

36th Annual Meeting of the  
**International Society of Chemical Ecology**

Hosted virtually from South Africa 5-10 September 2021



**CHEMICAL ECOLOGY AND  
SUSTAINABLE DEVELOPMENT**

**PROGRAMME &  
BOOK OF ABSTRACTS**



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## Welcome Message from ISCE President,

Andrés González Ritzel



Dear colleagues and friends,

Welcome to the 36<sup>th</sup> Annual Meeting of the International Society of Chemical Ecology!

At last, here we are! Ready to enjoy great talks and meet with old and new friends of the great chemical ecology community. First, I want to thank our hosts, Jeremy Allison (Canadian Forest Service), Christian Pirk (University of Pretoria), Francois Roets (Stellenbosch University) and Bernard Slippers (FABI, University of Pretoria) for all your hard work. These have not been easy times to organize an international conference, and you made it happen.

We all wanted to be there with you in the beautiful

Stellenbosch; we postponed the meeting in 2020 hoping for the best, but things were more complicated than expected. Unfortunately, we had no option but to meet virtually, and I am sure we all will give our best to have a great meeting with plenty of good science and personal interactions. We will learn from this virtual experience, as has the entire academic community, and I hope we will make the best of the two worlds in future hybrid meetings, combining the joy of personal interactions with the wider participation possibilities associated with virtuality. For now, let's enjoy a new annual meeting of our treasured ISCE. The Society has remained strong, we have made some progress and we have suffered some losses, but here we are to share, as the meeting theme states, the immense potential of chemical ecology for a more sustainable world.

Andrés González

President of ISCE

## Message from the ISCE 2021 Symposium Organising Committee:

**Dumela - Lotjhani - Bhota - Sawubona - Ndaa - Avuxeni - Hello - Hallo**

A warm welcome to the 36<sup>th</sup> ISCE Meeting in South Africa!

The local organising committee would like to take the opportunity to welcome you to the first ISCE meeting taking place in Africa. The theme of this year's meeting is "Chemical Ecology and Sustainable Development" which links fundamental research with its potential applications. In light of the ongoing pandemic, it is unfortunate that we cannot welcome you in person to Stellenbosch as planned or to workshops at the University of Pretoria prior to or after the meeting. Or that you cannot enjoy the magnificent scenery of the Garden Route, Kruger Park or the Kalahari during spring. Nevertheless, we hope that despite the virtual character of this year's meeting you will find the talks and presentations as well as the resulting exchange of ideas and thoughts more than inspiring. We hope it lays the foundation for new collaborations, strengthens established ones and results in new and exciting links among members of this global family of Chemical ecologists. We hope that at some stage in the not-too-distant future we will be able to welcome you in person to South Africa.

**Jeremy Allison, Bernard Slippers, Francois Roets, Christian Pirk**

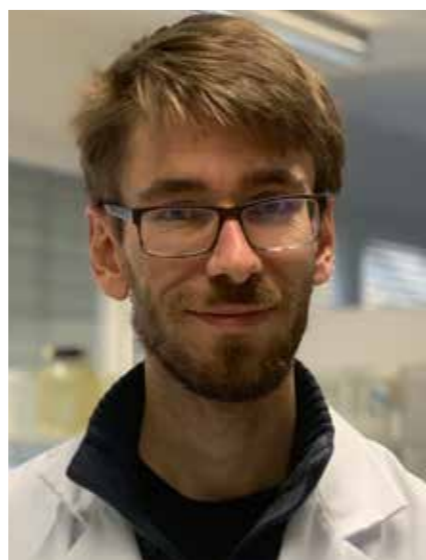
(ISCE 2021 Organizing Committee)

## Tobias Züst

### 2020 Early Career Award

Tobias Züst earned his PhD degree from the University of Zürich in 2012 under the supervision of Dr. Lindsay Turnbull. His thesis focused on the role of 'Aphids as drivers of natural selection on plants' and resulted in several publications, including a highly cited article integrating molecular and chemical ecology, evolution, and biogeography published in *Science*. Following his PhD, he was awarded a 3-year postdoctoral fellowship by the Swiss National Science Foundation (SNSF), which he spent in the lab of Prof. Anurag Agrawal at Cornell University. In 2015, Tobias returned to Switzerland and joined the University of Bern on a competitive SNSF Ambizione fellowship, where he established an independent research program on the role of novel plant defense compounds in plant-insect coevolution. Recently, he was awarded a prestigious ERC Starting Grant and a concurrent SNSF Eccellenza professorship to continue his research at the University of Zürich.

Throughout his career, Tobias has developed a research avenue on understanding the functional role of plant defensive chemicals in plant-herbivore interactions. Driven by this overarching goal, he has worked on a diversity of model systems and developed substantial expertise in analytical chemistry, metabolomics, molecular biology, and statistics, while combining small-scale laboratory approaches with larger-scale experimental and field studies.



### Novel chemical defences as an escape from co-adapted herbivores

University of Zürich / Department of Systematic and Evolutionary Botany

Phytochemical diversity is thought to result from coevolutionary cycles as specialization in herbivores imposes diversifying selection on plant chemical defences. Plants in the speciose genus *Erysimum* (Brassicaceae) produce both ancestral glucosinolates and evolutionarily novel cardenolides as defences. Across- and within-species, we could demonstrate that concentrations, inducibility, and diversity of the two chemical defences varied independently, with no evidence for trade-offs. Closely related, geographically co-occurring species shared similar cardenolide traits, but not glucosinolate traits, likely as a result of specific selective pressures acting on each defence trait. Herbivore feeding is significantly reduced on *Erysimum* plants compared to other Brassicaceae plants, and several herbivores that specialize on Brassicaceae plants are unable to survive on *Erysimum*. For the subset of herbivores that still feed on *Erysimum*, glucosinolates remained the most effective defence against non-adapted herbivores, while cardenolides specifically impaired glucosinolate-resistant herbivores. Ancestral and novel chemical defences in *Erysimum* thus provide complementary rather than redundant functions for the plant, and the dual defence system may be a primary driver of the evolutionary success of this genus.

Keywords: cardenolides; coevolution; glucosinolates; phytochemical diversity; plant-herbivore interactions



## Stefan Schulz

### 2019 Silver Medal Award

Prof Schulz was born in Hamburg, where he studied chemistry and completed his PhD in 1987 under the supervision of Prof Wittko Francke. This was followed by a year in Prof. Meinwald's laboratory at Cornell University. Since then he worked as a researcher in various institutions in Germany before accepting the call of the Technical University Braunschweig as a full professor in 1997, where he is also serving as the head of the Institute of Organic Chemistry. His research revolves around Natural Product Chemistry with a focus on Chemical Communication and Chemical Ecology. His research covers the identification and isolation of compounds, their stereoselective synthesis and analysis, and studies on their biosynthesis in the living organism with the aim is to understand why nature produces such compounds and to find potential uses. Prof Schulz is a long-term member of the ISCE and served on the council, the board and the executive from 1992 to 2014. He was elected vice president of the society in 2012 and president in 2013-14. He also received the ISCE Silver medal in 2020. He received many other prestigious awards during his career and is a Member of St. John's College. Prof Schulz is also very engaged in the promotion of early career scientists and has been awarded the "Joachim Jungius Gesellschaft fuer Wissenschaften zu Hamburg" in recognition of his efforts to support budding scientists during their formative years. He has also significantly contributed to the scientific literature with many outstanding publications

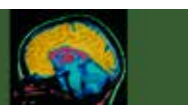


### The Interaction of Chemistry and Chemical Ecology

*Technische Universität Braunschweig, Institute of Organic Chemistry, Hagenring 30, 38106 Braunschweig, Germany*

In this lecture I will focus on three research topics of my group in chemical communication and will discuss old and new results in a comparative manner. The great importance of cooperation and interdisciplinary research will be shown and aspects of future developments discussed. Spider pheromones interested my group from its start and despite on and off during the years some results accumulated that enhance our understanding of spider pheromone communication. In a second topic the Chemistry of chemical signals of butterflies will be discussed starting from danaid butterflies to arrive at *Heliconius*. *Heliconius* serves as model for speciation, evolution and mimicry is currently intensively studied. Finally, the structural diversity of bacterial volatiles will be discussed together with aspects on their identification and function. Recently a multitude of functions have been associated with bacterial volatiles. In all cases chemical methods will be addressed providing the basis of our work including analytical methods such as GC/MS, GC/IR, derivatizations and synthesis. Synthetic material is of major importance to verify structure assignments, verify stereochemistry, and supplies material for testing. The understanding of biosynthetic aspects is further important to understand why a certain compound has evolved to become a chemical signal.

Keywords: Pheromones, Spiders, *Heliconius*, Bacterial volatiles, GC/IR



## Gerhard Gries

### 2020 Silver Medal Lecture

Gerhard Gries is a Professor in the Department of Biological Sciences at Simon Fraser University (SFU). He received his PhD in forest entomology from the Georg-August-Universität in Göttingen (Germany) in 1984. Reaching the rank of Full Professor in 2000, he is currently in the 17th year of an Industrial Research Chair on “Multimodal Animal Communication Ecology” supported by BASF, Scotts Miracle-GRO, and the Natural Sciences and Engineering Research Council of Canada. The Chair’s research embraces most sensory modalities (olfaction, vision, audition, vibration, magneto-reception, infra-red sensing). Gerhard has published 298 peer-reviewed research articles (including >50 with undergraduate students as co-authors), graduated 61 students, has been granted 15 patents, and produced 13 scientific films on insects in collaboration with the Institute of Scientific Film in Germany. He has received over \$11 million dollars of research support as a principal investigator and currently runs a large laboratory with 12 graduate students, 4 Research Associates, and many undergraduate students, often recruited from his Insect Biology class. Gerhard is Fellow both of the Entomological Society of Canada (ESC) and America (ESA) and received multiple (international) awards for his research

[Nan-Yao Su Award for Innovation and Creativity in Entomology (ESA); Gold Medal (ESC); Woodworth Award (with Regine Gries) Pacific Branch (ESA); Recognition Award in Insect Physiology, Biochemistry and Toxicology (ESA)]. His passion for teaching was recognized by SFU in 1994 through an Excellence-in-Teaching-Award and by a Distinction in Student Mentoring Award from the ESA (Pacific Branch) in 2019.



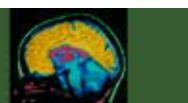
### Thirty Years of Entertainment in Chemical Ecology Research

Departments of Biological Sciences, Simon Fraser University, Burnaby, British Columbia, Canada

Rather than talking about projects from my lab’s research program that required challenging semiochemical identifications (e.g., pheromones of cecidomyiid midges or *Limonius* click beetles), I will present previous and ongoing studies that portray the intricacy, diversity, and thrill of research in chemical ecology. I will talk about (i) sexual communication systems in Norway rats (*Rattus norvegicus*) and house mice (*Mus musculus*), (ii) an eavesdropper’s network involving mice and bumble bees (*Bombus spp.*), (iii) pheromonal communication in false black widow spiders (*Steatota grossa*), revealing new pheromone components, their mechanisms of dissemination, and effects on both males and females, (iv) pheromone-mediated aggregation behaviour in bed bugs (*Cimex lectularius*), and (v) life in the fast lane – sexual communication of a strepsipteran parasite (*Xenos peckii*). Time permitting, I will touch on potential applications arising from some of these projects.

Keywords: Norway rats; eavesdropper’s network; bumble bees; strepsipteran parasite

Fondation Jean-Marie Delwart



## Aleš Svatoš

### 2019 Silverstein-Simeone Lecture

Prof Svatoš was born in Prague (Czech Republic) and graduated from the Prague Institute of Chemical Technology with a PhD in chemistry in 1985. Following his graduation he was a research associate at the Institute of Organic Chemistry and Biochemistry (IOCB) at Czechoslovak Academy of Sciences for five years. This was followed by a 2-year postdoctoral fellowship at Cornell University (Profs Meinwald and Eisner) and 1-year Alexander von Humboldt fellowship in Bonn/Jena (Prof. Boland), before he was appointed as a Senior Research Associate at the IOCB. Since 2002 he is the group leader of the Mass Spectrometry/Proteomics Research Group at the Max-Planck-Institute for Chemical Ecology in Jena, Germany, where he is in charge of the MS laboratory of the institute. The primary objective is to measure mass spectra (MS) for structural elucidation and new compound characterization (molecular composition) in cooperation with the scientific departments of the MPI-CE. In addition to service, which is aimed especially at structure confirmation of synthetic compounds, projects on structure elucidation of natural products and the elucidation of biosynthetic pathways are carried out. New analytical methods are also developed with a focus on new MALDI matrices and small molecule in situ distribution visualization/imaging. Prof Svatoš is also very involved in teaching and training of students, and has been organizing summer schools with topics in mass spectrometry of small molecules and proteomics. He has an outstanding publication record and has significantly contributed to many publications in high-ranking journals.



### Modern mass spectrometry methods for chemical ecology

*Mass spectrometry / Proteomics research group, Max-Planck Institute for Chemical Ecology, Jena, Germany*

Chemical signaling in nature is based on specialized metabolites occurring in minute amounts or produced only in specific context. Structure elucidation of so called semiochemicals was always significant challenge for chemists. Previously, large amounts of individuals were collected from field or reared in laboratory and then semiochemicals extracted and purified. Recent technological advances in mass spectrometry enable analytical chemist determine chemical structures on individual level. Semiochemicals are typically produced in specialized glands and there can be mapped using mass spectrometry imaging methods. It could support ecological observations and confirm the site of semiochemical biosynthesis.

In my lecture I will discuss example from my laboratory and highlighted the importance of using modern spectrometric methods in chemical ecology.

Keywords: analysis, mass spectrometry, metabolomics, identification, imaging

**SPRINGER NATURE**

## John Borden

### *Applied Chemical Ecology Lecture*

John Borden graduated from high school in Vancouver, BC, Canada in 1955, flunked out of pre-med at the University of BC in 1957, was honorably discharged from the United States Marine Corps in 1961, and finally graduated from Washington State University in 1963, with a BSc in entomology. He received an MSc in forest entomology from the University of California, Berkeley in 1965, and a PhD in 1966, both under the supervision of pioneering chemical ecologist David L. Wood. That year, he joined Simon Fraser University in Burnaby, BC, where for 37 years he taught entomology and pest management, supervised 101 graduate students, and received over \$11 million in external grant support. He has published 404 refereed papers, mainly on insect chemical ecology and development of semiochemical-based integrated pest management (IPM) programs for forest, agricultural and urban insect pests. John has also received 11 patents. His work on ambrosia beetles led to the world's longest-running semiochemical-based IPM program (1982-present). In 2003, the day after mandatory retirement, he joined the dark side, becoming Chief Scientific Officer at Contech Enterprises Inc. (then Phero Tech Inc.). Following Contech's bankruptcy in 2015, he got a new job and a welcome demotion on being rescued by Scotts Miracle-Gro. In 2017, he established a sole proprietorship consulting company, JHB Consulting, which now demands his full attention. With industry, he has worked on developing new products for bark beetles, honey bees, yellowjackets, fruit flies, bed bugs, synanthropic flies, ticks, mosquitoes and rodents. John has received many honors and awards, among them the ISCE Silver Medal, and election as Fellow of the Entomological Societies of America and Canada, and the Royal Society of Canada. In 2009 he received an Honorary Doctor of Laws from the University of Northern British Columbia. Still breathing at the age of 83, he finds that work is a great retirement hobby.



### **NEW AWARD OLD STORY:**

#### *The World's Longest-Running Semiochemical-Based IPM Program*

Ambrosia beetles mine in the sapwood of conifer logs and newly sawn lumber in British Columbia, causing annual economic loss in lumber degrade, disrupted manufacturing and export restrictions estimated at \$85-189 million. By 1970 aerial application of insecticides to prevent attack on logs stored in fresh water lakes and salt water inlets was suspended, leading to receptive potential clients for alternative management methods. By that time, research had already begun on aggregation pheromones for the three most important species, leading to the identification of sulcatol for *Gnathotrichus sulcatus*, retusol for *G. retusus* and lineatin for the most important pest, *Trypodendron lineatum*. Replacement of sticky traps with the multiple-funnel (Lindgren) traps met another essential prerequisite. Captures of 2,200,000 *T. lineatum*, 500,000 *G. sulcatus* and 15,000 *G. retusus* at a dryland log sort in 1981 fulfilled the last prerequisite, i.e. that mass trapping could be used to reduce populations and protect logs and lumber from attack. Area-wide integrated pest management (IPM) of ambrosia beetles, with mass trapping as its cornerstone, was implemented in logging sites, log sorts and sawmills in 1982. The IPM program integrates three main tactics: 1) prevention of beetle population build-up at all stages of logging, 2) limitation of beetle transportation to new areas, and 3) removal of populations at every storage site. As in most successful IPM programs, only the third tactic involves semiochemicals, i.e. mass trapping in dryland sorts and sawmill yards. After misadventures in the first year of operation, Pherotech hired Eveline Stokkink in 1983 to run the contract IPM service. This became a family enterprise. Eveline soon moved the service to her own company, and 38 years later at the age of 73 she is now transitioning the business to her daughter Sharlene.





## Paulo Henrique Zarbin

### *2020 Silverstein-Simeone Lecture*

Paulo H. G. Zarbin is a full professor at the Department of Chemistry at Federal University of Paraná/Brazil (UFPR), where he coordinates the Laboratory of Semochemicals. Graduated in chemistry at Federal University of São Carlos/Brazil (1993), obtained his master's (1995) and PhD degrees in Organic Chemistry (1998) at the same institution, having completed part of his doctorate at the National Institute of Sericultural and Entomological Science, in Tsukuba, Japan (1996-1997). He was coordinator of the graduate program in chemistry at UFPR in the 2002-2004 biennium. Member of several scientific societies, he was president of the International Society of Chemical Ecology - ISCE (2011-2012), vice president of the Entomological Society of Brazil - SEB (2010-2012), secretary of the Paraná region of the Brazilian Chemical Society - SBQ (2002-2004) and president of the Latin American Association of Chemical Ecology - ALAEQ (2016-2018). He is currently associate editor of *Scientific Reports* (Nature) and a member of the editorial board of the *Journal of Chemical Ecology* (Springer). Prof. Zarbin has been a CNPq/Brazil productivity fellow since 1999, and his research interest is focused on the structural identification and synthesis of insect pheromones and the chemical ecology of insect-plant interactions.



### **Insect Chemical Ecology: A Tropical Approach**

*Department of Chemistry, Federal University of Parana, 81531-900, Curitiba/PR - Brazil*

In the last 20 years, our laboratory in Brazil has been developing a series of works focusing on the identification and synthesis of pheromones from several families of insects; special emphasis has been given to neotropical agricultural pests belonging to Curculionidae and Pentatomidae.

Extensive work has been done on the pheromone chemistry within these two families. Most pheromones reported in curculionids are produced by males and usually attract both sexes, so they are known as aggregation pheromones. To date, the compounds that make up the pheromones of Curculionidae, basically, correspond to two classes: monoterpenoids (mostly cyclic) and possible fatty acids derivatives (alcohols, ketones, ester, etc).

The Pentatomidae show a high diversity in the chemical structure of the sex pheromones identified so far. However, structural similarities in pheromones have been found for species of the same genus. Many molecules already identified are sesquiterpenes or structures derived from fatty acids containing different amounts of methyl branches.

In this talk, the main results we have achieved on identification of pheromones will be presented and discussed. Highlights will be given to the works that culminated in the identification of new chemical structures. Also, identification of an unexpected and still unpublished class of compounds in curculionids will be presented.

Keywords: Agricultural Pest; Biological Control; Entomopathogenic Nematodes; Experimental Evolution; *Heterorhabditis* spp.; *Photorhabdus* spp.

**SPRINGER NATURE**

## Ricardo Machado

### 2021 Early Career Award

Ricardo Machado did his bachelor studies in Agronomic Engineering at Universidad Nacional de Colombia (Medellin, Colombia) and his master studies in Microbiology at the Friedrich Schiller University of Jena (Jena, Germany). Then, he conducted his PhD at the Max Planck Institute for Chemical Ecology under the supervision of Prof. Matthias Erb and Prof. Ian Baldwin on the role of inducible changes in primary metabolites and stress hormones in plant-herbivore interactions. His PhD research resulted in >10 highly cited peer-reviewed publications. After his PhD, Ricardo successfully transitioned into a Project Leader position within the Research Section Biotic Interactions at the University of Bern (Switzerland) in 2015, where he investigated the molecular and chemical aspects of belowground multitrophic interactions in the context of entomopathogenic nematodes and their symbionts and also in the context of host location and foraging behavior of root herbivores. His discoveries were published in more than 20 peer reviewed papers within 5 years in top-tier interdisciplinary journals such as Nature Biotechnology, PLoS Biology, eLife, and PNAS. In 2020, Ricardo was awarded an Ambizione Fellowship to build his own independent research group in the University of Neuchâtel (Switzerland), where he continues investigating different aspects of the chemical and molecular ecology of terrestrial ecosystems and also the taxonomic and phylogenetic relationships of entomopathogenic nematodes and their symbiotic bacteria



### Engineering bacterial symbionts of nematodes improves their biocontrol potential to counter the western corn rootworm

*Experimental Biology Research Group, Institute of Biology, University of Neuchâtel, Switzerland*


Bacterial symbionts benefit their hosts by providing auxiliary biological functions. Despite their prevalence and importance across the tree of life, the potential to enhance their services through targeted biological engineering remains largely untapped. Here, we use an experimental evolution approach to generate *Photorhabdus* symbionts that resist plant toxins and thereby improve the capacity of their entomopathogenic nematode hosts to control an important agricultural herbivore pest. The efficacy of the nematodes to control the western corn rootworm, one of the most damaging maize pests on the planet, is reduced by benzoxazinoids, which are produced by plants and sequestered by the western corn rootworm for self-defense during feeding. We show that selecting *Photorhabdus* bacteria on benzoxazinoids results in genetic alterations that are associated with enhanced benzoxazinoid resistance. Selected bacteria are more efficient at killing benzoxazinoid-containing western corn rootworm larvae. The evolution of benzoxazinoid resistance leads to a reduction of growth and symbiosis in some strains. However, we identified a *Photorhabdus* strain that is more resistant to benzoxazinoids and does not suffer from any reduction in growth or symbiosis. Reestablishing symbiosis between this bacterial strain and its ancestral nematode host creates an enhanced entomopathogenic nematode strain that is more effective at killing benzoxazinoid-containing western corn rootworm larvae. Hence, this work provides an avenue for the improvement of biological control agents and demonstrates the power of forward evolution for the improvement of bacterial symbionts.

Keywords: Agricultural Pest; Biological Control; Entomopathogenic Nematodes; Experimental Evolution; *Heterorhabditis* spp.; *Photorhabdus* spp.



CHEMICAL ECOLOGY AND  
SUSTAINABLE DEVELOPMENT

## Day 1: Sunday 5 September 2021

Time SAST	Time UTC				
16:30 - 16:40	14:30 - 14:40	Introduction and Welcome		Orientation for delegates	
16:40 - 17:30	14:40 - 15:30	2020 Early Career Award		Tobias Zuest: Novel chemical defences as an escape from co-adapted herbivores	
17:30 - 17:35	15:30 - 15:35	Short break		Delegates navigate to session of choice	
17:35 - 18:50	15:35 - 16:50	Three concurrent sessions			
Time SAST	Time UTC	Session #1: Chemical ecology of disease vectors: from fundamental to applied research Symposium Chair: Marcelo Lorenzo		Session #2: Chemical ecology of marine holobionts Symposium Chairs: Catherine Leblanc & Soizic Prado	
17:35 - 18:05	15:35 - 16:05	Baldwyn Torto	Insights into Use of Semiochemicals for Management of Disease Vectors in Kenya	Georg Pohnert	Infection and resistance in complex microbial communities of the plankton - small scale processes with global impact
18:05 - 18:20	16:05 - 16:20	David Tchouassi	Non-human primate and human-derived attractants for <i>Aedes</i> mosquitoes	Alexia Lourtie	Anthraquinones produced by crinoids allow host selection for the symbiotic snapping shrimp <i>Synalpheus stimpsonii</i>
18:20 - 18:35	16:20 - 16:35	Rickard Ignell	Malaria mosquitoes take the piss	Benoît Paix	A multi-omics approach deciphers how temperature and copper stress shape seaweed-microbiota interactions at the surface of <i>Taonia atomaria</i>
18:35 - 18:50	16:35 - 16:50	Sharon Hill	Age matters: gene regulation in the mosquito antenna during the first gonotrophic cycle	Bertille Burgunter-Delamare	Exploring the microbiota of <i>Saccharina latissima</i>
18:50 - 19:00	16:50 - 17:00	Break			
Time SAST	Time UTC	Session #1: Chemical ecology of disease vectors: from fundamental to applied research Symposium Chair: Rickard Ignell		Session #2: Chemical ecology of marine holobionts Symposium Chairs: Catherine Leblanc & Soizic Prado	
19:00 - 19:15	17:00 - 17:15	Tristram Wyatt	Life scientists in chemical ecology can benefit from psychology's responses to its own 'reproducibility crisis'	Emilie Adouane; Soizic Prado	Deciphering the marine algal holobiont interactions for the discovery of new eco-compatible antifoulings
19:15 - 19:30	17:15 - 17:30	Marcelo Lorenzo	The ionotropic co-receptor IR8a is required for the expression of robust sexual behavior in males of the Chagas disease vector <i>Rhodnius prolixus</i>	Hendrikje Jorissen	Coral larval settlement preferences linked to crustose coralline algae with distinct chemical and microbial signatures
19:30 - 19:45	17:30 - 17:45	Nicoletta Faraone	Tick Repellents: Modulators of Electrophysiological and Behavioural Activities	Joost Mansour	Carbon and nitrogen uptake and translocation between the single cell marine protist <i>Acantharia</i> and their symbionts
19:45 - 20:00	17:45 - 18:00	Anaïs Tallon	Functional analysis of two odorant receptors related to host seeking in <i>Aedes aegypti</i>	Qikun XING	Studies of the oxylipin pathway regulation in brown algal kelps
20:00 - 20:15	18:00 - 18:15	Laura Pickett	Electrophysiological responses of <i>Ixodes scapularis</i> to host volatiles	Dolma Michellod	Exploring chemical diversity in the deep sea: linking bacterial symbionts to changes in mussel metabolome
Conclusion		Networking and socialize			
20:30 - 23:30	18:30 - 21:30	ISCE Executive Committee Meeting			

## Day 2: Monday 6 September 2021

11:15 - 12:00	09:15 - 10:00	Morning Q&A		Opportunity for delegates to meet with and talk to speakers			
12:00 - 12:10	10:00 - 10:10	Introduction and Welcome		Orientation for delegates			
12:10 - 13:00	10:10 - 11:00	2019 Silver Medal Lecture		Stefan Schulz: The Interaction of Chemistry and Chemical Ecology			
13:00 - 13:05	11:00 - 11:05	Short break		Delegates navigate to session of choice			
<b>Time SAST</b>	<b>Time UTC</b>	<b>Session #4: Insect Microbe Interactions Symposium Chair: Marko Rohlf</b>		<b>Session #5: Insect Olfactory Neuroethology Symposium Chair: Bill Hansson</b>		<b>Session #6: Using chemical ecology in push-pull control of insect pests: from theory to implementation Symposium Chair: John Pickett</b>	
13:05 - 13:35	11:05 - 11:35	Tobias Engl	Nutritional endosymbionts' Shikimate pathways are susceptible to glyphosate inhibition	Silke Sachse	Sensory processing in the fly brain	Zeyaur Khan	The success of the push-pull system for controlling lepidopterous pests and parasitic striga weeds in small-holder cereal farming
13:35 - 13:50	11:35 - 11:50	Jurgen Gross	Tritrophic interactions between plant pathogens and their vector insects mediated by primary and secondary plant metabolites	Wei Xu	Chemosensory Proteins (CSPs) in Cotton Bollworm <i>Helicoverpa armigera</i>	Islam Sobhy	Deciphering the chemical ecology of push-pull intercropping system in mitigating fall armyworm, <i>Spodoptera frugiperda</i> , herbivory
13:50 - 14:05	11:50 - 12:05	Marko Rohlf	Insect-induced changes in fungal chemical exudate composition impact key properties of an experimental decomposer system	Bill Hansson	Alcohol boosts fly attractiveness	Christina Conroy	'Pushing' towards a new IPM strategy. <i>Drosophila suzukii</i> , the search for effective repellents
14:05 - 14:20	12:05 - 12:20	Paul Becher	Fruit, flies and fungi – studies of <i>Drosophila suzukii</i> ecology for pest control	David Ruel	Olfactory indolergic receptors in <i>Drosophila melanogaster</i>	Casper Nyamukondiwa	New opportunities for a push-pull system in brassica crop production
14:20 - 14:30	12:20 - 12:30	Comfort Break					
<b>Time SAST</b>	<b>Time UTC</b>	<b>Session #4: Insect Microbe Interactions Symposium Chair: Marko Rohlf</b>		<b>Session #5: Insect Olfactory Neuroethology Symposium Chair: Silke Sachse</b>		<b>Session #6: Using chemical ecology in push-pull control of insect pests: from theory to implementation Symposium Chair: John Pickett</b>	
14:30 - 14:45	12:30 - 12:45	Almuth Hammerbacher	Bark beetle-associated fungi assist in the detoxification of host flavonoids and provide a fitness benefit the Eurasian spruce bark beetle	Thomas Baker	An OR having previously unrecognized wide-field ligand affinities explains the behavioral activity of a new, cryptic, redundant sex pheromone component in the moth, <i>Ostrinia nubilalis</i>	John Pickett	Exploiting Push-Pull pest insect management by biotechnology
14:45 - 15:00	12:45 - 13:00	Martin Kaltenpoth	Outsourcing immunity: Evolution, chemical ecology, and genomics of protective insect-bacteria symbioses	Sonja Bisch-Knaden	Neuroethology of odor-guided behavior in the hawkmoth <i>Manduca sexta</i>	Vincent Jacob	Chemical interaction between the sugarcane spotted borer <i>Chilo sacchariphagus</i> (Lepidoptera: Crambidae) and a dead-end trap plant
15:00 - 15:15	13:00 - 13:15	Dineo Mailula	Catabolism of branched chain and aromatic amino transferases, route to fusel alcohols and acetates by the Ceratocystidaceae	Victoria Ivey	Molecular and neural plasticity in the sex pheromone response of the corn earworm ( <i>Helicoverpa zea</i> )	Jimmy Pittchar	Socio-economic issues of developing push-pull technologies
15:15 - 15:30	13:15 - 13:30	Anna Jirošová	Biosynthesis of aggregation pheromone component in spruce-killing bark beetle <i>Ips typographus</i>	Erika Plettner	Gypsy Moth Pheromone-Binding Proteins: Kinetic, Structural and Functional Aspects of Interaction with Pheromones	Frank Chidawanyika	Controlling the Fall armyworm, <i>Spodoptera frugiperda</i> , using the push-pull system: an update
15:30 - 15:45	13:30 - 13:45	Refreshment Break					
<b>Time SAST</b>	<b>Time UTC</b>	<b>Session #7: Chemical Mimicry Symposium Chairs: Andreas Jürgens &amp; Katherine Goodrich</b>		<b>Session #8: Commemorating Prof. Kenji Mori - Synthesis and Chemical Ecology Symposium Chair: Stefan Schulz</b>		<b>Session #9: Evolution of Plant Chemical Defenses Symposium Chair: Shuqing Xu</b>	
15:45 - 16:15	13:45 - 14:15	Steven Johnson	Long-horned beetles and single orchids: discovery of a new class of sexual mimicry in plants	Karl Gademann	NONO compounds: A New Chemical Language in Bacteria?	Shuqing Xu	Did plant chemical defences evolve like the spandrels of San Marco?
16:15 - 16:30	14:15 - 14:30	Thomas Rupp	Scent-mediated deceptive strategies in fly-pollinated <i>Aristolochia</i>	Yunfan Zou	Identification and synthesis of a novel sesquiterpene from several species of leafhopper bugs	Priscila Mezzomo	Herbivore order and life stage, but not specialization, drive defensive responses in the crack willow
16:30 - 16:45	14:30 - 14:45	Annemarie Heiduk	Revolver flowers fool freeloaders – pollination strategies of trap and non-trap flowers in Stapeliinae (Apocynaceae)	Hirosato Takikawa	Synthesis of semiochemicals with the guidance of Professor Kenji Mori	Bianka Siewert	Light-activated Defense Strategies in Mushrooms
16:45 - 17:00	14:45 - 15:00	Katherine Goodrich	Flies, flowers, and fermentation	Robert Britton	Total Synthesis of Tetrahydrofuran-Containing Marine Macrolides	Valentin Gfeller	Impact of benzoxazinoid-dependent plant-soil feedbacks on plant performance and food quality within a crop rotation
17:00 - 17:15	15:00 - 15:15			Richmond Sarpong (30 min)	Strategies for Chemical Synthesis Inspired by Complex Natural Products	Maria da Silva	Synthesis of Ruthenium and Magnesium-Flavonoid Complexes and their effect <i>in vivo</i> on Citrus with CVC
17:15 - 17:45	15:15 - 15:45	Dinner Break					
17:45 - 18:35	15:45 - 16:35	2020 Silver Medal Lecture		Gerhard Gries: Thirty Years of Entertainment in Chemical Ecology Research			

## Day 3: Tuesday 7 September 2021

11:15 - 12:00	09:15 - 10:00	Morning Q&A					
12:00 - 12:10	10:00 - 10:10	Introduction and Welcome		Orientation for delegates			
12:10 - 13:00	10:10 - 11:00	2019 Silverstein-Simeone Lecture <b>SPRINGER NATURE</b>		Ales Svatos lecture and discussion, Modern mass spectrometry methods for chemical ecology			
13:00 - 13:05	11:00 - 11:05	Short break		Delegates navigate to session of choice			
13:05 - 14:50	11:05 - 12:50	Three concurrent sessions					
<b>Time SAST</b>	<b>Time UTC</b>	<b>Session #10: Insect pest management - Part I Symposium Chair: Aijun Zhang</b>		<b>Session #11: Plant-pollinator interactions - Part I Symposium Chair: Stefan Dötterl</b>		<b>Session#12: Recent discoveries and new approaches to the study of plant volatiles and their roles in plant communication - Part I Symposium Chair: Andrea Clavijo-McCormick</b>	
13:05 - 13:20	11:05 - 11:20	<b>Anat Levi-Zada</b>	Pheromone identification by SSGA – an analytical method using circadian rhythms of volatile emissions	<b>Manfred Ayasse</b>	Sexual dimorphism in floral scents of the neotropical orchid <i>Catasetum arietinum</i> and its possible ecological and evolutionary significance	<b>Velemir Ninkovic</b>	Plant-Plant Communication via Volatiles Triggers Growth and Defense Synchronization among Plants
13:20 - 13:35	11:20 - 11:35			<b>Adam Shuttleworth</b>	Composition and EAD-active components of the scents of flowers pollinated by <i>Hemipepsis</i> (Pompilidae) wasps		
13:35 - 13:50	11:35 - 11:50	<b>Bruna Czarnobai de Jorge</b>	Nanofibers as carrier of push substances against pear psyllids	<b>Yudai Okuyama</b>	Pollinator-mediated speciation in endemic flora of Japan associated with dynamic changes of floral scents	<b>James Blande</b>	Perception and Response in Hybrid Aspen: Volatile-Induced Defence Traits
13:50 - 14:05	11:50 - 12:05	<b>Salvatore Guarino</b>	Volatiles of <i>Capsicum annuum</i> L. as attractant source for <i>Lasioderma serricorne</i> F. (Coleoptera: Anobiidae)	<b>Magali Proffit</b>	Chemical signal is in the blend: bases of plant-pollinator encounter in a highly specialized interaction	<b>Mirian Michereff</b>	Differential defence responses of Neotropical maize genotypes to Fall Armyworm, Green Belly Stink bugs, herbivore-induced volatiles and the plant elicitor indole
14:05 - 14:20	12:05 - 12:20	<b>Tugcan Alinc</b>	Host sex discrimination by egg parasitoids of stink bugs	<b>James Ryalls</b>	Diesel exhaust and ozone pollution reduce insect-mediated pollination services	<b>Anne Jones</b>	<i>Helicoverpa zea</i> Caterpillars Manipulate Volatile Emission from Maize Through Salivary Glucose Oxidase Activity
14:20 - 14:35	12:20 - 12:35	<b>Elisa Pal</b>	Characterization of the alarm pheromone of <i>Bathycoelia distincta</i> (Pentatomidae), a major pest of macadamia	<b>Isabel Alves dos Santos</b>	Nocturnal bee pollination systems mediated by floral scents	<b>Po-An Lin</b>	Silencing the alarm: An insect salivary enzyme closes plant stomata and inhibits volatile release
14:35 - 14:50	12:35 - 12:50	<b>Johannes Joubert</b>	Bioassay and mass spectrometry-guided identification of constitutive plant metabolites associated with the feeding preference of the <i>Eucalyptus</i> snout beetle, <i>Gonipterus</i> sp. n. 2.			<b>Amanuel Tamiru</b>	Fall Armyworm Oviposition Suppresses Volatile Emission in Maize: Effects on Recruitment of Egg Parasitoid
14:50 - 15:20	12:50 - 13:20	Meal Break					
15:20 - 16:05	13:20 - 14:05	Poster session #1 (3 minute pre-recorded posters presentations)					
16:05 - 17:10	14:05 - 14:10	Short Break					
17:10 - 17:55	14:10 - 14:55	Poster session #2 (3 minute pre-recorded posters presentations)					
17:55 - 18:05	14:55 - 15:05	Short Break					
18:05 - 18:55	15:05 - 15:55	Applied Chemical Ecology Lecture		John Borden lecture and discussion, New Award Old Story: The World's Longest-Running Semiochemical-Based IPM Program			
<b>Conclusion</b>		Networking and socialize					

## Day 4: Wednesday 8 September 2021

11:15 - 12:00	09:15 - 10:00	Morning Q & A	Opportunity for delegates to meet with and talk to speakers
12:00 - 12:10	10:00 - 10:10	Introduction and Welcome	Orientation for delegates
12:10 - 12:55	10:10 - 10:55	Poster session #3 (3 minute pre-recorded posters presentations)	
12:55 - 13:00	10:55 - 11:00	Short break	Delegates navigate to session of choice
13:00 - 14:45	11:00 - 12:45	Three concurrent sessions	
<b>Time SAST</b>	<b>Time UTC</b>	<b>Session #13: Insect Pest Management - Part II Symposium Chair: Jian Chen</b>	<b>Session 14: Plant-pollinator interactions - Part II Symposium Chair: Steve Johnson</b>
13:00 - 13:15	11:00 - 11:15	<b>John Byers</b> Field experiments and simulations of semiochemical-based mass trapping and push-pull of <i>Euwallacea fornicatus</i> , an ambrosia beetle pest of avocado	<b>Hannah Burger</b> Attractants and filters in a wasp- and a bee-pollinated <i>Gomphocarpus</i> species
13:15 - 13:30	11:15 - 11:30	<b>Marc Bouwer</b> The sex pheromone of the pine brown-tail moth, <i>Euproctis terminalis</i> (Lepidoptera: Erebididae)	<b>Katharina Brandt</b> Unravelling the olfactory biases of male euglossine bees: Species-specific antennal responses and their evolutionary significance for perfume flowers
13:30 - 13:45	11:30 - 11:45	<b>Sean Moore</b> Detection of infested fruit: a dog's nose and a wasp's antenna	<b>Markus Knaden</b> Learning of feeding and oviposition cues in a hawkmoth
13:45 - 14:00	11:45 - 12:00	<b>Wayne Kirkman</b> Postharvest detection of infested fruit: The solution is hanging in the air	<b>Rebecca Hofer</b> Effects of water stress on floral scent, morphology and reproductive success
14:00 - 14:15	12:00 - 12:15	<b>Cesar Rodriguez-Saona</b> Potential novel sources of repellents/oviposition deterrents for spotted-wing drosophila management	<b>Karin Gross</b> Why so variable? Polyploidy and floral volatiles in the coevolving, nursery pollinated <i>Lithophragma bolanderi</i> (Saxifragaceae)
14:15 - 14:30	12:15 - 12:30	<b>Falko Drijfhout</b> Cuticular hydrocarbons (CHC) as recognition cues in thrips (Thysanoptera: Thripidae)	<b>Robert Raguso</b> Moths in flowers: volatile mediated interactions from obligate mutualism to seed predation
14:30 - 14:45	12:30 - 12:45		
14:45 - 15:15	12:45 - 13:15	Meal Break	
15:15 - 16:00	13:15 - 14:00	Posters session #4 (3 minute pre-recorded posters presentations)	
16:00 - 16:05	14:05 - 14:55	Short Break	
16:05 - 16:55	14:05 - 14:55	2020 Silverstein-Simeone Lecture <b>SPRINGER NATURE</b>	Paulo Zarbin lecture and discussion

## Day 5: Thursday 9 September 2021

11:15 - 12:00	09:15 - 10:00	Morning Q&A	Opportunity for delegates to meet with and talk to speakers				
12:00 - 12:10	10:00 - 10:10	Introduction and Welcome	Orientation for delegates				
12:10 - 13:55	10:10 - 11:55	Three concurrent sessions					
<b>Time SAST</b>	<b>Time UTC</b>	<b>Session #16: Insect pest management - Part III Symposium Chair: Jian Chen</b>		<b>Session #17: Sexual Communication in the Hymenoptera - Part I Symposium Chair: Tamara Pokorny</b>			
12:10 - 12:25	10:10 - 10:25	Wei Xu	Electroantennogram responses of the parasitoid wasp, <i>Diadegma semiclausum</i> , to host-related odours	Joachim Ruther	Pheromones in the <i>Nasonia</i> group – behavior and biochemistry		
12:25 - 12:40	10:25 - 10:40	Md Mostafiz	Insecticidal efficacy of three benzoate derivatives against <i>Aphis gossypii</i> and its predator <i>Chrysoperla carnea</i>				
12:40 - 12:55	10:40 - 10:55	Mary Angelique Tavera	Semiochemical based approach for the control of <i>Helopletis bakeri Poppius</i> (Hemiptera:Miridae)	Hao Xu	How parasitic wasps use a combination of plant volatiles and sex pheromones to locate mates		
12:55 - 13:10	10:55 - 11:10	Divina Amalin	On Farm Trial of Cacao Pod Borer Sex Pheromone Trapping System in the Philippines	Tamara Pokorny	Similar, but not the same - mate recognition based on cuticular hydrocarbons		
13:10 - 13:25	11:10 - 11:25	Raimondas Mozūraitis	(S)-(-)- $\delta$ -Heptalactone, an aggregation pheromone of fruit fly <i>Rhagoletis batava</i> , a <i>Hippophae rhamnoides</i> berries pest	Aidan Williams	<i>Doublesex</i> regulates pheromone communication and odour-guided behaviour in <i>Nasonia</i> wasps		
13:25 - 13:40	11:25 - 11:40	Chales Kwadha	Sub-Saharan Africa, a new home for <i>Drosophila suzukii</i>	Alejandro Ibáñez	Chemical characterization and interspecific variation assessment of sexually dimorphic mental glands in three species of turtles (family Geoemydidae)		
13:40 - 13:55	11:40 - 11:55			Li Chen	How ants efficiently stabilize their mutualism with aphids? A cross-species function of ant's trail pheromones		
13:55 - 14:05	11:55 - 12:05	Comfort Break					
<b>Time SAST</b>	<b>Time UTC</b>	<b>Session #18: Insect pest management - Part IV Symposium Chair: Jian Chen</b>		<b>Session #19: Chemical ecology in the Anthropocene - Part I Symposium Chair: Shannon Olsson</b>		<b>Session #20: Insect physiological responses to plant toxins - Part I Symposium Chairs: Susanne Dobler &amp; Franziska Beran</b>	
14:05 - 14:20	12:05 - 12:20	Aijun Zhang	Green Pesticide: Methyl Benzoate and its Analogs	Shannon Olsson	Chemical ecology as inspiration for global change	Georg Petschenka	Evolutionary Physiology of Insect-Plant Interactions on a Tritrophic Scale
14:20 - 14:35	12:20 - 12:35	Gadi Reddy	Application of pheromones for the management of insect pests in Montana	Robbie Girling	Field-scale effects of air pollution on invertebrate community composition in wheat		
14:35 - 14:50	12:35 - 12:50	Jian Chen	Toxicity and Repellency of Naturally Occurring Isothiocyanates against Red Imported Fire Ants	Candice Dubuisson	Effect of ozone pollution on the chemical signal emitted by a Mediterranean tree to attract its specific pollinator	Felix Feistel	The role of quinolizidine alkaloids from <i>Genista</i> plants (Fabaceae) in aphid-plant interactions
14:50 - 15:05	12:50 - 13:05	Matthew Ethington	Benzaldehyde mediates host colonization by the peach bark beetle, <i>Phloeotribus liminaris</i>	Joyshree Chanam	Impact of elevation and global warming on plant-pollinator relationships in the Eastern Himalayas	Marlena Winter	Functional characterization of Na,K-ATPase subunit combinations reveals the adaptive strategy of cardenolide-resistant large milkweed bugs
15:05 - 15:20	13:05 - 13:20	William Morrison	Improving semiochemical resources and behaviorally-based management strategies for stored products: from the laboratory to food facilities	Florian Straub	Land-use stress and pesticides alter the chemical communication of wild bees	Nikolaos Deligioris	Convergent recruitment of detoxification enzymes: The flavin-dependent monooxygenases of <i>Longitarsus jacobaeae</i>
15:20 - 15:35	13:20 - 13:35			Maryse Vanderplanck	Impact of ozone on the perception of olfactory signal by generalist pollinators	Prayan Pokharel	Dietary cardenolides enhance growth and change the direction of the fecundity-longevity trade-off in specialized sequestering milkweed bugs (Heteroptera: Lygaeinae)
15:35 - 16:00	13:35 - 14:00	Comfort Break					

## Day 5: Thursday 9 September 2021 (continues)

Time SAST	Time UTC	Session #21: Insect Pest Management - Part V Symposium Chair: Jian Chen		Session #22: Chemical ecology in the Anthropocene - Part II Symposium Chair: Shannon Olsson		Session #23: Insect physiological responses to plant toxins - Part II Symposium Chairs: Susanne Dobler & Franziska Beran	
16:00 - 16:15	14:00 - 14:15	Andrés González	Effect of <i>Drosophila suzukii</i> on blueberry VOCs: chemical cues for a pupal parasitoid	Guaraci Cordeiro	Highly diverging effects of increased temperatures on floral scents of crop species	Franziska Beran	The horseradish flea beetle selectively absorbs glucosinolates across the foregut
16:15 - 16:30	14:15 - 14:30	Junwei Zhu	New discoveries of natural products for controlling agricultural, medical and veterinary pests	Conxita Avila	Natural products from Antarctic marine benthic invertebrates		
16:30 - 16:45	14:30 - 14:45	Emily Lemke	Communication ecology of pest <i>Limonius</i> species (Coleoptera: Elateridae) in North America			Anshika Singh	Sponge Watch Program- An initiative towards 'one ocean-one health' approach in the age of Covid-19
16:45 - 17:00	14:45 - 15:00	Kendal Catherine Singleton	Identification of candidate sex-pheromone components of two Nearctic <i>Agriotes</i> species (Coleoptera: Elateridae)	Gabrielle Nevitt	Anthropogenic influences on marine food webs: the case of plastic ingestion	Carla Arce	<i>Diabrotica virgifera virgifera</i> females can sequester multiple plant toxins to protect their eggs against natural enemies
17:00 - 17:15	15:00 - 15:15	Wim van Herk	Use of sex pheromones for wireworm (Coleoptera: Elateridae) management in Canada			Vojislava Grbic	Rapid specialization of counter defenses enables two-spotted spider mite to adapt to novel plant hosts
17:15 - 17:30	15:15 - 15:30	Kyle Arriola	Identification of Brassicadiene, a Diterpene Hydrocarbon Attractive to the Invasive Stink Bug <i>Bagrada hilaris</i> , from Volatiles of Cauliflower Seedlings, <i>Brassica oleracea</i> var. <i>botrytis</i>	Joerg Bohlmann			Chemical & Physical Defenses of Conifers and Insect Counter Strategies
17:30 - 17:45	15:30 - 15:45						
17:45 - 18:15	15:45 - 16:15	Short break					
18:15 - 19:30	16:15 - 17:30	Q & A	Time to network: Opportunity to interact with speakers who presented on Sunday, Monday and Tuesday				
Conclusion		Networking and socialize					



## Day 6: Friday 10 September 2021

13:45 - 14:30	11:45 - 12:30	Drop in Q&A for speakers	Opportunity for delegates to meet with and talk to speakers
14:30 - 14:40	12:30 - 12:40	Introduction and Welcome	Orientation for delegates
14:40 - 15:30	12:40 - 13:30	2021 Early Career Award 	Ricardo Machado: Engineering bacterial symbionts of nematodes improves their biocontrol potential to counter the western corn rootworm
15:30 - 15:35	13:30 - 13:35	Short break	Delegates navigate to session of choice
15:35 - 16:50	13:35 - 14:50	Two concurrent sessions	
<b>Time SAST</b>	<b>Time UTC</b>	<b>Session #24: Insect Pest Management - Part V Symposium Chair: Jian Chen</b>	<b>Session #25: Sexual communication in the Hymenoptera - Part II Symposium Chair: Joachim Ruther</b>
15:35 - 15:50	13:35 - 13:50	<b>Bradley Higbee</b> Success in progress; mating disruption for navel orangeworm ( <i>Amyelois transitella</i> ) is becoming part of the foundation for IPM in California nut crops	<b>Falko Drijfhout</b> Next-generation cuticular hydrocarbon analysis in insects
15:50 - 16:05	13:50 - 14:05	<b>Larry Gut (Peter McGhee)</b> Pheromone-based mating disruption: From conceptualization to commercialization	<b>Nathan Derstine</b> Using sublethal pesticide exposure to examine honest signaling properties of sexual communication in bees
16:05 - 16:20	14:05 - 14:20	<b>Qing-He Zhang</b> Semiochemical-based Traps and Repellents for Pestiferous Social Wasps	<b>Andreas Fischer</b> Sexual signalling in a widow spider – Unravelling contact and airborne pheromone components and the role of an enzyme in their dissemination
16:20 - 16:35	14:20 - 14:35	<b>Gary Judd (Don Thomson)</b> Codling Moth Mating Disruption and the Mechanistic Principles Underpinning its Success	<b>Gerhard Gries</b> Sex pheromone components and potential sex recognition cuticular hydrocarbons of yellowjackets
16:35 - 16:50	14:35 - 14:50	<b>Don Thomson</b> Codling Moth: A Look Back with an Eye on the Future	<b>Jocelyn Millar</b> Interspecific variation in the sex pheromones of slave-making ants in the genus <i>Polyergus</i>
16:50 - 17:05	14:50 - 15:05	<b>Pavlina Kyjakova</b> Do we choose our partner based on smell?	<b>David Sillam-Dussès</b> Courtship behavior confusion in the invasive termite species <i>Coptotermes formosanus</i> and <i>C. gestroi</i>
17:05 - 17:35	15:05 - 15:35	Short Meal Break	
17:35 - 18:35	15:35 - 16:35	Q & A	Time to network: Opportunity to interact with speakers who presented on Wednesday, Thursday and Friday
18:35 - 18:45	16:35 - 16:45	Short break	Preparation for closing business meeting
18:45 - 19:45	16:45 - 18:45	ICSE Closing Business Meeting	

## ISCE Posters 2021

**Poster Session 1**

Tuesday 7 Sept 12:55 - 14:40 UTC

**Poster Session 2**

Tuesday 7 Sept 15:10 - 15:55 UTC

**Poster Session 3**

Wednesday 8 Sept 10:10 - 10:55 UTC

**Poster Session 4**

Wednesday 8 Sept 12:55 - 14:40 UTC

**Chemical ecology of disease vectors: from fundamental to applied research (Poster Session 1)**

1	Federico Galassi	Head Louse Feces: Chemical and Behavioural Analysis
2	Daniel Leybourne	Landscape-Vector-Virus Interactions: Do farms surrounded by diverse landscapes benefit from enhanced virus suppression?
3	Haozhe Wang	Repellent and acaricidal effects of basil essential oil and rock dust on ticks

**Chemical ecology of marine holobionts (Poster Session 1)**

4	Alexia Lourtie	Does the chemical environment impact the survival of a host-specific symbiont? A transcriptomic approach of a "host-separation syndrome"
5	Emily Claereboudt	Distinct saponin profile drives an olfactory-mediated aggregation in <i>Holothuria scabra</i> (Holothuroidea)

**Eusocial Insects: chemical ecology of social organisation at the genetic, behavioural and ecological levels (Poster Session 1)**

6	Rocío Lajad	Avoidance response to low-quality pollen in foraging honeybees
7	Anne-Geneviève Bagnères-Urbany	Heterogeneous chemical profiles of <i>Vespa velutina nigrithorax</i> alarm pheromone

**Insect microbe interactions (Poster Session 1)**

8	Keamogetswe Maswanganyi	Stressed-induced changes in the microbial community of <i>Populus nigra</i>
9	Emily Puckett	Exploring the potential nutritional symbiosis between <i>Ips typographus</i> and its fungal associates
10	Laima Blazyte-Cereskiene	Behavioural responses of <i>Rhagoletis cerasi</i> flies to volatiles from the yeasts populating cherry berries
11	Rosa Knoppersen	The role of the gut microbiome in overcoming the chemical defenses of <i>Eucalyptus</i> species in the <i>Eucalyptus</i> snout beetle, <i>Gonipterus sp. n. 2</i>

# CHEMICAL ECOLOGY AND SUSTAINABLE DEVELOPMENT

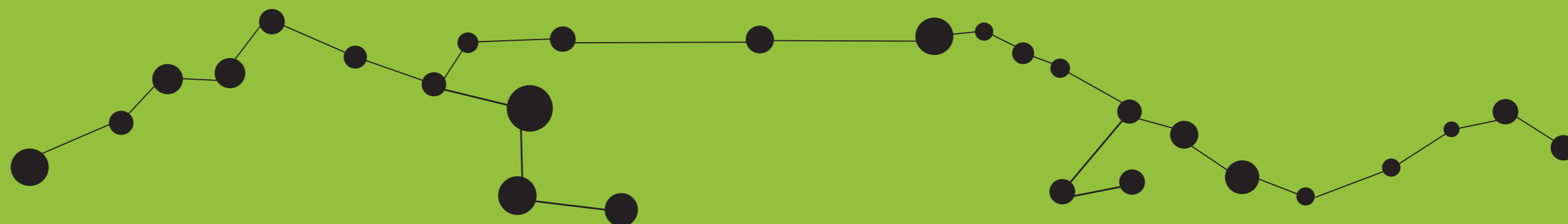


Using chemical ecology in push-pull control of insect pests: from theory to implementation (Poster session 1)		
12	Bruna Sartório de Castro	Chemical profile of non-volatile compounds from seeds, leaves and roots of <i>Crotalaria spectabilis</i>
13	Magdolna Szelényi	Olfactory background of stimulo-deterrent pest management strategy in the sugarcane-borer <i>Eldana saccharina</i>
Chemical Mimicry (Poster session 1)		
14	Melanie McClure	Can colour pattern be a “magic” trait in transparent aposematic and mimetic butterflies?
Insect Olfactory Neuroethology (Poster session 2)		
16	Gaëlle Ramiaranjatovo	Shared volatile compounds among the various host-fruits drive fruit flies olfactory system (Diptera:Tephritidae)
17	Vincent Jacob	Three-point electroantennography: a setup to exhaustively explore the olfactory sensitivity of bulbous antennae
18	Yiftach Golov	Integrated effect of biological and physical factors on chemo-sexual communication in moths
19	Neil Hillier	Blend reception and plasticity in moth olfaction
21	Sarah Koerte	Investigating the semiochemistry underlying host selection and oviposition of <i>Mythimna unipuncta</i>
23	Twinkle Biswas	Characterization of olfactory sensory neurons in <i>Trypodendron lineatum</i>
Commemorating Prof. Kenji Mori - Synthesis and Chemical Ecology (Poster session 2)		
24	Moritz Gerbaulet	Identification of Methyl Branched 1-Methoxyalkanes from Tetragnatha Spiders, Potential Species Recognition Signals
25	Rajarajan Ramakrishnan	Understanding pheromone biosynthesis in spruce forest vital pest <i>Ips typographus</i> .
Evolution of Plant Chemical Defenses (Poster session 2)		
26	Barbora Stříbrská	Forest edge induced physiological and chemical changes increases the susceptibility of Norway spruce ( <i>Picea abies</i> ) to bark beetle ( <i>Ips typographus</i> ) attack.
28	Fernanda Moreira Andrade	Neutral effect of multiple herbivory by prey and non-prey on indirect defense of coffee plants
29	Maria Fernanda Peñaflores	Transgenerational effects of maternal herbivory by a sap-sucking insect on plant resistance
Sexual Communication in the Hymenoptera (Poster session 2)		
60	Nils Schöfer	Sublethal effects of insecticides on partner and host finding of <i>Nasonia vitripennis</i>

# CHEMICAL ECOLOGY AND SUSTAINABLE DEVELOPMENT



Plant-pollinator interactions (Poster session 3)		
30	Danny Kessler	Attraction and defense - The dual role of floral scent compounds in <i>Nicotiana attenuata</i>
31	Kim Heuel	Full of impatience - nectar scents in <i>Impatiens</i> flowers visited by <i>Bombus impatiens</i>
32	LI CAO	Phylogenetic constraints on the evolution of floral scents in a nursery pollination mutualism
33	Olabimpe Okosun	Interactions of insect pest pheromones and insect pollinators
Recent discoveries and new approaches to the study of plant volatiles and their roles in plant communication (Poster session 3)		
34	Amanuel Tamiru	Direct and Indirect Defense Induction on Maize by Neighbouring Molasses Grass
Insect physiological responses to plant toxins (Poster session 3)		
35	Carmen Rossini	Preference of the bronze bug <i>Thaumastocoris peregrinus</i> (Hemiptera: Thaumastocoridae) for eucalyptus species: correlation with its secondary chemistry
36	Guillermo Bragunde	Effect of essential oils on young honeybee gustatory response
Chemical ecology in the Anthropocene (Poster session 3)		
37	Sílvia Borrull and Conxita Avila	Climate change and chemical ecology: Determination of natural products in an Antarctic and a Mediterranean bryozoan species
38	Lente van Zyl	Production of defensive metabolites by <i>Pinus patula</i> x <i>Pinus tecunumanii</i> hybrids in response to <i>Fusarium circinatum</i> infection
39	Paula Schirmmacher	Structural and Functional Comparison of 2-Phenylethylamine and Dopamine as Infochemicals for Hermit Crabs under Climate Change
40	Gemma Llàcer	Natural products in Antarctic sponges: the case of <i>Mycale acerata</i>
41	Pere Monràs	The effect of temperature in the chemical defense of the nudibranch <i>Doris kerguelenensis</i> (Mollusca: Heterobranchia)



# CHEMICAL ECOLOGY AND SUSTAINABLE DEVELOPMENT

## Insect pest management (Poster session 4)

42	Aletta van Tonder	TV PheroLure®: The influence of a semiochemical lure on the volatile profile of a commercial tomato field
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ISCE 2021

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## Deciphering the marine algal holobiont interactions for the discovery of new eco-compatible antifoulings

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Biofilms are complex communities of microorganisms in contact with a surface and included in a matrix that they secrete. These microbial biofilms provoke many problems for military, industrial, and recreational activities. Most of the used antifouling compounds are particularly toxic biocides that have a significant impact on the natural environment. However, no sustainable, environmentally-friendly alternative has been developed. However, such chemical compounds exist in Nature, and many marine species have developed strategies to protect themselves from biofouling. This is particularly the case of macroalgae that secrete molecules targeting quorum sensing, a mode of intercellular communication involved in the formation of biofilms. The search for “quorum quenching” compounds, mimicking this key mechanism, is thus an innovative strategy to identify new antifouling substances.

The knowledge of the algal holobiont has revealed the existence of microbiota associated with macroalgae capable of effecting the fitness of the host alga. Indeed, the algal epimicrobiota is the place of intense chemical communications, which finely regulates the organization of algal surface biofilms but whose mechanisms have been little studied. However, we have demonstrated that the epimicrobiota of the alga *Saccharina latissima*, a key specie of the northern European coasts, is the seat of the production of chemical mediators capable of inhibiting bacterial QS and by extension inhibiting the formation of biofilms. The molecular dialogue which occurs within the algal holobiont appears thus as an efficient bioinspired target to better decipher chemical interactions within the holobiont and to find new sustainable natural compounds.

**Keywords:** algal microbiota; antifouling, chemical communication; holobiont; metabolites

## Host sex discrimination by egg parasitoids of stink bugs

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Egg parasitoids foraging for suitable hosts scattered in the environment rely mainly on chemical cues. During the host location process, egg parasitoids use a wide range of stimuli, such as long-range kairomones from the host/plant complex and short-range contact cues after landing on an infested plant. Egg parasitoids associated with stink bugs can detect chemical traces left by their hosts while walking on the leaves. Remarkably, chemical traces do not only provide information about potential presence of the stink bug host in the surrounding areas but also provide information on the host sex. In several stink bug-egg parasitoid systems, in fact female wasps spend more time on the traces left by female bugs which would normally be of higher hierarchical value, being more likely associated with egg presence. Such host sex discrimination strategy based on exploitation of host chemical traces has likely evolved by egg parasitoid species in order to avoid wasting time and ensure access to freshly laid eggs. Here, we provide a comprehensive overview of host sex discrimination ability in egg parasitoids of stink bugs and discuss the role of cuticular hydrocarbons as key compounds mediating the discrimination behavior.

**Keywords:** host-foraging behavior; cuticular hydrocarbons; kairomone; semiochemicals

## On Farm Trial of Cacao Pod Borer Sex Pheromone Trapping System in the Philippines

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The Philippines is one of the countries in Asia with a competitive advantage for cacao production with its strategic location and favorable soil and climatic conditions. Despite its competitive advantage, cacao production in the country does not meet the current growing industry requirements. There are a number of constraints for the low yield, but predominantly due to pest and disease infestations. Among the most important pests of cacao in the Philippines is the cacao pod borer (CPB), *Conopomorpha cramerella*. Current management of CPB heavily relies on chemical control but very expensive and not safe to human and environment. Integrated Pest Management (IPM) program for cacao in the Philippines was launched in 2016 focusing on the use of biologically-based approaches. The use of sex pheromone for monitoring and control of CPB is one of the strategies in the cacao IPM program. The new blend of the synthetic sex pheromone of CBP showed was pilot tested in the field with the participation of the farmers. Our result is suggesting that 0.5m above the canopy could be most appropriate placement of the trap. A follow-up study is underway to verify this result and also to determine the optimum number of traps for one (1) hectare of cacao farms. We will also evaluate the efficiency of the lure with regards to the length of time of exposure in the field.

**Keywords:** *Conopomorpha cramerella*; cacao pests; farmers; IPM; sex pheromone

## Reproductive signaling in bees – The mighty queen, the power of peers and the role of brood

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Pheromones inducing sterility in the worker caste of social insects are one of the greatest puzzles in social evolution since they seemingly negate the worker reproductive interests and evolved in the absence of a shared interest between the producer and the receiver of the signals - a situation which calls for additional mechanisms ensuring signal honesty. While the role of the queen in regulating worker reproduction has grabbed most of the attention in selected model organisms, the roles of chemical signals produced by other players, such as the brood and nestmates, were poorly studied.

Bumble bees are primitively eusocial insects that go through several solitary and social phases, allowing the unique opportunity to study changes in regulatory patterns of reproduction as the colony develops. In my talk I will discuss some of latest progress in the study of reproductive division of labor in bumble bees. Particularly, the regulation of reproduction by multiple players in the colony (queen, nestmates and brood), the interplay between the use of behavioral and chemical means to control reproduction, the importance of social context in preventing cheating, and the genetic mechanisms underlying sterility-inducing pheromones.

**Keywords:** Pheromones, Reproduction, Social context, Mechanisms, Bumble

## Plant-Associated CO<sub>2</sub> Mediates Long-Distance Host Location and Foraging Behaviour of a Root Herbivore

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Insects use different cues to locate and select suitable hosts. What are the plant metabolites that guide and are used by root herbivores to locate their host plants from a distance? There is abundant literature on the topic, which is plenty of predictions, but little direct evidence for the involvement of individual metabolites. The development of modern methods to study how root volatiles modulate foraging behaviour of root herbivores are urged to tests these predictions and provide direct evidence in this context. We developed an RNAi system to impair the perception of CO<sub>2</sub> in the larvae of the western corn rootworm and use it to study the importance of plant emitted CO<sub>2</sub> to modulate host location and larval foraging behaviour. We found that the expression of a carbon dioxide receptor, *DvGr2*, is specifically required for larval responses to CO<sub>2</sub>. Impairing CO<sub>2</sub> perception has no effect on the ability of WCR larvae to locate host plants at short distance (<9cm), but strongly impairs host location at greater distances. We also found that WCR larvae preferentially orient and prefer plants that grow in well-fertilized soils compared to plants that grow in nutrient-poor soils, a behaviour that has direct consequences for larval growth and depends on the ability of the larvae to perceive root-emitted CO<sub>2</sub>. This study unravels how CO<sub>2</sub> serves as a distance-dependent host location cue and provides evidence for the power of modern genetic manipulation approaches to study plant volatiles and their roles in plant communication and ecological interactions in general.

**Keywords:** Plant-herbivore interactions; foraging; volatile perception; behavior; host location.



## ***Diabrotica virgifera virgifera* females can sequester multiple plant toxins to protect their eggs against natural enemies**

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Various herbivorous insects sequester defensive secondary metabolites from their host plant and use them against their natural enemies. The known examples of sequestration involve one specific type of chemical defense and this is well documented for insect larvae and adults. However, it is still rarely shown that metabolites can be transferred to eggs and to our knowledge there are no examples of insects sequestering multiple types of defense metabolites. In this study, we: i) investigate sequestration by *Diabrotica virgifera virgifera* beetles, ii) whether sequestered metabolites are transferred to beetle eggs, and iii) whether those metabolites serve defensive functions. *Diabrotica* beetles feed on various host plants, including maize, cucumber and beans, containing defensive benzoxazinoids, cucurbitacins, and cyanogenic glucosides, respectively. Chemical analyses revealed that *D. v. virgifera* beetles sequester and transfer benzoxazinoids and cucurbitacins to their eggs, but not cyanogenic glucosides. To test whether the two sequestered toxins protect eggs against predators, we fed *D. v. virgifera* beetles with toxin-free or toxin-containing plants, and offered their eggs to the rove beetle *Atheta coriaria* and the minute pirate bug *Orius laevigatus*. In choice experiments, both predators consumed more toxin-free eggs than toxin-containing eggs. Moreover, survival assays confirmed the toxic effects of benzoxazinoid-containing eggs on the predators, but, surprisingly, cucurbitacins had no apparent effect. Our results reveal a unique ability of *D. v. virgifera* to use multiple plant defensive chemicals against higher trophic levels, which may in part explain the extraordinary success of this invasive pest.

**Keywords:** biological control; maize; predators; progeny; western corn rootworm.

## **Identification of Brassicadiene, a Diterpene Hydrocarbon Attractive to the Invasive Stink Bug *Bagrada hilaris*, from Volatiles of Cauliflower Seedlings, *Brassica oleracea* var. *botrytis***

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Brassicadiene, a novel tricyclic diterpene hydrocarbon, was identified by a combination of mass spectrometry, microchemical tests, analysis of NMR spectra, and polarimetry. The compound constitutes >90% of the volatile organic compounds produced by cauliflower seedlings, *Brassica oleracea* var. *botrytis*. The invasive stink bug *Bagrada hilaris* is strongly attracted to brassicadiene, providing a mechanism for this herbivore, which specializes on cruciferous plants, to locate its hosts in a nutrient-rich and vulnerable stage. The isolation and process of structural elucidation of the compound including its relative and absolute stereoconfiguration, will be described, accompanied by physical data and bioassay data confirming the role of brassicadiene in attracting *B. hilaris* to the vulnerable *Brassica* spp. seedlings.

**Keywords:** cue; phytochemical; structure-elucidation

## Natural products from Antarctic marine benthic invertebrates

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Recent research in our lab is focusing at new approaches to study the natural products from Antarctic marine benthic invertebrates and their chemical ecology. A part of the description of the new compounds found and their bioactivities, we are looking at the production of these metabolites under situations of stress, trying to see changes in gene expression related to natural compounds synthesis. Global change is one of the key factors that may affect benthic communities, inducing stress (for example, water temperature increase), and understanding how this may interact with production and use of natural products in the relationships among organisms is of crucial relevance. We are using temperature increase and predation pressure to determine changes in natural products presence and diversity in several benthic invertebrates. On the other hand, we are using CADD (Computer-Aided Drug Design) techniques to help us in the search for bioactivities for these compounds, and experimentally validating these results. For all these, we are studying selected tunicate and gastropod molluscs compounds, but also sponges, bryozoans, and others. In this talk, a summary of these recent developments will be provided.

**Keywords:** Marine chemical ecology, marine benthos, CADD, temperature stress, bioactivity.

## The evolution of caste-specific chemical profiles and of queen pheromones in halictid bees

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In eusocial insects, chemical communication is crucial for mediating many aspects of social activities, especially the regulation of reproduction. Though queen signals are known to decrease ovarian activation of workers in highly eusocial species, little is known about their evolution. In contrast, some primitively eusocial species are thought to control worker reproduction through physical aggression by the queen rather than via pheromones, suggesting the evolutionary establishment of chemical signals with more derived sociality. However, studies supporting this hypothesis are largely missing. Socially polymorphic halictid bees, such as *Halictus rubicundus*, with social and solitary populations in both Europe and North America, offer excellent opportunities to illuminate the evolution of caste-specific signals. Here we compared the chemical profiles of social and solitary populations from both continents and tested whether (i) population or social level affect chemical dissimilarity and whether (ii) caste-specific patterns reflect a conserved queen signal. Our results demonstrate unique odour profiles of European and North American populations, mainly due to different isomers of n-alkenes and macrocyclic lactones; chemical differences may be indicative of phylogeographic drift in odour profiles. We also found common compounds overproduced in queens compared to workers in both populations, indicating a potential conserved queen signal. However, North American populations have a lower caste-specific chemical dissimilarity than European populations which raises the question if both use different mechanisms of regulating reproductive division of labour. Therefore, our study gives new insights into the evolution of eusocial behaviour and the role of chemical communication in the inhibition of reproduction.

**Keywords:** Halictid bee, social behaviour, chemical communication, regulation of reproduction, population dialect

## Sexual dimorphism in floral scents of the neotropical orchid *Catasetum arietinum* and its possible ecological and evolutionary significance

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Species of the neotropical orchid genus *Catasetum* produce unisexual flowers characterized by a morphological sexual dimorphism and emit floral perfumes that act as both signal and reward for male euglossine pollinators. Although the role of floral perfumes of *Catasetum* in attracting euglossine pollinators is well investigated, little is known about whether perfumes differ between floral sexes and, if they do, whether this could influence the pollination ecology of the plants. Our aim was to observe the behaviour of pollinators, such as *Euglossa nanomelanotricha* and *E. securigera* in flowers of *Catasetum arietinum* and to compare scent properties (i.e. chemical composition, total amount and temporal fluctuation) of male and female flowers. Floral scent samples were collected using dynamic headspace methods and analyzed via gas chromatography coupled with mass spectroscopy (GC-MS). Bees approached male and female inflorescences in similar proportions but landed significantly more often on female flowers, which emitted more scent than male flowers. Furthermore, the total amount of scent emitted varied across the day, corresponding to the pattern of the diel foraging activity of pollinating bees on flowers of both sexes. The chemical composition of scents differed significantly between sexes. The two major compounds (*Z*)-methyl-p-methoxycinnamate and (*E*)-geranyl geraniol contributed most to this difference. This is the first case of sexual dimorphism reported in orchid floral perfumes. We discuss the influence of sex-specific floral scents on the behaviour of euglossine pollinators and offer new insights into the ecological and evolutionary significance of divergence in floral scents among dioecious plants.

**Keywords:** Catasetinae; euglossine bees; perfume-rewarding orchids; pollination

## An OR having previously unrecognized wide-field ligand affinities explains the behavioral activity of a new, cryptic, redundant sex pheromone component in the moth, *Ostrinia nubilalis*

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The odorant receptor OnubOR3 in the sex pheromone communication system of E-strain European corn borers, *Ostrinia nubilalis*, was found to be more broadly receptive to analogs of *O. nubilalis* natural pheromone components than had been anticipated. This wide-field pheromone OR and the OSNs it was expressed on responded to the longer-chain compound, (*Z*)-11-hexadecenyl acetate in addition to its natural 14-carbon pheromone component, (*Z*)-11-tetradecenyl acetate. Most importantly, (*Z*)-11-hexadecenyl acetate evoked high levels of upwind flight and pheromone source location in E-strain males when substituted for (*Z*)-11-tetradecenyl acetate as a minor component in the E-strain *O. nubilalis* pheromone blend. This behavioral result indicates that (*Z*)-11-hexadecenyl acetate now should be classified as a cryptic redundant minor pheromone component in the E-strain *O. nubilalis* sex pheromone system because it has been found in the gland but seems to have behavioral activity only when the normal minor pheromone component, (*Z*)-11-tetradecenyl acetate, is missing. Cryptic redundant pheromone components such as this might prove to be more prevalent than previously recognized, and may provide a new and more fluid, natural selection pathway toward the evolution of novel pheromone blends than had been previously considered.

**Keywords:** Behavioral bioassay, Biosynthetic enzymes, European corn borer, Mutual replaceability, Olfactory sensory neurons

## Fruit, flies and fungi – studies of *Drosophila suzukii* ecology for pest control

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Insects and microorganisms use fruit as a substrate for their development. We studied the ecological relation between the fruit-infesting dipteran pest, the spotted wing drosophila, *Drosophila suzukii*, yeast and mold. Behavioral assays with larval and adult *D. suzukii* showed that infestation of fruit is influenced by different factors related to fruit quality, presence of yeast or the development of mold. Interestingly, various of the factors that determine the suitability of fruit as a substrate for *D. suzukii* oviposition and development are interconnected. Understanding the ecological interactions underlying fruit infestation might help to develop tools for management of *D. suzukii*. Specifically, the association between *D. suzukii* and the yeast *Hanseniaspora uvarum* appears promising for application in pest management based on the strong attraction behavior induced by yeast odors. Based on our findings from the laboratory and field we think that formulations of the yeast *H. uvarum* could be of use for monitoring and control of *D. suzukii* in horticultural production.

**Keywords:** bait; egg-laying; fermentation; habitat; symbiosis

## The horseradish flea beetle selectively absorbs glucosinolates across the foregut

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The ability to accumulate (sequester) plant defense compounds for protection from predators is widespread in herbivorous insects. Yet, the absorption mechanisms for polar defense compounds that cannot passively diffuse through cell membranes are not well understood. We investigated the uptake of glucosinolates, the characteristic defense compounds in Brassicaceae, from the gut lumen into the hemolymph of the horseradish flea beetle (*Phyllotreta armoraciae*). By allowing beetles to ingest an aqueous mixture of different plant glucosides (including glucosinolates and non-host glucosides), we demonstrate a selective absorption of glucosinolates across the gut. In addition, beetles accumulated sinigrin, the major glucosinolate in their natural host plant horseradish, at a higher rate than other glucosinolates. Together, these findings show that glucosinolate absorption in *P. armoraciae* is highly selective. We next determined where exactly glucosinolates are absorbed from the gut lumen, and detected a much higher uptake rate across the foregut compared to the midgut. Since the foregut is of ectodermal origin and, therefore, lined with a chitin-containing cuticle, we hypothesized that morphological adaptations are necessary for glucosinolate uptake to occur. Indeed, we observed a massive reduction of the procuticle thickness and chitin content of the *P. armoraciae* crop in comparison to a non-sequestering leaf beetle. In summary, we show that physiological and morphological adaptations enable *P. armoraciae* to sequester plant defense compounds. Our results lay the groundwork for the identification of glucosinolate-specific transporters in the foregut.

**Keywords:** adaptation, cuticle, plant defense, sequestration, transporter

## Neuroethology of odor-guided behavior in the hawkmoth *Manduca sexta*

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Female hawkmoths, *Manduca sexta*, use olfactory cues to locate nectar sources and oviposition sites. We investigated how a large set of ecologically important and chemically diverse odors is coded in the antennal lobe (*in vivo* calcium imaging), which behavioral relevance the same odors have (wind tunnel experiments), and analyzed the correlation between activity levels of single olfactory glomeruli and the observed feeding and oviposition behavior. Next, we aimed at identifying odorant receptors that target behaviorally relevant glomeruli, and therefore expressed individual odorant receptors of the hawkmoth in the antennae of the vinegar fly *Drosophila melanogaster*. Among the 72 odorant receptors present in *M. sexta*, we selected a cluster of five odorant receptors that are *M. sexta*-specific, and therefore might play a key role in the life history of the moth. Using the same odors as before, we were able to correlate response profiles of these odorant receptors with those of feeding-related olfactory glomeruli. Furthermore, we used CRISPR/Cas9 to disrupt the expression of single odorant receptors, and study their impact on feeding and oviposition. Our results are a step forward towards unraveling the neuronal circuits underlying odor-guided behaviors in the hawkmoth.

**Keywords:** antennal lobe; deorphanization; foraging; odorant receptors; oviposition

## Perception and Response in Hybrid Aspen: Volatile-Induced Defence Traits

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It has been convincingly demonstrated that plants can detect and respond to volatile organic compounds (VOCs) infiltrating their environment. Sources of response-eliciting VOCs include the plant itself, neighbouring plants, and herbivores. Despite a wealth of information accumulating to document elevated resistance in VOC-receiving plants, questions remain about the degree of the receiver plant response, the scope and flexibility of VOC-encoded information, and the general ecological significance of such interactions. Using hybrid Aspen, *Populus tremula x tremuloides*, as a model plant, and *Phratora laticollis* as a model herbivore, we investigated volatile-mediated intra-plant, inter-plant and herbivore-plant interactions. A combination of laboratory studies and fieldwork was conducted to investigate responses of the receiver plants to VOCs from different sources. Analyses of a suite of metabolic responses provides evidence that receiver plants prime emission of VOCs and secretion of extrafloral nectar in response to cues indicative of a future herbivore presence. We will discuss these results in the context of perception of volatiles by plants and the influence of environmental complexity.

**Keywords:** Herbivore; plant-herbivore interactions; plant-plant interactions; priming; salicylaldehyde.

## Chemical & Physical Defenses of Conifers and Insect Counter Strategies

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Conifers have evolved complex oleoresin-based chemical defenses against herbivores and pathogens. In co-evolved bark beetles, the terpene components of the conifer oleoresin also serve various chemo-ecological functions as pheromone precursors, chemical barcodes for host identification, or nutrients for insect-associated microbiomes. This presentation highlights the genomic, molecular and biochemical underpinnings of the large chemical space of oleoresin terpenes produced by trees. While oleoresin terpenoid defenses have contributed much to the evolutionary success of conifers, under new conditions of climate change, these chemical defenses may become inconsequential against range-expanding forest pests. Another line of conifer defense against insects involves the deposition of stone cells as a physical barrier against stem feeding insect larvae. Physical stone cell defenses can provide a durable resistance, that may be difficult to overcome by insects.

**Keywords:** Terpenes; oleoresin; stone cells, bark beetle; weevil

## The sex pheromone of the pine brown-tail moth, *Euproctis terminalis* (Lepidoptera: Erebidae)

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The pine brown-tail moth, *Euproctis terminalis* (Walker 1855), is a periodic pest in pine plantations in South Africa. The larvae feed on pine needles and can cause severe defoliation when population densities are high. Population densities can fluctuate temporally and spatially, complicating the prediction of potential growth loss and tree mortality. The aim of this study was to identify the sex pheromone of the pine brown-tail moth to provide stakeholders with a tool for monitoring this pest. GC-EAD and GC-MS analyses of female pheromone gland extracts identified the major compound as (Z,Z,Z,Z)-7,13,16,19-docosateraen-1-ol-isobutyrate. Traps baited with (Z,Z,Z,Z)-7,13,16,19-docosateraen-1-ol-isobutyrate were more attractive to males than unbaited traps. The delta trap was shown to be a superior design compared to the bucket funnel trap. This pheromone can now be used for monitoring *E. terminalis* in pine plantations.

**Keywords:** Detection; Pest; Plantation; Traps; (Z,Z,Z,Z)-7,13,16,19-docosateraen-1-ol-isobutyrate.

## Unravelling the olfactory biases of male euglossine bees: Species-specific antennal responses and their evolutionary significance for perfume flowers

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In the communication system of euglossine bees, strong selective pressures are expected to act on the olfactory system, which could lead to sensory specialization in favor of an increased sensitivity to specific chemical compounds. The floral scents of “perfume flowers” are hypothesized to have evolved in response to the sensory biases of male euglossine bees and group according to pollinating genera. However, this has never been investigated on the sensory level of bee pollinators. Here, we present a comparative analysis using electroantennography (EAG) of males euglossine bees testing whether floral scent compounds characteristic to perfume flowers elicit stronger responses in the peripheral olfactory system of pollinators relative to non-pollinating species. In support of this hypothesis, we found that antennal response profiles differ among pollinating genera corresponding to compounds that are typically found in the bouquets of perfume flowers either pollinated by *Euglossa* (e.g. ipsdienol) or *Eulaema* bees (e.g. (-)-(E)-carvone epoxide). Moreover, we conducted a comparative phylogenetic analysis on the macroevolutionary patterns of antennal responses. The results resemble the findings described for sexual signaling in euglossine bees and indicate presence of a phylogenetic signal, stabilizing selection and a more rapid evolution of antennal response profiles than expected under a Brownian-motion model. Altogether, our results suggest that 1) euglossine bee species exhibit species-specific antennal responses differing among taxonomic groups, 2) antennal responses diverged early after speciation events, and 3) scent composition of perfume flowers evolved in response to pollinator-mediated selection imposed by pre-existing sensory biases in euglossine bees.

**Keywords:** Antennal responses; Electroantennography; *Eufriesea*; *Euglossa*; Euglossine bees, *Eulaema*; *Exaerete*; perfume flowers

## Total Synthesis of Tetrahydrofuran-Containing Marine Macrolides

**Robert Britton**

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Several structurally distinct families of tetrahydrofuran (THF)-containing marine macrolides have been reported that demonstrate potentially useful properties as antibiotics or anticancer agents. While these macrolides vary in ring size, substitution patterns and ‘warhead’ functionality, there are remarkable similarities in the configuration of the embedded THF, which plays a central role in determining the dominant macrocyclic conformation. In recent years we have developed several practical and robust synthetic methods to access configurationally unique THFs from enantiomerically enriched  $\alpha$ -chloroaldehydes. The application of these strategies in the total synthesis of the THF-containing marine macrolide biselide A will be presented. In addition, extensive calculations have probed the unoccupied, but related, chemical space adjacent to the privileged families of THF-containing marine macrolides. Our efforts to better understand the structural parameters that define this space and design a convergent synthetic approach to efficiently sample biologically relevant regions of this space will be presented.

**Keywords:** chemical space; macrocyclic polyketides; natural products; phenotypic screening; chloroaldehydes

## Attractants and filters in a wasp- and a bee-pollinated *Gomphocarpus* species

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Floral traits function as attractants for pollinators and as mechanisms that filter out non-beneficial flower visitors. We studied two closely related South African *Gomphocarpus* species, that are similar in their general floral architecture but differ in their main pollinators, vespid wasps and honeybees. Our experiments showed that the floral scent of *G. physocarpus* is highly attractive for wasps and characterized by acetic acid as a main component. The wasp-pollinated flowers offer freely accessible nectar that is avoided by honeybees, probably because of its toxicity. Isolated nectar components caused a high mortality in bees but not in wasps. In bee-pollinated *G. fruticosus* flowers, wasps cannot reach the nectar as it is covered by a morphological structure that makes it difficult for short-tongued insects to access the reward. Altogether, pollinator specialization seem to be due to a combination of floral filter mechanisms and scent cues that flower visitors can use to differentiate between the two species.

**Keywords:** chemical defense; floral scent; honeybees; nectar; vespid wasps

## Exploring the microbiota of *Saccharina latissima*

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*Saccharina latissima* is an abundant kelp-forming species of brown macroalgae in Europe. Its current distribution ranges from Spain to Spitzbergen, although several populations are in decline. Despite its economic and ecological importance, little is known about the composition of its microbiome and the interactions of the latter with the host. To explore the microbiome of *S. latissima* and to determine if specific patterns in microbial communities are correlated with algal fitness, we sampled both healthy and diseased (signs of degradation, twisted stipe, etc.) individuals from different geographic locations (Brittany, Helgoland, Southern Norway, and Svalbard) and in different seasons. For each sampled individual, we assessed the microbiome composition by 16S metabarcoding and isolated live microbial cultures for future co-culture experiments. Amplicon Sequence Variant (ASV) analyses highlighted the *Proteobacteria* (*Alpha*- and *Gammaproteobacteria*) as dominant phylum and showed a separation between the apex and meristem bacterial communities for all samples. Furthermore, several ASVs were specific to either healthy or diseased individuals. Bacterial cultures (381 isolates in total, belonging to four phyla, 16 orders, 45 genera) were dominated by *Firmicutes* and *Gammaproteobacteria*. Among these isolates, 48 were found exclusively on diseased individuals and 37 exclusively on healthy ones. These data contribute to our understanding of the variability of the *S. latissima* microbiome, establish a basis for ongoing experimental evaluations of algal-bacterial interactions, and serve for comparisons with other model systems such as *Ectocarpus*.

**Keywords:** Holobiont; brown macroalgae; kelp; metabarcoding



## Field experiments and simulations of semiochemical-based mass trapping and push-pull of *Euwallacea fornicatus*, an ambrosia beetle pest of avocado

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*Euwallacea fornicatus* (Eichhoff), the polyphagous shot hole borer (PSHB), is an ambrosia beetle (Coleoptera: Curculionidae) infesting avocado limbs in North America, Israel and South Africa. Quercivorol is attractive in the field to female PSHB (males flightless) over a release range of five orders of magnitude. A dose-response curve over this range fit a kinetic formation function that predicts trap catch or effective attraction radius (EAR) of the baited trap (0.126 mg/day = 1.18 m EAR). Sticky traps covering naturally infested limbs of living avocado trees were attractive to PSHB (EAR = 1.17 m). Ethanol released from 7.5 to 480 mg/day moderately inhibited PSHB attraction to quercivorol. A field test of potential inhibitors of quercivorol was done using ~1 mg/day releases of monoterpene ketones: (-)-(S)-verbenone, (+)-(R)-verbenone, 3-methyl-2-cyclo-hexen-1-one (MCH or seudenone), piperitone, (+)-(S)-carvone, and racemic cryptone. Only piperitone and the two enantiomers of verbenone were strongly inhibitory. A blend of piperitone and verbenone tested together at different distances (0, 0.5, 1, 2, and 4 m) from a quercivorol-baited sticky trap became increasingly ineffective in inhibiting attraction as separation distance increased. Due to the relatively short-range repellency (<1 m), inhibitors would need to be released from several places on each tree to effectively repel PSHB. Push-pull simulations of moving beetles were performed in 1 ha orchards with 2, 4, or 16 quercivorol-baited traps with 0, 1, or 3 inhibitors per tree. The simulations indicate that push-pull methods would be more effective in reducing PSHB mating than simply using mass-trapping alone.

**Keywords:** Quercivorol; verbenone; piperitone; repellents; attractants

## Impact of elevation and global warming on plant-pollinator relationships in the Eastern Himalayas

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Climate and environmental change can significantly impact biotic interactions such as plant-pollinator relationships. The Himalayas have been identified as one of the most vulnerable areas in the world regarding climate change impacts. In our study, we examined how elevation and warming impact floral odor profiles and pollinator response in the Eastern Himalayas across three elevations (3000, 3500 and 4000 m above sea level). We selected four alpine meadow flower species, and also conducted an *in situ* warming experiment on a fifth species, using open top chambers that simulate future global warming. We also compared pollinator-visitation rates across elevations for three of the species. Our results show that both elevation as well as warming significantly affected the quantity and quality of floral odor profiles. However, in a cross-species comparison, the floral odor profiles of a particular species were more similar to each other across elevations than to other species at the same elevation. Further, pollinator-visitation rates on each of the flower species did not vary significantly with elevation. We therefore conclude that while elevation-related environmental factors significantly alter floral odors, they do not affect total pollinator-visitation rates in these environments. This indicates the potential for constancy in perception of floral odors by pollinators in a diverse floral community in the face of environmental change. Our study has implications in understanding how floral odor profiles change in response to environment and contributes to our understanding of plant-pollinator interactions in a changing climate.

**Keywords:** climate change; climate warming; floral VOCs; perceptual constancy; tropical alpine meadows

## Toxicity and Repellency of Naturally Occurring Isothiocyanates against Red Imported Fire Ants

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Isothiocyanates (ITCs) are naturally occurring compounds that can be generated in the glucosinolate-myrosinase system in plants of the Brassicaceae family. Isothiocyanates play an important role in plant resistance against herbivore insects. In this study, contact and fumigation toxicities of four isothiocyanates including allyl isothiocyanate (AITC), 3-butenyl isothiocyanate (3BITC), 3-(methylthio) propyl isothiocyanate (3MPITC), and 2-phenylethyl isothiocyanate (2PEITC) were evaluated against the red imported fire ant workers, *Solenopsis invicta*. The LD50 value of AITC, 2PEITC and 3MPITC were 7.99, 2.36, and 2.09  $\mu\text{g}/\text{ant}$  respectively. In addition, AITC and 3MPITC also showed fumigation toxicity, but not 2PEITC. The LC50 values of AITC, and 3MPITC were 32.49 and 57.6  $\mu\text{g}/\text{mL}$ , respectively. In contrast, 3BITC didn't exhibit any contact and fumigation toxicity even at 74.9  $\mu\text{g}/\text{ant}$  and 100  $\mu\text{g}/\text{mL}$  respectively. Esterase (EST), glutathione S-transferase (GST) and acetylcholinesterase (AChE) inhibiting activities were also assessed for three ITCs in *S. invicta* workers. All three ITCs inhibited both EST and GST activities, but not AChE. The in vitro IC50 values of AITC, 2PEITC and 3MPITC for GST were 3.32, 0.61 and 0.66  $\mu\text{g}/\mu\text{l}$ , respectively. A mound drench formulation using 3MPITC was formulated and its efficacy was evaluated in the field.

Keywords: isothiocyanates; red imported fire ants; toxicity

## Controlling the Fall armyworm, *Spodoptera frugiperda*, using the push-pull system: an update

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The push-pull system for maize Lepidopteran stemborers is an important conservation biological control approach that protects crops from pest damage through semiochemically mediated insect behavioural manipulation. This manipulation is achieved by intercropping maize with companion plants that repel the pests and recruit parasitoid wasps together with peripheral companion plants that attract pests away from the maize crop. The recent invasion by the devastating maize pest fall armyworm, *Spodoptera frugiperda*, in Africa calls for urgent development of effective control strategies. Recent studies showed how the push-pull system, although originally developed for controlling *Busceola fusca* and *Chilo partellus*, substantially reduces crop damage. Here, I will give an update on the key semiochemically mediated mechanisms underlying the crop defence against fall armyworm within the push-pull system, and progress on the characterisation of volatile emission profiles for candidate trap plants for integration within the push-pull.

## 'Pushing' towards a new IPM strategy. *Drosophila suzukii*, the search for effective repellents

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*Drosophila suzukii* is a worldwide pest that causes severe economic losses to producers of soft and stone fruit. It has a highly serrated ovipositor enabling it to lay eggs in ripening fruit. The summer morph *D. suzukii* are highly attracted to fruiting crops. Winter morph *D. suzukii* overwinter in woodlands and hedgerows surround crops and then disperse into fruit crops at the start of the growing season. These studies aimed to identify semiochemicals that elicit a repellent response in both the summer and winter morphs. Electroantennograms were used to determine if the *D. suzukii* antenna could detect the 14 chemicals. Laboratory bioassays found seven of the chemicals repellent to the summer morphs, and five chemicals were repellent to the winter morphs. Four chemicals caused a repellent response in both morphs. Semi-field trials in polytunnels were used to examine if the potential repellents could reduce the number of *D. suzukii* attracted to fruit held in traps compared to a control. Three of the four tested chemicals reduced the number of *D. suzukii* caught in traps and eggs laid in sentinel fruits. The final experiment was conducted in a strawberry crop and demonstrated, for the first time, two chemicals that reduced *D. suzukii* oviposition in fruit. The repellent chemicals offer an additional control strategy to the integrated pest management of *D. suzukii*.

**Keywords:** Crop protection; Push-pull; Spotted Wing Drosophila; *Drosophila suzukii*; Integrated pest management

## Highly diverging effects of increased temperatures on floral scents of crop species

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Global temperatures are expected to increase in this century by up to 5°C. However, little is known about the effect of increased temperatures on floral scent emissions and chemical communication between plants and their pollinators. Here we analyzed flower scents of three insect pollinated crop plants (buckwheat, *Fagopyrum esculentum*; oilseed rape, *Brassica napus*; strawberry, *Fragaria x ananassa*) at two different temperature scenarios: physiological optimal and 5°C warmer than optimal temperatures. Floral scents were sampled by dynamic headspace and analyzed using gas chromatography coupled to mass spectrometry. The floral scents of buckwheat and oilseed rape were dominated by 2- as well as 3-methylbutanoic acid and p-anisaldehyde as well as linalool, respectively, no differences of total amount and scent composition between the two scenarios. In strawberry, however, both the amount and composition of scent was strongly affected by increased temperatures. In the warmer scenario the emission decreased eightfold and p-anisaldehyde was replaced by 1,4-dimethoxybenzene as the strongly dominating compound. Our results highlight that responses of floral scent emissions to increased temperatures are crop species-specific. As next step we will test in strawberry how the detected changes in scent emission influence attractiveness of flowers to pollinators.

**Keywords:** climate changes; chemical communication; crop pollination; floral volatiles

## Synthesis of Ruthenium and Magnesium-Flavonoid Complexes and their effect *in vivo* on Citrus with CVC

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We explored the antimicrobial potential of two newly synthesized antimicrobials complexes against *Xylella fastidiosa*. Several nuclear magnetic resonance (NMR) assays with high resolution and sensitivity were developed to identify new diastereoisomers in the context of octahedral ruthenium [Ru(narin)(phen)<sub>2</sub>]PF<sub>6</sub> and magnesium naringenin 5-alkoxide [Mg(narin)(phen)<sub>2</sub>]OAc complexes, obtained in the present work. The NMR assays proved to be powerful tools for the identification of isomers in metal complexes. Moreover, a protocol for the *in-vivo* determination of the effects of these complexes against *X. fastidiosa* was developed. The main trunks of *X. fastidiosa* infected plants were injected with the two complexes using a syringe; the number of bacterial cells in the plants following treatment was estimated via real-time quantitative PCR (qPCR). Importantly, the administration of both complexes drastically reduced the number of *X. fastidiosa* cells *in vivo*. Hence, the generation of novel antimicrobial complexes, like [Mg(narin)(phen)<sub>2</sub>]OAc, [Ru(narin)(phen)<sub>2</sub>]PF<sub>6</sub>, represent an important step in the long and costly process that is the development of new marketable compounds for crop protection.

**Keywords:** Xylella; Ruthenium; Magnesium; Flavonoid; Bactericidal

## Convergent recruitment of detoxification enzymes: The flavin-dependent monooxygenases of *Longitarsus jacobaeae*

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Toxic secondary plant compounds impose a strong selective pressure on herbivores for their tolerance or detoxification. Convergenly evolved strategies to deal with plant toxins are emerging across an increasing number of species. In this study we focus on the physiological adaptations of insects to detoxify pyrrolizidine alkaloids (PAs). The flea beetle *Longitarsus jacobaeae* is one of only a few animals specialized to feed on the tansy ragwort (*Senecio jacobaea*). This common wildflower is highly toxic, containing pyrrolizidine alkaloids, which function as a defense mechanism against mammalian and insect herbivores. Transcriptomic analyses of *L. jacobaeae* revealed two genes similar to known flavin-dependent monooxygenases (FMOs) and highly conserved in their predicted crystal structure. These enzymes have been recruited in two other groups of herbivorous insects as PA-specific detoxification enzymes that reconvert the alkaloids to non-toxic *N*-oxides. In our functional tests using heterologously expressed proteins of *L. jacobaeae* FMOs we tested the ability of both FMOs to *N*-oxidize senecionine, the common PA of *S. jacobaea*. Tissue-specific gene expression analyses suggest that FMO 1 is responsible for primary tissue-protection throughout the entire body, whereas FMO 2 is restricted to the intestinal and nervous tissue. Phylogenetic analyses support that, unlike the situation in arctiid moths, the gene duplication in the genus *Longitarsus* predates the adaptation to PA plants and that both genes were recruited as detoxification genes in the beetles.

## Using sublethal pesticide exposure to examine honest signaling properties of sexual communication in bees

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Honest signaling theory predicts that pheromones convey true and reliable information and evolved this way to limit fitness losses due to cheating. As such, individuals of lower fitness potential, whether due to “bad genes” or environmental stressors such as disease or pesticide exposure, are predicted to display a reduction in pheromone titer or detectable alterations to the chemical blend of the signal. Here, we put this theory to test by examining the impacts of pesticides exposure on mating behavior and honest signaling properties of sexual communication in the social bee *Bombus impatiens*. Mating behavior was examined in both sexes following an oral administration of imidacloprid, a common neonicotinoid. We further examined how pesticide exposure affects the production of sex pheromones in the male labial glands, the virgin queen mandibular glands, the cuticular hydrocarbons of both sexes, the antennal responses to these pheromones and the fitness physiological consequences in both queens and males. Our data show that males prefer queens not exposed to imidacloprid while queens show no preference against imidacloprid exposed males. This effect is not mediated by chemical signaling, either at the level of the production or perception. Metrics related to diapause capability in virgin queens (lipid content and cold tolerance) and sperm viability in males were examined to evaluate the fitness consequences of neonicotinoid exposure and are discussed in the context of honest signaling.

**Keywords:** Bumble bees; social insects; cheating; sex pheromones; neonicotinoids

## Tissue-specific volatile-mediated defense regulation in maize leaves and roots

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Herbivore induced plant volatiles (HIPVs) can mediate plant defenses in neighboring plants. Yet, whether this phenomenon occurs belowground remains unknown. Given (i) that roots can emit a distinct bouquet of volatiles upon herbivory, (ii) that some of these volatiles can diffuse in the soil, and (iii) that some root volatiles can be perceived by neighboring plants, we hypothesized that HIPV-mediated defense regulation may occur in the roots as well. We infested maize plants with the root herbivore, *Diabrotica balteata*, and investigated the physiological responses of neighboring plants. Exposure to root HIPVs did not alter constitutive nor herbivore-induced levels of defense-related gene expression, phytohormones, volatile and non-volatile primary and secondary metabolites, growth and herbivore resistance in roots. Cross-exposure experiments between roots and leaves HIPVs revealed that maize roots, in contrast to maize leaves, neither emit nor respond strongly to known defense-regulating HIPVs. Together, these results demonstrate that volatile-mediated defense regulation is restricted to the leaves of maize plants. This finding is in line with the lower diffusibility of volatiles in the soil and the availability of other, potentially more efficient information conduits belowground.

**Keywords:** belowground plant-herbivore interactions, maize, plant-plant interactions, priming, volatiles, root defenses

## Nocturnal bee pollination systems mediated by floral scents

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Bees are the most important pollinators of angiosperms in both natural and crop areas. While this is true and well known for diurnal bees, there is a large gap about the role of nocturnal bees (c. 250 species) as pollinators and how these bees recognize their host plants at night. We used a multidisciplinary approach to investigate the pollination and chemical communication between nocturnal bees and various of their host plants, among them the crop plant *Campomanesia phaea* (cambuci, Myrtaceae) together with several other Myrtaceae and *Paullinia cupana* (guarana, Sapindaceae). We found that the plants open their flowers at night, and if studied, depend on animal pollen vectors. Nocturnal bees of the genera *Megalopta*, *Megommation*, *Ptiloglossa*, and *Zikanapis* heavily visited the plants and were effective pollinators at least for cambuci and guarana. The flowers released mainly aromatics (e.g., 2-phenylethanol, benzyl alcohol), aliphatics (e.g., 1-octanol, 1-hexanol), and terpenoids (e.g.,  $\alpha$ -copaene, (*E*)- $\beta$ -ocimene, linalool), with strong differences but also similarities in scents among species. Synthetic mixtures resembling the volatile profiles of cambuci and guarana both successfully attracted nocturnal bee pollinators. Overall, we show that a) the scents of plants pollinated by nocturnal bees are highly variable, b) nocturnal bees are generalists that respond to various compounds, and c) the scent profiles overlap with scents of moth (and bat) pollinated plants. It is likely that many other plants also use this group of bees as effective pollen vectors and attract them by floral volatiles.

**Keywords:** Chemical signals, flight orientation, flower visitors, floral volatiles, crepuscular-night bees.

## Cuticular hydrocarbons (CHC) as recognition cues in thrips (Thysanoptera: Thripidae)

**Falko P. Drijfhout, Anca D. Covaci, Fail József, William D.J. Kirk**

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Thrips are high priority agricultural pest species causing damage to commercial crops of flowers and vegetables, and some are vectors for plant disease such as tospovirus. Thrips are amongst the fastest growing group of invasive species in the world. Identification of pheromones unique to each thrips species improves the understanding of when to apply pest controls and provides an alternative to pesticide by attracting these insects away from the crops.

7-Methyltricosane, identified as a contact pheromone in the western flower thrips (*Frankliniella occidentalis*), appears to act as a male recognition pheromone but the exact function is unclear. Research on the cuticular hydrocarbon on another high priority thrips species, *Thrips tabaci* (Lindeman) also known as the onion thrips showed that their CHC profile is very different to *F. occidentalis*. In addition, *T. tabaci*, exist as both arrhenotokous and thelytokous populations, with significant biological differences, which can result in different levels of damage to crops, or ability to transmit viruses. Significant differences were observed between the CHC profiles of these populations, which seem to link to their mating behavior. This indicates that CHCs could function as a recognition cue and play a role in thrips' mating behavior.

Result from these analyses, as well results from other thrips species will be presented and discussed to elaborate on the use of CHCs in biocontrol.

**Keywords:** cuticular hydrocarbons, thrips, mating, recognition cues, chemotaxonomy

## Next-generation cuticular hydrocarbon analysis in insects

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During the last 50 years there has been an exponential growth in the analysis of cuticular hydrocarbons in (eusocial) insects (Web of Sciences data analysis). Although in the 70s only a handful of papers were published, in the last few years 70-90 papers are published each year. You might expect that we have a significant level of understanding of the function of these compounds.

Yet according to the latest review of cuticular hydrocarbons in insects in 2020, several crucial questions are still unanswered to establish the exact function of cuticular hydrocarbons within insects. One of the bottlenecks identified is our inability to fully separate structurally similar hydrocarbons, e.g. methyl branched hydrocarbons with methyl groups located in the centre of the carbon chain. This inability leads to great challenges in assigning function(s) to individual compounds, if they indeed exist.

The use of GCxGC is quite common these days in the analysis of mineral and petroleum samples, but far less used in chemical ecology. In this presentation I will show results from analysing cuticular hydrocarbons from thrips, ants and flies using GCxGC-TOF-MS and discuss the benefits in using this type of analysis compared to one-dimensional GC-MS. Unless we substantially change the analysis of these cuticular hydrocarbons, and rely more on multivariate statistical analysis, many more papers may be published, but without gaining any further insight into their function.

**Keywords:** cuticular hydrocarbons, GCxGC, TOF mass spectrometry, separation

## Effect of ozone pollution on the chemical signal emitted by a Mediterranean tree to attract its specific pollinator

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Since the industrial time, the drastic increase in atmospheric pollution contributes widely to the present global change and causes real pressures on species interactions, especially between plants and pollinators. Among these pollutants, tropospheric ozone (O<sub>3</sub>) is a strong oxidizing agent that has the potential to affect the quality of the chemical signals emitted by plants. We tested if O<sub>3</sub> affects the attraction of pollinators towards their host plants via (i) an effect on the blend of Volatile Organic Compounds (VOCs) emitted by flowers and/or (ii) an alteration of the chemical signal during its lifetime in the atmosphere. Our working model is the interaction between the Mediterranean fig tree, *Ficus carica*, and its unique pollinator, *Blastophaga psenes*. To evaluate these effects we simulated O<sub>3</sub> pollution peaks under controlled conditions. Thanks to Gas Chromatography-Mass Spectrometry and Proton Transfer Reaction-Time of flight-Mass Spectrometry analyses we (i) quantified VOCs emitted by fig trees exposed to a high O<sub>3</sub> concentration during 5h and (ii) we determined VOCs reactivity with O<sub>3</sub> as well as the secondary organic compounds formed by ozonolysis. Our results show (i) a decrease in the abundance of some VOCs emitted by the fig trees, and particularly some of the compounds attractive for the pollinator, and (ii) the degradation of some of these compounds and the formation of new VOCs. Thus the proportions of VOCs that constitute the attractive chemical signal for the pollinator are drastically modified. We are currently evaluating if these changes can alter pollinator attraction towards figs.

**Keywords:** VOCs emission; ozonolysis; atmospheric pollution; specific interaction; chemical communication

## Nutritional endosymbionts' Shikimate pathways are susceptible to glyphosate inhibition

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Glyphosate is widely used as a herbicide, but recent studies begin to reveal its detrimental side effects on animals by targeting the shikimate pathway of associated gut microorganisms. However, its impact on nutritional endosymbionts in insects remains poorly understood. Here, we sequenced the tiny, shikimate pathway encoding symbiont genome of the sawtoothed grain beetle *Oryzaephilus surinamensis*. Decreased titers of the aromatic amino acid tyrosine in symbiont-depleted beetles underscore the symbionts' ability to synthesize prephenate as the precursor for host tyrosine synthesis and its importance for cuticle sclerotization and melanization. Glyphosate exposure inhibited symbiont establishment during host development and abolished the mutualistic benefit on cuticle synthesis in adults, which could be partially rescued by dietary tyrosine supplementation. Furthermore, phylogenetic analyses indicate that the shikimate pathways of many nutritional endosymbionts likewise contain a glyphosate sensitive 5-enolpyruvylshikimate-3-phosphate synthase. These findings highlight the importance of symbiont-mediated tyrosine supplementation for cuticle biosynthesis in insects, but also paint an alarming scenario regarding the use of glyphosate in light of recent declines in insect populations.

**Keywords:** Bacteroidetes; cuticle; insect decline; *Oryzaephilus surinamensis*; symbiosis

## Benzaldehyde mediates host colonization by the peach bark beetle, *Phloeotribus liminaris*

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The peach bark beetle (*Phloeotribus liminaris* Harris, PBB) affects the health, quality, and value of black cherry (*Prunus serotina* Ehrh.) within the Central Hardwoods Forest Region of North America. When colonized by adult beetles, black cherry trees produce a defensive exudate, or 'gum', staining the wood and decreasing its value up to 90 percent. Current management tactics are inadequate to avoid extensive damage to most veneer-sized black cherry in the region. We test the hypothesis that PBB colonization behavior is chemically-mediated and determine the extent to which PBB is attracted to compounds associated with wounded or PBB-infested cherry wood. Through olfactometer and field bioassays, we determined that adult PBB were attracted to cherry branches infested with female beetles. We then used dynamic headspace sampling to collect and identify volatiles associated with wounded and infested bolts of black cherry. The volatile benzaldehyde dominated these collections and was more abundant in aerations of female-infested bolts than other odor sources. In subsequent field assays, we evaluated the bioactivity of benzaldehyde, as well as  $\alpha$ -longipinene, in combination with several chemical carriers. Traps baited with benzaldehyde captured more PBB than all other treatments, irrespective of other lure components. Moreover, PBB were not attracted to traps baited solely with ethanol, a common attractant for bark beetles that colonize hardwood trees. This is the first report of benzaldehyde as an attractant for a species of bark beetle and could aid in developing semiochemical-based management tactics for this important pest.

**Keywords:** attractant; cherry; management; *Prunus*; trapping



## Tick Repellents: Modulators of Electrophysiological and Behavioural Activities

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Understanding tick olfaction has relevant implications in animal and human health for the development of repellent alternatives. The effects on tick chemosensory system of long-term exposure to repellents is unknown, and no studies have investigated potential long-term disruption and inhibition of tick abilities to detect hosts. We investigated whether pre-exposure to repellents impact the chemosensory system of ticks. We recorded the electrophysiological response of *Ixodes scapularis* female ticks to a known attractant and host volatile (i.e., butyric acid), before (i.e., pre-) and after (i.e., post-) exposure through fumigation to selected known repellents (i.e., lemongrass, DEET). Behavioral Y-tube bioassays were performed to evaluate potential changes in attraction post-exposure to butyric acid. After 20 minutes of fumigation, exposure to repellents significantly inhibited tick's ability to detect host volatiles by decreasing electrophysiological and behavioural activities. These results demonstrate repellents can act as inhibitors, modulating tick chemosensory activity, and provide important information in better understanding mode of action of tick repellents.

**Keywords:** *Ixodes scapularis*; tick chemosensory system; essential oil; exposure; inhibition

## The role of quinolizidine alkaloids from *Genista* plants (Fabaceae) in aphid-plant interactions

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The pea aphid (*Acyrtosiphon pisum*), native to legume plants, comprises at least 15 genetically different biotypes, each adapted to just one or a very few different host plants. Only on its respective host plant, an aphid biotype can perform well. One of these biotypes is specialized on *Genista* plants, known to contain quinolizidine alkaloids (QAs). These compounds normally stimulate and paralyze the central nervous system of insects, ultimately leading to their deaths. This raises the question what enables *Genista* biotype aphids to perform on this chemically defended plant? Before answering this question, we needed to identify the most abundant QAs within extracts of *Genista tinctoria*. This was done using NMR spectroscopy and high-resolution mass spectrometry. Eight QAs were found, one of which new for *Genista* species and one previously unknown. To investigate whether the aphids are exposed to these, we evaluated the compounds' distribution within the aphid-plant system. The identified QAs were quantified by LC-MS/MS in *G. tinctoria* phloem sap (the aphids' food), leaf and stem extracts, as well as in *Genista* biotype aphid tissue and honeydew. Additionally, cross-sections of stems and leaves from *G. tinctoria* were investigated by MALDI-MS supported by microscopy and histochemical staining. Only a few of the identified QAs are present within the *G. tinctoria* phloem sap and, in turn, in the aphids and their honeydew. Additionally, we observed translocation of particular compounds into the aphids' hemolymph. These findings provide the basis for further investigations how aphids of the *Genista* biotype are able to avoid poisoning by QAs.

**Keywords:** *Acyrtosiphon pisum*; alkaloids; chemical defense; *Genista tinctoria*; sequestration

## Sexual signalling in a widow spider – Unravelling contact and airborne pheromone components and the role of an enzyme in their dissemination

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Females of web-building spiders deposit sex pheromone onto their web. Volatile pheromone components attract males, whereas contact pheromone components induce courtship behavior by males. Yet, only 12 spider pheromones have been identified thus far and the mechanisms underlying the release of airborne components are still not known. Here we studied pheromonal communication of the globally invasive building-dwelling false black widow spider, *Steatoda grossa*. Using gas chromatography-mass spectrometry (GC-MS), we analyzed methanol web extracts of sub-adult, adult virgin and mated females as well as males. We found seven compounds that were unique to virgin adult females, but none elicited courtship behavior by males. We then fractionated methanol web extract of virgin females by high-performance liquid chromatography (HPLC) and bioassayed each HPLC fraction for pheromonal activity. Compounds in bioactive fractions were isolated by HPLC for nuclear magnetic resonance (NMR) spectroscopy. Based on combined information of HPLC mass spectra and NMR spectroscopy spectra, we identified and synthesized three candidate pheromone components. The synthetic blend of these three components was as effective as methanol web extract in inducing courtship behavior by males. Using proteomics coupled with activity assays, the contact pheromone components were found to be enzymatically hydrolysed, resulting in the release of volatile pheromone components. Synthetic replica of these airborne components were tested in building hallways and shown to attract male spiders. Our study unravels the remarkable complexity and intricacy of pheromonal signalling in a web-building spider.

**Keywords:** metabolomics; mate calling; *Steatoda grossa*; pheromone; identification

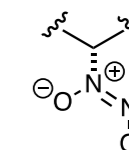
## NONO compounds: A New Chemical Language in Bacteria?

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Bacteria exchange information based on chemical signals in a process sometimes referred to as quorum sensing. This process has been demonstrated to be often central in different areas such as virulence, differentiation, luminescence, pathogenicity, etc. However, over the last decades, there have been only a few classes of compounds characterized. In this communication, we will report on the isolation and characterization of NONO compounds, so called diazeniumdiolates, from Gram-negative bacteria and their putative function as chemical signals. Akin to the discovery of a new language, we will discuss the structural features of the signal, and the effect on the receiving end. Evidence for a broader distribution of these signals in various eukaryote/bacterial systems will be presented.

Diazeniumdiolates



**A New Chemical Language  
in Bacteria?**

**Keywords:** Quorum sensing; chemical biology; organic synthesis; plant-microbe interactions; natural products

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## Impact of benzoxazinoid-dependent plant-soil feedbacks on plant performance and food quality within a crop rotation

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Plants are constantly interacting with myriads of soil-born microorganisms. Secondary metabolites that are exuded by plant roots alter the microbiota in and around the roots, which can affect the performance and resistance of the next plants grown in the same soil. How plant-soil feedbacks that are mediated by secondary metabolites influence the productivity of agricultural systems is not well understood. In an agronomically realistic two-year field experiment we evaluated the impact of maize benzoxazinoids on the performance and food quality of three winter wheat varieties. We found that maize benzoxazinoid soil conditioning increases wheat biomass accumulation as well as the final yield. In addition, insect infestation was reduced on wheat growing in benzoxazinoid conditioned soil. Analysis to elucidate possible effects on wheat food quality are in progress. Understanding how the exudation of secondary metabolites affects the performance of subsequent crops will help to assess their potential to promote sustainable agriculture in the context of plant-soil feedbacks.

**Keywords:** agricultural productivity; maize; plant-microbe interactions; secondary metabolites; wheat

## Field-scale effects of air pollution on invertebrate community composition in wheat

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Air pollution has the potential to affect invertebrates both directly (e.g. physiologically) and indirectly (e.g. via changing plant chemistry of host plants or by disrupting volatile organic compounds used in communication). This study determined the effects of two common air pollutants, diesel exhaust emissions (including nitrogen oxides – NO<sub>x</sub>) and ozone (O<sub>3</sub>), both individually and interactively, on the community composition of predominantly ground-active invertebrates within a field of winter wheat over two years. Pitfall traps within 8-meter diameter fumigation rings were used to collect invertebrates, which were then taxonomically identified and characterized by functional group (i.e. predator, parasitoid, herbivore, fungivore, detritivore or pollinator), diet specialization (i.e. generalist or specialist-feeding) and the presence of wings. Taxonomic richness and Shannon's diversity were calculated from the most precise taxonomic resolution identified. Moderate increases in the levels of NO<sub>x</sub> (diesel treatment) and O<sub>3</sub> had adverse impacts on invertebrate community composition, with greater declines in invertebrate abundance and taxonomic richness observed under diesel exhaust-pollution compared with O<sub>3</sub>-pollution. For both pollutants in combination, the effect on invertebrate abundance and taxonomic richness was reduced compared with diesel pollution alone, likely due to the reaction between atmospheric NO<sub>x</sub> and O<sub>3</sub>. Moreover, specialist-feeding and winged invertebrate species responded more negatively to air pollution treatments than generalist feeders and wingless species, respectively. Understanding how invertebrate groups respond to multiple interacting air pollutants such as diesel exhaust and O<sub>3</sub> facilitates predictions into how terrestrial environments will respond to future changes in anthropogenic emissions, especially as we shift away from fossil fuel dependence.

**Keywords:** Free-Air Diesel and Ozone Enrichment (FADOE); functional group; ground-active invertebrates; insect ecology

## Effect of *Drosophila suzukii* on blueberry VOCs: chemical cues for a pupal parasitoid

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Biocontrol agents such as parasitic wasps use long-range volatiles and host-associated cues from lower trophic levels to find their hosts. *Drosophila suzukii* (Diptera: Drosophilidae), also known as the spotted-wing drosophila (SWD), is a highly polyphagous fruit pest native to eastern Asia and recently arrived in South America. The aim of our study was to characterize the effect of SWD attack on the volatile organic compounds (VOCs) of blueberries, a common host fruit, and to correlate these odor changes with the olfactory-mediated behavioral response of resident populations of *Trichopria anastrephae* female parasitoids. Using fruit VOC chemical characterization followed by multivariate analyses of the odor blends of SWD-attacked blueberries, we showed that the development of SWD immature stages inside the fruit generates a different odor profile than control fruits (physically damaged and free of damage). These differences can be explained by the diversity, frequency and amounts of fruit VOCs. The behavioral response of female parasitic wasps showed that *T. anastrephae* has an innate attraction to volatile cues from infested host fruits, which may lead to successful location of their insect host. SWD infestation of blueberries resulted in an increase of typical volatile compounds that other *Drosophila* species use. It is then likely that *T. anastrephae* females use these same general odorant cues to locate their established drosophilid hosts. Since resident parasitoids are able to locate this novel potential host, biological control programs using local populations may be plausible and convenient as a SWD control strategy.

**Keywords:** SWD; *Trichopria anastrephae*; olfactometer; volatile cues; biocontrol agents

## Flies, flowers and fermentation

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Pollination systems involving floral mimicry are fascinating study arenas with which to explore floral phenotype evolution, pollinator sensory perception and behavior, and the interaction of the two. In most cases, the flower's chemical mimicry of the model's volatile chemistry is a key component of the mimic's pollination success. Floral mimicry of fermenting substrates is not well-documented, yet an increasing number of studies identify fermentation-associated volatiles in floral scents. Furthermore, naturally-occurring fermenting substrates (fruit, sap, etc.) attract a diversity of flies, beetles, and other insects which could be utilized as dupes (pollinators) by a flower mimicking fermentation. We investigate floral mimicry in *Asimina triloba* (Annonaceae), a woody species with spring-blooming, maroon, yeasty-smelling flowers that are pollinated by fermentation-loving species of Diptera. While the unusual floral phenotype and associated insect taxa suggest that the flower is mimicking a non-floral fermenting substrate, we seek to demonstrate (1) the occurrence of an ecologically-relevant model upon which the mimic is based, and (2) that the visitor identity and behavior at the model overlaps with the visitor identity and behavior at the flowers. Here we present data documenting the overlap of floral phenotype with several ecologically-relevant models. We also present data on pollinating insects and insect visitors to local fermenting substrates.

**Keywords:** *Asimina triloba*; Diptera; floral mimicry; pollination; volatiles

## Sex pheromone components and potential sex recognition cuticular hydrocarbons of yellowjackets

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Yellowjackets in the genera *Vespula* and *Dolichovespula* are prevalent eusocial insects of great ecological and economic significance, but only few compounds mediating sexual communication and sex recognition have been identified. The first part of the presentation will focus on the identification of sex attractant pheromone components of virgin bald-faced hornet queens (*Dolichovespula maculata*). We analyzed body surface extracts of queens by coupled gas chromatography–electroantennographic detection (GC-EAD), isolated the compounds that elicited responses from male antennae by high-performance liquid chromatography (HPLC), and identified these components by GC-mass spectrometry (MS), HPLC-MS, and NMR spectroscopy. In olfactometer experiments, synthetic (2Z,7E)-3,7-dimethyldeca-2,7-diendioic acid and (2Z,7E)-10-methoxy-3,7-dimethyldeca-10-oxo-deca-2,7-dienoic acid in binary combination attracted bald-faced hornet males. The second part of the presentation will provide evidence that cuticular hydrocarbons (CHCs) of four yellowjacket species (*D. maculata*; *Vespula squamosa*; *V. pensylvanica*; *V. alascensis*) differentiate sex, caste, and nest membership. We extracted cold-euthanized queens, gynes, workers, and males from 35 nests with pentane, and analyzed extracts by GC and GC-MS to identify and quantify CHC constituents (aliphatic alkanes and alkenes; mono-, di-, and tri-methyl-branched alkanes). To determine whether caste and sex differ in CHC profiles of wasps, we performed linear discriminant analyses. Wasps from each of the four species clustered into their respective groups (queens, gynes, workers, males), with significant differences in group centroids. Workers and males from most species also clustered according to nest. These data inspire future studies to determine the definitive role(s) that gyne- and male-specific CHCs play in the context of sexual communication.

**Keywords:** caste; *Dolichovespula*; gynes; sexual communication; *Vespula*

## Nanofibers as carrier of push substances against pear psyllids

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Pear decline (PD) is a bacterial disease that causes considerable financial losses for growers in pear-growing areas in Europe. Fighting this disease requires a reliable and inexpensive method for protecting orchards against pear psyllids, which are the major responsible of its transmission. The need for alternatives to insecticides has stimulated studies on the use of plant essential oils and plant extracts against pest insects. But, little is known about the efficacy of essential oils and their specific volatile organic compounds (VOCs) as repellents against the PD vectoring pear psyllid *Cacopsylla pyri*, although they may provide a safe alternative to conventional insect control. The major drawback of VOCs use in field conditions is the lack of long-lasting systems for dispensing repellent substances for crop protection. Thus, we investigated nanotechnology as an innovative tool for delivery systems of VOCs. We evaluate the repellent and/or deterrent activity of Cinnamon bark *Cinnamomum zeylanicum* and Cedarwood *Juniperus mexicana* essential oils on female psyllids. In Y-shaped olfactometer bioassays, *C. pyri* females avoided the olfactometer arm containing essential oil of Cedarwood and Cinnamon bark, while more than 70% and 80% preferred the control arm, respectively ( $P < 0.01$ ). Subsequently we formulated the essential oils in nanofibers made of biodegradable polymers as dispensers and evaluated their release performance and the longevity of the nano-encapsulated repellents. In this study, we discuss the development of nanoformulations to enable the use of VOCs for push-and-pull systems to reduce migration and proliferation of psyllids in fruit orchards, preventing new infections by phytoplasmas.

**Keywords:** Nanotechnology; Essential oils; volatile organic compounds; Repellents; Chemical ecology

## Tritrophic interactions between plant pathogens and their vector insects mediated by primary and secondary plant metabolites

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Phloem feeding insects from the genus *Cacopsylla* (Hemiptera: Psyllidae) are often vectors of plant pathogenic bacteria (phytoplasmas) causing severe diseases in fruit crops. We compared two closely related pathosystems, *Cacopsylla picta*-apple tree-apple proliferation phytoplasma and *C. pruni*-stone fruit-ESFY phytoplasma, regarding the behavior (oviposition, feeding) and fitness (mortality, developmental time, size) of the respective vector insect mediated by metabolites of infected and uninfected plants.

The influence of host plant infection status onto feeding behavior of the respective vectoring insect was assessed by electropetrography (EPG). Phytoplasma infection in the apple pathosystem resulted in changes in feeding and oviposition behavior of *C. picta*, whereas no behavioral changes were recorded in the stone fruit pathosystem. Analyses of both primary (amino acids, sugars) and secondary metabolites (sesquiterpenes) in the host plants by gas chromatography coupled with mass spectrometry revealed significant differences in both apple odor and phloem composition induced by the phytoplasma, but no changes in the stone fruit. Additionally, the fitness of *C. picta* but not of *C. pruni* was negatively influenced by phytoplasma infection of their host plant. The impact of the results for the understanding of the evolution of phytoplasma-vector pathosystems and for the application in plant protection are discussed.

**Keywords:** *Candidatus* phytoplasma; psyllid vector; EPG; phloem composition; VOC

## Why so variable? Polyploidy and floral volatiles in the coevolving, nursery pollinated *Lithophragma bolanderi* (Saxifragaceae)

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Floral volatiles important for highly specialised, coevolving plant-pollinator interactions are expected to be under strong stabilising selection. A recent study on the coevolutionary interaction between the plant genus *Lithophragma* (Saxifragaceae) and its highly specialised nursery pollinator *Greya politella* (Prodoxidae), however, found exceptionally high floral volatile variation among and within species. Although in most *Lithophragma* species, floral volatiles were more similar among geographically close populations and in populations interacting with the same pollinators, neither was the case in the highly variable species *L. bolanderi*. In this study, we investigate the effects of whole-genome duplications (polyploidisations) – the most dramatic mutation and a potential cause for diversification – on floral volatile variation. We collected seeds from natural populations throughout the distribution range of *L. bolanderi*, grew them in a greenhouse common garden environment (n = 29 populations, >1500 individuals) and then collected floral volatiles and quantified genome size. We found three major ploidy types: diploids, tetraploids, and hexaploids. We present data on the covariation of complex floral traits, such as floral volatiles, with the geographical distribution of ploidy types, and how this variation is linked to the geographic mosaic of specialised and generalised pollinators found in *L. bolanderi*. These results provide insights into the proximate and ultimate causes of the diversification of floral traits within species.

**Keywords:** floral evolution; geographic mosaic; plant-pollinator interactions; trait diversification; whole-genome duplication

## Volatiles of *Capsicum annuum* L. as attractant source for *Lasioderma serricorne* F. (Coleoptera: Anobiidae)

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The cigarette beetle, *Lasioderma serricorne* F. (Coleoptera: Anobiidae), is an important stored product pest increasingly impacting museums and herbaria. Monitoring methods make use of pheromone traps which can be implemented using chili fruit powder as food attractant. In detail, recent studies evidenced that *Capsicum annuum* fruit powders elicited the strongest attraction among other *Capsicum* species. In this study we present the results of laboratory and trap bioassays aimed at identifying the chemical cues involved in this attraction in order to select candidate semiochemicals for their use as co-attractant in pheromone traps. *Capsicum annuum* fruit powder was extracted and fractionated for chemical analysis and olfactometer experiments, which evidenced that the attraction is solely determined by the polar fraction. Further olfactometer tests indicated that the attractive effects toward the adults of the beetle is determined by the main components of the fruit powder (i.e.  $\alpha$ -ionone and  $\beta$ -ionone). Based on these data, a trap bioassay was carried out in a bread industry with pheromone traps implemented with  $\alpha$ -ionone or  $\beta$ -ionone at different doses, in order to compare the recorded captures with those obtained in traps loaded with the commercial formulation of the synthetic sex pheromone alone. The results obtained showed as the traps baited with the combination of pheromone and  $\beta$ -ionone captured significantly more individuals than those loaded with pheromone alone or to pheromone traps implemented with  $\alpha$ -ionone.

**Keywords:**  $\alpha$ -ionone;  $\beta$ -ionone; Cigarette beetle; chili; pheromone traps

## Pheromone-based mating disruption: From conceptualization to commercialization

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The possibility of controlling insects by dispersing large amounts of sex attractant into a crop in an effort to interfere with mating was first demonstrated for the cabbage looper, *Trichoplusia ni*, over 50 years ago. Advances in analytical and synthetic chemistry facilitated pheromone identification and synthesis for many insect species. The availability of synthetic insect pheromones stimulated a groundswell of research confirming the practical application of pheromone-mediated mating disruption. Commercialization of mating disruption, however, lagged the research. The first registered product in the United States was for the pink bollworm, *Pectinophora gossypiella*, in 1978. Registrations for additional species came slowly for at least a decade, but then accelerated in the early 1990s following regulatory relief by the US EPA. Through the combined efforts of researchers, private industry, extension educators, and others, a viable and expanding mating disruption industry is now well established. Worldwide, in excess of 1 million hectares are currently treated with this novel method of control. Mating disruption products have largely been developed for control of lepidopteran species targeting fruit, forest, cotton and vegetable pests. This presentation will review the key developments that led to mating disruption becoming an efficacious and commercially accepted control tactic. Progress in understanding moth behavior and other biological characteristics that influence the outcome of pheromone-based control programs were instrumental. This includes an improved understanding of the principal mechanisms responsible for mating disruption. Many crucial advances were technical or operational in nature, such as the development of devices that could deliver the optimal release of pheromone in space and time and new tools for monitoring pest populations in crops treated with mating disruption technologies. Commercial adoption of mating disruption was to a great extent driven by new regulations governing the use of insecticides, increasing environmental concerns, food and worker safety issues, and the regular occurrence of resistance to chemical controls. The presentation will conclude with an attempt to identify key areas of research needed to further enhance opportunities for successful mating disruption.

**Keywords:** application, control, mechanisms, pest management, sex attractant

## Bark beetle-associated fungi assist in the detoxification of host flavonoids and provide a fitness benefit the Eurasian spruce bark beetle

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Bark beetles are a major cause for the decline of conifer forests world-wide which are a sink for atmospheric carbon dioxide, and hence an important buffer against climate change. Bark beetles are associated with ophiostomatoid fungi which are thought to assist them to successfully complete their life cycle. The Eurasian bark beetle, *Ips typographus*, transports its associated fungi to a new host which establish in the tree when the beetle tunnels through the phloem to lay its eggs. The tree responds to beetle attack by producing resins which are defensive and can deter bark beetles from persisting in the host. However, it is not known if other host compounds also contribute to the tree's defense against *I. typographus*. Inoculations of Norway spruce saplings with the bark beetle-associated fungus, *Endoconidiophora polonica*, revealed that it responds to fungal infection by producing high concentrations of the flavonoids, catechin and taxifolin. These compounds reduced the tunneling rate and weight gain of *I. typographus* when applied *in vitro*. Furthermore, bark beetles avoided tunneling in substrates with high concentrations of catechin. However, this behavior was reversed when *E. polonica* was established on the medium first. *In vitro* growth assays of *E. polonica* revealed, that the growth of the fungus was also retarded by catechin and taxifolin, but that it could detoxify these compounds. The beetle's preference for substrates colonized by its associated fungi may indicate that fungal detoxification of flavonoids is beneficial to the beetle and contributes to its fitness during attacks on chemically well-defended hosts.

**Keywords:** taxifolin; catechin; *Endoconidiophora polonica*; flavonoids biosynthesis; tree defence

## Alcohol boosts fly attractiveness

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Attraction of vinegar flies, *Drosophila melanogaster*, towards alcohols, especially ethanol, has been extensively studied. Here, we show that there exists a simple yet vital biological rationale for alcohol contact by these insects. Flies display active attraction to alcohol-rich fruits and to pure alcohols, especially methanol, which results in an amplification of pheromone levels. Exposure to alcohols in turn elevates the probability that a male will successfully compete for a female. We identify three types of olfactory sensory neurons that detect ethanol and methanol. Moreover, we reveal their role in both attraction and aversion, where valence is balanced around mating status.

**Keywords:** *Drosophila*, olfaction, chemical ecology, alcohol, pheromone



## Queen pheromone(s) in higher termites: a long quest with a happy end

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Queens of advanced social insects maintain their reproductive dominance over sterile colony members using queen pheromones. While the empiric support for queen pheromone existence in termites is available for over half a century, little is known on the chemistry of these putative pheromones: the queen pheromone identified in 2010 in the lower termite *Reticulitermes speratus* remains the only termite queen pheromone characterized as yet. Current knowledge suggests that in small societies of socially primitive termites, the information on queen presence and fertility may be mediated via queen-specific signatures of cuticular hydrocarbons on the body surface of queens. By contrast, in socially advanced species, including higher termites, the queen pheromone is expected to be volatile so as to ensure the queen monopoly in the populous colonies inhabiting large nests. Indeed, the queen pheromone of *R. speratus*, living in large colonies, is a volatile blend. In my talk, I will overview our search for queen pheromones in higher termites, the most diversified and advanced clade of termites, during the past decade. I will show evidence of queen-specific volatiles in multiple South-American species of higher termites, including identification and synthesis of these queen pheromone candidates. I will show that these volatiles are secreted by the queens in high enantiomeric purity and in quantities correlated with the level of their fertility. And finally, I will report on the first experimental evidence that queen-specific volatiles in higher termites really do act as queen pheromones and inhibit the reproductive potential of nestmates.

**Keywords:** Termitidae; primer pheromone; queen dominance; (3*R*,6*E*)-nerolidol; (5*Z*,9*S*)-tetradec-5-en-9-olide

## Using Plant Odours for Early Detection of Disease: A Case Study in Bananas

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Plant diseases reduce the production and quality of food, fibre and biofuel crops. Globally almost 16% of all crops are lost to disease every year. Early detection of plant disease is vital to control spread and limit impacts on horticultural industries. Recently, several studies have investigated the use of odour for detection of disease, e.g. sniffer dogs to detect citrus greening disease (huanglongbing) in Florida and a smartphone-based VOC-sensing platform for detecting late blight in tomato. Panama disease (Fusarium wilt) is the most devastating disease of bananas of modern times, caused by the soil-borne fungus *Fusarium oxysporum* f.sp. *cubense* (Foc). When grown on rice, race 4 isolates of Foc that are infective in 'Cavendish' bananas produce distinctive odours, which are not produced by race 1 isolates to which 'Cavendish' bananas are not susceptible. We previously described how headspace volatiles from 'Lady Finger' bananas inoculated with Foc sub-tropical race 4 (STR4), were different from controls, before external symptoms were evident. The same was not true for 'Cavendish' bananas inoculated with Foc STR4. Fungal odours were detected in the corm of both varieties when they were destructively sampled. Here we describe studies on the impact of infection with the more aggressive strain of Foc (tropical race 4 - TR4), and the patterns of plant and fungal odours produced in glasshouse and field trials. Foc TR4 has rapidly spread through banana production worldwide and now threatens the major exporting countries in Central America. Early detection of the disease is critical to containment strategies.

**Keywords:** fungi, Fusarium wilt, GC-MS, *Musa*, volatiles

## Revolver flowers fool freeloaders – pollination strategies of trap and non-trap flowers in Stapeliinae (Apocynaceae)

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The Stapeliinae comprise outstanding floral diversity and complexity with multiple shifts from pitfall (*Ceropegia*) to non-pitfall (*Brachystelma*, stapeliads) flowers and emerging evidence of diverse chemical mimicry strategies. This diversity of form and strategies is, somewhat paradoxically, driven by a single, albeit functionally diverse pollinator group – flies. Understanding the evolution and diversification in Stapeliinae requires detailed understanding of both pollination ecology and mechanism (pollinator specificity), as well as identification of cues pollinators use to locate flowers. Thus far, studies have focussed on *Ceropegia* pitfall flowers where evidence exists that floral chemistry plays a key role in pollinator attraction and in achieving pollinator specificity. Many species are pollinated by Diptera with kleptoparasitic habits, i.e. they are freeloaders on prey of predatory arthropods. These flies locate such food sources using volatiles released by the prey when attacked or freshly killed. Some *Ceropegia* species chemically mimic injured insects to lure freeloader flies into their pitfall flowers and use them as non-rewarded pollinators. This remarkable strategy called kleptomyiophily seems widespread among *Ceropegia* though other strategies, i.e. yeast mimicry, have also been discovered. Recent studies on non-trapping *Brachystelma* species have revealed different strategies to *Ceropegia*, with some species mimicking carrion or dung and attracting different groups of fly pollinators. Intriguingly, however, some species also attract kleptoparasitic flies of the same genera as are attracted by *Ceropegia* species. Chemical and electrophysiological analyses suggest that the basis of initial attraction is similar to that in *Ceropegia*. The presence of rewards in *Brachystelma* seems to further promote successful pollination.

**Keywords:** chemical mimicry; deception; fly pollination; kleptomyiophily; strategy-shifts

## Success in progress; mating disruption for navel orangeworm (*Amyelois transitella*) is becoming part of the foundation for IPM in California nut crops

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Trécé, Inc.

The navel orangeworm, *Amyelois transitella* (Walker), is a generalist that attacks mature fruit of a wide variety of horticultural crops. It is the key pest of almonds and pistachios, both are among the 10 most valuable crops in California. Mating disruption for navel orangeworm (NOW) was first demonstrated to be an effective tool for damage reduction in almonds in the early 2000's. Because only the relatively unattractive major sex pheromone component ((Z,Z)-11,13 - hexadecadienal ) is used for disruption, a non-competitive mechanism is likely. A commercial product was introduced in 2005. From 2005-2015 it was used in a relatively low number of acres (3-6% of 700k ha of almonds and pistachios). The cost of this technology was initially an impediment, but as global demand increased, almond and pistachio grower returns also increased. At the same time, damage thresholds for NOW decreased. Insecticide programs in high pressure situations are often not able to reduce NOW damage below current damage tolerances. However, when NOW MD is used in combination with insecticides, results have been better than either approach used separately. An estimated 200,000 ha of almonds and pistachios were under MD for NOW in 2019 and it is projected that MD for NOW will be adopted in an additional 50,000 ha in 2020.

**Keywords:** Almonds; mating disruption; navel orangeworm; pistachios; sex pheromone

## Age matters: gene regulation in the mosquito antenna during the first gonotrophic cycle

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Mosquito behaviours associated with maturation, feeding and reproduction directly affect vectorial capacity. Odours mediate each phase of the gonotrophic cycle from host blood seeking through post-blood meal refuge seeking, oviposition site seeking and oviposition, and finally to post-oviposition reassertion of host seeking, while sugar seeking continues throughout. The age- and blood meal-induced regulation of chemosensory, neuromodulatory and other signal transduction genes was investigated in the antenna of female mosquitoes during maturation (1 and 3 days post-eclosion), and subsequently every 24 h from 5 to 10 days post-eclosion from two cohorts: 1) non-blood fed and 2) blood fed. Using a transcriptomic approach, we observed a concerted regulation of multiple genes within the sensory pathways of the antenna, which likely play a key role in modulating the behavioural changes observed with age and post-blood meal. Functional characterization of the proteins generated by the genes-of-interest identified in this study may provide a better understanding of the regulation of gonotrophic feeding and a pool of potential targets for vector control strategies.

**Keywords:** gene expression; olfaction; transcriptomics; blood feeding; host seeking

## Effects of water stress on floral scent, morphology and reproductive success

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Reduced precipitation and drought events can alter floral traits with cascading effects on flower-visitor interactions and plant fitness. However, the interplay of magnitudes of water stress on floral traits, especially scent and petal color, flower-visitor interactions and reproductive success are poorly understood. In a field experiment, we tested how reduced mean precipitation and prolonged dry periods affect floral scent bouquet, morphology, phenology, color, flower-visitor interactions and seed set. Plant individuals of multiple mother plants were randomly assigned to either of three treatments: mean precipitation, reduced mean precipitation and drought treatment. Individuals were planted under five rain-out shelters, in which each shelter served as a replicate. Plants in the precipitation treatments were watered daily using a drip irrigation system, whereas plants in the drought treatment were only watered when they showed severe signs of wilting. We found that the effects on floral traits were dependent on the strength of water stress – measured as leaf water potential– with increasing stress resulting in e.g., smaller growth and lower number of flowers. However, total scent emission per flower was not affected by water stress and plants were able to maintain scent bouquets and flower-visitor interactions, what retained the pollination success of the existing flowers. Nevertheless, as water stress reduced the number of flowers, which in turn led to fewer siliques and ultimately reduced seed set, we could show that water stress indirectly reduces plants' reproductive success.

**Keywords:** Brassicaceae; climate change; drought; floral traits; plant-pollinator interactions

## Chemical characterization and interspecific variation assessment of sexually dimorphic mental glands in three species of turtles (family Geoemydidae)

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Intraspecific chemical signaling in tortoises and turtles (i.e. chelonians) is little-known compared to other taxa such as insects or mammals. However, chelonians have a well-developed chemical sense that has the potential to detect chemosignals emitted by conspecifics. We studied chelonian mental glands (also called chin glands), i.e. organs situated in the gular part of the neck that are likely involved in the production of chemosignals. We examined the chemical composition of mental gland secretions using gas chromatography–mass spectrometry (GC-MS) in three species of turtles. First, chemical differences between male and female mental glands were examined in the turtle *Mauremys leprosa* in a recent study published by the authors. The main chemical compound classes found in mental gland exudates included carboxylic acids, carbohydrates, alkanes, steroids and alcohols. Many of these compounds were shared by males and females and just a few compounds were present in higher amounts in one of the sexes. We speculate that compounds found in relatively large amounts in males compared to females could be involved in sexual communication in *M. leprosa* but bioassays are needed to confirm the functionality of these. In addition, new data on mental gland chemistry from two other turtle species (*Cuora amboinensis* and *Siebenrockiella crassicollis*) is provided here. Preliminary comparisons show that many compounds are shared across species suggesting that interspecific differences may occur just in few compounds. Data on a larger number of species is necessary to robustly assess how specific chemical signals are across distinct turtle taxa.

**Keywords:** turtle, mental glands, GC-MS, lipids, secretions

## Malaria mosquitoes take the piss

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Feeding on the secretions or the excrement from vertebrates, behaviours included in the broader term ‘puddling’, has been described in a range of herbivorous and detritivorous terrestrial arthropods, but to date not in hematophagous insects. Supplementary feeding by females of the malaria mosquito *Anopheles arabiensis* on cattle urine, a rich source of nitrogen compounds, differentially enhances survival, flight behaviour and reproduction, an effect dependent on the age of the urine. These results extend our current understanding of which nutrients are limiting to these disease vectors. Female *An. arabiensis* are attracted to cattle urine odour, a behavioural response modulated by the age of the urine, reflecting the observed effects on life history parameters. Chemical, electrophysiological and behavioural analyses allowed us to identify bioactive volatile compounds, which when presented as blends in their natural ratio reproduce the behavioural response to differently aged cattle urine. One of these blends attracts both host-seeking and blood fed mosquitoes in the field. When assessed in a large-scale longitudinal study, a village equipped with outdoor traps baited with this blend, captured substantial numbers of mosquitoes leading to >60% and >80% reduction of indoor host seeking and resting *An. arabiensis*, respectively, and ca. 60% reduction of malaria prevalence compared to that of the control village. As a whole, this study is a proof of principle that an increased understanding of the ecology of disease vectors can lead to effective control measures of vectors and the disease they transmit.

**Keywords:** puddling, *Anopheles*, chemical ecology, attraction, vector control

## Molecular and neural plasticity in the sex pheromone response of the corn earworm (*Helicoverpa zea*)

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The corn earworm (*Helicoverpa zea*) is a major crop pest of the Americas. Larvae feed primarily on corn and sorghum in addition to various other commercial crops, resulting in billions of dollars in agricultural losses annually. Mating disruption using sex pheromones emitted by mature females to attract males provides an effective alternative control method to broad-spectrum pesticides. Males detect pheromones using olfactory receptors (ORs) on trichoid sensilla located on their antennae, with each sensillum containing two olfactory sensory neurons (OSNs). Particular ORs are sensitive to specific pheromone components. Research suggests that pre-exposure of moths to higher concentrations of pheromone components could cause alterations in the expression of corresponding ORs, OSN sensitivity, and mating behaviors. Our research investigates if such alterations are induced by pre-exposure to different concentrations of conspecific or heterospecific pheromone components over different time periods. Isolated, 2-4-day-old moths were exposed to different concentrations of conspecific or heterospecific pheromone components for 1 or 24 hours. Antennae were then removed and used for either electroantennograms to measure the sensitivity of OSNs or quantitative PCR to detect differences in OR gene expression between conditions. This research will provide insight into mechanisms underlying insect olfaction and potentially improve pheromone-based pest control methods.

**Keywords:** electroantennograms; gene expression; mating disruption; neuroplasticity; quantitative PCR

## Chemical interaction between the sugarcane spotted borer *Chilo sacchariphagus* (Lepidoptera: Crambidae) and a dead-end trap plant

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Sugarcane yield losses due to the sugarcane spotted stem borer, *Chilo sacchariphagus*, are reduced when plants of *Erianthus arundinaceus*, a close relative to sugarcane, are dispatched at the border of sugarcane fields. Previous studies concluded in a dead-end trap plant type of mechanism, since female borers preferentially lay their eggs on *E. arundinaceus* and the subsequently emerged larvae are unable to reach adulthood. We explored the chemical mechanisms involved in this plant-insect interaction. 71 compounds were identified through mass spectrometry in host and trap plant emissions at dusk (n=2x6). Among them, seven compounds, shared between the emissions of the two plants, elicited a significant electroantennographic detection in female borers (n=2x9, bootstrap p<0.05). This result suggests that *C. sacchariphagus* hardly distinguishes between the two plant species. In addition, the terpene (E)- $\beta$ -ocimene was solely observed in *E. arundinaceus* emissions (bootstrap p<0.001), elicited a dose-dependent antennal response (n=10, Wilcoxon's rank sum test p<0.01) and an attraction of female *C. sacchariphagus* in Y-tube bioassays (n=13, bootstrap p<0.001). Our data suggest that a marginal sensory difference is sufficient to induce a preference for egg-laying between two plants with otherwise similar emission profiles. We will discuss the evolutionary implication of this mechanisms, and why we believe that it might result in long-term protection of the crops. This work was published in J Chem Ecol (2021). <https://doi.org/10.1007/s10886-020-01240-z>.

**Keywords:** Stem borer; Trap crop; GC-EAD; GC-MS;  $\beta$ -ocimene

## Biosynthesis of aggregation pheromone component in spruce-killing bark beetle *Ips typographus*

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The main problem in the European forestry in the last decade is the epidemic population of European spruce bark beetle (*Ips typographus*, Coleoptera) causing the destruction of host habitat, Norway spruce, *Picea abies* forests. The successful aggregation of *Ips typographus* on host tree and ability to overcome the defense is triggered by the possessing of the potent aggregation pheromone. Active pheromone compounds (mainly 2-methyl-3-buten-2-ol and *cis*-verbenol) are produced by the pioneering males while invading the host tree and starting to bore in. Understanding the major precursors of the pheromone biosynthesis and molecular mechanisms over the beetle's developmental stadia is the straightforward approach to possible regulation of the aggregation behaviour. We chose a several methodological approaches to describe this process. We constructed dynamic production curve of pheromone components, intermediates and associated metabolites by the metabolomics analysis of different developmental beetle stadia by the GC/MS and UPLC/MS. We correlated transcriptomes of *Ips typographus* tissues in various life stadia using Differential Gene Expression (DGE) analysis with aim to target key genes involved in biosynthesis of main pheromone components and intermediates and we validated its regulation level by qRT-PCR.

**Keywords:** *cis*-verbenol; 2-methyl-3-buten-2-ol; European spruce bark beetle; terpene biosynthesis

## Long-horned beetles and single orchids: discovery of a new class of sexual mimicry in plants

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Sexual mimicry is a reproductive strategy used mainly by plants with flowers that imitate the mating partners of animals, in order to exploit them as pollinators. This form of deception is known mostly from orchids that exploit Hymenoptera or Diptera as pollinators. We recently discovered a case of floral sexual mimicry that involves the deception of male longhorn beetles (Cerambycidae). The Cape endemic orchid *Disa forficaria* is pollinated by males of a single species of beetle to which the flowers bear an uncanny resemblance. Male beetles show copulatory behavior on the flowers and microscopic examination of flowers showed that they ejaculate sperm onto the hairy tip of the floral lip. Using GC-EAD and mass spectrometry techniques we identified and then synthesized a single novel macrolide that is electrophysiologically active and highly attractive to male beetles in field bioassays. Structure-activity studies confirmed that chirality and other aspects of the structural geometry of the macrolide are critical for attraction of the male beetles. The orchid itself is extremely rare with only 11 individuals having been recorded in the past two centuries.

**Keywords:** Cerambycidae, chemical synthesis, GC-EAD, pheromone, pollination

## ***Helicoverpa zea* Caterpillars Manipulate Volatile Emission from Maize Through Salivary Glucose Oxidase Activity**

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The glucose oxidase (GOX) effector in the saliva of many herbivorous caterpillars may induce or reduce the emission of volatile organic compounds (VOCs) from host plants. However, the ability of this effector to either induce or decrease VOC emissions appears to be largely dependent on the plant itself. Since maize-produced green leaf volatiles (GLVs) and indole prime neighboring conspecifics and herbivory-induced terpenes act as signals for parasitoids, we examined the influence of GOX on the emission of maize GLVs, indole, and terpene volatiles. Maize treated with synthetic GOX emitted a lower quantity of GLVs and higher quantities of induced terpenes compared to PBS treated control plants. A similar pattern of volatile emission was observed from maize treated with salivary gland homogenates from wild-type *Helicoverpa zea* (with high GOX activity) and CRISPR-CAS9 gene edited *H. zea* unable to produce GOX. Interestingly, the synthetic GOX treatment did not induce indole from maize, while the application salivary gland homogenate from wild-type and mutant *H. zea* induced indole emission. Mediation of volatiles by GOX is likely mediated partially through stomatal closure as has been shown in several crop systems. The ramifications for GLV emission reduction through *H. zea* GOX activity are unknown in the context of the GLV-indole priming signal to nearby maize plants. Additionally, increased terpene emission from GOX-treated maize has unknown effects on the ability of parasitoids to learn the volatile signal emitted from caterpillar-damaged maize. Further study will examine the time-course of terpene synthase transcript induction with GOX treatment.

**Keywords:** Glucose oxidase; *Helicoverpa zea*; insect effectors; volatiles; plant-insect interactions

## **Coral larval settlement preferences linked to crustose coralline algae with distinct chemical and microbial signatures**

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The resilience of coral reefs is dependent on the ability of corals to settle after disturbances. While crustose coralline algae (CCA) are considered important substrates for coral settlement, it remains unclear whether coral larvae respond to CCA metabolites and microbial cues when selecting sites for attachment and metamorphosis. This study tested the settlement preferences of an abundant coral species (*Acropora cytherea*) against six different CCA species from three habitats (exposed, subcryptic and cryptic), and compared these preferences with the metabolome and microbiome characterizing the CCA. While all CCA species induced settlement, only one species (*Titanoderma prototypum*) significantly promoted settlement on the CCA surface, rather than on nearby dead coral or plastic surfaces. This species had a very distinct bacterial community and metabolomic fingerprint. Furthermore, coral settlement rates and the CCA microbiome and metabolome were specific to the CCA preferred habitat, suggesting that microbes and/or chemicals serve as environmental indicators for coral larvae. Several amplicon sequence variants and two lipid classes - glycolipids and betaine lipids - present in *T. prototypum* were identified as potential omic cues influencing coral settlement. These results support that the distinct microbiome and metabolome of *T. prototypum* may promote the settlement and attachment of coral larvae.

**Keywords:** *Acropora cytherea*; coral-algal interactions; coral recruitment; metabolome; microbiome

## Bioassay and mass spectrometry-guided identification of constitutive plant metabolites associated with the feeding preference of the *Eucalyptus* snout beetle, *Gonipterus* sp. n. 2.

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*Gonipterus* sp. n. 2 is one of three invasive cryptic species within the *Gonipterus scutellatus* species complex (Gyllenhaal, 1833 (*Curculionidae*, *Coleoptera*). These weevils, native to Australia and Tasmania, first invaded South Africa in 1916, and since then have spread to nearly every continent. Once *Gonipterus* sp. n. 2 infests a plantation, they can cause severe defoliation. These beetles feed selectively on young leaves and new shoots, and show a strong preference for certain *Eucalyptus* genotypes. Since the underlying cause of this host preference is poorly understood, we conducted laboratory choice assays to determine the relative levels of susceptibility of 59 *Eucalyptus* genotypes from 22 species to feeding by *Gonipterus* sp. n. 2. This revealed large intraspecific variation in susceptibility which exceeded the interspecific variation. Therefore, we selected 27 genotypes from *E. dunnii*, *E. grandis* x *urophylla* hybrids and *E. nitens* for detailed metabolite profile analysis. Using linear regressions, feeding behaviour was plotted against metabolite abundance, which identified ten highly correlated compounds. Eight of these compounds were tested for their ability to alter the behaviour of these beetles using a newly developed standardized artificial diet in an *in vitro* feeding preference assay. This revealed three phagostimulants (1,8-cineole, oxalic acid, and sucrose) and two deterrent compounds (shikimic acid and palmitic acid) for *Gonipterus* sp. n. 2. These results were then confirmed under field conditions, using 12 unique genotypes. Results from this study have the potential of being used as quantitative traits for resistance to *Gonipterus* sp. n. 2 in commercial *Eucalyptus* breeding programs.

**Keywords:** artificial diet; feeding behaviour; feeding deterrent; phagostimulant; plant-insect interactions

## Codling Moth Mating Disruption and the Mechanistic Principles Underpinning its Success

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Over the last thirty years mating disruption (MD) of codling moth (CM), *Cydia pomonella*, has emerged as a success story for applied chemical ecology and the pheromone industry. Research in Switzerland (1976-1987) laid the ground work for CMMD using reservoir-type dispensers, but when the first commercial CMMD product was registered in North America (1991), little was known about the underlying mechanisms by which it worked, or ways to improve its efficacy. At the time, several mechanisms for disrupting pheromone communication had been proposed, but postulating mechanisms proved easier than finding definitive proof they were important, or actually occurred. The need to understand the mechanisms underpinning CMMD was borne out of necessity rather than design, as the rapid commercial adoption of CMMD, a result of area-wide projects in 1990's, far outpaced our understanding of the technology. Ultimately, it was an understanding of insect behaviour, ecological mechanisms, and field assessments of the relative efficacy of various commercial products which helped researchers defend their support for one technology over another. Using a combination of field and laboratory tools, both behavioural and physiological, research eventually revealed the importance and interrelationship of point source density and dispenser release rates in eliciting competitive attraction, desensitizing moths, and maximizing CMMD. This understanding was later codified into mathematical models that defined competitive attraction and disruption. Today, CM is one of the best, if not only examples of where an understanding of the behavioural, physiological, and ecological principles underpinning MD have improved implementation and helped to optimize formulation technologies.

**Keywords:** attraction; behaviour; *Cydia pomonella*; pheromones; physiology



## Outsourcing immunity: Evolution, chemical ecology, and genomics of protective insect-bacteria symbioses

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Symbiotic associations with microbes are important driving forces of evolutionary innovation. Several insects engage in symbiotic alliances that protect their immature stages against pathogen infection through the production of antimicrobial compounds. I will report on novel findings of symbiont-provided antibiotic defense in beewolf wasps and darkling beetles, discussing the ecological dynamics and evolutionary implications of defensive symbiosis, as well as the genomic consequences for the bacterial partners.

**Keywords:** mutualism, *Streptomyces*, *Burkholderia*, antibiotic, entomopathogen.

## The success of the push-pull system for controlling lepidopterous pests and parasitic striga weeds in small-holder cereal farming

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The 'push-pull' system ([www.push-pull.net](http://www.push-pull.net)) effectively controls serious biotic constraints to cereal-livestock production in Africa, stemborers, fall armyworm, and striga weed, while improving soil health and biodiversity. It allows income diversification with sustainable livelihood components such as livestock farming. The companion cropping system makes smallholder farms more resilient often with a tripling of yields and has now been made more climate smart. It involves attracting stemborers with trap plants (pull) whilst stemborers and fall armyworm away from the main crop using a repellent intercrop (push). Chemicals released by intercrop roots induce abortive germination of the noxious parasitic striga weed. The companion plants provide high value animal fodder year-round, facilitating milk production. Furthermore, soil fertility is improved due to improved nitrogen fixation, carbon sequestration and phosphorus availability, reduced soil erosion and degradation. The technology improves gender equity and is appropriate for smallholders, and economical as it is based on locally available plants, not expensive external inputs. It fits well with traditional mixed cropping systems in Africa. The push-pull system has been adapted to drier and hotter conditions linked to climate change by identification and incorporation of drought tolerant companion crops. This climate-smart push-pull directly responds to rising uncertainties in Africa's rain-fed agriculture due to the continent's vulnerability to climate change. The new companion crops, *Desmodium intortum* and *Brachiaria* Mulato II hybrid, can withstand extended periods of drought stress with no water. To date push-pull has been adopted by over 250,000 smallholder farmers in 18 sub-Saharan African countries whose maize yields have increased from about 1 t/ha to 3.5 t/ha. Low-input technologies that address several production constraints and deliver multiple benefits are more relevant for African smallholder farmers but also proves useful lessons for agricultural systems in the developed world.

## Postharvest detection of infested fruit: The solution is hanging in the air

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The false codling moth (FCM), *Thaumatotibia leucotreta*, is a major pest of citrus in southern Africa, with zero tolerance in certain export markets. Nondestructive postharvest detection of the pest in fruit would greatly reduce the risk of market interceptions. Volatiles from infested and healthy fruit were effectively captured by Solid Phase Microextraction (SPME). Post extraction, compound detection was achieved by Gas Chromatography-Mass Spectrometry (GCMS). GCMS analysis was conducted on five major volatile compounds of interest, previously shown to be released by FCM infested oranges, namely D-limonene, 3,7-dimethyl-1,3,6-octatriene, (E)-4,8-dimethyl-1,3,7-nonatriene, caryophyllene and naphthalene. With Navel, Midnight and Valencia oranges, D-limonene levels decreased with time after infestation, while levels of a naphthalene derivative increased. The ratio of these compounds was significantly different between healthy and infested fruit 7, 14, 21 and 28 days after infestation. No differences were found in caryophyllene levels. Trials conducted on Clementine Mandarins showed an increase in beta-Ocimene levels with time after infestation, where levels were undetectable in the control fruit. Speed of detection is critical for practical application, so Select Ion Flow Tube Mass Spectrometry (SIFT-MS) was examined to evaluate volatile emissions, as it can measure emissions from 12 fruit simultaneously in real time. This technique could clearly differentiate between healthy fruit and fruit injured 24 hours prior to evaluation. The presence of D-Limonene could be identified after a few seconds of SIFT-MS analysis following 20 seconds of headspace collection. These techniques show potential for development of a system for online detection of infested fruit in a packing facility.

**Keywords:** citrus; Gas Chromatography-Mass Spectrometry, Select Ion Flow Tube Mass Spectrometry; false codling moth.

## Learning of feeding and oviposition cues in a hawkmoth

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The tobacco hawkmoth *Manduca sexta* nectar feeds from and oviposits on solanaceous plants including several *Nicotiana* and *Datura* species. Both, feeding and oviposition, are (at least partly) driven by innate attraction towards flower and/or leave volatiles. However, here we show, that the preference for specific flowers as well as for specific host plants is affected by learning. Moths can e.g. target a flower in the absence of innately attractive odours just by visual cues. If the moth then while feeding from this flower experiences an innately non-attractive odour, it will associate it with nectar reward after single-trial learning and will later follow this odour to target the next flower. Similarly, moths that have already oviposited on a given hostplant will later always prefer the odour of this host plant over odours of other host plant species. Interestingly, although the moths exhibit olfactory sensilla both on the antenna and the tip of the proboscis, only the antenna is involved in this odour learning process.

**Keywords:** flower constancy, host plant choice; *Manduca sexta*; olfaction; Anthropocene.

## Sub-Saharan Africa, a new home for *Drosophila suzukii*

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Native to Southeastern Asia, *Drosophila suzukii* is dispersing rapidly as a world-wide invasive pest of soft-fruits. With the serrated ovipositor, females lay eggs into the pulp of soft-skinned fruits which contributes to a high host plasticity. Additional features such as high fecundity and low temperature tolerance were helping *D. suzukii* to expand its territories to the Americas and Europe during the last 1-2 decades. Consequently, an overwhelming number of studies have been conducted to understand the biology and ecology of *D. suzukii*. While *D. suzukii* was reported in Morocco (North Africa), no similar account has been made in Sub-Saharan Africa. The suitability of the Sub-Saharan region to support the establishment of *D. suzukii* was already predicted in 2015. In 2019 and 2020, we therefore investigated if *D. suzukii* was present in Kenya, by carrying out a monitoring survey using traps baited with a blend of apple-cider vinegar and red wine. By catching *D. suzukii* in both years, we provide empirical evidence of the presence of *D. suzukii* in Kenya indicating its on-going dispersal into new territories. Currently, we are studying the possibility to disrupt *D. suzukii* behavior by use of semiochemicals. We will share preliminary results from our on-going laboratory assays.

**Keywords:** Expansion; global; invasive species; management; spotted-wing drosophila.

## Do we choose our partner based on smell?

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Partner choice is a crucial decision in human life. Among factors affecting partner preferences are parental characteristics remembered from childhood via sexual imprinting. Existing studies suggest that people tend to couple with partners similar to their opposite-sex parents, especially when they judge their early relationship with the parent as positive. We tested this hypothesis in fathers and long-term partners of 65 women, using perceived similarities of body odors between parents and actual partners and by instrumental comparison of their volatiles. When assessed by independent raters, axillary body odors of fathers and actual long-term partners of the women showed strongly significant perceived similarity when compared to randomly established father-partner pairs. Independently, we compared the axillary volatiles using comprehensive two-dimensional gas chromatography coupled with mass spectrometry (GC×GC-TOFMS) on a subset of 41 father-partner pairs, sampled separately in the left and right axilla (altogether 164 samples). Using a newly developed “tile-based” chromatographic alignment algorithm and optimized inclusion rules for individual analytes (signal to noise ratio and F-ratio comparison with blank samples), we retrieved the multiple thousands detected analytes to a set of 341 compounds characteristic for men’s axillary odor. These analytes covered the spectrum of typical human-emitted volatiles, such as fatty acids, alcohols, aldehydes, esters, sterols, wax esters, and others. We used multidimensional comparison of the relative abundances of these volatiles to establish “chemical distances” between all pairs of subjects and observed that genuine father-partner distances are highly significantly shorter than randomized father-partner pairs.

**Keywords:** axillary odor; chemical distances; GC×GC-TOFMS; human volatiles; partner choice

## Communication ecology of pest *Limonius* species (Coleoptera: Elateridae) in North America

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Wireworms, the larval form of click beetles (Elateridae), are significant agricultural pests of cereals, potato, corn, vegetable, sugarcane and other crops worldwide. In North America, four *Limonius* species cause severe economic losses, notably *L. californicus*, *L. canus* and *L. infuscatus* in western regions and *L. agonus* in eastern regions. Recently, (*E*)-4-ethyloct-4-enoic acid ('limoniic acid') was identified as the female sex pheromone for both *L. californicus* and *L. canus* and sex attractant for *L. infuscatus* and *L. agonus*. High numbers of male *Limonius* spp. beetles were captured in pitfall traps baited with limoniic acid deployed in the field. The three western species often co-occur, and since limoniic acid is highly attractive to them all, the mechanisms that impart species-specificity need to be determined so limoniic acid can be used effectively in pheromone-based monitoring or management tactics. Here, we present results from field studies conducted to determine if differences (or shifts) in seasonal occurrence periods and/or diel periodicity function as female tactics that impart heterospecific mating behaviour. Moreover, as some heterogeneric wireworm species co-occur and inflict similar crop damage in western North America, we tested whether the pheromones of various *Agriotes* spp. and *Limonius* spp. can be combined in a single lure without reducing trap captures of target species.

**Keywords:** Wireworms; click beetles; pheromone-based monitoring; integrated pest management; insect chemical communication

## Pheromone identification by SSGA – an analytical method using circadian rhythms of volatile emissions

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The SSGA (Sequential SPME/GCMS analysis) method uses circadian rhythms of volatile emissions for identifying insect pheromones. The history/background in development of the SSGA method will be presented along with its advantages compared to other established methods. The usefulness of the SSGA method and how it can be implemented to gain circadian information on semiochemicals and pheromones will be demonstrated with examples of different pest insects such as moths, fruit flies, and mealybugs. I will also show how these volatiles can be further tested for their biological activity in order to optimize effective attractants.

**Keywords:** Circadian rhythm; SPME; moth; mealybugs; fruit flies

## Silencing the alarm: An insect salivary enzyme closes plant stomata and inhibits volatile release

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Herbivore-induced plant volatiles (HIPVs) are widely recognized as an ecologically important defensive response of plants against herbivory. Although the induction of this “cry for help” has been well-documented, only a few studies have investigated the inhibition of HIPVs by herbivores, and little is known about whether herbivores have evolved mechanisms to inhibit the release of HIPVs. To examine the role of herbivore effectors in modulating HIPVs and stomatal dynamics, we conducted series of experiments combining pharmacological, surgical, genetic (CRISPR-Cas9) and chemical (GC-MS analysis) approaches. We show that the salivary enzyme, glucose oxidase (GOX), secreted by the caterpillar *Helicoverpa zea* on leaves, causes stomatal closure in tomato (*Solanum lycopersicum*) within 5 min, and in both tomato and soybean (*Glycine max*) for at least 48 h. GOX also inhibits the emission of several HIPVs during feeding by *H. zea*, including (Z)-3-hexenol, (Z)-jasmonol, and (Z)-3-hexenyl acetate, which are important airborne signals in plant defenses. Our findings highlight a potential adaptive strategy where an insect herbivore inhibits plant airborne defenses during feeding by exploiting the association between stomatal dynamics and HIPV emission.

**Keywords:** effector; HIPV; insect herbivore; plant defense; stomata

## The ionotropic co-receptor IR8a is required for the expression of robust sexual behavior in males of the Chagas disease vector *Rhodnius prolixus*

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The ionotropic receptor co-receptor IR8a is involved in diverse chemosensory-driven behaviors in arthropods. It has been reported as necessary for host-seeking in mosquitoes, foraging in *Drosophila melanogaster*, and shown to have a significantly higher antennal expression in males of the copepod *Eurytemora affinis*. Increased antennal expression of IR8a was also recently reported for male *Rhodnius prolixus*. Our current results show that significantly decreased *RproIR8a* expression (RNAi) affects the sexual performance in males of this species. We significantly knocked-down the expression of *RproIR8a* in male adults and subsequently used them to test their sexual responses. Firstly, *RproIR8a* knocked-down males showed a significantly decreased tendency to leave shelters in the presence of females in comparison to intact males. Furthermore, a lower proportion of *RproIR8a* knocked-down males approached females in the same experiment. A second experiment showed that *RproIR8a* knock-down induced a decreased mating frequency in single pair assays. This is the first study to incriminate *IR8a* in insect sexual behavior. Future studies should determine which sensory processes affected by *RproIR8a* knock-down relate to the negative effects here described in males of this species.

**Keywords:** triatomines, sex, pheromones, receptors, olfaction

## Anthraquinones produced by crinoids allow host selection for the symbiotic snapping shrimp *Synalpheus stimpsonii*

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Quinones are molecules produced by various organisms, including feather stars (Echinodermata, Crinoidea). Besides being one of the major pigment groups that provide their bright colors, they can also act as defensive molecules that make crinoids unpalatable and even repellent to most organisms. Conversely, these organisms are also known to shelter an important associated fauna, such as the ectocommensal snapping shrimp *Synalpheus stimpsonii*. Around the Great Reef of Toliara (Madagascar), *S. stimpsonii* can be found in association with two different species of crinoids: *Comanthus wahlbergii* and *Phanogenia distincta*. Our study investigated the chemical cues that allow host selection for this symbiont. The chemical attractiveness of the two crinoid hosts and a non-host species, *Cenometra bella*, was tested in an olfactometer. The three crinoids released cues in the ecosystem that were attractive for *S. stimpsonii*. Analyses of purified *P. distincta* extracts by mass spectrometry highlighted three kinds of anthraquinones (*i.e.* rhodoptilometrin, 3-propyl-1,6,8-trihydroxy-9,10-anthraquinone and a new crinoid anthraquinone). This mixture of anthraquinones seems specific to this species, as suggested by the current literature. Moreover, when tested in the olfactometer, these extracts induced a similar attractive behavior on the shrimp. Same results were also observed with an addition of pure commercial anthraquinones. Therefore, we suggest that crinoid anthraquinones are not confined to their traditional defensive function, but also act as kairomones involved into the host selection in the symbiotic association between *S. stimpsonii* and *C. wahlbergii* or *P. distincta*.

**Keywords:** Marine chemical ecology; Kairomones; Feather stars; Olfactometry

## Catabolism of branched chain and aromatic amino transferases, route to fusel alcohols and acetates by the Ceratocystidaceae

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Fungi in the family Ceratocystidaceae produce fusel alcohols and acetates that have fruity and floral odours. Since fusel alcohols are produced in fungi from amino acids via the Ehrlich pathway in a three-step catabolic process, we identified and characterized the genes encoding enzymes involved in catalysing the first and second steps in this pathway. We identified three putative branched-chain amino transferases, three putative aromatic amino transferases and a putative pyruvate decarboxylase in each of the *Bretziella*, *Berkeleyomyces*, *Ceratocystis*, *Davidsoniella*, *Endoconidiophora*, *Huntiella* and *Thielaviopsis* genomes. Using gas chromatography coupled to mass spectrometry (GC-MS), also revealed that all the strains included in this study produced high levels of isoamyl acetate. In contrast, only some members of *Bretziella*, *Berkeleyomyces*, *Ceratocystis* and *Huntiella* produced isobutyl acetate, while only the *Berkeleyomyces*, *Ceratocystis* and *Huntiella* strains produced 2-phenylethyl acetate in low quantities. Either by accepting the branched-chain amino acid substrates (valine and leucine) or accepting the aromatic amino acid substrate (phenylalanine). Fusel alcohols and acetates produced by fungi in the Ceratocystidaceae can therefore be used as additives of food products, perfumes and soaps. In addition, identified fusel alcohols can potentially be utilized as biofuels or biodiesels, bringing solutions to the problems associated with limited fossil resources and climate change.

**Keywords:** Fusel alcohols; Fusel acetate; Ceratocystidaceae; Ehrlich pathway; Gas Chromatography Mass Spectrometry

## Carbon and nitrogen uptake and translocation between the single cell marine protist *Acantharia* and their symbionts

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*Acantharia* are ubiquitous, heterotrophic single celled plankton in oceanic waters. Their contribution and roles in ecosystems have previously been underestimated, being elusive due to their broad size range and fragility. Yet, recent studies show that they are major components of the planktic community contributing greatly to, among others, the carbon flux. The difficulties inherent to the study of live *Radiolaria* make it that very little is known about their physiology. *Acantharia* are known to form a photosymbiosis with the microalgae *Phaeocystis* sp.. The photosymbiont is heavily modified in the *acantharian* host. These modifications seem to aim to exploit photosynthetic capabilities. Yet, how much the *Acantharia* relies on photosynthesis or feeding is not known, nor the metabolic interactions of host and symbiont. Here we aimed to elucidate the metabolic dialogue between these (endo)symbiotic partners. Therefore, we used single cell isolations of *Acantharia* incubated with stable isotopes of carbon and nitrogen. We then used chemical imaging to visualize carbon (nitrogen) uptake, incorporation, and photosynthate translocation between symbionts and host over time, and the effect of nitrogen ( $\text{NO}_3^-$  or  $\text{NH}_4^+$ ) thereon. With this, we aim to visualize how carbon is translocated in the holobiont and through which partitions (i.e. host or symbiont). Furthermore, we expect to resolve the capabilities of the host to itself directly take up  $\text{NO}_3^-$  or  $\text{NH}_4^+$ , as well as how increased nitrogen affects the carbon assimilation.

**Keywords:** carbon; plankton; *Phaeocystis*; photosynthates; photosymbiosis

## Plant Volatiles in Invasion Scenarios

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Climate change and biological invasions constitute two of the main threats to biodiversity. An essential aspect for developing sustainable management strategies and diminishing species loss is understanding the responses of organisms and ecological communities to changing environments. There is an increasing awareness of the impact of climate change on plants, including its effects on volatile organic compound (VOC) emissions and their ecological roles. However, less is known about plant VOCs in invasion settings. Interesting questions include: Do exotic plants behave chemically different in their native and invaded ranges? Which environmental factors influence the volatile emissions of these species in their invaded ranges? Do native plants respond to invaders by changing their chemical profiles? And; which are the potential impacts of these new chemical environments at the community level? This talk will use information obtained during five years of research on the invasive weed *Calluna vulgaris* (heather) in the Central Plateau of New Zealand to address some of these questions. We will also highlight knowledge gaps and propose new avenues for future research on VOCs in invasion scenarios.

**Keywords:** invasive species; volatile organic compounds; plant competition; plant-insect interactions, metabolomics.

## Herbivore order and life stage, but not specialization, drive defensive responses in the crack willow

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Plants produce diverse chemical defenses that protect them against insect herbivores. These defenses often show some specificity to individual herbivores, which probably promotes their efficacy. For example, producing specific blends of Herbivore Induced Plant Volatiles (HIPVs) can help plants attract natural enemies specific to the herbivores they suffer from. Here we aimed at testing what traits of insects govern the specificity in HIPV blends. We focused on *Salix fragilis*, that harbors diverse assemblages of herbivorous insects. In a greenhouse experiment, we exposed 209 willow cuttings to a set of 24 herbivore species. We used various insects including specialists and generalists, leaf-chewers and sap-suckers, beetles, caterpillars, and hemipterans. We detected 37 volatile compounds, including mainly sesquiterpenes, and decomposed variation in their blends elicited by the willows upon attack with multivariate methods. We used the herbivore's order, feeding guild, life stage, and specialization as explanatory variables. The variation in HIPVs was best explained by the herbivore order and life stage, with adult beetles eliciting responses significantly different from beetle larvae. Furthermore, we recorded significant differences between individual beetle and caterpillar species, suggesting specificity in HIPVs also within these two orders. In contrast, the HIPV profiles elicited by sap-sucking hemipterans did not differ from the controls. Specialization did not play a role either. In conclusion, our results show high specificity in HIPVs, with much variation being explained by herbivore orders and life stages. Such specificity can be informative to specialized natural enemies that typically use olfactory cues for host detection (e.g. parasitoids).

**Keywords:** Feeding guild; HIPVs; insect herbivores; leaf-chewers; *Salix*.

## Exploring chemical diversity in the deep sea: linking bacterial symbionts to changes in mussel metabolome

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Deep-sea symbioses represent an untapped world of chemical diversity. We investigated the metabolome of a variety of deep-sea mussel species to unravel chemical cues mediating the interaction between the partners. All 12 mussel species analyzed rely on intracellular chemosynthetic bacteria for their nutrition. They form single or dual symbioses with sulfide-oxidizing bacteria and methanotrophic bacteria. The mussels were collected at hydrothermal vents and cold seeps in two different oceans. Covering this broad sample diversity enabled us to determine the influence of the symbionts on the mussel metabolome and identify symbiont-induced changes. The LC-MS/MS data was analyzed using multivariate statistical analysis, clustering and classification. The data was further mined using GNPS tools to gain a more comprehensive chemical overview and visualize structural diversity through molecular networking. We followed the detected chemical cues with spatial metabolomics. Our mass spectrometry imaging approach helped to localize partner specific metabolites at a micrometer scale. The combination of bulk and spatial analyses revealed a new group of metabolites specific to mussels harboring a particular symbiont species. The elucidation of the symbionts-induced changes may help us to gain a better understanding of the establishment and maintenance of this beneficial symbiotic association in the deep-sea

**Keywords:** GNPS; HPLC-MS; Interaction; MALDI-MSI; Symbiosis



## Differential defence responses of Neotropical maize genotypes to Fall Armyworm, Green Belly Stink bugs, herbivore-induced volatiles and the plant elicitor indole

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Herbivore-induced plant volatiles (HIPVs) emitted by Neotropical maize can prime indirect defence in neighbouring plants, resulting in the recruitment of natural enemies. Using a Brazilian population of *Spodoptera frugiperda*, we investigated i) the development of *S. frugiperda* larvae on five Neotropical maize genotypes (Zapalote Chico, Mirt 2A, Sintético Spodoptera L3, BRS 4103 and BRS 1040) with differing benzoxazinoid (BX) levels and ii) the effect of HIPVs and the known plant elicitor indole upon BX production. Furthermore, using the stink bug *Dichelops melacanthus*, we explored whether or not other generalist herbivores are affected by varying BX levels in maize. When feeding on the selected maize genotypes, *S. frugiperda* larvae took an additional week to pupate in genotypes BRS 1040 and Mirt 2A, but larval survival was the same and high (> 70%) on all the genotypes. When *S. frugiperda* larvae fed on BRS1040 and Mirt 2A genotypes, production of Bxs in these genotypes was suppressed, suggesting that *S. frugiperda* larvae can alter maize defence plant responses in its favour. By contrast, when the SS genotype was exposed to HIPVs followed by *S. frugiperda* larvae or indole, BX levels were higher. Survival of *S. frugiperda* larvae on indole-treated plants was significantly reduced. In contrast to the effects seen with *S. frugiperda*, *D. melacanthus* feeding enhanced the production of Bxs in the SS genotype. Furthermore, survival of male and female *D. melacanthus* was also reduced by pre-exposure of the SS genotype to indole.

**Key words:** maize genotypes, benzoxazinoids, direct defence, fall armyworm, stink bug

## Interspecific variation in the sex pheromones of slave-making ants in the genus *Polyergus*

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Following a serendipitous observation that males of the North American slave-making ant *Polyergus breviceps* (now renamed *P. topoffi*) were highly attracted to an accidentally squashed queen, the queen's attractant pheromone was subsequently identified as a blend of 3-ethyl-4-methylpentanol and 6-methylsalicylate. The pheromone is produced in the mandibular glands, and is released to attract males when the virgin queen accompanies a raiding party attacking a host nest. Subsequently, the European species *P. rufescens* was found to produce a blend of the same two compounds. However, examination of additional North American species determined that there are at least two species groups, with queens of one group producing the same pheromone blend as above, whereas queens of the other group produce a blend of methyl 3-ethyl-4-methylpentanoate and 6-methylsalicylate as their pheromone. Most recently, a two-component pheromone blend has been described from the Asian species *P. samurai*. The regional variation in the queen pheromones will be discussed in light of speciation within this genus. This genus also is one of the few ant genera from which sex attractant pheromones have been identified.

**Keywords:** 3-ethyl-4-methylpentanol; methyl 3-ethyl-4-methylpentanoate; 6-methylsalicylate; speciation; queen pheromone

## Detection of infested fruit: a dog's nose and a wasp's antenna

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Citrus produced in southern Africa, can be infested by false codling moth (FCM), *Thaumatotibia leucotreta*, larvae. Due to the phytosanitary status of FCM for certain export markets, reliable postharvest detection of infested fruit is important. We investigated the ability of a sniffer-dog to detect FCM-infested fruit. Imprinting of the dog was conducted to detect fruit that had been infested 3 days previously, overcoming barriers such as waxing and wrapping of fruit and burying of infested fruit in a carton of healthy fruit. A series of trials was conducted where the dog achieved 98.9% true positive detections, of which 81.2% were on the first passing. Challenges associated with using dogs for this purpose are their lack of stamina, the need for competent handlers and the requirement for numerous dogs to service multiple packing facilities. Consequently, we investigated the use of the larval endoparasitoid of FCM, *Agathis bishopi*, as a sniffer-wasp. The response of female adult *A. bishopi* parasitoids to olfactory and visual cues associated with FCM infested fruit were evaluated using a Y-tube olfactometer and flight tunnel. Parasitoids were strongly attracted to infested over healthy fruit. Four synthetic compounds, associated with citrus fruit were also tested, eliciting up to a 92% response. The main problem associated with using these parasitoids is the difficulty in mass rearing them. Consequently, we are now investigating the antennal response of *A. bishopi*, for identifying key volatiles indicative of FCM fruit infestation, by using Coupled Gas Chromatography-Electroantennographic Detection (GC-EAD).

**Keywords:** *Agathis bishopi*; citrus; Coupled Gas Chromatography-Electroantennographic Detection; false codling moth; sniffer-dog

## Improving semiochemical resources and behaviorally-based management strategies for stored products: from the laboratory to food facilities

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There has been a concerted push to diversify integrated pest management (IPM) programs for stored products given the challenges to existing fumigation tools over the last few decades. One alternative approach is through the use of semiochemical-mediated, behaviorally-based management tactics. In reviewing the literature, we find that behaviorally-based management tactics have been attempted for eight insect species from two orders. The subset of these that are commercially available are restricted to mating disruption for just a handful of moths. We present a case study evaluating the development of a new attract-and-kill based trap to intercept insects along the perimeter of a food facility. We present intermediate steps of development, including attractant type and dose assessment. Over two years, we were able to trap over 3000 individuals from 14 taxa in a field experiment deployed at food facilities in Kansas and Arkansas. We also present evaluations of plume reach for the trap in simulated warehouses. We discuss future implications for use of behaviorally-based management tactics to protect stored products at food facilities, and highlight their importance for quarantine threats such as khapra beetle.

**Keywords:** attract-and-kill, stored products, insecticide netting, khapra beetle, red flour beetle

## Insecticidal efficacy of three benzoate derivatives against *Aphis gossypii* and its predator *Chrysoperla carnea*

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Plant-derived benzoates are known to have insecticidal and repellent activities; however, these effects have been evaluated thoroughly in only a few groups of insects. This research used three commercially available benzoates, methyl benzoate (MB); ethyl benzoate (EB), and vinyl benzoate (VB) were evaluated how to control *Aphis gossypii* Glover and its lacewing predator *Chrysoperla carnea* Stephan. In experiments rates used of three formulated benzoates were 0.1%; 0.25%; 0.5%; and 1%, and each experiment replicated three times and control. MB showed the highest contact toxicity against nymphs and adults of *A. gossypii*, as compared to those of EB and VB. Treatments with 1% MB, EB, and VB were 100.0%, 93.0% and 60.0% effective, respectively, against third-instar nymphs and 100.0%, 69.0%, and 39.0% effective, respectively, against adults of *A. gossypii*, as evaluated 24 h after application. A mixture of MB+EB showed higher efficacy than other benzoate combinations against *A. gossypii*. The efficacy of MB, EB, and VB against *A. gossypii* on cucumber plants under greenhouse conditions was 93.7%, 68.5%, and 56.6%, respectively. In addition, treatments with 1% MB, EB, and VB were 20.0%, 24.0%, and 12.0% effective, respectively, against first-instar larvae and 6.7%, 13.3%, and 6.7% effective, respectively, against adults of *C. carnea* at 24 h after treatment. Our results showed that the tested benzoates were less toxic to the predator *C. carnea* than to the pest *A. gossypii*. This study suggests that plant-derived benzoates are potential biopesticides for aphid control and are compatible with natural enemies in integrated pest management.

**Key words:** Aphids; benzoates; natural enemies; plant-derived pesticides; predators

## (S)-(-)- $\delta$ -Heptalactone, an aggregation pheromone of fruit fly *Rhagoletis batava*, a *Hippophae rhamnoides* berries pest

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Tephritid flies cause a large threat to fruit and vegetable production throughout the world. The sea buckthorn fly, *Rhagoletis batava* Hering (Diptera: Tephritidae), is a pest of economic importance of sea buckthorn (*Hippophae rhamnoides* L., Rosales: Elaeagnaceae) berries. The plantation area of *H. rhamnoides* is expanding in many countries due to the multiple ecological and medicinal benefits of sea buckthorn, thus creating suitable conditions for the rapid expansion of *R. batava*. However, no environmentally friendly means of pest control are available for this species; thus, insecticides are widely used to control pests. To decrease insecticide use, effective means for pest population monitoring are required, including the use of pheromones. Male fruit flies emit (S)-(-)- $\delta$ -heptalactone as revealed by gas chromatography-mass spectrometry analyses of samples obtained using headspace methods. The two enantiomers of  $\delta$ -heptalactone were synthesized using enantioselective synthesis. A gas chromatography-electroantennographic detection analysis of both stereoisomers revealed that only (S)- $\delta$ -heptalactone elicited electrophysiological responses, whereas (R)-(+)- $\delta$ -heptalactone proved to be inactive in fruit flies of either sex. In a field assay, traps baited with (-)- $\delta$ -heptalactone caught significantly more fruit flies compare with the unbaited traps. Our results are the first to demonstrate the efficacy of (S)- (-)- $\delta$ -heptalactone as a bait for trapping *R. batava*. As a behaviorally attractive compound to *R. batava* fruit flies of both sexes, (S)-(-)- $\delta$ -heptalactone constitutes an aggregation pheromone, the first one identified within the genus *Rhagoletis*.

**Keywords:** sea buckthorn; gas chromatography-electroantennography; enantioselective synthesis; field assay; enantiomer separation

## The role of the host queen in regulating reproductive parasitism in *Apis mellifera capensis* laying workers

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The honeybee queen mandibular gland pheromone (QMP) maintains reproductive dominance by inhibiting ovary activation and secretion of queen-like pheromones in workers. Biosynthesis of the mandibular gland (MG) fatty acids starts with stearic acid which undergoes caste-selective hydroxylation and oxidation to produce the queen-associated (*E*)-9-oxodec-2-enoic acid (9-ODA) and (*E*)-9-hydroxydec-2-enoic acid (9-HDA), and the worker-associated 10-hydroxy-decanoic acid (10-HDAA) and (*E*)-10-hydroxydec-2-enoic acid (10-HDA). In a comparative study, we measured the fatty acid profiles, ovary activation and expression of genes encoding two Cytochrome P450s responsible for the caste-specific hydroxylation of acylated stearic acid, and also genes encoding the enzyme alcohol dehydrogenase (Adh), in the intraspecific socially parasitic *Apis mellifera capensis* workers (“clones”) infesting *A. m. scutellata* colonies that were either queen-right (QR) or queen-less (QL). We show that clones infesting QL colonies primarily secreted the queen-associated 9-ODA and 9-HDA, had relatively low expression of the worker-associated Cytochrome P450s and fully activated ovaries. QR clones had an accumulation of 9-HDA and 10-HDA, relatively high expression of worker-associated Cytochrome P450 and inactive ovaries. This shows that while the QMP produced by *A. m. scutellata* queens chiefly inhibits dominance in *scutellata* workers at the level of hydroxylation of stearic acid, laying workers can bypass this inhibition. QMP acts on the hydroxylation products from these parasitic workers by inhibiting the oxidation of 9-HDA into the “queen substance” 9-ODA, shown by the significantly lower transcript levels of the enzyme alcohol dehydrogenase. This study contributes to our understanding of the evolution of reproductive division of labour in social insects.

**Keywords:** African honeybees; communication; mandibular gland; pheromones; social parasitism

## Anthropogenic influences on marine food webs: the case of plastic ingestion

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A hallmark of the Anthropocene is the ubiquitous presence of plastic waste in the world’s oceans. Plastic marine debris is of growing concern to ecologists, due, in part, to the deleterious impact it has on wildlife that consume it. Although it has commonly been assumed that ingestion occurs because organisms mistake plastic marine debris for food, this behavior has only recently been examined in relation to the chemical signals that are thought to drive foraging interactions. My laboratory has been investigating plastic ingestion in this context, focusing on the procellariiform seabirds as a model system. This is a diverse order of highly pelagic species, many of which have been shown to track prey using dimethyl sulfide (DMS) as a foraging cue. DMS is produced from phytoplankton and other marine algae, and is a critical info-chemical across a wide range of organisms and marine habitats. We and others are beginning to explore how attraction to this compound is associated with plastic ingestion. My presentation will provide a brief overview of these findings to illustrate how this keystone foraging cue contributes to this ecological trap.

**Keywords:** dimethyl sulfide; ocean; marine debris; pollution; seabird

## Plant-Plant Communication via Volatiles Triggers Growth and Defense Synchronization among Plants

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The capacity of individual plants to rapidly detect and respond to "true" signals that point to real upcoming threats from neighbours is essential as it determines their growth strategy and survival. Volatile organic compounds (VOCs) released from herbivory damaged plants are the most common signals that reveal emitter physiological status to neighbours. In our studies, we investigated whether VOCs emitted by undamaged plants may also be used as signals in the detection of competitive neighbours and whether plant response can be modified if the VOCs are changed by other types of plant interactions. We found that one undamaged barley variety exposed to VOCs of another variety allocated more biomass to the roots and become less attractive to aphids. Low red: far red (R:FR) light is signal that provides reliable information about the presence of competitors. Emitter barley exposed to R:FR allocated more biomass to above ground parts and changed the blend of VOCs released which induced the same response in exposed barely variety making it more competitive for the light-foraging, but not for defence. Touching between plants is another signal in detection of competitive neighbours. We have demonstrated that plants respond to touch by upregulation of early defence genes and by changes in the emission of VOCs that activated the same defence genes in neighbouring plants. Our studies show that highly specific signals embedded in the volatile profile of emitting plants can transfer information about their changed physiological status to neighbours, triggering growth and defence synchronization among nearby plants.

**Keywords:** acclimation; aphids; coexistence; plant-insect interactions; plant-plant interactions

## New opportunities for a push-pull system in brassica crop production

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The diamondback moth (DBM), *Plutella xylostella* (L.) is the major economic pest of brassica crops in the tropics. Ongoing climate change has significantly exacerbated its pest status through e.g. short generation time, increased abundance, fecundity and metabolic activity translating into increased feeding and pest pressure. Control of this pest in Africa is generically achieved by deploying hard synthetic pesticides. However, several reports suggest pesticide resistance in DBM, likely triggering environmental, public health and sustainability concerns owing to pesticide overuse. However, rich vegetational diversity (intercrops), may help sustainably manage specialist pest insects through obscuring volatile cues used for host plant identification, physical obstruction, increased pest emigration, reduced chances of appropriate landing and making host crop plants less apparent- and -attractive. Thus, through the manipulation of habitats and host finding behaviour using natural host-pest semiochemicals and aligning to Root's resource concentration and natural enemies' hypotheses, intercropping of brassica crops with plants that are rich in attractant and repellent volatiles could create a push-pull environment that may sustainably reduce DBM pest abundance and subsequent crop damage. Here, we propose the use of multipurpose plants in a push-pull system for the management of DBM in brassicas. Specifically, we propose Indian mustard, *Brassica juncea* L. (Czern). as an attractant crop and *Hyptis spicigera* Lam. as a repellent plant in a system that creates synergistic and complementary pest control effects. Use of multipurpose plants is highly sustainable through provision of other useful economic products e.g. human and livestock food (*B. juncea*) as well as livestock nutraceuticals (*H. spicigera*) and forms critical synergies in mixed crop-livestock production systems. The results have huge implications in reducing the use of hazardous synthetic pesticides, improving public and environmental health as well as improving sustainability in smallholder African horticulture and mixed cropping systems.

**Keywords:** *Brassica juncea*; horticulture; *Hyptis spicigera*; *Plutella xylostella*; push-pull

## Re-igniting the mustard oil bomb: knocking out key host plant adaptive genes in *Pieris* butterfly larvae

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*Pieris* butterfly larvae feed on plants containing diverse glucosinolates (GLS) with redirecting toxic breakdown products of GLSs to less toxic metabolites using gut-expressed nitrile-specifier proteins (*NSPs*). *NSP* is considered an evolutionary key innovation for Pieridae that enabled these butterflies to colonize GLS containing plants and allowed subsequent diversification. However, we still do not fully understand the roles of both *NSP* and its sister gene, major allergen (*MA*), in overcoming the wide range of host plant GLSs larvae encounter in the field. Here, we tested the ecological relevance of *NSP* and *MA* for *Pieris* larvae by knocking out both genes in *Pieris brassicae* using the CRISPR/Cas9 genome editing technique. We found that gut protein extracts of *NSP/MA* double KO larvae completely lost their activity against all GLSs tested. Moreover, we also found that *NSP* KOs and *MA* KOs showed lower performance on host plants with different GLS profiles, whereas *NSP/MA* KOs could not survive on host plants with higher GLS concentration. These results clearly suggest that both *NSP* and *MA* have different but complementary roles in defusing the mustard oil bomb in *Pieris* larvae, and that both genes are crucial for *Pieris* in overcoming their host plants' major chemical defense.

**Keywords:** Nitrile-specifier protein; Glucosinolate; counter-adaptation; genome-editing; substrate specificity

## Insights into reproductive processes and hierarchies within social insect societies

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The stability and integrity of the social insects' colony is established through effective pheromonal communication to regulate reproductive behaviour and dominance. Under normal conditions, the queen is the only one reproducing with group of workers that are functionally sterile. However, some honey bee workers do escape the reproductive regulatory mechanism and become reproductively active. This study investigated how honey bee workers that becomes reproductively active, exploit pheromonal communication to their advantage and how pheromones contribute to reproductive dominance and reproductive hierarchies. The results provide evidence for establishment of reproductive dominance through the use of pheromones from diverse glandular secretions acting synergistically or additively to regulate various processes in the colony. These complex interplay of pheromonal signals from different exocrine glands have both primer and releaser effects among the honey bee groups. This study provides additional understanding into how pheromones from various glandular secretions contribute to the evolution of reproductive dominance and reproductive division of labour within social insect societies.

**Keywords:** honey bees; behaviour; exocrine; evolution; secretions

## Pollinator-mediated speciation in endemic flora of Japan associated with dynamic changes of floral scents

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Species radiation in a geographically restricted area such as on certain islands serves as an excellent model for studying the history and mode of evolution. Japan archipelago harbors a rich flora with 7000 vascular plant species, one third of which are endemic. Among them, several plant lineages such as genus *Asimitellaria* (Saxifragaceae), genus *Asarum* sect. *Heterotropa* (Aristolochiaceae), and genus *Arisaema* sect. *Pistillata* (Araceae) are especially remarkable in both endemism and the floral diversity. A series of our recent studies have revealed that various dipterans are responsible for pollination of these plant species, while the patterns of their interactions are variable among the systems. In *Asimitellaria*, the principal pollinators are a limited species of fungus gnats (Mycetophilidae), and the multiple pollinator switches between long-tongued fungus gnats and short-tongued fungus gnats have taken place in association with the losses or gains of floral volatile containing lilac aldehydes. In *Heterotropa*, the plants interact with more diverse arrays of dipterans and the floral volatiles involved are also more diverse and complex, yet the losses or gains of a key compound, dimethyl disulfide might be important. In *Pistillata*, most species attract and trap specific species of fungus gnats (Mycetophilidae or Sciaridae) for pollination, while a clear exception was found in *A. sikokianum*, where obligately mycophagous drosophilids are the only pollinator and the derived floral traits for chemical/morphological mushroom mimicry has evolved in the species. Overall, dynamic changes of floral volatiles are likely to be responsible for pollinator-mediated diversification in these three plant lineages.

**Keywords:** Diptera; floral scents; Japan; plant speciation; pollinator

## Chemical ecology as inspiration for global change

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Humans are currently altering this planet more than any single species in the history of Earth. In fact, this phenomenon has led to the dawn of a new geological age, known as the Anthropocene. Humans not only impact the environment itself, but the ecological interactions between organisms, including chemical communication. The ubiquitous nature of chemical ecology across all life on Earth offers a unique opportunity to understand the impact humans are having on our natural world. Our field also allows us to potentially develop ways we can adapt to the impacts of humans on the very means by which organisms interact with each other. Here I will briefly outline our research on chemical communication in the current Indian context, particularly concerning pollination and environmental pollution, and discuss how the tenets of chemical ecology have inspired a new global network to increase the communication between science and society. We hope our journey inspires chemical ecologists to use their unique multidisciplinary knowledge to engage with their communities.

**Keywords:** *Urbanization; Pollution; Global change; Chemical communication; Outreach*

## Behavioral and social context is necessary for the full effect of queen semiochemicals in *Bombus impatiens*

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Regulation of reproduction via chemical signaling has been shown in a number of social insect species. But few chemicals with this capacity have been identified and it remains largely unknown how semiochemicals are regulated by the environment and the social context in which they operate. We previously showed that in *Bombus impatiens*, the queens' strategy to monopolize worker reproduction changes with life stage, shifting from overt aggression to chemical signaling as the queen gets older. Particularly, old egg-laying queens exhibited a higher ratio of short to long cuticular hydrocarbons (CHCs). Here we investigated whether CHCs alone are able to inhibit worker reproduction and whether this effect depends on the context provided by the queen's behavior and the presence of eggs she lays. We examined the effect of the queen's CHC secretion on worker reproduction in three scenarios: (1) without a behavioral context; (2) with a behavioral context of a free-moving virgin queen, and (3) at the presence of free-moving virgin queen and newly laid eggs. Our results indicate that queen CHCs affect worker reproduction only when combined with a behavioral context and that the presence of eggs is indispensable for the full effect of the queen's signal. Our findings highlight the complexity of queen-worker interactions in *B. impatiens* and the role of social context in reproductive signaling.

**Keywords:** pheromones, social insects, social context; queen-worker interactions; honest signal

## A multi-omics approach deciphers how temperature and copper stress shape seaweed-microbiota interactions at the surface of *Taonia atomaria*

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Although considered as holobionts, macroalgae and their surface microbiota share intimate interactions that are still poorly understood. In this context, the epibacterial communities of the Ochrophyta *Taonia atomaria* exhibited a high specificity whatever the five contrasted sites investigated on the North West Mediterranean coast. Using a multi-omics approach combining metabarcoding and metabolomics, the holobiont dynamics was found to vary as a whole in agreement with previous studies. During the occurrence period of *T. atomaria*, epibacterial densities and  $\alpha$ -diversity increased while the relative proportion of core and specific communities decreased. Pioneer bacterial colonizers (e.g. *Litorimonas* and *Granulosicoccus* genera) constituted a large part of the specific and core taxa and displayed potential functional features involved in adhesion, biofilm formation and adaptation to the epiphytic lifestyle. Then, the concomitant increase of temperature and several algal compounds, especially dimethylsulfoniopropionate could explain the bacterial diversification, especially with *Roseobacter* taxa specialized in the catabolism of this metabolite. Copper concentration constituted a second factor shaping the holobiont system. The resulting oxidative stress caused an adaptation of the algal surface metabolome with a higher expression of photo-pigment including chlorophyll derivatives and carotenoids ( $\beta$ -carotene and fucoxanthin), which could result in the selection of particular epibacterial taxa. In a context of global change, this study brought new insights on the dynamics of a Mediterranean holobiont submitted to heavy anthropic pressures.

**Keywords:** Holobiont; macroalgae, metabolomics; metabarcoding; environment



## Characterization of the alarm pheromone of *Bathycoelia distincta* (Pentatomidae), a major pest of macadamia

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*Bathycoelia distincta* (Pentatomidae) is the most dominant insect pest in South African macadamia orchards, where adults are responsible for causing severe yield losses. Similar to other hemipterans, *B. distincta* produce volatile compounds from their scent glands that can deter natural enemies, and act as alarm signal amongst conspecifics. These volatiles can be exploited for the development of pest management strategies. The overall aim of this study was to investigate the alarm pheromone of *B. distincta* in more detail. For this, we i) examined the scent gland extracts by gas chromatography coupled with mass spectrometry (GC-MS), ii) analysed volatiles released from live stink bugs after stress by GC-MS, and iii) evaluated the electrophysiological and behavioural activities of alarm pheromone compounds by dose-response experiments. A blend of sixteen compounds was identified in the scent gland extracts of adult stink bugs. Of these, six major compounds were released by live stink bugs after stress, namely (*E*)-2-hexenal, (*E*)-2-decenal, tridecane, dodecane, (*E*)-4-oxohex-2-enal and (*E*)-2-decenyl acetate. No qualitative and quantitative differences were observed between sexes. Tridecane was the most abundant compound, comprising ~50% of total secretions. All compounds elicited a response from both male and female antennae, but the response varied according to compound and dose tested. (*E*)-2-Hexenal and (*E*)-2-decenal at lower and higher doses, elicited larger responses in both sexes than tridecane and dodecane. 4-Oxohex-2-enal and (*E*)-2-decenyl acetate generally elicited large antennal responses in males and females. Preliminary results showed (*E*)-2-hexenal and (*E*)-2-decenal induce a higher deterrent response than tridecane and dodecane.

**Keywords:** alarm pheromone; macadamia; Pentatomidae; pest management

## Evolutionary Physiology of Insect-Plant Interactions on a Tritrophic Scale

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Plants produce a tremendous variety of toxic compounds to protect themselves against herbivores. Remarkably, many herbivorous insects not only cope with plant toxins but also store them in their bodies as a defense against predators and parasitoids (sequestration). Since sequestering insects exploit plants in at least two ways, as a dietary resource and as a source of defensive compounds, understanding the evolution of their host plant relationships require the integration of both traits. We study the physiological mechanisms underlying insect resistance to host plant toxins in the milkweed butterflies (Lepidoptera: Danainae) and found that resistance and sequestration can be intertwined. Specifically, sequestration of plant toxins requires resistance mechanisms different from those needed to cope with toxins in the diet. Hence, predators selecting for sequestration can spur the coevolutionary arms race between insects and plants. Moreover, in the milkweed bugs (Heteroptera: Lygaeinae) it seems likely that acquisition of plant toxins for defense directed specific associations with certain host plants. Although sequestration is common among herbivorous insects, the underlying mechanisms and the physiological constraints of sequestration are largely unknown. Using mechanistic approaches in a comparative evolutionary framework, our research revealed first insights how quantitative differences between sequestering species and non-sequestering relatives are mediated. Furthermore, we found that dietary exposure to toxins causes different physiological effects in closely related sequestering species suggesting that physiological constraints are highly context dependent. Based on our findings, we propose that detailed physiological analyses are required to understand the evolutionary forces directing plant-insect coevolution in sequestering insects.

**Keywords:** cardenolide; milkweed bug; milkweed butterflies; resistance; sequestration

## Exploiting Push-Pull pest insect management by biotechnology

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The 'push-pull' system [www.push-pull.net](http://www.push-pull.net) by a companion planting approach, effectively controls serious biotic constraints to cereal-livestock production in sub-Saharan Africa. This system fits well with traditional mixed cropping systems in Africa and has been adapted to drier and hotter conditions linked to climate change by identification and incorporation of drought tolerant companion crops. However, the push-pull control of the fall armyworm and striga weed now recommend use of elements of this technology for more industrialised production systems where expensive seasonal inputs have largely failed. Thus, incorporating the chemical ecological tool by which the push-pull controls pests and weeds sustainably is of growing interest. In the push-pull, lepidopterous pests are pulled to naturally attractive trap plants whilst these pests, including the fall armyworm, are pushed away from the main crop using specific repellent intercrops. Key compounds in this process are known as the homoterpenes: (*E*)-4,8-dimethyl-1,3,7-nonatriene (DMNT) and (*E,E*)-4,8,12-trimethyl-1,3,7,11-tridecatetraene (TMTT). Recently it was demonstrated that the protein OsCYP92C21 is responsible for homoterpene biosynthesis in rice and the ability of rice to produce homoterpenes is dependent on subcellular precursor pools. By increasing the precursor pools through specifically subcellular targeting of expression, genetic transformation and genetic introgression, the biosynthesis of the homoterpenes DMNT and TMTT were enhanced in rice. The final introgressed GM rice plants demonstrated strong attractiveness, without damage, to the parasitic wasp *Cotesia chilonis* because of the enhanced homoterpene emissions compared to the wild type rice. Thus, the way is obvious for the biotechnological enhancement of this "push" trait directly in crop plants by GM or GE. Furthermore, the natural chemicals released by intercrop roots that result in abortive germination of the noxious parasitic striga weed have now been elucidated. The unique feature of these compounds is the addition of two *C*-linked glycoside groups onto the widely produced plant flavone apigenin and the *C*-glycosyl transferases partially characterised. Thus, the other major trait determining the striga weed control by the push-pull is close to exploitation by GM or GE. However, although these biotechnologies for delivering some aspects of the push-pull are becoming available it must be remembered that the low-input and thereby sustainable technologies of the push-pull that address several production constraints and deliver multiple benefits are more relevant for smallholder farmers in sub-Saharan Africa but also proves useful lessons for agricultural systems in the developed world.

## Electrophysiological responses of *Ixodes scapularis* to host volatiles

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*Ixodes scapularis* is an important vector of *Borrelia burgdorferi*, the causative agent of Lyme disease, in North America. In recent years, the threat of disease transmission has risen significantly, resulting in an increased demand for environmentally safe, tick repellent products. To better understand and potentially disrupt host seeking behavior, host odorants can be identified and characterized, allowing for a more in depth understanding of what volatile organic compounds promote tick host-location. We identified key odorants/blends by collection and chemical characterization of volatile compound (VOC) from hosts (e.g., humans, cats, dogs, etc.) and the mechanism by which ticks detect them. Through a novel electrophysiological approach, we recorded tick responses to host VOCs using gas chromatography linked electrotarsography (GC-ETG). After identification of biologically-active chemicals, we further tested relative sensitivity to homologous series of carboxylic acids, aldehydes, and hydrocarbons at different concentrations. Ticks responded most strongly to compounds with 4-6 carbons, denoting a specific pattern of sensitivity. Behavioural assays assessed tick response to stimulation with electrophysiologically-active components. These results will contribute to defining key host volatiles that trigger host-seeking behaviour.

**Keywords:** electrophysiology; tick; host volatiles; carboxylic acids; aldehydes

## Reproductive dominance and the role of multiple pheromones sources in honey bees

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Honey bee workers are potentially reproductive females and they can display both behavioural and physiological traits similar to those of the queen caste. Such false-queens are frequent in African subspecies of *Apis mellifera* and very common in the Cape honey bee making reproductive workers an ideal model system to investigate the role of pheromonal signals during the competition for reproduction. One of the challenges is that the same pheromones that help an individual gain reproductive dominance are used by the individual's opponents to subdue it. We have shown that exogenously applied pheromones on workers stimulates them to become a false-queen rather than inhibiting them. Here we present evidence of the role of multiple signals originating from different glandular sources and the role of diet on gaining reproductive dominance. Not only is the perception of the pheromones context dependent, we also showed that pheromones from mandibular, tergal and Dufour's gland working in synergy to allow individual workers (false queens) to establish reproductive dominance. The complex role of pheromonal signals in honey bees is used to investigate the evolution of this pheromone based communication system. Furthermore, understanding the role of pheromones in establishing dominance hierarchies in social insects like the honey bee deepens our understanding of the ultimate and proximate drivers shaping eusociality.

**Keywords:** competition; context dependent; communication; false-queens; hierarchies

## Socio-economic issues of developing push-pull technologies

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Agricultural production in sub-Saharan Africa (SSA) does not match its people's food needs and contributes to environmental degradation. The food production system is limited by insect pests, parasitic weeds, aflatoxins, water stress, and climate change, which cause yearly yield losses worth over US\$ 14 billion, and affecting livelihoods of >500 million people. Push-pull technology rebuilds sustainable local production systems, improves farm incomes, and contributes to food and nutrition in SSA, by increasing grain yields 3 to 5-fold, with minimum reliance on external inputs, and is more resilient and sustainable under changing climate conditions. The economic performance of push-pull technology has consistently shown benefit-cost ratios greater than those for other recommended cereal-cropping options or for farmers' traditional practices. The technology consistently posts higher factor productivity and yields with significant net economic gains. Push-pull has been disseminated and widely adopted by farmers in SSA. The main drivers of adoption of the push-pull practice are the farmers' need to control Striga, fall armyworm and stemborers, to increase cereal and livestock fodder yield, to control soil erosion, and improve soil fertility. Several dissemination pathways have been deployed: mass media, print media, and interpersonal pathways such as field days, farmer field schools (FFSs), farmer teachers/trainers, fellow farmers, and public meetings. Demonstrative pathways which catalyze interactive learning e.g., field days triggered have the highest impact on both the probability and intensity of push-pull adoption, followed by FFSs, and farmer teachers. Farmer-to-farmer extension models drastically increased awareness and adoption of the practice.

**Keywords:** adoption; Africa; dissemination; productivity; Push-pull

## Gypsy Moth Pheromone-Binding Proteins: Kinetic, Structural and Functional Aspects of Interaction with Pheromones

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The gypsy moth, *Lymantria dispar*, uses a chiral pheromone, the hydrocarbon epoxide 2-methyl-(7*R*, 8*S*)-epoxyoctadecane ((+)-disparlure) to attract mates. Recognition of this compound by male moths is highly specific: even a few percent of the opposite enantiomer ((-)-disparlure) will cancel male upwind flight behavior. Small structural alterations to the pheromone also result in a decrease or loss of attractiveness. The sensor for the disparlure enantiomers, the odorant receptor, is on the dendrites of olfactory neurons that innervate hollow sensory hairs on the antennae. These dendrites are surrounded by sensory lymph, which contains pheromone-binding protein (PBP), the first species-specific gene product in the olfactory mechanism to selectively interact with the pheromone. The gypsy moth has two PBPs: PBP1 and PBP2, which differ in sequence, selectivity and kinetic regime of the interaction with pheromone and other ligands. At equilibrium *in vitro*, PBP1 binds (-)-disparlure more strongly than (+)-disparlure, whereas PBP2 has the opposite preference. PBP1 associates and dissociates faster than PBP2. Here we present our newest kinetic and structural results with these two PBPs. We interpret these in terms of the structures of these proteins and the two roles PBPs could serve simultaneously: 1) solubilization of the hydrophobic pheromones in the lymph, to prevent adsorption of pheromone molecules prior to their arrival at odorant receptors, and 2) scavenging of excess odorant molecules to prevent sensory saturation.

**Keywords:** fluorescent pheromone probe; molecular recognition; olfaction; protein dynamics; protein structure

## Infection and resistance in complex microbial communities of the plankton - small scale processes with global impact

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Planktonic communities are complex microbial assemblages with continuous changes in species composition. Seasonal species succession is highly predictable, but the underlying regulative principles are only poorly understood. We hypothesize that chemical mediators released by the interacting partners explain a substantial part of the community dynamics. Here we introduce how chemical signals and defensive metabolites shape the interaction of unicellular algae and pathogens in the plankton. Flagellated oomycetes frequently infect unicellular algae, thus limiting their proliferation. Using single cell mass spectrometry, bioassays and infection models we show how the marine oomycete *Lagenisma coscinodisci* rewires the metabolome of the bloom-forming diatom *Coscinodiscus granii*. The algal alkaloids  $\beta$ -carboline and 2,3,4,9-tetrahydro-1H-beta-carboline-3-carboxylic acid are induced during infection and support the success of the pathogen. Single-cell profiling with AP-MALDI-MS and confocal laser scanning microscopy reveals that algal carbolines accumulate in the reproductive stages of the parasite. The compounds arrest the algal cell division, increase the infection rate and induce plasmolysis in the host. In another example we show how infection of algae with lytic bacteria is dependent on dynamic induction of resistance factors using approaches from single cell resolution up to ecosystem wide manipulations in mesocosms.

**Keywords:** Single Cell Mass Spectrometry, Metabolomics, Oomycete Infection, Diatoms, Bioassays

**Dietary cardenolides enhance growth and change the direction of the fecundity-longevity trade-off in specialized sequestering milkweed bugs (Heteroptera: Lygaeinae)**

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Milkweed bugs (Heteroptera: Lygaeinae) sequester high amounts of plant-derived cardenolides as a defense against predators. Although being inhibitors of the ubiquitous animal enzyme  $\text{Na}^+/\text{K}^+$ -ATPase, milkweed bugs can tolerate cardenolides by means of resistant  $\text{Na}^+/\text{K}^+$ -ATPases. Both adaptations, resistance and sequestration, are ancestral traits shared by most species of the Lygaeinae. Using four milkweed bug species and the related European firebug *Pyrrhocoris apterus* showing different combinations of the traits 'cardenolide resistance' and 'cardenolide sequestration', we tested how the two traits affect larval growth upon exposure to dietary cardenolides in an artificial diet system. While cardenolides impaired the growth of *P. apterus* nymphs neither possessing a resistant  $\text{Na}^+/\text{K}^+$ -ATPase nor sequestering cardenolides, growth was not affected in the non-sequestering milkweed bug *Arocatus longiceps*, which possesses a resistant  $\text{Na}^+/\text{K}^+$ -ATPase. Remarkably, cardenolides increased growth in the sequestering specialists *Caenocoris nerii* and *Oncopeltus fasciatus* but not in the sequestering generalist *Spilostethus pandurus*, which all possess resistant  $\text{Na}^+/\text{K}^+$ -ATPases. Furthermore, we investigated the effect of dietary cardenolides on life-history traits in *O. fasciatus*. Interestingly, nymphs developed faster and lived longer as adults. However, adults raised on cardenolide-containing diet produced less offspring when maintained on the same diet, while no effect was observed when adults were transferred to sunflower seeds. We speculate that the resistant  $\text{Na}^+/\text{K}^+$ -ATPase of milkweed bugs is selected for working optimally in a 'toxic environment', i.e. when cardenolides are sequestered in the body tissues. Our results indicate a trade-off between longevity and fecundity, whose direction can be altered by the availability of cardenolides in the diet.

**Keywords:** Fitness costs; Life-history traits;  $\text{Na}^+/\text{K}^+$ -ATPase; Sequestration; Trade-offs

**Similar, but not the same - mate recognition based on cuticular hydrocarbons**

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Insects often use chemical cues and signals to recognize prospective mates. While some sex pheromones attract conspecifics from a distance, others serve for mate recognition at close range. Among the latter, cuticular lipids are likely the most commonly used compounds. They comprise mainly cuticular hydrocarbons (CHC), but often more polar compounds are present as well. Typically, the composition of the cuticular profile is species-specific. Additionally, many species show differentiation between the sexes, caused by specific compounds and/or compound ratios. Such a clear sexual differentiation in the cuticular lipid profiles is often considered a prerequisite and indication for a sex pheromone function. In parasitic wasps, single compounds or the sex-specific profile as a whole have been shown to serve as close-range sex pheromones. Here, we studied the parasitoid wasp *Tachinaephagus zealandicus* (Encyrtidae, Hymenoptera). Chemical analyses of cuticular extracts showed males and females produce the same set of compounds in similar relative amounts. We investigated whether this species uses cuticular lipids for mate recognition despite the observed close similarity. Males showed copulation behaviour exclusively towards freeze-killed females, but neither towards dead males nor dead females from which the cuticular lipids had been removed. Reapplication of female whole-body extracts restored bioactivity completely. When testing lipid fractions, only the CHC fraction was bioactive on its own. Again, males reacted to female, but not to male CHC, showing that these wasps are indeed using CHC for close range sex recognition despite the overall similarity of male and female profiles.

**Keywords:** behaviour, cuticular lipids, Encyrtidae, parasitic wasp, sex pheromone

## Chemical signal is in the blend: bases of plant-pollinator encounter in a highly specialized interaction

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In several highly specialized plant-insect interactions, scent-mediated specificity of pollinator attraction is directed by the emission and detection of species-specific volatile organic compounds (VOCs). Although some plants engaged in highly specialized interactions with their pollinators emit singular compounds, others emit mixtures of common VOCs. In the present study, we investigated the chemical ecological bases of host recognition in one of the most specific interactions between plants and pollinators: the interaction between the dioecious *Ficus carica*, and its obligate and specific pollinator *Blastophaga psenes*. We show that females of *B. psenes* are attracted by VOCs of receptive figs of both sexes and do not exhibit preference for VOCs of either male or female figs (in which they cannot reproduce). Of all the VOCs emitted by receptive figs, only five were found to be physiologically active on female pollinator antennae. Behavioral tests revealed that, in contrast to VOCs presented alone, only a blend with a specific proportion of four of these VOCs is as attractive as the odor of receptive figs, and a very small change in this blend proportion impedes pollinator attraction. Therefore, this study revealed that in highly specialized mutualistic interactions specificity could be mediated by a particular and unique blend of common plant-emitted compounds. Variation in emission of VOCs by receptive figs in natural populations of *F. carica* needs to be investigated to establish whether pollinator-mediated selection is maintaining the proportion of VOCs emission in this strictly mutualistic system.

**Keywords:** Dioecy; *Ficus*; floral scent; mutualism; nursery pollination; specificity.

## Moths in flowers: volatile mediated interactions from obligate mutualism to seed predation

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Moth pollination includes textbook examples of specialization, from Darwin's star orchid to the yucca-yucca moth mutualism. Closer examination reveals a continuum between these extremes, including plants whose pollinators also are herbivores (e.g. *Datura* plants and *Manduca* moths) and plants that attract both generalized pollinators and specialized florivores (e.g. *Silene* plants, *Deilephila* and *Hadena* moths), all of which are mediated by floral scent. Like figs, *Yucca* (Agavaceae) have species-specific pollinators (*Tegeticula* spp., Prodoxidae) that oviposit into pollinated flowers, producing larvae that consume the resulting seeds. Both sexes of yucca moths orient to *Yucca* volatiles, which are chemically unique and phylogenetically conserved. *Lithophragma* (Saxifragaceae) also are pollinated by prodoxid moths (*Greya* spp.) that oviposit into flowers, but specialization on these moths varies locally in ways that inspired Thompson's "Geographic Mosaic Theory of Coevolution". Like yuccas, *Lithophragma* flowers use scent to attract *Greya* moths, but unlike yuccas, *Lithophragma* scents are blends of conventional compounds that vary at geographic and phylogenetic scales. Female *Greya* moths show olfactory site fidelity in attraction and oviposition. Finally, *Oenothera* plants (Onagraceae) include over 140 spp., many of which are pollinated by hawkmoths with generalized foraging habits. However, *Oenothera* flowers also are visited by *Mompha* moths (Momphidae), which lay eggs into buds or developing fruits but do not pollinate flowers. Selective pressure by *Mompha* moths drives geographic variation in a key floral volatile, (R)-(-)-linalool, through preferential oviposition and seed predation. A key remaining question is how floral volatiles might mediate the evolutionary transition of seed predators into obligate mutualists.

**Keywords:** eavesdropping; geographic mosaic; nursery pollination; private channels, VOCs

## Application of pheromones for the management of insect pests in Montana

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The cereal and pulse crops are major crops grown in Montana. Wheat stem sawfly, wireworms, wheat Head Armyworm, wheat midge on cereal crops and pea leaf weevil on pulse crops are causing significant damage. While the chemical control is one option, often do not provide adequate control. Therefore, pheromone-based work on these pests were undertaken from 2012 to 2020. Although, pheromone-based traps caught sufficient number of wheat stem sawfly adults, colored traps caught more sawflies than the bucket and the delta traps. Pitfall traps are found to be passive traps that catch click beetles (adult forms of wireworms) that happen to fall into them. The European based click beetle pheromone lures were not effective in catching the click beetles in Montana. Pheromone traps attracted wheat head armyworm males at all the study sites indicating the presence of this pest in Montana and lower dose (1 mg) of lures are adequate for monitoring this pest. The prevalence of wheat midge and its natural enemy was also well documented in Montana using pheromone baited sticky traps. In pulse crops, pheromone-baited pitfall and ramp traps caught significantly more pea leaf weevils than ground or delta traps. Pitfall traps baited with gray rubber septa captured significantly more adults than traps baited with membrane formulations. All these findings can be used to improve pest monitoring and should be taken into consideration when developing an integrated pest management program.

**Keywords:** Pheromones; cereal crops; pulse crops; Montana

## Potential novel sources of repellents/oviposition deterrents for spotted-wing drosophila management

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Native to Southeast Asia, the spotted-wing drosophila, *Drosophila suzukii* (Matsumura), has become a major pest of soft, thin-skinned small fruit crops worldwide. Here, we investigated three potential sources of repellents/oviposition deterrents for *D. suzukii* management. First, we determined the repellent/oviposition deterrent effects of anthracnose, an important disease of blueberries, and its causal agent, the fungus *Colletotrichum fioriniae*, on *D. suzukii*. In choice tests, *D. suzukii* females were repelled by fruit treated with anthracnose solutions containing spores either from field-collected infected fruit ('fruit') or a laboratory *C. fioriniae* culture ('colony'). *Drosophila suzukii* laid fewer eggs in fruit treated with the 'fruit' or 'colony' anthracnose solutions than in untreated fruit in no-choice tests. We also evaluated the effects of five commercially-available elicitors of plant defenses (Actigard, Blush, LifeGard, Oxidate, and Regalia) on *D. suzukii* behavior. We found a negative effect of the elicitors on *D. suzukii* oviposition, with the lowest numbers of eggs laid in fruits treated with Actigard, LifeGard, and Oxidate. When given a choice, fewer eggs were laid in fruits treated with the elicitors, except for Blush, compared with untreated fruit. However, only Regalia repelled *D. suzukii* females. Lastly, we evaluated methyl benzoate, a volatile from fermented apple juice, and nine analogs on *D. suzukii* behavior. One of these compounds (methyl 2-methoxybenzoate) repelled females and reduced oviposition under laboratory and semi-field conditions. These studies provide information on potential new natural sources of repellents/oviposition deterrents that could be used in combination with attractants to develop push-pull strategies to manage *D. suzukii*.

**Keywords:** *Drosophila suzukii*; plant defense elicitors; fungal volatiles; invasive pest

## **Insect-induced changes in fungal chemical exudate composition impact key properties of an experimental decomposer system**

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Secondary metabolite biosynthesis is a pervasive and often environmentally induced process by which organisms influence ecosystem properties. Understanding the causes and consequences of these biochemical dynamics is a central challenge in ecology. Although fungi have crucial functions in ecosystems, the involvement of their secondary metabolites in affecting community members remains elusive. We present experimental evidence that insect-induced changes in the chemical composition of fungal exudates released into the environment can have community-wide consequences. Fungal exudates resulting from a complex interaction between fungal genotype and insect attack not only affected insect foraging patterns and survival, but also the population growth of key microbes in a model decomposer system. Therefore, induced changes in the composition of fungal exudates may provide a critical but hitherto overlooked link between plasticity in chemical phenotype of fungi and dynamics in ecological communities.

## **Olfactory indolergic receptors in *Drosophila melanogaster***

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Indoles are ubiquitous compound known to be inter-kingdom signalling molecules. Their ecological significance in insects is unclear. In mosquitoes, indoles may indicate a human host or an oviposition site. Until the recent discovery of a 3-methylindole odorant receptor in the housefly *Musca domestica*, indole-sensitive odorant receptors (indolORs) were thought to be mosquito-specific. This finding lead us to explore the potential occurrence of indolergic odorant receptors in *Drosophila melanogaster*, another significant brachyceran species. In this work, we used a sequence homology-based approach to identify putative indolergic odorant receptors in this species. We operated the two-electrode voltage clamp system of *Xenopus laevis* oocytes expressing one of the three *D. melanogaster* candidate indolORs, among the larval-expressed OR30a, and the adult-expressed OR43a and OR49b. We assessed their selectivity toward a panel of 32 compounds, including 27 indole derivatives. We found that indole is the most potent ligand for OR30a and OR49b, while OR43a is highly sensitive to 3-methylindole.

**Keywords:** brachyceran; indole; olfaction; pharmacology



## Scent-mediated deceptive strategies in fly-pollinated *Aristolochia*

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Deceptive flowers trick pollinators into visiting them by advertising a reward, which they do not provide. Numerous deceptive plants are pollinated by Diptera and rely on floral scent for attracting these insects. Apart from sapromyophilous pollination systems, the chemical ecology of such interactions remains largely unstudied. We worked on seven deceptive *Aristolochia* (Aristolochiaceae) species in the Mediterranean, which are mainly pollinated by the dipteran families Phoridae, Drosophilidae and Chloropidae. Their peculiarly shaped flowers temporarily trap their pollinators and release them loaded with pollen. To resolve the mechanisms of pollinator attraction and deceptive strategies, we identified pollinators and applied chemical-analytical methods, chemical synthesis, electroantennography, and behavioral assays. We show that pollinators are, as expected, attracted by floral scent, the composition of which being strongly different among the studied species. Scent blends mostly consist of only a small number of compounds, including widespread floral volatiles, but also compounds previously unknown from floral scents. The scents of the different *Aristolochia* species resemble fermenting fruits (acetoin and derivatives), alarm pheromones of bugs (aliphatic esters), carrion (sulfides and pyrazines), as well as possibly sex pheromones of Phoridae (known and novel aliphatic compounds). Attractiveness of synthetic mixtures of these scents to pollinators was tested in field bioassays. Our results suggest that the studied *Aristolochia* species exploit various deceptive strategies, including chemical mimicry of food-sources of kleptoparasitic flies, oviposition-sites (most likely invertebrate carrion), and possibly sexual deception.

**Keywords:** chemical mimicry; electroantennography; deceptive pollination; floral scent; fly pollination

## Pheromones in the *Nasonia* group – behavior and biochemistry

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During the past decades, the genus *Nasonia* has become a model system for the study of all aspects of parasitoid wasp biology. Together with the closely related genera *Trichomalopsis* and *Urolepis* they have been suggested to form the so-called “*Nasonia* group”. Males of all species studied so far in this group produce substrate-borne sex pheromones to attract virgin females. Mated females exhibit dopamine-mediated olfactory plasticity and do no longer respond to the pheromone. Male sex pheromones in all *Nasonia* species are synthesized de novo in the rectal vesicle and are derived from fatty acid metabolism. A chiral hydroxylactone shared by all *Nasonia* species is synthesized from saturated fatty acids by the interplay of  $\Delta 9$ - and  $\Delta 12$ -desaturases, a putative cytochrome P450, the epoxide hydrolase NasviEH1 and enzymes catalyzing  $\beta$ -oxidation. The cosmopolitan species *N. vitripennis* has evolved an additional pheromone component, which allows females to discriminate against males of sympatric *Nasonia* species. Key enzymes for the evolution of the novel pheromone component in *N. vitripennis* are short chain dehydrogenases/reductases that epimerize the pre-existing pheromone component. Identification of the sex pheromone in *U. rufipes* revealed that a biosynthetic switch must have occurred during pheromone evolution in the *Nasonia* group. Males of this species produce a di-hydroxylated monoterpene as sex pheromone that is produced via the mevalonate pathway in the same gland and is used in a similar manner as in *Nasonia*. Hence, pheromone diversification in the *Nasonia* group occurred by both the modification of pre-existing pheromone components and the deployment of an unrelated biosynthetic pathway.

**Keywords:** biosynthesis; parasitoid wasp; pre-zygotic isolation; sex pheromone; *Urolepis*

## Diesel exhaust and ozone pollution reduce insect-mediated pollination services

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There have been significant declines in pollinator abundance and diversity during the last century, caused by factors such as agricultural intensification and climate change, with further declines predicted. Recent research has proposed a role for common tropospheric pollutants, e.g. diesel exhaust (particularly nitrogen oxides - NO<sub>x</sub>) and ozone (O<sub>3</sub>), in these declines. Small-scale behavioural and chemical studies, combined with atmospheric model simulations, demonstrate that such pollutants chemically alter or deplete many of the volatile organic compounds (VOCs) emitted by flowers to attract pollinators, and this process is expected to reduce pollinator foraging efficiency. However, no in-situ evidence exists demonstrating the effects of air pollution on wild or managed pollinators or the services they provide. Using free-air fumigation over two years, we show that elevation of NO<sub>x</sub> and O<sub>3</sub>, to concentrations commonly recorded in the lower troposphere, had significant effects on the foraging behaviour of field-populations of insect pollinators. These pollutants, individually and in combination, significantly reduced pollinator counts and flower visits, of our study plant (*Brassica nigra*), by at least 62% and 83%, respectively. These reductions, which were consistent over two years, were driven by responses of major pollinator groups (honey bees, bumble bees, solitary bees and hoverflies) and coincided with significant decreases in pollination and yield metrics. Moreover, O<sub>3</sub> reduced the effects of NO<sub>x</sub> on pollinator and plant metrics, likely due to the counteraction between atmospheric NO<sub>x</sub> and O<sub>3</sub>. These results provide the first evidence of substantial negative field-scale effects of NO<sub>x</sub> and O<sub>3</sub> pollution on insect-mediated pollination services.

**Keywords:** air pollution; field-scale; odour cues; pollinator; volatiles

## Sensory processing in the fly brain

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Most animals rely on their olfactory system to accomplish behavioral tasks that guarantee their survival and reproduction. Since the odor space consists of an enormous, nearly infinite number of possible stimuli, olfactory systems require special strategies to perceive, identify and evaluate the highly diverse odor information from the environment. The vinegar fly *Drosophila melanogaster* represents a premier model system for studying olfactory processing mechanisms since it exhibits a stereotyped architecture which is similar to its mammalian counterpart, but is less complex and highly tractable as well as susceptible to genetic manipulations. By exploiting these genetic techniques and linking them to neurophysiological, molecular and behavioral methods, my group is dissecting the neural circuits that are involved in coding, processing and perception of odors. We identified and dissected the neuronal correlates to specific behavioral outputs resulting from the perception of odor mixtures, we demonstrated that the neural composition of every olfactory glomerulus is unique and correlated to its functional relevance, and we were able to show that higher brain centers decode the behavioral value of an odor. We are currently examining whether the olfactory circuitry is hardwired or can be modulated by previous experience and/or associative learning. The talk will summarize our recent insights into coding and processing strategies of the olfactory circuitry of *Drosophila*.

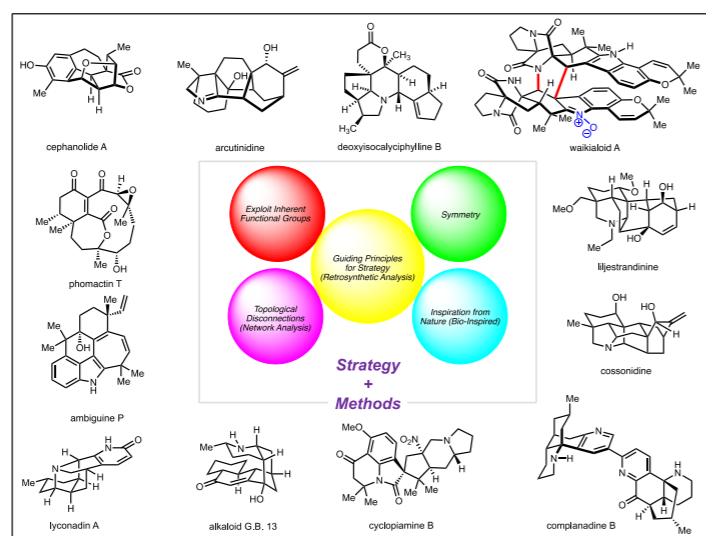
**Keywords:** *Drosophila*; odor coding; olfaction; neurogenetics; optical imaging

## Strategies for Chemical Synthesis Inspired by Complex Natural Products

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Natural products continue to inspire and serve as the basis of new medicines. They also provide intricate problems that expose limitations in the strategies and methods employed in chemical synthesis. Several strategies and methods that have been developed in our laboratory and applied to the syntheses of architecturally complex natural products will be discussed. In particular, new ways to employ C–C bond cleavage (i.e., break-it-to-make-it strategies) in complex molecule synthesis will be presented.



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## Towards Spatial Analyses of Community Composition: What Role for Plant Volatiles?

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Essential biodiversity variables (EBV's) set priorities, sampling and measurement standards for global monitoring. Only two EBVs exist for community composition: taxonomic diversity, and species interactions. We aim to develop scalable approaches for both of these using remote sensing and spatially resolved sampling. As plants are the trophic base of many communities, and sessile, we are interested in plant properties which can serve as useful indicators. Plant specialized metabolites – metabolites not required for cell growth and differentiation, which have diversified over the course of evolution – can be indicative of plant identity and condition, as well as strong determinants of ecological interactions and community structure. One example are the volatile compounds released by plants under stress. For example, a tree eaten by caterpillars will release volatiles which may be specific to the caterpillar species, and severity of attack. These volatiles can attract natural enemies of the caterpillars and activate defensive responses in neighboring trees. Laborious, time-resolved sampling and sample processing have hindered testing of community-level hypotheses. We aim to establish remote sensing and high-throughput, spatially resolved sampling approaches for metabolites of interest and lift this barrier.

**Keywords:** essential biodiversity variables (EBV's); plant-plant interactions; plant responses; plant specialized metabolites; species interactions

## Courtship behavior confusion in the invasive termite species *Coptotermes formosanus* and *C. gestroi*

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Congeneric species that live in sympatry may have evolved various mechanisms that maintain reproductive isolation. However, with the spread of invasive organisms, some species that evolved in allopatry can now be found outside their native range and may have the opportunity to interact in the absence of mechanisms for reproductive isolation. In South Florida, where the Asian termite *Coptotermes gestroi* and the Formosan termite, *C. formosanus* (Blattodea: Rhinotermitidae) are invasive, the two species can engage in heterospecific mating behavior as their distribution range and their dispersal flight season both overlap. In this study, by using gas chromatography coupled to mass spectrometry (GC-MS), microscopy and behavioral assays, we showed that females of both species produce (3Z,6Z,8E)-dodeca-3,6,8-trien-1-ol (DTE) from their tergal glands. Both species rely on an inundative dispersal flight strategy to find a mate. DTE is used as a contact pheromone to initiate and maintain tandems between males and females. The preference of *C. gestroi* males for *C. formosanus* females during tandem resulted from the higher amount of DTE produced by tergal glands of *C. formosanus* females than those of *C. gestroi* females. This results in confusion of mating during simultaneous dispersal flights and potential hybridization. Such observations imply that no prezygotic barriers emerged while the two species evolved in allopatry for ~18 Ma.

## Composition and EAD-active components of the scents of flowers pollinated by *Hemipepsis* (Pompilidae) wasps

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Floral scent can play an important role in maintaining highly specialized interactions between flowers and their specific pollinators, although well-understood examples of this remain scarce. In South Africa, spider-hunting wasps in the genus *Hemipepsis* pollinate a guild of 24 species of grassland plants, most of which are pollinated exclusively by these wasps. Although several studies have demonstrated the importance of floral scent as a key pollinator attractant, the particular compounds mediating wasp attraction remain to be established. We used Gas Chromatography-Electroantennographic Detection (GC-EAD) experiments with *Hemipepsis* wasp antennae to explore the active compounds in headspace samples and dichloromethane (DCM) extracts from the flowers of various guild members. Antennae responded to various saturated alkanes as well as aliphatic alkenes and aldehydes present in DCM extracts. In headspace samples, various methoxy aromatics elicited particularly strong antennal responses. The scents of all guild members are currently being scrutinized for active compounds identified thus far, while behavioural experiments testing the attractiveness of particular active compounds and combinations of compounds remain ongoing.

**Keywords:** spider-hunting wasp; specialized pollination; GC-EAD; private channel; pollination guild.

## Light-activated Defense Strategies in Mushrooms

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Whenever insects nibble on fruiting bodies of the green *Cortinarius austrovenetus* the bite site turns reddish. The chemistry behind it is as easy as it is fascinating: Hypericin is formed by oxidation from the fungal pigment austrovenetin as soon as the fungal tissue is damaged. Inspired by this striking observation, we started to systematically explore phototoxicity in fruiting bodies. Studying over fifty different species by the means of photocytotoxicity assays and feature-based molecular network (FBMN) showed that pigments of the polyketide pathway are especially interesting. Furthermore, we could show that phototoxicity is a common trait in the subgenus Dermocybe. Photoactivity guided isolation strategies yielded several so-called photosensitizers from fruiting bodies. 7,7'-Biphyscion (BP), as example, was isolated from *Cortinarius uliginous* and – in a nanomolar range – induced apoptosis in cancer cells solely under irradiation. Moreover, we started to investigate the ecological relevance of these photo-active pigments. We hypothesized that – according to the optimal defense hypothesis – phototoxicity should be concentrated in the gills. Statistical analysis of more than forty individual fruiting bodies proved an enriched of phototoxicity in the gills by inter alia the accumulation of emodin. Here we will present our most recent photo-activated defense strategy insights from an overlooked Kingdom, from fungi.

**Keywords:** Cortinarius; Fungi; Photosensitizer; Pigments; Singlet Oxygen; FBMN-analysis

## Sponge Watch Program- An initiative towards 'one ocean-one health' approach in the age of Covid-19

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In the Anthropocene epoch (also known as 'Age of Plastics'), plastic pollution has become a global problem, contributing to 80% of marine litter. India produces an estimated 25,940 tonnes of plastic waste every day, 40% of which remains uncollected and ends up in our oceans. These plastics are further broken down into tiny pieces (= Microplastics, MPs) that enter the marine food web affecting >70% of marine species and humans as well. At present, we do not sufficient techniques to efficiently detect or remove MPs from our oceans. But what if the answer lies in our world's oldest animal – the marine sponge. Marine sponges are the most efficient filter-feeders with the capacity to filter more than six times their volume per minute and the ability to thrive in polluted waters. We used a self-designed combinatorial approach to isolate and characterise MPs from common species of sponges found in coastal regions of the Gulf of Mannar, India. It was found that marine sponges can accumulate significantly higher MPs ( $p < 0.05$ ) as compared to ambient seawater and sediment. Moreover, different species of sponges showed varied bioaccumulation of MPs inside their body, suggesting species-specific responses to MP. We aim to understand the mechanisms behind MP bioaccumulation and explore the potential bioindicator and/or bioremediation capabilities of sponges. This will offer unique insights for marine conservation, sanitation, and waste management, and has the potential to find new bioinspired ways to remove microplastics from our water and understand how it is affecting our living world, especially in the age of COVID-19. We aim to establish these marine sponges as a suitable system to monitor environmental health such as microplastics pollution. It is important to identify monitoring parameters and to determine the most appropriate biological indicators for long-term microplastics monitoring. These parameters can be listed out and designed in the form of user-friendly kits to allow the common public to take part in such environmental monitoring programs. Our integrated approach will help in developing a science-citizen program to take care of our ocean and our health.

**Keywords:** marine pollution; biofiltration; bioindication; bioremediation; marine chemical ecology and health, one ocean-one health concept

## Identification of candidate sex-pheromone components of two Nearctic *Agriotes* species (Coleoptera: Elateridae)

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Click beetles are a diverse family that contain several important agricultural pests, some of which have reemerged in high numbers in recent decades. While some species have identified sex-pheromones, particularly those in the Palearctic *Agriotes* pest complex, little is known about the sex-pheromones of Nearctic species, and native *Agriotes* species had not been previously studied. This paucity of knowledge has severely limited the development of effective monitoring and semiochemical based management tactics for Palearctic pest species. Species-specific, pheromone-based monitoring tools are desirable as the beetle stage is typically much easier to capture and identify than the soil-dwelling larvae. Semiochemical based management tools target the beetle stage before they mate or oviposit. Moreover, the spread of invasive European *Agriotes* species in Canada threatens to displace native *Agriotes* pest species such as *A. mancus* and *A. ferrugineipennis*. Hence having pheromones for both native and invasive *Agriotes* species in Canada will allow us to understand the composition of elaterid pest species in crops, monitor pest spread and species composition changes over time, and inform mass trapping and pesticide application decisions. Here we discuss the candidate sex-pheromone components of *A. mancus* and *A. ferrugineipennis*. Headspace odorants from female *A. mancus* and *A. ferrugineipennis* were collected on Porapak Q and aliquots of Porapak extract were analyzed by gas chromatographic-electroantennographic detection (GC-EAD) and GC-mass spectrometry. Components were field tested in Vernon Pitfall Traps in the Spring of 2020 and 2021 and showed strong preliminary attraction. We compare pheromonal structures with those of European *Agriotes*.

**Keywords:** agriculture; click beetles; insect-trapping; integrated pest management; pheromone-based monitoring

## Novelty seeking, attention and collective behaviour in the honeybee, *Apis mellifera*

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Individual differences in learning can influence how animals respond to and communicate about their environment, which may nonlinearly shape how a social group accomplishes a collective task. There are few empirical examples of how differences in collective dynamics emerge from variation among individuals in cognition. Here, we use a naturally variable and heritable learning behaviour called latent inhibition (LI) to show that interactions among individuals that differ in this cognitive ability drive collective foraging behaviour in honeybee colonies. We artificially selected two distinct phenotypes: high-LI bees that ignore previously familiar stimuli in favour of novel ones and low-LI bees that learn familiar and novel stimuli equally well. We then provided colonies differentially composed of different ratios of these phenotypes with a choice between familiar and novel feeders. Colonies of predominantly high-LI individuals preferred to visit familiar food locations, while low-LI colonies visited novel and familiar food locations equally. Interestingly, in colonies of mixed learning phenotypes, the low-LI individuals showed a preference to visiting familiar feeders, which contrasts with their behaviour when in a uniform low-LI group. We show that the shift in feeder preference of low-LI bees is driven by foragers of the high-LI phenotype dancing more intensely and attracting more followers. Our results reveal that cognitive abilities of individuals and their social interactions, which we argue relate to differences in attention, drive emergent collective outcomes.

## Deciphering the chemical ecology of push-pull intercropping system in mitigating fall armyworm, *Spodoptera frugiperda*, herbivory

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The Fall armyworm, *Spodoptera frugiperda*, is a serious invasive pest currently spreading across the world after having previously been confined to the Americas. Intercropping maize with companion plants in a push-pull system has been shown to substantially reduce *S. frugiperda* infestation, but the underpinning mechanisms are still unknown. We hypothesised that companion crop volatiles repel herbivores (push) while attracting natural enemies (pull). Plant volatiles collected from companion plants (*Desmodium intortum*, *Desmodium uncinatum* and *Brachiaria Mulato II*) were used in bioassays and electrophysiological recordings with *S. frugiperda* and key larval endoparasitoids (*Cotesia icipe* and *Coccygidium luteum*). Coupled GC-electroantennogram (GC-EAG) recordings from the antennae of *S. frugiperda* and both parasitoids *C. icipe* and *C. luteum* showed robust responses to aromatic and terpenoid volatile compounds from companion plants. In wind tunnel bioassays, moths made significantly fewer source contacts with maize volatiles when mixed with desmodium compared to maize alone. In no choice oviposition bioassays, while a significantly lower proportion of eggs was laid at the bottom of the oviposition compartment when *S. frugiperda* were positioned above companion plants, significantly more eggs were laid at the top of these compartments, indicating that female moths were repelled by the volatiles of the companion plants. The parasitoid wasps were attracted to the scent of both *Desmodium* spp. (intercrop) and the *Brachiaria* border crop in an olfactometer bioassay. Field experiments revealed that push-pull plots (*i.e.* maize intercropped with *D. intortum* and surrounded by *Brachiaria*) had fewer *S. frugiperda* larvae, lower damage and higher parasitism rate than monocropped maize. Our findings decipher the underpinning mechanisms of how push-pull companion cropping mitigates *S. frugiperda* herbivory in sub-Saharan Africa maize agroecosystem.

**Keywords:** Behavioural assays; companion crops; GC-EAG recordings; maize fields; oviposition; tritrophic interaction; volatile profiles.

## Land-use stress and pesticides alter the chemical communication of wild bees

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In the last years, insect pollinators are significantly declining in agricultural areas by evidence. These declines are due to many factors e.g. habitat and food loss, emergent diseases, pesticides and climate change. In intensified agricultural areas pollinators are chronically exposed to various pesticides that were found to negatively affect foraging bees by altering orientation and learning behaviour. Effects of pesticides or other stressors on chemical communication, however, have rarely been investigated. We studied the effects of stressors, which affect pollinators in intensified agricultural areas, on chemical communication of wild bees. *Bombus lapidarius* workers were caught in grasslands with different land-use intensities in Germany. In chemical analyses of cuticle surface odours with a function in chemical communication, we found a significant effect of land-use intensity on scent bouquets. In a further study, we tested the effect of single stressors such as neonicotinoids, on communication and antennal sensitivity for semiochemicals. Bumblebees and mason bees were treated with field realistic doses of neonicotinoids. Workers of *B. terrestris* differed significantly in their chemical profile after treatment with thiamethoxam or clothianidin. In the mason bee *Osmia bicornis*, clothianidin decreased antennal sensitivity to common floral volatiles. In our study, we found an effect of neonicotinoids on the production and perception of semiochemicals in bumblebees and mason bees. For *B. lapidarius*, it is not finally clarified, which stressor led to the changes in the scent bouquet. However, our results indicate that agricultural management and practice may have a negative effect on chemical communication and pollination.

**Keywords:** agricultural management, *Bombus*, cuticular hydrocarbons, electrophysiology, *Osmia*

## Synthesis of semiochemicals with the guidance of Professor Kenji Mori

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The late Professor Kenji Mori published over 850 original papers, including 264 on "Pheromone Synthesis". About two-thirds of these papers were written in his home laboratory at the University of Tokyo. I am now the professor of the very laboratory where Prof. Mori had accomplished so many great things. I started my career as an organic chemist under his supervision and have learned a lot from him. Thus, his excellent guidance is an irreplaceable treasure for me and will always be with me. In this symposium, I would like to talk about my research on the synthesis of semiochemicals with the guidance of Mori-sensei.

- 1) Synthesis of the sex pheromone of the German cockroach
- 2) Synthesis of limatulone, the defensive triterpene metabolite of the limpet *Achmeia limatula*
- 3) Structural revision of alectrol, a strigolactone isolated from cowpea

Since I was one of the people closest to Mori-sensei in his last years, except for his family, I would like to mention his struggles and dedication to science in his last half year.

**Keywords:** defensive metabolite; natural product; pheromone; stereoselective synthesis; strigolactone

## Functional analysis of two odorant receptors related to host seeking in *Aedes aegypti*

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The mosquito *Aedes aegypti* is the primary urban vector of several highly infectious human pathogens. Disease transmission heavily relies on the ability of the female to locate and feed on the blood of infected hosts, which are primarily odour-mediated behaviours. Olfactory sensory neurons, housed in sensilla, express a wide array of chemosensory genes that are likely to play central functions in these behaviours. The emergence of heterologous systems to express and screen odorant receptors (ORs) with known behaviour-modulating volatiles has permitted the reliable identification of key ligands for these receptors. Three ORs-of-interest, *AaegOr4*, *AaegOr103* and *AaegOr117*, were previously described by our group as potentially involved in the evolution of human host preference and the modulation of onset host seeking in female *Ae. aegypti*. While *AaegOr4* was upregulated in human-preferring mosquitoes and showed a consistent response to sulcatone, a volatile compound highly abundant in human odour, the function of the two other receptors remains unknown. Through GC-SSR, we monitored odour-evoked responses in *AaegOr103* and *AaegOr117*, heterologously expressed in the *Drosophila* ab3A empty neuron system, and revealed both antennal inhibition and activation to certain volatile compounds collected from humans and host plants. We speculate that these two receptors are candidate genes involved in the evolution of host preference and the modulation of odour-mediated behaviours in *Ae. aegypti*. A better understanding of the function of these and other ORs could enable the development of future control strategies against vector borne diseases.

**Keywords:** deorphanization; enantiomers; olfaction; reverse chemical ecology; sensillum



## Fall Armyworm Oviposition Suppresses Volatile Emission in Maize: Effects on Recruitment of Egg Parasitoid

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Plants subjected to insect infestation often increase volatile emission to attract natural enemies, although some arthropods have evolved ways to suppress volatile profiles of host plants. Concurrently, natural enemies have developed a finely tuned olfactory system to locate plants on which their prey is present. Our earlier studies showed that egg deposition by stemborer moth (*Chilo partellus*) elicited increased levels of volatile emission from certain maize genotypes which attracted natural enemies of the pest. In this study, we investigated whether similar effects could be observed due to egg deposition by an alien invasive pest to Africa, the fall armyworm *Spodoptera frugiperda* (J.E. Smith) (Lepidoptera: Noctuidae) and the multitrophic consequences thereof. Interestingly, *S. frugiperda* oviposition suppressed volatile emission in maize when compared to control intact plants and changed the ratio of components in the emitted blend resulting in distinct volatile profiles. In an olfactometer bioassay, the egg parasitoid *Telenomus remus* Nixon (Hymenoptera: Platygasteridae) was significantly attracted to volatiles from egg-infested maize plants compared to uninfested controls. Our findings imply that egg deposition by *S. frugiperda* triggers an early herbivore alert signal involving suppression of maize volatile emission; however, the herbivore's key egg parasitoid is tuned in to the cue. The implications of the findings in exploiting native parasitoids for biological control of the invasive pest, *S. frugiperda*, are discussed.

**Key words:** Behavioural bioassay; *Telenomus remus*; biocontrol; *Spodoptera frugiperda*; oviposition-induced plant volatiles (OIPVs)

## Semiochemical based approach for the control of *Helopeltis bakeri* Poppius (Hemiptera:Miridae)

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Cacao, an agriculturally important crop, is grown and cultivated in the Philippines due to its strategic location and weather conditions. However, pests and diseases threatens the sustainability of the cacao plantations in the local regions. The cacao mirid bug, *Helopeltis bakeri* Poppius (Hemiptera:Miridae), is an emerging pest of cacao in Southeast Asia. One effective method of Integrated Pest Management (IPM) is the use of semiochemicals to trap and bait these pest insects. One of the semiochemicals that attract the mirid bugs for feeding is  $\beta$ -caryophyllene that was identified by headspace extraction using 100  $\mu$ m Polydimethylsiloxane (PDMS) and 50/30  $\mu$ m CAR/PDMS/DVB solid phase micro extraction (SPME) fibers coupled with gas chromatography-mass spectrometry analysis. Preliminary olfactory analysis with an effective concentration of 23  $\mu$ g elicited a 60-90% attraction of the CMBs. With wind tunnel bioassay, an effective concentration of 90  $\mu$ g in the impregnated lure showed 75% positive attraction of the CMBs. Different trap designs were also tested to show which is best for field deployment. Data gathered from this study will substantially help in the development of lures and traps for field testing.

**Keywords:** cacao mirid bug; caryophyllene; kairomone; SPME; trap design

## Non-human primate and human-derived attractants for *Aedes* mosquitoes

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Yellow fever (YF) and dengue (Den) share a common niche mainly transmitted by *Aedes* (*Stegomyia*) species. Surveillance of vector populations is a critical component of risk assessment for YF and Den transmission and outbreaks. Implementing this effectively requires the use of efficient trapping tools for monitoring vector populations which can be achieved by employing lures through knowledge of their blood-seeking behavior. Blood-seeking mosquitoes use host-derived cues including breathe (e.g. CO<sub>2</sub>) and body chemicals for host location. So far, identification of host-derived odors as attractants has been attempted for domestic anthropophilic *Aedes aegypti*. However, similar data remain lacking for non-human-biting *Aedes* vectors in a sylvatic setting where non-human primates (NHP) serve as reservoir hosts. This presentation will highlight progress in our efforts to develop attractive lures for improved surveillance of adult *Aedes* vectors of YF and Den in both sylvatic and domestic environments, employing chemical ecology approaches and a series field experiments.

**Keywords:** *Aedes*-borne viruses; host-seeking; lures; sylvatic environment; domestic environment

## Codling Moth: A Look Back with an Eye on the Future

**Donald Thomson**

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The sex pheromone of codling moth *Cydia pomonella* was identified in 1971. Research by scientists affiliated with public institutions laid the foundation for the commercialization of codling moth mating disruption (CMMD) technologies starting 30 years ago. Initially, adoption was slow with many hurdles to overcome but CMMD is now an integral part of pest management programs for pome fruit worldwide. Dispenser technology has improved in the last 30 years to ensure season long release of pheromone. Despite dispenser improvements, CMMD is still not a stand-alone technology and requires careful monitoring and supplemental controls to ensure that growers get acceptable control. Due to extensive research, we now better understand the mechanisms of CMMD. The physiological and subsequent behavioural responses to pheromone are different than for other insects such as oriental fruit moth *Grapholita molesta* where mating disruption is a largely stand-alone technology. Pest management systems and technologies have changed dramatically over the last 25 years. High density plantings creating more open and exposed environments are common. Contact insecticides have been replaced by technologies that need to be ingested requiring careful attention to timing and coverage. The success of CMMD requires an understanding of codling moth's physiological and behavioural responses to its pheromone and the strategies needed to best position CMMD in pest management systems.

**Keywords:** behaviour; commercialization; *Cydia pomonella*; pheromones; physiology

## Insights into Use of Semiochemicals for Management of Disease Vectors in Kenya

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In recent years, previously controlled vector-borne diseases have resurged or reemerged in new geographic areas. Few vaccines are available to control these vector-borne diseases. Reports of resistance to drugs and insecticides by vector-borne pathogens and vectors, respectively, have become of major concern. To effectively tackle these vector-borne diseases, we at the International Centre of Insect Physiology and Ecology, Nairobi, Kenya, argue in a recent article entitled “Grand Challenges in Vector-Borne Diseases Targeting Vectors” published in *Frontiers in Tropical Diseases*, that given the existing constraints, development of vector control tools should be intensified to reduce vector-human contact. We identified several priority research areas to facilitate the development of innovative control tools, among them the chemical ecology of disease vectors. In this lecture, I will highlight some of the progress made in the development of these tools based on understanding and exploitation of weak links in the chemical ecology of vector-host interactions, focusing on a few diseases; sleeping sickness, malaria, dengue and yellow fever, Rift Valley fever and leishmaniasis in Kenya. I will also highlight a few research areas to aid development of more effective semiochemical-based tools for disease vector management.

**Keywords:** disease vectors, sleeping sickness, malaria, leishmaniasis, Rift Valley fever

## Use of sex pheromones for wireworm (Coleoptera: Elateridae) management in Canada

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There are approximately 20 pest wireworm species in Canada, of which three have been introduced. Until recently, these three (*Agriotes lineatus*, *A. obscurus*, and *A. sputator*) were the only species for which sex pheromones had been identified and used for monitoring and wireworm risk assessment purposes. However, since 2010 there has been a renewed interest in identifying the sex pheromones of native North American pest species, and to date structures have been described for various species in at least five genera, including *Agriotes*, *Melanotus*, *Selatosomus*, *Cardiophorus* and *Limonius* spp. Here we discuss results of field testing some of these new compounds, the potential of combining different pheromones for managing co-occurring heterogeneric species, the distribution of different wireworm pest complexes in Canada and species for which pheromone structures (if present) still need to be elucidated, and work done to date to develop semiochemical-based management strategies (mass trapping and mating disruption) in Canada. Several knowledge gaps will be identified, as well as suggestions and proposed work to address these.

**Keywords:** click beetle; Integrated Pest Management; mass trapping; mating disruption; monitoring.

## Impact of ozone on the perception of olfactory signal by generalist pollinators

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Concentration of tropospheric ozone (O<sub>3</sub>) has dramatically increased since pre-industrial times and could threaten the functioning of ecosystems. Negative effects on both emission and transport of plant-emitted volatile organic compounds (VOCs) have been already highlighted, which does not augur well for the attraction of insects that relies on these chemical signals, particularly pollinators. Surprisingly direct effects of O<sub>3</sub> on the attraction of insect itself have not been considered so far. To investigate the effects of O<sub>3</sub> on the VOC detection and the behavioral response of *Bombus terrestris* foragers, electrophysiological experiments and behavioral assays were carried out under controlled conditions. In order to simulate a peak of O<sub>3</sub>, naïve foragers were exposed to ozone at a given concentration (i.e. 0, 80, 120 or 200 ppb) for 60 min or 180 min. Following the exposition, the responses of bumblebee antennae were measured for different concentrations (i.e. 0.1, 1, 10 and 100 µg/µL) of three synthetic VOCs mimicking compounds generally found in floral scents (i.e. (-)-linalool, benzaldehyde and nonanal). Our results show that ozone exposition affects volatiles perception by bumblebees but that this effect depends on the O<sub>3</sub> exposure (i.e., concentration and duration) as well as on the VOC and its concentration. Besides behavioral assays using glass Y-tube olfactometer showed a modification of their behavioral response to a VOC stimulus (i.e. benzaldehyde) after ozone exposition, depending on both concentration and duration of O<sub>3</sub>. These results indicate that ozone-rich environment can affect plant-bumblebee interactions, interfering with both host detection and attraction.

**Keywords:** Air pollution; Antennal detection; Behavioral response; Bumblebees; Plant-pollinator interactions

## *Doublesex* regulates pheromone communication and odour-guided behaviour in *Nasonia* wasps

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Parasitoid Hymenoptera have evolved a comprehensive capacity to sense chemical cues for sexual communication and host localisation. The underlying olfactory and evolutionary mechanisms, however, remain poorly understood. Evolutionary changes frequently arise from modifications in regulatory elements of existing genes that contain binding sites for transcription factors such as *Doublesex*. We hypothesise that *Doublesex* directly regulates pheromone production and chemosensory differentiation in *Nasonia* wasps. Silencing *Doublesex* in *Nasonia* males by RNA interference (RNAi) significantly reduced both long-range pheromones and cuticular hydrocarbons (CHCs) essential for mate recognition. The altered CHC profile made RNAi-treated males attractive to wild-type males, which prompted us to further research communication based on sexually-dimorphic CHC. In addition, females failed to become receptive to RNAi-treated males, and we hypothesise that this is due to the absence of an oral pheromone produced by males. We therefore carried out a preliminary analysis of the oral pheromone through dynamic headspace sampling to determine its chemical composition. We also hypothesise that *Doublesex* regulates the chemosensory system, and we confirmed a reorganisation of sex-specific glomeruli in the antennal lobe of RNAi-treated males and females. The formation of these glomeruli was traced to sexually-dimorphic sensilla and neural innervation, giving rise to differential electroantennogram and behavioural responses to both host and pheromone chemicals. We found *Doublesex* to play a pivotal role in regulating pheromone production and sex-specific differentiation of the chemosensory system. We aim to identify *Doublesex* target genes to establish whether modifications in *Doublesex* regulation are fundamental for the evolution of pheromone communication and speciation in parasitoid wasps.

**Keywords:** courtship; glomeruli; hydrocarbons; neuroecology; sensilla

## Functional characterization of Na,K-ATPase subunit combinations reveals the adaptive strategy of cardenolide-resistant large milkweed bugs

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The evolutionary arms race between plants and specialized herbivorous insects has been widely studied but the myriad mechanisms involved in this race still pose fascinating riddles. Here, we present one facet of coevolution from the insect perspective: the evolution of three Na,K-ATPase  $\alpha$ -subunit gene copies with strongly differing resistance towards cardenolides in the large milkweed bug (*Oncopeltus fasciatus*). The large milkweed bug ingests high concentrations of cardenolides by sucking the seeds of *Asclepias* plants, and does not suffer any harm in the process. We identified different amino acid substitutions in the cardenolide binding site of three  $\alpha$ -paralogs (A,B,C), and we found that each paralog had altered enzymatic activity and resistance. We further identified four different  $\beta$ -subunits that modulate the Na,K-ATPases' behavior. We heterologously expressed nine possible  $\alpha/\beta$ -subunit combinations of *O. fasciatus* Na,K-ATPases in Sf9 cells with the baculovirus expression system. The recombinant enzymes were exposed to increasing concentrations of two cardenolides, which differ in source, structure, and steric conformation: ouabain and calotropin. Calotropin, synthesized in the bug's host plants, showed a much stronger inhibitory effect on the Na,K-ATPase than ouabain. The conserved and most enzymatically active  $\alpha C$ -copy was completely inhibited at high cardenolide concentrations, whereas  $\alpha B$  maintained moderate residual activity at the same concentrations. We found evidence that specific  $\alpha/\beta$ -combinations can enhance the activity ( $\alpha C\beta 3$ ) or resistance to cardenolides ( $\alpha A\beta 1$ ) of the Na,K-ATPase. Our results thus reveal that adaptation to the host plant toxins tuned the duplicated paralogs to trade-off the pleiotropic effects of resistance versus ion transport.

**Keywords:** recombinant enzymes; inhibition; calotropin; ouabain;  $\alpha/\beta$ -subunit combinations

## Life scientists in chemical ecology can benefit from psychology's responses to its own 'reproducibility crisis'

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Reproducibility failures have affected all parts of the life sciences relevant to chemical ecology, from animal behaviour to molecular biology. Psychologists have responded constructively and creatively to their own field's very public 'reproducibility crisis'. The solutions include new ways of doing experiments, such as Registered Reports and aspects of Open Science. To illustrate some of our own challenges in chemical ecology, I will use the story of the 'putative human pheromones' androstadienone and estratetraenol which, despite never having been shown to be pheromones, have been the subject of some 60 studies claiming 'significant' positive results. These are quite possibly false positives, part of the 'reproducibility crisis', sadly common in the rest of the life and biomedical sciences, which has many instances of whole fields based on false positives. Chemical ecological research would benefit from vigorously adopting the proposals made by psychologists to enable better, more reliable science, with an emphasis on enhancing reproducibility. A key change is the adoption of study pre-registration and/or Registered Reports, which will also reduce publication bias. A growing number of journals covering chemical ecology offer Registered Reports, including *BMC Biology*, *Nature Communications*, *Scientific Reports*, and *Royal Society Open Science*.

**Keywords:** pre-registration; reproducibility; false positives; p-hacking; open science

## Studies of the oxylipin pathway regulation in brown algal kelps

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In intertidal zones, brown algal kelps are key components of marine forests, and potential sources of ecologically important chemical compounds. Indeed, similarly to land plants and animals, kelps have been shown to feature inducible metabolic responses upon both abiotic and biotic stress, such as the release of free polyunsaturated fatty acids (PUFAs), oxidized derivatives from both C18- and C20- PUFAs, and aldehydes. However, in the brown algal lineage, their biosynthesis pathway has been hardly explored neither their biological and ecological roles in defense or distance signaling. Preliminary results obtained in our laboratory have suggested that some products of PUFA oxidative pathway such as oxylipins and aldehydes might induce defense reactions against grazers in some kelps. To explore their signaling roles in kelps, different chemicals such as aldehydes (4-Hydroxy hexenal, 4-HHE) and oligoguluronate (GG) elicitors were applied on young kelp plantlets of *Saccharina latissima* and untargeted metabolomic analysis revealed the regulation of several oxylipin-derived compounds. In parallel, large-scale transcriptomic analyses were conducted to identify both molecular short- and longer-term responses in *S. latissima*. RNA-seq data were acquired at four time points (0.5h, 1h, 4.5h and 12h) after application of GG. Preliminary result showed significant gene regulation compared to control, including 7 LOX genes mostly up-regulated after 4.5 hours. This indicated an early activation of oxylipin pathway. Altogether, the integration of metabolic and transcriptomic data will help us to decipher the molecular bases of oxylipin pathway regulation in brown algae.

**Keywords:** Oxylipins; aldehydes; defense signaling; gene expression; transcriptomics; metabolomics

## How parasitic wasps use a combination of plant volatiles and sex pheromones to locate mates

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Plant volatiles are not only used by herbivorous insects to find their host plants, but also by predators and parasitoids to find plants that carry prey or hosts, respectively. There is also increasing evidence that plant volatiles, in addition to species-specific pheromones, help these insects to find mating partners. In the case of parasitoids, we found that wasps of four braconid species are strongly attracted by herbivore-induced plant volatiles (HIPVs) independently of the wasps' sex and mating status. Each of the four species seems to use sex pheromones differently (released by virgin females or males). However, in all cases, compared to HIPVs, the pheromonal compounds appeared to attract wasps over relatively shorter distances, probably because they are less volatile and released in smaller amounts. The solitary endoparasitoid *Microplitis mediator* (Haliday) appears to be the most extreme, likely relying exclusively on HIPVs for finding mates over longer distances, since we found no evidence for volatile pheromonal cues in the species. Host-damaged plants are probably used as rendezvous sites by each of the studied parasitoids, whereas pheromones are more likely used for mate recognition over shorter distances. There is increasing evidence that also in other insects the mate-finding process is mediated by a combination of plant volatiles and pheromones. These insects (e.g. herbivores, pollinators and parasitoids) may have evolved this combined use of cues as an efficient foraging strategy to simultaneously locate food and mates.

**Keywords:** plant-insect interactions; mate finding; hymenopteran parasitoids; volatile cues; insect foraging strategies.

## Did plant chemical defences evolve like the spandrels of San Marco?

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Many plants produce chemical defences that can reduce damages from herbivores and pathogens. While the evolution of chemical defences have been mainly considered to be driven by herbivores and microbes, recently advances in illustrating the biosynthesis and function of plant chemical defences suggested that some of them might have evolved from side effects of other selection pressures, the phenomenon termed by Stephen Gould and Richard Lewontin as “the spandrels of San Marco”. In this talk, I will use some of our recent findings to illustrate how plant chemical defences might have evolved as side effects of other evolutionary processes. I will also discuss the future directions of studying the evolution of plant chemical defences.

**Keywords:** Chemical defences; evolution; the spandrels of San Marco; detoxification; biosynthesis; cellular signaling

## Chemosensory Proteins (CSPs) in Cotton Bollworm *Helicoverpa armigera*

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Chemosensory proteins (CSPs) are a group of small soluble proteins conventionally known as odorant and pheromone carriers in insect chemosensory system. Recent studies reveal that they also function in development, nutrient metabolism and insecticide resistance. In depth and systematic characterization of previously unknown CSPs will be valuable to investigate more detailed functionalities of this protein family. Here we identified 27 CSP genes from the genome and transcriptome sequences of cotton bollworm, *Helicoverpa armigera* (Hübner). The expression patterns were studied by using transcriptomic data obtained from different tissues, stages and whole larvae reared on various host plants. The results demonstrate that *H. armigera* CSP genes are not only highly expressed in chemosensory tissues such as antennae, mouthparts and tarsi, but also in salivary glands, cuticle epidermis and hind guts. The mRNA expression levels of HarmCSP1, 2, 5, 9, 11, 19, 21/22, 23, 24 and 25 can be significantly regulated when larvae feed on certain host plant species, suggesting CSPs may play a role in insect plant feeding behaviors. HarmCSP6 and HarmCSP22 were further expressed, purified and utilized as “baits” to develop a reverse chemical ecology strategy to “fish” candidate ligands from host plants. This study advances our understanding of insect CSPs, their functions and insect-plant interactions.

**Keywords:** chemosensory protein, CSP, cotton bollworm, *Helicoverpa armigera*, gene expression, olfactory

## Electroantennogram responses of the parasitoid wasp, *Diadegma semiclausum*, to host-related odours

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An increase in insecticide resistance have been reported broadly in the diamondback moth (DBM) (*Plutella xylostella*), promoting interest in the biological control using natural enemies. Parasitoid wasps have long been used as biocontrol agents of DBM, which use blends of volatiles released from attacked plants to localize hosts. To identify and analyze these compounds and their specificity are the first key step to understand the mechanisms how natural enemies localize host insects within crops. In this study, canola seedlings were used as the host plant, which was consumed by *P. xylostella* to identify the volatile compounds. *Diadegma semiclausum* was studied as the DBM parasitoid wasp to examine its antennal responses to various volatile compounds. Scanning electron microscope (SEM) was used to investigate the olfactory sensilla of the male and female *D. semiclausum*. The results identified six compounds that were of significant changes in their amounts from DBM-infested canola. Electroantennogram (EAG) results demonstrated a group of physiologically active compounds which can elicit antennal responses of male or female *D. semiclausum*, which might be candidate attractants for *D. semiclausum* to localize the DBM. Interestingly, male and female *D. semiclausum* showed different responses to certain tested compounds in EAG. SEM results revealed seven types of olfactory sensilla from *D. semiclausum* adult antennae. This study identified candidate attractant compounds for *D. semiclausum* and improves our understanding of their olfactory systems, which will help optimize our biological control strategies to control *P. xylostella* in future.

**Keywords:** Insect olfaction, volatile compounds, Diamondback moth, *Diadegma semiclausum*, Electroantennogram

## Eavesdropping into host communication: the bee louse *Braula coeca* selects its host using kairomones

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The bee louse *Braula coeca* had until recently a global distribution that coincided with its host the Western honey bee *Apis mellifera* L. The adult fly usually attaches itself to a worker and steals food out of the host's mouth. However, not all worker bees carry *Braula* and the mechanism used by the bee louse to select a particular host is poorly known. We sampled and analysed using gas chromatography, the mandibular gland secretions (MDG) of worker bees that were carrying and those not carrying *Braula* from queenright colonies of *A. m. scutellata*. MDG profiles were qualitatively identical containing the five main MDG components, but workers carrying *Braula* had proportionately more methyl p-hydroxybenzoate (HOB) and the queen substance 9-oxo-2(E)-decanoic acid (9-ODA). Quantitatively, bees with *Braula* had higher amounts of the pheromones with a mean of 6.02 µg per bee, compared to 3.62 µg per bee for those not carrying *Braula*. A multiple comparison between all the components in the MDG profiles shows that, irrespective of the colony sampled, bees carrying and those not carrying *Braula* are different in both the proportions and concentrations of pheromones except for the worker component 10-hydroxy decanoic acid (10-HDAA). *Braula* is thus capable of using kairomones as cue that allows it to benefit from throphallactic dominance by selecting individuals that have a higher probability of being fed so as to get enhanced access to food.

**Keywords:** Bee louse, pheromonal communication, Mandibular gland secretions, honeybee



## Green Pesticide: Methyl Benzoate and its Analogs

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Benzoate methyl ester, also known as methyl benzoate (MB), is a volatile organic compound that exists naturally as a floral fragrance in many plants. Our behavioral bioassays show that MB and some of its naturally occurring and synthetic analogs kill insects at different life stages. Compared to commercial pesticides containing pyriproxyfen and acetamiprid, MB and some analogs are 1.3 to 3.4 times more toxic to gypsy moth larvae and brown marmorated stinkbug nymphs. The arthropod repellent DEET is also a benzoate ester and shares the same chemical skeleton with MB. They differ by the diethylamide ester and a methyl group on the benzene ring in DEET. However, unlike MB, DEET does not kill insects; instead, it deters or repels them. Exactly how DEET causes the repellent effect in target organisms is still a mystery. Due to the MB's structural similarity to DEET, exploring the structure – activity relationship (SAR) of the MB analogs will provide useful information for the discovery of the mode and mechanistic actions of DEET as an insect repellent. In addition, the SAR will allow researchers to modify the chemical structure of the MB molecule, leading to the development of more efficient, safe, and environmentally – friendly green pesticides.

**Keywords:** Natural product; volatile organic compound; toxicity; integrated pest management

## Semiochemical-based Traps and Repellents for Pestiferous Social Wasps

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Eusocial vespid wasps include several subfamilies, such as Polistinae, Vespinae, and Polybiinae from Vespidae (Hymenoptera), commonly referred to as paper wasps, yellowjackets, and hornets in North America. They are important predators of other arthropods, but they can be pestiferous for people as well as pets and livestock. Several species of Vespidae also prey on honeybees (such as *Vespa velutina*) or damage fruit. In cooperation with USDA-ARS scientists (especially the late Dr. Peter J. Landolt), Sterling International, Inc. (SII) has developed and commercialized several reusable and disposable traps for catching all the major social wasp species baited with either heptyl butyrate or combination of acetic acid and 2-methyl-1-butanol for the consumer market in North America. These traps include Rescue!<sup>®</sup> reusable yellowjacket trap, disposable yellowjacket trap, W·H·Y trap and OrnamenTrap<sup>™</sup>, and play an important role in combating these nuisance social wasps. In addition, two essential oil-based spatial repellent devices for pestiferous social wasps, Rescue!<sup>®</sup> GoClips and DecoShield were developed by SII for the consumer market. Such repellent devices can be deployed near the target activity or event centers to push the social wasps away in combination with attractant-baited traps that are set up at a distance around the target activity or event to pull the social wasps away in a “push-pull” fashion, which might dramatically enhance the pest control efficacy against these pestiferous social wasps.

**Keywords:** attractant; hornet; paper wasp; push-pull; yellowjacket

## New discoveries of natural products for controlling agricultural, medical and veterinary pests

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Plant derivatives, or botanical-based crude extracts have been used against arthropods for at least two millennia in ancient China, Egypt, Japan and India. In Europe and North America, the documented practice of using botanicals as the major classes of synthetic chemical insecticides has shown with more than 150 years. Recent studies have confirmed their practical applications in agricultural and veterinary pest management. In addition, natural products such as plant essential oils have been used throughout history for therapeutic purposes, such as topically for its antimicrobial and anti-inflammatory effects. In this presentation, I will report some novel discoveries using natural products including catnip, osage orange, coconut oil as bleeding-sucking insect repellents, larvicides, oviposition deterrents, as well as some antibacterial activities against bacterial food source for stable fly larval development and other common public health pathogens.

**Keywords:** repellent, essential oil, larvicide, antimicrobial activity, pest management

## Identification and synthesis of a novel sesquiterpene from several species of leaf-footed bugs

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Sexually mature males of several leaf-footed bug species in the genus *Leptoglossus* produce blends of sex-specific compounds as possible aggregation pheromones. These include sesquiterpenes such as bergamotenes and  $\beta$ -caryophyllene, and at least three species produce a novel sesquiterpene which elicits strong antennal responses in electroantennogram assays. This compound was isolated from volatiles collected by entrainment, followed by liquid and gas chromatographic purification. Based on NMR (<sup>1</sup>H, COSY, NOE) and GC-MS analysis, the unknown structure was narrowed down to 2 most likely possibilities: a 5,7- or a 4,8-fused ring structure. The 5,7-fused ring structure with trans ring junction was first synthesized employing an enyne metathesis strategy. Its <sup>1</sup>H NMR spectrum and EI-MS fragmentation pattern did not match those of the insect-produced compound, but showed a high degree of similarity. Careful reexamination of all the NMR data confirmed that a 4,8-fused structure was the only remaining skeletal possibility. The synthesis of the alternate, 4,8-fused ring structure was achieved in 4 steps from  $\beta$ -caryophyllene. The spectra of the synthesized compound matched those of the insect-produced compound, proving the structure unambiguously. Bioassays to test the activity of the new compound, given the common name leptotriene, are in progress.

**Keywords:** aggregation pheromone; bergamotene; *Leptoglossus occidentalis*; *Leptoglossus zonatus*; leptotriene

# Poster Presentations

ISCE 2021

## Trapping of *Retrachydes thoracicus thoracicus* and other South American cerambycid beetles in combined pheromone and plant kairomone traps

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Sex-aggregation pheromones in longhorn beetles show remarkable chemical parsimony. Similar structural motifs such as  $\alpha$ -hydroxyketones have been found in numerous species, sometimes working in combination with plant volatile kairomones. *Retrachydes thoracicus thoracicus* (Olivier, 1790) is a polyphagous South American cerambycine beetle with unknown pheromone chemistry. Over the past 4 years, we have been conducting field studies with cerambycid pheromones in citrus orchards located in southern Uruguay, with significant incidental captures of *R. thoracicus thoracicus* in cross-vane traps lured with racemic 3-hydroxy-2-hexanone. In the 2020-21 summer season, an experiment was performed to compare the attraction of lures composed of neat 3-hydroxy-2-hexanone, 3-hydroxy-2-hexanone plus lemon essential oil, and 3-hydroxy-2-hexanone plus ethanol. An absolute control with empty lures was also performed. After eight weeks of captures, the results showed a remarkable increase in *R. thoracicus thoracicus* trap captures when 3-hydroxy-2-hexanone was added with ethanol ( $43 \pm 6$  insects) compared to 3-hydroxy-2-hexanone plus citrus volatiles ( $0.6 \pm 1.1$ ) and 3-hydroxy-2-hexanone alone ( $1 \pm 1$ ). Consistently, more females (132) than males (3) were caught. Smaller numbers of eight other native cerambycid species were also caught in pheromone-lured traps, suggesting that they either produce 3-hydroxy-2-hexanone for intraspecific communication, or they “eavesdrop” on the pheromone communication system of other guild members, as has been reported for other species. The strong synergistic effect of ethanol in the attractiveness of 3-hydroxy-2-hexanone is likely explained by its kairomonal role as a cue for plant stress or ripeness.

**Keywords:** Longhorn beetles; Cerambycinae; kairomone-pheromone synergism; 3-hydroxy-2-hexanone; ethanol

## Neutral effect of multiple herbivory by prey and non-prey on indirect defense of coffee plants

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Plants under attack by multiple herbivores often release a distinct volatile blend than that emitted under attack by single herbivore. These quantitative/qualitative changes can exert a positive, negative or neutral effect on the attractiveness to the third trophic level. In a previous work, we found that herbivory by the red spider mite *Oligonychus ilicis* on coffee plants facilitates the infestation by the white mealybug *Planococcus minor*. Here, we examined whether multiple herbivory by the mite and mealybug on coffee plants influences the emission of herbivore-induced plant volatiles and their attractiveness to the predatory mite *Euseius concordis*. In olfactory behavioral tests, *E. concordis* was attracted by the volatiles emitted by prey-infested plants (mite) when contrasted to uninfested plants or non-prey infested plants (mealybug). The predatory mite preferred volatiles from multiple-infested plants over those of uninfested or non-prey infested plants. However, *E. concordis* did not distinguish the odors of prey-infested plants from those of plants under multiple herbivory. Multivariate analysis showed that volatile composition of prey-infested plants was distinct from those emitted by uninfested and non-prey infested plants, but similar to the emission of multiple-infested plants. Therefore, our results show that infestation by the white mealybug on mite-infested coffee plants does not change the composition of herbivore-induced plant volatiles emitted nor interfere on the attractiveness of the predatory mite *E. concordis*.

**Keywords:** *Coffea arabica*; *Euseius concordis*; herbivore-induced plant volatiles; olfactory behavior.

## A novel olfactory protein-based concept for the Red Palm Weevil early detection and control

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The Red Palm Weevil (RPW) *Rhynchophorus ferrugineus* (Olivier) is reported in almost all the dominant palm tree-growing countries worldwide. RPW recently attained quarantine category – 1 pest status, which, if not eradicated, would cause significant damage to the palm trees. Here we propose a novel olfactory concept that, combined with functional genomics approaches, will help long-term pest management solutions for the RPW control. Recently, we reported the molecular basis of pheromone, (4*RS*,5*RS*)-4-methylnonan-5-ol (ferrugineol), detection in RPW, through functional characterization of ferrugineol-specific odorant-binding proteins – OBP (*RferOBP1768*). Here, we complete our view of ferrugineol detection by the identification of the corresponding odorant receptor (*RferOR1*) via RNAi and heterologous expression. These data are being used to develop ferrugineol-specific OBP/OR- based biosensors. Through an international network of interdisciplinary research, we aim at immobilizing *RferOBP1768* and/or *RferOR1* on Quartz Crystal Microbalance biosensors for the precise detection of the palm weevil pheromone, with the long-term practical application for early detection of RPW adults in the fields.

**Keywords:** Red palm weevil, olfactory proteins, odorant-binding protein, odorant receptor, biosensor.

## Climate change and chemical ecology: Determination of natural products in an Antarctic and a Mediterranean bryozoan species

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The marine environment is exposed to global change and one of its effects is the increase of the water temperature, affecting all marine living species, even changing their metabolism. Many benthic organisms produce secondary metabolites (natural compounds) which are used as a defensive system against predation, competitors, microorganisms, pathogens, fouling, etc. In our study, our goal is to determine if there are variations in the natural product composition of two species of bryozoans when temperature is higher than usual. Therefore, two species of bryozoans were selected, representative of two different environments, *Himantozoum (Himantozoum) antarcticum* (Calvet, 1909) from Antarctica, and *Chartella tenella* (Hincks, 1887), from the Mediterranean Sea. The experiments consisted in keeping the animals at three different temperatures, 15°C, 20°C, and 25°C for the species *C. tenella*, and 0°C, 5°C, and 10°C for the species *H. antarcticum*. The experiments were done in filtered seawater aquaria, with a total of 40 organisms, 20 of each species for a total of two weeks for the Mediterranean samples and four weeks for the Antarctic samples. After organic extraction and clean-up of the samples, the determination of the different compounds was done using chromatography techniques coupled to ultraviolet-visible and a mass spectrometry detector (HPLC-MS). Chromatogram profiles show different composition between bryozoan extracts coming from the two environments, and samples kept at different temperatures present different chemical pattern, and therefore an effect of temperature on bryozoan natural products is observed.

**Keywords:** *Chartella tenella*, *Himantozoum antarcticum*, HPLC-MS, Marine benthos, Temperature.

## Natural products in Antarctic sponges: the case of *Mycale acerata*

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Many benthic invertebrates, including sponges, are known to develop defensive strategies against potential predators and fouling. The constant and accelerated global change of our planet could have an effect on these functions, altering the normal relationships within communities. The aim of this study is to evaluate the potential effect of rising temperatures due to climate change on the Antarctic demosponge *Mycale acerata*. Furthermore, the influence of predation in the natural products of this species is tested too. Samples were collected in Livingston Island, an Antarctic island in the Southern Ocean (South Shetlands Archipelago). Sponges were kept alive at different temperatures (0°C, 5 °C, 10°C) and with two predation situations (macropredation with starfish, micropredation with amphipods), against some controls, in Antarctica. After 4 weeks samples were frozen and transferred to the University of Barcelona. Organic solvents were used for extracting the natural products and HPLC for further analysis and quantification. Variations related to temperature and/or predation may produce significant changes in the chemical defense compounds of *M. acerata*, and thus affect their ability to survive in the field. Since Porifera are significant components of Antarctic biodiversity, the entire ecosystem could be compromised.

**Keywords:** Marine chemical ecology, marine benthos, climate change, temperature stress, predation, bioactivity.

## The effect of temperature in the chemical defense of the nudibranch *Doris kerguelenensis* (Mollusca: Heterobranchia)

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*Doris kerguelenensis* is an Antarctic marine mollusc possessing a wide range of natural products, including many terpene glycerides, used for defense against potential predators. The ability to produce those chemicals is paramount for its survival and it could be compromised by climate change or enhanced by predators. Therefore, we studied here the impact of rising temperatures and predator's presence on the chemical defense of this slug. Nudibranchs were collected at Deception Island (South Shetland Islands, Antarctica). The experiments were carried out in water tanks with three different controlled temperatures: 0°C, 5°C and 10°C, as well as a control, and 5 replicates each. For predation pressure experiments, the omnivorous macropredator, the sea star *Odontaster validus* was placed with the nudibranchs in the tanks. After 4 weeks samples were frozen and transported to the University of Barcelona for further analysis. Chemical extractions with organic solvents and HPLC were used to assess the chemical variations between the different treatment groups. Any variations respect to the controls may affect the survival of the species and thus, affect the interactions in their habitat and by extension, the ecosystems where these animals live in.

**Keywords:** Marine chemical ecology, marine benthos, climate change, temperature stress, predation, bioactivity.

## Heterogeneous chemical profiles of *Vespa velutina nigrithorax* alarm pheromone

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As major communication signals, pheromones have long been studied. In colonial organisms such as social insects, volatile compounds play a crucial role in interspecific interactions by helping regulate colony behavior. In certain circumstances, it is desirable to detect or suppress insect populations, as invasive species. Management's techniques have included lures, baits, and traps. Synthetic or natural insect pheromones are widely in use around the world for pest control. Such molecules can be used for pest management and or control strategies. Because of their success in establishment and ecological dominance, Asian hornets are considered as severe economic and ecological pests. Native to Southeast Asia *Vespa velutina nigrithorax* has spread throughout Europe. To identify the composition of the species' alarm pheromone and assess differences in chemical profiles among queens, foundresses, gynes, and workers, we employed gas chromatography-mass spectrometry. Twenty six compounds were identified in the venom gland (chain lengths: C<sub>8</sub> to C<sub>12</sub>), the organ that produces the alarm pheromone. Venom gland composition differed quantitatively among the females (workers, gynes and queens). These results could help in the development of a pheromone-based trap for the control of this invasive hornet species.

**Keywords:** Yellow-legged hornet; Invasive species; Chemical communication; Vespidae; Defence

## Characterization of olfactory sensory neurons in *Trypodendron lineatum*

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The striped ambrosia beetle *Trypodendron lineatum* (Coleoptera, Curculionidae, Scolytinae) is a major economic pest in the Holarctic region. This species uses odor cues such as aggregation pheromones and host volatiles to invade the host tree and to maintain insect-fungus relationships. These beetles are mostly attracted to stressed and dying conifer trees, where they bore into the xylem and make their fungal galleries. Fungal symbiont *Phialophoropsis ferruginea* is the main source of food for *T. lineatum*. Insects primarily detect odorants with the help of odorant receptors (ORs) present in the olfactory sensory neurons (OSN) inside the antennal sensilla. Single sensillum recordings (SSR) is used to characterize OSN responses, and it is an efficient way to test which odorants are detected by the insect and how the odor environment is encoded by the peripheral neurons. OSN responses to pheromones, host tree and fungal volatiles are poorly understood in *T. lineatum*. Hence, we screened 150 sensilla using SSR with odor stimuli consisting of pheromones, host and non-host compounds, and fungal volatiles on male and female *T. lineatum*. The results show that OSNs responding to lineatin (aggregation pheromone) are abundant on the antennae. Additional OSN classes were identified, including neurons tuned to green leaf volatiles (GLVs) and fungal compounds. Our data also show that *T. lineatum* responds to other bark beetles' pheromones, including 2-methyl-3-buten-2-ol and ipsdienol.

**Keywords:** Aggregation pheromone; Ambrosia beetle; Fungal symbiont; Single sensillum recording (SSR)

## Development of Odorant Binding Protein based Biosensor for early detection of Red Palm Weevil

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The Red Palm Weevil (RPW) *Rhynchophorus ferrugineus* is one of the most invasive and globally important quarantine pest of palm trees. Early detection of such infestations is highly important for commercial crops but no such systems yet exist. Male produced aggregation pheromones may be involved in coordination of mass attacks on trees. Odorant Binding Proteins (OBPs) are small soluble proteins present in the olfactory systems of vertebrates and insects having affinity to various volatile organic molecules. Because their high conformational stability, OBPs are attractive biorecognition elements to create sensors for different applications. The pheromone-specific OBPs (*RferOBP1768* and *RferOBP23*) were recently identified from the Red Palm Weevil. These were incorporated onto Quartz Crystal Microbalances (QCMs) to produce sensors for detection of the aggregation pheromones, 4-methyl-5-nonanol (ferrugineol) and 4-methyl-5-nonanone (ferruginone), together with a kairomone, ethyl acetate to be used for early detection of RPW infestation in date palms. The tertiary structures of the OBPs were modelled to study the ligand binding sites *in silico* and the proteins were expressed and purified. The expressed OBPs were characterized by ligand binding studies in solution. The proteins were immobilised on QCMs using a self-assembled monolayer approach. The resulting sensors were exposed to pulses of vapours from target analytes and were able to selectively and sensitively recognise in vapour phase the two aggregation pheromones ferrugineol and ferruginone at very low concentrations (ppb levels). The biosensors remain stable and respond to analyte vapours for up to one year.

**Keywords:** Red Palm Weevil; Biosensor; Odorant Binding Protein; pheromone detection; aggregation pheromone

## Behavioural responses of *Rhagoletis cerasi* flies to volatiles from the yeasts populating cherry berries

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The European cherry fruit fly, *Rhagoletis cerasi* (L.) (Diptera: Tephritidae) is the most important pest of sweet and sour cherry (*Prunus avium* L. and *Prunus cerasus* L., Rosales: Rosaceae) berries in Europe. Without insecticide treatment, up to 100% of fruits can be infested. Chemical and biological control means as well as formation of mechanical barriers around trees are currently used for cherry fruit fly control. Up to our knowledge, no efficient semiochemicals have been identified for attraction of *R. cerasi* flies. The goal of this study was to identify volatiles released by yeasts colonizing cherry berries that could have behaviour modulating effect on *R. cerasi* flies. Yeasts of five species attributed to *Pichia*, *Hanseniaspora*, *Metschnikowia*, *Aureobasidium*, and *Cryptococcus* genera emitted around eighty volatiles as shown by gas chromatography-mass spectrometry analyses of samples obtained using headspace methods. A gas chromatography-electroantennographic detection analysis of yeast volatiles revealed six electrophysiological active compounds classified as esters and alcohols. Two esters and an alcohol showed the most pronounced activity in Y-tube olfactometric tests and were selected for tests in cherry orchards. In the field assay, traps baited with alcohol caught more fruit flies compare with the un-baited traps. Our results demonstrated the efficacy of yeast volatiles as baits for trapping *R. cerasi* flies.

**Keywords:** fruit fly; sweet cherry; sour cherry; chromatography-electroantennography; field assay

## Effect of essential oils on young honeybee gustatory response

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During their life cycle, honeybees are constantly exposed to xenobiotics from the environment and from in-hive products applied as pesticides. Many of these products do not produce honeybee mortality at the applied doses, however their sublethal effects in physiological processes such as gustatory responsiveness are not often evaluated. Among other potential new acaricides for honeybee colonies, plant essential oils (EO) have been found effective and safe for honeybees. However, EO intake can produce sublethal effects as has been shown for cuticular hydrocarbon profiles. To further characterize the potential chronic effects of the exposure of honeybees to EO, we here studied the effect of the consumption of EO from *Eupatorium buniifolium* (Asteraceae), a known acaricide, on the sucrose sensitivity of young honeybees. We characterized the sucrose response threshold (lowest concentration to which honeybees extend their proboscis) of young bees at 2/3, 5/6 and 9/10 days old, using imidacloprid as a positive control given its known effect on the gustatory response of honeybees. Our results showed that the intake of *E. buniifolium* EO does not reduce sensitivity to a sucrose reward at any age (one-way ANOVA,  $p=0.526$ ,  $p=0.985$ ,  $p=0.645$ , respectively for each age group). Contrarily, the intake of imidacloprid in our experimental setup reduced the sensitivity on bees at 5/6 and 9/10 days old (one-way ANOVA,  $p=0.034$ ,  $p=0.044$ , respectively). These results suggest that the use of EO as acaricides may be safer than other products, and highlights the need for testing sublethal effects of potential sanitary products for honeybees.

**Keywords:** Asteraceae; *Eupatorium buniifolium*; Imidacloprid; Sublethal effect; Xenobiotics



## Oviposition cues for the Asian citrus psyllid, *Diaphorina citri* (Hemiptera: Liviidae)

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The Asian citrus psyllid (ACP) is the vector of Huanglongbing, the most destructive citrus disease. During host plant search, ACP uses visual, odor and contact cues from flushing shoots. A preference greenhouse experiment performed using 6 citrus species: Murcott and Clemenules Tangerine; Valencia, Navelina and Lanelate Oranges, and Lisbon Lemon shown that ACP prefers to oviposit on tangerines, being the number of eggs at least two-fold than on the other varieties (GLMM,  $p < 0.05$ ,  $N=16$ ). Considering the number and length of shoots by variety, tangerines developed more ( $29 \pm 7$  vs.  $17 \pm 9$ ,  $p < 0.05$ ) and shorter shoots ( $3.3 \pm 1.5$  vs  $4.2 \pm 1.8$ cm,  $p=0.05$ ). In turn, the oviposition preference ( $N^\circ$ eggs/species) at the end of the bioassay positively correlates to the number of shoots ( $R^2=0.30$ ,  $p=0.003$ ). Searching for biomarkers that may serve as kairomones for ACP females, the volatile organic compounds (VOCs) and the epicuticular waxes from shoots of all varieties ( $N=8$ /species) were analyzed (GCMS). All data were processed using PARADISE software. Eighty four peaks were detected in VOCs profiles and 140 in wax profiles. Univariate analyses allowed to identify, among other terpenes, that limonene was in higher amounts and caryophyllene in lower amounts in tangerine VOCs compared to all other varieties. Besides tangerine waxes exhibited a lower amount of a still unidentified fatty alcohol and a higher amount of an unidentified diterpene ( $p < 0.05$  in all cases). These results suggest that under experimental conditions, the ACP could use a combination of biological (number of suitable shoots for oviposition) and chemical cues to choose its oviposition plants.

**Keywords:** Epicuticular waxes; Kairomones; Metabolomics; Preference; Volatile organic compounds VOCs.

## Phylogenetic constraints on the evolution of floral scents in a nursery pollination mutualism

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Most studies of chemical mediation between plants and pollinators stress the direct impact of selection by pollinators on the composition of floral scents. Nevertheless, phylogeny may constrain scent composition and thereby the evolution of the emitted signal. Using a model system for obligate pollination interactions, those between figs and their species-specific pollinating fig wasps, we studied whether phylogenetic history constrains the composition of plant chemical signals mediating interactions with pollinators. In this 'nursery pollination mutualism', the pollinators can breed only in receptive figs of their host tree, which depends in turn on the wasp as its sole pollinator. The encounter of the pollinator and the receptive fig is mediated by volatile organic compounds (VOCs). We collected floral scents from receptive figs using in situ headspace extraction of odours from 32 species of several sub-genera of *Ficus* from different tropical and subtropical regions, and analysed their chemical composition by gas chromatography / mass spectrometry (GC-MS). Using phylogenies available for *Ficus*, we analysed the phylogenetic signal in semi-quantitative patterns of floral-scent data using phylogenetic principal component analysis and several multivariate indices of phylogenetic signal. Our results revealed a strong phylogenetic signal in the VOCs emitted by receptive figs, probably due to constraints in the biosynthetic pathways of volatile compounds. Using the same analysis, we found no effect of the pollinator phylogeny. These findings constitute one of the first demonstrations, on a wide scale, that phylogenetic constraints play a significant role in the diversification of VOCs signals emitted by receptive flowers.

**Keywords:** Floral scents, mutualism, pollination, phylogeny, chemical mediation

## Distinct saponin profile drives an olfactory-mediated aggregation in *Holothuria scabra* (Holothuroidea)

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In echinoderms, research on chemical communication has been often limited to pre-spawning aggregation. However, sea cucumber farmers have long observed year-round adult aggregation as a potential source of disease propagation, and sub-optimal use of available sea pen acreage and food resources.

In this study, through spatial distribution statistics, we demonstrated the significant aggregation of the aqua cultivated sea cucumber, *Holothuria scabra*, both as adults in large sea-based pens, and as juveniles in laboratory-based aquaria, proving that aggregation in these animals is not only observed during spawning (*i.e.* fertilization). The role of chemical communication in aggregation is investigated using olfactory experimental assays. Our study established that sediment that *H. scabra* feeds on as well as water pre-conditioned by conspecifics induced the positive chemotaxis on tested sea cucumbers. More specifically, through comparative mass spectrometry, a distinct triterpenoid-saponin profile/mixture was identified as a pheromone allowing sea cucumber intraspecific recognition and aggregation. This aggregation-inducing saponin profile was however not conserved in starved conspecifics who were no-longer attractive to conspecifics.

In summary, this study brings new light onto pheromones in echinoderms. It highlights the complexity of chemical signals detected by sea cucumbers and suggests a role of saponins well beyond that of a simple toxin.

**Key Words-** Saponins, *Holothuria scabra*, sea cucumber, aggregation, behavior, pheromone.

## Chemical profile of non-volatile compounds from seeds, leaves and roots of *Crotalaria spectabilis*

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*Crotalaria spectabilis* (Fabaceae) is commonly used as a green manure for nitrogen fixation. Previous studies have shown that this plant produce, as secondary metabolites, several flavonoids and the toxic compounds pyrrolizidine alkaloids. In a field experiment using *C. spectabilis* as a border crop with maize, the results showed a significant decreased in population level of *Spodoptera frugiperda* larvae in maize. Therefore, the aim of this study was to identify the chemical profile of *C. spectabilis* to help to understand the chemical interaction between *S. frugiperda* and *C. spectabilis*. For this, roots and leaves of *C. spectabilis* plants with 45 days after germination and seeds were submitted to a liquid extraction using ethanol/water (8:2). After extraction, the material was filtered and submitted to HPLC (Flexar, Perkin Elmer) with a photodiode detector and LC-ESI-MS/MS analysis (Eksper ultraLC 100 coupled to a TripleTOF 5600+, SCIEX). The LC-MS/MS data were submitted to the web-based mass spectrometry ecosystem called GNPS, to deconvolution of spectra and the identification of the compounds, that were conducted using the spectral fragmentation pattern comparison with the GNPS public library. The GNPS analysis of data identified 19 compounds among the seeds, roots and leaves samples. The major compound in the seeds was monocrotaline, followed by flavonoids and other pyrrolizidine alkaloids. The major compound in the roots and leaves are still being identified.

**Keywords:** chemical interaction; crotalaria; liquid chromatography; mass spectrometry; non-volatile compounds.

## Behavioral responses to specific preys by predator coccinellids

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Coccinellids are important biological control agents, and despite that some species have generalist habits, food specialization occur within some tribes such as Scymnini. Also, prey preference or pre-imaginal conditioning may be accentuated when coccinellids are subjected to mass rearing. Then, predator-prey interaction could be a conditioned response to stimuli of the prey in which predator was reared. To assess the impact of the pre-imaginal experiences, the coccinellids *Cryptolaemus montrouzieri* and *Tenuisvalvae notata* were fed either *Ferrisia dasyliirii* or *Planococcus citri*, respectively, for at least eight generations. Moreover, we measured the behavioral responses of the predators to partially treated arenas with the volatiles and trail produced by preys, regarding: i) residence time, ii) walking distance, and iii) walking speed. Finally, we investigated feeding preference in laboratory and semi-field conditions, offering both types of prey in Petri dishes, and directly on cotton plants, respectively. Coccinellids were not conditioned by specific rearing preys, as both species responded to the volatiles associated with either prey. Also, there was no difference in their responses between the control area and the trail left by prey, suggesting that trails are not enough to trigger predator response. Finally, both predator species consumed more *P. citri*, regardless of the prey they were reared. Therefore, *C. montrouzieri* and *T. notata* respond to prey's cues regardless of the prey species in which they were reared in laboratory conditions, without pre-imaginal conditioning. These results suggest that both predators are effective in the biological control of different mealybug species.

**Keywords:** coccidophagous predator; food specificity; massive rearing; pre-imaginal condition; prey recognition

## Host selection responses of Asian citrus psyllid (*Diaphorina citri*) to salinity stressed and unstressed citrus seedlings

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The Asian citrus psyllid (ACP), *Diaphorina citri*, vectors *Candidatus Liberibacter asiaticus* and *Ca. Liberibacter americanus*, the putative causal agents of Huanglongbing (HLB), the most destructive citrus disease in the world. Currently no therapies exist for treating infected trees, which typically become unproductive and die. Growers rely entirely on insecticides to control ACP, and sustainable control methods are needed. ACP incidence is dependent on the presence of shoots, where it reproduces and develops. Because salinity stress affects shoot physiology, we hypothesized that that moderate salinity stress could interfere with host selection by ACP. Here, we evaluated ACP host preference when presented with a pair of Rangpur lime (cv. 'Cravo Santa Cruz') seedlings that had been exposed to either low saline (1.7 dS m<sup>-1</sup>) solution or highly saline (10 dS m<sup>-1</sup>) solution. At 15 and 20 days following the imposition of salinity stress, pairs of unstressed and stressed seedlings were placed inside screened cages (45 cm length, 42 cm width, 42 cm height) containing 30 adult ACP. After 48 h, the number of ACP on each seedling was evaluated. Significantly higher proportions of ACP settled in the unstressed seedlings than in the salinity-stressed seedlings. Key physiological responses, such as net photosynthesis, transpiration and osmotic potential, were significantly lower in salinity-stressed seedlings. These physiological changes may have influenced ACP host selection behavior. Strategies for the insect management can be developed based on this knowledge, especially in semi-arid and arid areas where citrus is irrigated with water that has low to moderate salinity.

**Keywords:** abiotic stress; Asian citrus psyllid; biotic stress; greening; plant-insect interaction

## The hydrocarbon footprint profiles of three predaceous Coccinellidae (Coleoptera) are species and gender-specific

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*Eriopis connexa* (Germar) and *Tenuisvalvae notata* (Mulsant) are coccinellids native to South America, whereas *Cryptolaemus montrouzieri* Mulsant is an exotic species, introduced in the 90s. These species are important biological control agents of aphids and mealybugs, respectively. However, when they occur simultaneously in an area, they may compete for food niche. Footprints of these species can influence intraspecific and interspecific recognition, affecting their behaviour. Thus, the objective of this study was to evaluate the chemical profiles of footprints of these three coccinellid species. The footprints were extracted from glass Petri dishes with hexane, identified and quantified by GC-FID and GC-MS. Fifty-two different aliphatic hydrocarbons have been identified in all tree species. In *E. connexa* footprints, twenty-two saturated hydrocarbons were identified, most of them methyl-branched hydrocarbons (ranging C<sub>23</sub> to C<sub>31</sub>). In *T. notata* footprints, seventeen hydrocarbons were identified (ranging C<sub>25</sub> to C<sub>35</sub>), ten unsaturated. Finally, in *C. montrouzieri* there were twenty-three hydrocarbons (ranging C<sub>21</sub> to C<sub>31</sub>), twelve of them unsaturated. Two linear hydrocarbons, pentacosane and heptacosane, were common to all species, but their quantity was species-specific. In addition to the differences between species, there was a qualitative and quantitative difference in hydrocarbons between the genders. The differences on the chemical profile of the footprint hydrocarbons can indicate that the behaviour recognition may be related to specific stimuli. These results can contribute to the adequate management of the studied coccinellids, aiming the control of pests that damage production.

**Keywords:** behaviour; biological control; chemical communication; ladybugs; semiochemicals.

## Head Louse Feces: Chemical and Behavioural Analysis

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Human head lice *Pediculus humanus capitis* (De Geer) (Phthiraptera: Pediculidae) are insect parasites closely associated with humans, feeding on the blood of their hosts and causing them skin irritation and probable secondary infections. Despite being a severe nuisance, very few studies have reported on intraspecific chemical communication in head lice. Here, we evaluated the behavioral responses of head lice to the volatile compounds and solvent extracts from their feces. We also chemically analysed the main volatile components of these feces by CG-MS and those of the feces extracts by HPLC-MS. Head lice were attracted to the methanol extract of their feces but not to the hexane or dichloromethane extracts, suggesting the polar nature of bioactive chemicals present in head louse feces. Follow-up chemical identifications, in fact, showed the presence of hypoxanthine, uric acid, and another purine tentatively identified as either guanine or iso-guanine. Additionally, head lice were significantly attracted by volatiles emitted from samples containing feces. The volatiles emanated from feces alone contained 19 identified substances. The major compounds found were decanal, nonanal, hexanal, and acetic acid, together representing approximately 60% of the identified compounds. This work represents the first chemical evidence of intraspecies communication among head lice. The results support the existence of active substances present in the feces of *P. humanus capitis* that may be involved in its aggregation behaviour.

**Keywords:** Head lice, feces, behavioral activity, chemical analysis

## Identification of Methyl Branched 1-Methoxyalkanes from Tetragnatha Spiders, Potential Species Recognition Signals

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Only a few pheromones of spiders have been characterized so far. All known spider pheromones are small compounds. Lipids from spiders have been analyzed repeatedly, consisting mostly of hydrocarbons and long chain methyl branched 1-methoxyalkanes.

Extracts of the silk and body of males and females of the species *Tetragnatha extensa* and *Tetragnatha versicolor* were analyzed by GC/MS and chemical derivatizations. A species-specific mixture of one major and one or two minor compounds was found on the bodies and silk. The four different compounds were identified as 1-methoxyalkanes, with a chain length of C<sub>29</sub>. Methyl groups were located at C-6 or C-8, C-12 or C-14, and C-20. These lipids seem to play a role in species recognition.

To further elucidate and confirm the proposed structure of the four different compounds identified, synthesis has been carried out. A palladium catalyzed Negishi coupling has been used as the key step in a convergent synthesis.

**Keywords:** lipids, GC/MS, derivatization, synthesis, arachnids

## Integrated effect of biological and physical factors on chemo-sexual communication in moths

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Male moths locate their mates by following their emitted volatile signal. The properties of the olfactory signal may be influenced by the phenotypic condition of the sender and by the physical condition of the flow. Here we tested the effect of the signal properties (biological and physical) on sexual communication in moths. Experiments were conducted in a wind tunnel, using the pink bollworm (*Pectinophora gossypiella*) as a model insect. Four calling females, in a small, cylindrical screen cage, were used as the source of the signal, upwind at the tunnel's entrance. The signals, sugar-fed and/or water-fed females (biological attributes), were presented to males either as one source (no choice) or two sources at a time (choice test). The flow's condition was either under low (no manipulation) or high level of physical disruption by placing a vertical cylinder behind the source (only behind one source in the choice test). The physical disturbance had a significant effect on the success rate of the males in reaching a single source, regardless of its quality. A significant reduced success rate was observed when the males were faced with a high level of flow disruption. When the two volatile sources were presented simultaneously, males significantly preferred the signal of sugar-fed females. This tendency was inverted when the signal of sugar-fed females was physically disrupted. These results provide new knowledge of the integrated effect of biological and physical factors on chemosexual communication in moths.

**Keywords:** moths; disruption; flow; volatile; sex-pheromone, source

## Full of impatience - nectar scents in *Impatiens* flowers visited by *Bombus impatiens*

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*Impatiens* flowers have been of high interest for floral ecologists for decades but there's almost no study on the floral scent patterns of different species. In this study we worked on *Impatiens pallida* and *Impatiens capensis*, which grow in sympatry and are both frequently visited by *Bombus impatiens*. The two species have conspicuous yellow and orange tubular flowers that end in a typical nectar spur. We analysed scent and colour patterns within the flowers focussing on nectar scents and performed behavioural assays to understand the role of the scent components. Our results show that the species-specific scent bouquet and colour pattern enables pollinators to differ between the species. They also show that nectars are strongly scented and differ completely from the scent emitted by whole flowers, without giving signalling reward status of a flower to an approaching pollinator. First bioassays revealed different functions of nectar volatiles and those of whole flowers. The study system is ideal to further investigate the role of nectar constituents and flower constancy behaviour in plant-pollinator interactions.

**Keywords:** bumble bees; floral colour; floral scent; foraging behaviour

## Blend reception and plasticity in moth olfaction

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Heliothine moths are ubiquitous, representing some of the most serious agricultural pests on the planet, causing massive annual crop losses particularly in the developing world. We are developing a global initiative to investigate comparative evolution of olfactory systems in this important group of agricultural pests to develop this species complex as a model system for evolution of complex pheromone communication. Using comparative physiology, chemistry and molecular tools to characterize odorant receptor shifts associated with communication, this study examines blend complexity in pheromone production, detection and behavioural response from amongst sympatric and allopatric species. Furthermore, we are examining plasticity of olfactory receptor (OR) expression and physiological function, as well as the spectrum of blends which initiate agonistic and antagonistic behaviours. The overall goal is to identify shifts in communication which may contribute to larger trends in speciation (both sympatric and allopatric) and evolution in insects. Overall, this initiative will determine genetic factors mediating shifts in female pheromone production, male behavioural pheromone.

**Keywords:** Heliothine, pheromones, plasticity, receptor expression, speciation

## Plants detect and respond to the presence of the third trophic level

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Interactions between plants, herbivores and herbivore natural enemies, described as tritrophic interactions, shape ecological processes in (agro)ecosystems. Recently, evidence has accumulated that plants may directly perceive and respond to the presence of herbivore natural enemies. However, this plant response and impact on the tritrophic system remain overlooked. To address this gap of knowledge, we used a tritrophic system involving maize plants (*Zea mays*), the banded cucumber beetle herbivore (*Diabrotica balteata*), and entomopathogenic nematodes (*Heterorhabditis bacteriophora*). We report a comprehensive depiction of the maize metabolomic response to entomopathogenic nematodes. We further highlight the specificity of this response and external factors that modulate it. Finally, we explore how the plant response feeds back into tritrophic interactions by showing the plant, herbivore and nematode performance.

Overall, we argue that the plant response to the presence of natural enemies may be a crucial, but underestimated, process shaping tritrophic interactions in nature, and biological control success in agriculture.

**Keywords:** Tritrophic interactions; Belowground interactions; Soil ecology; Entomopathogenic nematodes; Plant defenses: Root herbivory

## Three-point electroantennography: a setup to exhaustively explore the olfactory sensitivity of bulbous antennae

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Chemical Ecology studies typically rely on electroantennography (EAG) to estimate the olfactory sensitivity of insects to putative semiochemicals. However, such recordings mostly sample the activity of a subset of the olfactory receptor neurons on an antenna. This is especially problematic in species, such as flies with bulbous antenna, with an inhomogeneous distribution of receptors on the antennal surface. To exhaustively explore insect's olfactory sensitivity, multiprobe EAG might be necessary. With this aim, we developed a novel experimental setup composed of three simultaneous EAG recordings dispersed on an insect antenna. Maximized spacing between the microelectrodes enhances mechanical stability and ensures covering the activity of most olfactory receptors. Data are analyzed through an adapted model of current source density. The setup is coupled with a gas chromatograph through a system chopping the effluent gas at a frequency of 1 Hz. Recordings were successfully performed in several Tephritidae species with various antennal size and shape. The chemical specificity depended on the recording position, which validated an improved scanning of antennal responsiveness. Characterization and calibration revealed significant responses to general plant compounds down to injected amount of 0.1 ng. This new setup allows quantitatively determining the olfactory sensitivity of an insect antenna and will be an asset in future research on flies Chemical Ecology.

**Keywords:** Chopper stabilization; electroantennogram; fruit flies; GC-EAD; Olfactory Receptor Neurons

## Attraction and defense - The dual role of floral scent compounds in *Nicotiana attenuata*

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Plant volatiles may have multiple functions even within a single plant species. By using wild tobacco plants (*Nicotiana attenuata*) silenced in the expression of the biosynthetic gene of benzyl acetone, the dominant floral volatile in this species, we investigated the attractive and deterrent functions of this compound in the plants' native habitat in Southwestern Utah. We found that by emitting benzyl acetone on the corolla limb, *N. attenuata* is able to attract hawkmoth pollinators (*Manduca sexta*), as well as prevent the establishment and resulting floral damage by a florivore, the cucumber beetle (*Diabrotica undecimpunctata*), at the same time. Benzyl acetone functions to increase the fitness of individual flowers not only by increasing detectability but also by enhancing the pollinator's foraging efforts, all of which is governed by olfactory neurons on the tip of the moth's proboscis. Additionally, plants lacking benzyl acetone emissions were not only more often colonized by florivores, but also suffered significantly more damage than control plants, which did produce benzyl acetone. A single floral volatile can thus simultaneously function as an important floral attractant for pollinators and as effective feeding deterrent against florivores in the same plant species and the same plant organ.

**Keywords:** florivory; pollination; benzyl acetone, *Manduca sexta*, floral damage

## The role of the gut microbiome in overcoming the chemical defenses of *Eucalyptus* species in the *Eucalyptus* snout beetle, *Gonipterus sp. n. 2*

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The *Eucalyptus* snout beetle is an oligophagous invasive insect of *Eucalyptus* species. The beetle is attracted to emerging leaves containing high concentrations of essential oils consisting of a complex mixture of mono and sesquiterpenes. In *Eucalyptus*, terpenoids can repel insects through powerful odours and bitter taste. Furthermore, on a molecular scale, terpenoids are involved in breaking down cell membranes and affecting ion transport negatively. Regardless of the elaborate chemical defences of *Eucalyptus* species, the *Eucalyptus* snout beetle is able to tolerate and overcome varying concentrations of terpenoids. We hypothesize that the environmentally acquired gut microbial community of *Gonipterus sp. n. 2* is involved in the detoxification of *Eucalyptus* secondary metabolites. *Gonipterus* beetles were reared on two *Eucalyptus* host genotypes with significantly different biochemical profiles. The metabolomes of frass collected from reared *Gonipterus* beetles were compared with leaf metabolomes. Metabolomic analysis indicated vast differences in the metabolite profiles between the leaf material and the beetles' frass. Additionally, between the leaf and the frass profiles, terpenoid concentrations from leaf to frass dramatically decreased. Furthermore, several biotransformation products, detoxified by cytochrome p450s and dioxygenases, were identified in the frass, including numerous oxygenated and hydroxylated products of 1,8-cineole,  $\alpha$ -pinene and limonene. These findings suggest that the gut microbiome of *Gonipterus sp. n. 2* may facilitate in the detoxification of plant secondary metabolites.

**Keywords:** essential oils; microbial communities; plant secondary metabolite; terpenoids



## Investigating the semiochemistry underlying host selection and oviposition of *Mythimna unipuncta*

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Throughout North America, the true armyworm, *Mythimna unipuncta* (Haworth) (Lepidoptera: Noctuidae), occurs in sporadic large outbreak populations. As a generalist herbivore, the enormous numbers of emerging larvae cause considerable economic damage to cereal and forage crops such as barley, oats, corn, and alfalfa during such infestations. While work has been done on the host preference of the armyworm females, little is known on how the moths synchronize oviposition timing and mechanisms which cause females to oviposit their egg clusters in close vicinity to each other, initiating mass outbreaks. In this study, we want to learn more about the host plant odors attracting the armyworm females, their oviposition preference between infested and unoccupied plants, and possibly identify putative host marking pheromones. We will collect extracts and the headspace of various host plants which are either intact, infested (eggs) or damaged (larvae) and measure the electrophysiological response of the armyworm females using Gas Chromatography–Electroantennographic detection (GC-EAD) and Single Sensillum Recordings (SSRs). Furthermore, we will look at the preference of females to oviposit on host plants in various conditions (intact, infested, damaged) during two-choice behavioral assays in wind-tunnel experiments and free-flight screen cages. Single compounds of interest identified during preliminary experiments will be tested for their behavioral activity in future wind-tunnel or free-flight cage bioassays.

**Keywords:** host marking, host volatiles, *Mythimna unipuncta*, olfaction, oviposition

## Avoidance response to low-quality pollen in foraging honeybees

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Deterrent substances present in food, like toxic and/or bitter compounds, can exert repelling responses. Pollen, the main protein resource for *Apis mellifera*, may present compounds that induce distasteful and/or malaise experiences. Although honeybee colonies avoid collecting some low-quality pollens, evidence supports that foragers themselves are not able to make foraging decisions based on pollen composition at the food sources. We hypothesize that assessment occurs after pollen is processed inside the nest, likely mediated by young bees. To unveil the mechanisms that enable foragers to avoid low-quality pollens, we performed dual-choice experiments with flying bees confined in cages (9x3x2m). We compared foragers' preferences for two monofloral-pollen sources before and after one of them was adulterated with amygdalin. The adulterated pollen was offered either: i) to all the bees inside the hive; ii) to foragers at the pollen source or iii) to young bees transiently isolated from the colony during the treatment. Controls with unadulterated pollens were included. Foragers significantly reduced their preferences for pollens that had been experienced as adulterated inside the hive (i). Interestingly, they could not avoid the adulterated pollen experienced directly at the food source (ii), but they did after the pollen was incorporated into the nest. Experienced young bees could not modify responses of inexperienced foragers (iii). Altogether, results suggest that pollen assessment requires the resource to be processed in the colony and rule out that experienced young bees alone could bias foraging preferences.

**Keywords:** amygdalin; *Apis mellifera*; foraging preferences; in-hive experiences; pollen avoidance

## **Landscape-Vector-Virus Interactions: Do farms surrounded by diverse landscapes benefit from enhanced virus suppression?**

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It has been well documented that agricultural fields surrounded by a more diverse and/or more complex landscape often benefit from an increase in the provision of ecosystem services, including the suppression of agriculturally important herbivorous insect species. However, it is unclear whether this benefit extends to the suppression of plant diseases that are transmitted by these herbivorous insects. Barley yellow dwarf virus (BYDV) is an important aphid-vector-borne plant virus that can cause significant yield losses in winter cereal crops. BYDV control is generally achieved through the suppression of BYDV vectors via pesticide application. However, emerging insecticide resistance in BYDV vectors has increased the need for alternative avenues of BYDV vector control. One potential avenue for alternative BYDV vector control is to promote the delivery of ecosystem services that facilitate natural pest/virus suppression. In this project, 17 winter wheat fields, selected along a landscape diversity gradient, were assessed for BYDV symptoms at multiple timepoints from tillering and during stem elongation. The extent of BYDV infection was determined across each field and was assessed in relation to 1) landscape diversity (i.e. the diversity of land use types around fields), 2) landscape complexity (i.e. amounts of non-crop habitats around fields), and 3) natural enemy activity. The results of this project will give further insight into landscape-vector-virus interactions and will improve our understanding of the potential to promote beneficial ecosystem services through increased landscape diversity.

**Keywords:** Ecosystem Services; Entomology; Landscape Ecology; Pest Suppression; Virology

## **Controlling insect pests in rice by manipulating defense responses using chemical elicitors**

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Upon infestation by herbivores, plants perceive herbivore-associated molecular patterns and damage-associated molecular patterns and produce defence responses, including the release of herbivore-induced volatiles and the accumulation of non-volatile defensive compounds, by activating a defense-related signaling network. This network mainly consists of mitogen-activated protein kinase cascades and pathways mediated by jasmonic acid, salicylic acid and ethylene. The defence responses have been shown in the lab and field to reduce the fitness of herbivores directly and indirectly by attracting natural enemies of herbivores. Our previous studies with rice have found that the manipulation of defence responses, by genetic modification or application of synthetic chemical elicitors, has great potential for the control of pest populations. Here, we will show our recent findings from a novel synthetic chemical elicitor WJ-72. We found that exogenous application of WJ-72 enhances the resistance of rice and other major cereals such as wheat and barley to piercing-sucking insect pests by inducing a novel resistance mechanism. The increased herbivore resistance reduces the population of herbivores and enhances crop yield in the field.

**Key Words:** defensive compounds; ethylene; jasmonic acid; salicylic acid; tritrophic interaction

## Does the chemical environment impact the survival of a host-specific symbiont? A transcriptomic approach of a “host-separation syndrome”

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Symbioses are intimate and durable associations between at least two heterospecific organisms, commonly named host and symbiont. Coevolution allows intrinsic adaptations that induce advantages for the symbionts, which may become strongly host dependent. Moreover, the chemical environment produced by the host can be crucial for symbiosis. Recent research has shown that polyhydroxynaphthoquinones, also called spinochromes, are kairomones produced by the sea urchin *Echinometra mathaei* which are involved in host recognition by the symbiotic shrimps *Arte indicus* and *Tulearicoaris holtuisi*. They may also be involved in other functions for the latter: when they are isolated from its host, a “host separation syndrome” is induced. This syndrome is defined as an alteration of the health status of a host-specific symbiont that may rapidly induce its death. Spinochromes seem to be linked to the symbiont survival rate, as their addition into the environment of an isolated symbiont can improve drastically its survival. Our study aims to identify key genes and molecular pathways that may be involved in the “host-separation syndrome” occurring in the symbioses cited above. Three conditions were tested: (i) symbiont with the host; (ii) isolated symbiont and (iii) isolated symbiont with an addition of spinochromes. We performed transcriptomic analyses and observed an important number of differentially expressed genes between the different conditions. Some gene candidates have been identified as genes coding for histone-binding proteins, heat shock proteins and metallothioneins, specifically expressed in the “isolation” condition. Additional analyses are currently in progress to better understand molecular pathways leading to the host-separation syndrome.

**Keywords:** Crustacea; Gene expression; Kairomone; Spinochrome; RNA-seq

## Stressed-induced changes in the microbial community of *Populus nigra*

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The plant microbiome can benefit plants by promoting their growth and defence against pests and diseases. The structure and function of the microbiome is very dynamic and is affected by the host genotype and the environment. Plants produce various secondary metabolites and volatiles in response to abiotic and biotic stressors that may facilitate specific interactions including with microbial partners. In this study, we investigated changes in the metabolome of poplar in response to infection by *Fusarium euwallaceae* and *Graphium euwallaceae*. The plant volatiles collected using a “pull-push” dynamic headspace collection system were analysed using gas chromatography coupled to mass spectrometry (MS). Semi-polar secondary metabolites were extracted with methanol and analysed using liquid chromatography coupled to MS. We found quantitative differences in the volatiles and secondary metabolites in plants inoculated with fungi compared to control plants. Furthermore, there were differences in the volatile composition of the plants inoculated with different fungal species. Using these results, this study aims to explore whether these fungus-induced compounds affect the bacterial community. Furthermore, we aim to determine if the bacterial community structure changes when a fungal competitor is introduced, and if there is a gradual shift in community structure from young tissue to older tissue.

**Keywords:** dichloromethane; GC-MS; LC-MS; metabolites; quantitative; semi-polar.

## Behavioral response of *Sirex noctilio* towards its symbiotic fungus *Amylostereum areolatum* grown on different substrates.

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The woodwasp, *Sirex noctilio* (Fabricius) (Hymenoptera: Siricidae) is an invasive species that has invaded most of the regions of the world with *Pinus spp.* Since its establishment in Argentina in the 1980s, the wasp has become one of the main insect pests affecting forestry industry. To date, an environmentally sound species-specific monitoring and control tool based on semiochemicals is lacking for the pest. In this sense, a species-specific monitoring method requires, as a basis, knowledge on the sensory ecology of the species, specifically in terms of the chemical volatiles involved in eliciting attractive responses of the species. For instance, previous studies carried out in our laboratory have shown a strong attraction of *S. noctilio* females to the obligate symbiotic fungus *Amylostereum areolatum*.

In this context, the objective of my study was to evaluate the behavioral response of *S. noctilio* females to different volatile stimuli, focusing on those elicited the fungus *A. areolatum* grown in different substrates (artificial agar-based culture medium or two of the pine species most widely cultivated in Patagonia: *Pinus contorta* and *Pinus Ponderosa*). Through 4-way olfactometric bioassays, I was able to determine that there is a clear hierarchy in relation to the preferences of the females towards the different stimuli evaluated, being the semiochemicals emitted by the fungus cultivated in *Pinus contorta* the most important. The results are discussed in the context of possible species-specific semiochemical-based monitoring and control tools and the directions of proposed future research.

**Keywords:** pests, semiochemicals, monitoring, control, integrated management.

## Can colour pattern be a "magic" trait in transparent aposematic and mimetic butterflies?

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Despite being transparent to some degree, clearwing butterflies are not entirely cryptic as they possess bold and colourful markings on their wings. They also engage in mimetic interactions with other unpalatable butterfly species, and as Müllerian mimicry is the result of convergent selection on a warning display, this suggests that the colour pattern in this system is both recognized and avoided by predators. Although wing colour pattern in aposematic butterflies is a trait that shows rapid evolution with a known role in reproductive isolation, it is unclear whether this is also true for transparent Ithomiini species. We set out to test factors that drive mating isolation, and therefore speciation, between two parapatric and ecologically similar taxa of the transparent *Ithomia salapia* found in north-eastern Peru. Our data shows nearly complete mating isolation, despite being the early stages of divergence. In stark contrast to non-transparent Ithomiini, recent divergence is accompanied with strong differentiation in pheromones. This may reflect a general need for further reproductive barriers fairly early in the speciation process as a result of the more inconspicuous nature of their colour pattern, but diversification may still be initiated by changes in colour pattern associated with changes in mimicry ring. That is not to say that this is true for all clearwing butterflies, as different evolutionary scenarios are possible and further studies are needed to shed further light on why some taxa are prone to diversify more than others and what circumstances are conducive for this.

**Keywords:** assortative mating; behaviour; divergent selection; pheromone; prey defenses

## Interactions of Insect pest semiochemicals and insect pollinators

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Plants and insects being among the most abundant groups of organisms on earth have diverse interaction among them with resultant major effects on agroecosystems production. The main role of induce plant volatiles due to herbivory is to mediate different range of ecological interactions between plants, and different visitors such as arthropods, microorganisms, and natural enemies for ultimate plant reproductive success. The floral scent use for attracting pollinators can also be used by insect herbivores as kairomones with subsequent impacts on flower numbers, nectar production, flowers size, flowering timing, and pollinator's behavior. Hence, semiochemicals based monitoring traps for insect pests in crop fields often accidentally captures pollinators leading to pollinator limitations and assemblage. An integrated approach that include the use of semiochemicals involved in plants-herbivores-pollinator-predators' interactions for integrated pest pollinator management is discussed.

**Keywords:** Attractions; kairomones; florivory; induced volatiles; herbivory

## How does the combined use of humic substances and beneficial bacterial affect coffee plant resistance to mealybugs?

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Humic substances (HS) and beneficial microorganisms have been shown to promote greater plant growth and ameliorate abiotic and biotic stresses. However, little is known about effects of the combined use of these two bioestimulants on plant resistance to insects. Here, we investigated how the combined and single use of HS and the plant growth-promoting bacterium (PGPB) *Enterobacter tabaci* influenced resistance of *Coffea arabica* to the white mealybug *Planococcus minor*. We evaluated resistance by assessing the mealybug's host selection and performance assays and by quantifying total phenols of HS-treated, PGPB-treated, HS+PGPB-treated and untreated coffee plants as a putative direct defense. Treatment with any of the biostimulants promoted greater shoot growth relative to that of untreated coffee plants. In four-choice assays, mealybugs were found in lower numbers on HS-treated, PGPB-treated and HS+PGPB-treated plants compared to the control, but the lowest infestation was on HS-treated plants. The mealybug performed poorly on HS-treated and HS+PGPB-treated compared to untreated control. Despite the greater resistance in some of the bioestimulant treatments, the quantification of total phenols was similar among the treatments, suggesting that their levels do not play a role in coffee resistance against the white mealybug. In conclusion, our results showed that treatment with HS promotes increased resistance of coffee plants to the white mealybug, while the PGPB showed a discrete positive effect on resistance. The combined used of the bioestimulants did not yield a synergistic nor an antagonistic effect on coffee resistance against the mealybug.

**Keywords:** bioestimulants; herbivore performance; host selection; phenols; plant growth promoting bacteria.

## Transgenerational effects of maternal herbivory by a sap-sucking insect on plant resistance

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Maternal plants under herbivory pressure can transfer induced defense traits to offspring through epigenetic effects. While this phenomenon has been well studied in plant resistance against leaf feeder insects, little is known about transgenerational resistance induced by sap sucking insects. Here we studied whether maternal herbivory by the green peach aphid *Myzus persicae* (Hemiptera: Aphididae) influences offspring resistance in sweet pepper plants (*Capsicum annuum*). Mother plants were infested from the vegetative stage until fruit ripening in a greenhouse. Aphids preferred to colonize and settle on the offspring plants from uninfested plants than those from infested plants. Increased resistance of the offspring from infested plants coincided with the elevation of 60% in constitutive levels of total phenols relative to the offspring from uninfested plants. Our results showed that maternal herbivory by *M. persicae* on sweet pepper increases resistance of the offspring and levels of a putative chemical defense.

**Keywords:** *Capsicum annuum*; induced defenses; *Myzus persicae*; total phenols; transgenerational resistance.

## Exploring the potential nutritional symbiosis between *Ips typographus* and its fungal associates

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The European spruce bark beetle (*Ips typographus*) is one of the most infamous European forest pests. Despite this beetle's obvious relevance to the forest industry, fairly little is known about its ecology. The bark of conifer trees represents a harsh environment for insects, not only because it is fortified with layers of defenses meant to keep them out, but also because it lacks nutritional compounds to support insect development. Microbes have been found to assist insects in both these situations, either by detoxifying host defensive compounds or by supplementing insects' diets with nutrients that may be missing from or in an unusable form in the host tree. *Ips typographus* is known to be associated with fungal exosymbionts, although the role or roles these fungi play remains unclear. We hypothesized that a major role could be symbiosis through nutritional supplementation. To investigate this, we first analyzed concentrations of macronutrients in these fungi using LC-MS/MS and performed elemental analysis to determine their carbon-to-nitrogen ratios. We also used an artificial rearing system to observe the effect of fungi on bark beetles from the time of hatching to eclosion as callow adults. Results from these assays suggest that exposure to certain fungal symbionts influences bark beetle development, possibly via nutritional supplementation. Understanding the nutritional impact these symbionts may have will lead to a better understanding of bark beetle ecology and their management as a pest species.

**Keywords:** Coleoptera; forestry; microbe; mutualism; pest

## Understanding pheromone biosynthesis in spruce forest vital pest *Ips typographus*

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*Ips typographus*, also known as European spruce bark beetle, considered common pest to native species *Picea abies* in Eurasian forest. The beetle's various life stages and respective pheromones production plays vital role in successful attack over the host. Aggregation pheromones of *I. typographus* such as 2-methyl 3-buten-2-ol and cis-verbenol is important in their co-ordinated mass attack. Genes families such as *Isoprenyl-di-phosphate synthase* (IPDS) and *cytochrome P450* (CyP450) were found responsible for aggregation pheromone production in relative bark beetle species. However, pheromone biosynthesis of *I. typographus* is understudied at both physiological level and molecular level. Identifying key biosynthetic pathway genes involved in the aggregation pheromone of *I. typographus* was our priority. Involvement of mevalonate pathway genes such as geranyl-di-phosphate synthase, 3-hydroxy-3-methylglutaryl Co-enzyme A reductase and few CyP450 genes were found in the beetle's gut tissue during attack stage in new host. Further method of characterizing the found candidate gene/s will lead us to narrow down responsible gene/s for the mentioned biosynthesis.

**Key words:** Bark beetle; aggregation pheromone; 2-methyl 3-buten-2-ol; biosynthesis; mevalonate pathway.

## New opportunities for a push-pull system in brassica crop production

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The diamondback moth (DBM), *Plutella xylostella* (L.) is the major economic pest of brassica crops in the tropics. Ongoing climate change has significantly exacerbated its pest status through e.g. short generation time, increased abundance, fecundity and metabolic activity translating into increased feeding and pest pressure. Control of this pest in Africa is generically achieved by deploying hard synthetic pesticides. However, several reports suggest pesticide resistance in DBM, likely triggering environmental, public health and sustainability concerns owing to pesticide overuse. However, rich vegetational diversity (intercrops), may help sustainably manage specialist pest insects through obscuring volatile cues used for host plant identification, physical obstruction, increased pest emigration, reduced chances of appropriate landing and making host crop plants less apparent- and -attractive. Thus, through the manipulation of habitats and host finding behaviour using natural host-pest semiochemicals and aligning to Root's resource concentration and natural enemies' hypotheses, intercropping of brassica crops with plants that are rich in attractant and repellent volatiles could create a push-pull environment that may sustainably reduce DBM pest abundance and subsequent crop damage. Here, we propose the use of multipurpose plants in a push-pull system for the management of DBM in brassicas. Specifically, we propose Indian mustard, *Brassica juncea* L. (Czern). as an attractant crop and *Hyptis spicigera* Lam. as a repellent plant in a system that creates synergistic and complementary pest control effects. Use of multipurpose plants is highly sustainable through provision of other useful economic products e.g. human and livestock food (*B. juncea*) as well as livestock nutraceuticals (*H. spicigera*) and forms critical synergies in mixed crop-livestock production systems. The results have huge implications in reducing the use of hazardous synthetic pesticides, improving public and environmental health as well as improving sustainability in smallholder African horticulture and mixed cropping systems.

**Keywords:** *Brassica juncea*; horticulture; *Hyptis spicigera*; *Plutella xylostella*; push-pull

## Re-igniting the mustard oil bomb: knocking out key host plant adaptive genes in *Pieris* butterfly larvae

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*Pieris* butterfly larvae feed on plants containing diverse glucosinolates (GLS) with redirecting toxic breakdown products of GLSs to less toxic metabolites using gut-expressed nitrile-specifier proteins (*NSPs*). *NSP* is considered an evolutionary key innovation for Pieridae that enabled these butterflies to colonize GLS containing plants and allowed subsequent diversification. However, we still do not fully understand the roles of both *NSP* and its sister gene, major allergen (*MA*), in overcoming the wide range of host plant GLSs larvae encounter in the field. Here, we tested the ecological relevance of *NSP* and *MA* for *Pieris* larvae by knocking out both genes in *Pieris brassicae* using the CRISPR/Cas9 genome editing technique. We found that gut protein extracts of *NSP/MA* double KO larvae completely lost their activity against all GLSs tested. Moreover, we also found that *NSP* KOs and *MA* KOs showed lower performance on host plants with different GLS profiles, whereas *NSP/MA* KOs could not survive on host plants with higher GLS concentration. These results clearly suggest that both *NSP* and *MA* have different but complementary roles in defusing the mustard oil bomb in *Pieris* larvae, and that both genes are crucial for *Pieris* in overcoming their host plants' major chemical defense.

**Keywords:** Nitrile-specifier protein; Glucosinolate; counter-adaptation; genome-editing; substrate specificity

## Insights into reproductive processes and hierarchies within social insect societies

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The stability and integrity of the social insects' colony is established through effective pheromonal communication to regulate reproductive behaviour and dominance. Under normal conditions, the queen is the only one reproducing with group of workers that are functionally sterile. However, some honey bee workers do escape the reproductive regulatory mechanism and become reproductively active. This study investigated how honey bee workers that becomes reproductively active, exploit pheromonal communication to their advantage and how pheromones contribute to reproductive dominance and reproductive hierarchies. The results provide evidence for establishment of reproductive dominance through the use of pheromones from diverse glandular secretions acting synergistically or additively to regulate various processes in the colony. These complex interplay of pheromonal signals from different exocrine glands have both primer and releaser effects among the honey bee groups. This study provides additional understanding into how pheromones from various glandular secretions contribute to the evolution of reproductive dominance and reproductive division of labour within social insect societies.

**Keywords:** honey bees; behaviour; exocrine; evolution; secretions



## The major pheromone component identity of the Pine Emperor moth, *Nudaurelia clarki* (Lepidoptera: Saturniidae), is confirmed

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*Nudaurelia* spp. (Saturniidae) are sporadic pine plantation pests in South Africa. The development and implementation of pheromone-based management tactics in South Africa has been limited by taxonomic uncertainty with this group. We compared *Cytochrome oxidase subunit I* (COI) gene sequences from historical and contemporary samples of moths from the KwaZulu-Natal (KZN), Western Cape and Mpumalanga regions that have previously been reported as *Nudaurelia clarki* and *N. cytherea* in literature. Female pheromone gland extracts were also analyzed with gas-chromatography electroantennographic detection (GC-EAD) and gas-chromatography-mass spectrometry (GC-MS). COI gene barcoding sequences were identical for sampled moths from all considered regions suggesting that these populations may be the same species. The samples collected for this study were considered to belong to *N. clarki*. Male *N. clarki* antennae respond to two compounds in female extracts. One of these compounds was confirmed with a synthetic standard to be (*Z*)-dec-5-en-1-yl-3-methylbutanoate, the sex pheromone previously identified from *N. cytherea*. The identity of the second compound could not be confirmed. Both male and female antennae responded to four structurally related compounds in the synthetic pheromone standard. Custom-made traps fitted with polydimethylsiloxane and polyisoprene lures loaded with synthetic (*Z*)-dec-5-en-1-yl-3-methylbutanoate confirmed attraction of *N. clarki* males to the pheromone in field trials. The shared pheromone chemistry between populations previously considered as *N. cytherea* and *N. clarki* further promotes the hypothesis that they are the same species.

## Structural and Functional Comparison of 2-Phenylethylamine and Dopamine as Infochemicals for Hermit Crabs under Climate Change

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2-phenylethylamine (PEA) is a known predator cue in terrestrial environments. Our previous results, however, show an attraction of hermit crabs (*Pagurus bernhardus*) to this biogenic amine. This raises the question of the ecological role of PEA for current and future marine environments. Considering the close structural similarities between PEA and dopamine, this study relates functional to structural common features between the two neurotransmitters as infochemicals. Similarly to PEA, the role of dopamine for external signalling is largely unknown, but internal dopamine levels are known to be high during courtship and agonistic behaviour in crustaceans. We study their potential signalling function for hermit crabs in paired dose-dependent choice experiments, whereby different motion parameters are compared. Our experimental design also accounts for the influence of the hermit crab's sex and size on its response to the tested compounds. These are important factors in the context of courtship behaviour and also known to play a role in establishing dominance hierarchies in crustaceans. As the continuing uptake of atmospheric carbon dioxide reduces oceanic pH conditions, marine chemical environments are changing. We therefore additionally explore the pH-dependency of the hermit crab's behavioural responses. Using quantum chemical calculations, we compare structural features of PEA and dopamine in different protonation states and relate these to the animal's responses. This study provides an insight into the potential roles of neurotransmitters and structurally related compounds as external chemical signals for crustaceans in current and end-of-the-century oceanic pH conditions.

**Keywords:** Chemoattractant; Computational Chemistry; Neurotransmitters; Ocean Acidification; *Pagurus bernhardus*

## Sublethal effects of insecticides on partner and host finding of *Nasonia vitripennis*

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The use of insecticides is common in modern agriculture. However, insecticides can also have negative effects at sublethal doses on beneficial non-target species such as pollinators and parasitic wasps. We studied the sublethal effects of the insecticides ACETamiprid, DIMethoate, FLUPyradifurone and SULfoxaflo, all targeting cholinergic neurons of insects, on the chemically mediated mate and host finding in the model organism *Nasonia vitripennis*. Sublethal doses of ACE ( $\geq 1.05$  ng), DIM ( $\geq 0.105$  ng) and FLU ( $\geq 21$  ng) interrupted the female response to the male sex pheromone, while SUL did not at the doses tested (0.52-2.63 ng). Copulation rates decreased at the highest tested dose of any substance (ACE: 6.3 ng, DIM: 1.05 ng, FLU: 21 ng, SUL: 2.63 ng) when exclusively males were treated, while only FLU and SUL had an effect at these doses when females were treated. The strongest drop in copulation rate was found when both partners were treated with FLU ( $\geq 2.63$  ng). Females no longer preferred host odor when treated with sublethal doses of any of the tested insecticides, however, active doses varied widely between substances (ACE:  $\geq 1.05$  ng, DIM:  $\geq 0.105$  ng, FLU:  $\geq 5.25$  ng, SUL:  $\geq 0.52$  ng). Interestingly, the preference for host odor switched to avoidance in females treated with DIM at any of the tested doses ( $\geq 0.21$  ng). Parasitic wasps may take up sublethal doses of insecticides by contact with treated plants or feeding contaminated nectar, honeydew, or guttation water. Our results suggest that this may hamper olfactory orientation, thus reducing their reproductive success and effectiveness as natural enemies in ecosystems.

**Keywords:** insecticides; *Nasonia*; olfaction; parasitic wasp; sublethal effects

## Forest edge induced physiological and chemical changes increases the susceptibility of Norway spruce (*Picea abies*) to bark beetle (*Ips typographus*) attack

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Forests of the Czech Republic have experienced devastating damages in Norway spruce monocultures caused by bark beetle mass outbreaks in recent years. Total volume of trees killed mainly by the Eurasian spruce bark beetle (*Ips typographus*) exponentially increased from 1.5 million m<sup>3</sup> timber annually (in 2003-2015) to 23 million m<sup>3</sup> timber in 2019. In response to current calamities, our research focused on plant-insect interaction between mature Norway spruce and *Ips typographus*. The research explored why certain spruce trees in the forest are more susceptible to bark beetle attack, and how external stressors, such as drought, solar radiation, and rising temperatures affect the tree defence mechanism. In a comprehensive field study, stress levels of trees were manipulated by the cutting fresh forest edges leading to sudden sun exposure. Physiological features of the trees such as sap and resin flow and secondary metabolites profile in the bark were monitored for studying the tree defence. These records were related to bark beetle attacks observed in field bioassays, which conclude the differences in tree defence between trees at the fresh forest edge and forest interior.

**Keywords:** European spruce bark beetle; fresh cut trees; herbivore defence; Norway spruce; resin flow; secondary metabolites

## Olfactory background of stimulo-deterrent pest management strategy in the sugarcane-borer *Eldana saccharina*

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The African sugarcane borer (*Eldana saccharina*) is one of the most important pests of South Africa's sugarcane cultivation. The larval damage causes significant financial losses for farmers leading to unsustainable cultivation. The moth is indigenous to the wetland sedges of Africa, where it occurs in great abundances. One of the original hostplants of *E. saccharina* is *Cyperus papyrus*, but since then it has been reported that the moth feeds on various gramineous plants, most importantly sugarcane. The sensory adaptation behind the host shift from wetland sedges to sugarcane and behind the success of push-pull strategy is poorly understood. In our experiment, we collected and analyzed the volatile bouquet from the moth's original host plant, *Cyperus papyrus* and the invaded hosts such as two types of sugarcane varieties (N21 and 51NG146), as well as push-plant *Melinis minutiflora*. We pointed out physiological active volatiles from the volatile profiles on adult female and male moths by gas chromatography coupled electroantennography.

**Keywords:** *Eldana saccharina*; hostplants; plant volatiles; electrophysiology; push-pull strategy

## Direct and Indirect Defense Induction on Maize by Neighbouring Molasses Grass

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Plants have evolved intricate defence strategies against herbivore attack including mechanisms to eavesdrop on herbivore- or wound-induced volatile organic compounds (VOCs) emitted by neighbouring plants to activate direct and indirect defences. VOCs released by molasses grass, *Melinis minutiflora* (P. Beauv.) has been shown to repel stemborer *Chilo partellus* (Swinhoe) (Lepidoptera: Crambidae), a major pest of maize in sub-Saharan Africa, and enhance larval parasitism by *Cotesia sesamiae* Cameron (Hymenoptera: Braconidae). However, the role of VOCs signalling in molasses grass-maize interaction and any subsequent effect on the stemborer pest and its natural enemy (parasitoid) behaviour remains unknown. This study examined the effect of plant-plant signalling on *C. partellus* oviposition and *C. sesamiae* attraction by intercropping *M. minutiflora* with locally adapted maize cultivars (landraces), Nyamula and Jowi-red. In two-choice oviposition bioassays, *C. partellus* preferred non-exposed maize landraces for egg deposition to those exposed to molasses grass. Meanwhile, the parasitic *C. sesamiae* wasp was significantly attracted to volatiles from *M. minutiflora* exposed maize compared to unexposed control in a four-way olfactometer bioassay. The coupled chromatography (GC)-mass spectrometry and GC-linked electroantennography analyses of headspace samples from maize landraces exposed to *M. minutiflora* revealed strong induction of bioactive compounds such as (*E*)-4,8-dimethyl-1,3,7-nonatriene. Our findings suggest that constitutively emitted *M. minutiflora* VOCs can induce direct and indirect defence responses in neighbouring maize plants. The plant-plant volatile signalling could offer a novel and ecologically sustainable strategies of crop protection against the destructive stemborer pest that are relevant to smallholder maize farmers in the region.

**Key Words:** *Chilo partellus*, induce defence, volatile organic compounds, maize landraces, *Melinis minutiflora*.

## TV PheroLure®: The influence of a semiochemical lure on the volatile profile of a commercial tomato field

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Pheromone-based or semiochemical lures for insect detection and monitoring in agriculture is common practice. Many countries exempt these devices from regulatory requirements, however, not South Africa. The question arises whether the pheromone/semiochemical lures influence the naturally occurring compounds significantly, to justify concern for human toxicity and ecotoxicity. T.V. PheroLure® is a novel five-component lure developed by Insect Science (Pty) Ltd. used for monitoring African bollworm, *Helicoverpa armigera* (an important insect pest on tomatoes). T.V. PheroLure® is a volatile organic compound (VOC) blend impregnated in a polyethylene bulb. The influence of T.V. PheroLure® on the volatile profile of a tomato field was evaluated in a commercial growing area of South Africa. Tomato VOCs were collected before, during, and after the application of six T.V. PheroLures® in yellow bucket funnel traps randomly distributed over one hectare. VOCs were collected from planting until harvest (22 weeks) at five randomly selected sites at different heights (0 cm, 30 cm and 60 cm above ground level). Collection also took place in adjacent tomato fields where no T.V. PheroLure® was applied. The constituents of T.V. PheroLure® had no significant influence on the naturally occurring VOCs observed in the tomato field, irrespective of sampling height. Suggesting that the concern for toxicity and ecotoxicity is unjustified when using semiochemical devices for monitoring purposes. The natural physiology of the plant, rather than T.V. PheroLure®, influenced the VOCs observed in a tomato field.

**Keywords:** Insect monitoring; African bollworm; South Africa; Toxicity; Volatile organic compound (VOC)

## Production of defensive metabolites by *Pinus patula* x *Pinus tecunumanii* hybrids in response to *Fusarium circinatum* infection

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Pine trees defend themselves from attack by insect pests and microbial pathogens through a complex system of physical and chemical defences. These defences can be constitutive, present in the tree before an attack, or inducible, produced in response to an attack. Defensive chemicals induced upon fungal infection include terpenes and phenolics, large groups of structurally diverse compounds with known antifungal and fungistatic properties. However, the specific chemical defence response in pines to *Fusarium circinatum* infection is unknown. In this study, the defensive secondary metabolites produced by *Pinus patula* x *Pinus tecunumanii* hybrids in response to infection by *F. circinatum* were tested under different experimental parameters. Infection by a more virulent strain of *F. circinatum* correlated with longer lesion lengths on the stems of infected seedlings. However, LCMS analysis revealed that stronger virulence did not lead to a significant increase in production of defensive phenolic compounds. Furthermore, a general increase in production of defensive phenolics occurred as the time post-inoculation increased. GCMS analysis will determine whether similar trends can be observed in the production of defensive terpene compounds. Ongoing research will reveal whether low elevation and high elevation *Pinus* hybrids differ in their chemical defence response, as well as the effect of greenhouse temperature on secondary metabolite production in *Pinus* hybrids. This study also aims to reveal whether chemical markers for *F. circinatum* resistance or susceptibility can be identified and utilized in pines.

**Keywords:** defense; terpenes; phenolics; pine trees; disease resistance

## Repellent and acaricidal effects of basil essential oil and rock dust on ticks

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Ticks, such as blacklegged ticks (*Ixodes scapularis*) and American dog tick (*Dermacentor variabilis*) are vectors of several pathogens that negatively impact animal and human health. In recent years due to global warming, the threat of disease transmission has risen significantly, resulting in an increased demand for environmentally safe, tick repellent and acaricidal products. Natural products, such as essential oils and inert rock dust, are prospective alternatives to manage these pests. Basil (*Ocimum basilicum*, L.) has been reported to have promising pest repellent activity. We extracted and characterized essential oils from different basil varieties and tested them for long-term repellent activity towards nymphs of blacklegged ticks using horizontal bioassays at different concentrations. In addition, we combined basil essential oils with an inert material (i.e., granite rock dust) with known insecticidal properties to assess acaricidal activities against adult ticks. Among the tested basil varieties, Jolina essential oils (15% v/v) repelled 96% of tested ticks up to 2 hours post-treatment. In acaricidal tests, the combination of essential oils from Aroma2 var. at 10% w/w with rock dust resulted in 100% tick mortality after only 24 hrs post-exposure. The use of essential oils alone, and in combination of rock dust, represents an innovative and environmentally friendly approach to manage ticks and the spread of vector-borne diseases.

**Keywords:** ticks; essential oil; acaricidal; repellent; granite dust