromatography-Mass Spectrometry and High Pressure Liquid Chromatography were used for chemical analysis. Maize plants grown in soil from push-pull fields emitted higher amounts of (E)-4,8-dimethyl-1,3,7-nonatriene,  $\beta$ -caryophyllene,  $\beta$ -farnesene,  $\alpha$ -humulene, (E,E)-4,8,12-trimethyl-1,3,7,11-tridecatetraene and DIMBOA compared to those grown in soil from non-push-pull fields. These results show that push-pull cropping system-associated changes in soil quality affect plant metabolism and increase maize crop secondary metabolite production with effects on plants' direct and indirect defences against herbivores.

Key words: Maize, plant volatiles, cropping system, soil

Session/symposium: Practical applications/independent presentation

**ORAL PRESENTATION** 

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Müller C.<sup>1</sup>\*, Schweiger R.<sup>1</sup>, Bonte A.<sup>2</sup>, Voelsen J.<sup>1</sup>, Pons C.<sup>1</sup>, Brühl L.<sup>2</sup>, Matthaus B.<sup>2</sup> Moist storage of *Brassica napus* seeds induces rapid changes in the metabolic profiles of stored material and the resulting oils

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Cold-pressed rapeseed oils are usually prepared from non-germinated seeds and characterised by a seed-like and nutty smell and taste, which is caused by aroma-active degradation products of seed metabolites such as aldehydes, alcohols, esters and isothiocyanates. However, if seeds start to germinate and microorganisms grow due to unfavorable conditions during storage, oil pressed from this material may show drastically decreased quality. We used a multi-analytical metabolomics approach to characterise the profiles of carbohydrates and organic acids (GC-MS), amino acids (LC-FLD) and glucosinolates (LC-DAD) of non-germinated seeds and seeds kept moist for a few hours up to four days. Additionally, we measured the activities of myrosinases, which are the enzymes that hydrolyse glucosinolates to various breakdown products, including isothiocyanates and nitriles. Moreover, oil was pressed from this material after drying and volatile profiles measured from the resulting oils (GC-MS). The concentrations of several primary metabolites rapidly increased with moist storage, likely indicating the breakdown of storage compounds to support this energy-demanding process. Concentrations of indole glucosinolates increased with a slight time offset, suggesting that amino acids released from proteins were used for their biosynthesis. Myrosinase activities likewise changed over moist storage time and showed a higher affinity towards aliphatic compared to indole glucosinolates. In line with these changes, glucosinolate breakdown products increased in concentrations in the volatile profiles of the oils, negatively affecting their sensory quality. Our metabolomics approach thus provides a direct time-resolved link between changes in metabolism of seed material under moist storage conditions causing germination and the chemo-sensory quality of the resulting oils.

Session/symposium: Interspecific relationships/Metabolomics approaches in the Brassicaceae

ORAL PRESENTATION

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## Maize chlorotic mottle virus induced host plant volatiles attract vector thrips species

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Plant viruses are known to manipulate their vectors' behaviour through host plant nutrients or odours to enhance their transmission and spread. Uncovering mechanisms mediating interactions between pathogens, host plants and vectors could provide useful insights for designing effective vector management strategies. In this study, we examined the chemical ecology of Maize chlorotic mottle virus (MCMV)- maize (host)-thrips vectorsmulti-trophic interactions to better understand the vector ecology and disease epidemiology. The behavioural responses of the two vector thrips species, i.e. Maize thrips (Frankliniella williamsi) and Onion thrips (Thrips tabaci), to maize volatiles inoculated with MCMV and healthy plants were studied, and the volatile profiles compared by gas chromatography (GC) and GC coupled mass spectrometry (GC-MS). In the bioassay, both sexes of F. williamsi and male T. tabaci were significantly attracted to volatiles from maize plants infected with MCMV when compared to healthy plants and solvent controls. Moreover, chemical analysis revealed strong induction of (E)-4,8-dimethyl-1,3,7-nonatriene, methyl salicylate and (E, E)-4,8,12-trimethyltrideca-1,3,7,11-tetraene in MCMV-infected maize volatiles. Our findings demonstrate MCMV induces changes in the volatile profiles of host plants to elicit attraction of thrips vectors. The increased vector contact rates with MCMV-infected host plants could enhance virus transmission if thrips feed on the infected plants and acquire the pathogen prior to dispersal. Strategies for exploiting virus induced plant volatile semiochemicals in integrated vector management are considered.

Session/symposium: Interspecific relationships/independent presentation

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# Metabolic profiling of benzoxazinoids in weed suppressive and early vigour wheat genotypes

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Wheat (Triticum aestivum L.) cultivar trials were conducted in moderate to low rainfall zones at Wagga Wagga and Condobolin NSW in 2014-2016. At each site, crop and weed growth was monitored at tillering, vegetative, grain filling, harvest and post-harvest. Wheat roots, shoots, rhizosphere and bulk soils were collected from June to November for metabolomic profiling and biomass evaluation. Plant samples were extracted in methanol using an automated Buchi high pressure extractor while soil samples were extracted by rotary shaker. Extracts were analysed using untargeted analysis by UPLC-ESI MS QToF (Agilent 6530) and data analysed using Mass Profiler Professional (Agilent). Benzoxazinoids (BXs) were profiled using targeted analysis in negative ion mode while key microbiallyproduced metabolites (APO,AAPO,AAMPO) were profiled in positive ion mode. Targeted metabolic profiling resulted in detection of up to 20 individual BXs including BX glycosides,lactones,hydroxamic acids and related microbial metabolites. Qualitative and quantitative differences in BXs were observed and were cultivar-, growth stage- and locationdependent. Plant part and rhizosphere location (distance from root) also impacted BX concentration. Microbially-produced metabolites with phytotoxic activity were detected in rhizosphere soils. Metabolic profiling provided critical knowledge of seasonal impacts on wheat metabolism, as well as the biosynthesis and release of metabolites associated with weed suppression in commercial wheat cultivars, in contrast to rye (Secale cereale L.) and a heritage wheat cultivar Federation, both recognized for their potent ability to suppress weeds. Certain cultivars maintained high yield potential and were significantly more weed suppressive, depending on year and location, potentially due to their vigorous early growth habit, canopy architecture, and the release of BX and related microbially produced metabolites into the rhizosphere over time.

Session/symposium: New chemical structures/Omics in chemical ecology ORAL PRESENTATION

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Chemical ecology of the water vole *Arvicola terrestris* (Cricetidae) for sustainable population control in Europe

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The water vole *Arvicola terrestris* (= *Arvicola amphibious* L.) is endemic in Europe where its outbreak generates severe economic losses for farmers. Our project aims at deciphering the modalities of chemical communication in this species, to develop new sustainable methods for populations control. The water vole, as well as other rodents, uses specific urination sites as territorial and sex pheromone markers, the chemical and biochemical identification of which is still unknown. Lateral scent glands and urine samples were collected from wild males and females caught in the field, at different periods of reproduction. Volatile signals were searched in urine by SPME/GC-MS, and in lateral scent glands by solvent extraction followed by GC-MS.

The volatile composition of urine was analyzed for each individual and not on pooled samples, showing no significant difference between males and females. Lateral scent glands contained some volatile components (pyrazines, alcohols, terpenes), and mostly long chain fatty acid esters, again without quantitative and qualitative differences between sexes. Conversely, the urinary protein content, analyzed by 1D- and 2D-electrophoresis is totally different, as only males secrete high levels of lipocalins, whatever the reproduction period. The proteins were identified by "de novo" sequencing by mass spectrometry (Orbitrap), which provided enough information to design specific primers for PCR amplification of the sequences. The identified protein sequences are closed to those of *Myodes glareolus*, a closely related species of Cricetidae (Stopkova et al., 2010; Loxley et al., 2017).

Session/symposium: Intraspecific relationships/independent presentation ORAL PRESENTATION

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Comparing the efficiency of pheromone traps for three main click-beetle (Elateridae: *Agriotes* sp.) pests of Hungary

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Wireworms, the larvae of Elateridae species are harmful soil-dwelling pests due to their broad host plant preference and abundance in arable lands. Their distribution and abundance can be monitored only with labour-intensive soil-sampling and trapping, while the adults can easily be sampled by species-specific pheromone traps. To evaluate the damage risk, data of the species sampled separately should be summarized, but the result can be biased by the unknown efficiency of pheromone traps.

The efficiency of pheromone traps for *Agriotes sputator*, *A. rufipalpis* and *A. ustulatus*, the three dominant elaterid pests of Hungary, was compared with mark-recapture method in 2017 near Debrecen (East Hungary). In case of each species at least 10 groups of 25 marked beetles were released in a circle (r=6m) formed by 6 pheromone traps, and the recapture rate and time of recapture were recorded.

All recaptured *A. rufipalpis* and *A. ustulatus* specimens were recorded within 8 days after release while many *A. sputator* were recaptured after this period. The last *A. sputator* was recaptured 39 days after, which showed the different vagility and habitat use of this species.

The mean recapture rate of the three studied species varied between 32.5 and 34.5%. The efficiency of the pheromone traps of the three studied species was nearly similar thus their catches can be summarized for risk assessment without weighting.

Session/symposium: Intraspecific relationships/independent presentation

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Navarro M.<sup>1</sup>, Amat C.<sup>1</sup>, Bau J.<sup>2</sup>, Gemeno C.<sup>1</sup>\* **Sublethal effects of neonicotinoid on Tortricid moth pheromone communication** 

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The neonicotinoid thiacloprid was topically applied to adult male and female tortricid moths (*Cydia pomonella*, *Grapholita molesta* and *Lobesia botrana*) to determine the effect of sublethal insecticide doses (LC0.001, LC1, LC10, LC20) on sex pheromone communication. The percentage of calling females decreased dramatically with insecticide application, starting at lowest concentration tested (LC0.001), however the calling period and the composition and quantity of sex pheromone were minimally affected by the insecticide. The percentage of males responding to the sex pheromone in the wind tunnel also decreased dramatically, starting at LC1, and several flight track parameters were also affected (starting LC0.001), but the electroantennogram responses of intoxicated males was unaffected by the insecticide. The effect of thiachloprid was not equal in all three species. Our results could be relevant in the semiochemical control of these three important fruit-tree pests.

Session/symposium: Practical applications/independent presentation

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#### Allelopathic effects of Solidago canadensis on seed germination and seedling growth

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Solidago canadensis is native plant to North America, but in recent decades it spread throughout Europe, large parts of Asia, Australia, and New Zealand (Wang et al., 2016). The spread of the plant is possible due to the release of allelopathic substances. The allelopathic effects of leaf extracts on seed germination have been studied extensively, but the effects of blossom and root extracts are not well evaluated.

The aim of this study was to determine the activity of leaf, flower buds, and root extracts of the Canada golden-rod to seed germination an seedling growth. Under laboratory conditions allelopathic properties of three active fractions (acidic, neutral and alkaline) were tested on *Lactuca sativa* and *Lepidium sativum* seeds. Results shown, that the inhibitory allelopathic effect of leaf, flower buds, and root extracts differs. All fractions of flower buds extract in comparison to other vegetative parts are the most active and can inhibit seed germination completley.

Wang C. et al. (2016) Ecotoxicology 25:555–562.

Session/symposium: Interspecific relationships/independent presentation

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Nevo O.<sup>1</sup>\*, Razafimandimby D.<sup>1</sup>, Jeffrey J.A.J.<sup>1,3</sup>, Schulz S.<sup>4</sup>, Ayasse M.<sup>1</sup> Is fruit scent an adaptation to primate seed dispersal?

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Many angiosperms rely on animals for pollination and seed dispersal. This resulted in an evolution of a plethora of floral and fruit traits that attract animal mutualists. In flowers, various studies demonstrated the importance of both visual and olfactory signals in pollinator attraction. In fruits, visual signals have evolved to attract seed dispersers, primarily birds, which possess excellent color vision. Yet with the exception of a few studies the role of olfactory signals through fruit scent has remained understudied. Primates are now known to possess an excellent sense of smell which is primarily used for fruit selection. Thus, if fruit scent is under selection exerted by primate feeding behavior, fruits of species that specialize on primate seed dispersal are expected to be selected to shift their scents upon ripeness to allow primates to discriminate ripe from unripe fruits. In contrast, in species that rely on olfactory less -oriented frugivores, a change in scent profile upon ripeness may result from fruit maturation processes but is expected to be substantially less marked. We conducted a comparative study of changes in fruit scent in a plant community in Madagascar. We show that plants that specialize on seed dispersal by lemurs - the local primates - tend to substantially increase their scent production upon ripeness while bird specialists or generalists do not. More strikingly, the difference in the chemical composition of ripe and unripe fruits is significantly higher in lemur-specialist species. These effects are independent of phylogeny. Further, in a natural foraging setting, wild lemurs increase their reliance on olfaction when feeding on species which show more substantial differences between the scents of ripe and unripe fruits. These results indicate that fruit scent is an evolved signal whose function is to allow seed-dispersing primates to identify ripe fruits and thus facilitate plant reproduction.

Session/symposium: Interspecific relationships/independent presentation

**ORAL PRESENTATION** 

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# The origin of the odorant receptor gene family within the Hexapoda

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Crawling onto land and taking flight each changed the demands on arthropod chemosensory systems and these new opportunities promoted the origins and expansions of several chemosensory gene families. One of these gene families - the hexapod-specific odorant receptors (ORs) - is among the largest and most dynamically evolving multigene families within hexapod genomes. However, information on the early stages in the evolution of this gene family and particularly functional data on early OR-based olfactory systems are sparse.

We are currently undertaking genomic and transcriptomic surveys from basal hexapods to understand the origin/s and patterns of expansion of this multigene family. Consistent with a previous report in the firebrat *Thermobia domestica*, we find multiple homologs of Orco, the obligate OR co-receptor, but no ligand-specific ORs in antennal transcriptomes of the silverfish, *Lepisma saccharina*, and the basal forest silverfish, *Tricholepidion gertschi*. In contrast, Orco and a low number of putative ligand-binding ORs were detected in the genome and antennal transcriptome of the giant bush dragonfly, *Uropetala carovei*. Our results thus suggest that multiple Orco homologs were present in the terrestrial common ancestor of Zygentoma and Pterygota and that the functional separation into an obligate coreceptor Orco with separate ligand-specific ORs evolved in parallel with the evolution of insect flight.

The functional characterization of identified ORs and Orco homologs is underway and will shed light on the ligand spectra of early ORs and the factors that helped to establish these receptors as one of the largest gene families in most extant insect lineages.

Session/symposium: Interspecific relationships/Arthropod chemoreceptors KEYNOTE LECTURE

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# Behavioural response of bean flower thrips (Megalurothrips sjostedti) to male-produced aggregation pheromone

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Insects commonly use chemical signals to communicate information vital for their life processes such as feeding, mating, and egg-laying. Recently, we have observed active aggregation behaviour of male bean flower thrips (BFT), *Megalurothrips sjostedti* (Trybom) (Thysanoptera: Thripidae) on cowpea (*Vigna unguiculata*). To understand the mechanism underpinning the aggregation, we studied the behavioural responses of *M. sjostedti* to headspace volatiles from conspecifics in a four-arm olfactometer bioassay. Results showed that both male and female BFT were attracted to male odour but not to female odour. Gas chromatography-mass spectroscopy (GC-MS) analyses of M. sjostedti headspace volatiles revealed the presence of two distinct compounds (major and minor) in male headspace which were absent in female headspace. The study suggests that these male specific compounds (pheromones) may be responsible for observed aggregation in BFT. These findings could offer an excellent opportunity to develop a non-toxic, cost-effective and ecological sustainable monitoring and control tools for bean flower thrips management in Africa, where the pest causes widespread damage.

Session/symposium: Intraspecific relationships/independent presentation

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## Complexity of plant-plant interactions between undamaged and effects on aphids

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Plants coexist together in communities composed of one or more species that communicate through a variety of often complex signals. Due to their immobile lifestyle the capacity of individual plants to rapidly detect and respond to above or belowground neighbor signals is essential as it determines their growth strategy and survival. Volatile organic compounds released by undamaged plants are the most common signals that reflect present physiological status of emitter. These signals are constantly changing due to biotic or abiotic factors or plant physiological status of emitter inducing different responses in receivers to prepare for future competition. For instance, barley plants of variety Kara exposed to VOCs of variety Alva allocated more biomass to their roots comparing to controls. But, when grown in low red:far-red conditions Alva allocated biomass to shoots and changed blend of VOCs emitted. Changes in volatiles emission of Alva plants altered by low R:FR light induced a typical shade avoidance response of exposed Kara plants which in this case accumulated more resources into above ground biomass than to roots. Mechanical contact is another signals that provide an extra indication of the presence of potential competitor. Plant response to mechanical contact by biomass distribution between different organs from which they may benefit and by changes in the chemical composition of the emitted compounds with the influence aphids. The fact that the behaviour of aphids and their population development can be affected gives this phenomenon even wider ecological significance.

Session/symposium: Interspecific relationships/Semiochemistry of aphidophagous insects

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Methyl-2,4,6-decatrienoate identified as a potential male- produced aggregation pheromone of Antestia bug (Heteroptera: Pentatomidae: *Antestiopsis thunbergii*): a coffee pest native to Africa

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Antestia bug, Antestiopsis thunbergii also known as the coffee stink bug is a major pest of Arabica coffee in Africa. In addition to causing high yield losses, the pest infestation on coffee berries lead to low quality produce, a condition often referred to as "potato taste defect", making it unmarketable in the international trade. Although Antestia bug exhibit gregarious distribution patterns in coffee plantations, the mechanisms underlying this behavior are unknown. This present study investigated (1) the aggregative behavior of Antestia bugs, (2) pheromone contributing to aggregation of Antestia bugs, (3) the performance of synthesized and commercial standard of Antestia bug aggregation pheromone in field for control of the pest. In olfactory-laboratory based behavioral tests, sexually mature-unmated Antestia bug males attracted both sexes but neither sex responded to females. Chemical analysis of headspace volatile emissions from Antestia bug males and females led to the identification of the ester methyl-2,4,6-decatrienoate (MDT) in male samples only. Literature shows that MDT is a widely known male produced aggregation and sex pheromone of various important stink bug species and is currently commercially produced for monitoring and mass trapping pests in USA and Japan. Hence, we conducted electrophysiological and behavioral tests with synthesized MDT (95%), and found that both sexes of A. thunbergii responded positively to the treatment. Recently, we deployed synthesized MDT and commercial MDT in coffee plantations in Kenya to investigate the potential of using the pheromone in surveillance and mass trapping of A. thunbergii. We shall discuss in detail the performance of MDT in the control of this native pest of Africa as well as the ecological significance of our findings at the meeting.

Session/symposium: Intraspecific relationships/independent presentation ORAL PRESENTATION

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Electrophysiological and behavioral responses of pumpkin fruit fly, *Bactrocera depressa*, to plant volatile compounds

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A series of GC-EAD experiments and behavioral bioassays were conducted to identify the olfactory-active compounds and evaluate the behavioral activities of the EAD-active compounds in pumpkin fruit fly, *Bactrocera depressa*, a serious pest of Curcurbitaceae crops such as pumpkin and zucchini in Korea. In the GC-EAD experiments using the mixture of synthetic plant volatile compounds and the headspace extracts of pumpkin plants, 18 synthetic compounds and several compounds present in the pumpkin headspace elicited strong EAD responses from male and female B. depressa, and some of the synthetic compounds exhibited sex-specific EAD responses. Among the 18 EAD-active synthetic compounds, seven compounds appear to be produced by Curcurbitaceae plants. In laboratory bioassays using traps and a time-lapse web-cam recording system, a blend of EAD-active synthetic volatile compounds displayed significant attraction to male and female *B. depressa*. Attractants for *B. depressa* have not been available, and any of the known *Bactrocera* fruit fly attractants such as methyl eugenol, cue lure and raspberry ketone is not attractive to this species. Our results provide a strong foundation for developing semiochemical baits for *B. depressa*.

Key words: antenna, attractant, GC-EAD, plant volatile, semiochemical

Session/symposium: Practical applications/independent presentation

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Okosun O.O.\*, Yusuf A.A., Crewe R.M., Pirk C.W.W.

# Tergal gland pheromones of honey bee workers

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The primer and releaser effects of dominant honey bee workers' tergal gland pheromones are not known under queenless conditions. The Cape honey bee, *Apis mellifera capensis*, is the ideal model to investigate such question since workers normally reproductively dominate workers of all other subspecies. We determined the effects that short and long-term exposure to pheromone blends from dominant *A. m. capensis* workers had on subordinate workers of *A. m. scutellata*. Three putative pheromonal blends were tested and all the three putative pheromonal blends elicited releaser effects in the form of retinue formation and primer effects by suppressing ovarian activation in workers. The resultant effects indicate that these pheromonal blends appear to play a role in establishing dominance among workers and hence regulating opportunities to reproduce.

Session/symposium: Practical applications/independent presentation

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Effect of dimethyl disulfide, present in larval frass odors, on host selection by female adults of *Pieris rapae* 

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Phytophagous insects use various plant volatiles to find and locate suitable host plants. In addition to plant volatiles, however, conspecific larval frass odors often have great infuluences on their host selection. In many moth species, chemicals contained in larval frass are reported to repel conspecific females and deter their oviposition. The ecological significance of these chemicals is considered to be the regulation of larval population to avoid intraspecific competition for diets. However, few studies have been conducted on the olfactory responses of adult butterflies to larval frass. In the present study, we assessed the effects of conspecific larval frass odors on the host selection of *P. rapae* females.

With virgin and mated *P. rapae* females reared in our laboratory, we conducted two-choice bioassays using an olfactometer, in which individual butterflies were exposed to two different odors, with no visual cues available. Both virgin and mated females were significantly more attracted to intact cabbage odor than an odorless air plume. However, the odor of a cabbage infested by conspecific larvae was less attractive to them than that of an intact cabbage, and they significantly more frequently chose an odorless plume than the odor of conspecific larval frass. These results suggest that the larval frass contains some olfactory repellents against *P. rapae* females. Because our chemical analyses clarified the predominance of dimethyl disulfide (DMDS) in the larval frass odors, we examined the effect of its authentic chemical on female selection. As a result, DMDS was found to cause repellency against *P. rapae* females in two-choice bioassays, indicating that this compound was responsible for the repellency of larval frass odors. By means of the olfactory detection of DMDS, female butterflies may assess the conditions of host plant and avoid oviposition on damaged plants.

Session/symposium: Interspecific relationships/independent presentation

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## Equids as potential sources of repellents for Savannah tsetse fly control

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Tsetse flies are hematophagous insects occupying 37 sub-Saharan African countries and primary vectors of trypanosome pathogens causing African trypanosomosis in human (sleeping sickness) and livestock (nagana in cattle). Animal African trypanosomosis alone leads to annual losses over USD 4.5 billion in the agricultural and livestock production sectors. The use of natural repellents, identifiable from non-preferred vertebrates (e.g. waterbuck, zebra), to interfere with vector-host interactions represents a sustainable vector control approach. Identification of tsetse repellents has previously been attempted from skin odour of waterbuck and a repellent for tsetse (waterbuck repellent compounds, WRC) developed. Here, we investigated the potential of skin odours of zebra and their close relatives in the family Equidae (donkey and horse) as sources of new repellents for tsetse flies. In Latin square designed field trials, we showed that crude skin odours of zebra and donkey evaluated in the NGU tsetse traps in Nguruman, Kenya caught Glossina pallidipes numbers similar to the known tsetse repellent WRC, confirming the presence of putative tsetse repellent compounds in equid skin odours. In laboratory analyses using coupled gas chromatography-electroantennographic detection (GC/EAD) and GC-mass spectrometry (GC/MS), we identified seven physiologically-active compounds of the equid skin odour reproducibly detected in the antennae of adult female G. pallidipes. Further field evaluation of blends of these seven compounds, formulated in their proportion of natural occurrence in skin odours of the three equids also demonstrated efficacious repellency to G. pallidipes, comparable to that of WRC. Our results show the presence of repellents for tsetse in equid skin odour, which can be exploited for integrated management of tsetse and African trypanosomosis.

Session/symposium: Interspecific relationships/Ecology and evolution of toxins in vertebrate animals

**ORAL PRESENTATION** 

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Finding maximum gypsy moth population density controlled by mating disruption

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Mating disruption is the dominant tactic used against expanding gypsy moth populations in the United States, it has been proven to be safer, more economical and more effective than any other control method. Historically, mating disruption has been assumed to be most effective against low-density gypsy moth populations ( $\leq 30$  males/trap/season). However, the threshold gypsy moth population density above which mating disruption is no longer effective remains unknown, therefore, populations with higher densities are controlled using more expensive and less benign methods. To optimize gypsy moth management programs we analyzed historical gypsy moth mating disruption treatment data in the Slow the Spread Program and used this information to guide our field studies to evaluate efficacies of mating disruption treatments against populations of various densities (0-116 males/trap/day).

A novel mathematical model relating daily, weekly and season-long abundances of gypsy moth males was developed to analyse experimental data and relate our results to the management program.

Threshold gypsy moth population density appropriate for control using mating disruption tactic was estimated to be twice as high as previously thought. These findings open up the possibility of expanding mating disruption tactic to areas currently treated with harmful insecticides thus reducing negative effects on non-target organisms as well as the cost of gypsy moth control.

Session/symposium: Practical applications/Comparison of semiochemical control methods of pest insects

**ORAL PRESENTATION** 

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The curious case of black truffle beetle (*Leiodes cinnamomeus*) attractant based on host volatiles

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The truffle beetle, *Leiodes cinnamomeus* (Panzer, 1793) (Coleoptera: Leiodidae), is the most important pest of the valuable black truffle (*Tuber melanosporum*) in Europe. Through their feeding and oviposition habits, both the adults and the feeding larvae, contribute to economic losses by the destruction of truffles, but little is known about chemical communication between the beetle and its host fungus.

Attractants based on plant volatiles have been used to monitor and control insect pests, so our main objective was to develop a host volatile-based lure and test its effect on capture of *L. cinnamomeus* in field trapping experiments. The truffles release a chemical profile (40-50 components) which changes over time mainly due to maturation of fruit body, genetic variability or environmental factors. Before truffle-hunting dogs can sniff the ripe fruiting bodies, truffles are infested by *L. cinnamomeus*, thus some compounds given off by immature truffles are crucial for attracting beetles.

Volatiles compounds released by unripe and ripe truffles were collected by DHS on Tenax-TA or SPME adsorptions. GC-MS analysis of released volatiles from unripe and ripe truffles showed 34 compounds in noticeable amounts and, from a quantitative point of view, immature truffles emits tens of times higher quantities of dimethyl sulphide (DMS) than mature. Coupled GC-EAD revealed that 4 of these identified compounds elicited reproducible antennal signals in the tested adults and EAG recording showed a significantly higher response to DMS by both sexes. Surprisingly, field tests showed that synthetic DMS-baited traps captured significantly more *L. cinnamomeus* adults than traps loaded with a blend of EAG active plant volatile compounds. Therefore, probably a high concentration of DMS is essential for host location by the beetle, while other volatiles are of secondary importance.

Session/symposium: Interspecific relationships/independent presentation
ORAL PRESENTATION

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Osei-Owusu J. <sup>1,2</sup>\*, Vuts J. <sup>2</sup>, Caulfield J. <sup>2</sup>, Woodcock C. <sup>2</sup>, Osafo S. <sup>1</sup>, Birkett M. <sup>2</sup> Investigating cowpea, *Vigna uncuiculata*, herbivore-induced defence and floral volatiles for low-input cowpea pest management

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Cowpea, Vigna unguiculata, is an important crop in Ghana and provides an accessible source of protein. Crop yields, however, are reduced by many biotic factors, especially the pod-borer Maruca vitrata that can cause up to 80% losses. When plants are damaged by herbivores, they release blends of volatile organic compounds (VOCs) referred to as Herbivore-Induced Plant Volatiles (HIPV), which attract natural enemies and/ or deter herbivory attack. To characterize the chemical ecology of cowpea-pest interactions, VOCs were collected from plants that were challenged with five herbivores ie. Maruca vitrata, Aphis craccivora, Ootheca spp., Latoia vivida and Myzus persicae and their induced signals compared to mechanically-damaged cowpea plants. Induced compounds included green leaf volatiles (GLVs), 1-octen-3-ol, indole, methyl salicyclate, limonene,(E)-β-farnesene, DMNT, linalool, (E)-ocimene,  $\beta$ -sesquiphellendrene, myrcene and nerolidol. Induction was shown to be herbivore-specific eg. A. craccivora, a specialist aphid pest of cowpea, did not induce any chemistry, whereas the generalist aphid pest Myzus persicae, induced the production of DMNT,  $\beta$  -sesquiphallendrene, myrcene and (E)- $\beta$ -farnesene. Mechanical damage to plants induced only GLV production. In addition to investigating herbivore-induced defence in cowpea, floral VOCs were also collected from intact cowpea flowers. Analysis of the floral VOCs revealed the presence of nonanal, benzaldehyde, methyl benzoate, acetophenone, decanal, a-pinene, limonene, cinnamaldehyde, (E)-ocimene, octanal and traces of DMNT and methyl salicylate. Future work will explore the role of the identified compounds in deterring M. vitrata and recruiting M. vitrata parasitoids, and which compounds are important for *M. vitrata* host location.

Session/symposium: Interspecific relationships/independent presentation

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Ossowicki A.\*, Jafra S., Garbeva P. **Plant protection from a distance** 

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Soil microbiome is essential to maintain plant growth, health and protection against biotic and abiotic stresses. The phenomenon called soil suppressiveness, where plants show significantly reduced disease symptoms even if the pathogen and favourable conditions are present is strongly related to the microbial activity and secondary metabolites production. Among various modes of action of soil bacteria, one of the remarkable but less studied is the production of Volatile Organic Compounds (VOCs). Due to their physicochemical properties, VOCs diffuse easily through gas- and water-filled pores in soil and rhizosphere environments, allowing long-distance interactions. Many bacterial VOCs were found to possess strong antimicrobial activity. We have studied the volatile-mediated antimicrobial activity of tomato rhizosphere isolate *Pseudomonas donghuensis* P482 against fungal and oomycete plant pathogens (*R. solani, V. dhaliae* and *F. culmorum* and *P. ultimum*). In the VOCs blend produced by *Pseudomonas* P482, we identified several compounds with sulfurcontaining compounds as dominant. We observed clear fungistatic effect caused by the VOCs produced by P482 as well as by the pure compounds *S*-methyl thioacetate and dimethyl disulfide that were identified in the blend.

However, can we correlate fungistasis with soil suppressiveness? Our recent findings based on screening 28 soils from the Netherlands and Germany indicate that soil suppressiveness and strong soil fungistasis does not always correlate with each other. At present we are exploring the mechanisms involved in both soil fungistasis and suppressiveness.

Session/symposium: Interspecific relationships/Volatile-mediated microbe-plant interactions ORAL PRESENTATION

Papazian S.<sup>1</sup>\*, Khaling E.<sup>2</sup>, Bonnet C.<sup>3</sup>, Lassueur S.<sup>3</sup>, Reymond P.<sup>3</sup>, Moritz T.<sup>1</sup>, Blande J.D.<sup>2</sup>, Albrectsen B.R.<sup>1</sup>

Untangle complexity: metabolomics of plant-insect interactions in black mustard under a multiple biotic and abiotic stress scenario

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Complex chemical networks connect plants to their environment driving basic and specialized processes such as growth, reproduction, defence, and communication.

In this ecological framework, plants and insects play one of the oldest and most intricate interactions. The goal of eco-metabolomics is thus to help ecological research to untangle and understand such complexity. By looking at the plant metabolome in its entirety, rather than at single metabolites, metabolomics can improve our ability to predict how constant shifts in the environment affect plant physiology and ecology. By taking as an example the interaction between the wild black mustard Brassica nigra L. (Brassicaceae) and its specialized insect herbivore, the large white Pieris brassicae L. (Pieridae), here I will illustrate how we can use metabolomics to study plant responses and adaptation against a multiple biotic and abiotic stress scenario, such as herbivory (i.e. leaf-chewing caterpillars) and atmospheric pollution (i.e. ozone). I will show how we can investigate plant metabolic responses in depth by combining different targeted and untargeted metabolomics approaches and analytical techniques such as gas or liquid chromatography coupled to massspectrometry (GC-MS and LC-MS) for the high-throughput screening of hundreds of central 'primary' and specialized 'secondary' metabolites (amino acids, carboxylic acids, fatty acids, glucosinolates, phenolics, and volatiles). Finally, I will discuss metabolomics in the more comprehensive context of systems biology and show how connecting changes in the realized metabolome to changes in gene regulation using transcriptomics can help us to predict the emergence of higher-level physiological regulation and phenotypes, and thus guide us in the generation of new hypotheses for biological functions and ecological processes in plants.

Session/symposium: Interspecific relationships/Metabolomics approaches in the Brassicaceae

ORAL PRESENTATION

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Electrophysiological and behavioral responses of tomato-potato psyllid, *Bactericera cockerelli*, to plant volatile compounds

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We carried out single sensillum recording (SSR) experiments to identify the olfactory-active compounds for individual olfactory sensory neurons (OSNs) and laboratory bioassays to evaluate the behavioral activities of the olfactory-active compounds in tomato-potato psyllid, *Bactericera cockerelli* (Hemiptera, Triozidae), a serious pest of Solanaceae plants. In the SSR study to investigate the responses of the OSNs present in two placoid sensilla located in the first and second antennal flagella, respectively, to 35 synthetic plant volatile compounds, nine compounds elicited strong responses from the OSNs. The OSNs in the placoid sensilla of the first antennal flagellum exhibited specialized responses to octanal, nonanal, linalool and some green leaf alcohols and acetates. In contrast, the OSNs in the placoid sensilla of the second antennal flagellum displayed specialized responses to green leaf aldehydes. In behavioral bioassays using an open arena with a time-lapse video recording system, some SSR-active volatile compounds increased the behavioral activities of *B. cockerelli*. Taken together, our results suggest that the behavioral attraction of *B. cockerelli* to plants is mediated in part by a set of specialized antennal OSNs.

Session/symposium: Interspecific relationships/Arthropod chemoreceptors

Parpinelli R.<sup>1</sup>\*, Alves J.<sup>2</sup>, Toledo V.<sup>1</sup>, Nunes E.<sup>2</sup>, Hoffmann-Campo C.<sup>2</sup>, Gazzoni D.<sup>2</sup>\* **Nectar of soybean flowers as attractiveness factor for pollinators** 

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Pollination is an important regulatory ecosystem service for production of food, as pollinated flowers often produce more and better quality fruits and seeds. The attractiveness to the pollinator agents depends on factors such as floral morphology and colour intensity as well as the number of flowers per plant, olfactory, visual-tactile cues and seasonal availability. Bee body size in relation to the flower and nectary location is also important. To attract pollinators, the flower must secrete a high volume of nectar. Such insect-plant interaction is a symbiosis, and the floral nectar an important reward for the pollinating visitor. Several studies were carried out but, but still, there is no consensus among scientists regarding the role of pollinators in soybean (Glycine max L.) and many questions remain unanswered, including the control of the factors involved with such attraction. This study aimed to test and validate a technique for soybean flower (white/purple) nectar collection and to define the nectar sugar profile of soybean produced under normal growing conditions. Different collection techniques have tested the microcapillary with a capacity of 0.5µL was the only viable alternative. The determination of the nectar sugar profile was performed by HPLC-RI. The average volume of the nectar was of 0.25μL and 0,30μL for purple and white flowers, respectively. The sugar profile varied in qualitative and quantitative terms. A nectar sampling technique, simulating the pollinator suction process, were established without damaging the structure of the soybean flowers.

Keywords: pollination, soybean, nectar, sugar Project supported by a grant from Bayer Crop Science

Session/symposium: Practical applications/independent presentation

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Paudel S., Lin P., Rajotte E.G., Felton G.W.

Facing the heat: How global warming might affect tomatoes and their interactions with insect pests?

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Maintaining agricultural sustainability must change as the environment changes. As the global average temperature continues to rise, we need to better understand the role of temperature in shaping plant and insect metabolism, growth, development, and their interactions. Despite considerable research on insect-plant interactions, overall impacts of increasing temperature are poorly understood and very few studies have focused on agriculture crops like tomatoes. Using quantifiable methods and enzymatic assays, we explore the consequences of elevated temperature on insect-plant interactions, particularly with respect to a plants' life history and defense traits using tomato (Solanum lycopersicum var Better Boy) plants and a generalist herbivore, tomato fruit worm, Helicoverpa zea. In a series of growth chamber and greenhouse studies, we measure plant and herbivore growth, and various plant defense strategies, i.e. direct chemical defenses (defensive chemicalspolyphenol oxidase (PPO) and trypsin protease inhibitors (TPI), trichome density), and tolerance (the ability to regrow). Simultaneously, we measure leaves' relative water content and soluble protein thought to be an important factor in insect nutritional ecology. The results from the study are expected to identify a trend in tomato plants' defense and life history strategies in response to abiotic (high temperature) and biotic (herbivory) stresses so that the results could be used to help farmers and extension officers design effective pest management strategies and formulate and prepare the agricultural community for future global climate change dynamics.

Session/symposium: *Practical applications/independent presentation*ORAL PRESENTATION

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Chemical interaction and induction of secondary metabolites in leafcutter ants's symbiont: mutualistic fungi, parasite *Escovopsis*, actinomycetous and black yeast

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The group of Attine ants is part of a complex symbiosis. There is a complex ant-microbe mutualism and other microbes have evolved in this system. The ants have an obligate mutualism with fungi that they cultivate for food, Leucoagaricus gongylophorus; fungal parasites (genus Escovopsis) can be harmful to the garden. Hence, to protect against the parasite, ants have a mutualism with actinomycetous bacteria that produces important antibiotics to avoid parasites. Besides the new symbiont associated with leaf cutting ants: black yeast. Therefore, the aim of our study is to analyze chemical interactions of the microbial symbionts of leafcutter ants through co-cultive studies on solid media with these microbes. The strains of microbes used in this study were isolate from the nest of Atta and Acromyrmex ants. All the microbes were cultivated in axenics and co-culture in petri dish of maltose yeast extract agar medium (YEMA), incubated at 25°C. The agar with the microbial were extracted with ethyl acetate, in a water-bath sonicator for 20 min. The samples were analyzed by UPLC-QTof and 1H-NMR. The data were exported using Microsoft Excel® and multivariate analyses using Matlab® analyzed by Principal Component Analysis (PCA). The results showed that fungal interactions produces differences in the fingerprinting and can be used as a source of natural products. Some co-cultures showed a distance inhibition or a visible color change of the culture media. From these preliminary results it was possible to observe when there are interactions among other microbials, the induction of different compounds in co-cultures can change the secondary metabolism and the biological activity. Therefore, the identification of these chemical constituents can collaborate to the understanding of the complex chemical interactions in these microorganisms. Thus, further studies are currently underway aiming this investigation.

Session/symposium: Interspecific relationships/independent presentation

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#### Pickett J.A.

#### The influence of aphid chemical ecology on the aphidophagous insects

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In contrast to the diminutive appearance of aphids (Homoptera: Aphididae), particularly relative to their plant hosts, sophisticated life cycles and host interactions are typical for this insect family. Underpinning much of this sophistication is aphid chemical ecology. By accessing the phloem of plants from most parts of the kingdom, aphids exploit a vast food resource and, of the many organisms in turn exploiting this resource via aphids, the aphidophagous insects, including predators and parasitoids, are spectacular for their diversity and natural impact on aphid populations. The aphid pheromones, and other semiochemicals involved in host interactions, provide evolutionarily valuable foraging cues for the aphidophagous insects faced with the difficulty of locating their hosts in an often exceedingly high predominance of plant biomass. In spite of the gift of so many chemical ecological tools, aphid pest management relies almost entirely on insecticides and the attendant problems of rapid selection for resistance. However, as a consequence of recent in depth studies on the chemical ecology relating to aphidophagous insects, new opportunities are emerging for conservation biological control by semiochemical based manipulation of aphidophagous insects. Such approaches will be discussed in terms of problems but also new opportunities.

Session/symposium: *Interspecific relationships/Semiochemistry of aphidophagous insects* KEYNOTE LECTURE

#### Pickett J.A.

Integrated management of pest and beneficial insects by manipulating natural products using companion crops, GM and synthetic biology

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New tools delivering more sustainable integrated management of pest and beneficial insects are being developed from natural products, particularly those involved in secondary metabolite based signalling. For agroecological approaches, including use of companion plants, and GM, plant secondary metabolism is now, with new molecular tools, readily exploited. The tetranorterpenes (homoterpenes) acting as plant defence signals, particularly in the powerful enhancement of biological control of insects, and external plant activators, related to plant hormone systems, are targeted by selecting plants, from species biodiversity studies, and by GM, for releasing such natural products. Evidence of field effectiveness comes from development in regions relying on resource poor farming and where less sustainable seasonally applied chemical toxicants, still the main intervention in industrialised agriculture, are not available. Although the natural biosynthetic pathways are utilised in selecting or breeding smart companion plants and in GM approaches, synthetic biology is now being explored for developing new pathways based on novel synthetic genes for improved plant stress signalling molecules.

Session/symposium: Practical applications/Natural products for integrated pest management

ORAL PRESENTATION

Pickett J.A.<sup>1</sup>\*, Khan Z.R.<sup>2</sup>, Midega C.A.O.<sup>2</sup>

The Push-Pull system for controlling lepidopterous pests in cereals: new developments in exploiting GM in delivering the Push-Pull strategy

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We have developed the highly successful push-pull system for raising dramatically the food production on small-holder cereal farms. However, we have a long way to go to ensure take up by the range of particularly small-holder farms that would benefit. The generic term push-pull refers to the original approach of using companion plants to push insect pests, specifically lepidopterous stemborers, to the pull of trap plants and to pull in parasitoid wasps to attack the pests. The system employs a specific genus of livestock forage legumes Desmodium as the intercrop also to remove completely the parasitic weed Striga hermonthica from the farm. The perennial push-pull companion plants with no further inputs provide, in addition, ground cover, fixed nitrogen, help scavenge phosphorus and control plant pathogens. However, some funding agents and practitioners find the prospect of such a knowledge intensive system, in spite of its sustainability, potentially difficult to transfer into the 300 million farms that could be targeted. Recent development of climate smart push-pull, including drought tolerant *Desmodium* spp. and trap species in the Brachiaria genus, demonstrates the application to degraded and aridified land. However, the wider application could be facilitated by delivering all of the push-pull companion plant traits by using GM approaches in the crop plants. This talk will briefly update the Push-Pull by icipe and explain the routes by which GM delivery can be achieved.

Session/symposium: Practical applications/Comparison of semiochemical control methods of pest insects

ORAL PRESENTATION

Pokharel P.\*, Petschenka G.

#### Exposure to plant toxins causes a putative physiological benefit in an adapted insect

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Milkweed bugs (Hemiptera, Lygaeinae) cannot only cope with cardenolides but also sequester these toxins as a defense against predators. Cardenolides inhibit the ubiquitous Na+/K+-ATPase, an enyzme necessary for physiological functions. Milkweed bugs possess a Na+/K+-ATPase which is highly resistant towards cardenolides (target site insensitivity). Here, we studied how the traits cardenolide resistance and sequestration interact with larval growth in response to dietary toxins. We compared hemipteran species having different combinations of traits; i.e. unable to sequester and not possessing a resistant Na+/K+-ATPase (Pyrrhocoris apterus, Pyrrhocoridae), resistant but not sequestering (Arocatus longiceps, Lygaeinae), and resistant and sequestering (Oncopeltus fasciatus, Lygaeinae). We raised the bugs on cardenolides (ouabain and digitoxin) using an artificial diet and assessed the body mass over larval development. After reaching the adult stage, we determined the amount of sequestered cardenolides using HPLC. Dietary toxins compromised the growth of P. apterus substantially. A. longiceps, in contrast, grew equally well under all conditions and were not affected by the presence of cardenolides. Cardenolides had a dose-dependent positive effect on growth in O. fasciatus which sequestered cardenolides in a dose-dependent manner. We speculate that the resistant Na+/K+-ATPase was selected for working optimally in a 'toxic environment' i.e. when cardenolides are sequestered. When cardenolides are not available for sequestration, resistant Na+/K+-ATPase produces a physiological cost and this effect is alleviated on a cardenolide containing diet. Our data suggest that insect adaptations to host plant toxins can escalate so that the absence of plant defenses in a diet can lead to physiological cost.

Session/symposium: Interspecific relationships/independent presentation

Pokorny T.\*, Bogenberger K., Ruther J.

Mate recognition in *Muscidifurax raptorellus* 

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Mate recognition is a key factor for the perpetuation of premating isolation. Species and sex specific cues and signals can be perceived via different sensory modalities (e.g. visually, acoustically, olfactory). In insects, chemical information plays a dominant role in mate recognition. Usually, the male will initiate courtship behaviour after recognizing a potential mate, which is then followed by either acceptance or refusal to mate by the other individual. We studied mate recognition in the parasitoid wasp *Muscidifurax raptorellus* (Hymenoptera, Pteromalidae). In this species, courtship behaviour consists of wing fanning (high frequency wing beats while standing or walking), and, once the male has positioned himself on the female, stereotypical antennal movements followed by initiation of copulation itself. We investigated the chemical signals involved in mate recognition by evaluating the reaction of male *M. raptorellus* towards dummies treated with full body extracts or extract fractions of conspecific males and females. Our results indicate that visual stimuli are important in addition to chemical ones. Also, despite sex specific cuticular hydrocarbon profiles, these compounds alone are not sufficient for mate recognition. Compounds from at least two fractions in combination are required to elicit male wing fanning.

Session/symposium: Intraspecific relationships/independent presentation

Poliseli C.<sup>1</sup>, Veronezzi G.<sup>2</sup>, Meurer E.<sup>1</sup>, Hoffmann-Campo C.B.<sup>2</sup>, Nunes E.O.<sup>2\*</sup>, Gazzoni D.L.<sup>2</sup>

#### Anthocyanins and its influence in the color of soybean flowers

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Soybean is an autogamous plant and thus allowing self-pollination. One of the factors for increasing productivity is the use of bees in pollination, attracted by chemical and visual stimuli. Apis mellifera is responsible for 80% of entomophilous pollination. The soybean flower uses three strategies, which directly influence the selectivity and attractiveness: aroma, flower colour and the nutritional value of nectar and pollen. Initially, the flower colour is discriminated from the green of the leaves, after the bee is guided by visual signals on the petals from distinct pigments in the flower tissue. Anthocyanins play a key role in the colour of soybean. Although more than 600 types of anthocyanins are described only a few are synthesized by individuals of each plant species. Likewise, few studies regarding floral characters refer to a closer relationship with the sensory capabilities of pollinators. They cite as an example the colour of flowers and most of them in qualitative terms. Thus, this work investigates the anthocyanins profile of white soybean and purple flowers as an attractiveness factor. After acid extraction, the anthocyanin profile was determined by UPLC-PDA, l=520 nm, using Shield® (Waters) column. An average of 8 and 12 compounds was detected in extracts of white and purple flowers, respectively. The method developed for multi-detection of anthocyanins in soybean flowers had higher efficiency than those observed in the literature, in both qualitative and quantitative terms.

Keywords: UPLC-PDA, multi-detection method, extraction, separation and identification Project supported by a grant from Bayer Crop Science

Session/symposium: Practical applications/independent presentation

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Pradit N.\*, Rodriguez-Saona C.

#### Phytoplasma infection in cranberries benefits non-vector phytophagous insects

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Disease infection often changes the chemical composition in plants, which in turn may affect phytophagous insects. Therefore, I tested the hypothesis that non-vector phytophagous insects benefit from changes in the chemical composition of host plants caused by disease infection. In cranberries, phytoplasma infection causes false blossom disease; a disease vectored by the blunt-nosed leafhopper (Limotettix vaccinii). Three leaf-chewing caterpillars: spotted fireworm (Choristoneura parallela), Sparganothis fruitworm (Sparganothis sulfureana), and gypsy moth (Lymantria dispar) are non-vector insects of this disease in cranberries. In this study, I measured the performance of these caterpillars as well as the changes in nutrients and chemical defenses on infected and non- infected plants. All caterpillars grew 2-3 times bigger and consumed more leaves of phytoplasma-infected plants than those feeding on non-infected plants. Nutrient levels (e.g. N, P, K, Ca, S, Mn, Fe, B, Al, and Na) were higher in infected plants. Moreover, among chemical defenses, proanthocyanidin levels were lower in infected plants while flavonoid levels were similar between infected and non-infected plants. These results indicate that phytoplasma infection in cranberries facilitates feeding by non-vector phytophagous insects. This study provides new insights on the ecological interactions between plants, pathogens, and non-vectors insects and their possible mechanisms.

Session/symposium: Interspecific relationships/independent presentation

Proffit M.\*, Lapeyre B., Buatois B., Arnal P., Aubier P., Gouzerh F., Opsommer M., Staudt M., Hossaert-McKey M.

## Ozone pollution alters the olfaction and behavior of a specialized pollinator

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Volatile organic compounds (VOC) play a key role in the relationship between plants and their abiotic and biotic environments. For instance, pollinators usually rely on floral scents to locate their host plants. This chemical communication between plant and pollinator can be disturbed by exposure to pollutants such as ozone (O<sub>3</sub>), whose levels have increased in the troposphere and are predicted to further increase over the coming decades. In the present study, we evaluated the impact of O<sub>3</sub> concentration on the behavior of the highly specific pollinating wasp Blastophaga psenes towards the chemical signal emitted by its host plant the dioecious Mediterranean fig, Ficus carica, and on its detection of plant VOC. Firstly, using gas chromatography coupled with electroantennographic recordings and behavioral tests, we found that a particular ratio of three VOC is sufficient to attract B. psenes to the inflorescences of F. carica. Secondly, exposing individuals of B. psenes to different O<sub>3</sub> concentrations and then testing them in ambient O<sub>3</sub> conditions we evaluated the direct effect of O<sub>3</sub> on the pollinator behavior and olfaction. We observed that O<sub>3</sub> exposure, above a concentration of 120 ppb, affects the detection of VOC by antennae of B. psenes, as well as its attraction towards the attractive mixture of VOC. If O<sub>3</sub> effects on the emission of plant VOCs, and on the integrity of the chemical signal during its transport in the atmosphere have been already demonstrated, these results are the first evidence that O<sub>3</sub> pollution can directly alter the detection of flower VOC by pollinators, and therefore the crucial ecosystem services they provided.

Session/symposium: Interspecific relationships/independent presentation
ORAL PRESENTATION

Quaghebeur C., Serteyn L., Stouvenakers G., Sarles L., Ongena M., Ramirez C., Francis F.\* Effect of plant growth promoting rhizobacteria on plant selection by aphids

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Some soil bacteria belonging to the Bacillus and Pseudomonas genera are particularly efficient biocontrol agents as Plant Growth Promoting Rhizobacteria (PGPR) by showing strong antagonistic activity toward plant pathogens and inducing systemic resistance (ISR). Beside plant pathogen microbial agents, phytophagous insects should also be impacted by ISR in host plants. Particularly, aphids are considered as herbivore models closed to fungal and bacterial plant pathogens when comparing host plant induced defense mechanisms after aphid attacks. Then, PGPR could play a primary role in interspecific interactions related to plants and aphids including changes in aphid behaviour. Bioassays were developped to assess the impact of the application of various Bacillus as plant defense elicitor sources on the behaviour and biological parameters of different aphid species and clones, such as Acyrthosiphon pisum and Sitobion avenae. Also, electropenetrography (EPG) technique was developed to assess the changes of aphid sucking feeding behaviour related to PGPR application. Different responses according to the selected PGPR and dose were observed. According to the choice tests (inappetence and olfactory assays), significant variations in plant selection were determined not only according to the aphid species but also to the aphid clones. The results of these trials were discussed in relation to the complexity of multitrophic relations including microbials and insects regarding to the host plant defense mechanisms.

Keywords: rhizobacteria, aphid, microbiome, plant defense, behaviour

Session/symposium: Interspecific relationships/independent presentation

Ranger C.M.<sup>1</sup>\*, Biedermann P.H.W.<sup>2</sup>, Phuntumart V.<sup>3</sup>, Beligala G.U.<sup>3</sup>, Ghosh S.<sup>3</sup>, Mueller R.<sup>4</sup>, Schultz P.B.<sup>5</sup>, Reding M.E.<sup>1</sup>, Benz J.P.<sup>6</sup>

#### A new perspective on the affinity of fungus farming Ambrosia beetles for ethanol

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Ambrosia beetles are among the true fungus farming insects, whereby they cultivate fungal gardens within host trees on which the larvae and adults feed. Stress-induced ethanol emitted from weakened trees acts as strong attractant for many opportunistic ambrosia beetles. Ambrosia beetles specifically selecting host tree tissues containing a potent antimicrobial agent to cultivate their fungal gardens would therefore seem counterproductive. Here we present the first evidence that *Xylosandrus germanus* and probably many other ambrosia beetles rely on ethanol within tissues of living trees for optimizing production of their fungal gardens and therefore successful offspring production. We conducted a series of experiments to assess the influence of ethanol on fungiculture by ambrosia beetles. More than 300 attacks by X. germanus and other species were triggered by baiting trees with ethanol lures, but none of the foundresses established fungal gardens or produced broods unless tree tissues contained in vivo ethanol resulting from irrigating with ethanol solutions. More X. germanus brood were also produced in a rearing substrate containing ethanol. Selected Ambrosiella and Raffaelea fungal isolates from ethanol-responsive ambrosia beetles were subsequently determined to exhibit higher biomass on media containing ethanol, while the growth of "weedy" fungal garden competitors (Aspergillus, Penicillium) were suppressed. Ambrosiella and Raffaelea fungal symbionts also exhibited fasteralcohol dehydrogenase enzymatic activity compared to fungal competitors. These results demonstrate that ambrosia beetles rely on ethanol for reproduction because it benefits their fungus farming by promoting the growth of their fungal gardens while inhibiting the growth of "weedy" fungal competitors.

Session/symposium: New chemical structures/Invasions under the bark: chemical ecology of woodboring beetles
KEYNOTE LECTURE

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<sup>&</sup>lt;sup>3</sup>Bowling Green State University;

<sup>&</sup>lt;sup>4</sup>Hawkesbury Institute for the Environment;

<sup>&</sup>lt;sup>5</sup>Virginia Tech;

<sup>&</sup>lt;sup>6</sup>Technical University of Munich

Ray A.M.<sup>1</sup>\*, Francese J.A.<sup>2</sup>, Zou Y.<sup>3</sup>, Watson K.<sup>4</sup>, Crook D.C.<sup>2</sup>, Millar J.G.<sup>3</sup> **Detection of velvet longhorned beetle populations using attractant baited traps** 

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The velvet longhorned beetle, Trichoferus campestris (Faldermann)(VLB; Cerambycidae: Cerambycinae: Hesperophanini), is native to east Asia where it feeds on a wide range of tree species, including orchard and timber trees. Larvae of VLB can be transported in wood packing material, and individuals are frequently intercepted in quarantine facilities. Populations of VLB have established outside of the native range of the species, including near Salt Lake City, UT USA. Adults are nondescript and nocturnal, and monitoring and control efforts have been hindered by a lack of attractant lures. We recently isolated and identified a novel variant of the conserved 2,3-alkanediol/ hydroxyketone chemical structure from headspace volatiles of males but not females. In field bioassays, this compound attracted significantly more adult beetles than did commercially available highrelease ethanol lures or solvent control. We here describe results of subsequent bioassays evaluating the effects of trap design, trap color, and trap height on numbers of beetles captured. We also describe the results of a dose-response bioassay, the first results from field evaluation of commercial lures, and efforts to detect VLB populations in areas where the species was not known to occur. The results of our work will assist regulatory personnel and land managers in developing monitoring surveys for VLB throughout the world.

Session/symposium: *Practical applications/Semiochemical application for invasive species*ORAL PRESENTATION

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<sup>&</sup>lt;sup>4</sup>Utah Department of Agriculture and Food

Ren L.L.<sup>1</sup>, Balakrishnan K.<sup>2</sup>, Luo Y.Q.<sup>1</sup>\*, Schütz S.<sup>2</sup>

EAG response and behavioral orientation of *Dastarcus helophoroides* (Fairmaire) (Coleoptera: Bothrideridae) to synthetic host-associated volatiles

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Dastarcus helophoroides Fairmaire (Coleoptera: Bothrideridae) is an effective predatory beetle of larvae and pupae of several cerambycid beetles including *Monochamus alternatus* and *Anoplophora glabripennis*. Electroantennography (EAG) and a dynamic two-choice olfactometer were respectively used to measure the antennal and behavioral responses of both sexes to selected volatile compounds. Female and male *D. helophoroides* exhibited similar EAG and behavioral responses. Significant dose-dependent EAG responses in both sexes were elicited by nonanal, octanal, cis-3-hexenol, 3-carene, (R)-(+)- $\alpha$ -pinene, (R)-(+)-limonene and (S)-(-)-limonene. Female and male beetles were repelled at high concentration by cis-3-hexenol and (S)-(-)-limonene, respectively. Both sexes of *D. helophoroides* were significantly attracted to nonanal, cis-3-hexenol, 3-carene and (R)-(+)-limonene even at low concentrations. These compounds might be used either individually or in mixtures for developing biological control methods to attract this predatory beetle into forest stands threatened by cerambycid beetles.

Session/symposium: Intraspecific relationships/Importance of multicomponent mixtures for chemical cues

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Revadi S.V. <sup>1,2</sup>\*, Hunger G.M. <sup>1</sup>, Jacquin-Joly E. <sup>2</sup>, Anderson P. <sup>1</sup>, Walker W.B. <sup>1</sup>, Becher P.G. <sup>1</sup> **Differential expression of odorant receptors during two larval stages of** *Spodoptera littoralis* 

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For a lepidopteran larva, finding a suitable feeding site is a challenging task, particularly soon after hatching. Larvae rely on the sensory inputs, mainly olfaction for locating the food source. However, foraging behavior is not stable and may change due to increase in the body size, changing digestive physiology and the dietary needs across larval instars. This reflects on the larval foraging behavior and food preferences. To understand the molecular basis of food preferences during larval development, we studied the olfactory receptor repertoire in the larvae of the cotton leafworm *Spodoptera littoralis* (Lepidoptera: Noctuidae) during the first and fourth instar. We identified odorant receptors that are differentially expressed during the larval development. Furthermore, we identified through heterologous expression, one of the odorant receptor that is expressed only in first instar larvae. First and fourth instar larvae differ in their attraction behavior towards the ligand of the first-instar-specific receptor. We suggest molecular changes in the peripheral chemosensory system as a basis for the larval behavioral changes.

Session/symposium: Interspecific relationships/Arthropod chemoreceptors

Rischer M.<sup>1</sup>\*, Raguz L.<sup>1</sup>, Keiff F.<sup>1</sup>, Guo H.<sup>1</sup>, Diekert G.<sup>2</sup>, Goris T.<sup>2</sup>, Beemelmanns C.<sup>1</sup>

Insights into microbe-Cnidaria interactions – genome-mining inspired and activity-based isolation of morphogenetic biomolecules and defensive metabolites

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Natural products of marine microbial origin have been a rich source of structurally diverse metabolites with a broad range of bioactivities. Recent studies suggested that particularly host-associated microbes represent one of the most prolific sources of bioactive molecules in the marine environment as they e.g. protect the host from other settling or competing organisms by secretion of antimicrobial or anti-biofouling molecules.

We have chosen the marine polyp *Hydractinia echinata* to study the chemical communication between the eukaryotic host and its associated microbiome. The life cycle of the hydroid polyp includes the bacterially induced transition of the motile larvae to the sessile reproductive phase (polyp). The larva develops into a primary polyp only in response to a chemical cue/specific molecule provided by associated environmental bacteria.

To identify associated microbes and the morphogenic factors, we first profiled the microbial community using Illumina 16S rRNA. Subsequently, we isolated and characterized bacterial strains associated with *Hydractinina* using antimicrobial activity and larvae settlement assays. Bioassay guided analysis of the bacterial culture extracts showed that the bacterial signal is part of the microbial biofilm and stable to a broad range of physical and enzymatic treatments.

To analyze the genomic basis of morphogens and defensive metabolites, we sequenced the genomes of selected associated bacteria and co-occurring fungi. Subsequent genome mining and activity-guided analysis allowed us to identify several active components including antimicrobial N-acylaminoacids, selective cysteine protease inhibitors with rare moieties as well as new fungal hybrid molecules. The discovery of novel compounds highlights the chemical potential of *Hydractinia*-associated microbes.

Session/symposium: New chemical structures/independent presentation

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Roberts R.E.<sup>1</sup>\*, Powell D.<sup>1</sup>, Wang T.<sup>1</sup>, Hall M.H.<sup>2</sup>, Motti C.A.<sup>2</sup>, Cummins S.F.<sup>1</sup> **Putative chemosensory receptors are differentially expressed in the sensory organs of male and female Crown-of-thorns starfish**, *Acanthaster planci* 

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Chemosensation is a critical signalling process for all organisms and is achieved through the interaction between chemosensory receptors and their ligands. The Crown-of-thorns starfish, Acanthaster planci species complex (COTS), is a predator of coral polyps and Acanthaster cf. solaris is currently considered to be one of the main drivers of coral loss on the Great Barrier Reef in Queensland, Australia. This study is the first to describe the presence of variant Ionotropic Glutamate Receptors in any Echinoderm, which are differentially expressed in the olfactory organs of COTS. Several other types of G proteincoupled receptors such as adrenergic, metabotropic glutamate, cholecystokinin, trace-amine associated, GRL101 and GPCR52 receptors have also been identified. Based on their differential expression within the olfactory organs and presence in multiple tissues, it is possible that several of these receptor types have expanded within the Echinoderm lineage. Many are likely to be species-specific with novel ligand-binding affinity and a diverse range of functions. Several receptors display male-biased expression within the sensory tentacles, indicating possible reproductive significance. This is only the second study to investigate chemosensory receptors in any starfish or marine pest, and represents a significant step forward in understanding the chemosensory abilities of COTS.

Session/symposium: Interspecific relationships/Arthropod chemoreceptors
ORAL PRESENTATION

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Rodriguez-Saona C.<sup>1</sup>\*, Sanchez F.<sup>2</sup>, Guisti M.<sup>3</sup>, Zhou Y.<sup>3</sup>, Benrey B.<sup>4</sup> Effects of domestication of blueberries on the invasive vinegar fly *Drosophila suzukii* 

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Highbush blueberry is a crop native to the northeast USA that has been domesticated for about 100 years. In the present study, we investigated the susceptibility of wild and cultivated blueberries to an invasive vinegar fly, the spotted wing drosophila, Drosophila suzukii. This insect was introduced from Asia into the continental USA in 2008 and was first reported in the northeast USA in 2011. We tested the hypotheses that cultivated blueberry fruits are preferred by D. suzukii for oviposition and are more suitable hosts than their wild ancestors; and that these fruits differ in physical and chemical traits. Fruit from wild and cultivated blueberries was sampled from twelve different sites in the Pinelands national reserve in New Jersey (USA), a region where blueberries were first domesticated. Each sampled site had wild and cultivated blueberries growing in close proximity. Fruit from wild and cultivated blueberries was collected weekly from June through August in 2014 and 2015 from each site. The preference and performance of D. suzukii on wild and cultivated blueberries were studied in choice and no-choice bioassays. In addition, we compared size, <sup>o</sup>Brix (total sugars), penetration force, and phenolic and anthocyanin content between wild and cultivated fruit. In choice and no-choice bioassays, 2x more eggs were oviposited in, and 2x more flies emerged from, cultivated than in wild blueberries. Cultivated blueberry fruit was 2x bigger in size, had 20% lower °Brix levels, was 45% firmer, and had >1.5x lower phenolics and anthocyanins as compared with their wild ancestors. These results show that breeding for agronomic traits has made fruit more susceptible to D. suzukii. Increased susceptibility was correlated with larger fruit size and less secondary defenses in fruit. This study documents the potential positive effects of crop domestication on an invasive frugivorous insect pest.

Session/symposium: *Practical applications/Semiochemical application for invasive species*ORAL PRESENTATION

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Rohlfs M.

Volatiles from mutualist yeast protect saprophagous insects against invasion by chemically defended mould fungi

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Filamentous mould fungi can behave like serious pathogens invading the breeding sites of saprophagous insects. Using *Drosophila* fruit flies as model system, we demonstrate that an induced chemical response helps fungi to resist insect physical attack. The effect of this chemically mediated resistance is strong in bipartite insect-fungus interactions, whereas under more natural conditions that include the metabolic activity of facultative mutualist microbes (dietary yeasts), *Drosophila* larvae can permanently suppress mould growth. In a series of experiments, we found that mould fungi exposed to yeast metabolites were more susceptible to insect damage and had reduced insecticidal properties. Additionally, the volatile-exposed fungi were impaired in secondary metabolite formation and unable to activate "chemical defence" genes upon insect damage. Therefore, besides providing essential nutrients, external and facultative mutualist microbes can have an important function in stabilising insect habitats, without the need for co-evolved defensive microbial symbionts.

Session/symposium: Interspecific relationships/Insect-microbe interactions
ORAL PRESENTATION

Romero-Frías A.1\*, Bento J.M.S.2, Sinuco D.C.3

Male-produced aggregation pheromone of the Molytinae *Heilipus lauri* Boheman (Coleoptera: Curculionidae)

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The big avocado seed weevil, *Heilipus lauri* Boheman (Coleoptera: Curculionidae), is one of the most significant pests of avocado in Colombia and other countries, such as Mexico. The volatile compounds produced by males and females were collected by headspace-solid phase microextraction (HS-SPME) and dynamic headspace collection (DHS) on a sorbent. Comparative analysis of these volatile compounds was performed using gas chromatography coupled with mass spectroscopy (GC-MS). Four male-specific compounds were identified, in a ratio of 94.8:3.0:1.8:0.4. (1*R*,2*S*)-grandisol was the major component, and the chemical structures of the other three compounds seem to be derived from it: (1*R*,2*S*,6*R*)-papayanol, (1*R*,2*S*,6*R*)-papayanal and (1*R*,2*S*)-grandisal. These results may help in integrated pest management of this insect through the use of semiochemicals, specifically its pheromone.

Session/symposium: Practical applications/Natural products for integrated pest

management POSTER number: 108

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Identification of male hairpencil pheromone components in *Choristoneura fumiferana* Clemens (Lepidoptera: Tortricidae): chemistry and function

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In Lepidoptera, male pheromones may serve to indicate the location of the male, induce female acquiescence, or advertise male quality. During courtship, males may also release anti-aphrodisiac pheromones to dissuade competing males from locating or attempting to court a target female. Recently, the importance of pheromones associated with the male hair pencil organs in Choristoneura fumiferana (Clemens) (Lep.: Tortricidae) to mating success was confirmed; however, the behaviorally important chemical components were unknown. We investigated the possibility of male pheromones acting as anti-aphrodisiacs on conspecific males. GC/MS analyses and electroantennogram detection identified two compounds within the male pheromone blend, nonanal (9:Ald) and decanal (10:Ald); both elicited strong dose-dependent antennal responses in the antennae of adult males. When females were marked with nonanal, the proportion of mating pairs in copula was significantly lower as compared to when females were unmarked or marked with either a control solvent or decanal. Addition of nonanal to synthetic 2-component female pheromone plumes significantly decreased the occurrence of wing fanning in males stimulated by female pheromone in both close-range and flight tunnel bioassays. Interestingly, similar quantities of nonanal were also found in both virgin adult females and males. Our findings suggest that nonanal is used by courtship-initiating males (M') to 'mark' females during mating so that the possibility of extra copulations with competing males (M'n+1) is reduced, and to counteract male courtship behaviors in additional males initiated by the perception of female pheromone. This represents the first behavioral evidence of male anti-aphrodisiac pheromones in C. fumiferana.

Session/symposium: Intraspecific relationships/Importance of multicomponent mixtures for chemical cues

**ORAL PRESENTATION** 

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Rusalepp L.\*, Sober A.

Hypericum spp. and humidity: which is better, drier or wetter?

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Hypericum spp. are widespread and well-known for their medicinal properties. Their secondary metabolite profile is extensively studied and not all species are suitable for medicinal use. Less is known about the environmental effects on the metabolite profile. Water deficit can enhance the secondary metabolite production, but can there also be too much of water? With a view to find out, samples of aboveground parts of Hypericum maculatum Crantz where gathered from a free air humidity manipulation (FAHM) experiment in Rõka village, Estonia, and comparative samples of H. maculatum and H. perforatum L. where gathered from various locations in Estonia on different years. Monthly precipitation data was acquired from the Estonian Environment Agency. The concentrations of phenolic acids, flavanols, flavonols, naphthodianthrones, and phloroglucinols were determined by LC-DAD-MS/MS. Sample collection from the FAHM experiment happened to an extremely wet year and consequently the humidification effect was only seen on the contents of flavonols biapigenin and quercitrin, where control plants had higher metabolite yield than treatment plants. Previous data was taken into account to estimate whether the weather was already too wet for the humidification treatment to have an effect at all. Samples and precipitation data from three different years suggest that the connection between water availability and secondary compound production is with an optimum and too high water content in soil and air is detrimental for the secondary metabolite production.

Session/symposium: Intraspecific relationships/independent presentation

## Ryu C.M.

## Sniffing bacterial volatiles for healthier plants

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In the phytobiome world, microbial metabolic activity includes the secretion of diverse infochemicals that influence interspecific plant and microbes. Previously the metabolites have been utilized upon improving plant health. Certain plant growth-promoting rhizobacteria (PGPR) elicit induced systemic resistance (ISR) and plant growth promotion in the absence of physical contact with plants via bacterial volatile compound (BVC) emissions. In this presentation, I review the recent progress made by research into the interactions between PGPR and BVC, focusing on BVC emission by PGPR strains in plants. Particular attention will be given to the mechanisms by which these bacterial species elicit ISR. I provide an overview of recent progress in the elucidation of BVC interactions from studies utilizing transcriptome, metabolome and proteome analyses. BVCs also confer induced systemic tolerance to abiotic stresses, such as drought and heavy metals. Furthermore, to assess potential utilization of BVC for crop plants, volatile suspensions were applied to crops like pepper and cucumber and were found to be effective at protecting plants against plant pathogens and insect pests in the field. Taken together, these studies provide further insights into the biological and ecological potential of BVCs for enhancing plant self-immunity and/or adaptation to biotic and abiotic stresses in modern agriculture.

Session/symposium: Interspecific relationships/Volatile-mediated microbe-plant interactions KEYNOTE LECTURE

Salvador M.C.<sup>1</sup>, da Graca J.P.<sup>2</sup>, Ueda T.E.<sup>3</sup>, Lopes I.O.N.<sup>4</sup>, De Oliveira M.C.N.<sup>4</sup>, Neumaier N.<sup>4</sup>, Farias J.R.B.<sup>4</sup>, Ventura M.U.<sup>3</sup>, Hoffmann-Campo C.B.<sup>4</sup>\*

Temperature and CO<sub>2</sub> concentration: effects on soybean metabolism and implications on *Anticarsia gemmatalis* biological aspects

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Climatic changes may alter the metabolism of plants, as possible insect-plant interaction. Experiments were carried out in growth chambers at constant temperatures (25 ° C, 28 ° C, 31 ° C and 34 ° C ) and  $CO_2$  ( $\approx$  380ppm and 456 ppm) to estimate C: N ratio and flavonoid concentrations in BRS 359RR soybean leaves. The effect of those environments parameter on biological aspects of Anticarsia gemmatalis was also evaluated at elevated CO<sub>2</sub> (ECO<sub>2</sub>, 456 ppm). Contents of C and N were estimated by GC and flavonoids by HPLC. The ratio of C: N increased with temperatures and CO<sub>2</sub>; dropping in plants maintained at 34° C, in both CO<sub>2</sub> concentrations. Highest concentrations of isoflavones malonyl daidzin and malonyl genistin were detected on the leave of soybean grown at 25 ° C, under ECO2, but their content reduced as the temperature increased. Higher mortality of A. gemmatalis occurred at 34 ° C and ECO<sub>2</sub>. This parameter was strongly related to C, as well as genistein and daidzein conjugates contents. Insect-mass was lower and the female laid fewer eggs, but lifetime span was shorter at 34 ° C. We discuss that dropping of isoflavonoid concentration and the possible alterations in the content of primary and secondary metabolites caused by CO<sub>2</sub> and temperature increase may provide a better performance to A. gemmatalis development in soybean field, but not before gradual adaptation of the insect to the new temperature conditions.

Keywords: C: N ratio, isoflavonoid, biological parameters, velvetbean caterpillar

Session/symposium: Interspecific relationships/independent presentation

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Sampson C.

Mass trapping with pheromone incorporated sticky traps makes integrated thrips management programmes more robust

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The western flower thrips, *Frankliniella occidentalis* (Pergande) (Thysanoptera: Thripidae) is difficult to control with chemical insecticides due to pesticide resistance. In semi-protected strawberry crops, mass trapping of *F. occidentalis* using blue sticky roller traps reduced adult thrips numbers per flower by 61% and fruit bronzing by 55%. The addition of the *F. occidentalis* aggregation pheromone, neryl (S)-2-methylbutanoate, to the traps doubled the trap catch, reduced adult thrips numbers per flower by 73% and fruit bronzing by 68%. The addition of blue sticky roller traps to an integrated pest management programme using predatory mites, maintained thrips numbers below the damage threshold and increased grower returns by a conservative estimate of £2.2k per hectare. The system has been adapted for use in different cropping systems with consistent results.

Despite the success of mass trapping of thrips, there are limitiations. Huge numbers of thrips need to be caught to make an impact on a population, requiring high densities of sticky traps. The aggregation pheromone increases the activity of *F. occidentalis*, but thrips landed in response to an attractive colour rather than flying directly to a pheromone source. This limits the potential increase in trap-catch as thrips may land on flowers before reaching the traps. Neither pheromone nor an attractive colour draw thrips far away from a flowering crop. Tyically the traps are not sufficient to control thrips on their own but are a versatile tool that can be used in combination with existing biological, chemical, or semio-chemical control methods, making IPM programmes more robust.

Session/symposium: Practical applications/Comparison of semiochemical control methods of pest insects

ORAL PRESENTATION

Santana A.E.G. <sup>1</sup>\*; da Silva K.B. <sup>1</sup>, de L. Costa D. <sup>1</sup>, Tinoco R.S. <sup>2</sup>, Lins P.M.P. <sup>3</sup>, Goulart H.F. <sup>1</sup> Chemical composition of the female ovipositor and morphology of antennas of *Eupalamides cyparissias* (Fabricius, 1776) (Lepidoptera: Castniidae)

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Eupalamides cyparissias is one of the main pests of the Arecaceae family, with emphasis on palm oil (Elaeais guineensis) and coconut (Cocos nucifera). E. cyparissiasis classified as a lepidobroca due to its food habit, which causes galleries inside the strains of the plant causing serious damages that lead to heavy economic loss. The present study aims to verify the chemical composition of extracts of the female ovipositor and to analyze the morphological structure of the antennas of both sexes of E. cyparissias. The extract was obtained from 10 virgin females with 24 h of age, with excision of the ovipositor for extraction of the volatile organic compounds (VOCs) with doubly distilled hexane grade HPLC, for 20 minutes. The chemical composition of the extract was analyzed by gas chromatograph GC-FID and by gas chromatograph coupled with mass spectrometry GC-MS. The compounds were identified in the extract by analyzes of individual mass spectra and compared with Kovat index of compounds, reported in data bases as Pherobase and Nist and also using reference standards. The antennas of males and females with 24 h of age (n = 5 of each sex) had their morphological structures analyzed through the images obtained by scanning electron microscopy (SEM) at 5KV. Saturated aliphatic hydrocarbons, esters of fatty acids and terpenes were identified in the extract. The antennae of males and females are structurally similar, as well as, the sensory chemoreceptors, identified as caetic, tricoid, styloconic, squamiform, böhm bristles. The present data contribute with relevant information to E. cyparissias, helping to understand the chemical composition of the female reproductive organ and the olfactory system of both sexes of this insect. The chemoreceptor sensilla acts in the recognition of distinct chemical signals that contribute to the survival and reproduction of this insect in the field.

Session/symposium: Intraspecific relationships/independent presentation

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Sarles L.<sup>1</sup>\*, Verhaeghe A.<sup>2</sup>, Verheggen F.J.<sup>1</sup>

## Semiochemicals to control the Walnut Husk Fly Rhagoletis completa

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Most European walnut producers have to deal with the recent introduction of the Walnut Husk Fly, *Rhagoletis completa* (Diptera, Tephritidae), that causes severe economic losses, especially in biological productions. In orchards where *R. completa* is present and uncontrolled, losses in walnut yields can reach up to 80%. As a consequence, there is a need for developing environment-friendly methods of control.

In this research, we evaluated the efficiency of two different semiochemical blends to trap R. completa in walnut orchards. The first semiochemical blend consisted in walnut fruit volatiles, previously collected and identified from two walnut varieties. The second semiochemical blend was made of the putative male sex pheromone, made of two lactones, and previously collected from *R. completa* males. In field both semiochemical blends were associated with yellow sticky traps. The assays were conducted in France, in a total of 53 orchards, in 2015, 2016 and 2017.

The first blend (made of fruit kairomones) was highly attractive for both male and female R. completa in a laboratory assay, but did not enhance the number of fly captures in the field. On the other hand, sticky traps associated with the putative sex pheromone captured up to 10 times more fruit flies each week during the entire season. The total number of captured flies was also more important than with a mass trapping system baited with food attractant. These field results are promising for *R. completa* monitoring and mass trapping.

Session/symposium: Intraspecific relationships/independent presentation

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Epizingiberene synthase: a template for bioactive chemical space underpinning insect olfaction

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Semiochemical-based pest management uses signalling chemicals to manipulate pest behaviour for the reduction of crop plant infestation. Semiochemicals are highly specific olfactory ligands and only need to be deployed in small amounts at levels not causing toxicity. However, semiochemicals can be expensive to synthesise and chemically unstable. A rational design of analogues of these semiochemicals by chemoenzymatic synthesis can help to overcome these challenges. In the approach described here, the acceptance of unnatural substrate by the specifically responsible biosynthesis enzyme leads to analogues of the natural product which might have superior properties. This synthetic biology approach is investigated for the enzyme epizingiberene synthase in the BBSRC-funded project "Epizingiberene synthase: structure, mechanism and a template for design of bioactive chemical space underpinning insect olfaction". This enzyme is a member of a newly identified class of sesquiterpene synthases using (Z,Z)-farnesyl diphosphate as substrate instead of (E,E)-farnesyl diphosphate. The natural product of this enzyme, 7-epizingiberene, and its oxidation product (R)-curcumene show repellent properties against pest insects, such as whiteflies. The bioactivity of the analogues of both sesquiterpene hydrocarbons is studied by comparing the electrophysiological and behavioural responses of the relevant pest insects to the natural products, 7-epizingiberene and (R)-curcumene. The project will be introduced in more detail and the current progress will be presented.

Session/symposium: Practical applications/independent presentation

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## Schlyter F.

#### Host selection in *Ips typographus*: have we been barking up the wrong tree?

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Following the numerous successful elucidation of highly attractant pheromone and kairomone blends in chemical ecology, the specific cues has long been sought for "primary attraction" to weak, non-resistant host trees.

Contrasting, for Ips typographus and some "aggressive" tree killing bark beetles, clear positive data are elusive for long-distance volatiles attractants indicating low resistant trees. However, other olfactory signal as the strongly and fast acting aggregation pheromone are well documented. Likewise, anti-attractants from non-host trees or habitats (non-host volatiles) are strongly active and has specific receptors been since long demonstrated, as has old-host overcrowded signals such as verbenone. Similarly, a resistance indicator volatile 1,8-cineol, interfering with a pheromone component detection, is established. Trace host compound 4-thujanol, an oxygenated host monoterpene associated with more resistant (younger) trees, has similar activity and several such compounds are GC-EAD active and under behavioural testing. Several non-volatile phenolics compounds are also possible resistant indicators and are anti-feedant semiochemicals. It is observed, but not yet published, that *Ips typographus* adults land and walk on trees remaining non-attacked, as shown for N American *Dendroctonus*.

We may conclude that for basic understanding of the host selection process and possible semiochemical and genetic applications in forest management, we should study chemical and molecular biology resistance cues and characters, active after landing, in "the last trees standing" rather than in the first casualties.

Session/symposium: New chemical structures/Invasions under the bark: chemical ecology of woodboring beetles

**ORAL PRESENTATION** 

Schlyter F.\*, Turcani M.

From gene to landscape level: a 6-year project on chemical ecology and control of *Ips typographus* under climatic change

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Spruce decline in Europe is rampart, an effect of increasing temperature, droughts, and storm combined with the aggressive bark beetle *Ips typographus*. It is now 40 years since the discovery of a new key pheromone component allowed mass-trapping as a game-changing management tool [1]. We attempt to rely on modern molecular biology in combination with classical chemical ecology and forest science, including remote sensing, to produce both new basic science and new management tools.

We work on the tree-beetle interactions at three levels of scale: Gene, Tree, and Landscape.

Among studies, we are sequencing the genome of the Ips typographus, which will be first used for functional analysis of olfactory receptor (OR) proteins. Factors for tree resistence will be observed on Norway spruce trees (of genetically known decent) directly, by remote sensing, and by physio-chemical manipulation, while landscape polycultures' resilience will be modelled, genetically screened, and improved by increased semiochemical diversity. These and the in total 14 tasks of the project, will allow testing the Semiochemical Diversity Hypothesis (SDH)[2].

More details here: https://extemit.fld.czu.cz/en/

Apart from the science to be communicated, the project goals are to:

- · Increase international cooperation among CULS and other institutions.
- $\cdot$  Build the infrastructure, including a laboratory of chemical ecology for forest insect research.
- · Build an excellent research team including post-docs and PhD students.

Keywords: Genome, transcriptome, proteome, remote sensing, drones, stress, pheromone, non-host volatiles, SDH

- [1] Bakke A. et al. (1977) Naturwiss 64: 98.
- [2] Zhang Q.-H., Schlyter F (2003) Oikos 101: 299-310.

Session/symposium: Practical applications/Natural products for integrated pest

management
POSTER
number: 153

Schulz S.<sup>1</sup>\*, Bello J.<sup>1</sup>, Schmidt W.<sup>1</sup>, Steinbiss M.<sup>1</sup>, Schlawis C.<sup>1</sup>, Gerbaulet M.<sup>1</sup>, Leinaas H.P.<sup>2</sup>

## The unique chemistry of springtails from a chemical ecology perspective

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Collembola (springtails) are the oldest hexapods on earth and closely related to insects. Although this group contains only about 6,000 known species, they are likely the most abundant hexapods on earth. We are interested in the chemistry of these animals from a chemical ecology perspective. Therefore, we analyzed several species looking at three major topics: 1) cuticular lipids, 2) chemical defense, and 3) pheromones. Major differences between insects and springtails are found especially in cuticular lipids. While insects predominately use hydrocarbons derived from the fatty acid biosynthetic pathway, springtails often use terpenes instead. This difference leads also to functional differences. While in insect complex mixtures of cuticular compounds biosynthetically derived from C2 (acetate)building blocks, allowing fine-tuning of properties and development of chemical communication traits based on these mixtures, collembolan cuticular lipids are less structurally diverse. Usually few compounds dominate the mixtures. Whether this composition leads to functional differences compared to insects is unknown. Springtail cuticular hydrocarbons often do not fit in the conventional terpene classification, displaying rare and unprecedented terpene classes. Chemical defense of springtails is also unique. Several highly insect-repellent or toxic compounds produced by them are not known from other organisms. These compounds belong to varying classes, especially alkaloids, but also aromatic compounds and polyketides. Finally, pheromones are the least well-explored area. Although few pheromones have been characterized, the volatile emissions of springtails indicate that pheromone compounds might be not much different structurally compared to those used by insects. Some results of our work will be presented in the lecture.

Session/symposium: New chemical structures/independent presentation ORAL PRESENTATION

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Friends and foes, elevational trends in plant defences and pollinator attractants mirror insect community structure and gene flow along a mountain gradient

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Insects on plants are diverse and of profound importance. Vascular plant diversity is clumped into 'hotspots', especially tropical mountains. It is logical to ask, why are these mountain ranges so diverse and how does the diversity of vascular plants drive the diversity of their insect associates? We studied the genus Ficus, along an elevational gradient at the centre of its diversity in Papua New Guinea. Species of Ficus are pollinated by species specific pollinating wasps while their leaves are consumed by a rich community of lepidopteran larvae and adult beetles. Firstly, we tested the hypotheses that i) geneflow between populations of *Ficus* with large elevational ranges would be decrease with elevation, resulting in genetically structured parapatric populations, ii) that pollinating wasps would show even more genetic structure and be most attracted to fig fruit from their natal elevation and that iii) volatile organic carbon emissions from fig fruit would show significant levels of dissimilarity between elevational populations. Secondly, we tested a set of hypotheses related to insect herbivores, iv) the compound level diversity and content of alkaloids and polyphenols in fig leaves would decline with elevation, v) this decrease in defensive investment would result in a decrease in similarity of insect herbivore community with elevation. We combined population genomics with targeted LC-MS/MS and GC-MS analyses. We demonstrated that there is a genetic cline in many fig species and their pollinators. Pollinating wasps are attracted to elevation specific blends of VOC. We suggest that limited dispersal of pollinating wasps and elevation specific volatile emissions act together with local adaptation to facilitate parapatric speciation in this system. Insect herbivore communities are also strongly influenced by local chemical defences such that a turnover in secondary metabolites results in a turnover in insect herbivores.

Session/symposium: Interspecific relationships/independent presentation

ORAL PRESENTATION

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Field identification of biochemical biomarkers for screening plant resistance to insects: an example from the pollen beetle – oilseed rape interaction

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Pollen beetle (Brassicogethes aeneus syn. Meligethes aeneus) is one of the main insect pest affecting oilseed rape crops. Efficiency of insecticides used to control this pest is decreasing due to the development of resistance to compounds such as pyrethroids in many populations. Breeding oilseed rape for resistance to pollen beetle attacks could be an interesting strategy to find alternative control methods. However, screening plants for insect resistance remains complicated as it often involves field tests on large genotype collections which are complicated to carry out without biases. Current knowledge on the chemical ecology of interactions between oilseed rape and pollen beetles could help finding biochemical markers of this resistance and bypass this problematic field screening phase thus allowing an indirect breeding approach. Previous laboratory tests have shown that variations in attack levels among a small set of oilseed genotypes could be explained by the biochemistry of bud tissues. The present study aimed at validating this link under field conditions. For that purpose, we conducted a multi-site experiment in France with 19 genotypes exposed to pollen beetle attacks. We phenotyped pollen beetle damage and sampled buds in the field to assess their chemical composition. Large variability in pollen beetle attacks was observed over the genotypes. These attack levels were consistent between locations. Bud chemistry was highly variable but most compounds were well correlated between locations. Potential biomarkers previously identified in laboratory experiments were not confirmed but new compounds which may be considered interesting markers for resistance screening against the pollen beetle emerged. This study allowed to better identify constraints related to plant breeding based on biochemical biomarkers.

Session/symposium: Interspecific relationships/Metabolomics approaches in the Brassicaceae

ORAL PRESENTATION

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Sex pheromones and sex attractants of North American click beetles in the genus Cardiophorus

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Until 2018, all known or suspected pheromones of click beetles (Coleoptera: Elateridae) had been identified only from species native to Europe and Asia. Reports of identifications from North American species dating from the 1970s have since proven to be incorrect. In 2016, while testing longhorned beetle (Coleoptera: Cerambycidae) pheromones, we serendipitously discovered that males of two Cardiophorus species were specifically attracted to the cerambycid pheromone fuscumol acetate [(E)-6,10-dimethylundeca-5,9-dien-2-ol acetate], suggesting that this compound might also be a sex pheromone for Cardiophorus species. Further field trials with the enantiomers of fuscumol acetate determined that males of the Cardiophorus species were specifically attracted by the (R)enantiomer of fuscumol acetate. Furthermore, only the (R)-enantiomer elicited strong antennal responses from male Cardiophorus in electroantennogram assays. Female Cardiophorus beetles were collected for verification of the pheromone, but to our surprise, analysis of the resulting extracts showed that female Cardiophorus actually produced a different compound, methyl (3R,6E)-2,3-dihydrofarnesoate [(3R,6E)-3,7,11-trimethyl-6,10dodecadienoic acid methyl ester]. The bioactivity of the pheromone was verified in field trials of the synthetic pheromone. Potential reasons for the strong and specific attraction of Cardiophorus males to fuscumol acetate will be discussed. Two additional structural analogs, fuscumol propionate [(E)-6,10-dimethylundeca-5,9-dien-2-ol propionate] and methyl citronellate (methyl 3,7-dimethyloct-6-enoate) are being tested this field season to determine if they will also act as sex attractants due to the similarities in structure and spatial relationships of the functional groups between the true pheromone and these analogs.

Session/symposium: Intraspecific relationships/Chemical ecology of click beetles (Elateridae): practical applications and advances in the field.

ORAL PRESENTATION

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## Tibor Jermy and host shifts: are those shifts easy or hard?

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Tibor Jermy tilted at windmills, specifically Ehrlich and Raven's theory of coevolutionary arms races between plants and insect herbivores. Jermy began by contending that insect herbivores are incapable of exerting natural selection on plant chemistry. I disagree! However, time has proven Jermy prescient in other respects: the reciprocal coevolution that E&R proposed surely isn't the most common pattern. As a counter-example, in Agrawal et al's phylogenetic analysis of North American milkweeds, increases in plant defense were associated with reduced speciation rates, the opposite of E&R's prediction that innovative defenses should cause escape from herbivory and spur speciation. On the insect side of the coevolutionary scenario, E&R viewed host shifts as so difficult to achieve that a successful shift was typically followed by adaptive radiation of the insects. Again, Jermy balked at this, and he wasn't alone. Strong showed that species richness of insects on cacao was explained by abundance of cacao, independently of whether cacao was introduced or native, implying that host shifts onto cacao by native insects were easy and quick, occurring soon after the plants had been introduced. Our own work supports the view of host shifts as easy and frequent. We followed the diets of >50 populations of Edith's checkerspot butterfly for 50 years, and in so doing observed this species to perform 6 independent host shifts between plant genera. Three of the shifts involved rapid evolution of insect host preference. Two of these evolutionary shifts occurred after anthropogenic environmental change had generated potential novel hosts on which insects could immediately achieve higher fitness than on their traditional hosts, despite clear maladaptation to the novel resources. I suggest that evolution despite maladaptation has been undervalued and that estimating its prevalence would contribute to better understanding of host shifts in general.

Session/symposium: Interspecific relationships/Tibor Jermy's Legacy in insect-plant evolution

KEYNOTE LECTURE

Sivcev I., Sivcev L.

#### Further results of research on Bothynoderes punctiventris aggregation attractant

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The effectiveness of lower trap densities in mass trapping in newly sown sugar beet fields was explored. In a trial in 2014 there were significantly more beetles caught/trap on layout 30 x 30 m. However, in trials in 2015 and 2016, there were no significant differences between a number of weevils caught in trap density of 11 traps/ha (layout 30m x 30m) and 4 traps/ha (layout 50 m x 50 m).

Effects of mass trapping in sugar beet crop is tested in 2016 with 25 traps in the layout of 30m x 30m. Another plot, 100 m apart, with 25 traps in layout 50m x 50m was also set. There were no significant differences between a mean number of caught weevils in the plot with traps layout 30 x30 m (15.52 $\pm$ 1.669) and in a plot with traps layout 50 x 50 (11.92 $\pm$ 1.446) (p= .059). Assessment of the effects of mass trapping was during next year. The assessment showed mean of 36.6 weevils/trap in last year 30 x 30 m plot, 57.9 weevils/trap in last year 50 x 50 m plot and 164.2 weevils/trap in last year check plots. There was no significant difference in a mean number of caught weevils that between treatment plots (p= .155). But both treatments were significantly different to mean a number of weevils on untreated plots (p= .002).

Effects of mass trapping on a large scale plot was tested in 2017 when 4 plots comprising of 25 baited pitfall traps at a distance of 30m x 30m were deployed. During 2 months in total 2739 weevils were caught in traps  $(27.39\pm1.1\ /\ trap)$ . Effect of this mass trapping is assessed next year when weevils were at crawling stage. On 9 ha with mass trapping in 2017 it was caught  $195.5\pm8.99$  weevils /trap which was significantly higher than 77.26 weevils/trap in untreated plots (p<.001). Thus, large scale mass trapping in 2017 attracted and caught the weevils which remained alive in the field after insecticide treatment but also resulted in 2.5 fold higher weevil density next year.

Session/symposium: *Practical applications/independent presentation*KEYNOTE LECTURE

Sloggett J.J.\*, Oudendijk Z.

Intraspecific variation in ladybird beetle chemical defences: implications for theories of honest signalling

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Ladybirds beetles (Coccinellidae) are well known for their bright colours and spotted patterns, which advertise distasteful or toxic alkaloid defences. Over the last decade intraspecific variation in the detail of these patterns has been proposed as an honest signal of the strength of ladybird chemical defences i.e. the amount of alkaloid that a ladybird contains. I here discuss such hypotheses based on a knowledge of ladybird ecology and work on how chemical defence in ladybirds varies within species. In particular, I address whether the amount of alkaloid carried by ladybirds is sufficiently stable over time to make the signal truly honest.

Session/symposium: Interspecific relationships/Semiochemistry of aphidophagous insects
ORAL PRESENTATION

Soares A.M.L., Franca P.H.B., Triana M.F., dos Santos J.M., Goulart H.F., Santana A.E.G.\* **Reproduction of electrophysiological activity of** *Anthistarcha binocularis* (Meyrick, 1929) to (*E*)-6-dodecenol

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Early dwarf cashew is a locally important perennial plant in Brazil. A. binocularis, is considered a key cashew crop pest, because larval feeding disrupts the passage of sap to the floral branches, thereby directly impeding fruit development. The species is a moth with endophytic habit that belongs to order Geleciidae. In addition, there are few reports on the biology and behavior of this pest. Therefore, this study aims to describe specific aspects of the sexual behavior of A. binocularis. Observations of calling behavior, pre-copulation and copulation behaviour were set up with groups of A. binocularis containing virgin couples of 2d-old. The period of attraction start near the fifth hour of scotophase, with agitation behaviors, such as flights and hiking by the females and the males. Then both moths stood still, stirring just to the antenna for about eight minutes, starting pleural distension of abdomen, with exposure of the sexual glands of the females and then the male starts mating attempts putting his body parallel the female and trying to touch the end portion of the abdomen of the same with its copulatory organ. After approximately one minute the mating is achieved, which lasts from 2:30h to 3:40h. Analysis of sexual gland extracts from virgin females by gas chromatography coupled to an electroanthenographic detectorshowed evidence of two electrophysiologically active compounds, one of whichhad its structure proposed as 6-dodecenol based on mass spectrometry and derivatization with DMDS. In order to confirm the chemical structure of the biologically active compound, we synthesized both stereoisomers with E and Z configuration for the double bond, which were co-injected with the extracts and led to confirmation of E configuration. We further expect to assess the biological activity of the reported compoundin behavioural bioessays and field tests to confirm itsattractiveness to males.

Session/symposium: Practical applications/Natural products for integrated pest

management

Sousa M.\*, Becher P., Green K., Birgersson G.

Odor signals for host identification in *Medetera* long-legged flies (Diptera: Dolichopodidae)

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The long legged flies (Dolichopodidae) are important in the biological control of "aggressive" bark beetles such as *Ips typographus* and the most important predatory species belong to *Medetera* genus. The attack sequence starts with *Medetera* females identifying a tree under bark beetle attack from a distance. After landing the females oviposit on the bark surface and the eclosed larvae mine through the bark and into the bark beetle larval galleries, where they mainly predate on the bark beetle brood during their development. *Medetera* larvae can consume between 5 to 20 individuals depending on bark beetle density (Beaver, 1966) and together with other Dolichopodidae predators, they can accounted for more than 80% of the bark beetle mortality (Wermelinger, 2002).

Although, the importance of these predators have long time ago been identified, little information is available regarding *Medetera* ecology. In this PhD project we will follow the entire period of attraction of *Medetera* and elucidate their host finding cues. Our hypothesis is that *Medetera* flies locate the bark beetle attacked trees by their sense of olfaction. We will collect odours from bark beetle attacked trees on adsorbent columns from active spots on the bark surface where *Medetera* females oviposit. The collected volatiles will then be analyzed by electrophysiology on *Medetera* antenna (GC-EAD) and chemically identified (GC-MS). Identified compounds will be tested as synthetics in bioassays, in both the laboratory and in the field. We will also try to deduce the origin of the active compounds and develop a bait to attract *Medetera*. Using this bait, it will be possible to monitor the populations of *Medetera* flies in areas with bark beetle attacks or attract *Medetera* to attacked trees, where salvage is hard or impossible, as in nature reserves.

Session/symposium: Interspecific relationships/independent presentation

Steiger S.<sup>1,2</sup>

## The chemistry of mating: how pheromones help to spread and receive sperms

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Over the last decades, mating patterns have been intensively studied by evolutionary biologist and behavioral ecologists and there is now rich evidence that males and females from many species do not mate randomly, but have evolved diverse strategies that allow them to control the transfer and receipt of sperm. Among other mechanisms, chemical cues and pheromones have been found to be of high importance when it comes to mating. By presenting data from several insect species, I will demonstrate a fascinating diversity of "chemical strategies" animals have evolved to spread and receive sperm or to avoid the costs of mating. I will highlight, how and which chemical traits facilitate polyandry and polygyny or function as anti-aphrodisiacs. Furthermore, I will show that mate choice can exert a complex pattern of sexual selection on chemical traits and that sex pheromones can be condition dependent and inform about a mate's quality. When we look at the receiver's side we will see that substances do not necessarily lead to a fixed response but a receiver's decision can be based on learning, self-referencing or the specific context.

Session/symposium: Intraspecific relationships/independent presentation KEYNOTE LECTURE

Steitz I., Ayasse M.\*

Macrocyclic lactones act as a queen pheromone in the primitively eusocial sweat bee *Lasioglossum malachurum* (Hymenoptera: Halictidae)

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One major precondition for the evolution of sociality within insects was the establishment of chemical communication systems which are thought to mediate e.g. interactions of individuals and the regulation of reproduction. Pheromones emitted by queens are known to influence worker behavior and reproductive physiology and are therefore key components in regulating complex social behavior. Queen recognition by means of fertility signals has been shown to occur in many social insects, however, their chemical structures have not yet been described in sweat bees that are especially suitable for study of the evolution of chemicals associated with sociality as these bees exhibit a high variability of social behavior. In order to offer new insights into the evolution of sociality, the major aim of our study was to identify queen pheromones in the primitively eusocial sweat bee *Lasioglossum malachurum*.

We investigated cuticle odor bouquets of *L. malachurum* and performed bioassays to examine the function of cuticle compounds as queen pheromones. In chemical analyses we found caste-specific differences in the odor bouquets mainly due to higher proportions of alkanes and macrocyclic lactones in queens. Moreover, in two different bioassays we report for the first time that macrocyclic lactones, not CHCs, act as a queen pheromone influencing worker behavior and reproductive physiology in a social sweat bee, *L. malachurum*.

Session/symposium: Intraspecific relationships/independent presentation ORAL PRESENTATION

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# Evolution of caste-specific odor bouquets in halicitid bees

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Chemical communication is crucial for the maintenance of colony organization in eusocial insects and chemical signals are known to mediate important aspects of their social life, including the regulation of reproduction. Sociality is therefore hypothesized to be accompanied by an increase in the complexity of chemical communication. However, little is known about the evolution of odor signals at the transition from solitary living to eusociality. Halictid bees are especially suitable models to study this question as they exhibit considerable variability in social behavior. Here we investigated whether the dissimilarities in cuticle chemical signals in females of different castes and life stages reflect the level of social complexity across halictid bee species. Our hypothesis was that species with a higher social behavior ergo obligate eusocial species possess a more distinct chemical profile between castes or female life stages. We analyzed cuticular chemical profiles of foundresses, breeding females and workers of ancestrally solitary species, of facultative and of obligate eusocial halictid species. We also tested whether social complexity was associated with a higher investment in chemical signals. Our results revealed higher chemical dissimilarity between castes in obligate than in facultative eusocial species, especially regarding macrocyclic lactones, which were the single common compound class overproduced in queens compared with workers. Chemical dissimilarities were independent of differences in ovarian status in obligate eusocial species but were dependent on ovarian status in facultative eusocial species, which we discuss in an evolutionary framework.

Session/symposium: Intraspecific relationships/independent presentation

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## Structural elucidation of photocytotoxic compounds from Biserrula pelecinus l.

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Biserrula pelecinus L. (biserrula) is an annual pasture legume introduced to the Australian farming community from the Mediterranean regions of Europe and North Africa in the early 1990s.1 Biserrula possesses many desirable traits, such as high seed production and tolerance to drought, soil acidity, grazing and a wide range of soil types.[1-3] Unfortunately, shortly after the commercialisation of the cultivar Casbah in 1997 there were numerous cases of photosensitisation in ewes and lambs grazing biserrula pastures, with the first case report published in 2015 [4-5]. Swinny and co-workers have attempted to identify the causal compounds, with no success to date.5 We present our efforts towards structural elucidation of photocytotoxins isolated from biserrula using an MTT-based bioassay directed separation.

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Session/symposium: New chemical structures/independent presentation

Stewart C.D.<sup>1</sup>, Barrow R.A.<sup>1</sup>\*, Latif S.<sup>2</sup>, Weston L.A.<sup>2</sup>

An allelopathic compound from *Festuca* spp. as a lead molecule for development of preemergent herbicides for weed management

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There is a great need for the development of herbicides with novel modes of action, due to increasing evolution of herbicide resistance in common weed species and the lack new modes of action in commercial herbicides introduced in recent years.[1-3] Certain cultivars of fine leaved fescue (*Festuca* spp.) grasses are known to interfere with the growth of other plants by exudation of a phytotoxic non-protein amino acid, m-tyrosine, into the rhizosphere.[4,5] However, m-tyrosine is highly susceptible to microbial degradation and so is potentially unsuited for use as a commercial soil-applied pre-emergence herbicide. We have synthesised several analogues based on the bioactive architecture of this natural product, with improved herbicidal activity and will evaluate extended environmental half-life, and investigate potential mode(s) of action.

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Session/symposium: *Practical applications/independent presentation*ORAL PRESENTATION

Stowers L.

## Circuit coding of innate olfactory behvavior

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Olfactory cues trigger innate behavior in the mouse, but the precise identity of the underlying neurons and their circuit logic has not been determined. Here we present new studies to elucidate the neural components that transform olfactory signals into mouse vocalization behavior (ultrasonic vocalizations). We use optogentics and chemogenetics to determine the necessity and sufficiency of the underlying neurons and will evaluate the nature of the artificially induced vocalizations compared to natural calls. The identity of these neurons provides a platform to study olfactory information coding, motivated behavior, and determine how chemical communication is transformed into auditory communication.

Session/symposium: Intraspecific relationships/Importance of multicomponent mixtures for chemical cues

ORAL PRESENTATION

Stritih Peljhan N.\*, Zunic Kosi A.

Olfactory signals of the cave cricket *Troglophilus neglectus* (Rhaphidophoridae; Orthoptera): a rare example of temporally modulated chemical threats

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Cave crickets are wingless Orthoptera with no long-range acoustic signaling for mate attraction. Males of *Troglophilus neglectus* possess protrusive abdominal scent glands, long thought to function in female attraction or sexual stimulation. We rejected this hypothesis, showing that gland protrusion was not an obligatory part of male pre-mating behavior and caused neither female attraction nor influenced courtship success. Males protruded the glands significantly more often while contacting another male than a female, and especially while being aggressive, which suggested the released odor to represent an agonistic signal. We tested its more precise function by staging the physically matched individuals in dyadic contests and correlating the levels of behaviorally expressed aggression and dominance status with the degree of gland tissue protrusion - supposedly indicating the amount of released odor. Males protruded the glands during and after encountering a rival, with the degree increasing with the intensity of the signaler's aggression. Gland protrusion degree increased most strongly with the occurrence of the elevated body posture directly preceding the attack, and was significantly higher in encounter winners than in losers, suggesting an important role of the released odor for contest resolution. Following encounters, glands were protruded almost exclusively by winners, apparently announcing victory. We tested for the odor function also directly, by preventing gland tissue protrusion in the contestants. Treating only the dominant individuals significantly decreased the percentage of encounters they won, while treating both contestants elicited a significant increase in the frequency and duration of fights. These data confirm the olfactory signals of T. neglectus to function as a highly effective threat that prevents maximal contest escalation, which is a unique example of such use of chemical signals in terrestrial animals.

Session/symposium: Intraspecific relationships/Complementary or predominant role of nonchemical based communication and orientation of insects

ORAL PRESENTATION Suckling D.M.

# Behavioural disruption for suppression presents different challenges in different insect orders

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Habituation and adaptation, or desensitisation, was first described after pre-exposure of male moths to congeneric female moth sex pheromone in the 1960s, and this led to mating disruption being tested against tens of pest species. Today, competitive attraction is argued as the prevailing mechanistic factor based on codling moth and a few other species, but most of the many multiple species mating disruption blends commercialised as blend combinations appear unlikely to be attractive to one or more target pest, through behaviourally active industrial isomeric impurities or antagonists included for related pests, making this mechanism unlikely. On the other hand, behavioral disruption is relatively rare outside the Lepidoptera, with fewer than 30 species of Coleoptera, Diptera, Hemiptera and Hymenotpera recorded. In contrast, experiments show lack of sex pheromone habituation in a mealybug and a gall midge and no trail pheromone habituation in Argentine ants after pre-exposure. A lack of habituation makes competitive attraction essential for mating disruption, and shifts the probability for success towards high stakes competitive attraction for male removal outside the Lepidoptera. Other considerations such as delayed and multiple mating need to be investigated also.

Keywords: habituation, mating disruption, competitive attraction, sex pheromone

Session/symposium: Practical applications/Comparison of semiochemical control methods of pest insects

ORAL PRESENTATION

Sun R. 1\*, Gols R. 3, Harvey J. 4, Reichelt M. 1, Vassao D. 1, Gershenzon J. 1, Pandit S. 2

Metabolomics of Brassicaceae plants and their herbivores help explain the costs and benefits of glucosinolate detoxification

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Glucosinolates and their hydrolytic enzymes, the myrosinases, are the characteristic chemical defense system of Brassicaceae plants against herbivorous insects. Although stored in separate compartments, glucosinolates and myrosinases come into contact upon herbivore damage with glucosinolates becoming deglucosylated to produce toxic isothiocyanates. The specialist herbivore Plutella xylostella, the diamondback moth, has evolved the detoxification enzyme glucosinolate sulfatase (GSS), which rapidly converts glucosinolates to their corresponding desulfated forms that are unable to form isothiocyanates. GSS seems to be an effective adaptation to feeding on glucosinolate-myrosinase plants, but the actual costs and benefits of this trait have not been investigated. We used plant-mediated RNAi to silence the genes encoding GSS in genetic backgrounds with and without aliphatic glucosinolates. Comprehensive metabolite profiling of the plants was carried out to insure that there were no unexpected changes. Silencing of GSS gene expression in diamondback moth larvae significantly reduced insect performance. There was a reduction in larval mass, larval survival and adult egg laying upon silencing when larvae fed on plants containing glucosinolates compared to unsilenced controls and dramatic changes in glucosinolate metabolism. However, silencing had no negative or positive effects on diamondback moth performance when larvae were fed on plants without glucosinolates. Turning to the next trophic level, silencing of GSS benefited larvae by causing poorer performance of a natural enemy, the specialist hymenopteran endoparasitoid, Diadegma semiclausum. Thus the levels of GSS in the diamondback moth may be determined by a balance of costs (both physiological and ecological) and benefits.

Session/symposium: Interspecific relationships/Metabolomics approaches in the Brassicaceae

ORAL PRESENTATION

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Svatoš A.

## Omics in chemical ecology: a gateway to synthetic ecology?

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Chemical ecology used diverse analytical methods for answering particular questions on the chemical base of communication or chemical defense in past. For example a combination of inset's antennae for picking up a chromatographic peak of specific activity leads to identifications of more than 600 pheromones. But by ignoring rest of the chemical diversity we lose a large portion of information on biosynthetic precursors, catabolic products etc.

Omics methods provide a global overview of genetic, proteomic or chemical diversity of studied species and with the rapid technological progress in NGS, proteomic and metabolomics instrumentation and software annotation we can start using the omics as a tool to understand chemical communications in global overview.

I will present several examples of our studies using omic technology and discuss how to get to synthetic ecology in near future.

Session/symposium: New chemical structures/Omics in chemical ecology

KEYNOTE LECTURE

Svatoš A.<sup>1,2</sup>\*, Fandino R.A.<sup>1</sup>, Lorenz S.<sup>1</sup>, Hupfer Y.<sup>1</sup>, Buček A.<sup>2</sup>, Pichová I.<sup>2</sup> *Manduca sexta* sex pheromone biosynthesis: desaturase point mutations and CRISPR/Cas8 genes editing

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Tobacco hornworm moth *Manduca sexta* uses complex pheromone blend of different fatty aldehydes, originating from unique mono-, di- and tri-unsaturated fatty acid (3UFA) precursors, is interesting species for study molecular basis of sex pheromone diversification and the role of fatty acid desaturases (FADs) in the shift of pheromone composition. Previously [1], we have identified the amino acid residue 224 (aa224) functioning as desaturase specificity determinant in tobacco hornworm mFADs MsexD2 and MsexD3.

Here, by constructing a set of MsexD2 and MsexD3 mutants with glycine, alanine, valine, isoleucine, phenylalanine, and threonine, respectively at position 224 and by determining the mutant mFAD substrate specificity, regio- and stereoselectivity we provide experimental evidence that mFAD enzymatic selectivities can be fine-tuned by the volume of aa224 side chain. Lower volumes of aa224 side chain (Ala, Thr) drives the desaturase specificity towards Z11-desaturation of 16:0 to Z11-16 and towards conjugation of Z11-16:1 to E10,E/Z12-16:2 whereas larger volumes of aa224 (Val, Ile) drives the specificity towards E/Z14-desaturation of the diunsaturated precursors to triunsaturated products.

Beside the closelly related MsexD2 and MsexD3 desaturases additional MsexD5 is potentially involved in the biosynthetic pathway. For detailed understanding of involvement of individual desaturases we are now performing CRISPR/Cas8 genes editing. So far we were able to delete MsexD2 gene and block the pheromone precursors biosynthesis completely.

The obtained results will be discussed from sex pheromone evolution point of view and how a single point mutation could dramatically change the pheromone blends in Lepidoptera.

[1] Bucek et al. (2015) PNAS, 112: 12586-12591.

Session/symposium: Intraspecific relationships/Importance of multicomponent mixtures for chemical cues

**ORAL PRESENTATION** 

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## Syed Z.

## Molecular and physiological correlates of peripheral olfactory modulation

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Olfaction in fruit flies plays a key role in locating food sources, mates, identifying suitable substrates for laying eggs, and avoiding harm. While the olfactory behaviors are robust, there is an increasing evidence for plasticity. An Asian fruit fly, *Drosophila suzukii*, offers an exciting model system to study olfactory plasticity during an adult's life wherein a saprophytic gravid female seeks out fresh fruits to lay eggs. Both males and females of this fly are otherwise saprophytic, as are most other members of drosophilids; however, females encounter a very different olfactory landscape as they seek out appropriate substrate to lay eggs. A chemical-analytical analysis of odor profiles constituting saprophytic and fresh-fruit landscapes revealed significant qualitative and quantitative variations in the odorants. Additionally, olfactory physiological measurements will be presented demonstrating the accompanying peripheral modulations in the antennal detection of gravid and non-gravid females. Finally, a detailed analysis of olfactory transcriptome demonstrated a significant modulation of critical olfactory receptors at the gravid or non-gravid stage. A comprehensive review of peripheral olfaction and its modulation will be presented based on our extensive chemo-analytical, physiological and molecular analyses.

Session/symposium: Interspecific relationships/Arthropod chemoreceptors
ORAL PRESENTATION

Szanyi Sz.<sup>1</sup>\*, Szarukán I.<sup>1,2</sup>, Tóth M.<sup>3</sup>, Varga Z.<sup>1</sup>, Nagy A.<sup>2</sup>

# Semisynthetic lure for baiting wide range of Noctuid moths (Lepidoptera: Noctuoidea)

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Noctuoidea, one of the most species rich Lepidoptera groups in the Palearctic region, has great importance for both plant protection and nature conservation. Monitoring and detection of these pest species are routinely performed by traps baited with sex pheromones, while in faunistical surveys light traps are widely used.

The attractivity of different synthetic lures (e.g. phenylacetaldehyde, eugenol, isoamylalcohol) for Noctuid and other Lepidoptera has being known for a long time, but most of them are not effective enough for practical use.

We tested the effect of the addition of vine extract or vine as a natural component on the attractivity of the synthetic lure of isoamyl-alcohol and acetic acid and made comparisons between this semisynthetic lure and light trap catches. The studies were carried out with CSALOMON® VARL+ traps in 10 sites of east Hungary and West Ukraine.

We found that the addition of the natural component increased the efficiency of the lure with respect to both numbers of individuals caught and the number of species. The semisynthetic lure attracted 64-155 Noctuoidea species per site belonging mainly to the Xyleninae, Noctuinae and Hadeninae subfamilies and there were several species that were sampled only with this lure. The species number depended on the biogeographical situation and landscape structure.

Based on our results the semisynthetic lure developed can be used for parallel monitoring of Noctuid pests and for complementing the light trap method in faunistical studies.

Acknowledgements – Szabolcs Szanyi was supported Hungarian Eötvös Scholarship (MÁEÖ2017\_30/156926)

Session/symposium: Intraspecific relationships/independent presentation

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Szentesi Á.

## A local perspective on Tibor Jermy's contributions to insect-plant evolutionary interactions

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Tibor Jermy's sequential evolution theory (SET) is an alternative to the presently predominant coevolutionary theories (CET). SET is an evolutionary process, during which plants evolve independently of insects, and the latter follow changes of plants, sometimes with considerable delay, without noticeably affecting plant evolution.

In a lecture dated as early as 1971, and presented in Wageningen (The Netherlands), Jermy set the stage for the SET. The criticism he aimed at the CET rooted in his life-long experience with numerous herbivorous insect species, in recognition of the importance of negative chemosensory information in feeding and oviposition, in the importance of botanical distribution of deterrent substances, and in insect behaviour as the primary interface of the interaction. The Colorado potato beetle was an especially rewarding research subject in this time to obtain insight on food specialization.

The premises of SET are partly answers to assumptions of CET, and partly arguments of his own based on experience collected over years with herbivorous insect species. Culminating in a book with co-workers in 1998, the arguments for SET were more and more elaborated by accepting criticism from colleagues and incorporating developments of the field.

For the lack of a larger impact of SET on insect-plant evolutionary research I see three important points. First, there is an elementary refusal of SET without evaluating its merits, due to being a heresy against the prevailing theory. Second, palaeobotanists do not consider insects driving the evolution of angiosperms , whereas CET associates the angiosperm diversification to herbivorous insects. Third, in contrast to the numerous mathematical models of CET, SET possesses none. They would be important in formulating predictions and providing testable hypotheses.

Session/symposium: Interspecific relationships/Tibor Jermy's Legacy in insect-plant evolution

KEYNOTE LECTURE

Szyszka P. 1\*, Egea-Weiss A. 1, Sehdev A. 1, Mohammed Y. 1, Renner A. 2 Odor source separation and rapid odor coding in insects

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In recent years, it has become evident that olfaction is a fast sense, and millisecond short differences in stimulus onsets are used by animals to analyze their olfactory environment. In contrast, olfactory receptor neurons are thought to be relatively slow and temporally imprecise. These observations have led to a conundrum: How, then, can an animal resolve fast stimulus dynamics and smell with high temporal acuity? Using parallel recordings from olfactory receptor neurons in *Drosophila*, we found hitherto unknown fast and temporally precise odorant-evoked spike responses, with first spike latencies (time between odorant arrival and first spike) as short as 3 ms and with a standard deviation (jitter) well below 1 ms. These data provide new upper bounds for the speed of olfactory processing and suggest that the insect olfactory system can use the precise spike timing for olfactory coding and computation, which could explain insects' rapid processing of temporal stimuli when encountering turbulent odor plumes.

Session/symposium: Intraspecific relationships/Importance of multicomponent mixtures for chemical cues

ORAL PRESENTATION

Tallon A.\*, Hill S., Ignell R.

Sex and age modulate the expression of antennal chemosensory-related genes linked to the onset of host seeking in the yellow-fever mosquito *Aedes aegypti* 

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The mosquito Aedes aegypti is the primary urban vector for several infectious diseases such as yellow fever, dengue, chikungunya and most recently Zika virus in most tropical regions of the world, where billions of people live in areas at risk for epidemic transmission. In mosquitoes, host-seeking, a behaviour that is primarily odour mediated, is a crucial trait to vectorial capacity since disease transmission heavily relies on the ability of females to host seek and successfully feed on the blood of infected humans. A better understanding of alterations of host seeking behaviour and associated changes in pathogen transmission, will enhance the development of novel control strategies against vector borne diseases. However, the correlation between individual tuning of chemoreceptors and host-seeking behaviours remains largely unknown in Ae. aegypti. Our work investigates changes in abundance levels of chemosensory receptors according to sex and age in order to identify promising candidates that may be responsible for the regulation of antennal sensitivity in mosquitoes. In the present study, we show that 1 day post-emergence (dpe) females are significantly repelled by human odour while 3 and 5 dpe are attracted. A quantitative single-end sequencing of antennal libraries revealed that the modulation of chemoreceptor expression in young Ae. aegypti mosquitoes is sexually dimorphic and age-dependent. The largest increase in expression levels of all the chemosensory genes identified is shown to occur between 1 and 3 dpe coinciding with the increase of host-seeking behaviour observed in females. We identified relevant and trustworthy receptor families to be further investigated as potential molecular targets of both age- and sexually-specific odour-driven behaviours in newlyemerged Ae. aegypti mosquitoes particularly relevant in disease transmission and future potential application in disease vector control.

Session/symposium: New chemical structures/Omics in chemical ecology

Tan C.W.\*, Peiffer M., Felton G.

### Symbiotic polydnavirus of a parasitoid manipulates caterpillar and plant immunity

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Plants possess diverse defense mechanisms against herbivores that include defensive proteins and secondary metabolites that impair herbivore growth and survival. However, the interaction between plant and insect is considerably more complex because other trophic levels are involved in nature. Microorganisms are abundant in the environment which can impact the interaction in many ways. Braconidae parasitoids possess obligate mutualistic viruses called polydnaviruses (PDVs). PDVs are injected by parasitoids with their eggs into host caterpillars, which suppress caterpillar immune responses and metabolism, thus allowing parasitoid eggs to hatch and develop. Our study showed that the PDV of parasitoid, Microplitis croceipes, is the main factor down regulating host caterpillar, Helicoverpa zea, salivary glucose oxidase (GOX) gene expression and enzyme activity. GOX is an important caterpillar oral cue that triggers plant defense responses. The reduced GOX activity in parasitized caterpillar caused lower defense responses in tomato compare with nonparasitized caterpillar treatment. Parasitoid perform better when parasitized caterpillars fed on lower defended (parasitized caterpillar treated) plant. This study provides evidence that parasitoid PDV indirectly attenuate plant defense responses which significantly benefit parasitoid performance.

Session/symposium: *Interspecific relationships/Insect-microbe interactions*ORAL PRESENTATION

Tebayashi S.<sup>1</sup>\*, Sumita H.<sup>1</sup>, Oikawa A.<sup>2</sup>, Maseda H.<sup>3</sup>, Ishihara A.<sup>4</sup>

Induced resistance on the root of rice plant by plant hormones against root aphid.

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Induced resistance against pathogens and pest insects has been investigated in many plant species, and research on its application to crop protection has also been conducted. This resistance is caused by accumulation of secondary metabolites, expression of PR proteins and other processes. An exogenous treatment with various chemical regent including plant hormones can induce the resistances as well as an attacking by pathogens and pest insects. Among them, effect of application of jasmonate and salicylate, which were known as hormones related to plant resistance, were well investigated, however resistances induced by abscisic acid (ABA) or auxin (indole-3-acetic acid: IAA) against insect pest was a few reported. Here, we report induced resistance on root of rice plant against rice root aphid by ABA and IAA.

Reproduction of rice root aphid, *Rhopalosiphum rufiabdominalis*, on the root of rice seedling treated with ABA or IAA solution were evaluated. An inhibition of aphid growth was observed on the root treated with both hormones at the concentration of more than 0.1 mM solution since 4day after treatment. HPLC analysis of ABA treatment root revealed an increase of serotonin which strongly inhibits aphid reproduction. Thus, it was concluded that exogenous ABA treatment induced the resistance caused by accumulation of serotonin against an aphid. On the other hand, HPLC analysis of IAA treatment root revealed an inducible accumulation of a compound, which was putative identified as glucosylindol-3-carboxyrate by LC-MS analysis of the peak. Therefore, ABA and IAA can be used for plant protection, although further studies for biological effect of the compounds would be needed.

Session/symposium: Intraspecific relationships/independent presentation

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Temirbekova S.K.<sup>1</sup>\*, Afanasyeva Y.V.<sup>2</sup>, Metlina G.V.<sup>3</sup>, Vasilchenko S.A.<sup>3</sup> **Protection oilcrops safflower from diseases and weeds** 

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The results of long-term studies of the biological, morphological and phenological features of the introduced new culture of safflower in the Central, Volga and North Caucasus regions are presented. Optimum parameters of depth of seeding (5-6 cm), seeding rates (12-14 kg/ha), ensuring high productivity, oil content and quality of seeds are established. For the first time, the relationship between moisture availability of vegetation periods with accumulation of oil content and a change in the fatty acid composition was established. Oilseed (in untreated seeds) in the regions was from 14.5 to 31.2%, in excessively wet 2013 - 6.4% in the Moscow region and 8.6% near Saratov. Fatty acid composition revealed a high content of oleic acid in Krasa Stupinskaya variety – 13.6-16.8%, linoleic acid – 68.5-75.7%. The yield of oil in the Moscow region was 240 kg/ha. The yield of Krasa Stupinskaya in the Moscow Region was 0.6 t/ha, the Rostov Region 0.8 t/ha and Saratov Region 1.2 t/ha, with an average weight of 1000 seeds, respectively, by regions: 40.0 g, 47.3 g and 40.9 g. It has been established that excessive moistening during the flowering and seed filling period increases the harmfulness of enzyme-mycosis seed depletion (EMIS) - biological injury during maturation (enzymatic stage), followed by the seeding of the seeds with the phytopathogen Alternaria carthami Chowdhury. In the protection of safflower against harmful organisms used seed treatment for 1-3 months before sowing preparation Vincit, KS or Maxim, KS-1.5-2 l/t of seeds. A seedling of safflower does not have the ability to fight weeds. Were treated with pre-emergence soil herbicide is a new generation Dual gold EC of 1.3-1.6 l/ha. In a production environment used only a post-emergent drug Harmony-6-8 g/ha at a rate of 200-300 liters of water. Drugs stop the growth of monocotyledonous and dicotyledonous weeds, which gradually wither and die. Drugs do not affect the accumulation of oil and protein content.

Session/symposium: New chemical structures/independent presentation

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Features of cultivation of oilseeds – safflower in contrasting soil and climatic conditions of Russia

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The results of long-term studies of the biological, morphological and phenological features of the introduced new culture of safflower in the Central, Volga and North Caucasus regions are presented. Optimum parameters of depth of seeding (5-6 cm), seeding rates (300-350 thousand pieces/hectare or 12-14 kg), ensuring high productivity, oil content and quality of seeds are established. For the first time, the relationship between moisture availability of vegetation periods with accumulation of oil content and a change in the fatty acid composition was established. Oilseed (untreated seeds) in the regions was from 14,5 to 31,2%, in excessively wet 2013 - 6,4% in the Moscow region and 8,6% in the Saratov region. Fatty acid composition revealed a high content of oleic acid in Krasa Stupinskaya variety – 13,6-16,8%, linoleic acid – 68,5-75,7%. The yield of oil in the Moscow region was 240 kg/ha. The yield of Krasa Stupinskaya in the Moscow Region was 0,6 t/ha, the Rostov Region 0,8 t/ha and Saratov Region 1,2 t/ha, with an average weight of 1000 seeds, respectively, by regions: 40,0 g, 47,3 g and 40,9 g. The growing season for growing seeds was 105 days in the Moscow Region, 94 days in the Rostov Region and 95 days in the Saratov Region. It has been established that excessive moistening during the flowering and seed filling period increases the harmfulness of enzyme-mycosis seed depletion (EMIS) biological injury during maturation (enzymatic stage), followed by the seeding of the seeds with the phytopathogenic Alternaria carthami Chowdhury. In the breeding programs for productivity and oil content, it is recommended to use the varieties Moldir (Kazakhstan) and Krasa Stupinskaya (FGBNU VSTISP), the fatty acid composition of which is characterized by an increased content of oleic and linoleic acid.

Session/symposium: Practical applications/independent presentation

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Thöming G.\*, Knudsen G.K.

A semiochemical attractant for common green lacewings combined with floral buffer stripes to enhance biological control of aphids in barley

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There is a great potential to manage the agricultural landscape to better support the naturally occurring beneficial insects. These natural enemies are important tools in pest management. However, the impact of beneficials is often limited due to the lack of essential resources like food, habitat and hibernation shelter in the crop environment.

Our research focuses on a conservation biological control strategy based on a volatile blend attracting the common green lacewing *Chrysoperla carnae* s.l. to enhance biological control of aphids in cereals. Toth et al. (2006, 2009) and Koczor et al. (2015) have developed a ternary attractant for lacewings consisting of methyl salicylate, phenylacetaldehyde and acetic acid, which attracted lacewings and increased the egg laying. Based on these findings, we developed a CBC strategy resting on three approaches: (1) applying the attractant in the crop to attract lacewings and direct the egg laying, (2) establishing floral buffer stripes to enhance food and habitat sources for adult lacewings and, (3) applying the blend in hibernation chambers to increase the overwintering survival of adult lacewings. The overall aim of this strategy is the establishment of a robust lacewing population in the agroecosystem to keep aphid infestation in cereals below damage threshold.

In a field study, we were able to support the previous findings on the ternary blend, but we are also able to show that the increased oviposition and increased overwintering survival greatly increases the biological control of the two main aphid pests in barley (*Sitobion avenae, Rhopalosiphum padi*). Flower stripes have shown an additional positive effect on lacewing population in the fields and on the biological control of aphids.

Koczor S. et al. (2015) J Appl Entomol 139: 201-206 Tóth M. et al. (2006) Eur J Entomol 103: 267-271 Tóth M. et al. (2009) J Chem Ecol 35: 449-458

Session/symposium: Interspecific relationships/Semiochemistry of aphidophagous insects
ORAL PRESENTATION

#### Tolasch T.

#### Click beetle pheromones - more than pest control

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The click beetles (Elateridae) are a family with almost 10.000 described species, approximately 170 of them occurring in Central Europe. Click beetles are easily recognizable by their typical elongated habitus and their ability to "click", to turn themselves over, when lying on their back; by flipping in the air with a clearly audible clicking sound. The larvae – known as wireworms - develop occasionally predacious in decaying wood or phytophagous in the soil, were single species reach pest status. On the other hand, many click beetle species are red-listed today due to modern forestry and agriculture. Click beetle pheromones are female-produced and often stored in a paired gland like organ in the abdominal tip, and they are highly attractive to conspecific males. For a long time, sex pheromones have only been known for a few agriculturally important Agriotes species, where the females produce one or two geranyl- and/or (E,E)-farnesyl esters of fatty acids with 2 to 8 carbon atoms. During recent years, we identified sex pheromones of various "non pest" click beetles, and they turned out to be valuable tools in integrative taxonomy: The structural properties fit the higher taxonomic ranks in many cases, i.e. species of the same genus, tribe or subfamily often show similar pheromone structures. It has been possible to identify and resolve mixtures of cryptic species, not only by using their pheromones' structural features as additional characteristics, but also to gain males of questionable species in higher numbers in the field. Other "species", which were believed to be distinct, could be unmasked as mere colour forms the same way. Finally, for entomofaunistic purposes, the use of synthetic click beetle pheromones provides an excellent monitoring tool for rare and threatened species, especially in places, where they are considered extinct, but perhaps still exist below the limit of detection offered by conventional collecting methods.

Session/symposium: Intraspecific relationships/Chemical ecology of click beetles (Elateridae): practical applications and advances in the field.

KEYNOTE LECTURE

Toshova T.B. <sup>1</sup>\*, Tóth M. <sup>2</sup>, Furlan L. <sup>3</sup>, Vuts J. <sup>4</sup>, Velchev D.I. <sup>5</sup>, Subchev M. <sup>1</sup>

Detection and seasonal monitoring of *Agriotes* species (Coleoptera: Elateridae) in different vegetation habitats in Bulgaria by pheromone traps

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Larvae of the genus Agriotes are important soil pests damaging a wide range of cultivated plant species. Synthetic sex pheromones have been used by traps to study the abundance and swarming periods of click beetle species in Bulgaria in different vegetation types since 2000. Pheromone lures for the following species were used: A. brevis, A. lineatus, A. litigiosus, A. obscurus, A. sputator, A. rufipalpis/ sordidus and A. ustulatus. All target species were captured in the course of the study excepting A. sordidus (absent in Bulgaria) and A. litigiosus (doubtful presentation in Bulgaria). In addition to them, the following species were also recorded – A. acuminatus, A. gurgistanus, A. proximus, Agrypnus murinus, Athous hilfi, Athous sp., Cidnopus minutus, C. pilosus, Drasterius bimaculatus and Hemicrepidius sp. The highest numbers of click beetles were caught in maize crops while the lowest numbers were recorded in tobacco field. A. ustulatus and A. sputator were common in all plant habitats studied. In meadow, the most numerous species were A. lineatus and A. brevis. A. rufipalpis, A. proximus and A. ustulatus were the dominant species in maize, potato and tobacco, respectively. The swarming periods of the most abundant species were: A. acuminatus: early May - late June, A. brevis: early April - early October, A. gurgistanus: middle of June middle of July, A. lineatus: late April - middle of August, A. proximus: late April - middle of July, A. rufipalpis: late April - middle of September, A. sputaror: late March - end of August and A. ustulatus: early June – end of August.

Session/symposium: Intraspecific relationships/Chemical ecology of click beetles (Elateridae): practical applications and advances in the field.

ORAL PRESENTATION

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# Grandisol – aggregation attractant for *Bothynoderes affinis* Schrank (Curculionidae: Lixinae) and a possible attractant for other Lixinae species

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In field tests conducted in Bulgaria and Hungary, CSALOMON® TAL traps (PPI CAR HAS, Budapest, Hungary) baited with grandisol captured both sexes of *Bothynoderes affinis*, indicating that this compound functions as an aggregation attractant. Dose-response experiments showed that catches of *B. affinis* adults were positively associated with attractant dose. In a subsequent experiment studying the effect of trap colour (white, blue, yellow, fluorescent yellow and transparent) highest number of adults was recorded in transparent and yellow baited traps, and the mean catches in these traps were significantly higher than these in the control (transparent unbaited) traps. In addition to the target species, 16 other Lixinae species were recorded during the field experiments, demonstrating for the first time the possible role of grandisol in chemical communication systems of some of these species. TAL traps baited with grandisol might be useful tool for detecting and seasonal monitoring of *B. affinis* adults as well as for surveying Lixinae diversity in different biotopes.

Session/symposium: Practical applications/independent presentation

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# Toxin replenishment following experimental depletion of toxin reserves in *Bufo bufo* tadpoles

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Possessing toxins can contribute to an efficient defence against various threats in nature, yet we generally know little about the energy- and time-demands of toxicity. This is surprising as the time required to become protected against re-occurring predator attacks can substantially influence the efficiency of inducible chemical defence and its trade-off with other risk-induced phenotypic responses. In this study we examined how submergence into norepinephrine solution, administration of a mild stress mimicking predator attack and simple handling during the experimental procedure affects the amount and number of bufadienolides compared to control individuals in common toad (Bufo bufo) tadpoles, and investigated how fast animals can replenish their toxin reserves over time. We found that the total bufadienolide quantity (TBQ) of tadpoles significantly decreased in the norepinephrine treatment group immediately after treatment compared to the control, but not in the handling and stress treatment groups. This difference, however, disappeared after 12 hours, and there were no consistent differences in TBQ between treatments at subsequent sampling times. Interestingly, the amount of some bufadienolide compounds characterized by lower or higher mass signal values responded to the norepinephrine treatment in an opposite way: approximately half of the compounds characterized by >700 m/z values showed the same pattern as TBQ, but several bufadienolides characterized by <500 m/z values had higher quantities immediately after treatment. The number of bufadienolide compounds was not affected by any of the applied treatments, but was positively related to TBQ. Our study represents the first experimental evidence that common toad tadpoles can replenish their depleted toxin reserves within a short period of time, and provide some additional insights about the physiological background of inducible chemical defence in a vertebrate model species.

Session/symposium: Interspecific relationships/Ecology and evolution of toxins in vertebrate animals

**ORAL PRESENTATION** 

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Tu K.Y.<sup>1</sup>, Wu D.R.<sup>2</sup>, Tsai S.F.<sup>1</sup>, Guo T.W.<sup>1</sup>, Lin H.<sup>1</sup>, Yang Z.W.<sup>3</sup>, Liao C.T.<sup>4</sup>, Chuang W.P.<sup>1</sup>\* Dissecting the interaction between *Cnaphalocrocis medinalis* and its host plant under environmental effect

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Atmospheric temperature increases along with increasing atmospheric CO<sub>2</sub> concentration is a major concern for agroecosystems. Although the impact of an elevated temperature or increased CO<sub>2</sub> has been widely reported, there are few studies investigating the combined effect of these two environmental factors on plant-insect interactions. In this study, plant responses (phenological traits, defensive enzyme activity, secondary compounds, defenserelated gene expression and phytohormone) of Cnaphalocrocis medinalis (Guenée) susceptible and resistant rice under various conditions (environment, soil type, variety, C. medinalis infestation) were used to examine the rice- C. medinalis interaction. The results showed that leaf chlorophyll content and trichome density in rice were variety-dependent and were not affected by other factors. Plant defensive enzyme activity was affected environment, variety, or C. medinalis infestation. In addition, total phenolic content of rice leaves was affected by environment and C. medinalis infestation. Defense- related gene expression was affected by environment, soil type, or C. medinalis infestation. Plant phytohormones (ABA, JA, SA) were affected by C. medinalis infestation. Furthermore, under elevated CO<sub>2</sub> and temperature, rice plants had higher ABA content than plants under ambient conditions. The adult morphological traits of C. medinalis also were affected by environment. Under elevated CO<sub>2</sub> and temperature, C. medinalis adults had greater body length in the second and third generations. Taken together these results indicated that elevated CO<sub>2</sub> and temperature not only affects plants but also the specialized insects that feed on them.

Session/symposium: Interspecific relationships/independent presentation

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Targeting insect pests in greenhouses by recruiting specified native parasitoid wasps with herbivory-induced plant volatiles (HIPVs)

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To control target insect pests biologically in greenhouses, we studied the possible use of herbivory-induced plant volatiles (HIPVs) that specifically attracted the native natural enemies of insect pests. We focused on a tritrophic system involving cruciferous crops, diamondback moth (DBM; *Plutella xylostella*) larvae, and their specialist parasitoid wasps, *Cotesia vestalis*. We set dispensers of the attractant (DBM-HIPVs) together with feeders for *C. vestalis* in commercial greenhouses (treated greenhouses), and periodically observed the number of DBM and *C. vestalis*. In two years' experimental periods, the DBM occurrence rate (the proportion of greenhouses with at least one detected DBM per month) in the treated greenhouses was significantly lower than that in the control ones. Further, the *C. vestalis* occurrence rate (the proportion of greenhouses with at least one detected *C. vestalis* per month) was significantly higher than the DBM occurrence rate in the treated greenhouses, but they were not significantly different in the control greenhouses in either year. Based on these data together with our previously reported data, we will discuss the use of the attractant for biological control DBM in greenhouses.

Session/symposium: Practical applications/independent presentation

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### Bark beetle pheromones are produced by their associated symbiotic fungi

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The ruling theory is that bark beetles produce pheromones to aggregate on host trees. Thereby overpowering the defense of their host trees. These pheromones have been identified from the head space over the beetles and the compounds have been found physiologically active in electroanntennography. The pheromones attracked the beetles to traps so it was concluded that the identified compounds are pheromones, i.e chemical signals between individuals of the same species.

We have studied the fungi that are associated with the bark beetles that they bring into the trees and that generally help to kill the host trees by stopping the flow of resin that constitute the main defense of the trees.

Here we show that pheromonal compounds were consistently produced as the major metabolite by the symbiotic fungus when cultured on malt agar or spruce bark-containing agar. We found that not only is it the same pheromone compound but also the exact same enantiomer (stereoisomer) as the beetles react to that the fungi produces. We also report of other Scolytidae pheromones that are produced by their symbiotic fungi. Our results demonstrate that an apparent co-evolution of bark beetles have occurred so that the bark-beetle-associated fungi have evolved to produce exactly the same stereo-isomers of complicated acetal pheromones. The results rise a question; can it be examples of mistaken pheromone identification? That fungi on or inside the beetles actually is the organism that produces the compounds? These questions are not addressed in this presentation. We just present the striking finding that almost all of the the bark beetle-associated fungi investigated, produced pheromonal compound in high amounts.

Session/symposium: Interspecific relationships/Insect-microbe interactions
ORAL PRESENTATION

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Changes in the chemical defence of common toad (Bufo bufo) tadpoles experiencing complex environmental challenges

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Organisms living in heterogeneous environments face a plethora of selective factors, against which multiple responses may be adaptive. On the other hand, when multiple factors influence the expression of a single trait, disentangling the effect of single factors may be problematic. The chemical defence of amphibians may be such a trait, being selected to provide protection from various enemies including predators and competitors. In previous experiments we found that tadpoles of the common toad (Bufo bufo) increase their bufadienolide toxin production when exposed to limited food and increased density, however, a similar response to predator cues could only be demonstrated when tadpoles were raised alone. Here we test the hypothesis that the response to conspecific competitors makes a further adjustment of chemical defence to predators either unnecessary or physiologically impossible. To explicitly test how the presence of competitors and of chemical cues on predation risk interact in shaping toxin production, we raised toad tadpoles at low, medium and high densities and in the presence or absence of predator cues originating from European perches (Perca fluviatilis) in an outdoor microcosm experiment. Toxin composition and quantity was analysed using HPLC-DAD-ESI-MS. Our results confirm that both predator presence and conspecific competition influence toxin production of toad tadpoles, but in a complex way. Our study is the first to demonstrate that vertebrates are able to adjust their chemical defence to simultaneous challenges similar to many other life-history traits.

Session/symposium: Interspecific relationships/Ecology and evolution of toxins in vertebrate animals

**ORAL PRESENTATION** 

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Investigation of cellular heterogeneity in phytoplankton communities with comparative metabolomics and high resolution mass spectrometry imaging

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Phytoplankton communities are composed of diverse unicellular, autotrophic eukaryotes. During the spring and autumn seasons, these algal cells thrive and divide by millions to form blooms in marine and freshwater environments that fuel the aquatic food web. Despite the global importance of these algae our understanding of bloom development and termination is still in its infancy. Here we address how chemically mediated interactions contribute to the balancing of complex marine plankton communities. We developed analytical techniques to study cellular heterogeneity at the single cell level and link the observed variability to chemical interactions with flagellate parasites such as chytrids and oomycetes that often infect algal cells. These parasites are suggested to be involved in the bloom termination, but resistance of certain algae is observed.

In this context, we developed a new approach using laser desorption-ionization coupled to high-resolution mass spectrometry (LDI-HRMS) to profile the metabolome of live algal cells and hence investigate the heterogeneity in algal populations at the single-cell level. This lead to a reliable monitoring of the effects of nutrient depletion and cell ageing on the cellular metabolome. We could also link metabolic fluctuations to parasitic infection on the algal cell in a pathosystem model and highlight the functional role of algicidal molecules in a bioassay. Structural identification of the chemical mediators was performed with tandem mass spectrometry and bioinformatics tools. With this approach, we demonstrated the role of infochemicals in the control of phytoplankton bloom and we are now able to address the cellular heterogeneity encountered in algal population under stress. This will serve as a basis to characterize the species diversity and potential parasites outbreaks in wild phytoplankton blooms.

Session/symposium: *New chemical structures/Omics in chemical ecology* KEYNOTE LECTURE

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Defending a fortress under siege: optimizing glucosinolate allocation upon root and shoot herbivory in *Brassica* species

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Glucosinolates (GSLs) are a diverse class of well-studied defence compounds in Brassica species. They are constitutively produced, but are also induced locally and systemically by above- and belowground herbivory. We found that both constitutive and induced glucosinolate allocation patterns follow optimal defence theory (ODT) predictions ([1, 2]); constitutive and root-induced GSL levels are the highest in the most valuable and vulnerable organs. Molecular analyses showed that, next to biosynthesis, GSL transporter genes (GTR)[3] contribute to the allocation of GSLs upon root herbivory. When both root and shoot herbivores are feeding simultaneously, signaling pathways involved in induced responses can interact [4]. We hypothesized that aboveground-belowground interactions differ depending on the host specialization of the aboveground herbivore. We analyzed phytohormone levels and marker gene expression, together with measurements of GSLs, GTRs and GSL biosynthesis genes in B. rapa plants challenged with cabbage root fly and either one of four aboveground herbivore species (two specialists, two generalists). In addition, we assessed herbivore performance on plants with and without root fly feeding. Based on the results we concluded that the interaction between root and shoot herbivores is reflected on all defence levels. However, the interaction and its effect on the aboveground herbivore is species specific, rather than depending on specialization level.

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- [2] Tsunoda T. et al. (2018) Functional Ecology in press
- [3] Nour-Eldin H.H et al. (2012) Nature 488: 531-534.
- [4] Papadopoulou, G.V., van Dam, N.M. (2017). Ecological Research 32: 13-26.

Session/symposium: Interspecific relationships/Metabolomics approaches in the Brassicaceae

**ORAL PRESENTATION** 

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van Herk W.\*, Vernon B.

Trap-based estimates of click beetle populations can vary depending on duration of trap placement

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In areas where wireworms are causing extensive damage to field crops (e.g. in Prince Edward Island, Canada), pheromone traps are used to determine both the size of click beetle populations and when to apply control measures. For trap-based estimates to be reliable, however, it is important to know how representative the catches are of the actual beetle population present. Since many pest click beetle species disperse primarily by walking, it is possible that keeping a pheromone trap in a permanent location causes the population immediately around it to be depleted. This would lead to underestimations of the actual population in the field. In this talk we demonstrate that this can occur, but that it varies among species.

Session/symposium: Intraspecific relationships/Chemical ecology of click beetles (Elateridae): practical applications and advances in the field.

ORAL PRESENTATION

van Herk W., Vernon B.

## Development of a pheromone-based IPM program to manage *Agriotes* spp. click beetles in Canada

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Three European species of *Agriotes* click beetles have become established in eastern and western Canada and are now serious pests of potato and other field crops. Managing, and assessing the risk of economic damage from these species remains a challenge. In this talk we describe an efficient pheromone trap-based monitoring program that can be used to assess the risk of wireworm injury in fields and guide management decisions. Areas of fields that have consistently high populations and serve as reservoirs from which adjacent areas are infested can be targeted for adult beetle control using mating disruption, mass trapping, and/or other management approaches. Initially developed for *A. sputator*, the main wireworm pest species in eastern Canada (Prince Edward Island), this monitoring program is now also used for *A. obscurus* and *A. lineatus* in British Columbia. With the identification and development of pheromones for other pest species, this approach can be extended to other pest wireworm species in Canada and elsewhere.

Session/symposium: Intraspecific relationships/Chemical ecology of click beetles (Elateridae): practical applications and advances in the field.

ORAL PRESENTATION

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# Chemicals produced by males and transferred to females during mating prevent multiple matings

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Fire ant (*Solenopsis invicta*) female sexuals usually mate only once, even though female fire ant sexuals fly into a population-wide male lek 150 m in the air. We review the possible mechanisms, then discuss recent work that identifies male-specific compounds produced in the male reproductive system that elicits repellent activity to males in an olfactometer bioassay. We further show that males have control over when these compounds are released during the mating process. This ensures maximal repellent effects on other males attemping to mate with an already inseminated female sexual.

Session/symposium: Intraspecific relationships/Importance of multicomponent mixtures for chemical cues

**ORAL PRESENTATION** 

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Enhancing structural diversity in terpenoid biosynthesis: enzymes, substrates and cofactors

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The remarkable chemical diversity of terepnoids can be attributed to the combinatorial biosyn-thetic chemistry that starts from prenyldiphosphate precursors. Besides the numerous single product enzymes from all type of organisms, the recently discovered multiproduct terpenoid cyclases add another structural dimension, as they convert single prenyldiphos-phates into a multitude of structurally defined, often even enantio-merically pure products.

For example, incubation of the MtTPS5 enzyme from Medicago truncatula with (E,E)-FDP provides 27 different sesquiterpenes, all of them chiral and with high optical purity. Furthermore, this enzyme not only accepts (E,E)-FDP as a substrate, but also converts (E,Z)-FDP into a series of new chiral products. Other terpenoid synthases from corn plants show a clear prefer-ence and higher turnover with either E- or Z-isomers. Apart from substrate geometry, variations in specific assay conditions such as assay pH and metal cofactors additionally control the structural diversification. Metal cofactors (Co2+, Mn2+, Mg2+) may control the chain length of prenyldiphosphates in terpenoid biosynthesis, but also the product composition of multiproduct terpenoid cyclases. Novel X-ray data for a Co(II)/Mg(II)dependent terpenoid synthase from leaf beetles will be presented. Sometimes even a change in the pH affects the product distribution. In consequence, simple alterations in the cellular environment provide the organism (e.g. plant, insect or microorganism) with an enhanced structural plasticity without the need for investing into long-term evolutionary modifications. This ver-satility to ad hoc modulate product diversity on demand grants the producing organisms with access to an enhanced defensive repertoire by simply altering cofactors, pH level and substrate geometry.

Session/symposium: Intraspecific relationships/independent presentation ORAL PRESENTATION

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### Design of a multi-purpose pheromone trap for pest elaterids in Canada

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A number of pheromone-based trap designs have been developed or adopted for collecting various Elaterid pest species (e.g. *Agriotes* spp.) in North America, Europe and Asia. These traps have been used variously for: a) field-specific and national delimitation surveys; b) general scientific studies; c) monitoring click beetles spatially and temporally in IPM programs; and d) mass trapping and/or mating disruption. Some of these traps perform well in addressing some of these objectives, but under-perform in others. Here we describe the development of a new trap design for three exotic European wireworm species invasive to Canada (*Agriotes lineatus*, *A. obscurus* and *A. sputator*) that performs well for all objectives listed, taking into account: trap catch efficacy; ease of use in the field (assembly; installation and inspection); cost; and other variables (e.g. durability; consistency; non-target catch; water-proofing; field transportability).

Session/symposium: Practical applications/independent presentation

Vidkjaer N.H.\*, Kryger P., Fomsgaard I.S.

### Can nature's pharmacy influence the health of honey bees?

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Declining honey bee (hereafter bee) populations are receiving increasing attention especially because bees are important pollinators of our food crops. The underlying cause of the decline is hypothesized to be multifactorial and bees face many stressors including pathogens, xenobiotics, and changes in floral resources.

Multitudes of bioactive plant secondary metabolites (PSMs) – widely utilized in human medicine – are present in the bees' pollen/nectar diet. Some PSMs have reduced the levels of deformed wing virus [1] in bees [1] whereas others are known inducers of their detoxification- and immune system [2,3] but the influence of PSMs on bee health remain largely unexplored. Detailed studies of the impact of PSMs on bee health are thus essential and PSM uptake/metabolization requires clarification since limited knowledge presently exists.

In a controlled feeding experiment, bees were exposed to different PSMs including alkaloids, cyanogenic glycosides, iridoid glycosides and diterpenoids. A global overview of the bees' response to individual PSMs was attained using a combination of untargeted GCTOF-MS metabolomics focusing on effects on the bees' primary metabolism and qPCR targeting expression of genes related to the detoxification- and immune system. Uptake/metabolization of selected PSMs were investigated with LC-MS using both targeted and untargeted approaches. The results of this experiment establish the foundation for a larger field experiment focusing on the implication of PSMs in bee disease resistance.

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Session/symposium: Interspecific relationships/independent presentation ORAL PRESENTATION

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### Phylogenetic escalation and divergence of plant defense and their effects on insect herbivores

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Escalation (macroevolutionary increase) or divergence (disparity between relatives) in trait values are two outcomes of the plant-herbivore arms race. We examined the impacts of evolution of host-plant defences on communities in a case study on *Ficus* and discuss broader patterns across large tropical genera. We studied the defenses and caterpillars associated with 21 sympatric New Guinean figs. The evolution of proteases was conserved, alkaloid diversity has escalated across the studied species, polyphenol oxidative activity has escalated within one clade, and trichomes have diverged across the phylogeny. Herbivore specificity was correlated with their response to host defenses: escalating traits largely affected generalists and divergent traits specialists; but the effect of escalating traits on extreme specialists was positive. As such, the evolution of defenses in *Ficus* seems to be directed towards both escalation and divergence in individual traits, in combination providing protection against a broad spectrum of herbivores and increasing chemical diversity of the hosts on the whole.

The diversification of host plant defenses is, in turn, likely to promote the diversity of insect herbivores themselves. The divergence in defences may be especially important driver of insect diversity on a community level as it promotes heterogeneity among closely related host species. Our results across several plant genera illustrate that insect community dissimilarity due to secondary metabolite disparity between congeneric hosts can be of a similar magnitude as insect community dissimilarity between various plant genera. This is largely due to covariation between chemical traits and host phylogeny, which explained large part of caterpillar community variability between *Ficus*, *Macaranga*, and *Syzygium*. Such processes, rather than a tight coevolution, may promote global diversity of insects.

Session/symposium: Interspecific relationships/Tibor Jermy's Legacy in insect-plant evolution

**ORAL PRESENTATION** 

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### Exploring the molecular basis for species-specific ascaroside signaling in nematodes

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Recent research has shown that nematode development and behavior are regulated by ascarosides, glycolipids of the 3,6-dideoxysugar L-ascarylose linked to fatty acid derived side chains. Ascaroside signaling is highly conserved and represents a key modulator in nematode chemical ecology.

We have previously shown that the diversity of basic ascaroside signals originates from the co-option of a primary metabolic pathway, the peroxisomal  $\beta$ -oxidation of fatty acids, to generate various homologous aglycone series. Here we present highly sensitive mass spectrometric (MS) screens that facilitate the comprehensive analysis of known as well as yet unidentified ascaroside components in crude nematode exo-metabolomes. Using a phylometabolomic approach a large diversity of novel structures were detected. Dominating components were enriched by chromatography and their structures identified by one and two-dimensional nuclear magnetic resonance (NMR) spectroscopy. Structure assignments were subsequently confirmed by total synthesis of representative examples.

Our results demonstrate that several basic ascarosides are highly conserved in a large number of closely related nematode species. Furthermore, we identified various species-specific modifications, such as hydroxylation of ascaroside aglycones, epimerization, or the attachment of additional building blocks from diverse primary metabolic pathways to the ascaroside core structure. Taken together, these species-specific modifications downstream of the  $\beta$ -oxidation cycle generate a highly complex modular library, which forms the molecular basis of nematode communication.

Session/symposium: New chemical structures/independent presentation ORAL PRESENTATION

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### Enhancement of click beetle surveillance using female attractants

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Integrated pest management of economically important click beetle species (Coleoptera: Elateridae) affecting, among others, maize, wheat and potato crops in Western and Central Europe relies significantly on the deployment of pheromone-baited traps for the detection and monitoring of adult male populations. However, male trap catches must usually be interpreted in terms of the behaviour of the females, thus adding to the complexity of that interpretation.

As female click beetles directly determine the size of larval populations, traps that are capable of capturing females could provide more reliable data on the timing of oviposition, and thereby contribute to more precise control decisions against larvae. The development of plant-based attractive blends has recently been reported for *Agriotes ustulatus* and *A. brevis*, and research is underway in other pest species. Furthermore, the discovery in *A. brevis* and *A. sordidus* that female-emitted pheromones can also be perceived by emitters opens up new avenues in the development of monitoring tools targeting females.

The first demonstration of trapping females with pheromone-baited traps is now complemented by studies on the interaction between pheromones and plant volatiles. Such research has already revealed in *A. brevis* that these two chemical communication channels influence each other in a way that results in enhanced trap catches. Ongoing work on *A. ustulatus* is yielding similarly promising results.

Session/symposium: Intraspecific relationships/Chemical ecology of click beetles (Elateridae): practical applications and advances in the field.

ORAL PRESENTATION

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Expression of odorant receptors in the antennae of male and female *Rhagoletis* fruit flies.

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RNA-Sequencing methdology has been used to generate transcriptomes from antennae of male and female *Rhagoletis indifferens* (Western Cherry Fruit Fly) and *Rhagoletis pomonella* (Apple Maggot Fly). Qualitative and quantitative analyses have been conducted on the odorant receptors (ORs) expressed in the antennae of these two species. Phylogentic context across species provide evolutionary insights towards the functional capacities of olfactory detection in these species. Differential expression patterns of ORs in male and female antennae suggest underlying mechanisms for sexually dimorphic olfactory-based behaviours. Function Characterization of these ORs may lead to identification of novel ecologically relevant odorant ligands, with implications towards sustainable pest control strategies.

Session/symposium: Interspecific relationships/Arthropod chemoreceptors
ORAL PRESENTATION

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### "Push and pull" experiment for the spruce bark beetle in Sweden

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Bark beetles are integral components in forest ecosystems. Several bark beetles may cause extensive tree mortality through larvae attacks and their symbiotic blue stain fungi, both blocking the flow of water and nutrient. The beetle normally attacks injured, old and storm felled Norway spruce (*Picea abies*) but during epidemic outbreaks, they can cause stand level mortality. In 2011 and 2013 major storm events in central Sweden created high volumes of dead wood and was followed by dry and warm summers. Afterwards, there has been large episodic outbreaks of bark beetle Ips typographus and the beetle is today a serious pest in the Swedish forestry. The area in central Sweden still have large populations of bark beetles that continue to kill large amount of standing spruce forest.

Since 2008 the use of pheromone-baited trap logs is used to reduce beetle-related tree mortality. The pheromone is an aggregation pheromone that attract other individuals of the beetle. The pheromone traps attract, "pull", the beetles to the trap logs. The logs are then removed from the forest before the new generation of beetles fly off. This strategy has been evolved for a long time. Now we want to combine this with semiochemicals that repel, "push" the insect away from exposed and sensitive spruce forests. The semiochemicals is chemical volatile components derived from unsuitable and non-host tree species.

Our research is to merged these two strategies into a "push and pull" strategy which push the beetles out of the spruce forest by using semiochemicals and pull the beetles in to pheromone-baited trap logs outside the forest edge.

The experiment will be set up during May 2018 and some preliminary results will be showed in August.

Session/symposium: Practical applications/independent presentation

Wallin E. 1\*, Rahmani R. 1, Viklund L. 1, Schroeder M. 2, Hedenström E. 1 *Polygraphus punctifrons* (Coloptera, Curculinidae) in northern Sweden

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During recent years there have been several storms in northern Sweden which has lead to outbreaks of relatively large populations of *Ips typographus* and *Polygraphus poligraphus*. While investigating *P. poligraphus* a sibling species, *P. punctifrons*, was identified in the same geographical area of Sweden. *Polygraphus punctifrons* has previously been reported in northern Europe and Russia. In 2015 our group reported on volatiles emitted by *P. poligraphus* and similar methods to evaluate the emitted volatiles of *P. punctifrons* was applied. Solid phase micro extraction (SPME) was utilized to collect volatile organic compounds from insects feeding on spruce logs. Volatiles from males and females was compared with emission from the logs and structures of volatiles was elucidated by means of GC-MS. Male produced compounds was tested in field trials to monitor flight seasons and to monitor populations sizes.

Session/symposium: New chemical structures/Invasions under the bark: chemical ecology of woodboring beetles

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A receptor-neuron correlate for the detection of attractive plant volatiles in *Helicoverpa* assulta (Lepidoptera: Noctuidae)

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Plant volatiles are vital cues in the location of hosts for feeding and oviposition for Lepidoptera moths. The noctuid *Helicoverpa assulta* is a typical polyphagous moth, regarded as a good model for studying the olfactory reception of plant volatiles. In this study, four full-length genes encoding odorant receptors HassOR24, HassOR40, HassOR41, and HassOR55 expressed in antenna in *H. assulta* were functionally characterized. The highly expressed HassOR40 was narrowly tuned to a few structurally-related plant volatiles: geranyl acetate, geraniol and nerolidol. By systematically analyzing responses of single neuron in both trichoid sensilla and basiconic sensilla using single sensillum recording, the specific neuron B in one type of short trichoid sensilla was found to be mainly activated by the same chemicals as HassOR40 with high sensitivity, and with no significant difference between male and female neurons. Thus, a clear "receptor-neuron" relationship in H. assulta was demonstrated here, suggesting that HassOR40/HassOrco are expressed in neuron B of short trichoid sensilla. The active tobacco volatile nerolidol, recognized by this receptor-neuron line, elicits significant behavioral attraction of both sexes in H. assulta adults. The results indicate that we identified a receptor-neuron route for the peripheral coding of a behaviorally relevant host volatile in H. assulta.

Session/symposium: Interspecific relationships/Arthropod chemoreceptors
ORAL PRESENTATION

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

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The Asian longhorned beetle (ALB) Anoplophora glabripennis (Motschulsky) and citrus longhorned beetle (CLB) A. chinensis (Forster) are native to Asia and East-Asia respectively. These two dangerous pests in forestry are polyphagous xylophage and have been introduced to North America and Europe, causing considerable economic and ecological losses. These affinis species share many semiochemicals, and we found both beetles ecological niche are same in some provinces of China, for example damaging willow and elm simultaneously. Therefore, based on olfactory accurate host tree and pheromone sense are of utmost importance for fitness and reproductive isolation. Through antenna transcriptome analysis of A. glabripennis and A. chinensis, we identified 42 odorant-binding proteins (OBPs), 12 chemosensory proteins (CSPs), 14 pheromone-degrading enzymes (PDEs), 1 odorantdegrading enzymes (ODE), 37 odorant receptors (ORs), 11 gustatory receptors (GRs), 2 sensory neuron membrane proteins (SNMPs), and 4 ionotropic receptor (IR) in A. glabripennis; and identified 46 OBPs, 16 CSPs, 44 ORs, 19 GRs, 23IRs and 3SNMPs in A. Chinensis. Expression profile of all studied CSPs and PBPs in both species showed all expressed in antenna. Vast majority of CSPs of both beetles were highly expressed in multiple chemosensory tissues, suggesting their participation in olfactory recognition in almost all olfactory tissues. Intriguingly, the AglaPBP2 and AchiPBP2 was preferentially expressed in antenna, indicating that it is the main protein involved in efficient and sensitive pheromone recognition. This study establishes a foundation for determining the chemoreception molecular mechanisms of A. glabripennis and A. chinensis, which will also provide a new perspective for controlling longhorned beetle populations through olfactory interference.

Session/symposium: Intraspecific relationships/Complementary or predominant role of non-chemical based communication and orientation of insects

Wang M.Q.

Structural transformation detection contributes to screening of behaviorally active compounds: dynamic binding process analysis of DhelOBP21 from *Dastarcus helophoroides* 

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In light of reverse chemical ecology, the fluorescence competitive binding assays of functional odorant binding proteins (OBPs) is a recent advanced approach for screening behaviorally active compounds of insects. In this study, only (+)-β-pinene proved attractive to unmated adult beetles. The compound (+)-β-pinene and DhelOBP21 formed a stable complex through a secondary structural transformation of DhelOBP21. On the other hand, camphor could not efficiently induce a stable structural transformation. The other compound,  $\beta$ -caryophyllene, only collided with DhelOBP21 and could not be positioned in the binding pocket. Studying structural transformation of these proteins through examining the dynamic binding process rather than using approaches that just measure binding affinities such as fluorescence competitive binding assays can provide a more efficient and reliable approach for screening behaviorally active compounds. The structural transformation of OBPs was investigated using well-established approaches for studying binding processes, such as fluorescent quenching assays, circular dichroism, and molecular dynamics. The dynamic binding process revealed that the flexibility of DhelOBP21 seems conducive to binding specific ligands, as opposed to broad substrate binding. The compound  $(+)-\beta$ -pinene and DhelOBP21 formed a stable complex through a secondary structural transformation of DhelOBP21, in which its aminoterminus transformed from random coil to an α-helix to cover the binding pocket. On the other hand, camphor could not efficiently induce a stable structural transformation, and its high binding affinities were due to strong hydrogenbonding, compromising the structure of the protein. The other compound,  $\beta$ -caryophyllene, only collided with DhelOBP21 and could not be positioned in the binding pocket.

Session/symposium: *Practical applications/Semiochemical application for invasive species*ORAL PRESENTATION

Weber D.C.<sup>1\*</sup>, Wallingford A.K.<sup>1,2</sup>, Morrison III.W.R.<sup>3,4</sup>, Leskey T.C.<sup>4</sup>, Khrimian A.<sup>1</sup> Invasive stink bugs: semiochemical patterns of field-based attraction, cross-attraction, synergy, and application

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Stink bug importance as pests has increased in recent years because of the replacement of broad-spectrum insecticides with more selective suppression tactics for control of other key pests, such as the deployment of Bt-transgenic traits in field crops, use of mating disruption and insect growth regulators in control of tree fruit pests, and Lepidoptera- and Homoptera-selective control tactics used in vegetable and fruit crops.

All species of stink bugs for which pheromones have been identified (about 45) have male-produced pheromones which attract females, and in many cases also males and nymphs. Of these, approximately half are sex pheromones (attracting females only), and half are aggregation pheromones (attracting both sexes and, where tested, nymphs). These pheromones are currently used mainly for monitoring only a few important species.

In this presentation, we review identifications and syntheses of several pheromones, particularly sesquiterpenoids, and discuss their current and potential practical applications, including prospects for attract-and-kill or related tactics for suppression of pest bugs. Attraction to other stink bug pheromones (cross-attraction), as well as to plant volatiles, is widespread, and important for some species.

Expanded use of stink bug pheromones and other attractants in pest management in the future will depend on careful assessment and mitigation of vicinity effects on pest populations and damage, based on bug behavior resulting in spillover and/or halo effects from attractant sources. Successful implementation will require reliable and economically viable integration into grower crop management. We present two examples from the USA: brown marmorated stink bug (*Halyomorpha halys*) in apples and harlequin bug (*Murgantia histrionica*) in cole crops.

Session/symposium: Practical applications/Semiochemical application for invasive species
ORAL PRESENTATION

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Shoot herbivory on tomato affects the root metabolome and resistance to root knot nematode

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In the past, aerial plant parts like leaves and flowers have been the main focus of research in plant herbivore interactions. In recent years the belowground compartment has gained much more attention and thus as well the interactions of soil dwelling organisms and plant roots as well. Unfortunately both compartments, aboveground and belowground, have been rarely examined together. Here we present an untargeted ecometabolomics study that connects aboveground herbivory to belowground plant resistance to nematodes. We show that in tomato (*Solanum lycopersicum*) aboveground feeding by the tobacco hornworm (*Manduca sexta*) induces changes in the root metabolome. Furthermore shoot herbivore feeding enhances the plant's resistance to the southern root knot nematode (*Meloidogyne incognita*). We will present some of the root metabolites that are changing as a consequence of aboveground herbivory and discuss their potential role in nematode resistance.

Session/symposium: *New chemical structures/Omics in chemical ecology*ORAL PRESENTATION

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Weinstein A.M.<sup>1</sup>\*, Bohman B.<sup>1,2,3</sup>, Phillips R.D.<sup>1,4</sup>, Flematti G.R.<sup>3</sup>, Peakall R.<sup>1</sup> Chemical clues help resolve the Warty Hammer Orchid complex

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Sexually deceptive orchids effect pollination by luring male insect pollinators to flowers through visual and chemical mimicry of conspecific females. Due to the high specificity of insect sex pheromones, each species of orchid typically attracts a single pollinator species. We investigated the pollination ecology and chemistry of the sexually deceptive Drakaea livida complex (Orchidaceae), which attracts multiple pollinators across its geographic range. Floral baiting trials revealed three morphologically indistinguishable ecotypes, each of which attracts a single different species of thynnine wasp pollinator. Multivariate analysis of GC-MS data revealed three discrete chemotypes that each correlated with the species of pollinator attracted. Compounds that are electrophysiologically active to the ecotype-specific pollinator species could be used to accurately discriminate between the ecotypes. The specific alkyl- and hydroxymethylpyrazines that are electrophysiologically active to the ecotype one and two pollinators respectively occurred exclusively in flowers attracting these pollinators. We confirmed 4-hydroxy-3(methylthio)benzaldehyde (methylthio)benzene-1,4-diol to be electrophysiologically active to Zaspilothynnus dilatatus, and to occur exclusively in flowers attracting this pollinator. In an interesting case of convergence, these methylthiophenol compounds have also been found to function as sexual lures in a distantly-related spider orchid. The morphological crypsis of the D. livida ecotypes impedes their effective identification, and thus prevents any potential conservation efforts. In the D. livida complex, using floral chemistry as a taxonomic trait offers an effective solution in delimiting cryptic taxa. This methodology may be applicable to resolving both other cryptic sexually deceptive orchid taxa, as well as cryptic taxa in other systems based on chemical attraction.

Session/symposium: Interspecific relationships/independent presentation ORAL PRESENTATION

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Integration of olfactory and visual cues for detection of low density populations of sweetpotato weevil, *Cylas formicarius* (Fabricius)

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Sweetpotato is one of the most important staple crops in the world, and the sweetpotato weevil, Cylas formicarius (Fabricius), is the major pest of sweetpotato in most areas of cultivation. The sweetpotato weevil feeds primarily on the storage root, where its feeding and oviposition induce the production of extremely bitter-tasting and toxic sesquiterpenes which can render the roots unfit for consumption. A significant step towards improved management of this pest was the identification and commercial availability of a female-produced sex pheromone [(Z)-3-dodecenyl (E)-2-butenoate] to which males are highly attracted. However, other sensory modalities, such as vision, are also important in host finding. Previous field studies in areas with relatively high C. formicarius densities report a nearly 5-fold increase in male catch in traps baited with this pheromone and a green light-emitting diode versus traps baited only with the pheromone. Thus, the combination of olfactory and visual cues significantly enhanced trap effectiveness for this nocturnal species. Furthermore, other studies suggest that certain plant volatiles are also attractive to the sweetpotato weevil. The present study evaluated the effect of pheromone, green LED, and a plant volatile on sweetpotato weevil attraction in areas with relatively low weevil densities. Our results suggest that multimodal cues may provide improved C. formicarius detection and management over a wide range of weevil densities.

Session/symposium: Practical applications/Natural products for integrated pest management

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13-Tetradecenyl acetate, a female-produced sex pheromone component of *Melanotus communis* (Gyllenhal) (Coleoptera: Elateridae)

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The corn wireworm, Melanotus communis (Gyllenhal), is an important crop pest in much of the central and eastern USA, where it attacks corn, small grains, sugarcane, and tuber, root, and vegetable crops. The corn wireworm also occurs in most major corn growing regions of the world, and is on the EPPO A1 action list of quarantine pests. In an effort to increase opportunities for pest control, we conducted a study to identify the sex pheromone of this species by analysis of extracts with coupled gas chromatography-mass spectrometry (GC-MS), and gas chromatography with electroantennographic detection (GC-EAD). GC-MS analysis of the volatiles from crushed abdomens of females gave two pheromone candidates: 13-tetradecenyl acetate and tetradecyl acetate. We also considered two additional unsaturated 14 carbon acetates, (E)-11-tetradecenyl acetate, and (E)-11,13-tetradecadienyl acetate, as pheromone candidates due to their previously reported attractiveness to North American Melanotus spp., and structural similarities to sex pheromones found in Japanese Melanotus spp., respectively. Two of these compounds, 13-tetradecenyl acetate and (E)-11,13-tetradecadienyl acetate, elicited consistent responses from antennae of male M. communis in GC-EAD trials. In a 2-year field evaluation of the 14 carbon acetates, traps baited with 13-tetradecenyl acetate captured more than 50% of the male beetles trapped, whereas traps baited with tetradecyl acetate captured less than 1% of the male beetles trapped. Binary mixtures (1:1 and 4:1 13-tetradecenyl acetate:tetradecyl acetate) of these two compounds gave intermediate catches, as did a quaternary mixture (1:1:1:1) of all four compounds. The identification of a highly attractive sex pheromone for M. communis will enable new strategies for monitoring and management of this pest.

Session/symposium: Intraspecific relationships/Chemical ecology of click beetles (Elateridae): practical applications and advances in the field.

ORAL PRESENTATION

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Semiochemical regulation of interactions between *Tomicus minor* and *Tomicus yunnanensis* during the shoot-feeding phase

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The pine shoot beetles, Tomicus minor and Tomicus yunnanensis, are the most destructive pests of *Pinus yunnanensis* in southwestern China. We investigated pheromone communication within and between these species during shoot-feeding using walking The volatiles released by beetles were collected by static solid phase microextraction and extracted from hindgut. All chemicals were identified by gas chromatography-mass spectroscopy. Both species were significantly attracted by their own species and sex, and attraction was inhibited by exposure to additional beetles or to hindgut extracts. Female and male T. minor and T. yunnanensis hindguts contained 0.66-1.07, 0.01-0.015, 0.3–1.13, and 0.03–0.04 ng/individual of (-)-trans-verbenol, respectively; interactions with additional beetles increased the release levels to 16.74–292.71 ng/individual in T. minor females. The average amounts of verbenone detected in the hindguts of female and male of T. minor and T. yunnanensis under natural conditions were 0.16, 0.06, 0.03, 0.05 ng/individual, respectively, while that increased to 5.90, 2.43, 0.06, 0.19 ng/individual, respectively after exposure to additional insects. The levels of cis-verbenol and (-)-transverbenol most attractive to walking T. yunnanensis and T. minor from a distance of 19 cm were 0.1 and 1.0 ng/μL, respectively. Addition of verbenone to cis-verbenol or (-)-transverbenol decreased the attraction responses. High concentrations of (-)-trans-verbenol and verbenone are produced to realize intraspecific inhibition, avoid overcrowding and interspecific inhibition.

Session/symposium: New chemical structures/Invasions under the bark: chemical ecology of woodboring beetles

ORAL PRESENTATION

Xia Y.

# Current status of developing sex pheromone and plant volatile as attractants for the Chinese citrus fly

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The Chinese citrus fly, Bactrocera minax (Enderlein) (Diptera: Tephritidae), is a severe citrus pest in China. This univotine species feeds exclusively on fruit of citrus plants. Our three-year study concluded that the commonly-used lures for tephritid fruit flies are either no attractiveness to the species at all (e.g. methyl eugenol and cuelure), or very poor attraction (all food-based lures). Works had been conducted to explore sex pheromone as more potent lure. Overall speaking, these works were primarily early stage lab work, and results were often inconsistent. For instance, a laboratory study suggested that males of B. minaxresponded to gland and/or rectum extracts of females, and to a lesser extent of the same sex. One field study confirmed the observation that female rectum extract had the highest attraction to both males and females, compared to other three commercial lures. However, another field test failed to demonstrate that the female rectum extract attracted males. Several patents claiming as sex pheromones of B. minax have been approved in China, although the efficacy data of these compounds are scanty. These compounds include such as 2, 6-di-tert-butyl-p-cresol (BHT) and undecanol. In terms of work in plant volatile as potential attractant for B. minax, one laboratory study was conducted, suggesting that female adults were attracted to nonanal, citral, limonene and linalool from citrus fruit. However, no field test of these compounds was conducted. More recently, a USDA-APHIS sponsored study is being carried out in the lab and field. An exploratory field test shows a promising result in developing a sex pheromone for trapping both males and females of the pest.

Session/symposium: independent lecture/independent presentation

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# Effects of kin-recognition through water-soluble chemical substances of root-exudation on clonal reproduction of *Kalanchoë daigremontiana*

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Despite many plant species recognize kin or non-kin, our understanding about effects of kin-recognition on reproduction in plants is limited. Moreover, the cue of kin-recognition has not been sufficiently investigated. To examine the effects of the kin-recognition on clonal reproduction, we used Kalanchoë daigremontiana, a common invasive clonal plant. This species asexually produces many plantlets that grow on leaf margins, and these plantlets detach. Therefore, we can easily evaluate the effects of kin-recognition on clonal reproduction. First, we investigated the dry biomass of above-, below-ground to evaluate growth and resource allocation pattern, and plantlets and number of plantlets per plant as clonal reproduction associated with kin and non-kin plants in Kalanchoë daigremontiana. Next, to reveal the effects of root exudates on root allocation patterns associated with kin and non-kin plants, split-root experiment was conducted. As a result, plants developed their root for non-kin individual, but did not for kin-individual. Similar root allocation pattern was found in the sprit-root experiment. Plants competing with a kin individual produced more clonal plantlets than plants competing with a non-kin plant. These our findings provide evidence that kin-recognition through root exudation is adaptive for K. daigremontiana plants. The kin-recognition in clonal populations may play an important role in population growth through its enhancement of reproduction in each individual, especially in introduced regions, because invasive species often have low genetic diversity due to the bottleneck effect. Future studies concerning the relationships between self or kin-recognition and biological invasion are needed. In addition, the analyze of root exudate chemicals may contribute the biological control of invaded K. daigremontiana.

Session/symposium: Intraspecific relationships/independent presentation

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Yan F.\*, Li J., Song D., Tang X., Lu S.

## Chemical and behavioral ecology of virus-vector-plant interactions

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Cucurbit chlorotic yellows virus (CCYV) is a newly reported virus occurring on cucurbit plants and many other plant species. CCYV is transmitted specifically by B and Q biotypes of tobacco whitefly, Bemisia tabaci (Hemiptera: Aleyrodidae), in a semipersistent manner. In this study, we investigated whether or how a semipersistent plant virus manipulates the orientation and feeding behaviors, defense enzyme activities of its whitefly vectors, and change the plant features (including volatile profiles, JA related enzymes, and optical spectrum). (1) Impacts of CCYV on vector whitefly: Orientation behaviors of whiteflies were, to some extent, changed when carrying CCYV, and host choice of virus-carrying whiteflies also depends on plant status (healthy or infected, symptom development) and vector biotypes (B or Q). Activities of protective and detoxifying enzymes in CCYVcarrying whiteflies were changed in various degrees. we used electrical penetration graph (EPG) technique to record feeding behaviors of B. tabaci carrying CCYV. The results showed that CCYV altered feeding behaviors of both biotypes and sexes of B. tabaci with different degrees. (2) Impacts of CCYV on plants: Expression levels of 3 genes in JA pathway, LOX23, PLD and AOS, were regulated in CCYV-infected cucumber plants. The volatile profiles of CCYV-infected plants were different from those of heathy plants. Optical spectrum was measured for CCYV-infected plants, and absorption at 254 nm, the wavelength for whitefly attraction, was closely related to the virus titers in the CCYVinfected plants. From the present results we can conclude that CCYV can change the vector feeding behaviors and host plant features, thereby modify the plant-insect interactions. These modifications may also change the populations of non-vectors, natural enemies and other plant species, and may result in the changes of community structure in agroecosystems.

Session/symposium: Interspecific relationships/Insect-microbe interactions
ORAL PRESENTATION

Yusuf A.<sup>1</sup>\*, Demares F.<sup>1,2</sup>, Pirk C.<sup>1</sup>

Effect of brood pheromone on survival and nutrient intake of African honey bees (*Apis mellifera scutellata*) under controlled conditions

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The influence of pheromones on insect physiology and behaviour has been thoroughly reported for numerous aspects, such as attraction, gland development, aggregation, mate and kin recognition. Brood pheromone (BP) is released by honey bee larvae to indicate their protein requirements to the colony. Although BP is known to modulate pollen and protein consumption, which in turn can affect physiological and morphological parameters, such as hypopharyngeal gland (HPG) development and ovarian activation, few studies have focused on the effect of BP on nutritional balance. In this study, we exposed newly emerged worker bees for 14 d and found that BP exposure increased protein intake during the first few days, with a peak in consumption at day four following exposure. BP exposure decreased survival of caged honey bees, but did not affect either the size of the HPG acini or ovarian activation stage. The uncoupling of the BP releaser effect, facilitated by working under controlled conditions, and the presence of larvae as stimulating cues are discussed.

Session/symposium: Intraspecific relationships/independent presentation ORAL PRESENTATION

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Zainuddin N.\*, Ali J.G.

Getting to the root of plant-parasitic nematode chemotaxis: manipulation of plantnematode interactions

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Nematode olfaction is a critical component of host-seeking behavior and has been extensively studied in our free-living nematodes model organism, Caenorhabditis elegans. Moreover, olfaction has been postulated to play an important role in the host-seeking behavior in parasitic nematodes (Rasmann et al. 2012). However, only a few nematode olfaction mechanisms have been studied in various parasitic nematodes (Perry, 2001). Most of the parasitic nematodes studied were using entomopathogenic nematodes (EPN). Even though EPN are distantly related to plant-parasitic nematodes (PPN), have similarities in host-seeking and host-invasion behavior (Hallem et al., 2011). Thus, investigation on PPN olfaction was investigated by using easily control environment such as in the laboratory, greenhouse or growth chamber because little is known about PPN olfaction in natural ecologically environments condition. Here we investigate the olfactory responses of three diverse species of nematodes (Caenorhabditis elegans, Heterorhabditis bacteriophora and Pratylenchus penetrans) to five different major crops plant roots (carrot, wild carrot, tomato, potato and corn), and one known natural repellent plant host (marigolds). Understanding the differences in nematode chemotaxis will enable us to provide an alternative approach to manipulating of nematode populations. Volatile organic compound (VOC) emitted from the plant roots may be used as a push-pull strategy which ultimately involves manipulation of the nematodes host-seeking behavior in the agricultural fields (Cook et al., 2007). We show that all three nematodes respond differentially to the odor blends emitted by all major crops plant roots as well as to marigold odorants. It is crucial to identify which plant VOCs are the most highly attractive or repellent. This research is the first study comparing such responses across Nematode species and various plant roots.

Session/symposium: Interspecific relationships/independent presentation

Zhang A.\*, Feng Y.

Activity evaluation of attractants for spotted wing drosophila *Drosophila suzukii* in the field

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Drosophila suzukii, popularly known as the spotted wing drosophila (SWD), is an exotic fruit fly from Southeast Asia that was introduced to the United States in 2008. It attacks a wide variety of fruits and has become a devastating pest of soft-skinned fruit crops. Due to the rapid spread of SWD across the United States, fresh fruit markets have a zero tolerance policy regarding D. suzukii infestation. Specific and efficient D. suzukii detection tools are urgently needed so that farmers can deliver timely management interventions to meet market demands. Since SWD is known to be attracted to damaged and rotting fruits, headspace volatiles from fresh and fermented apple juices were collected and analyzed by gas chromatography – mass spectrometry. Special attention was given to the compounds produced and/or enriched during the fermentation process. After performing a series of field tests, we identified a quinary blend, which is more efficient and selective for D. suzukii than the currently standard apple cider vinegar and commercially available SWD lure under field conditions. It will help growers accurately detect D. suzukii adult infestations in orchards, thereby allowing for timely pest management interventions while reducing conventional insecticidal usage to protect our crops, environment, and ecosystem.

Session/symposium: *Practical applications/Semiochemical application for invasive species*ORAL PRESENTATION

Zhang D.D.<sup>1\*</sup>, Wang H.L.<sup>1</sup>, Hou X.<sup>1</sup>, Groot A.<sup>2</sup>, Krieger J.<sup>3</sup>, Löfstedt C.<sup>1</sup>

Male hairpencil polyenes activate receptors located in sensilla housing receptors for female-produced sex pheromones in noctuid moths

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Moth pheromone receptor (PR) genes constitute a specialised subfamily in the olfactory receptor gene family, responsive to the female-produced sex pheromones. From the PR subfamily, four orthologous clusters are expanded in noctuids. Among these, Cluster III includes receptor genes highly conserved in noctuid and geometrid moths. Our recent study showed that ObruOR1 from Cluster III is the receptor for the polyene pheromone in the geometrid moth Operophtera brumata. However, the ligands of Cluster III receptors in noctuid moths remained unknown. In the current study, we investigate the representative noctuid species Agrotis segetum, Heliothis virescens and Spodoptera exigua and found that polyene compounds are present in the male hairpencil extracts. We re-investigated the function of Cluster III receptor genes of these species, AsegOR3, HvirOR11 and SexiOR11 in the Xenopus oocyte recording system and found that they are responsive to the hairpencil polyene of the respective species. Different from the male-biased pheromone receptor genes, the Cluster III receptor genes have similar expression levels in the antennae of both sexes, as indicated by qPCR results from this and previous studies. By fluorescence in situ hybridization and single-sensillum recordings, we confirmed that the receptors for male hairpencil polyenes are co-localised with receptors for the major female-produced sex pheromone component in one type of sensilla trichodea. Taken together, our results provide intriguing insights into how chemical information about conspecifics of the same and opposite sex is received in the peripheral olfactory system of moths. Behavioural assays are needed to further reveal the biological significance of the male hairpencil polyenes.

Session/symposium: Interspecific relationships/Arthropod chemoreceptors
ORAL PRESENTATION

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## Zhang Q.H.

## Food-based fruit fly trap for the consumer market

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The common fruit fly (or vinegar fly), *Drosophila melanogaster* Meigen, is a well-known model organism widely used for biological research in genetics, physiology, microbial pathogenesis, and life history evolution. This fruit fly is also a common nuisance pest in restaurants, grocery stores, fruit markets, canneries, homes, and other locations with fermenting or rotting vegetative or fruit matter.

There are several commercially available disposable fruit fly traps on the markets (instore or online) with different trap colors/shapes and attractants. In the current presentation, I will introduce our newly developed reusable Rescue® Fruit Fly Trap with food-based attractants, and I will present electrophysiological data [GC-EAD and electroretinogram (ERG) recordings], trap design data (shape/color)[1-3G and comparative bioassay results (using cage olfactometer) against various other commercially available fruit fly traps. Our Rescue® Fruit Traps caught 3-20 times more fruit flies than did the competitor traps, and are available in all major the U.S. and Canadian retail stores.

The RESCUE!® Fruit Fly Trap can be placed indoors on a counter, table, windowsill or other stable surface near the fruit fly problem. Each trap comes with a 30-day supply of attractant and is reusable with RESCUE!® Fruit Fly Trap Attractant Refills.

#### References:

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- [2] Zhang Q.-H., Chapin M. (2014) Fruit fly trap entry structure. U.S. Patent # D715,892.
- [3] Zhang Q.-H. et al. (2014). Fruit fly trap. U.S. Patent # D716,907.

Session/symposium: Practical applications/Natural products for integrated pest management

ORAL PRESENTATION

Zhang X. 1,2, Ran W. 1,2, Zhang J. 1,2, Li X. 1,2, Sun X. 1,2\*

Cloning, expression and enzymatic characterization of a cystatin gene involved in herbivore defense in tea plant (Camellia sinensis)

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Plant cystatins play crucial roles in the production of plant defenses against herbivorous insects. In this study, a cDNA clone, designated as CsCPI2, was isolated from the tea plant (*Camellia sinensis* cv. Longjing 43) using a 5'-/3'-RACE extension. We characterized the transcriptional and biochemical capacity of this gene. The full-length sequence of CsCPI2 is 618 bp, encodes 205 amino acid residues and has a deduced molecular weight of 23.07 kDa. Multiple sequence alignments with cysteine protease inhibitors (CPIs) showed that two glycine residues and the LARFAV-like motif were located at the N-terminus, while the reactive site QVVAG and the tryptophan residues were located at the C-terminus in CsCPI2. The fusion protein CsCPI2-MBP was over-expressed in Escherichia coli, and its inhibitory effect on ficin was approximately 1.17 and 3.46 times that on papain and chymopapain, respectively. The mRNA accumulation of CsCPI2 was higher in seeds, roots and flowers than in leaves and stems. Moreover, higher cystatin activity was found in the leaves of tea plants infested with *Myllocerinus aurolineatus* than in unharmed tea plants. Finally, we found CsCPI2 was a competitive inhibitor of the cysteine proteinases in the tea weevil's gut.

Session/symposium: Interspecific relationships/independent presentation

Zhao T. 1,2,3\*, Ganji S. 1, Hammerbacher A. 4, Krokene P. 5, Borg-Karlson A.K. 2, Unelius C.R. 1 Fungal symbionts of the spruce bark beetle catabolize tree defense chemicals and synthesize aggregation pheromones

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Norway spruce (Picea abies) have well-developed defence mechanisms against invaders, including the production of a resin containing terpenes and phenolic compounds. To survive inside this well-protected tree species, spruce bark beetles have evolved several strategies to overcome the tree's defence. The symbiotic relationship with blue-stain fungi, together with effective aggregation phero-mones, is thought to be the key to the beetles' ability to kill healthy trees. However, how fungi exhaust tree defences and contribute to tree-killing is not clear. In this study, five major fungal associates of the spruce bark beetle were incubated on Norway spruce bark and malt agar, or inoculated to living Norway spruce trees. Hereafter we characterized the volatiles present in the headspace of each fungal culture and quantified the phenolic compounds in live spruce bark. Fungal associates of spruce bark beetles produced a large array of metabolites including bark beetle aggregation pheromones and other semiochemicals. In addition, the fungi also significantly decreased the concentrations of defence chemicals in spruce bark. The virulent fungi metabolized the tree defence terpenes and phenolics more efficient than weak fungi. These results offer evidences that fungi associated with spruce bark beetles may contribute to the aggregation behavior and facilitate tree colonization by the spruce bark beetle. Furthermore, it provides new insights into the possible ecological roles of fungal symbionts over three Kingdoms in the ecological system of bark beetles, their symbiotic fungi, and the host plant.

Session/symposium: Interspecific relationships/Insect-microbe interactions

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Zhao X.C.\*, Liu X.L., Ma B.W., Xie G.Y., Chen W.B., Tang Q.B. Adaption of ovipositor sensilla to the egg-deposition patterns in moths

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Egg-laying behaviors of insects are mediated by the sensation of ovipositors, which may also be related to egg-deposition patterns. A variety of egg-deposition patterns might be guided by different ovipositor sensilla. In the present study, we compare the morphology of ovipositor sensilla in three different moth species, the cotton bollworm *Helicoverpa armigera*, the Asian corn borer, *Ostrinia furnacalis*, and the oriental armyworm, *Mythimna separata*. The moth of *H. armigera* deposits eggs singly on the surface of leaves, while *O. furnacalis* and *M. separata* deposit eggs in masses. Furthermore, *O. furnacalis* deposits eggs on the smooth surface of leaves, while *M. separata* in the dry and curled leaf edges. The morphology of the ovipositor sensilla of these three species were observed by using the scanning electronic microscopy. The morphology and distribution of the sensilla are different between species, which may be in relation to their different egg-deposition patterns.

Grand sponsor: National Science Foundation of China (31471830).

Session/symposium: Interspecific relationships/independent presentation

Zhu J.

Discovery and development of natural products for managing blood-sucking insect vectors

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It is well-known that insect bites can cause local or systemic effects that lead to infectious or inflammatory responses in human and animals. Many blood-sucking insects (mosquitoes, ticks, biting flies and bedbugs) can transmit many pathogens including plasmodium (malaria), viruses causing West Nile, Zika, yellow fever, and dengue in human, as well as equine infectious anemia, African swine fever, Lumpy Skin Disease and Rift Valley viruses in animals. Plant derivatives, or botanicals are so-called natural products have been studied widely to develop to insecticides and repellents against arthropods with at least two millennia in ancient China, Egypt, and India. Even in Europe and North America, the practice of using botanicals dates back more than 150 years. The use of repellents has become one of the most efficient ways to prevent disease transmission and the discomfort of insect bites. In this presentation, I will report our recent findings of some repellent compounds from various natural products that present stronger repellency to four different types of insect vectors (mosquitoes, ticks, biting flies and bed bugs). This is also the first time to report that the longevity and effectiveness of these identified repellent compounds are better than the gold standard repellent, DEET. In laboratory bioassays, these compounds repelled biting flies and bed bugs for two weeks after application and ticks for one week. In addition, strong repellency was also found against mosquitoes. Repellency was stronger and with longer residual activity than that of DEET, the most effective and long-lasting repellent currently available commercially. An aqueous starch-based formulation of natural coconut oil fatty acids protected pastured cattle from biting flies up to 96-hours in hot summer, which is the longest from natural repellent products discovered.

Session/symposium: Practical applications/Natural products for integrated pest management

ORAL PRESENTATION

Žunič Kosi A.<sup>1</sup>\*, Stritih Peljhan N.<sup>1</sup>, Zou Y.<sup>2</sup>, McElfresh S.<sup>2</sup>, Millar J.<sup>2</sup>

A male-produced pheromone of *Arhopalus rusticus* may be useful in managing this invasive longhorn beetle

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Forest ecosystems provide numerous services and benefits to society, including providing wood, climate regulation, fresh water supply, and biodiversity, and many others. However, forests face increasing natural and anthropogenic threats, including the invasion of exotic pest species. The wood-boring longhorn beetles (Cerambycidae) are some of the most common and most successful invaders; They are easily transported in wood and wood packaging materials, and over the past few years, invasive longhorn beetles have been detected in new regions worldwide. For example, cerambycids from the genus Arhopalus have been recorded as invaders in many countries, and these may be particularly dangerous because they are likely vectors of the invasive pine wood nematode. Here, we collected volatile chemicals released by males and females of the European species Arhopalus rusticus (L.). Analyses of the resulting headspace extracts revealed the presence of two male-specific compounds, identified as (S)-fuscumol and geranyl-acetone. In field bioassays in Slovenia, traps baited with the synthetic compounds indicated that at least one of them functions as an aggregation-sex pheromone for this species. The identified compounds are common pheromone components from species in the subfamilies Lamiinae and Spondylidinae. The identification of an attractant pheromone for A. rusticus should be of benefit in ongoing monitoring and control efforts in invaded regions and countries.

Session/symposium: New chemical structures/Invasions under the bark: chemical ecology of woodboring beetles

ORAL PRESENTATION

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Züst T.

Novel defenses as an escape from co-adapted herbivores: cardenolides in the Brassicaceae

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Plants express a diverse array of defensive chemicals to fight off enemies. Complex chemical mixtures may interact with more target sites in herbivores, or may harbor more potential for facilitation among compounds to increase toxicity. Despite this potential benefit of chemical diversity, most plants are highly phylogenetically constrained in their chemotype. For example, plants of the Brassicaceae produce diverse mixtures of defensive compounds, but most belong to a single compound class, the glucosinolates. Such phylogenetic conservatism in chemical defense may facilitate specialization of herbivores, as a gain of tolerance to one compound will increase the likelihood of tolerance to related compounds as well.

Plants of the genus *Erysimum* (Brassicaceae) have gained the ability to synthesize toxic cardenolides in addition to evolutionary basal glucosinolates, making them at least partially resistant to several specialist herbivores of the Brassicaceae. Such events of gain-of-function provide unique opportunities for the study of the selective forces that drive phytochemical diversification, as they involve mostly non-adapted herbivores and thus allow us a peek at co-evolutionary processes as they are ongoing. Here I show how a community of herbivores with varying degrees of host specialization responds to the suite of defenses produced by *Erysimum*, and I highlight evolutionary patterns of phytochemical diversification that arose following the gain of the novel defense trait.

Session/symposium: Interspecific relationships/independent presentation

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