# ISCE2015
## International Society of Chemical Ecology

## Stockholm, Sweden
## 29th June - 3rd July 2015

# Contents

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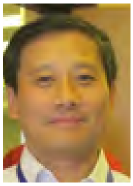

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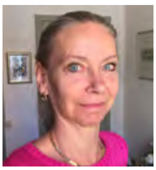

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# Sponsors

We would like to thank our partners for their invaluable support for ISCE 2015.

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C I

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IC-E3

# Stockholm University


# Conference Dinner

## *Skansen: Solliden, Thursday 2nd July, 19:00*

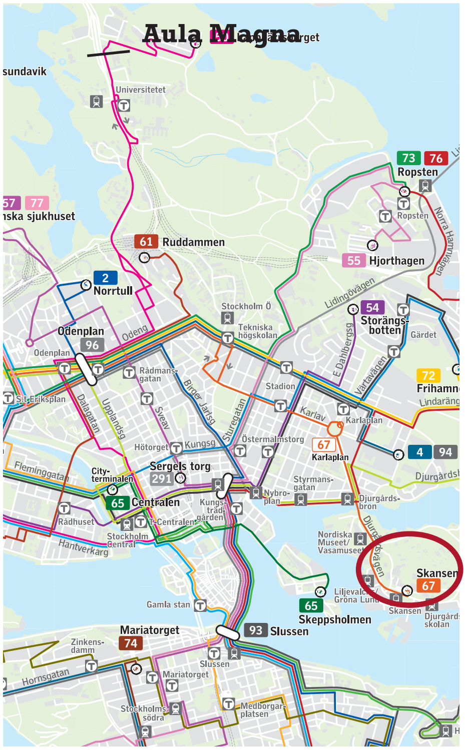
Welcome to the conference dinner in the Solliden building at Skansen. Skansen is located on the green island of Djurgården in the east part of Stockholm.

To get to Skansen - take bus 67 from Tekniska Högskolan or Karlaplan metro stations, or the tram from the city center. It is also a pleasent walk of about 20-30 minutes along the water from *e.g.* Sergels Torg/T-centralen.

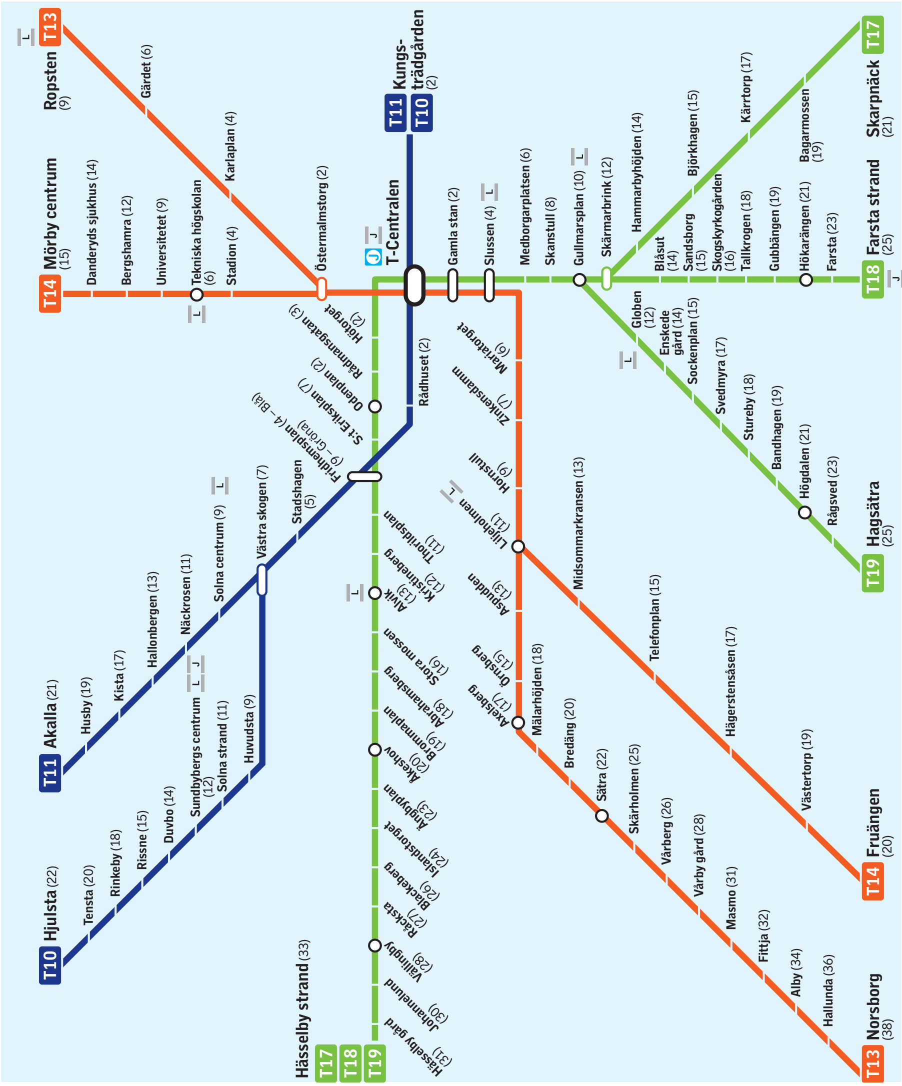
At the gates of Skansen, inform the staff that you are taking part in the conference dinner of **ISCE** - and they will let you in at no charge.

The dinner starts with a pre-dinner cocktail on the terrace, and dinner is served from 19:30.

If you have special dietary requirements, please be so kind to inform your waitor of your name and needs, and they will make sure you get the right food.



# Stockholm Metro





Stockholm is known for its convenient, efficient and safe public transportation system. Stockholm Public Transport (SL) manages buses, the metro, commuter trains, trams and some of the ferry lines in Greater Stockholm. Tickets can be purchased at SL Centres, metro platform barriers, news agent kiosks but not by busdrivers.

Numbers in parenthesis in the map above are travel time in minutes to the hub ”T-centralen”. Trains run from 05:00 am - 00:30 am during weekdays, and about every 30 minutes night time Fridays and Saturdays. Metro stations are marked with a blue T.

# Aula Magna

Meeting locations: Hall A (Left hall); Hall B (Right hall); Hall C (Bergsmannen); Hall D ("Spelbomskan")

EXIT **Emergency Exit**

**Coffee Station**

**Lecture Hall Exit**

Hall A **Hall A** (600 seats)

Hall B **Hall B** (600 seats)

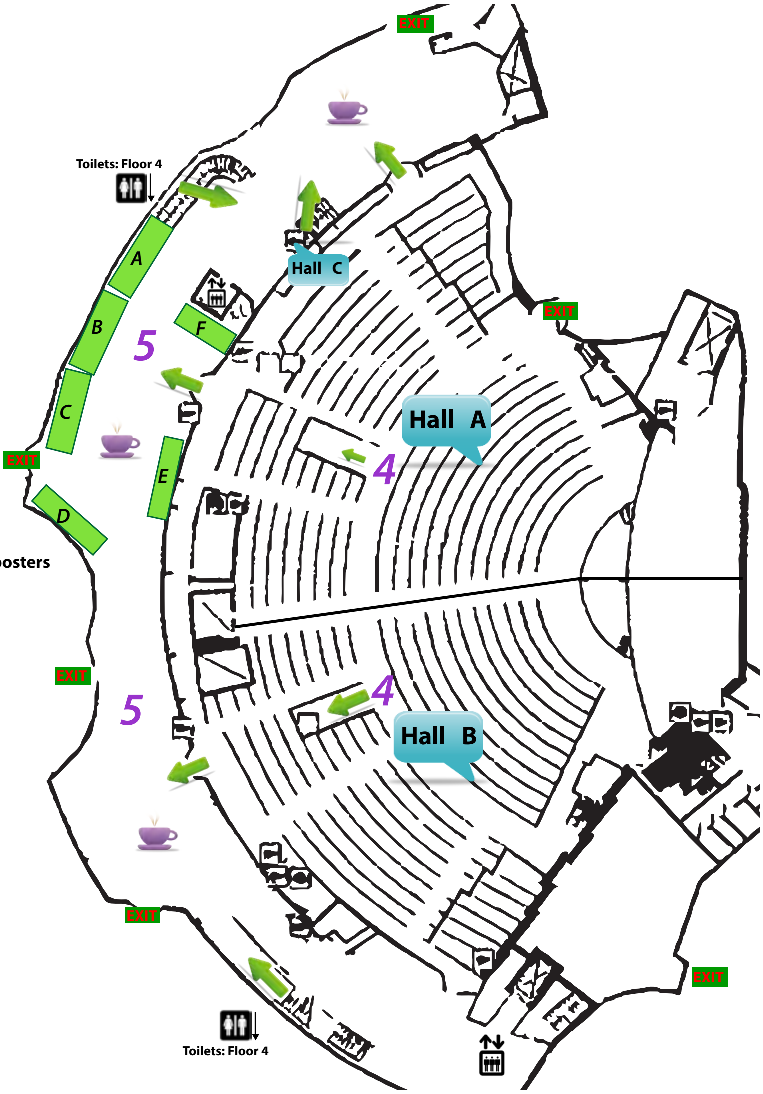
Hall C **Hall C** (60 seats), on floor 6

**Toilets** (Floor 4)

**Elevator**

5 **Floor 5**
Exhibition, coffee, posters

4 **Floor 4**
Main Entrance, information, posters




## Exhibitors

| | |
|---|---|
| A | Saveen Werner |
| B | Springer |
| C | NinoLab |
| D | MSCi / Gerstel |
| E | Bergman Labora |
| F | Apotekarsocieteten |

# 31st Conference for the Internati

## MONDAY 29 JUNE

| Hall A | Hall B | Hall C |
|---|---|---|
| | | 09:30 - 12:00 ISCE Executive Committee |
| 12:00-12:40 **Welcome!** | | |
| 12:40-13:45 **Silver Medal Lecture** *Ritsuo Nishida* | | |

**13:45 - 14:00 Short Break**

| Hall A | Hall B | Hall C |
|---|---|---|
| **Unraveling the chemistry of chemical ecology** Chairs: Paulo Zarbin, Jan Bello | **Chemical neuroecology** Chairs: Silke Sachse, Markus Knaden | **Pheromone communication** Chair: Irena Valterova |
| 14:00-14:30 John Clardy | 14:00-14:30 Ilona Grunwald Kadow | 14:00-14:30 Kirk Hillier |
| 14:30-14:45 Josef Vuts | 14:30-14:45 Dennis Mathew | 14:30-14:45 Denis Willett |
| 14:45-15:00 Nadja Nagel | 14:45-15:00 Richard Newcomb | 14:45-15:00 Alvin Hee |

**15:00-15:30 Coffee break**

| Hall A | Hall B | Hall C |
|---|---|---|
| 15:30-15:45 Stefanos Andreadis | 15:30-15:45 Markus Knaden | 15:30-15:45 Astrid Groot |
| 15:45-16:00 Marilia Trapp | 15:45-16:00 Silke Sachse | 15:45-16:00 Irena Valterova |
| 16:00-16:15 Nanna Hjort Vidkjær | 16:00-16:15 Eduardo Hatano | 16:00-16:15 Helena Larsdotter Mellström |
| 16:15-16:30 Stefan Schulz | 16:15-16:30 Sebastien Lebreton | 16:15-16:30 Margarita Orlova |
| 16:30-16:45 Göran Birgersson | 16:30-16:45 Thomas Baker | 16:30-16:45 Thomas Butterfield |

**16:45 - 19:00 Reception Mingle Poster Session I**

## TUESDAY 30 JUNE

| Hall A | Hall B | Hall C |
|---|---|---|
| 08:30-09:15 Sex pheromone chemistry and evolution in Thynnine wasps Rod Peakall | | |
| 09:15-09:45 Plant toxins induce defensive signals Matthias Erb | | |

**09:45-10:15 Coffee Break**

| Hall A | Hall B | Hall C |
|---|---|---|
| **Chemical ecology of invasive species** Chairs: Swantje Enge, Gabor Szocs | **Induced resistance of plants and prey** Chairs: Gunilla Toth, Robert Glinwood | **Unraveling the chemistry of chemical ecology** Chairs: Paulo Zarbin, Jan Bello |
| 10:15-10:45 Max Suckling | 10:15-10:45 Mark Hay | 10:15-10:30 Wittko Francke |
| | | 10:30-10:45 Remington Poulin |
| 10:45-11:00 Mirka Macel | 10:45-11:00 Erik Selander | 10:45-11:00 Hong Lei Wang |
| 11:00-11:15 Salvatore Guarino | 11:00-11:15 Fiia Havisto | 11:00-11:15 Christine Lembke |
| 11:15-11:30 Boyd Mori | 11:15-11:30 Mike Birkett | 11:15-11:30 Jana Krasulová |
| 11:30-11:45 Sofia Branco | 11:30-11:45 James Blande | 11:30-11:45 Ivan Galis |
| 11:45-12:00 Helga Pankoke | 11:45-12:00 Brett Younginger | 11:45-12:00 Åsa Emmer |
| 12:00-12:15 Eva Castells | 12:00-12:15 Anjel Helms | 12:00-12:15 Ashot Khrimian |

**12:15-14:00 Lunch**

| Hall A | Hall B | Hall C | Hall D |
|---|---|---|---|
| **Chemical ecology of invasive species** Chairs: Swantje Enge, Gabor Szocs | **Induced resistance of plants and prey** Chairs: Gunilla Toth, Robert Glinwood | **Chemical Neuroecology** Chairs: Silke Sachse, Markus Knaden | **MSCi Workshop** Chairs: Jens Glastrup |
| 14:00-14:30 Steven Seybold | 14:00-14:15 Lina Castano Duque | 14:00-14:15 Martin Andersson | 14:00-15:00 Jens Glastrup |
| | 14:15-14:30 Ricardo Machado | 14:15-14:30 Yoanne Yew | |
| 14:30-14:45 Gaylord Desurmont | 14:30-14:45 Guillermo Jimenez-Aleman | 14:30-14:45 William Walker | |
| 14:45-15:00 Rainee Kaczorowski | 14:45-15:00 Jenny Lazebnik | 14:45-15:00 Jacob Corcoran | |

**15:00-15:30 Coffee Break**

| Hall A | Hall B | Hall C | Hall D |
|---|---|---|---|
| 15:30-15:45 David Hall | 15:30-15:45 Anthony Clarke | 15:30-15:45 Caroline Nieberding | 15:30-16:30 Jens Glastrup |
| 15:45-16:00 Florian Weinberger | 15:45-16:00 Velemir Ninkovic | 15:45-16:00 Alan Soffan | |
| 16:00-16:15 Stephane Greff | 16:00-16:15 Daniel Ballhorn | 16:00-16:15 Dan-Dan Zhang | |
| 16:15-16:30 Robert Vander Meer | 16:15-16:30 Vartika Mathur | 16:15-16:30 Kyung San Choi | |

**16:30 - 18:00 Poster Session II**

**19:30 JCE Editorial board meeting**

## W

| Hall A |
|---|
| 08:30-09:15 A molecular rationale for the unpredictable nature of female behaviour Lisa Stowers |
| 09:15-09:45 Thousands and one DEET receptor Walter Leal |
| |
| **Pharmacognosy meets chemical ecology** Chairs: Lars Bohlin |
| 10:15-10:30 Christina Wedén |
| 10:30-10:45 Ingemar Struwe |
| 10:45-11:00 Jennifer Lohr |
| 11:00-11:15 Ulf Göransson |
| 11:15-11:30 Georg Petshenka |
| 11:30-11:45 Paco Cardenas |
| 11:45-12:00 Mika Zagrobelny |
| 12:00-12:15 Nahid Moghbel |
| **Excursion I** Boat trip to Drottningholm Royal Palace |

## WEDNESDAY 1 JULY

### Hall B

**09:45-10:15 Coffee Break**

**Vertebrate chemical ecology**
Chairs: Lisa Stowers, Raimondas Mozuraitis

- *10:15-10:45* Ivan Rodriguez
- *10:45-11:00* Tomas Bozza
- *11:00-11:15* Bruce Kimball
- *11:15-11:30* Carlos Grau Paricio ○
- *11:30-11:45* Marianne Gabirot ○
- *11:45-12:00* Raimondas Mozuraitis
- *12:00-12:15* Micaela Thoß

**Excursion II**
Bus trip to forest fire affected area in Västmanland

### Hall C

**09:45-10:15 Coffee Break**

**Plant-insect interactions**
Chairs: Magne Friberg, Glenn Svensson

- *10:15-10:45* Peter Andersson
- *10:45-11:00* Felipe Yon
- *11:00-11:15* Tao Li
- *11:15-11:30* Camila C Filgueiras ○
- *11:30-11:45* Fernandez / Buteler
- *11:45-12:00* Renja Romey-Glüsing
- *12:00-12:15* Christina Crava

**Excursion III**
Guided tour in the Botanical Gardens

No pre-registration needed - Sign up at conference

## THURSDAY 2 JULY

### Hall A

- *08:30-09:15* Chemical ecology of bark beetles in expanding ranges made assessible by climate change
  Ken Raffa
- *09:15-09:45* Exploring the white spruce giga-genome
  Melissa Mageroy

**09:45-10:15 Coffee Break** (Halls A, B, C)

### Hall A

**Forest chemical ecology**
Chairs: Göran Nordlander, Malin elfstrand, Benedicte abrectsen

- *10:15-10:45* Jonathan Gershenzon
- *10:45-11:00* Paal Krokene
- *11:00-11:15* Tao Zhao
- *11:15-11:30* Almuth Hammerbacher
- *11:30-11:45* Niklas Björklund
- *11:45-12:00* Elisabeth Eilers
- *12:00-12:15* Jennifer Klutsch ○

### Hall B

**Chemistry of mutualism and deception**
Chairs: Jette Knudsen, Stefan Dötterl

- *10:15-10:45* Steven Johnsson
- *10:45-11:00* Birgit Oelschlägel
- *11:00-11:15* Rosalie Burdon ○
- *11:15-11:30* Pratibha Yadav ○
- *11:30-11:45* Philipp Schlüter
- *11:45-12:00* Robert R. Junker
- *12:00-12:15* Björn Bohman

### Hall C

**Microbial chemical ecology**
Chair: Birgit Piechulla

- *10:15-10:45* Birgit Piechulla
- *10:45-11:00* Victoria Challinor
- *11:00-11:15* Sandra Aragon ○
- *11:15-11:30* Lakshy Katariya ○
- *11:30-11:45* Paolina Garbeva
- *11:45-12:00* Ander Achotegui-Castells ○
- *12:00-12:15* Dajana Domik

**12:15-14:00 Lunch**

### Hall A

**Forest chemical ecology**
Chairs: Göran Nordlander, Malin elfstrand, Benedicte Albrectsen

- *14:00-14:30* Luis Sampedro
- *14:30-14:45* Robert Mitchell
- *14:45-15:00* Sibylle Unsicker

### Hall B

**Chemistry of mutualism and deception**
Chairs: Jette Knudsen, Stefan Dötterl

- *14:00-14:15* Magne Friberg
- *14:15-14:30* Manfred Ayasse
- *14:30-14:45* Sara Leonhardt
- *14:45-15:00* Matthew R Goddard

### Hall C

**Applied chemical ecology**
Chairs: Atle Wibe, Erik Hedenström

- *14:00-14:30* Jocelyn Millar
- *14:30-14:45* Mattias Larsson
- *14:45-15:00* Päivi Lyytikäinen-Saarenmaa

**15:00-15:30 Coffee Break**

### Hall A

- *15:30-15:45* Carsten Kulheim
- *15:45-16:00* Zhang Zhen
- *16:00-16:15* Andrés González
- *16:15-16:30* Malin Elfstrand

### Hall B

- *15:30-15:45* Rachelle Adams
- *15:45-16:00* Danny Zemeitat ○
- *16:00-16:15* Xavier Martini
- *16:15-16:30* Sara Hermann

### Hall C

- *15:30-15:45* Olle Anderbrant
- *15:45-16:00* Benjamin Creton
- *16:00-16:15* Paul Cunningham
- *16:15-16:30* Jürgen Gross

**16:30 - 18:00 Poster Session III**

***19:00 -***
**Conference Dinner**
Skansen
Solliden

## FRIDAY 3 JULY

### Hall A

- *09:00-09:45* Silverstein-Simeone Award Lecture
  Exploiting plant distress signals for crop protection
  Ted Turlings

**09:45-10:15 Coffee Break** (Halls A, B)

### Hall A

**Applied chemical ecology**
Chairs: Atle Wibe, Erik Hedenström

- *10:15-10:30* Sándor Koczor
- *10:30-10:45* Angélique Porciani
- *10:45-11:00* Monique Rivera ○
- *11:00-11:15* Vera Thoss
- *11:15-11:30* Jette Knudsen & Erika Wallin
- *11:30-11:45* Junwei Zhu
- *11:45-12:00* Abdullahi Yusuf
- *12:00-12:15* Owen Jones

### Hall B

**Chemical ecology of blood sucking animals**
Chairs: Sharon Hill, Mikael Carlsson

- *10:15-10:45* Ken Haynes
- *10:45-11:00* Rickard Ignell
- *11:00-11:15* Elin Isberg
- *11:15-11:30* Jenny Lindh
- *11:30-11:45* Mark Mescher
- *11:45-12:00* Adena Why ○
- *12:00-12:15* Noushin Emami ○

**12:15-13:15 Lunch**

### Hall A

**Concepts in chemical ecology**
Chairs: Jeremy Allison, Stephen Foster

- *13:15-13:45* Bill Hansson
- *13:45-14:00* Joachim Ruther
- *14:00-14:15* Joe Elkinton
- *14:15-14:30* Rayko Halitschke
- *14:30-14:45* Michael Domingue

### Hall B

**Chemical ecology of blood sucking animals**
Chairs: Junwei Zhu, Sharon Hill

- *13:15-13:45* Marcelo Lorenzo
- *13:45-14:00* Mandela Fernandez-Grandon
- *14:00-14:15* Daniel Kline
- *14:15-14:30* Prasad Doddala
- *14:30-14:45* Niels Verhulst

**14:45-15:15 Coffee Break**

### Hall A

- *15:15-15:30* Dan Hare
- *15:30-15:45* Jeremy Allison
- *15:45-16:15* Ring Cardé

### Hall B

- *15:15-15:30* Baldwin Torto
- *15:30-15:45* Victoria Soroker
- *15:45-16:00* Larry Gut
- *16:00-16:15* Amy Parachnowitsch

**16:15-16:20**

**16:20-17:00 ISCE Business Meeting** (Hall A)

○ This symbol marks contestants for the Best Student Presentation Award

# Programme - Monday 29 June

10:00-12:00 Hall C
ISCE Executive Meeting

12:00-12:40 Hall A
Welcome! Opening of ISCE2015
Anna-Karin Borg-Karlson and Christer Löfstedt

12:40-13:45 M1 ISCE Silver Medal 2015
Ecological significance of plant secondary metabolites in insect–plant interactions
Ritsuo Nishida, Kyoto University, Japan

## Unravelling the chemistry of chemical Ecology - Hall A

*Chairs: Paulo Zarbin and Jan Bello*

14:00-14:30 UNR01
Keeping it lonely at the top: competition between *Pseudonocardia* strains in fungus-growing ant systems
Jon Clardy, Harvard Medical School, USA

14:30-14:45 UNR02
Chemical ecology of the dried bean beetle: first results and future prospects
Jozsef Vuts, Rothamsted Research, UK

14:45-15:00 UNR03
Harmonia axyridis' Defense Alkaloid Harmonine – Synthesis and Bioactivity
Nadja Nagel, Max Planck Institute for Chemical Ecology, Germany

15:30-15:45 UNR04
Isolation of a behaviorally active sex pheromone component of the mushroom fly *Lycoriella ingenua*, using GC/EAG and an unusual, coupled GC/Behavior technique
Stefanos Andreadis, Pennsylvania State University, USA

15:45-16:00 UNR05
MS in chemical ecology: From characterization of oxylipins to their role in plant-microbes interactions
Marilia Trapp, Federal University of São Carlos, Brazil

16:00-16:15 UNR06
Ecological chemistry of weaver ants – nutrient cycling and search for novel chemical characteristics
Nanna Hjort Vidkjær, Aarhus University, Denmark

16:15-16:30 UNR07
Synthesis and mass spectra of macrolides used in chemical communication systems
Stefan Schulz, TU Braunschweig, Germany

16:30-16:45 UNR08
Catch and release – how to efficiently trap, analyze and formulate plant odors
Göran Birgersson, Swedish University of Agricultural Sciences, Sweden

# Programme - Monday 29 June

## Chemical Neuroecology - Hall B

*Chairs: Silke Sachse and Markus Knaden*

14:00-14:30 NEU01
Chemosensation between environment and internal state
Ilona Grunwald Kadow, Max-Planck Institute of Neurobiology, Germany

14:30-14:45 NEU02
Functional Contributions of Olfactory Receptor Neurons in *Drosophila melanogaster* larva
Dennis Mathew, University of Nevada, Reno, USA

14:45-15:00 NEU03
Insect odorant receptors: The state of play on how these novel integral membrane proteins mediate odour detection in insects
Richard Newcomb, Plant & Food Research, New Zealand

15:30-15:45 NEU04
Escaping death by sensing your enemy's sex odor
Markus Knaden, Max Planck Institute for Chemical Ecology, Germany

15:45-16:00 NEU05
Elucidating the neuronal architecture of olfactory glomeruli in the *Drosophila* antennal lobe
Silke Sachse, Max Planck Institute for Chemical Ecology, Germany

16:00-16:15 NEU06
A herbivore-induced volatile interferes with host plant and mate location in moths through jamming signaling pathways in the brain
Eduardo Hatano, Swedish University of Agricultural Sciences, Sweden

16:15-16:30 NEU07
Starvation modulates coding of pheromone and food signals in *Drosophila*
Sebastien Lebreton, Swedish University of Agricultural Sciences, Sweden

16:30-16:45 NEU08
Atomic Force Microscopy of Moth Trichoid Sensilla Across Species Reveals Common Themes and Specializations for Odorant Capture
Thomas Baker, Pennsylvania State University, USA

# Programme - Monday 29 June

## Pheromone Communication - Hall C

*Chairs: Irena Valterova and Kirk Hillier*
14:00-14:30 PHE01
Decoding evolution of pheromone communication in Heliothine moths
Kirk Hillier, Acadia University, Canada

14:30-14:45 PHE02
Aggregation in entomopathogenic nematodes
Denis Willett, University of Florida Citrus Research and Education Center, USA

14:45-15:00 PHE03
Chemical Ecology of Fruit Flies Provided Primary Impetus in Successful Synonymization of Four Major Pest Species in the Bactrocera dorsalis complex
Alvin Kah-Wei Hee, Universiti Putra Malaysia, Malaysia

15:30-15:45 PHE04
The genetic basis underlying sex pheromone variation in moths
Astrid Groot, University of Amsterdam/Max Planck Institute for Chemical Ecology, The Netherlands

15:45-16:00 PHE05
Biosynthesis of isoprenoids in the male marking pheromone of bumblebees: Through regulation of gene expression to speciation?
Irena Valterova, IOCB AS CR, Czech Republic

16:00-16:15 PHE06
Male butterflies use an anti-aphrodisiac pheromone to tailor ejaculates
Helena Larsdotter Mellström, University of Western Australia, Australia

16:15-16:30 PHE07
Quality control: Honeybee workers assess queens by pheromonal and genetic correlates of quality
Margarita Orlova, Tel Aviv University, Israel

16:30-16:45 PHE08
Chemical profiling and temporal biochemical polymorphism in *Lasius* ants
Thomas Butterfield, University of Sussex, UK

## Reception Mingle and Poster Session I

16:45-19:00
Reception Mingle and Poster Session I

# Programme - Tuesday 30 June

08:30-09:15 PLE01
Sex pheromone chemistry of thynnine wasps and the evolution of its exploitation by sexually deceptive orchids
Rod Peakall, Australian National University, Australia

09:15-09:45 M2 Early Career Award
Plant toxins induce defensive signals- Evolutionary explanations for a functional paradox
Mattias Erb, Bern University, Switzerland

## Chemical Ecology of Invasive Species - Hall A

*Chairs: Swantje Enge and Gábor Szőcs*

10:15-10:45 ISP01
How can chemical ecology contribute to biosecurity against invasive Arthropods?
Maxwell Suckling, University of Auckland, New Zealand

10:45-11:00 ISP02
Admixture in invasive plants: effects on herbivory and chemistry
Mirka Macel, University of Tübingen, Germany

11:00-11:15 ISP03
Impact of an invasive pentatomid on ecophysiology and volatile organic compounds emission in *Brassica oleracea L.* var Botrytis
Salvatore Guarino, CNR, Italy

11:15-11:30 ISP04
Untangling the role of yeasts in attraction and oviposition site selection in an invasive frugivorous insect, *Drosophila suzukii*
Boyd Mori, Swedish University of Agricultural Sciences, Sweden

11:30-11:45 ISP05
Chemical cues of the interaction Gonipterus platensis – *Eucalyptus globulus*
Sofia Branco, Universidade Nova de Lisboa, Portugal

11:45-12:00 ISP06
Polar secondary plant metabolites provide a high resistance against natural enemies to native and invasive populations of *Buddleia davidii* in the invasive range
Helga Pankoke, Bielefeld University, Germany

12:00-12:15 ISP07
Are invasive plants more toxic than native plants? An example of rapid evolution after invasion
Eva Castells, Autonomous University of Barcelona, Spain

14:00-14:30 ISP08
Chemical Ecology of Subcortical Insects in New Contexts: The Roles of Climate Change and Invasion of Naïve Habitats
Steven Seybold, University of California, Davis, USA

14:30-14:45 ISP09
The true cost of exotic perfumes: impact of invasive herbivores on native infochemical networks.
Gaylord Desurmont, University of Neuchâtel, Switzerland

14:45-15:00 ISP10
The effects of Tree tobacco nectar alkaloids on Palestine sunbird foraging behavior and performance
Rainee Kaczorowski, University of Haifa – Oranim, Israel

15:30-15:45 ISP11
Chemical Ecology of *Monochamus galloprovincialis*, Vector of Pine Wood Nematode in Europe
David Hall, University of Greenwich, UK

15:45-16:00 ISP12
Selection for increased production of Prostaglandin E2 and related deterrents during the invasion history of a seaweed, *Gracilaria vermiculophylla*
Florian Weinberger, Helmholtz Center for Ocean Science, Germany

16:00-16:15 ISP13
A chemical ecology approach to assess the proliferation of the red alga *Asparagopsis taxiformis*: Metabolomics, natural toxicity and biological effects
Stephane Greff, Aix Marseille University, France

16:15-16:30 ISP14
Biochemical evidence for cryptic fire ant species in Argentina
Robert Vander Meer, USDA, USA

## Induced Resistance of Plants and Prey - Hall B

*Chairs: Gunilla Toth and Robert Glinwood*
10:15-10:45 RES01
The dynamics of offense and defense: Induced defenses, intraspecific variance, and the ecological important of plasticity
Mark Hay, Georgia Institute of Technology, USA

10:45-11:00 RES02
Lipid signaling in marine plankton
Erik Selander, University of Gothenburg, Sweden

11:00-11:15 RES03
Induced and constitutive phlorotannins as defenses in a perennial brown alga
Fiia Haavisto, University of Turku, Finland

11:15-11:30 RES04
Inducible Plant Signalling: Opportunities for Real Time Management of Pests
Michael Birkett, Rothamsted Research, UK

11:30-11:45 RES05
Volatile-mediated plant-plant interactions: requirements for induced resistance
James Blande, University of Eastern Finland, Finland

11:45-12:00 RES06
Fungal endophytes prime pathogen resistance in wild lima bean (*Phaseolus lunatus*)
Brett Younginger, Portland State University, USA

# Programme - Tuesday 30 June

12:00-12:15 RES07
Eavesdropping plants: Insect odors prime plant defenses
Anjel Helms, Pennsylvania State University, USA

14:00-14:15 RES08
Caterpillar and rootworm feeding differentially affects defense protein accumulation in corn
Lina Castano-Duque, Pennsylvania State University, USA

14:15-14:30 RES09
Jasmonate-dependent depletion of plant carbohydrates constrains resistance and tolerance against herbivores
Ricardo A.R Machado, Max Planck Institute for Chemical Ecology, Germany

14:30-14:45 RES10
JA-Ile-Macrolactones induce nicotine accumulation in *Nicotiana attenuata* leaves and reduce *Manduca sexta* mass gain and survivorship
Guillermo Jimenz-Alemán, Max Planck Institute for Chemical Ecology, Germany

14:45-15:00 RES11
The stressed-out potato
Jenny Lazebnik, Wageningen University, The Netherlands

15:30-15:45 RES12
The 'Red Bull'® effect: an additional explanation for response to phenylpropanoids by male *Bactrocera* fruit flies
Anthony Clarke, Queensland University of Technology, Australia

15:45-16:00 RES13
Volatile interactions between undamaged plants affect herbivore insects and their natural enemies
Velemir Ninkovic, Swedish University of Agricultural Sciences, Sweden

16:00-16:15 RES14
Herbivore damage induces a transgenerational increase of cyanogenesis in wild lima bean (*Phaseolus lunatus*)
Daniel Ballhorn, Portland State University, USA

16:15-16:30 RES15
Does better growth imply better immunity? Effect of biofertilizers on induced responses of *Brassica juncea*
Vartika Mathur, University of Delhi, India

# Programme - Tuesday 30 June

## Unravelling the chemistry of Chemical Ecology - Hall C

*Chairs: Paolo Zarbin and Jan Bello*

10:15-10:30 UNR09
Spiros are Everywhere
Wittko Francke, University of Hamburg, Germany

10:30-10:45 UNR10
Chemical components of urine mediate predator-prey interactions
Remington Poulin, Georgia Institute of Technology, USA

10:45-11:00 UNR11
Identification of the sex pheromone in three heliozeliid leafminer species infesting grapevine in South Africa and Italy
Hong-Lei Wang, Lund University, Sweden

11:00-11:15 UNR12
A sex-inducing pheromone triggers cell cycle arrest and pheromone production in *Seminavis robusta*
Christine Lembke, Friedrich-Schiller-University Jena, Germany

11:15-11:30 UNR13
The perfume of termite queens: quest for termite queen pheromones
Jana Krasulová, IOCB AS CR, Czech Republic

11:30-11:45 UNR14
Emerging role of phenolamides as universal plant defense metabolites
Ivan Galis, Okayama University, Japan

11:45-12:00 UNR15
High performance separation and mass spectrometry for chemical ecology research
Åsa Emmer, KTH Royal Institute of Technology, Sweden

12:00-12:15 UNR16
Stereoisomeric Libraries for Pheromone Identifications: 1-Bisabolen-3-ols
Ashot Khrimian, U.S. Department of Agriculture, USA

# Programme - Tuesday 30 June

## Chemical Neuroecology - Hall C

*Chairs: Silke Sachse and Markus Knaden*

14:00-14:15 NEU09
The physiological and ecological significance of olfactory sensory neuron co-localization
Martin N Andersson, Lund University, Sweden

14:15-14:30 NEU10
Neuropeptide control of pheromone detection in a gustatory neural circuit
Joanne Yew, University of Hawaii at Manoa, USA

14:30-14:45 NEU11
Whole Transcriptome Analysis of Chemosensory Receptor Expression in the Codling Moth, *Cydia pomonella*
William Walker, Swedish University of Agricultural Sciences, Sweden

14:45-15:00 NEU12
Pheromone reception in the lightbrown apple moth, *Epiphyas postvittana*
Jacob Corcoran, Lund University, Sweden

15:30-15:45 NEU13
Transcriptomics of olfactory communication mediated by male sex pheromone in a butterfly
Caroline Nieberding, University of Louvain-la-Neuve, Belgium

15:45-16:00 NEU14
Molecular cloning and RNAi demonstration of olfactory co-receptor gene from two palm weevils species
Alan Soffan, King Saud University, Saudi Arabia

16:00-16:15 NEU15
Functional characterization of a receptor for a Type II sex pheromone in the winter moth, *Operophtera brumata* (Lepidoptera: Geometridae)
Dan-Dan Zhang, Lund University, Sweden

16:15-16:30 NEU16
Carbon dioxide receptor involved in the perception and production of the sex pheromone
Kyung San Choi, Rural Development Administration, Korea

## Poster Session II

16:30-18:00
Poster Session II with complementary drinks and snacks

19:00-
JCE Editorial board meeting
Restaurant "The Flying Elk" - Mälartorget 15, Metro: "Gamla Stan"

# Programme - Wednesday 1 July

08:30-09:15 PLE02
A molecular rationale for the unpredictable nature of female behavior
Lisa Stowers, Scripps Research Institute, USA

09:15-09:45 PLE03
Reverse chemical ecology with a cast of thousands and one DEET receptor
Walter Leal, University of California, Davis, USA

## Chemical Ecology Meets Pharmacognosy - Hall A

*Chairs: Lars Bohlin*

10:15-10:30 PCY01a
Truffle fungi and their volatile substances in vector mediated spore dispersal - an overview
Christina Wedén, Uppsala University, Sweden

10:30-10:45 PCY01b
Diptera attracted to the truffle *Tuber aestivum* and dimethylsulfide in Sweden.
Ingemar Struwe, Ingemar Struwe Entomologiska Uppdrag, Sweden

10:45-11:00 PCY02
Biological role of triplicated Na, K-ATPase1α genes in the large milkweed bug, with regard to target-site insensitivity against cardiac glycosides
Jennifer Lohr, University of Hamburg, Germany

11:00-11:15 PCY03
Peptides from plants and animals: ecology connected to drug discovery
Ulf Göransson, Uppsala University, Sweden

11:15-11:30 PCY04
Phytochemical diversity: structure-activity-relationships in the toxin-receptor interaction between milkweeds and monarchs
Georg Petschenka, Cornell University, USA

11:30-11:45 PCY05
Sponge taxonomy 2.0 meets pharmacognosy and chemical ecology
Paco Cardenas, Uppsala University, Sweden

11:45-12:00 PCY06
Volatiles from the burnet moth *Zygaena filipendulae* (Lepidoptera) and associated flowers, and their role in mating communication
Mika Zagrobelny, University of Copenhagen, Denmark

12:00-12:15 PCY07
Chemical and molecular characterization of nicotine to nornicotine conversion phenotype in Australian *Nicotiana* species used as chewing tobacco
Nahid Moghbel, University of Queensland, Australia

# Programme - Wednesday 1 July

## Vertebrate Chemical Ecology

*Chairs: Lisa Stowers and Raimondas* Mozūraitis

10:15-10:45 VER01
The sick sense is in the nose
Ivan Rodriguez, University of Geneva, Switzerland

10:45-11:00 VER02
Of mice and amines: genetic analysis of odor perception
Tomas Bozza, Northwestern University, USA

11:00-11:15 VER03
Host Immune Function and the Volatile Metabolome
Bruce Kimball, USDA-APHIS-WS-NWRC, USA

11:15-11:30 VER04
Does Fel d 1, the main cat's allergen, have a kairomone role?
Carlos Grau Paricio, Research Institute in Semiochemistry and Applied Ethology, France

11:30-11:45 VER05
How blue Petrels find their scented burrow?
Marianne Gabirot, Cardiff University, UK

11:45-12:00 VER06
Sex pheromone dynamics during oestrous cycle in dairy cows
Raimondas Mozūraitis, KTH Royal Institute of Technology, Sweden

12:00-12:15 VER07
Major urinary protein (MUP) profiles show dynamic changes rather than individual 'barcode' signatures
Michaela Thoß, University of Veterinary Medicine, Austria

# Programme - Wednesday 1 July

## Plant-Insect Interactions - Hall C

*Chairs: Magne Friberg and Glenn Svensson*

10:15-10:45 INS01
Experience to plant cues affects host plant choice behaviours in a moth
Peter Anderson, Swedish University of Agricultural Sciences, Sweden

10:45-11:00 INS02
Floral scents to the touch: Scents inform moth's proboscis for probing and foraging, thus determining the plant fitness
Felipe Yon, Max Planck Institute for Chemical Ecology, Germany

11:00-11:15 INS03
Ozone pollution compromises within-plant signalling via volatiles
Tao Li, University of Eastern Finland, Finland

11:15-11:30 INS04
Aboveground application of elicitors recruits an entomopathogenic nematode belowground
Camila C Filgueiras, Universidade Federal de Lavras, Brazil

11:30-11:45 INS05
Both volatiles and epicuticular plant compounds determine oviposition of the willow sawfly nematus oligospilus on leaves of Salix spp. (Salicaceae)
Patricia Fernandez and Micaela Buteler, INTA EEA Delta del Parana/Conicet, Argentina

11:45-12:00 INS06
Become a specialist with an enzyme – The senecionine-monooxygenase of *Longitarsus jacobaeae*
Renja Romey-Glüsing, University of Hamburg, Germany

12:00-12:15 INS07
Keeping your food fresh: active manipulation of cytokinin-metabolism by a cell content feeder
Cristina Crava, Fondazione Edmund Mach, Italy

# Excursions

## Bus to Forest Fire

## Bus transfer to Boat to Drottningholm Palace

## Walk to Botanic Garden

# Programme - Thursday 2 July

08:30-09:15 PLE04
Chemical ecology of bark beetles in expanding ranges made assessible by climate change
Kenneth F. Raffa, University of Wisconsin–Madison, USA

09:15-09:45 PLE05
Exploring the white spruce giga-genome for biosynthetic pathways of novel insect defense metabolites
Melissa Mageroy, University of British Columbia, Canada

## Forest Chemical Ecology - Marcus Wallenberg Symposium - Hall A

*Chairs: Göran Nordlander, Malin Elfstrand and Benedicte Albrectsen*

10:15-10:45 FOR01
Terpenoid resin defenses in Norway spruce: Herbivore induction and insensitivity
Jonathan Gershenzon, Max Planck Institute for Chemical Ecology, Germany

10:45-11:00 FOR02
Defense priming in Norway spruce: chemical and molecular evidence
Paal Krokene, Norwegian Institute of Bioeconomy Research, Norway

11:00-11:15 FOR03
Fungal symbionts of the spruce bark beetle synthesize aggregation pheromone and reduce tree defense monoterpenes
Tao Zhao, KTH Royal Institute of Technology, Sweden

11:15-11:30 FOR04
A common fungal associate of the spruce bark beetle metabolizes the stilbene defenses of Norway spruce
Almuth Hammerbacher, Max Planck Institute for Chemical Ecology, Germany

11:30-11:45 FOR05
Improved forest regeneration by triggering the induced defense of conifer seedlings against bark-feeding insects
Niklas Björklund, Swedish University of Agricultural Sciences, Sweden

11:45-12:00 FOR06
Insect egg deposition – a warning signal enhancing sex-biased anti-herbivore defence in a tree
Elisabeth Johanna Eilers, Free University of Berlin, Germany

12:00-12:15 FOR07
Impact of interactions among native biotic disturbances on range expansion of mountain pine beetle into novel jack pine forests
Jennifer Klutsch, University of Alberta, Canada

14:00-14:30 FOR08
Intraespecific variation in constitutive and inducible defensive allocation in Maritime pine, a model Mediterranean species
Luis Sampedro, Consejo Superior de Investigaciones Cientificas, Spain

14:30-14:45 FOR09
The mesquite borer *Megacyllene antennata* produces an aggregation-sex pheromone composed of floral and green leaf volatiles
Robert Mitchell, University of Arizona, USA

# Programme - Thursday 2 July

14:45-15:00 FOR10
Black poplar volatiles in biotic interactions
Sybille Unsicker, Max-Planck Institute for Chemical Ecology, Germany

15:30-15:45 FOR11
Chemical and transcriptome analysis of resistant and susceptible Eucalyptus genotypes to the insect pest *Leptocybe invasa*
Carsten Kulheim, Australian National University, Australia

15:45-16:00 FOR12
Semiochemicals regulating intraspecific and interspecific relationships of three Tomicus species in *Pinus yunnanensis* Franch
Zhen Zhang, Chinese Academy of Forestry, China

1600:-16:15 FOR13
Studies on the chemical communication of the bronze bug, *Thaumastocoris peregrinus* (Heteroptera: Thaumastocoridae), a pest of Eucalyptus
Andrés González, Universidad de la República, Uruguay

16:15-16:30 FOR14
Norway spruce ATAF1-like NAC transcription factors modulate stress
Malin Elfstrand, Swedish University of Agricultural Sciences, Sweden

# Programme - Thursday 2 July

## Chemical Ecology of Mutualism and Deception - Hall B

*Chairs: Jette Knudsen, Stefan Dötterl*

10:15-10:45 MUT01
Concepts and Challenges in Mimicry Research
Steven D. Johnson, University of KwaZulu-Natal, South Africa

10:45-11:00 MUT02
Flowers of Aristolochia rotunda mimic pheromones of true bugs to attract and trap fly pollinators
Birgit Oelschlägel, TU Dresden, Germany

11:00-11:15 MUT03
Functional role of the floral volatile, S-(+)-linalool, in P*enstemon digitalis*
Rosalie Burdon, Uppsala University, Sweden

11:15-11:30 MUT04
Knock, knock. Who is there? Host location mechanisms in non-pollinating fig wasps
Pratibha Yadav, Indian Institute of Science, India

11:30-11:45 MUT05
Odour genes and pollinator-driven speciation in sexually deceptive *Ophrys* orchids
Philipp Schlüter, University of Zürich, Switzerland

11:45-12:00 MUT06
Floral microbial ecology – patterns, mechanisms, consequences and functions of bacterial colonization on flowers
Robert R. Junker, University of Salzburg, Austria

12:00-12:15 MUT07
Choosy wasp cheated by copycat orchid chemistry
Björn Bohman, Australian National University, Australia

14:00-14:15 MUT08
Floral scent in a geographic mosaic of coevolution
Magne Friberg, Uppsala University, Sweden

14:15-14:30 MUT09
Antennal sensitivity to floral scents of Campanula: A comparative study of polylectic and oligolectic bees
Manfred Ayasse, University of Ulm, Germany

14:30-14:45 MUT10
How to attract your seed disperser: The chemistry behind an unusual mutualism between and Australian eucalypt and stingless bees.
Sara Leonhardt, University of Würzburg, Germany

14:45-15:00 MUT11
The Chemical Context of Interspecific Communication: Ecological Chemistry as a Driver of Mutualisms
Matthew R. Goddard, University of Auckland, New Zealand

15:30-15:45 MUT12
Chemically armed ants pillage and protect fungus-farming societies
Rachelle Adams, University of Copenhagen, Denmark

15:45-16:00 MUT13
Chemical communication in a mutualistic system – The myrmecophilous Australian butterfly *Jalmenus evagoras* (Lepidoptera: Lycaenidae)
Dany Zemeitat, University of Melbourne, Australia

16:00-16:15 MUT14
The fungus, *Raffaelea lauricola*, manipulates release of host plant odors causing initial repellency and subsequent attraction of trees to its symbiont and vector, the redbay ambrosia beetle (*Xyleborus glabratus*).
Xavier Martini, University of Florida Citrus Research and Education Center, USA

16:15-16:30 MUT15
Volatile Predator Cues Drive Non-consumptive Effects
Sara Hermann, Cornell University, USA

## Chemical Ecology of Microbes - Hall C

*Chairs: Birgit Piechulla*

10:15-10:45 MIC01
Trichoderma volatile reduces fungal phytopathogenic symptoms in *Arabidopsis*
Birgit Piechulla, University of Rostock, Germany

10:45-11:00 MIC02
Identification and biosynthesis of new bicyclic alkaloids from the entomopathogenic bacteria *Xenorhabdus*
Victoria Challinor, Goethe University, Germany

11:00-11:15 MIC03
Not visible, but highly effective: Plant volatile manipulation by endophytic fungi and responses of herbivores
Sandra Aragón, Georg-August University, Germany

11:15-11:30 MIC04
Sniffing out the enemy: Fungus-growing termites can differentiate between mutualistic and parasitic fungi using volatiles
Lakshy Katariya, Indian Institute of Science, India

11:30-11:45 MIC05
Volatile affairs in microbial interactions
Paolina Garbeva, Netherlands Institute for Ecology, The Netherlands

11:45-12:00 MIC06
Terpene arms race in the *Seiridium cardinale – Cupressus sempervirens* pathosystem
Ander Achotegui-Castells, CREAF, Spain

12:00-12:15 MIC07
Elucidation of the secondary metabolite of the rhizobacterium *S. plymuthica* 4Rx13
Dajana Domik, University of Rostock, Germany

## Applied Chemical Ecology - Hall C

*Chairs: Atle Wibe, Erik Hedenström*

14:00-14:30 APP01
Insect Pheromones for Insect Management: Promise versus Reality
Jocelyn Millar, University of California, USA

14:30-14:45 APP02
Pheromone monitoring as a game changer in insect biodiversity and conservation research
Mattias Larsson, Swedish University of Agricultural Sciences, Sweden

14:45-15:00 APP03
Monitoring the common pine sawfly populations with pheromone traps in managed boreal forests
Päivi Lyytikäinen-Saarenmaa, University of Helsinki, Finland

15:30-15:45 APP04
Detection, monitoring, and forecast using pheromone traps for three spruce seed feeding moths
Olle Anderbrant, Lund University, Sweden

15:45-16:00 APP05
Use of a vertebrate's semiochemical for long-term protection of horses' ears against Black flies (Diptera : Simuliidae)
Benjamin Creton, Research Institute in Semiochemistry and Applied Ethology, France

16:00-16:15 APP06
Fruit ripening volatiles act synergistically as host cues in a pest tephritid fruit fly.
Paul Cunningham, Queensland University of Technology, Australia

16:15-16:30 APP07
Innovative control strategies for phytoplasma vectoring insects by infochemicals
Jürgen Gross, Julius Kühn-Institut, Federal Research Centre for Cultivated Plants, Germany

19:00-01:00
Conference Dinner at Skansen (Solliden Building)

# Programme - Friday 3 July

09:00-09:45 M3 Silverstein-Simeone Medal 2015
Exploiting plant distress signals for crop protection
Ted Turlings, University of Neuchâtel, Switzerland

## Applied Chemical Ecology - Hall A

*Chairs: Atle Wibe, Erik Hedenström*
10:15-10:30 APP08
Studies on interference between different lacewing attractants: new perspectives for Central European species (Neuroptera: Chrysopidae)
Sándor Koczor, Plant Protection Institute CAR HAS, Hungary

10:30-10:45 APP09
Insecticide resistance mutation (Kdr) in *Anopheles gambiae* modulates host choice and olfaction in presence of pyrethroid-treated net
Angélique Porciani, IRD, France

10:45-11:00 APP10
Can herbivore induced plant volatile compounds be used to enhance attraction of entomopathogenic nematodes?
Monique J. Rivera, Rugers University, USA

11:00-11:15 APP11
Observations from a Bluebell and Bracken Climax Vegetation and Approaches for Bluebell Conservation
Vera Thoss, Bangor University, UK

11:15-11:30 APP12
Chemical tools for training dogs to find bed bugs
Erika A Wallin, Mid Sweden University, Sweden
Jette Knudsen, Nattaro Labs, Sweden

11:30-11:45 APP13
Semiochemical-based technologies for fly management
Junwei Zhu, USDA-ARS, USA

11:45-12:00 APP14
Prospects of managing a social parasite using pheromone supplements
Abdullahi Yusuf, University of Pretoria, South Africa

12:00-12:15 APP15
Commercial Applications of Semiochemicals – Current status and future prospects
Owen Jones, Suterra UK, UK

# Programme - Friday 3 July

## Chemical Ecology of Blood Sucking Animals - Hall B

*Chairs: Sharon Hill and Mikael Carlsson and Junwei Zhu*

10:15-10:45 BLO01
Chemical Ecology of Bed Bugs
Kenneth Haynes, University of Kentucky, USA

10:45-11:00 BLO02
Grass volatiles partially explain oviposition site selection by Anopheles mosquitoes
Rickard Ignell, Swedish University of Agricultural Sciences, Sweden

11:00-11:15 BLO03
Identification of Host Volatiles and their Role in the Behavioural Modulation of Host-Seeking Culicoides Biting Midges
Elin Isberg, Swedish University of Agricultural Sciences, Sweden

11:15-11:30 BLO04
Discovery of an odor bait for gravid malaria vector mosquitoes of the *Anopheles gambiae* species complex
Jenny Lindh, KTH Royal Institute of Technology, Sweden

11:30-11:45 BLO05
Parasite-induced olfactory cues influence mosquito attraction to malaria-infected hosts
Mark Mescher, ETH Zurich, Switzerland

11:45-12:00 BLO06
Behavioral responses of Culex tarsalis to fish-associated semiochemicals in wind tunnel bioassays
Adena Why, University of California, USA

12:00-12:15 BLO07
The Malaria whistle for immediate transfer
S. Noushin Emami, Stockholm University, Sweden

13:15-13:45 BLO08
Transcriptomic analysis of the antennae of a Chagas disease vector
Marcelo Lorenzo, CPqRR-FIOCRUZ, Brazil

13:45-14:00 BLO09
Heritability of Attractiveness to Mosquitoes
Mandela Fernandez-Grandon, University of Greenwich, UK

14:00-14:15 BLO10
Utilization of flowers and their volatiles for surveillance and management of mosquitoes and other biting flies.
Daniel Kline, USDA-ARS, USA

14:15-14:30 BLO11
Gustatory synergy between sugars and amino acids in the yellow fever mosquito, *Aedes aegypti*
Prasad Doddala, Swedish University of Agricultural Sciences, Sweden

14:30-14:45 BLO12
Difference in mosquito attraction; between humans, within humans and between humans and great apes
Niels Verhulst, Wageningen University, The Netherlands

15:15-15:30 BLO13
Plant volatiles in perspective of mosquito disease vector control
Baldwyn Torto, International Centre of Insect Physiology and Ecology, Kenya

15:30-15:45 BLO14
Disruption of Varroa-honeybee association by chemosensory inhibition
Victoria Soroker, ARO, Volcani center, Israel

15:45-16:00 BLO15
Optimizing pheromone aerosol emitters for codling moth mating disruption
Larry Gut, Michigan State University, USA

16:00-16:15 BLO16
Unifying the field of floral chemical ecology
Amy Parachnowitsch, Uppsala University, Sweden

# Programme - Friday 3 July

## Concepts in Chemical Ecology

*Chairs: Jeremy Allison, Stephen Foster*
13:15-13:45 CON01
Insect Olfaction – Quo vadis?
Bill Hansson, Max Planck Institute for Chemical Ecology, Germany

13:45-14:00 CON02
Insect cuticular hydrocarbons: exciting functions of boring chemicals?
Joachim Ruther, University of Regensburg, Germany

14:00-14:15 CON03
Estimating rates of spread of invasive insects with pheromone-baited traps
Joseph Elkinton, University of Massachusetts, USA

14:15-14:30 CON04
Mating system and induction shape the volatile emissions of wild potatoes
Rayko Halitschke, Justus Liebig University, Germany

14:30-14:45 CON05
Advances in material science allow a fully integrative study of visual and chemical ecology
Michael Domingue, Pennsylvania State University, USA

15:15-15:30 CON06
Critical Themes in Research on Tritrophic Interactions
J. Daniel Hare, University of California, USA

15:30-15:45 CON07
Stabilizing Selection on Moth Pheromone Blends: Fact of Fiction?
Jeremy Allison, Canadian Forest Service, Canada

15:45-16:15 CON08
Functional, evolutionary and teleological perspectives on finding a source of odor
Ring Cardé, University of California, USA

16:15-16:20
Short Break

16:20- 17:00 Hall A
ISCE Business Meeting

# EuCheMS
## European Chemical Sciences

EuCheMS, the European Association for Chemical and Molecular Sciences, aims to nurture a platform for scientific discussion and to provide a single, unbiased European voice on key policy issues in chemistry and related fields.

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# PLENARY

# Sex pheromone chemistry of thynnine wasps and the evolution of its exploitation by sexually deceptive orchids

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**Bjo** Erik Jacobsson, Uppsala university, Uppsala, Sweden; Camilla Eriksson, Uppsala university, Uppsala, Sweden; Martin Hedström, Lund University, Lund, Sweden; Henrik **avin** Seth, Gothenburg University, Gothenburg, Sweden; Eric McEvoy, Liverpool John Moores University, Liverpool, United Kingdom; Per Sundberg, Gothenburg University, Gothenburg, Sweden, Malin Strand, Swedish Species Information Centre, Swedish University of Agricultural Sciences, Uppsala, Sweden<br /><br />Ulf Göransson, Uppsala university, Uppsala, Sweden

Ants, bees and wasps (*Hymenoptera*) include as many species worldwide as the moths and butterflies (*Lepidoptera*), or the flies (*Diptera*), however, our knowledge of sex pheromone chemistry lags behind. The hyperdiverse Australian thynnine wasps exhibit unique mating behaviour and a reliance on sex pheromones to attract mates. Intriguingly, several hundred orchids lure male thynnine wasps as specific pollinators by sex pheromone mimicry. We have previously reported that two chemically unrelated sex pheromone systems are each exploited by orchids: chiloglottones and hydroxymethylated pyrazines. Here we report the discovery of three additional systems: a thiophenol ether that operates both on its own, and in combination with hydroxymethylpyrazines; and another dual system involving hydroxymethylacetophenone and citronellol. Sex pheromones consisting of chemicals that originate from distinct biosynthetic pathways appear to be rare in nature. Yet, we have found both single and dual pheromone systems within a single wasp genus. Remarkably, both pheromone systems have also been co-opted by sexually deceptive orchids. An overlay of the emerging knowledge of sex pheromone chemistry onto the wasp phylogeny reveals phylogenetic structure and correlated bias in exploitability by orchids. Conversely, an overlay of sex pheromone mimicry onto the orchid phylogeny reveals there has been multiple independent origins of sexual deception, with major switches in chemistry even occurring within some orchid clades. These exciting findings highlight the Australian thynnines as unique research subjects for the discovery of many new and chemically diverse sex pheromone systems, and ideal candidates for evolutionary studies of both wasps and the orchids that exploit them.

Bohman B, Philips RD, Menz MHM, Berntsson BW, Flematti GR, Barrow RA, Dixon KW, Peakall R (2014) Discovery of pyrazines as pollinator sex pheromones and orchid semiochemicals: implications for the evolution of sexual deception. New Phytologist 203, 939–952.

Peakall R, Ebert D, Poldy J, Barrow R, Francke W, Bower C, Schiestl F (2010) Pollinator specificity, floral odour chemistry and the phylogeny of Australian sexually deceptive Chiloglottis orchids: Implications for pollinator-driven speciation. New Phytologist 188, 437-450.

# A molecular rationale for the unpredictable nature of female behavior

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We are studying how chemosensory cues generate emotion. While it is understood that mouse pheromones regulate social behavior, it is less well appreciated that these behaviors are tightly coupled with emotion to motivate the behavior. When we undertook this research no mouse pheromones had been isolated. Mouse urine and saliva each contain hundreds of odor molecules. How would one recognize a pheromone, let alone deduce its function? We focused on robust behaviors and evaluated fractions of native secretions to identify the underlying bioactive molecule. This method enabled us to discovered the elusive ligands, and also immediately know their function. We repeated this approach and now have a repertoire of ligands that stimulate rage, attraction, and fear behavior. These pheromones/kairomones elicit behavior without associated learning, indicating that they activate hardwired, experimentally tractable, circuits. We are now beginning to identify and study these neural circuits in order to learn how an olfactory signal is transformed into a behavioral response, and how the circuit encodes emotional intensity and valence.

In my talk I will demonstrate how pheromones/kairomones elicit robust, emotion-linked, behavior in the mouse and outline our expectations for the underlying neural code. However, I will focus most of the talk on how the use of purified pheromones enabled us to discover that the female's sensory receptors are directly regulated by sex-steroids. It had been known that females display dramatically different behavior depending on their state of ovulation. This is thought to occur through sex-specific hormones acting on behavioral centers in the brain. We asked here whether sensory activity also differs across the ovulation cycle to alter behavior and I will show data to indicate that female mouse vomeronasal sensory neurons (VSNs) are temporarily and specifically rendered 'blind' to a subset of male-emitted pheromone ligands during diestrus, yet fully detect and respond to the same ligands during estrus. VSN silencing occurs through the action of the female sex-steroid progesterone. Not all VSNs are targeted for silencing; those detecting cat ligands remain continuously active irrespective of the estrous state and I will show how progesterone targets specific subsets of male-pheromone responsive neurons for inactivation. These findings indicate that internal physiology can selectively and directly modulate sensory input to produce state-specific behavior.

## Reverse chemical ecology with a cast of thousands and one DEET receptor

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Insect repellents have been used since antiquity as prophylactic agents against diseases transmitted by mosquitoes and other arthropods, including malaria, dengue fever, and encephalitis. They evolved from smoke generated by burning plants (e.g.: lemon gum) and topical applications of essential oils (e.g: lemon eucalyptus extract) into repellent substances, including those isolated from plants (e.g.: p-menthane-3,8-diol, PMD) and a broad-spectrum synthetic repellent N,N-dimethyl-3-methylbenzamide (DEET), which was discovered in the early 1940s from a screening of thousands of compounds. Thereafter, other synthetic repellents have been developed (e.g: IR3535, 3-(N-acetyl-N-butyl)aminopropionic acid ethyl ester), but DEET remains the most widely used repellent substance world-wide. Molecular modeling led to the development of picaridin ((RS)-sec-butyl 2-(2-hydroxyethylpiperidine) (2), but progress in drug design has been slow, in part because of the unknown mode of action. Although it is well established that DEET acts on the insect’s gustatory and olfactory systems, its mode of action as an odorant was a matter of considerable debate, with two dichotomous hypotheses. One school suggests that DEET modulates the activity of many odorant receptors (ORs), whereas there is growing evidence suggesting that repulsion is triggered by excitatory responses of odorant receptor neurons and that OR are involved. As adult mosquito ORs remained elusive, it has been postulated that the ionotropic receptor IR40a could account for the widespread effect of DEET olfactory repellency. To thoroughly test these hypotheses, we carried out molecular, behavioral, and electrophysiological studies mostly with the vector of West Nile virus in California, the southern house mosquito, Culex quinquefasciatus. Here, we report on a DEET receptor, which is also sensitive to plant-derived semiochemicals.

## Chemical ecology of bark beetles in expanding ranges made assessible by climate change

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Warming temperatures are allowing many species to expand their ranges into higher elevations and latitudes. These shifts pose many unanswered questions, which have important ramifications to ecosystem processes and socioeconomic well-being. How insect herbivores will behave in new habitats, the threats they may pose, and our optimal approaches to mitigation will be determined by a complex set of factors. Many of these factors relate to chemical signaling, so recent advances by chemical ecologists in fields such as plant defense, host selection, and tritrophic interactions will prove particularly informative. This presentation focuses on native bark beetle-microbial complexes in North American conifers as an example, and compares features of their chemical ecology in historical, semi-naïve, and naïve habitats.

# Exploring the white spruce giga-genome for biosynthetic pathways of novel insect defense metabolites

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Recent advancements in conifer genome and transcriptome assembly are enabling new discoveries in forest chemical ecology as shown in our work on the chemical and anatomical defense of spruce and pine species against stem boring and foliage feeding insects. Spruce budworm (SBW) is one of the most detrimental native pests of spruces in North America. White spruce (*Picea glauca*), for which we published a 20 Gbp genome assembly (Birol et al. 2013), is heavily attacked by periodic outbreaks of SBW. Previous studies identified natural resistance to SBW in a population of P. glauca. Four acetophenone compounds, the glucosides picein and pungenin and their respective aglycons piceol and pungenol, are important in the difference between resistance and susceptibility. In susceptible trees, only the glucosides are present while resistant trees also produce the aglycons. A microarray analysis of 23,853 P. glauca genes identified a β-glucosidase, PgβGlu-1, 770-fold more highly expressed in resistant trees. Enzyme assays with the recombinant PgβGlu-1 showed this protein is responsible for the hydrolysis of picein and pungenin releasing the aglycons. The combined genomic and biochemical analysis revealed PgβGlu-1 as an important contributor to SBW resistance by activating defensive acetophenones, and is now being developed as a potential biomarker to enhance breeding for insect resistance (Mageroy et al., 2014). To identify additional picein and pungenin biosynthetic genes, we explored temporal patterns of metabolite accumulation. Acetophenone glucosides appeared after approximately 8 weeks following bud burst. RNA-seq on young shoots from 6 to 9 weeks after bud burst is revealing gene candidates whose expression correlated with the appearance of the acetophenone glucosides. Several of these genes have been expressed in N. benthamiana for functional characterization. In summary, developments in conifer genomics are helping to advance our knowledge of uncharted specialized metabolite biosynthetic pathways of conifer defense against insects.

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# MEDALISTS

ISCE SILVER MEDAL 2015

# Ecological significance of plant secondary metabolites in insect–plant interactions

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Plants produce a diverse array of secondary metabolites mainly as chemical defense against herbivores. Many phytophagous insects are well adapted to these allelochemicals; and may use them as specific host-finding cues (kairomones), own defensive substances (allomones), and as sex pheromones by selectively sensing, incorporating and/or processing specific plant metabolites. Insects also serve as pollinators often effectively guided by specific floral fragrances (synomones) in the mutualistic interactions. Various insect-plant interactions mediated by such plant allelochemicals and their ecological significance will be discussed in this presentation, particularly on the following topics:

1. Phytochemicals for host-finding (kairomone): Butterfly females find their host plants by sensing specific phytochemicals through contact chemoreceptors on their forelegs. A series of oviposition stimulants have been systematically characterized in the swallowtail family (Papilionidae).

2. Phytochemicals for defense (allomone): A number of phytophagous insects sequester plant allelochemicals into their body tissues and/or integuments, thereby, obtain a potent defense mechanism without producing their own noxious chemicals.

3. Phytochemicals for sexual communication (pheromone): Insects produce a diverse assemblage of sex pheromones. In some instances, insects employ specific plant secondary metabolites effectively to attract and excite conspecific partners during courtship. Such pheromone systems may often be associated with defense mechanisms as in topic 2.

4. Phytochemicals for mutualistic associations (synomone): Pollination syndromes between higher plants and insects have resulted in the rich flora and fauna on the planet earth. Unique mutualistic interactions via floral synomones between a group of orchid species and pollinator fruit flies belonging to the genus of Bactrocera (Diptera: Tephritidae) will be highlighted.

Nishida, R. 2014. Chemical ecology of insect-plant interactions: Ecological significance of plant secondary metabolites. Biosci. Biotechnol. Biochem. 78: 1-13.

**EARLY CAREER AWARD 2015**

# Plant toxins induce defensive signals- Evolutionary explanations for a functional paradox

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Plant metabolites fulfil a number of important roles in plant defense, ranging from hormonal signaling to induced toxicity. While signals typically induce toxins, evolutionary theory predicts that the former may have evolved from the latter. This prediction again leads to the counterintuitive assumption that transition states should exist in which toxins induce signals [1]. I will present our own work on indole and its derivatives to illustrate that toxins can indeed have signal-like properties. Indole by itself for instance acts as an induced volatile feeding toxin against a generalist herbivore, but also functions as an essential volatile priming signal in maize which increases the herbivore-induced jasmonate burst [2]. Furthermore, indole derived benzoxazinoids act as defensive metabolites against herbivores, but are also required for aphid-induced callose deposition and resistance [3,4]. Apart from lending support to current evolutionary scenarios on the evolution of chemical communication, these findings suggest that strengthening the production of plant toxins can lead to positive knock-on effects on induced resistance signaling in plants.

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[2] Erb, M., Veyrat, N., Robert, C.A.M., Xu, H., Frey, M., Ton., J., Turlings, T.C.J. Indole is an essential herbivore-induced volatile priming signal in maize (2015). Nature Communications, 6273.

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**SILVERSTEIN-SIMEONE MEDAL 2015**

# Exploiting plant distress signals for crop protection

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Seven billion and counting…. the ever-increasing human population is putting unprecedented pressures on our natural resources. Arguably the biggest contemporary challenge for humanity is to meet world's current and future food security. About one third of the potential crop yield is still lost to insect pests and pathogens, but rapid advances in the fields of metabolomics and genomics offer new opportunities to explore plant traits that may help to develop strategies to combat these pests. During my talk I will make a plea for a greater contribution by chemical ecologist to contribute to these efforts.

Our own research uses a chemical ecological approach to better understand the interactions among maize plants, pest insects and the natural enemies of the pests. For example, with the latest chemical analytical techniques and with the use of mutant maize plants we think we have found an explanation for the voraciousness of one of the most important pests to maize, the Western corn rootworm or Diabrotoca virgifera virgifera. Larvae of this beetle were found to prefer to feed on nutritious crown roots of maize, which are also very well-defended by benzoxazinoids (1), toxic compounds that normally deter herbivores from feeding on the roots. The rootworm larvae, however, are not affected by the toxins and use them to identify these roots (2), which are rich in sugars and amino acids.

One of the solutions to fight corn rootworm are entomopathogenic nematodes (EPN), tiny parasitic worms that kill the larvae within days. We have discovered that the nematodes use a chemical signal that is specifically emitted from maize after rootworm attack(3). However, this signal, the sesquiterpene E-(β)-caryophyllene is not emitted American maize varieties (4), which makes these varieties far less attractive to the beneficial EPN. By genetically transforming an American maize line with a caryophyllene-synthase gene from oregano, caryophyllene emission was restored in this line and field experiments revealed that this resulted in enhanced protection by EPN against rootworm damage (5).

The research shows the great potential of EPN as biological agents to control rootworm larvae and other soil-borne pests, but, as yet, their application is too expensive and labor intensive. Our research efforts now focus on the development of novel ways to apply EPN to crop fields. The latest approach involves the encapsulation of EPN in alginate capsules or beads (6). By adding specific chemicals to the beads we can put the EPN in a state of quiescence (7), which significantly prolongs their shelf-life and ensures that the EPN are in optimal condition when the beads dissolve or are consumed by target insects. We are trying to isolate and identify such a quiescence factor from root extracts. Moreover, to encourage the insects to eat the beads we wish to incorporate plant-derived attractants and feeding stimulants. I will use these examples of our research to show that a good understanding of chemically mediated interactions among plants, insects and their natural enemies can lead to novel strategies for crop protection.

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# UNRAVELING THE CHEMISTRY OF CHEMICAL ECOLOGY

# Keeping it lonely at the top: competition between *Pseudonocardia* strains in fungus-growing ant systems

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The complex multilateral symbioses involving fungus-growing ants, their fungal crops, specialist fungal pathogens (*Escovopsis* spp.), and the Actinobacteria (*Pseudonocardia* spp.) that biosynthesize chemical defenses are maintained by multiple exchanges of small molecules. After a brief introduction to the general system and some of the already established interactions, the talk will focus on two features: 1) the genetic basis creating the spectacular biosynthetic diversity of the symbiotic *Pseudonocardia* spp. and 2) the vertical transmission of the *Pseudonocardia* from worker to worker and the existential threat posed by their closest relatives.

# Chemical ecology of the dried bean beetle: First results and future prospects

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The dried bean beetle, *Acanthoscelides obtectus* Say (Coleoptera: Chrysomelidae, Bruchinae), is a pest of stored legumes, mainly *Phaseolus* spp. (Fabaceae). Due to the level of damage it causes, *A. obtectus* was studied several decades ago by chemical ecologists in search of novel semiochemical-based management methods.

Methyl (*E,R*)-2,4,5-tetradecatrienoate was identified as a male-produced sex pheromone more than 40 years ago. More recently, octadecanal was identified from males and found to synergize the activity of the ester as a sex attractant for females. Using GC-MS and GC-EAG, we recently identified additional compounds from headspace extracts of males, comprising two methyl esters of unsaturated C12 carboxylic acids and two farnesene isomers. In olfactometer bioassays, pure methyl (*E,R*)-2,4,5-tetradecatrienoate was only weakly attractive to unmated females. However, a synthetic blend of the six identified volatiles released in physiologically relevant ratios and doses proved to be as active as headspace odours collected from live males.

Other studies on *A. obtectus* suggested that males use contact chemoreception to recognise sexes. They antennate conspecifics, which then results in the sequence of chasing, mounting and copulation with females. We set up a series of laboratory choice tests to establish if certain cuticular compounds aid contact sex recognition in *A. obtectus*. We found that methyl (*E,R*)-2,4,5-tetradecatrienoate, besides being a male-produced sex pheromone, is present on the cuticle of males, but not of virgin females, and acts as a male-recognition signal. Males also transfer it onto females during mating, resulting in mated females being avoided by courting males.

# *Harmonia axyridis'* Defense Alkaloid Harmonine – Synthesis and Bioactivity

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More than 5200 species belong to the lady beetle family Coccinellidae. Most of them are carnivorous demonstrating an enormous appetite for aphids and mites and have therefore been used as biological control agents. However, species like *Harmonia axyrids* have become invasive threatening the native lady beetle assemblage.

The enormous invasive success is likely due to its resistance against various pathogens. The main defense compound of *H. axyridis* is harmonine ((*17-R, 9-Z*)-1,17-diaminooctadec-9-ene). Studies have proven that this alkaloid possesses a broad activity spectrum against fast-growing mycobacteria, such as *Mycobacterium tuberculosis* and the malaria parasite *Plasmodium falciparum* (1). Recently microsporidia were discovered in the hemolymph of *H. axyridis* and were shown to infect intra-guild predators. In this context, harmonine was postulated to protect the beetle against self-infection (2).

With continuing research more possible applications of harmonine are discovered raising the interest in easily accessible, synthetic harmonine and in convenient derivatives to design new lead compounds for medicinal use or industries. Therefore we developed a highly flexible synthesis providing access to the racemic form via reductive olefination of a macrocyclic lactone. This "one-pot Wittig-type" reaction yields the harmonine skeleton with an excellent cis/trans ratio (≥ 98/2). By enantioselective saponification of the lactone both enantiomers of harmonine become available in good yields.

Minor changes of the synthesis allow the generation of related derivatives to study structure-activity relationships. Initial biotests confirmed an altered activity profile for harmonine-derivatives.

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# Isolation of a behaviorally active sex pheromone component of the mushroom fly *Lycoriella ingenua*, using GC/EAG and an unusual, coupled GC/behavior technique

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*Lycoriella ingenua* (Dufour) (formerly known as *L. mali* Fitch) (Diptera: Sciaridae) is acknowledged as the major pest species of the white button mushroom, *Agaricus bisporus*, throughout the world. Control of *L. ingenua* primarily relies on applications of conventional synthetic pesticides. However, insecticide options are limited due to numerous reasons. It is therefore essential to develop alternative control strategies including the use of semiochemicals. Previous reports concerning the identification of a sex pheromone comprised of C15 – C18 saturated straight-chain hydrocarbons useful for trapping *L. mali*, with the major component declared to be heptadecane (Kostelc et al. 1980), have proven to be questionable (Gotoh et al. 1999). Thus, the purpose of our present study has been the collection of extract from unmated females and thereafter the isolation and identification of behaviorally active fractions of these extracts for evoking upwind flight in males. In behavioral assays using a Y-tube, our female extract evoked wing fanning, upwind flight toward the source, and abdomen curling in males. Further investigations using GC/EAD, plus especially a rarely used GC-coupled behavioral assay, resulted in a behaviorally active pheromone component being isolated and partially characterized via GC/MS. This component was found definitively to not be heptadecane or any of the other C15-C19 hydrocarbons previously erroneously identified (Kostelc et al. 1980). Once this compound is finished being fully chemically characterized by our laboratory, it will have potential utility for monitoring populations of *L. ingenua* and reducing their density and damage to the mushroom crop.

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# Mass spectrometry in chemical ecology: from characterization of oxylipins to their role in plant-microbes interactions

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Mass spectrometry is a powerful technique for both identification and quantification of bioactive molecules, particularly when they are present in small amounts and in complex samples. These features make mass spectrometry a useful tool in chemical ecology studies. In the present work, we used high throughput screening analysis in order to characterize and identify the antibiotic oxylipins present in the Brazilian medicinal plant *Alternanthera brasiliana*. A bioguided-assay fractionation and HPLC-HRMS analysis leaded to the identification of 17 antibiotic oxylipins (oxidized octadecanoic acids).1 These compounds are mainly identified in the *A. brasiliana* stem, which is also colonized by a large number of endophytic bacteria. These oxidized fatty acids are well known as stress response compounds to both physical damage and infection. Moreover, they have also been reported as bacterial metabolites.2 Therefore, in order to understand the role of these oxylipins in plant-microbes interaction we developed and validated an HPLC-MS/MS method to accurately quantify oxylipins in different plant tissues. The quantification of both fatty acids and bacterial cells strongly suggest that the higher is the concentration of bacterial cells higher is the amount of some oxidized fatty acids present in these tissues. We are now studying bacterial metabolism and performing re-inoculation experiments to understand the biosynthetic origin of such oxylipins. These results show the great potential of mass spectrometry to identify new compounds involved in ecological interactions and understand their ecological function.

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# Ecological chemistry of weaver ants – nutrient cycling and search for novel chemical characteristics

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The tropical weaver ants nest in the canopies of trees and have for centuries been used in pest control in tropical orchards where they protect several crops against more than 50 insect pests. Experiments have suggested that these ants not merely control insect pests by preying on them, but produce repellent semiochemicals (Offenberg 2014). The chemistry potentially underlying the repellence has hitherto not been investigated. In the literature limited knowledge on the chemistry and semiochemicals of the weaver ants was found. In ongoing experiments we therefore not only perform targeted investigations of potentially repellent semiochemicals, but also an extensive exploration of the chemistry of the weaver ants, which could potentially uncover important new chemical characteristics of these ants.

Field studies of weaver ant-hosting trees have also illustrated that the feces they generously deposit onto the leaves of their host trees supply nutrients. Nutrient cycling of especially nitrogen from other ant species to plants have previously been demonstrated. Our chemical analyses established that the feces from weaver ants contain valuable nitrogen nutrients such as amino acids and the known foliar nutrient urea. In an untargeted GC-TOF-MS metabolomics experiment greenhouse *C. arabica* plants hosting the Asian weaver ant *Oecophylla smaragdina* displayed metabolic changes analogous to plants supplied with increased levels of nitrogen (Vidkjær et al. 2015). The results additionally suggested that nitrogen nutrients were not only assimilated, but were also translocated, thereby instigating systemic effects in the plants. This was later confirmed by uptake and translocation of urea in biologically relevant concentrations in *C. arabica* plants.

It would greatly enhance the benefits of using weaver ants in pest control if these ants through their dietary intake of pests can transform these from a threat to crop health into high value nutrients and repellent semiochemicals via their metabolic machinery.

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# Synthesis and mass spectra of macrolides used in chemical communication systems

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Macrolides are used by various animals as pheromones, including beetles [1] , butterflies [2], and frogs [3]. Contrary to related open chain compounds, e. g. aliphatic esters, ketones, alcohols, alkanes etc., no straightforward interpretation of their mass spectra is possible, hindering their proper identification. The elucidation of structural features as the ring size, the location of methyl branches, or the position of double bonds from EI-mass spectra remains challenging. Identification still requires tedious trial and error, synthesis of putative target compounds and comparison with the natural sample. Isolation for NMR experiments is only possible in favorable cases. In the presentation we will discuss synthetic approaches to various macrolides used by hyperolid and mantellid frogs, as well as *Oryzaephilus* beetles. An economic approach is the synthesis of libraries of compounds, basing on potential biosynthetically likely compounds. These macrolide libraries allow systematic investigation of mass spectra of the macrolides hoping to decipher basic fragmentation rules. The current progress of this approach will be presented, leading to the identification of several macrolides not known from nature before.

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## Catch release and release – How to efficiently trap, analyze and formulate plant odors

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Volatile collection for chemical analysis of plant odor can be done in various ways. The volatiles can be collected in situ, i.e. in the field, or the plants can be brought to the lab. Either way, we try to do our aerations as similar as possible, without method bias. The plant material is enclosed in 'odorless' cooking bags, in order to minimize background contaminants. Charcoal filtered air is introduced at the bottom of the bag, passes over the plant and brings the released volatiles across the bag to the adsorbent column. The adsorbet, Porapak ® Q or Tenax ® GR, is packed in Teflon ® tubes (ID 3mm), and connected to small vacuum pumps. Only glass or Teflon inside the cooking bag, and silicon tubes only between the columns and the pumps. During field work we use 6V battery-operated membrane pumps, while in the lab, we use 12V membrane pumps with AC-adapters. The volume of the cooking bag is minimized, and the air flow through the column is around ten per cent of the enclosure volume per minute, to reduce the risk for break-through of the most volatile compounds during the aeration.

After elution of volatiles, and eventually concentration, the qualitative and quantitative analyses are made by combined gas chromatography and mass spectrometry (GC-MS). Not only the identification, but also the proportions of the collected volatiles are often necessary for optimal bioassay of a formulation.

So, how to formulate compounds with different volatility at constant proportions over a period of time, and especially so when a bait has to work for days or even a week? When compounds are put in a specific proportion on a static device, the most volatile compounds will diffuse first, leaving the less volatile ones to volatilize later, giving very biased mixtures over a period of time. The method we have successfully used for decades are cotton lined Teflon tubes: "wick-baits". The compounds to be tested are dissolved and homogenized in a 'carrier', i.e. an alkane; pentane, hexane, … decane: depending on for how long the bait is supposed to last. The 'wick' is inserted through the lid of a vial, and the 'carrier' delivers the compounds to the tip of the wick, where they volatilize. Depending on the alkane chosen and the temperature, the release rate can vary from mL/h to mL/week. With known evaporation rate of the 'carrier', the release rate of each compound to test can be set with high accuracy, by a certain concentration (i.e. ng/µL) even in complicated mixtures, or with the aeration extract, to certify that all active compounds were collected during the aeration.

# Spiros are everywhere

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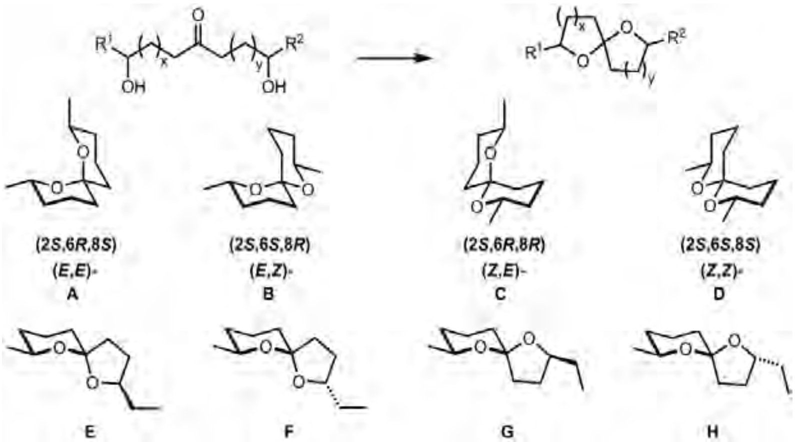
Dihydroxyketones (ketodiols), bearing the two hydroxy groups on either side of the carbonyl group may form bicyclic products (spiroacetals = spiroketals) upon intramolecular cyclisation (see sketch). Showing a multitude of biological activities, these compounds are widespread in nature and have been found in microorganisms, plants (tree odors, flower volatiles), and animals (insects, mammals). Depending on the number of methylene groups between the carbonyl carbon and alcohol functions in the acyclic precursors, the formed spiro compounds will show different ring sizes. Up to now, five different systems have been found in nature (see sketch: X and Y = 1,2,3): compounds showing two 5- to 7-membered rings and mixed structures. Apart from very few exceptions, the carbon skeletons of these volatiles are unbranched, indicating an origin from the acetate pool. Due to the two oxygens adjacent to the spiro center, the stability of the possible diastereomers is different, and therefore the natural products may show very different proportions. Compound A (s. sketch) usually co-occurs with B, C, and D, however, amounts of D do not exceed 1-2% of that of A. In contrast, E and F, which may be associated with A-D, usually form a ca 1:1-mixture. Reasons of this phenomenon will be discussed. Mass spectra of A-D look very similar, and so do those of spiros E-H (s. ref.) Knowing principles of the mass spectrometric fragmentation pattern of spiros strongly facilitates their identification by GC/MS.




W. Francke, W Kitching, 2001, Spiroacetals in insects. Current Organic Chemistry 5, 233-251

# Chemical components of urine mediate predator-prey interactions

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The Atlantic blue crab, *Callinectes sapidus*, is a top predator in the Southeastern US reef system and a primary predator of the Atlantic mud crab, *Panopeus herbstii*. Previous research has shown that the urine of blue crabs causes reduced foraging of mud crabs through non-consumptive predator-prey interactions. Non-consumptive effects and resulting trophic cascades are well established as a critical prey interaction in many ecosystems. Responses to chemical cues by prey have been shown to be dependent on predator diet in some cases and independent of diet in others, however, the identity of cues are unknown in most systems. Utilizing 1H NMR metabolomics of urine samples from blue crabs fed different diets, we showed that metabolite profiles are unique to each diet and distinguishable from one another. Utilizing partial least squares discriminant analysis (PSL-R) of spectral data in conjunction with a laboratory bioassay to detect behavior of mud crabs in response to chemical cues from blue crab urine, we have tentatively identified 10 metabolites from blue crab urine whose concentrations correlated with behavioral responses in mud crabs. We are currently confirming the molecular structures of these metabolites and measuring their deterrent abilities as individual compounds as well as the deterrent ability of a complex mixture as components of the natural cue in blue crab urine. Further analysis of urine samples, including MS- based metabolomics, will help identify minor components in blue crab urine. Ultimately, we aim to understand the role of chemical signaling in predator-prey interactions and community structure of the oyster reef habitat.

# Identification of the sex pheromone in three heliozeliid leafminer species infesting grapevine in South Africa and Italy

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Leafmining insects from the moth family Heliozelidae may cause severe damage to their host plant belonging to the grape family Vitaceae, thus seriously affecting grape production (Duso et al., 2011). Various pesticides have been used over the past decades in grape production, resulting in residues contaminating grapes and eventually migrating into the wine. Due to the increasing public concern over the table grape and wine safety, the use of pesticides is not recommended, especially in the period around harvest when the leafminer may be highly abundant. Sex pheromones may serve as the foremost alternative to traditional chemical pesticides for pest control. However, pheromones have not been identified so far for heliozeliid moths, partly due to the challenge of collecting the airborne volatiles from these tiny moths. Here we report the sex pheromones from three heliozeliid species, *Holocacista capensis*, a newly identified species that recently infested grapevine in South Africa (van Nieukerken and Geertsema, 2015), and *Antispila oinophylla* and *Holocacista rivillei* that infest Italian grapevine severely. The female-produced pheromones were collected by solid phase micro extraction (SPME) and analyzed by gas chromatography with electroantennographic detection (GC-EAD). The compounds eliciting electrophysiological activity from the male antenna were subsequently identified by coupled gas chromatography-mass spectrometry (GC-MS) analysis. Field trapping experiments in a South African and Italian vineyards confirmed that both (*5Z*)-5-tetradecenal and (*7Z*)-7-tetradecenal are essential for the attraction of male *H. capensis* and *A. oinophylla* but in different blend ratios, whereas (*5Z*)-5-dodecenal and (*7Z*)-7-tetradecenal are essential for the attraction of male *H. rivillei*. The identification of the Type I pheromones, i.e. structurally belonging to long-chain fatty alcohols and corresponding aldehydes and acetates, from this relatively basal moth lineage provides further insight into the evolutionary history of lepidopteran pheromones.

Duso C, Pozzebon A, Baldessari M, Angeli G (2011) Current status of grapevine leafminers in North-eastern Italy. IOBC/wprs Bulletin 67:203-206.
Nieukerken EJ van, Geertsema H (2015) A new leafminer on grapevine and *Rhoicissus* (Vitaceae) in South Africa within an expanded generic concept of *Holocacista* (Insecta, Lepidoptera: Heliozelidae). ZooKeys (submitted).

# A sex-inducing pheromone triggers cell cycle arrest and pheromone production in *Seminavis robusta*

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Diatoms shape the marine environment as major primary producers and are the basis of aquatic food webs. The chemical cues that mediate their life cycle and mating are poorly understood. A first attraction pheromone was identified in the pennate diatom Seminavis robusta. This proline derived diketopiperazine L-diproline mediates the chemoattraction of the mating partners [1]. However, it is obvious that it is not the only signaling molecule of relevance in sexual reproduction.

Here we introduce further investigations on the pheromone system that regulates mating in *S. robusta*. We found signaling molecules that induce a cell-cycle arrest in the pairing cells and the production and perception of the attraction pheromone. The identification of these sex-inducing pheromones was done using a metabolomics approach. The exometabolomes of the two mating types of *S. robusta* were analyzed by LC-MS and upon comparison of their metabolic profiles candidate molecules were found and verified in bioassays. A sulfated, polyhydroxylated compound was identified as the L-diproline inducing pheromone underlining a cost-efficient, multistep pheromone system in this benthic diatom.

[1] J. Gillard et al., Angew. Chem. Int. Ed. 2013, 52 (3), 854-857

# The perfume of termite queens: quest for termite queen pheromones

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The way how termites perceive the world and communicate is not based on visual signals but on sounds, vibrations and predominantly on chemical signals, the pheromones. After many years of studies on these ‘dwellers in dark‘, the knowledge of central molecules signaling the presence of reproductives - kings and queens - and ensuring their dominance in the colony is still very poor. Up to now, a single queen pheromone has been identified in a single species out of almost 3000 known species.

Since our research aims at the most advanced termite lineage, the higher termites, we tried to consider the hypothesis on the presence of a volatile fertility signal produced by the queens in the populous colonies of these species. Using GC×GC/MS we identified the sesquiterpene alcohol, (*E*)-nerolidol to be the queen-specific volatile in body washes and headspace of the queens of the neotropical termite species *Embiratermes neotenicus* (Termitidae: Syntermitinae). The same compound was also found on the surface of eggs laid by the queens. We further observed an increase of the production of the compound with age and with the fertility of queens. Interestingly, the same compound was detected in three other related species from the subfamily Syntermitinae (*Silvestritermes holmgreni*, *Labiotermes labralis*, and *Cyrriliotermes angulariceps*).

To conclude, we have in hand a compound emitted specifically by the queens, which is a candidate for the queen fertility signal. Since many different functions are attributed to these fertility signals, the ultimate goal of our research is to unravel the real biological role of nerolidol in E. neoternicus and related species.

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Matsuura et al. 2010. Proc. Natl Acad. Sci. USA 107: 12963-12968.

# Emerging role of phenolamides as universal plant defense metabolites

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Coevolution of plants and insects has contributed largely to the presently existing diversity of natural products in plants. Many of these metabolites are known to accumulate in response to wounding, insect feeding or both stimuli. Previously, we found a strong accumulation and defense function of two phenolamides, caffeoylputrescine and dicaffeoylspermidine, against Lepidopteran larvae in native tobacco *Nicotiana attenuata* plants. To investigate potential function of phenolamides in monocot plants, we now examined the spectrum of phenolamides in cultivated rice (*Oryza sativa*). Rice seedlings accumulated two major phenolamides, *p*-coumaroylputrescine (CoP) and feruloylputrescine (FP) in response to herbivore feeding. Interestingly, both chewing and sucking herbivores from different feeding guilds, together with mechanical wounding, could significantly increase the content of CoP and FP in challenged leaves. With independently demonstrated broad function and/or associations of phenolamides with microbial disease resistance, we propose that these compounds may serve multiple as well as unique protective roles in plants against biotic stressor that co-exist and simultaneously attack plants in the natural environment.

# High performance separation and mass spectrometry for chemical ecology research

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High performance separation and mass spectrometric techniques are vital in many biological, biomedical, and environmental research areas. With the aid of optimized methods new routes could also be taken in ecological chemistry. Here, a few examples are given, where analytical schemes have been utilized in order to gather new information about and knowledge of samples from the flora and fauna. Alternatives to control insect attacks in the forests are needed and the biodegradable substance methyl jasmonate is known to induce tree defence against the pine weevil. Therefore, there is a need for analytical methods to study the content in the conifer bark of e.g. amino acids. Thus, a capillary electrophoresis method was developed for analysis of bark from untreated plants and plants treated with methyl jasmonate. Liquid-liquid extraction was used and indirect UV detection simplified the procedure. In a parallel project single wood cells were analyzed regarding the composition of monosaccharides. Preconcentration performed in microchannels on a silicon chip was utilized to render the low amounts of analytes detectable. This pretreatment was directly interfaceced with capillary electrophoretic separation and indirect UV detection. Capillary electrophoresis is a technique that offers efficient separation with very low sample volume requirements. The technique has hence also been used for analysis of samples obtained from the small spermatophores transfered from male to female butterflies during mating. The reproduction proteins that are among the spermatophore content arouse a lot of interest due to effects on remating, egg laying, female life span etc. The proteins were extracted with different solvents and separated with capillary electrophoresis. To acquire more information about the proteins capillary electrophoresis was coupled to matrix assisted laser desorption/ionization mass spectrometry (MALDI-MS).This makes possibles determination of molecular weight of the separated peptides and proteins and by using tandem mass spectrometry information of molecular sequence could also be obtained. Performing miniaturized on-target tryptic digest could contribute to further knowledge about the substances. Above, some examples of specific analytical schemes have been given. However, the procedures could be optimized for a wide variety of samples and analytes especially when sensitive and selective analysis of minor samples is demanded.

# Stereoisomeric libraries for pheromone identifications: 1-Bisabolen-3-ols

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The detailed stereoisomeric structures of many natural sesquiterpenes with the bisabolane skeleton were previously unknown because of the absence of stereoselective syntheses of individual stereoisomers. We developed a novel and straightforward route to all stereoisomers of 1,10-bisaboladien-3-ol (total eight) and 10,11-epoxy-1-bisabolen-3-ol (total sixteen) via the rhodium-catalyzed asymmetric addition of trimethylaluminum to diastereomeric mixtures of cyclohex-2-enones. Preparation of these complete libraries became pivotal for the identification of the aggregation pheromones of two economically important pentatomid bugs, the brown marmorated stink bug, *Halyomorpha halys*, and the harlequin bug, *Murgantia histrionica*. While these bugs share (*3S,6S,7R,10S*)-10,11-epoxy-1-bisabolen-3-ol as their major pheromone component, the minor components, needed for maximum attractiveness, were different: (*3R,6S,7R,10S*)-10,11-epoxy-1-bisabolen-3-ol for the *H. halys* and (*3S,6S,7R,10R*)-10,11-epoxy-1-bisabolen-3-ol for *M. histrionica*.

We also found that the natural zingiberenol isolated from ginger oil has (3R,6R,7S) absolute configuration, identical with the zingiberenol identified as a sex pheromone of the rice stink bug, *Oebalus poecilus*. We will discuss syntheses, assignments of relative and absolute configurations using single-crystal X-ray crystallography, as well as pheromone identifications aided by a gas-chromatography on enantioselective columns.

The three 10,11-epoxy-1-bisabolen-3-ols have proven useful for field attraction and trapping of *H. halys* and *M. histrionica*, and will serve as important tools for semiochemical management of these major pests.

# CHEMICAL NEUROECOLOGY

## Chemosensation between environment and internal state

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When interacting with their environment animals constantly have to make decisions. These decisions usually aim at maximizing reward while avoiding negative consequences such as energy costs, pain, or long-term disadvantages. Faced with a choice animals consider and integrate several parameters such as their internal state as well as other external stimuli. Therefore, even innate preferences need to be evaluated in a context-dependent manner and hence, context strongly impinges on behavior. While it is generally accepted, that context influences behavior our knowledge of the neural mechanisms of how internal state and external conditions alter behavioral outcomes is scarce. We are interested in understanding how context influences neural processing and behavior to odors and tastes. To this aim, my lab combines behavioral analysis, *in vivo* functional imaging, optogenetics, and electrophysiology with state of the art genetic methods to understand how context influences neural processing and ultimately behavior. At this symposium, I will present recent projects of the lab.

# Functional contributions of olfactory receptor neurons in *Drosophila melanogaster* larva

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The ability of an animal to detect, discriminate, and respond to odors depends on the function of its olfactory receptor neurons (ORNs). The *Drosophila melanogaster* larva contains 21 ORNs, most expressing a single odor receptor (Or). An intriguing question in the field concerns the roles of different ORNs and receptors in driving behavior. Preliminary evidence indicates that each ORN contributes differently to the olfactory circuit. We hypothesize that during larval olfactory response, each discrete behavioral element, including runs, pauses, turns, and head swings, is encoded in the activities of individual ORNs. To investigate the contributions of individual ORNs to the olfactory circuit, we used molecular genetic approaches in combination with strong, specific activation of individual ORNs in the larva. Behavioral responses of the larva upon ORN activation was characterized. Our results suggest that larval ORNs do not belong to a single functional class; strong activation of some ORNs elicited strong behavioral responses while strong activation of others elicited weak behavioral responses. Activity of individual ORNs affect different subsets of discrete behavioral elements during larval behavioral response. ORN contributions to behavior are affected not only by the identity of the odor receptor expressed but also by the temporal dynamics of ORN response. Differences among ORN contributions adds a layer of complexity to the mechanism for translating different signals into different behavioral responses. Our analysis of olfactory function at the front end of a simple olfactory circuit may be useful in elucidating the circuitry and principle by which such translation occurs.

# Insect odorant receptors: The state of play on how these novel integral membrane proteins mediate odour detection in insects

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Insects have co-opted a unique family of seven-transmembrane proteins for odour sensing. Odorant receptors evolved from gustatory receptors somewhere at the base of the hexapoda and have expanded substantially to become the dominant class of odour recognition elements within the insects. These odorant receptors comprise an obligate co-receptor, Orco, and one of a family of highly divergent odorant "tuning" receptors. The two subunits are thought to come together at some as yet unknown stoichiometry to form a functional complex that is capable of both ionotropic and metabotropic signalling. While there are still no 3D structures for these proteins, site directed mutagenesis, resonance energy transfer, and structural modelling efforts, all mainly on *Drosophila* odorant receptors, are beginning to inform hypotheses of their structure and how such complexes function in odour detection. Some of the loops, especially the second extracellular loop that probably forms a lid over the binding pocket, and the extracellular regions of some transmembrane helices have been implicated in ligand recognition in tuning receptors. The interaction between Orco and tuning receptor subunits through the final intracellular loop and the adjacent transmembrane domains is thought to be important for transducing ligand binding into receptor activation. Phosphorylation sites and a calmodulin binding site in the second intracellular loop of Orco are also thought to be involved in regulating gating. A number of new methods have recently been developed to express and purify insect odorant receptor subunits in recombinant expression systems. These approaches are enabling high throughput screening of receptors for agonists and antagonists in cell-based formats, as well as producing protein for the application of biophysical methods to resolve the structures of the subunits and their complexes.

## Escaping death by sensing your enemy's sex odor

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Detecting danger is one of the foremost tasks for a neural system. Larval parasitoids constitute a clear and present danger to *Drosophila* as up to 80% of fly larvae become parasitized in nature. We show that *Drosophila melanogaster* larvae and adults avoid sites smelling of the main parasitoid enemies, *Leptopilina* wasps. This avoidance is mediated via a highly specific olfactory sensory neuron (OSN) type. While the larval OSN expresses the olfactory receptor Or49a and is tuned to the *Leptopilina* sex pheromone iridomyrmecin, the adult expresses both Or49a and Or85f and detects the wasp odors iridomyrmecin, actinidine and nepetalactol. The information is transferred via projection neurons to a specific part of the lateral horn known to be involved in mediating avoidance. *Drosophila* has thus developed a dedicated circuit to detect and avoid an enemy based on the smell of its sex pheromone. Such a danger-detecting olfactory circuit has earlier only been demonstrated in the mouse.

NEU05

# Elucidating the neuronal architecture of olfactory glomeruli in the Drosophila antennal lobe

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Species of quite diverging animal phyla with an advanced olfactory system share an important similarity, which is the presence of olfactory glomeruli. During the last decades the wiring properties of these spherical compartments has been elucidated in great detail while only little is known about the numerical neuronal composition of individual glomeruli. The lack of exact numbers leads to a common basic assumption of glomerular uniformity, although different glomeruli do not accomplish a uniform function. In order to scrutinize whether each glomerulus possesses a unique neuronal architecture or whether glomeruli are uniform structural units, we characterized the detailed neuronal architecture of individual glomeruli and correlated these anatomical features with their functional properties in the model organism *Drosophila melanogaster*. We report a complete quantitative mapping of all receptor-specific sensory neurons that innervate a certain glomerulus, including sexually dimorphic distributions and glomerular volumes. Our data disprove the so far assumed universal 30:1 convergence and demonstrates for the first time the impact of OSN number on glomerular dimensions. Moreover, we show sex-specific differences in neuron number and glomerular volume also for fruitless negative glomeruli. In addition, we demonstrate a glomerulus-specific projection neuron innervation. Finally, we correlate these morphological features with functional properties and provide evidence for a unique neuronal architecture of glomeruli encoding behavioral relevant odors.

# A herbivore-induced volatile interferes with host plant and mate location in moths through jamming signaling pathways in the brain

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Plants under herbivore attack release volatiles that attract natural enemies, and herbivores in turn avoid such plants. Which volatiles herbivores use and how deterrence is coded in the olfactory system are largely unknown. Here we demonstrate that herbivore-induced cotton volatiles suppress orientation of the moth *Spodoptera littoralis* to host plants and mates. We found that (*E*)-4,8-dimethyl-1,3,7-nonatriene (DMNT), an induced volatile, is key in herbivore deterrence: DMNT suppressed plant odor- and pheromone-induced behaviors. We then dissected the neurophysiological basis of this interaction. This revealed that moths possess no DMNT-specific sensory channel. Instead, DMNT suppressed responses to pheromone and (*Z*)-3-hexenyl acetate, a host-plant attractant. Olfactory sensory inhibition, which has previously been reported without reference to an animal's ecology, can be at the core of coding of ecologically relevant odors. As DMNT attracts natural enemies and deters herbivores, it may be useful in the development or enhancement of push-pull strategies for sustainable agriculture.

## Starvation modulates coding of pheromone and food signals in *Drosophila*

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Modulating behaviour according to physiological requirements is crucial for animal survival and reproduction. In the fruit fly *Drosophila melanogaster*, the male-produced pheromone *cis*-vaccenyl acetate (cVA) regulates many aspects of social and sexual behaviour. Here we show that starvation differently affects cVA perception in male and female flies. While males become attracted to cVA when starved, females are only attracted when fed. This sexual dimorphic effect of starvation on behaviour is correlated with the activity in the fly antennal lobes (ALs), the first olfactory centre in the insect brain. Starvation increases sensitivity to food odours (vinegar) in both males and females. In contrast, using functional imaging we could show that starvation reduces the response to cVA in the ALs of females. The effect of feeding on female attraction to cVA is mediated by the insulin-signalling pathway within olfactory neurons converging onto specific glomeruli in the ALs, including the glomerulus innervated by cVA receptive OSNs, DA1, as well as the glomerulus VM2 which responds to vinegar. Female sexual receptivity is also dramatically affected by starvation. However, this effect is independent from the insulin-signalling pathway. This suggests that starvation regulates pheromone perception and sexual receptivity through different mechanisms. In conclusion, insulin signalling controls pheromone perception in *Drosophila* females to match their sexual receptivity in response to changes in food availability.

# Atomic force microscopy of moth trichoid sensilla across species reveals common themes and specializations for odorant capture

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The functional olfactory unit on the antennae of male moths for adsorbing and reporting the abundance of sex pheromone molecules is the trichoid sensillum. The surface of each of these sensilla is festooned with hundreds of pores that facilitate entry of pheromone molecules into the sensillar lumen. If the pores do not allow pheromone molecules to enter the lumen, then the dendrites of olfactory sensory neurons cannot receive molecules and nothing else neurophysiologically can happen. Thus, understanding how these pores and their associated ridges work is important to understanding pheromone olfaction. We used two new kinds of Atomic Force Microscopy, PeakForce Tapping mode and Kelvin Probe Force Microscopy (PF-KPFM) to characterize the topographic and chemical features of the trichoid sensilla of males of *Manduca sexta*, *Lymantria dispar*, *Helicoverpa zea*, and the "*E*" and "*Z*" pheromonal strains of *Ostrinia nubilalis*. PeakForce Tapping mode allowed us to collect information on the topography as well as changes in adhesion, deformation, and modulus ("sponginess") across the sensillar surfaces of these species. Our recordings showed details regarding the adhesion and deformation related to these features for all four species, giving us information on the surface-lipid adhesive forces and hence their relative hydrophilic vs. lipophilic chemistries. The pores and the ridge structures were always intimately associated in a stereotypical way across species, and thus the ridges must play an important role in odorant capture and transport to the pores. The pores of all four species were found to be more hydrophilic than the surrounding surfaces. We used PF-KPFM to collect information on topography and surface potential, and found how surface potential changes significantly across pores compared to the rest of the sensillar surfaces. This change in potential may somehow be related to the transport of pheromone molecules into the sensillum lumen.

# The physiological and ecological significance of olfactory sensory neuron co-localization

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Insect olfactory sensory neurons (OSN) are stereotypically grouped into sensilla located on the chemosensory organs. The functional significance of OSN co-localization is poorly understood, but two hypotheses have been proposed. First, co-localization might allow for signal modulation in the periphery via cross talk between OSNs sharing the same extracellular environment. Secondly, co-localization allows for coincidence detection of odour filaments, which should improve the discrimination of closely separated odour sources. We performed electrophysiological studies and field experiments on the European spruce bark beetle (*Ips typographus*), and found support for both hypotheses. In this species, the aggregation pheromone component *cis*-verbenol is detected by an OSN that is co-localized with an OSN detecting the host-derived behavioural antagonist 1,8-cineole. To test whether co-localization allows for signal modulation in the periphery, we studied the responses of these OSNs to binary mixtures of the compounds. In support of the hypothesis, the response of the *cis*-verbenol neuron was strongly inhibited by the simultaneous response of the 1,8-cineole neuron. To test whether co-localization also improves odour source discrimination, we designed a field trapping experiment to investigate the beetles' response to odour source spacing. We separated the aggregation pheromone source from the 1,8-cineole source (0-48 cm), and compared the catches with those obtained in response to the same spacing between the pheromone and verbenone – a behavioural antagonist detected by OSNs that are not co-localized with pheromone OSNs. Consistent with the hypothesis, trap catch increased with distance between odour sources more for 1,8-cineole than for verbenone. In summary, our results suggest that (I) co-localization of OSNs allows for peripheral signal modulation, and (II) it also improves the discrimination of closely separated odour sources. Thus, selection for peripheral mixture processing and improved odour source discrimination ability could both underlie the strict co-localization of OSNs that is seen across the Insecta.

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# Neuropeptide control of pheromone detection in a gustatory neural circuit

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Gustatory cues are one of the primary sources of information that shape an organism's behavior. However, little is known about the processing of taste in higher brain centers. This work describes a peptide-modulated gustatory circuit in *Drosophila* that underlies the programmed behavioral response to the anti-aphrodisiac sex pheromone, (*3R*,*11Z*,*19Z*)-3-acetoxy-11,19-octacosadien-1-ol (CH503). Using behavioral analysis, genetic manipulation, and live calcium imaging, we show that a population of sexually-dimorphic gustatory neurons on the forelegs of male flies responds physiologically to the pheromone. Information from the forelegs is relayed to the central brain via neuropeptidergic pathways. The release of the neuropeptide tachykinin from 4 cells residing within the protocerebrum and 10 cells within the subesophageal zone is required for the courtship suppression behavioral response. Since tachykinin has also been shown to regulate metabolism and aggression, this central peptide circuit could serve as a nexus for balancing opposing physiological drives with the decision to initiate and sustain courtship.

# Whole transcriptome analysis of chemosensory receptor expression in the codling moth, *Cydia pomonella*.

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The sense of smell, or olfaction, plays a critically important role in the life history of insects. Vital behaviours, such as food and mate seeking as well as predator and parasitoid avoidance, are strongly guided by olfactory cues detected from an insects' environment. Owing to the importance of this chemical sensory system, decades of research on insect olfaction has facilitated novel pest control strategies aimed at reducing the damage of economically and medically important insect pests, including the Codling moth, *Cydia pomonella*, which is a leading world wide pest of apples and other pome fruits.

In order to better understand the chemical ecology of *C. pomonella*, we have produced and analyzed an Illumina-based RNA-Seq transcriptome derived from the antenna of male and female adult moths and also neonate larval head tissue of *C. pomonella*. In our first analysis of this transcriptome, we report here a more comprehensive repertoire of chemosensory receptor genes from the odorant receptor (OR), gustatory receptor (GR) and ionotropic receptor (IR) gene families. Importantly we have identified several OR genes that display sex specific expression in either males or females. Also, we have identified larval specific, or enriched genes from the OR and GR families that may be ecologically relevant to unique aspects of olfactory-guided neonate larval behavior.

# Pheromone reception in the lightbrown apple moth, *Epiphyas postvittana*

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Pheromone receptors (PRs) have been identified from several moth species and functionally characterized in vitro through expression in *Xenopus laevis* oocytes and Human Embryonic Kidney (HEK) 293 cells. These studies have shown that some PRs are relatively specific for certain compounds while others are more broadly tuned to several pheromone compounds, including both behavioral agonists and antagonists. Here, we express previously identified candidate PRs from the pest moth *Epiphyas postvittana* in HEK293 cells along with EposOrco and EposSNMP1 and test for responsiveness to a panel of 62 pheromone-related compounds. We found that EposOR1, EposOR6 and EposOR45 all respond to *E. postvittana* pheromone components and to pheromone compounds used by other moths, some of which have known function as behavioral antagonists in this species. None of the EposORs tested in these experiments showed responses to *E. postvittana* pheromone components alone. Responses obtained for EposOR1 and EposOR6 to pheromones in HEK293 cells were confirmed through expression and functional testing in *Xenopus* oocytes. Finally, we show that activation of EposOR6 with the major pheromone component, E11-14:OAc, or the behavioral antagonist, Z11-14:OAc, prevents subsequent re-activation by either compound in this cell-based system, implying that a pheromone-degrading enzyme may be required to reset the system. These results lead us to propose that in *E. postvittana* behavioral agonism and antagonism may be mediated through a peripheral molecular mechanism.

# Transcriptomics of olfactory communication mediated by male sex pheromone in a butterfly

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Sex pheromones were recently shown to play a role in mate choice in the model butterfly *Bicyclus anynana* (1-2) and in reproductive isolation across the *Bicyclus* genus (3). Here we build on knowledge on the molecular bases of olfaction acquired for moths, and investigate the whole sequence of steps involved in sex pheromone communication in a butterfly. We 454-sequenced the transcriptome associated to i) olfactory perception, ii) sex pheromone biosynthesis, iii) regulation of sex pheromone biosynthesis, as well as iv) development of androconia, the sex pheromone-producing wing structures. We could not only identify which genes are expressed in association with different steps and tissues, but also compare levels of expression between sexes and/or tissue sections. We followed this up with a more detailed characterization of the expression (qPCR) and phylogenetic analysis (comparison to data available for moths and butterflies) of selected candidate genes involved in sex pheromone synthesis (by males) and reception (by females). We assess the conservatism of sex pheromone communication between moths and a first butterfly.

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# Molecular cloning and RNAi demonstration of olfactory co-receptor gene from two palm weevils species

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The palm weevil genus, *Rhynchophorus*, known as one of the most damaging invasive insect species in the world. The red palm weevil, *Rhynchophorus ferrugineus*, and Asian palm weevil, *R. vulneratus*, historically had a taxonomic status dispute due to the lack of differences in the response to aggregation pheromone (4-methyl-5-nonanol and 4-methyl-5-nonanone). However, recent studies confirmed both as distinct species. *R. ferrugineus* and *R. vulneratus* are distributed in Saudi Arabia and Indonesia respectively as well as having host plant differences, date palm and toddy palm respectively. Disrupting palm weevil olfactory system is a promising approach to control this pest, especially by targeting an expression of an olfactory coreceptor (Orco) gene. Here, we reported the cloning and sequencing of Orco genes from *R. ferrugineus* (RferOrco) and *R. vulneratus* (RvulOrco) and validation of RNAi technique to study the knockdown of RferOrco. RferOrco gene consists of 1961 bp full length nucleotides with an open reading frame (ORF) encodes 482 amino acids showing 99.17 % identity with RvulOrco. However, less similarity was found in the untranslated region (UTR), especially in the 5 end region. Phylogenetic tree located both RferOrco and RvulOrco in the same clade with other coleopterans, and tissue specificity study showed that Orco genes expressed only in the antenna of both male and female. Injection of RferOrco double–stranded RNA (dsRNA) on the first dorsal abdominal segment of late *R. ferrugineus* pupae showed a significant decrease of RferOrco transcripts in the adult antennae compared to the control and untreated one. Further validation and study is in progress that lead us to know whether Orco gene knockdown strategy might become an efficient technique for palm weevil management.

# Functional characterization of a receptor for a Type II sex pheromone in the winter moth, *Operophtera brumata* (Lepidoptera: Geometridae)

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Female moths produce two major types of species-specific sex pheromones along distinct biosynthetic pathways. Type I pheromones consist of C10-C18 straight-chain alcohols, acetates and aldehydes, whereas Type II pheromones are C17-C23 straight-chain polyenes and corresponding epoxymonoenes or epoxydienes (Millar, 2000; Ando et al., 2004). Female released pheromone signals are detected by the distinct receptors hosted in the antennal olfactory sensory neurons of conspecific males. To date, all functionally characterized pheromone receptors are tuned to Type I pheromones, and it is unknown how receptors matching Type II pheromones evolved. Here we report the identification of the first receptor, namely ObruOR1 tuned to a Type II pheromone, (*1,3Z,6Z,9Z*)-nonadecatetraene from the winter moth, Operophtera brumata. Similar to previously identified receptors for Type I pheromones, ObruOR1 is found in neurons housed in antennal sensilla trichodea and is expressed in a male-biased pattern. Phylogenetic analysis shows that ObruOR1 nests within the subfamily of receptors for Type I pheromones, however in a particular cluster containing a number of ligand-unknown orthologues. The strong purifying selection on this cluster suggests a conserved polyene-responding profile, which is supported in the present study by the response of a noctuid orthologous gene to a selection of polyenes. Our results suggest that moths did not evolve an entirely new type of receptors, but recruited a cluster from the existing pheromone receptor gene tree to match the novel Type II pheromone compounds.

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# Carbon dioxide receptor involved in the perception and production of the sex pheromone

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The effect of increased carbon dioxide ($CO_2$) concentration in atmosphere was examined on the pheromone system of *Helicoverpa armigera*. $CO_2$ gas was treated at three identical rearing room (2×2×2 m) where the $CO_2$ concentration was 450 ppm (Control), 601 ppm (600 ppm), 971 ppm (1000 ppm), respectively.

Elevated $CO_2$ affected the EAG response decreased and lowered attractancy to sex pheromone lure at 1000 ppm in adult males. While the sex pheromone production increased in female adult. We hypothesized that $CO_2$ receptor in labial palp is involved in those processes and examined on the EAG response and attractancy of the male and the sex pheromone production of the female with/without $CO_2$ receptor. EAG responses of normal head were coincided with the antennal responses at all different $CO_2$ levels, while the responses of head without $CO_2$ receptor were apart from the antennal response. The attractancy of the males without $CO_2$ receptor was lowered than that of the normal males in wind tunnel. Therefore, $CO_2$ receptor is involved in the process in the perception of sex pheromone by tuning the signal from antenna at different $CO_2$ environments. Sex pheromone production of the female without $CO_2$ receptor decreased and showed an altered diel rhythm particularly in the low $CO_2$ levels. we speculate that $CO_2$ receptor is involved in controling a valve for the emission of PBAN from SOG.

# PHEROMONE COMMUNICATION

# Decoding evolution of pheromone communication in *Heliothine* moths

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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 genomic tools to characterize odorant receptor shifts associated with communication, we are developing a comprehensive comparative database of olfaction for this group. The long term goal of this study will be the establishment of a global network of collaborators using heliothines as a model system to correlate shifts in communication manifested by differential olfactory receptor (OR) expression with 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 behavioral pheromone preference and physiological processing of these odorants as these species diverge.

# Aggregation in entomopathogenic nematodes

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Self-organized aggregation, observed across trophic levels and environments, is facilitated by conspecific recognition and communication that can be mediated by chemical cues. We investigate the potential chemical basis for aggregation in the entomopathogenic nematodes *Steinernema diaprepesi* and *Heterorhabditis indica*. In sand-filled six-arm olfactometers, S. *diaprepesi* aggregates in the presence of pregeijerene, an herbivore-induced plant volatile that is a known attractant, but not in its absence. In trials in the presence of pregeijerene, nematodes responded in greater numbers to and aggregated more in sand moistened with water taken from nematode cultures (aqueous culture media) than in sand moistened with plain water. In the absence of pregeijerene, nematodes responded in slightly greater numbers to but did not aggregate to the same degree in sand moistened with aqueous culture media. Additionally when added sequentially to olfactometers over the course of 48 hours, *H. indica* presence was highly correlated with *S. diaprepesi* aggregation; *H. indica* tend to follow *S. diaprepesi.* These results suggest that aggregation in entomopathogenic nematodes is both chemically mediated and that the chemicals mediating such aggregation may be shared across species.

# Chemical ecology of fruit flies provided primary impetus in successful synonymization of four major pest species in the *Bactrocera dorsalis* complex

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A significant breakthrough was recently achieved when four highly invasive fruit fly pest species - the Oriental fruit fly (*Bactrocera dorsalis*), African invasive fruit fly (*B. invadens*), Asian fruit fly (*B. papayae*) and the Philippine fruit fly (*B. philippinensis*) was recognized as belonging to the same biological species *B. dorsalis*. This success is particularly important to many countries in Asia and Africa that are potential exporters of fresh fruit and vegetable commodities but have been subjected to severe quarantine restrictions due to having the presence of one more of those pest species. Through lifting of those restrictions, this is expected to notably facilitate global agricultural trade amongst those affected countries. At the heart of this synonymization success is the role of chemical ecology as an integral impetus leading to international efforts in resolving the longstanding controversy over the correct identification of those former putative species. We have shown that following consumption of methyl eugenol (ME), a common phenylpropanoid found in over 450 plant species, males of *B. dorsalis*, *B. invadens*, *B. papayae* and *B. philippinensis* produced identical sex pheromone components - (E)-coniferyl alcohol and 2-allyl-4,5-dimethoxyphenol that are sequestered into the rectal gland for storage prior to emission during courtship and mating at dusk. This paper highlights how in answering a simple question of why male Oriental fruit flies are so uniquely attracted to and compulsively feed on ME had led to a FAO/IAEA-sponsored coordinated research project involving almost 50 researchers from over 20 countries in resolving the confusion over the identities of those four pest taxa, originally distinguished based on genitalia length and/or scutum color, to a single biological species.

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# The genetic basis underlying sex pheromone variation in moths

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Evolutionary diversification of sexual communication systems in moths is difficult to understand, because signal and response seem to be under stabilizing selection in many species, which is expected to constrain evolutionary change. To unravel this evolutionary mystery, we take a genetic approach to identify the gene(s) underlying inter- and intraspecific variation in signal and response. This has been a successful approach in *Ostrinia nubilalis*, where we identified the gene underlying the variation in the Z and E pheromone blend (Lassance et al. 2010), while for the male response we narrowed the location to two candidate genes (Koutroumpa et al. 2015 in prep; Unbehend et al. 2015 in prep). In the noctuid moth *Heliothis virescens* we found consistent variation in the female signal in every population sampled. After selecting on the extreme phenotypes, we genetically analyzed this variation and found one major locus and one candidate gene to be responsible (Groot et al. 2014). We now found the exact mutation in this candidate gene: a stop codon at the start of the coding sequence (Groot et al. 2015 in prep). In males, this mutation does not seem to have an effect on their physiological response, but it does seem to affect their behavioral response. This stop codon does not seem to be present in all populations sampled, but this gene is highly variable in its intronic regions, which is also correlated to the phenotype variation and likely affect its expression levels. Our combined analysis is thus a successful approach to identify the genetic basis of pheromone diversification and variation, with which their evolutionary trajectory can be unraveled.

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Lassance et al. 2010. Nature 466

# Biosynthesis of isoprenoids in the male marking pheromone of bumblebees: Through regulation of gene expression to speciation?

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*Bombus terrestris* and *Bombus lucorum* are important agricultural pollinators. These bumblebee species are closely related, but they employ very diverse pheromone bled for sexual communication. While *B. terrestris* males attract females mainly by terpenic compounds such as 2,3-dihydrofarnesol and geranylcitronellol, *B. lucorum* males use fatty acid derivatives as marking pheromone. To define possible regulatory mechanisms determining species-specific composition of pheromone, we investigated the isoprenoid biosynthesis in the labial gland (LG) of both bumblebee species. The RNA seq quantification and qPCR analysis of mevalonate and isoprenoid gene expression levels in LG of both species indicated a significantly lower expression level of all genes from isoprenoid biosynthesis in *B. lucorum*. The highest down regulation of gene expression in *B. lucorum* was observed for HMG-CoA reductase (HMGR), acetoacetyl-CoA thiolase (AACT), and farnesyl diphosphate synthase genes (FPPS). Maximum of AACT, HMGR, and FPPS gene expression was observed in three days old male LG of *B. terrestris*. Detection of AACT activity in LG of both species confirmed differences at the protein activity level in LG. In addition, incubation of labelled acetate with dissected LGs detected biosynthesis of terpenic compounds in *B. terrestris* only. Thus, the lack of terpenoid components in *B. lucorum* pheromone blend is a result of significant transcription down-regulation of key metabolic enzymes of mevalonate and isoprenoid biosynthesis in the male labial gland, which perhaps contributed to speciation in the subgenus *Bombus* s.str.

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## Male butterflies use an anti-aphrodisiac pheromone to tailor ejaculates

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When females mate with multiple partners, the risk of sperm competition depends on female mating history. To maximize fitness, males should adjust their mating investment according to this risk. In polyandrous butterflies, such as *Pieris napi*, males transfer a large, nutritious ejaculate at mating that also contains an anti-aphrodisiac that deters subsequent males. Larger ejaculates confer an advantage in sperm competition but is very costly to the male.

We tested if male ejaculate size in *Pieris napi* varies with female mating history and thus sperm competition, and whether males assess sperm competition intensity using the male-transferred anti-aphrodisiac methyl salicylate (MeS) as a cue.

First we examined olfactory sensitivity to MeS. Both sexes responded in a dose-dependent manner. Males; however, were an order of magnitude more sensitive to MeS than females.

Secondly, ejaculates transferred by males mating with previously mated females were on average 26% larger than ejaculates transferred by males mating with virgin females, which conforms to sperm competition theory and indicates that males tailored their reproductive investment in response to sperm competition. Furthermore, ejaculates transferred by males mating with virgin females with artificially added MeS were also 26% larger than ejaculates transferred to control virgin females.

In conclusion; Male-transferred anti-aphrodisiac pheromone not only functions as a male deterrent, but also carries information on female mating history and thus allows males to assess sperm competition intensity.

PHE07

# Quality control: honeybee workers assess queens by pheromonal and genetic correlates of quality

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One of the theories explaining worker sterility in social insects is worker self-restraint, which supposedly must be advantageous in terms of inclusive fitness. Therefore the most important condition underlying a worker's decision to forego reproduction is her ability to assess the quality of the queen – the individual to whom reproduction is conceded. Our study intended to test such ability in honeybee workers. We exposed groups of worker to pairs of queens, separated by a queen excluder, which differed in reproductive status and examined their attitude to each queen and measured presumed correlates of each queen quality, i.e., reproductive, pheromonal and genetic properties. Our results indicate that workers show attention preference toward queens with higher reproductive potential, and that display pheromonal and genetic parameters indicative of higher quality. These included queens that secreted higher amounts of QMS and of esters in the Dufour's gland (conveying information about quality), queens exhibiting higher expression of vitellogenin – a protein correlated with higher fertility and longevity, as well as higher expression of alcohol dehydrogenase – an enzyme participating in the QMP biosynthesis. Summarily, our findings suggest that worker possess an ability to distinguish between queens of different qualities in terms of reproductive potential, health and longevity, and that pheromonal composition plays an important role in such distinction.

# Chemical profiling and temporal biochemical polymorphism in *Lasius* ants

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*Lasius flavus* is one of the most abundant species of ant present throughout Northern Europe. Previous research has highlighted its importance as an ecosystem engineer, and shown that it is much more reliant on 'social' information (trail pheromones) when foraging than the much studied congeneric *Lasius niger*. Despite this, very little is known about its chemical ecology. Here we investigate the compounds present in 3 major exocrine glands of *L. flavus*, and attempt to identify the trail pheromone composition using a combination of HPLC, GCMS and liquid and solid sampling techniques.

Many ants from the *Lasius* genus of formicine ants exhibit temporal polyethism, whereby the tasks a worker performs tend to be influenced its age. Ants also possess a 'chemical toolkit'; the contents of different glands are used for different jobs. In formicine ants, the hindgut tends to be where trail pheromone is produced, the venom gland contains formic and acetic acid to use for defence, and the Dufour gland may contain large amounts of antibiotic chemicals. To our knowledge the biochemical polymorphism in the ant's toolkit has never been investigated with relation to the tasks they perform. We divided *Lasius niger* workers into two different castes (nurses and foragers) based on their aggregation behaviour and via GCMS we identified and quantified the amounts of compounds present in the hindgut. We found significant differences in the chemical composition of the gland and a trail-following bioassay was used to detect whether the variation in trail pheromone abundance was effective at a behavioural level.

# CHEMICAL ECOLOGY OF INVASIVE SPECIES

# How can chemical ecology contribute to biosecurity against invasive arthropods?

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How can Chemical Ecology Contribute to Biosecurity against Invasive Arthropods ? David Maxwell Suckling, The NZ Institute of Plant and Food Research Ltd and University of Auckland, New Zealand Biosecurity includes responses to new invasive species which have recently expanded their geographic range (by means of delimitation, containment or eradication), as well as long-term management tactics against established arthropod pests which cannot be eradicated. A sound knowledge of the chemical ecology of invasive species is fundamental to both areas and can contribute greatly to improving outcomes by minimising the long term costs of control, including economic, social and environmental costs of pests. Identification of the nature and role of many hundreds of semiochemicals involved in attraction has led to the largest number of practical applications. This has occurred through the delimitation of the distribution of new pests and assessment of the results of interventions for their containment or eradication, as well as the development of monitoring and decision support systems for established pests. Semiochemicals have also been developed into direct control tactics ! such as mating disruption, mass trapping and lure and kill systems against a wide range of insects including fruit flies, moths, beetles and some other groups. Straight chained lepidopteran sex pheromones have emerged as a source of market advantage in orchard pest management, with trapping systems and residue-free multiple species disruption systems being increasingly adopted to reduce insecticide applications and consequently residues on fruit. Despite some successes, for the majority of invasive arthropods pests, there are few tools that can meet current needs for practical and cost-effective solutions. Worldwide, there is a rising rate of incursions of alien invasive species and this has been matched by a rise in costly official eradication programs which strongly supports the need for further investment in the areas of discovery and development of surveillance and eradication technologies, from the science of chemical ecology.

Suckling DM (2015) Can we replace toxicants, achieve biosecurity, and generate market position with semiochemicals? Frontiers in Ecology and Evolution 3(17):1-7. doi: 10.3389/fevo.2015.00017

## Admixture in invasive plants: effects on herbivory and chemistry

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Admixture is the hybridization between previously isolated populations within one species. It can play an important role in biological invasions by creating novel genotypes, increasing genetic diversity and lifting inbreeding depression. Here I will present a study where we used the invasive plant *Lythrum salicaria* (Purple Loosestrife) as a model system to investigate evolution of chemical defenses and the effects of admixture on plant chemistry and herbivore resistance. *L. salicaria* is an outcrossing species. Crosses were made in the greenhouse between plants of the same population and between plants of different populations within the native and invasive range. Using a comprehensive untargeted LC-MS metabolomics approach we discovered new alkaloids in the invasive *L. salicaria* populations. These alkaloids were mostly undetected in the native European plants. Native populations contained higher concentrations of phenolic compounds. Generalist herbivores fed more from the native populations, while specialist herbivores fed more from the invasive populations. The total number of detected metabolites in our metabolomics analyses was higher in invasive populations compared to the native *L. salicaria*. Admixture increased the total number of metabolites in the native populations but not so in the invasive populations. We hypothesize that admixture in the introduced range of *L. salicaria* has led to the overall higher chemical diversity of the invasive populations. Generalist herbivory tended to be lower on the admixed plants. These results suggest evolution of chemical defenses of an invasive plant not only through selection by herbivores but also through admixture of native populations in the invasive range.

# Impact of an invasive pentatomid on ecophysiology and volatile organic compounds emission in *Brassica oleracea* L. var *Botrytis*

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*Bagrada hilaris* is an invasive pentatomid dangerous to the brassicacous crop production. In this study laboratory experiments were conducted to assess how the infestation of this invasive species can affect the host plant *Brassica oleracea* in terms of apparent damage, ecophysiological processes and VOCs emission. The impact of this species on its host plant was compared with the native species *Nezara viridula*, also feeding on brassicacous crops, and widespread worldwide. Non-choice bioassays were carried out to assess the apparent damages induced from a single bug of *B. hilaris* or *N. viridula* on *B. oleracea* leaves and of groups of individuals in affecting the photosynthesis and stomatal conductance of the host plant. Moreover air collection of volatiles of plant infested with *B. hilaris*, *N. viridula* or uninfested were carried out and subjected to GC-MS analysis. Results showed that a single *B. hilaris* determine a higher area of necrotic spots than *N. viridula*. Moreover the photosynthesis and stomatal conductance of the plant infested with 40 adults *B. hilaris* (high infestation) was reduced by the 43%. VOCs analysis revealed that the compounds emitted by *B. oleracea* changed significantly in response to *B. hilaris* and/or *N. viridula* infestation. Among the VOCs identified, limonene production was strongly reduced by infestation activity of the two pentatomids, while an increase in the emission of acetic acid and 1-hexanol-2-ethyl was observed in highly infested *B. hilaris* plants. *N. viridula* infested plants produced and higher amount of decanal rather than *B. hilaris* infested plants. This study showed how insects belonging to the same family can influence differently the same host plant and that the impact of the alien species *B. hilaris* can determine evident visual damage, photosynthesis reduction and change in VOCs profile of infested *B. oleracea* plants.

# Untangling the role of yeasts in attraction and oviposition site selection in an invasive frugivorous insect, *Drosophila suzukii*

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*Drosophila suzukii* Matsumara (Diptera: Drosophilidae) has the ability to oviposit on fresh, ripe fruit compared to the more ancestral drosophilid condition of overripe or rotten substrates. A key innovation contributing to the unique host shift of *D. suzukii* is a heavily sclerotized and serrated ovipositor that allows females to penetrate the skin of soft fruits and lay eggs below the surface. Due to its unique ovipositor *D. suzukii* has become a significant worldwide pest of ripe, soft fruits. Volatile cues are often used to locate suitable food, potential mates and oviposition sites, and it is hypothesized that an alteration in the olfactory system of *D. suzukii* may have contributed to the host shift from rotten to ripe fruit. Recent studies have focused on the attraction of *D. suzukii* to host fruit volatiles, but have understated the potential contribution of microorganisms, including yeast, on the surface of fruits. Here, we use a wind tunnel bioassay to investigate the long-range attraction of adult *D. suzukii* to volatiles from two known yeast associates, *Hanseniaspora uvarum* and *Pichia terricola*; the model organism, *Saccharomyces cerevisiae*; as well as a common host fruit, blueberry, *Vaccinium* spp. *Drosophila suzukii* were attracted to all odour sources; however, the highest attraction was to *H. uvarum*. We also investigate the oviposition preference of *D. suzukii* females and discovered berries inoculated with yeast are a preferred oviposition substrate compared to non-inoculated ripe and unripe berries. Furthermore, larvae are also attracted to yeast and have the highest preference for *H. uvarum*. Our results indicate that *D. suzukii* has an intricate relationship with yeast, and in particular *H. uvarum*, which may contribute to host recognition.

## Chemical cues of the interaction *Gonipterus platensis* – *Eucalyptus globulus*

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*Gonipterus* spp. weevils are worldwide pests of *Eucalyptus*. In Portugal serious losses are caused by *Gonipterus platensis*. Although biological control was attempted using the egg parasitoid *Anaphes nitens*, in cooler regions population densities remain above the economic threshold of damage. The chemical ecology of *Gonipterus* species and the use of semiochemicals for integrated pest management (IPM) are still largely unexplored.

In this work research was conducted to determine which compounds emitted by the host plants may be involved in the chemical ecology of *G. platensis*. For this purpose, *E. globulus* volatile organic compounds (VOCs) were collected by headspace solid phase microextraction (HS-SPME), MonoTrapTM disks and simultaneous distillation-extraction (SDE). Gas chromatography – mass spectrometry electroantennographic detection (GC/MS-EAD) was used to determine which compounds are detected by the antennal olfactory system of the insect. Further chemical analysis of the extracted compounds was conducted using two-dimensional gas chromatography coupled with time-of-flight mass spectrometry and GC with flame ionization detector (GCxGC-TOFMS and GCxGC-FID). Multi-dimensional gas chromatography (MDGC) with dean switch-heart cutting was used to clarify potential coelutions and identify the compounds present in chromatogram areas showing EAG response.

More than 30 compounds elicited EAG activity, including monoterpenes and monoterpenoids, sesquiterpenes and sesquiterpenoids, alcohols and ketones, among others. The EAG activity of the antenna was confirmed for 18 compounds employing commercial standards, which were then selected for behavioural bioassays. To test the effect of different concentrations of these bioactive compounds on insect behaviour, weevils of known age, sex and physiological condition were individually placed on a ten arm olfactometer coupled to video tracking and data analysis software. Weevil position was recorded for 20m and the time spent in each arm determined. A minimum of 20 insects were tested in each trial and paired samples t-test, or Wilcoxon signed ranks test were applied to reveal significant differences between the time spent in each arm for each group. Attraction was observed to five compounds: aromadendrene, α-gurjunene, camphene, *ρ*-cymene and benzeneethanol. While the chemical signature of eucalyptus responsible for its attractiveness to *Gonipterus* will most likely, consist of a specific blend of compounds, our results represent one step forward towards the design of an effective kairomonal lure for monitoring and/or mass-trapping these insects in the field.

# Polar secondary plant metabolites provide a high resistance against natural enemies to native and invasive populations of *Buddleia davidii* in the invasive range

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Invasive plants can cause severe ecological damage by reducing local biodiversity and by inferring high financial costs. Diverse ecological hypotheses try to explain the success of invasive plants by searching for particular traits that allow an exotic plant to become invasive in the future. In particular, two hypotheses are related to secondary plant defence compounds and their mediation of biotic interactions. While the 'novel weapon' hypothesis relates the higher competitive ability of successful exotic plants to possessing 'novel' toxic secondary metabolites, which provide the plants with enemy-free space, the 'shifting defence' hypothesis explains the success of invasive plants by rapid evolutionary changes of the plant metabolome that ultimately provide the plants with a higher resistance against natural enemies. Chemical ecology provides valuable tools to analyse these chemical plant traits that might facilitate establishment in new habitats. To test the hypothesis that phytometabolic traits are related to its invasive range expansion in Europe, we chose the invasive ornamental plant species, *Buddleia davidii* (Scrophulariaceae). To analyse the metabolic composition of ten native and ten invasive populations grown in a common garden experiment, we used a metabolic fingerprinting approach with ultra-high performance liquid chromatography coupled with time-of-flight-mass spectrometry. The chemical composition of invasive populations was most similar to two native populations, which however differed from the rest of the native populations. Bioassay-guided fractionation further revealed that specific secondary compounds are involved in plant resistance by providing enemy-free space against two polyphagous generalist herbivores and a generalist phytopathogen. As these particular compounds were generally more abundant in the invasive and two native populations, we conclude that different concentrations of these plant defence compounds did not result from rapid evolutionary changes, but preadaptively confer high resistance against natural enemies to *B. davidii* populations in the invasive range, and might thus be classified as 'novel weapons'.

ISP07

# Are invasive plants more toxic than native plants? An example of rapid evolution after invasion

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Biological invasions are excellent systems to study rapid evolution of plant chemical defenses. Current hypotheses predict a divergence of plant chemical defenses in response to a decrease in herbivory after invasion (e.g. EICA hypothesis) or in response to novel climatic conditions. Post-invasive changes in plant chemistry can modify the interactions with herbivores and facilitate invasion success. However, whether plant toxicity is changed after invasion remains to be evaluated.

*Senecio pterophorus* is a shrub native from Eastern South Africa and a recent invader in Western South Africa (~100 years ago), Australia (>70-100 years ago) and Europe (>30 years ago). These distributional regions of *S. pterophorus* differ in their summer drought stress and in their interactions with herbivores. As other Asteraceae, *S. pterophorus* contains pyrrolizidine alkaloids (PAs) toxic to vertebrate and invertebrate herbivores. Plants from 54 populations sampled throughout the entire known worldwide distributional area, including the native and three non-native ranges, were grown under controlled conditions. First, we analyzed the levels of chemical defenses and leaf morphological traits to determine whether plant genetically-based traits diverged between native and non-native populations. Second, we performed non-choice bioassays with generalist herbivores (e.g. *Spodoptera* sp) to evaluate changes in plant toxicity after invasion.

Plants from different origins diverged in their chemical and morphological traits. Levels of chemical defenses were higher in the introduced populations, including the highly toxic 1,2-unsaturated PAs and the less toxic 1,2-saturated PAs. These results, indicative of higher toxicity in the invasive range, were consistent with the lower larval growth when insects consumed non-native plants. We discuss what factors, either chemical or morphological, determine the increase in plant toxicity after invasion. By comparing the genetic similarity across the native and non-native areas obtained by neutral markers we evaluate whether changes in toxicity are result of a rapid evolution.

# Chemical ecology of subcortical insects in new contexts: The roles of climate change and invasion of naïve habitats

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California has been a hot bed of invasive species of bark and ambrosia beetles and other wood borers. These invasions have been facilitated in this region by a high level of international commerce, the wide climatic and topographic variation, and an attendant uniqueness and diversity of woody plants available as hosts. Approximately one-third of California is forested (13,450,941 ha), and its large metropolitan areas are adorned with major urban forests and parks. For example, of California's 86 native forest and woodland tree species, 21 species grow only in the State, and these forests contain 59 species of native conifers, which is more than any other U.S. state. California's urban forests are also wildly diverse and replete with a multitude of non-native tree species, e.g., 600 species of indigenous and introduced trees in 86 families have been recorded in the city of Santa Barbara alone. Threatening the health of standing trees in these urban, peri-urban, and wild land forests are at least six new subcortical insects (and in some cases insect/pathogen complexes) [*Agrilus auroguttatus* Schaeffer (Buprestidae), and *Orthotomicus* (*Ips*) *erosus* (Wollaston), *Hylurgus ligniperda* F., *Scolytus schevyrewi* Semenov, *Euwallacea near fornicatus* (Eichhoff), and *Pityophthorus juglandis* Blackman (all Scolytidae)]. This presentation will emphasize aspects of the chemical ecology of these invaders related to the host colonization behaviors in the new habitat. The extent to which climate change has facilitated the successful introduction and establishment of these invasive species and impacted the assessment of their risk will also be considered. Efficacious lures for detection have been developed for the Mediterranean pine engraver, *O. erosus*, and the walnut twig beetle, *P. juglandis*, and, coincidently, the lures are each based on a hemiterpenoid male-produced aggregation pheromone component (2-methyl-3-buten-2-ol and 3-methyl-2-buten-1-ol, respectively). Host (primary) attractants may contribute to the aggregation behaviors of both species, but their significance is under evaluation.

# The true cost of exotic perfumes: impact of invasive herbivores on native infochemical networks.

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Exotic herbivores invading new environments can pose a threat to the functioning of native foodwebs. In the context of the EUROVOL project InvaVol, we investigated the disruptive effects of invasions by exotic insect herbivores on insect-plant interactions mediated by plant volatiles. Using *Brassica rapa* and its complex of native herbivores and natural enemies as a study system, we conducted several studies investigating the effects of a range of exotic herbivores on the foraging behavior of native parasitoids.

Results showed that native parasitoids are in general very efficient at innately distinguishing between plant infested by their native host and plants infested by a non-host herbivore, independently of the origin of the non-host herbivore, and show a strong preference for host-infested plants. However, in case of double infestation (host and non-host herbivore feeding on the same plant), plants doubly infested with an exotic non-host became less attractive to parasitoids. This result was consistent for a range of 17 non-host herbivores, showing a general effect of exotic herbivores possibly leading to reduced fitness for native parasitoids. Further experiments focused on the native parasitoid *Cotesia glomerata* and the exotic herbivore *Spodoptera littoralis* showed that both herbivore density and parasitoid associative learning mediate the outcome of exotic herbivory on parasitoid attraction. In summary, the InvaVol project revealed that exotic herbivores can impact infochemical networks and that these effects can escalate through the food chain to the third trophic level.

# The effects of Tree tobacco nectar alkaloids on Palestine sunbird foraging behavior and performance

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Many plant species contain plant secondary metabolites (PSMs), like alkaloids, in their tissues for protection against herbivore attack, but PSMs can also be found in floral nectar. This could expose pollinators to potentially toxic rewards with possible negative effects. Some pollinators have been shown to discriminate against floral nectar with PSMs and consuming PSMs may have negative fitness effects on pollinators, but little is known about the effect of PSMs on pollinator foraging performance. Here, we addressed the question of whether the natural concentrations of the alkaloids, nicotine and anabasine, found in the invasive Tree tobacco (*Nicotiana glauca*) affect foraging performance in Palestine sunbird (*Nectarinia osea*) pollinators that use the plant's nectar as a food source. We trained foraging sunbirds to discriminate rewarding and non-rewarding artificial flowers based on color. We measured their accuracy at distinguishing the two colors immediately after training (pre-treatment), and again the following day after consuming sucrose solutions with or without alkaloids (post-treatment). We found that alkaloid consumption significantly decreased sunbird accuracy in distinguishing the rewarding color, suggesting reduced forging performance due to memory retention and/or other cognitive or physiological effects following alkaloid consumption. We found that birds were generally less active and visited fewer flowers per unit time in the later test (post-treatment). There was no significant difference in activity level between birds that consumed alkaloids and those that did not, while birds in the alkaloid-consuming group generally had a higher visitation rate across both test times. We also found that birds discriminated against higher concentrations of alkaloids and generally consumed less alkaloid solution when they had previous experience with alkaloids. Reduced foraging performance due to PSM ingestion could greatly affect a pollinator's foraging efficiency, which could, in turn, affect plant reproductive fitness.

# Chemical ecology of *Monochamus galloprovincialis*, vector of pine wood nematode in Europe

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Pine wood nematode, *Bursaphelenchus xylophilus*, is an invasive pest causing a fatal wilting disease in susceptible pine trees. Native of America, it was introduced from the Far East to Portugal in 1999. The nematode is vectored by Cerambycid beetles of the *Monochamus* genus, and the only confirmed vector in Europe is *M. galloprovincialis*, although related species are also potential vectors. As part of an EU project to restrict spread of the nematode in Europe, aspects of the chemical ecology of the vector were investigated to provide potential management tools for the nematode and the disease.

The aggregation pheromone produced by male *M. galloprovincialis* was identified and its attractiveness to both sexes was shown to be strongly synergised by host-plant volatiles and bark beetle kairomones. Traps baited with the attractant are being developed for measuring dispersal of the beetle and mass trapping around outbreaks of the nematode. The attractant attracts mature beetles and intensive work in field and laboratory has not produced an attractant for the immature beetles.

Cuticular compounds are involved in species and sex recognition in *M. galloprovincialis*, although key, non-hydrocarbon compounds produced by the males are responsible for this rather than hydrocarbons in the female, as reported for other Cerambycids.

*M. galloprovincialis* beetles tend to colonise stressed pines, including those damaged by fire. Electrophysiological studies and field trapping tests indicate the beetles can detect volatile compounds found in wood smoke and these may provide a new approach to attraction of the beetles.

# Selection for increased production of prostaglandin E2 and related deterrents during the invasion history of a seaweed, *Gracilaria vermiculophylla*

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Differences with respect to anti-herbivore defense were investigated in invasive and native populations of the seaweed *Gracilaria vermiculophylla*. Specimens from six native populations in East Asia and eight invasive populations in Europe and W-Mexico were maintained under identical conditions and offered in no-choice feeding assays to herbivorous snails from the native range (*Littorina brevicula*) and from Europe (*Littorina littorea*). *L. brevicula* consumed in total significantly larger amounts of *G. vermiculophylla* tissue compared to *L. littorea*. Further, both snail species consumed the least of seaweed specimens originating either from non-native populations or from native populations in the Korean East Sea/Sea of Japan. The Korean East Sea/Sea of Japan has previously been identified as putative donor region of all the invasive populations of *G. vermiculophylla*. Thus, populations in the donor region as well as non-native populations in different invaded realms feature an increased capacity to resist feeding pressure. Differences in nutrient content did not account for the observed patterns of consumption, as palatability and C:N-ratio were not significantly correlated. Thus, mechanical or chemical defenses or the content of feeding cues influenced the behavior of the snails.

# A chemical ecology approach to assess the proliferation of the red alga *Asparagopsis taxiformis*: metabolomics, natural toxicity and biological effects.

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In marine sub-tropical and tropical ecosystems, algal proliferations are impacting several species. The competition between algae and corals modify the functioning and the structure of the reefs. The genus *Asparagopsis* (Rhodophyta) is known to be cosmopolite, and may even be invasive in some locations. The lack of knowledge on these algae and their real impacts on the reefs require a global and multi-disciplinary study to better understand, and manage some invasion events (ERA-NET BIOME project www.seaprolif.ird). The main goals of this franco portuguese consortium are: i) to assess the worldwide distribution of *A. taxiformis*, and its dynamics in tropical/temperate sites; ii) to evaluate the link between the genotypes and their metabolic contents, in association with their microbial diversity; iii) to study mechanisms involved in the interaction between *A. taxiformis* and indigenous tropical/temperate corals. In this context, our work will focus on two particular points: i) previous studies highlighted five different lineages for *A. taxiformis* [1]. Using UHPLC-QqToF, metabolomic fingerprints of the different lineages were recorded to determine the link between specialized metabolism, genotypes and their bioactivities in order to explain their possible invasiveness; ii) the major specialized metabolites of this species were isolated and characterized in order to determine their contribution to algal toxicities. We also studied their implication in tropical and temperate coral interactions. The first studies allow the isolation and characterization of two new highly brominated cyclopentenones [2], while only highly halogenated compounds (C1 to C4) were identified previously. Algal extracts tested in situ on four tropical corals show only low effects on coral bleaching, while no change in the algal metabolism was highlighted in contact with the massive coral Porites. However, the alga in direct interaction with the Mediterranean orange coral *Astroides calycularis* develop a specific metabolism with a toxicity increase after 15 days in contact.

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Journal of Natural Products, 77 (5), 1150-1155, DOI: 10.1021/np401094h

# Biochemical evidence for cryptic fire ant species in Argentina

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The imported fire ant, *Solenopsis invicta*, is an economically important pest ant in several parts of the world. Its origin is thought to be northern Argentina. The classical taxonomic efforts have been likened to "walking through a mine field" or one "of the more knotty problems in ant taxonomy." Introduction of biocontrol agents for imported fire ants depends on accurate identification of *S. invicta* populations. We report the use of fire ant venom alkaloids, cuticular hydrocarbons, and recruitment pheromone analysis to define *S. invicta* in the USA and survey the chemical characters in an area in northern Argentina thought to be composed of *S. invicta* populations. Our results show three distinct chemotypes based on venom alkaloids and cuticular hydrocarbons: *S. invicta*, non-invicta, and Invictoid. *S. invicta* and non-invicta occur sympatrically with no chemical evidence for introgression. Similarly, non-invicta and Invictoid occur sympatrically without apparent introgression. In addition, the major *S. invicta* recruitment pheromone component, *Z,E*-α-farnesene, is absent in Invictoid and non-invicta worker ants. Thirty-six percent of 25 collection sites had atypical USA *S. invicta*, yet taxonomically they have not yet been distinguished. Successful biological control of *S. invicta* in the USA and other invaded locations depends on accurately mining USA-type *S. invicta* colonies in Argentina. These data help explain why some pathogen collections from Argentina could not be successfully transferred to USA *S. invicta*.

# INDUCED RESISTANCE OF PLANTS AND PREY

# The dynamics of offense and defense: Induced defenses, intraspecific variance, and the ecological important of plasticity

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Species are commonly treated as static, ideal forms. Any, population biologist, geneticist, crop breeder, or traveler looking at humans in an international airport knows, this is false. Every species is a variable cloud of phenotypes, some of which vary tremendously from the mean. Adult humans vary from 51 to 272 cm in height and from 10 to 630 Kg in mass – 5 and 63 fold differences, respectively. Variance in secondary metabolite composition and concentration may be similarly great in many species and can be quickly altered in response to biotic threats. Variance in natural enemy susceptibility to these compounds may be similarly great. Overlooking this variance results in tremendous loss of opportunity for ecological discovery and evolutionary insight.

Here, I overview the dynamics of chemical responses to natural enemies from both the defensive and offensive perspectives – reviewing what is known of induced defenses against consumers, competitors, and other natural enemies, as well as the offensive counter-measures taken by attackers. These responses may take the form of activated defenses, induced defenses, or potentially epigenetic changes that propagate through multiple generations. Variance occurs across spatial scales ranging from geographic (more potent chemical defenses at lower latitudes) to among different portions of a single individual (with induction occurring in some critical plant portions but not in others of that same individual). Temporal scales are equally variable, with alteration of chemical defenses sometimes taking seconds, and sometimes taking an annual cycle. Even prey as simple as seaweeds and phytoplankton not only induce defenses, but chemically recognize the attacking species and respond with the correct defense for that particular species of attacker – sometimes changing palatability by two orders of magnitude within only days. Understanding such variance offers critical ecological and evolutionary insight into the processes that have driven selection within natural systems.

# Lipid signaling in marine plankton

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Chemical signals between marine plankton are poorly understood. In recent years, however, several polar lipids signals have been identified from plankton organisms. Polar lipid signaling is found in diverse interactions including virus-phytoplankton, zooplankton-phytoplankton, and bacteria-choanoflagellate, which suggest that polar lipids may be an important set of chemical signals in the aquatic micro-environment. We recently identified a group of taurine conjugated lipids, the copepodamides, from copepod zooplankton. Harmful algae respond to pico- to nano-molar concentrations of copepodamides by increased toxin production and induced cryptic traits to evade predation. There are at least 12 different copepodamides, and copepods species have specific copepodamide composition that allows for a high level of specificity. We use copepodamides to explore the fundamentals of polar lipid signaling in aquatic micro environments. Which copepodamides are emitted from different species of copepods, how do these lipophilic compounds spread and distribute in seawater, what effects do copepodamides have on responding prey? Copepods are the most abundant metazoans on Earth and play a key role in the aquatic ecosystem. Copepodamide signaling may consequently have large scale implication for the function of the pelagic food web and biogeochemical cycling of elements in the oceans.

# Induced and constitutive phlorotannins as defenses in a perennial brown alga

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We explored the defensive role of inducible and constitutive phlorotannins in a perennial marine brown alga *Fucus vesiculosus* under natural herbivory. We manipulated access of herbivores on algae in a field experiment during a season of high natural herbivore density and measured grazing damage and both the induced and constitutive phlorotannins. We carried out herbivore feeding preference bio-assay to document resistance induction. We found that in the field experiment, algae lost 58 % of their biomass to herbivores. Herbivory induced resistance and triggered an 18 % increase in phlorotannins. Both the content of constitutive and induced phlorotannins varied among the algal genotypes and there was a trade-off between these, which suggests that phlorotannin production is costly. Grazing damage in the field decreased with constitutive phlorotannin content while induced phlorotannins tended to increase with the grazing damage. However, after the three week manipulation all the genotypes had increased phlorotannin content and were highly resistant to further herbivory in the preference bio-assays. Our results show that both the constitutive and induced phlorotannins act as resistance traits against herbivores in *F. vesiculosus* but partly as alternative strategies and partly on a different time scales. Under strong natural herbivore attack the genotypes owing high constitutive phlorotannins gained an immediate resistance while the genotypes relying on induced resistance suffered costs, in terms of higher grazing damage, due to delaying the full resistance. Induced resistance, however, proved to be a very efficient defense as all the genotypes turned into highly unpalatable to further herbivory. In a system with strong seasonality, induced resistance is likely very important to minimizing mortality risk during the periods of high grazing pressure and acts as a cost saving strategy during the low risk of herbivory.

# Inducible plant signalling: Opportunities for real time management of pests

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Early onset of damage in plants is accompanied by upregulation of defence genes that are responsible for the production and emission of semiochemicals, which are released even before normal and recognizable symptomology. Induced VOCs, as well as being exploited by natural enemies in the location of suitable hosts and prey, are involved in plant/plant communication. The discovery of cis-jasmone, a volatile semiochemical released by damaged plants, as an elicitor of defence in crops, provides an opportunity for the development of smart crop protection strategies, based on linking elicitation to the induction of plant defence either directly or via a new type of sentinel (guard) plant. Here, promoter sequences for defence-related genes upregulated by elicitors are either used, with appropriate engineering, to 'switch on' the production of semiochemicals in genetically engineered crops, or are linked to the upregulation of visual markers, which would allow precision application of appropriate control agents. Prospects for the development of real time management of pests via sentinel plants will be discussed.

Birkett and Pickett, Curr Opin Plant Biol, (2014), 19: 59-67 and references within

# Volatile-mediated plant-plant interactions: requirements for induced resistance

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Plants respond to stress, including damage by herbivorous insects, by emitting a blend of volatile organic compounds. This response provides community members with an airborne chemical plume that reflects the physiological condition of the emitting plant. Extraction of information from the chemicals, or stimulation of a response by them, requires the receiver to possess the physiological means to detect, process and respond to the whole, or part of, the chemical blend. Many different organisms respond to plant volatiles, but the responses of nearby plants are particularly intriguing, from both evolutionary and mechanistic perspectives. It is increasingly apparent that plants acquire resistance after exposure to herbivore-induced volatiles. However, full elucidation of the mechanisms underlying the detection of volatiles and the subsequent coordination of responses is yet to be achieved. Active responses in receiver plants have been investigated in a number of studies, but observations of passive interactions involving the adsorption of volatiles to receiver plant surfaces supports the potential co-occurrence of different mechanisms. Both mechanisms require volatile chemicals to pass outside the physiological boundaries of the plant. In the case of aboveground terrestrial interactions, the volatiles are airborne. The composition of the air substantially affects the longevity and fidelity of the chemical blend emitted by damaged plants. Consequently, the presence of air pollutants may affect volatile-mediated interactions between plants. I will discuss recent work on induced resistance mediated by plant volatiles, what we have learned about the mechanisms and complexities arising when the medium carrying chemical plumes is tainted by pollution.

# Fungal endophytes prime pathogen resistance in wild lima bean (*Phaseolus lunatus*)

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Horizontally transmitted type II endophytes have been shown to affect plant resistance to pathogens, yet a comprehension of how these fungi alter plant defense is lacking. Furthermore, studies aimed at discovering endophyte-mediated effects in planta frequently utilize plants experimentally inoculated with single endophyte strains. However, under natural conditions plants are colonized by multiple endophytes simultaneously. This may question the relevance of such experimental setups. To improve our understanding of the functional effects of endophytes on plant resistance and potential bias from unnatural experimental conditions, we examined variations in endophyte-derived pathogen resistance in both sterile, wildtype lima bean (*Phaseolus lunatus*) plants and also wild lima bean plants with natural endophyte communities. Treatment plants were inoculated with a mutualistic endophyte and later challenged with a fungal pathogen (*Colletotrichum lindemuthianum*). Several PR-proteins were quantitatively analyzed (polyphenol oxidase, chitinase, and β-1,3-glucanase). We found that one out of the 266 endophyte isolates obtained from lima bean plants in nature caused a priming of polyphenol oxidase activity, effective at reducing pathogenic lesion numbers in plants. While both enzyme activity and pathogen resistance were more pronounced in sterile plants, plants growing in nature also showed significant endophyte-mediated pathogen resistance. This study is the first to demonstrate i) that endophyte-mediated effects in experimental plants inoculated with one endophyte can be reproduced in wild plants harboring natural endophyte communities and ii) that an endophyte-mediated upregulation of plant-derived polyphenol oxidase is the causative agent for the observed pathogen resistance.

# Eavesdropping plants: Insect odors prime plant defenses

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Insect feeding damage is known to induce plant defenses. More recent discoveries have found that plants can also perceive environmental cues associated with the presence of insect herbivores, allowing them to prepare their defenses for future attack. For example, plants may detect insect footsteps or oviposition, and some even use olfactory cues to sense the presence of nearby herbivores. Several studies have found that undamaged plants can eavesdrop on volatiles emitted by their insect-damaged neighbors and respond by enhancing their own anti-herbivore defenses. In this study, we demonstrate for the first time that plants can also perceive and respond to olfactory cues emitted directly by insect herbivores. Our findings indicate that tall goldenrod (*Solidago altissima*) plants exhibit enhanced defense responses following exposure to the putative sex attractant of a specialist herbivore, the goldenrod gall fly (*Eurosta solidaginis*). In field and laboratory experiments, goldenrod plants previously exposed to the male fly emission suffered significantly less herbivore damage than unexposed controls. Moreover, goldenrod plants exposed to the fly odor induced higher amounts of the key defense signaling hormone jasmonic acid following damage. Additionally, goldenrod plants exposed only to the most abundant compound in the fly emission exhibited an enhanced defense response similar to plants exposed to the entire emission blend. These results suggest that goldenrod plants eavesdrop on the olfactory signals of their insect antagonists and exploit them as indicators of impending herbivory. These findings document a new class of olfactory-mediated interactions with broad significance for the evolutionary ecology of plant-insect interactions.

Helms, A.M., De Moraes, C.M., Tooker, J.F. and Mescher, M.C. 2013. Exposure of *Solidago altissima* plants to volatile emissions of an insect antagonist (*Eurosta solidaginis*) deters subsequent herbivory. Proc. Natl. Acad. Sci. 110: 199-204. DOI: 10.1073/pnas1218606110

# Caterpillar and rootworm feeding differentially affects defense protein accumulation in corn

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When corn is attacked by insect herbivores it responds by accumulating a suite of defense proteins. Two defense proteins that are produced in response to foliar feeding by fall armyworm (FAW) are the insecticidal cysteine protease Mir1-CP and ribosome inactivating protein-2 (RIP2). However, there is little information regarding the accumulation of these defense proteins in response to belowground feeding by the western corn rootworm (WCR). Furthermore, the effects of jasmonic acid (JA) and ethylene (ET) on the regulation of abundance of these defensive proteins and their gene expression is unknown. In this study, we show that the kinetics and levels of mir1 and rip2 transcript accumulation in whorls and roots is different depending on the herbivore attacking the plant. Immunoblot analysis indicated that foliar FAW feeding increased Mir1-CP abundance in both whorls and roots, and root feeding by WCR increased Mir1-CP abundance in these two organ suggesting a systemic response. On the other hand, RIP2 protein abundance increased only in the tissues immediately attacked by FAW or WCR. The effects of blocking JA synthesis or ET perception on Mir1-CP and RIP2 accumulation during FAW or WCR infestation suggests that these defense proteins could have a different hormonal regulation process in whorl and roots depending on the insect attacking the corn plant.

# Jasmonate-dependent depletion of plant carbohydrates constrains resistance and tolerance against herbivores

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While the behavior and function of plant secondary metabolites in herbivore-attacked plants is well studied, less is known about primary metabolites in this context. We found that *Manduca sexta* attack dramatically decreased carbohydrates, including glucose, fructose, sucrose and starch, in *Nicotiana attenuata* leaves and roots. This effect was not observed in jasmonate-deficient transgenic plants, suggesting that carbohydrate depletion is jasmonate-dependent [1, 2]. The reduction in sugars and starch may have two consequences for the plant: First, it may change its nutritional value for herbivores and thereby affect herbivore resistance. Second, it may reduce the capacity of non-attacked tissues, including for instance the roots, to supply energy for regrowth following herbivore attack. We addressed these two questions in detail in two separate sets of experiments [1, 2]. To understand the impact of carbohydrate depletion on herbivore resistance, we manipulated leaf carbohydrates through genetic engineering and in vitro complementation. Contrary to our expectation, both in planta and in vitro approaches showed that the lower sugar concentrations led to increased *M. sexta* growth, suggesting that carbohydrate depletion constrains rather than enhances herbivore resistance. To understand the impact of carbohydrate depletion on herbivore tolerance, we combined natural variation and genetic manipulation of jasmonate signaling and carbohydrate allocation and measured their regrowth capacity upon herbivore attack. The results suggest that the herbivory-induced, jasmonate-dependent depletion of root carbohydrates significantly constrains the plant´s regrowth capacity and fitness upon insect attack. Taken together, these experiments demonstrate that jasmonate-dependent carbohydrate depletion reduces both resistance and tolerance to foliar herbivory.

1. Machado, R.A.R., et al., Leaf-herbivore attack reduces carbon reserves and regrowth from the roots via jasmonate and auxin signaling. New Phytologist, 2013. 200(4): p. 1234-1246.
2. Machado, R.A.R., et al., Jasmonate-dependent depletion of soluble sugars compromises plant resistance to *Manduca sexta*. New Phytologist (in press), 2015.

# JA-Ile-Macrolactones induce nicotine accumulation in *Nicotiana attenuata* leaves and reduce *Manduca sexta* mass gain and survivorship

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Jasmonates are phytohormones involved in a wide range of plant processes including growth, 11 development, senescence, and defense. Jasmonoyl-L-isoleucine (JA-Ile), an amino acid conjugate of jasmonic acid (JA), has been identified as the bioactive endogenous jasmonate.[1] However, JA-Ile analogues can generate different responses in the plant. For example, the phytotoxin coronatine potentiates JA-signaling, methyloxime derivatives antagonize JA-Ile perception[2] and 12-OH-JA-Ile acts as “stop” signal in JA-signaling. On the other hand, the lactone derived from 12-OH-JA (jasmine ketolactone, JKL) occurs in nature, although with no known biological function. Based on the chemical structure of JKL, and in order to further explore potential biological activities of JA-Ile derivatives, we have synthesized two macrolactones derived from 12-OH-JA-Ile. We explored the ability of the synthetic lactones to induce nicotine production, a well-known jasmonate dependent secondary metabolite. The macrolactones showed strong biological activity. They induced nicotine production and reduced *M. sexta* growth and survivorship to a similar extent as methyl jasmonate. By using genetically modified plant genotypes, we are currently testing i) whether the observed M. sexta performance reduction is due to the increased nicotine content, and ii) whether these responses are mediated by the JA-Ile receptor complex (SCFCOI1). The understanding of the mechanism of action of these new synthetic jasmonates will shed light on the JA-signaling pathway and on the potential of these compounds to protect crop plants from herbivores.

# The Stressed-Out Potato

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Aphids, Colorado potato beetles and the late blight pathogen are among the potato plant's most important stressors. How do potato plants deal with multiple attacks, and how do previous attacks on potatoes affect the newcomers thereafter? Understanding how plants integrate their defence responses can provide valuable insights into ecological interactions in an agroecosystem. In the current research we are investigating how herbivorous insects of different feeding guilds (the leaf chewing beetle *Leptinotarsa decemlineata*, and the phloem-sucking aphid, *Myzus persicae*) affect potato defence responses; and also how the oomycete, late blight (*Phytophthora infestans*) affects these responses in the two phases of its hemibiotrophic life cycle. We measured plant responses by quantifying expression of genes involved in two important defence pathways. These are the salicylic acid signalling pathway, which can be triggered by stress from phloem feeders or biotrophic pathogens; and the jasmonic acid pathway, normally triggered by stress from chewing herbivores and necrotrophic pathogens. We also observed how each of these single stressors can affect a second attacker by measuring insect body mass and monitoring development; or quantifying *Phytophthora* biomass in leaf tissue with or without previous stress. Results demonstrate that plant responses to insects with different feeding strategies induce different phytohormonal pathways. The potato blight pathogen induces hormonal cascades at different rates than the insect stressors, and the pathogen infection can have opposite effects on insects with different feeding strategies

# The 'Red Bull'® effect: an additional explanation for response to phenylpropanoids by male *Bactrocera* fruit flies

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Male *Bactrocera* fruit flies (Diptera: Tephritidae) respond strongly and positively to a small group of phenylpropanoids, including zingerone, methyl eugenol and raspberry ketone/cuelure. For most *Bactrocera* studied, feeding on these chemicals results in increased male mating success through the production of a 'sexier' male pheromone. For the Queensland fruit fly, *B. tryoni* (Froggatt), feeding on both cuelure and zingerone enhances male mating success, but only for cuelure was the pheromone of lure fed males more attractive: no increased female attraction was found to the pheromone of zingerone fed males. In other systems, raspberry ketone and zingerone have been identified as metabolism enhancers and appetite depressors, i.e. they act like caffeine in providing energy while offsetting hunger. We thus hypothesised that the mating benefits derived from zingerone feeding by *B. tryoni* males were due to a general increase in male activity, rather than by pheromone enhancement. We tested this through a comparative transcriptomic approach (seeking evidence for differential up-regulation of targeted GO groups and genes) and behavioural and physiological studies. Zingerone feeding was found to significantly enrich GO groups and pathways associated with metabolic processing, as well as genes associated with male-male aggression, pheromone production and courtship regulation. In physiological studies lure fed males lost weight faster than non-lure fed males; and in behavioural assays zingerone feeding was associated with increased male locomotor activity, earlier male courtship initiation, and increased male progression from mounting to copulation. These findings lead us to propose the 'Red Bull®' effect to explain lure feeding in male *Bactrocera*, our analogy to the well-known caffeine energy drink which provides short-term energy 'hits' and lifts general activity. This hypothesis is not competitive with the enhanced male pheromone hypothesis, but is additional to it in species where the pheromone is enhanced, or alternate to it where the pheromone is not enhanced.

# Volatile interactions between undamaged plants affect herbivore insects and their natural enemies

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Volatile organic compounds (VOCs) released by herbivore damaged plants are involved in a wide range of trophic interactions. However, plants release VOCs even when they are not attacked and these volatiles are available as signals for neighbouring plants. Interactions between plants by volatiles emitted from undamaged plants have been previously overlooked as a factor that can affect tri-trophic interaction. We investigated whether volatile exchange between undamaged plants affects volatile emission and plant-insect interaction. Significantly greater quantities of (*E*)-nerolidol and TMTT were found in the headspace of potato plants previously exposed to volatiles from undamaged onion plants analysed by mass spectrometry (1). The altered potato volatile profile had a deterrent effect against host-seeking green peach aphid (1,2) and had attracting effect on ladybird (3). In the experiment, a natural occurrence of peach-potato aphid into a potato crop was significantly reduced when the onion plants were grown alongside the potato (1). Our findings broaden the ecological significance of the phenomenon; volatiles carry not only information on whether or not neighbouring plants are under attack, but also information on the competitive emitter plants themselves. As behaviour of insects was affected, the findings represent a novel bottom-up effect of plant co-existence on insect and provide new evidence of the role of chemically-mediated mechanisms.

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(3) Vucetic A, Dahlin I, Petrovic-Obradovic O, et al. (2014) Volatile interaction between undamaged plants affects tritrophic interactions through changed plant volatile emission. Plant Signaling & Behavior; 9:e29517.

# Herbivore damage induces a transgenerational increase of cyanogenesis in wild lima bean (*Phaseolus lunatus*)

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Plants constantly manufacture defensive biochemicals through a diverse suite of metabolic pathways, as a result of the co-evolutionary history plants share with their herbivorous attackers. In addition to permanently expressed (constitutive) chemical or mechanical defenses, plants generally show an array of inducible defenses against their attackers. There is increasing evidence that inducible defenses may persist on a long-term time scale, and across generations. However, transgenerational studies that quantitatively analyze plant defenses together with multiple growth and reproductive traits are rare. Studies testing these multiple effects under natural conditions are lacking. In this study we show for the first time, transgenerationally elevated cyanogenesis (release of hydrogen from cyanogenic precursors) in wild lima bean plants, *Phaseolus lunatus*, experimentally exposed to extensive damage by natural chrysomelid herbivores, *Gynandrobrotica guerreroensis*. Increased cyanogenesis resulted from elevated enzymatic activity of β-glucosidases (enzymes specifically decomposing cyanogenic precursors), rather than quantitative changes in cyanogenic glucoside content. Compared to plants grown in the laboratory from seeds collected from non-manipulated plants in nature, in-field experimental offspring from extensively damaged parents were significantly better protected against herbivores. However, this effect was detectable only at the seedling stage and vanished within 12 weeks. Nevertheless, the present study shows for the first time, a transgenerational effect of herbivore damage on plant cyanogenesis and resistance against herbivores in nature.

# Does better growth imply better immunity? Effect of biofertilizers on induced responses of Brassica juncea

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Biofertilizers are emerging as promising plant growth promoters for farmers. However, when plants are stressed, they undergo several morphological and chemical changes, known as induced responses, which may utilize a substantial share of plant nutrition.

We determined whether biofertilizers boost both plant growth and immunity or is biofertilizer-facilitated growth compromised as a tradeoff for immunity. For this, we studied various systemically induced responses occurring due to insect herbivory in Indian/Brown mustard (*Brassica juncea*), which was grown in soil supplemented with phosphate solubilizing bacteria (PSB) and vesicular-arbuscular mycorrhiza (VAM), separately and in combination. Each treatment was analyzed for morphological and chemical induced responses after damage by Tobacco cutworm (*Spodoptera litura*). We divided our experiments into quick (3 to 72 hours) and delayed responses (4th and 9th day). Antioxidants were found to increase significantly all through the experimental period in PSB supplemented induced plants, whereas they increased only after 24 hour in VAM supplemented induced plants. Overall increase in antioxidants was better in induced plants supplemented with the biofertilizers separately, as compared to induced plants supplemented with PSB-VAM combination. Trichome number and density increased more in induced plants supplemented with VAM than in other treatments.

We tested the effects of induced changes on orientation and feeding preferences of *S. litura*. Although plants supplemented with PSB-VAM combination attracted most larvae when intact, this attraction was significantly reduced when plants were induced. Conversely, orientation and feeding preference of *S. litura* increased significantly when the plants supplemented with PSB were induced.

Our study suggests that biofertilizers enhance induced plant responses along with their growth, but they do not necessarily elevate induced resistance. Therefore, fitness of induced and uninduced plants supplemented with biofertilizers should be compared in an environment that includes herbivores, before establishing a positive correlation between growth and immunity.

# PHARMACOGNOSY MEETS CHEMICAL ECOLOGY

# Truffle fungi and their volatile substances in vector mediated spore dispersal - an overview

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Fungi are known for their peculiar chemistry. The secondary metabolites are produced to aid establishment in the various ecological niches of different fungal life forms. A truffle is a fungal life form defined by its hypogeous fruiting bodies. The ability of active spore dispersal has been lost and is instead dependent on vertebrate vectors. At 100% spore maturity, not before, truffle fruiting bodies emit an array of volatile organic compounds (VOC), thus attracting mammals such as squirrels and wild boars to unearth and eat them, efficiently spreading the spores in faecal pellets. The truffle life form has developed multiple times in fungi and volatile and non-volatile components are being studied to understand the nature of the fungus-animal interactions. Most studies on truffle VOCs have been done on species belonging to the phylum Ascomycota, the so-called true truffles. A single species may produce more than 100 different VOCs. Although common in many species, the relative quantities of VOCs give each truffle species a unique, species-specific scent at full spore maturity. The edible truffles of the genus Tuber have historically been used as an aphrodisiac. In modern science small amounts of the steroid androstenol have been found in the black truffle Tuber melanosporum. Androstenol is a major component of the boar pheromone, excreted in the saliva during mating. Despite the peculiarity and abundance of VOCs produced by the truffles, the main substance involved in attracting both mammals and certain insects has been identified as dimethyl sulphide (DMS). Insect attraction to DMS is known from southern Europe, where the fly Suillia gigantea (Heleomyzidae) is known for laying its eggs next to mature truffle fruiting bodies. Unlike the flies themselves, their larvae are able to consume the truffle. Man has also used the hovering behaviour of these flies, in order to locate priced truffles.

# Diptera attracted to the truffle Tuber aestivum and dimethylsulfide in Sweden.

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The edible, hypogeous truffle fungus Tuber aestivum is widely distributed in Europe. Its most northern known locality is the Baltic island Gotland, where it was first recorded in 1977. At full spore maturity, truffles emit volatile organic substances (VOC). Dimethylsulfide (DMS) has been identified as a key VOC in the Tuber genus. Reports from Southern Europe have identified truffle-indicating flies as species of genus Suillia (Diptera, Heleomyzidae), and rearing of truffles have yielded Suillia species. Trap experiments with truffle VOC:s demonstrated the significant role of DMS in attracting insects, especially Heleomyzidae. The aim of the study was to evaluate the role of DMS in attracting flies and to compare the attracted fly fauna between DMS and truffle traps in order to prove the specific relation between Suillia flies and T. aestivum in Sweden. Field experiments were set-up in Gotland in October and November. Funnel-traps containing either fresh truffle with mature spores or DMS were placed in truffle localities and emptied daily. Netting of observed "truffle flies" was followed by confirming truffle presence with truffle dogs. A Malaise trap was used as reference. Species were morphologically identified. In DMS- as well as truffle-traps exclusively Suillia flies were caught; most numerously in November. The same species occurred in both trap lines and in field nettings. Only few flies of other families were trapped; some parasitic wasps. In the Malaise traps only few Suillia flies were caught. DMS is a specific attractant for Suillia spp. that are equally attracted to mature truffles. The same species are responsible for the indication of truffle presence in the field. Heleomyzidae are cold-resistant flies, and genus Suillia is mainly mycetophagous; thus the connection between Suillia and truffle is logical. The occurrence of parasitic wasps is interesting in respect to parasitoids' known use of host feromones to localize hosts.

# Biological role of triplicated Na, K-ATPase1α genes in the large milkweed bug, with regard to target-site insensitivity against cardiac glycosides

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Plants produce a wide variety of secondary metabolites, including alkaloids, terpenoids and glycosides, many of which serve as antiherbivore or antimicrobial defense compounds. In response, a substantial number of insect species have evolved adaptations specific to the particular defensive compounds of their host plants. The large milkweed bug, Oncopeltus fasciatus, feeds on cardiac glycoside containing plants, which might potentially block their Na, K-ATPase´s. As an adaptation to counter the toxic effects of these cardiac glycosides, milkweed bugs contain three copies of the Na, K-ATPα subunit coding gene (copies A-C). Molecular docking simulations, as well as genetically engineered Na, K-ATPase constructs, suggest that the three copies differ greatly in their sensitivity to cardiac glycosides. Moreover, a preliminary gene-expression study indicates that the two putatively less sensitive copies (A and B) are expressed in the gut where the cardiac glycosides are processed, whereas the putatively more sensitive copy (copy C) is localized to the brain, where the glial sheath likely acts as a barrier against cardiac glycosides. Here we systematically categorize the function and expression patterns of these gene copies using RNAi. We designed copy-specific dsRNA probes, approximately 350-400bp in length, and injected them into adult bugs. Differences in expression of the gene copies were quantified using qPCR. The copy-specific knockdowns were highly effective; there was an over 80% reduction in expression for each copy. Interestingly, the B copy was expressed at a much lower level than the A and C copies. Knockdowns of the A, B and C copies resulted in cross compensation, and knocking out of the C copy was lethal at 3 days post-injection.

# Peptides from plants and animals: ecology connected to drug discovery

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# Phytochemical diversity: structure-activity-relationships in the toxin-receptor interaction between milkweeds and monarchs

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Plants typically produce a stunning array of structurally similar toxins to defend themselves against herbivores. However, the physiological significance of this variation is not well understood and yet is critical to understand the selective forces generating and maintaining phytochemical diversity. The monarch butterfly (Danaus plexippus) sequesters cardenolides (cardiac glycosides) from its host plants (Asclepias spp.) and is exposed to a variety of these toxins which are specific inhibitors of the ubiquitous animal enzyme Na+/K+-ATPase. As a first step to dissect this complexity, we have applied 12 commercially available (non-Asclepias) cardenolides on monarch Na+/K+-ATPase in vitro to test for structure-activity relationships. We found that different cardenolides can have very different inhibitory strength on monarch Na+/K+-ATPase and glycosides act more strongly than the corresponding genins. In a second step we isolated and tested the impact of cardenolides occurring in Asclepias on monarch Na+/K+-ATPase as well as on the more sensitive Na+/K+-ATPase of a related species, Euploea core. Three of the Asclepias cardenolides tested share the genin uzarigenin which has a rare planar configuration. Interestingly, these cardenolides had a much stronger effect on monarch Na+/K+-ATPase than on E. core Na+/K+-ATPase relative to the standard cardenolide ouabain. This may indicate that the specific configuration of Asclepias cardenolides could be a coevolutionary response of plants to the monarch butterfly’s adaptations. Lastly, we have purified calactin and calotropin, the two cardenolides most abundantly sequestered by monarch caterpillars and tested their effect on Na+/K+-ATPase. We found them to be the most toxic ones among all cardenolides tested, suggesting that monarchs optimize their protection against predators by accumulating the most potent toxins. The high toxicity furthermore suggests that calactin and calotropin could have been the evolutionary drivers for Na+/K+-ATPase resistance in monarch butterflies.

# Sponge taxonomy 2.0 meets pharmacognosy and chemical ecology

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Proper species identification and classification is crucial to any scientific study. Naming a species and using a proper classification is the only way to make sure that all the data linked to conspecific specimens but produced by different researchers can be understood, associated and compared. Linking biological data (molecular, morphological, biochemical, ecological) to an incorrect species name or to no species name will result in these data losing tremendous value. This is an important issue in the field of sponge natural products or sponge chemical ecology since 1) the biodiversity of sponges is still poorly known and since 2) sponge taxonomy has been very unstable in the last decades. In fact, in the past 13 years, the classical taxonomy has been considerably overturned by an increasing number of molecular phylogenetic studies, with numerous polyphyletic groups revealed or confirmed and new clades discovered. Based on these results, Morrow and Cárdenas (2015) now propose a revised classification of the Demospongiae, hoping to convince end-users to 1) abandon the use of artificial groups (=polyphyletic), and to 2) use the new/resurrected names when referring to the new Demospongiae clades. I will illustrate with examples from previous studies and from my current research how this revised classification can facilitate communication between end-users, reduce taxonomically biased results, and shed a new light in the fields of sponge natural product and chemical ecology.

Morrow C. and Cárdenas P. (2015) Proposal for a revised classification of the Demospongiae (Porifera). Frontiers in Zoology.

# Volatiles from the burnet moth *Zygaena filipendulae* (*Lepidoptera*) and associated flowers, and their role in mating communication

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Emissions of volatiles play an important role in communication between male and female insects. The burnet moth *Zygaena filipendulae* contains the cyanogenic glucosides linamarin and lotaustralin, which can be degraded to the volatiles HCN, acetone and 2-butanone. Linamarin and lotaustralin have previously been shown to be transferred from male to female during mating and hypothesized to be involved in mating communication. In this study, the volatile emissions from different ontogenetic stages of Z. *filipendulae*, and from flowers inducing mating were measured using head space solid-phase micro-extraction (SPME) GC-MS. All *Z. filipendulae* life-stages emit HCN, acetone and 2-butanone. Virgin females show higher emissions than mated females, while mated males have higher emissions than virgin males. HCN emission was only rarely detected in the course of male-female copulation. This indicates a role of the cyanogenic glucoside derived volatiles during female calling as well as during male courtship behaviour, but not as defence during copulation. Males rejected for mating by a female were accepted after injection of linamarin or lotaustralin, demonstrating that cyanogenic glucosides are important for female assessment of the fitness of the male. Analysis of emissions from males and females as well as from flowers used during mate calling resulted in identification of putative pheromones in *Z. filipendulae.*

# Chemical and molecular characterization of nicotine to nornicotine conversion phenotype in Australian Nicotiana species used as chewing tobacco

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A range of Nicotiana species endemic to Australia are chewed by Aboriginal populations of Australia. Among the preferred species are N. gossei, N. excelsior, N. goodspeedii, N. benthamiana, N. cavicola and N. velutina. However alkaloids levels and nicotine to nornicotine conversion trait vary with species, environmental, and preparation factors. In tobacco research, nicotine to nornicotine conversion has a vital importance because nornicotine affects tobacco quality and has hazardous health outcomes. The molecular identity of the conversion factor in Nicotiana is of great importance. A group of cytochrome P450 genes is reported to be involved in the conversion process.

To determine the alkaloid chemical phenotype of chewed species of Australian Nicotiana spp. from different regions and the responsible locus for the conversion of nicotine to nornicotine in them.

A HPLC technique was validated to quantitate the alkaloids. Leaves were extracted in aqueous MeOH, followed by separation of the alkaloids on a C18 column with a mobile phase of acetonitrile and ethylammonium formate. Total DNA was extracted from the leaves and the conversion locus was PCR amplified. Agarose gel electrophoresis was performed subsequently. Regions of the gel containing the amplified DNA fragments of interest were excised and purified for sequencing.

The quantification results were used for determining the conversion trait of species. The studied species were classified into non-converters (no nornicotine detected), medium converters (<50% conversion) and high converters (>50% conversion). The results indicated that N. gossei the most important chewed species is a non-converter, while N. benthamiana and N. excelsior are medium converters and N. goodspeedii, N. cavicola and N. velutina are high converters of nicotine to nornicotine. Gel electrophoresis results confirmed the presence of the conversion locus in all of the studied species. Therefore, the expression level of the genes is responsible for determination of different conversion chemotypes in them.

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# CHEMICAL ECOLOGY OF VERTEBRATE

# The sick sense is in the nose

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Although sociability offers many advantages, a major drawback is the increased risk of exposure to contagious pathogens, like parasites, viruses, or bacteria. Social species have evolved various behavioral strategies reducing the probability of pathogen exposure. In rodents, sick conspecific avoidance can be induced by olfactory cues emitted by parasitized or infected conspecifics. Our aim is to identify the chemical stimuli and the neural circuits involved in this behavior.

# Of mice and amines: genetic analysis of odor perception

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Chemical stimuli are represented in the main olfactory pathway by over 1,000 odorant receptors, each of which is mapped to specific glomeruli in the olfactory bulb. The Trace Amine-Associated Receptors (TAARs) are a small, additional class of evolutionarily conserved olfactory receptors that may mediate innate responses to social cues or predator-derived odors in rodents. Using gene targeting in mice, we have mapped the TAAR projections to the olfactory bulb for the first time, and have characterized the functional properties of these receptors in vivo. Our data reveal that the TAARs define a genetically and anatomically distinct subsystem that is selectively activated by presence of volatile amines. Genetic manipulation of the TAAR gene family is further allowing us to assess the contribution of these receptors to olfactory driven behaviors.

# Host Immune Function and the Volatile Metabolome

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An animal's volatile metabolome contains information about individual health status. Volatiles indicative of illness are often avoided and may modulate mate choice, parental care and other behaviors. We hypothesized that disease related alterations of the volatile metabolome were controlled in some manner by the host's own immune system. This hypothesis was tested using two independent assays of urines collected from mice subjected to immunization, inflammation, or injury. In bioassays, animal biosensors were trained to discriminate between urine samples derived from treated and control donors on the basis of volatile odors. In chemical assays, urines were subjected to dynamic headspace gas chromatography mass spectrometric analyses and chemometric modelling of the chromatographic data. Both trained biosensors and linear discriminant analysis models yielded excellent discrimination of urines from treated mice versus controls on the basis of volatile metabolites. Alterations of the volatile metabolome resulting from i.p. administration of killed rabies vaccine, live-attenuated West Nile Virus vaccine, lipopolysaccharide (potent inflammatory activator), or lateral fluid percussion brain injury (a mouse model for moderate traumatic brain injury) were observed within days of treatment and persisted for several weeks. Alterations (both up and down regulation) of endogenous metabolites varied among treatments, but no novel metabolites were identified. These results support our hypothesis that volatile responses to injury or insult are mediated, in part, by the host's immune system and raise the possibility of monitoring health via changes in the volatile metabolome.

# Does Fel d 1, the main cat's allergen, have a kairomone role?

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Different models of samples and animals have been used to obtain a predatory response under controlled conditions in mice. These experiments relied largely on the use of cats (Apfelbach et al. 2005). Previous studies have shown a kairomone role of Fel d 4 (Papes et al. 2010), a minor cat's allergen from the lipocaline family. Fel d 1 is the main allergen and long lasting molecule released in the environment by cats (Nicholas et al. 2008). It belongs to the secretoglobine family and is produced in large amounts in the sebaceous glands of the skin, especially in the cheeks' area. May et al (2012) found an effect of the cheeks rubbing marks of domestic cats decreasing the feeding behaviour in rats.

The aim of our study was to determine if a solution containing high amounts of Fel d 1 extracted from washes of chest and cheek zones of cats could alter feeding and exploratory behaviour in mice.

Six cats (males, females and castrated males) were used for sampling. The pooled sample contained 18.6 µg/ml of Fel d 1 and three Fel d 1 molecular forms, according to ELISA and Western-Blot analysis respectively.

Twenty-one mice RjOrl:Swiss (males and females) were used for behavioural essays. Tests were conducted in an 8 arm rectangular maze, during 10 minutes. Every arms contained flour wheat as an attractive stimulus and Fel d 1 or placebo solution on a gauze at their entrance.

No significant differences were observed for the number of entrances in tubes (P=0.42), feedings (P=0.97), or remaining time (P=0.76). No significant differences were observed between sexes. Our results suggested that Fel d 1 did not trigger a predatory response and so did not have a kairomone role for mice. Conversely, Fel d 1 may play a role in intraspecific communication.

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# How blue Petrels find their scented burrow?

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Sensory ecology, bird olfaction and emissions of volatile organic compounds (VOCs) are understudied. The contribution of olfaction to avian behaviour has been largely ignored by ornithologists and emphasis has generally been placed on vocal and visual signals. However, recent studies provided evidence that olfaction plays a fundamental role in the avian ecology, especially in hypogean petrels.

Petrels are known to return by night to the same nest with the same partner in the same colony each year. This nocturnal behaviour is probably driven by predation pressure from skuas and gulls. Latest investigations strongly suggested that birds use at least olfactory cues in nest and partner recognition with emission of specific and complex chemical labels. Olfactory cues that lead petrels towards the burrow entrance, might have a variety of sources such as owner's feathers and glandular excretions and plants. The ultimate carrier of these secretions is the plumage and the characteristic musky scent of petrels emanates only from it. The chemical composition of nest odour, the nature of components that facilitate recognition even after a yearlong absence, as well as the extent and nature of variation between nests, years, seasons, etc. remain unknown. Surprisingly, no study to date has focused on these scents. We proposed in this work to characterize chemically the composition and identity of VOCs emanating from plumage and nests to assess presence of such scent profiles. Samples of plumage and VOCs from nest were collected from breeding blue Petrels in the Kerguelen Islands, Southern Indian Ocean and analysed using GC-MS methods. Results suggested that nests carry a specific bouquet, but differ from bird scents. However, this is not excluding that some components of individual scents stay in the nest and help to the recognition of burrow each year.

# Sex pheromone dynamics during oestrous cycle in dairy cows

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In many mammal species the oestrous female signals its receptivity by means of chemical signals which could be used in practice for determination of the optimal insemination time. In cows, a sex pheromone consists of acetic acid, propionic acid and 1-iodo undecane [1]. These compounds were extracted from faeces and were only detectable during oestrus. The aim of the present study is to determine changes in the amounts of pheromone components, during the oestrous cycle of cow. Faecal samples were collected from Holstein cows with naturally occurring and hormone induced oestrus for monitoring long and short term dynamics of the secretion of pheromone components, respectively. The ovulation period was determined by ultrasound technique or by measuring progesterone levels in a blood. Solid phase micro extraction and GC-MS techniques were applied for the collection and determination of amounts of pheromone components, respectively. Two of the three pheromone components, acetic and propionic acid were detected in the headspace of faeces. Contradictory to the results published [1], we have found that both acids were present in faecal samples throughout the entire oestrous cycle. In cows with clearly recognizable oestrus, random changes of the concentration of two pheromone components were observed until 24 hours before ovulation, than concentration of acetic and propionic acids rose, and remained at a high level for about 12 hours, dropping to dioestrus levels thereafter. A strong negative correlation was detected between the amounts of progesterone and the two sex pheromone components during pre-oestrus and at the first day of oestrus which is a critical period in respect to optimal insemination time. The comparison between cows with natural and hormone induced oestrus revealed that the changes in the amounts of acetic and propionic acids in the faecal samples obtained in pre-oestrous and oestrous periods occur in the similar manner.

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# Major urinary protein (MUP) profiles show dynamic changes rather than individual 'barcode' signatures

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Male house mice produce large quantities of major urinary proteins (MUPs), which bind and transport volatile pheromones to urinary scent marks. It has been suggested that MUP isoform profiles are highly polymorphic and provide a unique signature that mediates individual and kin recognition (barcode hypothesis). This hypothesis assumes that MUP profiles show high inter-individual variability and high intra-individual consistency, and we conducted the first study to test these assumptions. We analyzed urinary MUP profiles of 66 wild-caught house mice (*Mus musculus musculus*) from 8 populations using isoelectric focusing. We found that MUP profiles were not individually unique, and the variation depended on the type of isoform. Most proteins (the major isoforms) were surprisingly monomorphic, but the smaller ones were variable and therefore candidates for individual barcodes. To test this hypothesis, we examined changes in 58 mice over time, and found that individual MUP profiles were surprisingly dynamic and the minor isoforms showed the most fluctuation. Thus, most MUP isoforms were monomorphic, and though we found minor isoforms that were variable among individuals, they showed dynamic changes over time within mice. Nevertheless, we found more similarities in MUP profiles between siblings than non-siblings. Our findings challenge the hypothesis that MUPs contain individual signatures, though it is feasible that they mediate kin recognition in house mice.

# PLANT-INSECT INTERACTIONS

# Experience to plant cues affects host plant choice behaviours in a moth

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Selection of a suitable host plant is crucial for herbivorous insects. In many species host plant selection requires that they can handle and integrate information from many potential host plants. Earlier experiences to plant cues could facilitate host plant choice decisions in complex environments by focusing search behaviour and reducing effects of neural limitations.

We have studied experience-driven phenotypic plasticity in host plant choice in the polyphagous moth *Spodoptera littoralis*. The host plant preferences of experienced and naïve moths was studied. Female preferences were tested in oviposition experiments, while in males we tested their attraction to sex pheromone with a background of plant odour in windtunnel experiments.

We found that the adult moth host plant choice was guided by a stable plant preference hierarchy, which can be modified by larval experience. The larval rearing host plant was in general elevated to the most preferred plant for the adult. Furthermore we have demonstrated that mating experience on a plant modulates plant preference in subsequent reproductive behaviours, whereas exposure to the plant alone or plant together with sex pheromone does not affect this preference. When exposing individuals to both larval feeding and mating experience experiences, we found that both experiences modulate host plant preference.

Our results show that *S. littoralis* change their host plant preferences based on experience to plant cues during specific behavioural events. They also show that different experiences may interact to modulate host plant choice behaviours.

# Floral scents to the touch: scents inform moth's proboscis for probing and foraging, thus determining the plant fitness

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It is assumed that floral scent functions as long distance attractants, whereas flower fine handling is guided by visual and tactile cues (Goyret et al. 2007, Goyret 2010), but it is known that flowers emit volatiles from specific tissue areas. Field observations of *Nicotiana attenuata* show that visitation rates of scenting (EV) and non-scenting (CHAL) flowers are similar while nectar removal is significantly higher in plants producing floral scent, and a lack of floral scent leads to reduced fitness (Kessler et al. 2008). If visitation rate is the same then how differential fitness is explained? And will the scents work only at long distance?

We conducted tent and wind tunnel experiments to observe the behavior of moth *Manduca sexta*, in both scenarios visitation rate was the same but not the foraging rate. The moth spends more time in probing CHAL flowers, by inspecting the outer corolla limb, but similar foraging time compare to EV flowers. We assessed the fitness in the wind tunnel by manually adding pollen to the proboscis and letting the moth visit either EV or CHAL flower sets at a time. Pollinated flowers were allowed to set seeds and results showed a differential pollen transfer, with higher number of capsules and seeds in EV flowers compare to CHAL. We hypothesize that *M. sexta's* proboscis works as a chemo-tactile extension while probing the corolla limb surface for signals such as benzylacetone (BA), the major bouquet component. Electron microscopy imaging revealed several sensilla in the proboscis surface that can potentially fill this role. BA emission is very important for the flower handling efficiency of *M. sexta* and is essential to ensure pollination (maternal fitness). BA thus plays a large role as a short range cue, besides navigational cue.

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## Ozone pollution compromises within-plant signalling via volatiles

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In response to attack by herbivores, plants release a complex bouquet of volatile organic compounds (VOC), both locally and systemically. In addition to mediating a wide array of interactions between plants and their associated organisms, plant VOCs released from damaged parts have recently been shown to trigger a systemic defense response in as yet undamaged parts of a plant, which has long been documented to be initiated by internal vascular signals. However, until now, the phenomenon of within-plant signalling by VOCs has been described for only five plant species – sagebrush, lima bean, poplar, blueberry and birch, and among them only studies with sagebrush and lima bean were conducted in the field. Therefore, there is still ongoing debate on the commonness of VOC-mediated within-plant signalling in nature. Furthermore, the co-existence of abiotic factors may affect the likelihood of occurrence of this process. One such abiotic factor – tropospheric ozone – has been shown to not only alter VOC emission patterns, but also react rapidly with many VOCs in the atmosphere. As a consequence, VOC-mediated interactions between plants and other organisms are compromised in the ozone-polluted atmosphere. For example, several studies have found that elevated ozone concentrations reduce the foraging efficiency of arthropod herbivores and their natural enemies, as well as the distance over which airborne plant-plant communication occurs. Yet, no attempts have been made to assess the impacts of ozone pollution on VOC-mediated within-plant signalling. Here, we used a system consisting of hybrid aspen (*Populus tremula* × *tremuloides*) and a specialist leaf beetle (*Phratora laticollis*) to demonstrate that VOC-mediated within-plant signalling occurs in both laboratory and field conditions. More importantly, we will present both field and laboratory data to address the role that moderate ozone levels can play in influencing this process.

# Aboveground application of elicitors recruits an entomopathogenic nematode belowground

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Plant hormones play important roles in regulating developmental processes and signaling networks that mediate plant responses to a wide range of abiotic and biotic stresses. In particular, stimulation of the salicylic acid and jasmonic acid pathways are important plant responses to infection and herbivory. Here we investigate the effects of aboveground foliar application of the elicitors methyl salicylate (MeSA) and methyl jasmonate (MeJA) on belowground recruitment of the entomopathogenic nematode *Steinernema diaprepesi*. In four arm olfactometers, citrus plants treated with foliar applications of MeSA recruited *S. diaprepesi*. Citrus plants treated with foliar applications of MeJA did not recruit significantly more *S. diaprepesi*. Analysis of root volatile profiles of citrus plants receiving foliar application of MeSA revealed production of D-limonene that was absent in controls. Two choice olfactometer trials with D-limonene suggest that this compound may be responsible for recruiting *S. diaprepesi*. These results suggest that aboveground stimulation of citrus plant defenses may have ramifications for belowground multitrophic interactions.

# Both volatiles and epicuticular plant compounds determine oviposition of the willow sawfly *Nematus oligospilus* on leaves of *Salix* spp. (Salicaceae)

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Plant volatiles and contact cues play a role in selection and acceptance of host plants by herbivorous insects. Here we studied volatile and contact cues used by the willow sawfly *Nematus oligospilus* (Hymenoptera: Tenthredinidae) to seek and accept its host plant. First, we recorded behavioral orientation in a Y-tube olfactometer of willow sawfly females to volatiles of the highly preferred genotype *Salix nigra* and the non-preferred genotype *S. viminalis*. The volatiles released by undamaged plants were analyzed by coupled gas chromatography-mass spectrometry. Afterwards, we recorded oviposition preference between intact leaves, and leaves in which their cuticular wax layer was removed by means of Arabic gum treatment. Contact cues were evaluated by studying the micromorphology and chemical composition of abaxial and adaxial leaf surfaces. Willow sawfly females oriented preferentially to *S. nigra* volatiles, which contained significantly higher amounts of (*Z*) and (*E*)-β-ocimene, undecane, decanal, and β-caryophyllene. Once on the plant, sawflies laid fewer eggs on *S. nigra* leaves after Arabic gum treatment, showing the importance of cuticular wax layer. No differences were found among the micromorphology of the leaf surfaces between preferred and non-preferred genotypes. Chemical composition of the cuticular waxes showed higher quantity and diversity of long chain alcohols in the preferred genotype that might be related to oviposition. Our studies suggest that several cues act in concert to provide oviposition cues for *N. oligospilus*: females are attracted to volatiles from a distance and, once alighting on the plant, they seek specific chemical contact cues in order to lay eggs.

# Become a Specialist with an Enzyme - The Senecionine-Monooxygenase of *Longitarsus jacobaeae*

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The flea beetle *Longitarsus jacobaeae* is one of only a few animals specialized to feed on the tansy ragwort (*Senecio jacobaea*). This common wild flower is highly toxic, containing pyrrolizidine alkaloids, mainly senecionine-N-oxide, which function as a defense mechanism against herbivores. Ingestion of these compounds causes hepatotoxic or even lethal effects not only in insects, but in farm animals as well.

In this study we investigated which physiological adaptions allow *Longitarsus jacobaeae* to feed unharmed on *Senecio jacobaea*. The challenge in dealing with pyrrolizidine alkaloids lies in their conversion from N-oxides into tertiary alkaloids in the herbivores' gut, as this is where the toxicity normally originates. Once reduced to the lipophilic tertiary alkaloids, pyrrolizidine alkaloids can passively cross the gut membrane. One possible solution to this problem would be to immediately reconvert the tertiary alkaloids after intake. Flavin-monooxygenases are enzymes known to perform such conversions, and thus we search for and identified two sequences in the beetle's transcriptome that are similar to known flavin-monooxygenases. Based on this information we preformed tissue-specific gene expression analyses. We found clear tissue-specific expression for both genes. In addition we expressed and harvested the two genes as recombinant proteins in Sf9 cells. Activity tests with tertiary senecione and the co-factor NADPH +H+ demonstrated that the flea beetle has two senecionine-monooxygenases which are able to specifically convert tertiary senecionine into harmless N-oxides.

# Keeping your food fresh: active manipulation of cytokinin-metabolism by a cell content feeder

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Cytokinins play a central role in plant physiology, including regulation of senescence and nutrient translocation. Recent studies have revived interest in their role in plant-insect interactions. At the end of the sixties, some scientists suggested for the first time that phytophagous insects might manipulate cytokinin levels in the tissues where they feed to increase their sink strength (Engelbrecht et al., Nature, 1969). Here, we present our results on the interactions between the cell-content feeding mirid *Tupiocoris notatus* and the wild tobacco *Nicotiana attenuata*. Using highly sensitive LC-MS techniques, we detected two types of active cytokinins present in mirid bodies: isopentenyl-adenine (IP), and isopentenyl-adenosine (IPR). Surprisingly, the free base IP was ten to fifty times as concentrated in mirid bodies as in the leaf tissues where *T. notatus* normally feeds. By using $N^{15}$-labeled plants, we showed that *T. notatus* specifically transfers these two types of cytokinins into the leaves on which it feeds. The effects of *T. notatus* damage on the physiology of tobacco leaves was assessed by determining the concentration of soluble sugars, soluble proteins, free amino acids, and photosynthetic parameters over a time course during mirid attack. Responses were compared in wild-type plants and in transgenic plants with manipulated levels of cytokinins or impaired cytokinin perception. Even when insects had damaged the majority of the leaf-surface, levels of nutrients remained close to levels in undamaged controls. In contrast, plants with altered cytokinin metabolism and signalling showed larger changes in nutrient levels during *T. notatus* feeding. Our results suggest that *T. notatus* compensates for the damage it causes by manipulating cytokinin signalling in damaged leaves.

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# FOREST CHEMICAL ECOLOGY

# Terpenoid resin defenses in Norway spruce: Herbivore induction and insensitivity

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One of the best known anti-herbivore defenses of forest trees is the terpenoid-rich resin of conifers, colorfully described as a "sticky glop" of toxic and repellent compounds that exudes from ducts in needles and stems when these tissues are injured. A principal feature of resins is their inducibility by herbivory. A recent bark beetle outbreak in Sweden gave us a special opportunity to demonstrate the importance of inducibility in resistance to herbivory under natural conditions. Norway spruce (*Picea abies*) trees that were attacked by bark beetles (*Ips typographus*) but survived were shown to have significantly higher amounts of induced monoterpenes and diterpenes than trees killed by beetle attack.

To understand more about the mechanism of resin induction, we have studied the inducibility of genes, enzymes and intermediates of terpene biosynthesis. An isoprenyl diphosphate synthase that produces the precursors of both monoterpenes and diterpenes was found to be induced by damage and located in cells adjacent to the resin ducts. Over-expression of the corresponding gene in Norway spruce saplings increased the level of gene transcript, enzyme activity and intermediates, but not the level of resin terpenes. Instead, a group of C20 prenylated fatty acid esters accumulated in the transgenic saplings, which were also found to have anti-herbivore properties.

Herbivores that feed readily on terpene-rich conifer tissues must possess some sort of adaptations to avoid their toxicity. We are exploring the role of bacterial symbionts in detoxification of terpene resin compounds in the pine weevil (*Hylobius abietis*), a damaging pest of conifer seedlings in Sweden and elsewhere in Europe. Weevils treated with antibiotics metabolize terpenes at a lower rate and suffer reduced performance on terpene-rich diets. A better understanding of how terpene resin is induced and how herbivores cope with it should suggest new approaches to protecting conifers from herbivore damage.

# Defense priming in Norway spruce: chemical and molecular evidence

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Conifers have complex preformed and inducible defense mechanisms to protect themselves from attack by insects and pathogens. The tree's natural defenses are the most important factor maintaining the beetle-fungus complex at low, endemic levels. Recent work has shown that spraying of spruce trees with the plant hormone methyl jasmonate (MeJA) can enable trees to respond more quickly and strongly when challenged several weeks after treatment, although initial changes in defense responses is not always observed. This phenomenon, known as defense priming, is a physiological process by which a plant is prepared to respond more quickly or aggressively to future biotic or abiotic stress. Priming in itself does not lead to increased resistance, but rather initiates a state of readiness that allows for accelerated induced resistance if an attack occurs. In conifers, the underlying molecular mechanisms of defense priming are unknown, but primed trees seem to be in a state where they are able to respond very efficiently to subsequent attacks. In this presentation we will present some chemical and transcriptomal aspects of defense priming in Norway spruce and discuss the dual roles of methyl jasmonate as inducer of tree defenses and trigger of defense priming.

# Fungal symbionts of the spruce bark beetle synthesize aggregation pheromone and reduce tree defense monoterpenes

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Norway spruce have well-developed resistance mechanisms against invaders, including the production of abundant terpenes (Franceschi et. al 2005). To survive inside this well-protected tree species, spruce bark beetles have evolved several strategies to overcome tree resistance. The symbiotic relationship with bluestain fungi, together with effective aggregation pheromones, is thought to be the key to the beetles' ability to kill healthy trees (Christiansen and Bakke 1988). However, little is known about the precise mechanisms by which fungi exhaust tree defences and contribute to tree-killing. In this study, we incubated five major fungal associates of the spruce bark beetle on Norway spruce bark and malt agar, and investigated the volatiles present in the headspace of each fungus using solid phase microextraction (SPME) coupled to gas chromatography-mass spectrometry (GC-MS). Fungal associates of spruce bark beetles produced a large array of metabolites including bark beetle aggregation pheromone components 2-methyl-3-buten-2-ol. In addition, the fungi also demonstrated considerable abilities to reduce the concentrations of the defense monoterpenes in tree bark. *Grosmannia penicillata*, a fungi with the strongest ability to grow in Norway spruce bark, dramatically decreased monoterpene, but enhanced oxygenated monoterpene emission from the bark. These results suggest that fungi associated with spruce bark beetle contribute to the aggregation behavior and facilitate tree colonization of spruce bark beetle. This study thus provides new insights into the possible ecological roles of fungal symbionts in bark beetle system.

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Franceschi VR, Krokene P, Christiansen E, Krekling T. 2005. Anatomical and chemical defenses of conifer bark against bark beetles and other pests. New Phytologist 167(2):353-375.

# A common fungal associate of the spruce bark beetle metabolizes the stilbene defenses of Norway spruce

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Norway spruce (*Picea abies*) forests suffer periodic fatal attacks by the bark beetle *Ips typographus* and its fungal associate, *Ceratocystis polonica*. *P. abies* protects itself against fungal and bark beetle invasion by production of terpenoid resins, but it is unclear whether resins or other defenses are effective against the fungus. We investigated stilbenes, a group of phenolic compounds found in *P. abies* bark with a diaryl-ethene skeleton with known antifungal properties. During *C. polonica* infection, stilbene biosynthesis was up-regulated as evidenced by elevated transcript levels of stilbene synthase genes. However, stilbene concentrations actually declined during infection and this was due to fungal metabolism and use as a carbon source. Candidate genes involved in polyphenol catabolism which were up-regulated after infection were discovered. Biochemical characterization of the enzymes coded for by these genes revealed that they could form either ring-opened lactones, deglucosylated or dimeric products from spruce stilbenes. A correlation was also observed between the activities of these enzymes in vivo and the virulence of the fungus during spruce infection.

# Improved forest regeneration by triggering the induced defense of conifer seedlings against bark-feeding insects

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Only in Sweden 360 million conifer seedlings are planted each year but a substantial proportion of these are killed by pine weevils (*Hylobius abietis*) and black spruce beetles (*Hylastes cunicularius*). A high survival rate is essential for a high primary production of forest raw materials and biomass. There is currently no suitable non-insecticide plant protection option for a large part of these seedlings. One option may, however, be to trigger the seedlings´ own induced defense by treating them with a plant activator at the nursery so that they are prepared when they later becomes attacked by pine weevils in the field.

The induced defense is under natural conditions generally expressed after damage by, e.g. a pest insect, whereas constitutive defenses are permanently present. Constitutive defenses serves to inhibit an initial attack whereas the induced defense serves to actively and vigorously stop the attack. The induced defense is powerful but the time lag after an initial attack before the induced defenses are fully expressed may be detrimental for a plant when the pest pressure is high. This weakness of the induced defense can be circumvented by applying a plant activator to trigger the induced defense of the plant before it is exposed to attacks.

There is a suitable plant activator to trigger the induced defense of conifers available. That is methyl jasmonate which is a substance that also is produced naturally when a plant is attacked. It triggers the production of traumatic resin channels and different types of chemical defense substances. In this talk I will present some recent results related to the potential of this method to protect conifer seedlings against pine weevils.

# Insect egg deposition - a warning signal enhancing sex-biased anti-herbivore defence in a tree

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Insect eggs can be taken by plants as a warning signal of future herbivory and lead to intensified or accelerated plant defence responses. However, little knowledge is available on this phenomenon in perennial plants. We investigated if and how prior egg deposition by the chrysomelid beetle *Xanthogaleruca luteola* affects defence of the European field elm *Ulmus minor*. The elm leaf beetle is a multivoltine specialist herbivore of elm.

Insect performance parameters (survival and weight of larvae and adult beetles) were compared for egg-free and previously egg-deposited elms. Furthermore, chemical leaf parameters were analysed by especially HPLC and enzymatic assays. We compared untreated control leaves, egg-free and egg-deposited leaves with and without feeding damage.

Increased larval mortality, a reduced number of female beetles and reduced weight in adults resulting from surviving larvae were observed on egg-deposited elms. A comparative analysis of the amounts of the measured leaf chemicals consumed by larvae as well as a spectrum-wide comparison of these chemicals revealed that especially a decrease in total nitrogen content and increase in phenolic compounds contributed to these detrimental effects of egg-deposited elm leaves on insect performance.

The sex-biased increase in insect mortality with fewer females resulting from the surviving larvae may limit the abundance of later herbivore generations. These striking effects were caused by only minor shifts in the quantities of some leaf compounds resulting in clearly separable nutritional patterns of elm leaves. Our results indicate that only low metabolic investments are required by the perennial plant in order to successfully restrain performance of a multivoltine herbivore.

# Impact of interactions among native biotic disturbances on range expansion of mountain pine beetle into novel jack pine forests

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With recent range expansions of species due to climate change, it is important to understand the impact of endemic biotic disturbances on exotic species. Our research investigated the expansion of mountain pine beetle (*Dendroctonus ponderosae*) into the novel host jack pine (*Pinus banksiana*), which is an ecologically and economically important component of the Canadian boreal forest. We focused on plant secondary compounds to understand interspecies interactions and identified the impact of induced host defense compounds due to the infection of a widespread native parasitic plant (dwarf mistletoe, *Arceuthobium americanum*) on the success of mountain pine beetle. Although mistletoe-induced changes to host physical characteristics negatively affected mountain pine beetle, the chemical changes due to mistletoe infection reduced the competitive effect of the subcortical insect community on mountain pine beetle performance. Furthermore, the host susceptibility to mountain pine beetle-associated fungi was affected by the mistletoe-induced changes in chemical defense concentrations. We show that native plant pathogen-induced defense chemicals can influence interspecific interactions, which have important implications for community dynamics and the maintenance of mountain pine beetle in a novel host.

# Intraespecific variation in constitutive and inducible defensive allocation in Maritime pine, a model Mediterranean species

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Maritime pine has many isolated and highly differentiated populations, ranging from stressful Mediterranean climates to benign Atlantic coastal areas in Southwestern Europe. Differentiation processes in phenotypic traits such as drought tolerance, reproductive behaviour and growth potential has been suggested to be adaptive. However little is known about the differentiation in defensive traits and strategies. In this paper we will summarize the results from several field and greenhouse experiments focused on identifying the sources of phenotypic variability of constitutive and inducible allocation to chemical defences in this model species. We have studied genetic variation within and across populations, resource availability and other factors such as ontogeny, within plant variation and herbivore identity. We usually find significant variation in defensive strategies across populations, and as well that families within populations differ in the constitutive and induced expression of defensive chemical traits. Particularly when analyzing terpenoids, we usually find high narrow sense heritability for the concentration of many individual terpenoids species. We also found that inducibility of those compounds (in response to real herbivory or methyl jasmonate application) is also usually genetically variable. Constitutive and inducible allocation to chemical defences are tissue specific, part specific and also compound specific and as well depends on the nature of the herbivore damage. Altogether, these results suggest that, in spite the pressure exerted by herbivores, existence of genetic variation in defensive traits is the common pattern in pine trees, and it is quantitatively relevant. However, although we have found that families differ in effective field resistance against several pests and pathogens, we usually fail to find significant genetic correlations among individual chemical traits and effective field resistance. Trade-offs between defences, between defensive strategies, and between defences and other life history traits have likely influenced the co-differentiation processes in defensive traits and strategies in this species.

# The mesquite borer *Megacyllene antennata* produces an aggregation-sex pheromone composed of floral and green leaf volatiles

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The pheromone biology of the longhorned beetles (Cerambycidae) has been largely defined by a few highly conserved structures that may be shared by hundreds of species. In contrast, species of the cerambycid genus *Megacyllene* produce complex pheromone blends that are instead characterized by diverse monoterpenes and aromatic alcohols. Here, we identify the aggregation-sex pheromone of the mesquite borer, *M. antennata*, which is native to the mesquite forests of southwestern North America. Males produce at least eight sex-specific compounds: (*S*)-α-terpineol, (*E*)-2-hexen-1-ol, (*E*)-2-hexenal, 1-hexanol, (*R*)-1-phenylethanol, 2-phenylethanol, terpinolene, and (*S*)-limonene. The blend is dominated by α-terpineol and (*E*)-2-hexen-1-ol, but (E)-2-hexenal, 1-hexanol, and (R)-1-phenylethanol elicited strong antennal responses in electroantennography. Field trials identified the combination of α-terpineol and (*E*)-2-hexen-1-ol as a minimum attractive blend. Males do not produce these components in any consistent ratio, and additional bioassays confirmed that the dual component lure was attractive over a tenfold difference in ratio. These two compounds are ubiquitous floral and vegetative volatiles, and it remains unclear how this pheromone signal can retain its specificity against a backdrop of plant volatiles.

# Black poplar volatiles in biotic interactions

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Plants emit volatiles constitutively but specifically when they are attacked by insect herbivores. Numerous studies on short-lived herbaceous plant species have demonstrated that these herbivore-induced volatiles (HIPV) play an important role in direct and indirect plant defense and communication. In contrast, our knowledge on the defensive role of volatiles from long-lived woody plant species is limited and especially scarce with regard to natural field conditions. I will summarize the recent findings from my group on black poplar (*Populus nigra*) volatile emission with respect to temporal dynamics, herbivore-species specificity as well as the role in defense. We observed that black poplar trees release an enormous diversity of HIPVs some of which are also abundant in taxonomically unrelated plant species. Among these are DMNT, (*E*,*E*)-α-farnesene, (*E*)-β-caryophyllene, (*E*)-β-ocimene and green leaf volatiles like (*Z*)-3-Hexenyl acetate and (*Z*)-3-Hexenol. Contrary to expectations our results show that not the major compounds but a group of minor, nitrogenous volatiles play the most important role in the HIPV-mediated defense of poplar. I will discuss why we consider these minor volatiles as key players in poplar tree defense.

# Chemical and transcriptome analysis of resistant and susceptible *Eucalyptus* genotypes to the insect pest *Leptocybe invasa*

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Plants have evolved complex systems of defences to protect themselves against pests and pathogens. These include physical or chemical barriers with toxic, repellent or anti-nutritive properties. Little is known about the biotic defences of *Eucalyptus*, but they are known to produce a wide range of specialised metabolites, including terpenes, that vary quantitatively and qualitatively within a species and may affect biotic interactions. *Leptocybe invasa* is one of the most damaging pests in global *Eucalyptus* forestry and little is known regarding the molecular mechanisms governing the interaction between pest and host. The aim of this study was to investigate changes in the transcriptional profile and plant specialised metabolites of resistant and susceptible *Eucalyptus* genotypes in an effort to improve our understanding of this interaction.

Two genotypes were identified that exhibit differences in their response to *L. invasa*. One genotype (*Eucalyptus grandis* TAG 5) was fully susceptible and developed extensive galls, while the other (*Eucalyptus grandis* x *camaldulensis* GC 540) did not develop any galls. RNAseq was employed to investigate transcriptomical changes in uninfested and infested leaves from both genotypes. Quantitative RT-PCR was used to validate differential gene expression. Terpene concentrations in uninfested and oviposited leaves were investigated using gas chromatography coupled with mass spectroscopy.

We found 698 and 1115 significantly differentially expressed genes from the resistant and susceptible interactions, respectively. Gene ontology enrichment and Mapman analyses identified putative defence mechanisms including cell wall reinforcement, protease inhibitors, cell cycle suppression and regulatory hormone signalling pathways. There were significant quantitative and qualitative differences in mono- and sesquiterpene abundance both between genotypes and between control and infested material. We found that the changes in terpene profiles were accompanied by corresponding changes in the transcription of biosynthetic pathway genes. A model of the interaction between *Eucalyptus* and *L. invasa* was proposed from the transcriptomic and chemical data.

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

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The shoot beetles *Tomicus minor, Tomicus yunnanensis,* and *Tomicus brevipilosus* have been decimating *Pinus yunnanensis* trees for more than 30 years in southwest China. To understand the chemical ecological relationship between pines and Tomicus, and among the three beetle species, we compared the attraction of the damaged and undamaged Yunnan pine bolt bundles and shoots. We found that three species were attracted by the serious infested Yunnan pine bolt bundles than the un-infested control and the male *T. minor* and both sexes of *T. brevipilosus* were more strongly attracted to damaged shoots than to undamaged shoots and they showed attraction to shoots damaged by the same species. Female *T. minor* and both sexes of *T. yunnanensis* were attracted to shoots damaged by female *T. brevipilosus*. The three beetle species were attracted to shoot extracts and dynamic headspace volatiles from shoots damaged by the same species and sex. Female *T. minor* and male *T. yunnanensis* were also attracted to dynamic headspace volatiles from shoots damaged by both sexes of T. *brevipilosus*. The main semiochemicals which regulating the aggregation behaviours and interspecific interactions were identified by GC, GC-MS, EAG, walking bioassay and field bioassay.

Keywords: *Tomicus*, *Pinus yunnanensis*, walking bioassay, field bioassay, semichemical

# Studies on the chemical communication of the bronze bug, *Thaumastocoris peregrinus* (Heteroptera: Thaumastocoridae), a pest of *Eucalyptus*

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The bronze bug, *Thaumastocoris peregrinus* (Heteroptera: Thaumastocoridae), is an exotic emerging pest in *Eucalyptus* commercial forests in South America, Africa and southern Europe. Information on the chemical communication system and reproductive ecology of this insect is scant, and it may be relevant for designing management strategies for eucalypt plantations. Adults and nymphs usually aggregate in the field, possibly by means of chemical signals. We will summarize our recent findings on these chemical signals, as part of an ongoing effort to further understand the role of pheromones in the mating behavior of *T. peregrinus*, and the eventual practical applications that may be derived for the management of the insect in commercial *Eucalyptus* forests. Males emit large amounts of 3-methyl-2-butenyl butyrate, which attracts conspecific adult males but not females. Males emit this compound following a circadian rhythm, but the presence of females results in a decrease in the amount emitted by the males. Nymphs do not emit the compound, but late-instar male nymphs are attracted both to adult male volatiles and to synthetic 3-methyl-2-butenyl butyrate, whereas female nymphs show no attraction. The ecological role of this putative male aggregation pheromone remains unknown. The compound appears to play a role in the formation of nymph/adult aggregations, but the relevance of the specific intra-gender inter-stage attraction remains to be explained. Possible explanations are the exploitation of food resources, or a reproductive strategy for newly-emerged males. Also remains unknown why males emit 3-methyl-2-butenyl butyrate following a circadian rhythm, and why the presence of females decrease the amount emitted by the males, while they themselves show no attraction towards the compound.

# Norway spruce ATAF1-like NAC transcription factors modulate stress.

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Norway spruce (*Picea abies*) is an economically very important production tree species in Europe. Stem and root rot of Norway spruce caused by *Heterobasidion annosum s.l.* create significant economic losses, in Sweden alone the costs are estimated up to 2 million SEK every day! Thus breeding for resistance has a very high potential for economic and biodiversity gains.

We have previously identified metabolite and gene induction patterns that show correlation to the level of resistance to *H. annosum* in four genotypes of Norway spruce [1]. In the current study we focused on NAC transcription factors Norway spruce differentially regulated in response to *H. annosum* in these four Norway spruce genotypes.

Our analyses identified a number of transcripts with significant similarity to *Arabidopsis* clade III-3 NACs which include the well-characterized repressors of plant defense responses ATAF1, ATAF2, ANAC019 and ANAC055 [2]. Expression analyses show that the candidates NAC01210 and NAC02452 were induced in response to *H. annosum* as predicted by the transcriptome analysis. NAC01210 were selected for overexpression in Norway spruce. Results from the overexpression study in Norway spruce will be presented and discussed in the context of regulation of specialized metabolism and host defence responses.

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# CHEMISTRY OF MUTUALISM AND DECEPTION

# Concepts and Challenges in Mimicry Research

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Some of the best available examples of adaptation through natural selection involve organisms that mimic other organisms, thereby attaining either defence against predators or increased attractiveness to pollinators. Mimicry research has traditionally been focused on defence systems in animals, but there is now also a highly-developed and rapidly growing body of research on floral mimicry in plants. Floral mimicry of insect oviposition sites, insect mates and pollinator food sources is much more widespread among plants (even those that offer rewards) than was previously believed. Visual, olfactory and tactile signals can all be important in floral mimicry systems. The traditional focus of mimicry research has been on visual cues and these appear to be of key importance for floral food source mimicry. However, other forms of mimicry, notably sexual and oviposition site mimicry, are based largely on chemical cues. Do the evolutionary and ecological principles that were developed for defensive mimicry in animals also apply to floral mimicry in plants? Mimicry theory was developed around ideas of conditioning in signal receivers, but pre-existing sensory bias by pollinators may explain most cases of floral mimicry. Selection for traits that confer mimicry appears to have driven diversification in many different plant lineages and highlights the importance of interactions in a community context for explaining evolutionary processes and patterns.

# Flowers of *Aristolochia rotunda* mimic pheromones of true bugs to attract and trap fly pollinators

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Pollination of several angiosperms is based on deceit, i.e. their flowers signal a reward that is not provided. We report a new cheating pollination strategy that involves chemical mimicry in Mediterranean *Aristolochia rotunda*.

Pollinators were collected in the natural habitat. Chemical analyses (dynamic headspace, gas chromatography/mass spectrometry) together with electrophysiological (gas chromatography/electroantennographic detection) and behavioral tests on pollinators were used to identify the scent components that mediate the plant-pollinator interaction and reveal the model of the mimicry system.

The main pollinators of *A. rotunda* were female Chloropidae flies. They are kleptoparasites that feed on secretions of true bugs (Miridae) while these are eaten by arthropod predators. We found that *Aristolochia* flowers mimic the scent released by freshly killed mirids and used by chloropids to find their food sources to deceive their kleptoparasitic pollinators (Oelschlägel et al. 2014).

*Aristolochia* and other plants were believed to lure saprophilous flies and mimic brood-sites of pollinators. We demonstrate for *A. rotunda* the evolution of a different, kleptomyiophilous pollination strategy involving scent mimicry and the exploitation of kleptoparasitic flies as pollinators. Our findings suggest a reconsideration of plants assumed to show sapromyiophilous pollination.

Oelschlägel B, Nuss M, von Tschirnhaus M, Pätzold C, Neinhuis C, Dötterl S, Wanke S (2015). The betrayed thief - the extraordinary strategy of *Aristolochia rotunda* to deceive its pollinators. New Phytologist 206: 342-351.

## Functional role of the floral volatile, *S*-(+)-linalool, in *Penstemon digitalis*

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Adaptive functions for nectar volatiles are hypothesized to be attractants and honest cues of resources for pollinators, deterrents for antagonists or defenses against microbial growth. In North American *Penstemon digitalis*, we have detected natural selection to increase the nectar volatile *S*-(+)-linalool but did not determine the ecological agents driving selection. Therefore, we explore the ecological function of linalool for this bee-pollinated wild flower. Evidence that linalool attracts mutualists of *P. digitalis* is mixed. In the lab, bumblebees are innately attracted to linalool, are not deterred by its taste, and can learn to associate variation in emission with reward. In the field, *P. digitalis* linalool emission matches pollinator activity but lacks true honesty because reward-less flowers also emit linalool. Moreover, field foraging bees and antagonists appear indifferent to variation in emission strength. Interestingly, we also found that linalool in nectar affects the growth of bacterial communities found in *P. digitalis*, suggesting that it may instead function to safeguard nectar quality in nectar-microbe interactions. Our findings emphasize the ecological importance of hydrophilic floral volatile compounds in mediating interactions beyond attraction to pollinators.

## Knock, knock. Who is there? Host location mechanisms in non-pollinating fig wasps

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In the fig–fig wasp pollination system, low volatility compounds and surface chemistry of globular enclosed fig inflorescences (syconia) can serve as cues in short range host location by non-pollinating fig wasps such as gallers and parasitoids that parasitise the mutualism by ovipositing into syconia from the exterior. In behavioral assays, non-pollinating fig wasps exhibited the interesting dual behaviors of joining and avoidance in that they preferred to congregate on fig syconia previously exposed to oviposition by conspecifics but avoided prior oviposition sites on a syconium. This indicated the possible role of chemical signatures such as chemcial footprints and oviposition marking pheromone left by wasps on the surface of a syconium. We, therefore, analysed surface hydrocarbon profiles and changes in local volatile profiles of syconia with different oviposition histories. We also analysed footprint extracts of these wasps and recorded the response of wasps to these compounds. Surface hydrocarbon profiles and volatile profiles of these syconia with different oviposition histories were found to differ and a differential response of wasps to these profiles was recorded. Additionally, we also investigated the role of $CO_2$ and surface hydrocarbons of single galls as cues to assist parasitoids in host recognition via sensilla on their ovipositors. Our results show that change in surface chemistry and local volatile profiles of the host syconium as a result of the act of oviposition by wasps informs fig wasps about the contents of the syconium and induces them to avoid or engage in oviposition.

# Odour genes and pollinator-driven speciation in sexually deceptive *Ophrys* orchids

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Pollinator shifts may be one of the key drivers of speciation in the charismatic orchid genus *Ophrys*. These sexually deceptive orchids achieve a high specificity of pollination by chemical mimicry of the sex pheromones of female insects, and thereby attain pollination by insect males. Because of pollinator-mediated divergent selection, 'odour' genes underlying pseudo-pheromone production may be amongst the first to diverge in spite of a lack of genome-wide differentiation between diverging species. In a group of *Ophrys* species pollinated by solitary bees, floral alkenes with different double-bond positions and chain lengths are primarily responsible for specific pollination. Transcriptome data reveal candidate genes that likely control these alkene properties, and thereby pollination. In particular, acyl-ACP desaturases determine the location of alkene double-bonds. These genes evolved by gene duplication, followed by changes in both gene expression and catalytic function. Because of their large control over pollinator attraction, such desaturase genes may facilitate rapid pollinator-driven ecological speciation in Ophrys orchids.

# Floral microbial ecology – patterns, mechanisms, consequences and functions of bacterial colonization on flowers

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The composition of floral scent bouquets is assumed to be result of stabilizing or directed selection by pollinators and floral antagonists. Like all plant surfaces, flowers are colonized by microorganisms including bacteria, yeast and other fungi. Acknowledging the presence of microbes on reproductive tissues, we are currently investigating the composition of bacterial communities on flower tissues and testing the following hypotheses: 1.) Bacterial colonizers are often not commensalists but have positive and negative functions related to plant reproduction. 2.) Flower volatiles have both growth inhibiting properties but also serve as carbon source for bacteria. 3.) Bacteria metabolize volatiles emitted by flowers leading to altered floral scent phenotypes. 4.) Bacteria select for floral scents and thus need to be considered in the context of floral evolution.

Our data show that bacteria are non-randomly distributed across floral and vegetative tissues support hypotheses 1-3 and thus suggest that bacteria are driving forces in floral evolution (4). We were able to demonstrate that bacteria have the potential to decrease interaction frequencies with pollinators. Floral scents turned out to be Hutchinsonian niche dimensions for bacteria that determine the presence or absence of bacteria on plant surfaces by inhibiting the growth of some but provide carbon for other strains. Furthermore, in culture experiments we demonstrated that bacteria nearly deplete available volatiles, emit novel substances (depending on the medium) and thus may cause ecological relevant changes in floral scent bouquets. Our results thus provide novel insights into the volatile-mediated interactions between flowers and bacteria and strongly suggest that bacteria have profound effects on floral ecology and evolution.

# Choosy wasp cheated by copycat orchid chemistry

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Orchids are famous for their diverse and complex pollination strategies. One such strategy is sexual deception, where orchids attract male insect pollinators by mimicking the sex pheromone of the female mate. In Western Australia, sexually deceptive orchids are common in the genera *Drakaea* and *Caladenia*. In recent years we have been investigating the pollination chemistry of several species of *Drakaea* (hammer orchids), where novel pyrazine-based compounds, including hydroxymethylpyrazines, are used by the flowers to seduce their thynnine wasp pollinators.

In 2014 we broadened our research to *Caladenia* (spider orchids). In this talk I will reveal for the first time the pollination chemistry of the sexually deceptive *Caladenia plicata*. The sex pheromone of the pollinator, an undescribed species of *Zeleboria* wasp, was identified from female wasps and *C. plicata* floral extracts. Although the critical compounds were not new to science, the system is nonetheless highly unusual. The sex pheromone is a blend of two compounds, one aromatic ketone and one monoterpene. Bioassays revealed that neither compound on their own elicits attraction nor sexual behaviour. However, in combination, blends in the ratio range of 1:1 to 5:1 (but not 1:5 blends) elicit exceptionally strong mating behaviour. The specificity of this dual system is extreme, with bioassays involving other regioisomers of the ketone being completely unattractive in the field. Given emerging evidence for some chemical substitutability and flexibility in other thynnine wasp semiochemical systems, this finding was quite unexpected.

Björn Bohman, Ryan D. Phillips, Myles HM Menz, Ben W. Berntsson, Gavin R. Flematti, Russell A. Barrow, Kingsley W. Dixon, and Rod Peakall. "Discovery of pyrazines as pollinator sex pheromones and orchid semiochemicals: implications for the evolution of sexual deception." New Phytologist 203, no. 3 (2014): 939-952.

Björn Bohman and Rod Peakall "Pyrazines Attract Catocheilus Thynnine Wasps." Insects 5 no. 2 (2014):474-487.

## Floral scent in a geographic mosaic of coevolution

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As species coevolve, local populations diverge through adaptation and speciation forming geographic mosaics of coevolutionary interaction. Interactions between plants and phytophagous insects are potentially the most diverse forms of species interaction in nature and range from antagonism to mutualism. We study how a tight mutualistic interaction is mediated by floral scent signals in a chemically hypervariable plant genus. Whereas some species in the *Lithophragma* genus (Saxifragaceae) are pollinated by generalist pollinators (bees/flies), several species of two different clades are involved in a tight pollination mutualism with *Greya* moths (Prodoxidae). The moths pollinate the plants by ovipositing into the floral ovaries. Recent studies have shown qualitative and quantitative variation in floral scent among different *Lithophragma* species and a *Greya* preference to navigate towards and oviposit in flowers of the local plant species. Here, we present the results of a genus-wide scent collection effort from more than 90 *Lithophragma* populations and various Saxifragaceae outgroup species across the entire *Lithophragma* range in western North America. We use SPME-collection techniques and subsequent GC/MS-analysis to ask three specific questions: (i) what is the range of floral scent variation; (ii) how is this divergence partitioned within and among populations and species; and (iii) how is the floral scent variation affected by the coevolving interaction with *Greya* moths? We found tremendous floral scent variation at the level of clade, species and population. Interestingly, clade-specific analyses showed that the different plant clades pollinated by the moths show local convergence in floral scent profiles. This indicates that the tight mutualism has converging rather than diversifying effects on the floral scent variation, at least at a regional scale. Future studies should focus on understanding the ultimate processes responsible for the remarkable floral scent variation, and how these relate to geographic patterns of local coevolution of other plant and insect traits.

MUT09

# Antennal sensitivity to floral scents of *Campanula*: A comparative study of polylectic and oligolectic bees

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*Campanula* flowers are visited by polylectic and oligolectic bees and we found the floral scent to consist of common occurring and unusual volatiles (spiroacetals). This study compares the antennal responses of several bee species (three *Campanula oligoleges* of the genera *Chelostoma* and *Hoplitis*, three polylectic bees and the *Ranunculus oligolege Chelostoma florisomne*). We hypothesized *Campanula oligoleges* to be more sensitive to spiroacetals and the floral scent of *Campanula*, than non-*Campanula* specialists, while all tested bees were expected to respond similarly to common flower volatiles. Our results show that the *Campanula*-specialists as well as *C. florisomne* showed a higher sensitivity to either spiroacetals or the complete floral scent of Campanula compared to polyleges. In all bees the sensitivity to common volatiles was similar. The results indicate that *Campanula oligoleges* possess highly sensitive olfactory receptors for spiroacetals that offer a fitness advantage to locate their host-flowers. The sensitivity to the whole scent bouquet of *Campanula* might help to locate host flowers even from longer distances. Since phylogenetic investigations showed *C. florisomne* to share common ancestors with the *Campanula*-specialists of the genus *Chelostoma*, receptors for spiroacetals could be a pleisiomorphic trait.

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# How to attract your seed disperser: The chemistry behind an unusual mutualism between and Australian eucalypt and stingless bees.

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Many bees provide an essential mutualistic service to plants, the pollination of their flowers. But bees can also disperse seeds of plants, a rare seed dispersal mutualism called melittochory. Australian stingless bees (Apidae: Meliponini) disperse the seeds of an Australian endemic eucalypt (*Corymbia torelliana*, Myrtaceae) by collecting resin from its fruits. We investigated whether the resin chemistry of *C. torelliana* might be particularly well adapted to attract the bees by studying the importance of resin compounds as olfactory cues in this bee seed dispersal mutualism.

We performed several choice tests with pure resin extracts, fractionated extracts, extracts enriched with *C. torelliana* resin compounds and with single compounds, to investigate whether the stingless bee species *Tetragonula carbonaria* relies on olfactory cues when searching *C. torelliana* resin and which compounds of the blend they use to identify it.

We found that olfactory cues were sufficient to attract bees, but that they only landed on filter papers with pure resin extract, whereas they did not land on papers with fractionated or enriched extracts, nor with single compounds. This behaviour suggests that, instead of relying on just a few specific volatile compounds, the bees use a complex mixture as recognition cue as well as relative compound proportions. Moreover, they hardly tolerate even slight changes of concentrations of single components. Such high cue specificity is unusual for insects seeking resources. In the case of Australian stingless bees, it does however ensure detection and identification of the resin of their mutualistic partner.

# The chemical context of interspecific communication: Ecological chemistry as a driver of mutualisms

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We recently conducted a series of experiments which suggests that *Drosophila* flies and *Saccharomyces* yeasts may establish a mutualistic association, and that this is driven by chemical communication. While individual volatiles have been implicated in the attraction of *D. melanogaster*, the semiochemicals affecting the behavior of the sibling species *D. simulans* are less well characterised. Here, we comprehensively scrutinize a broad range of volatiles produced by attractive and repulsive yeasts to experimentally evaluate the chemical nature of communication between these species. When grown in liquid or on agar-solidified grape juice, attraction to *S. cerevisiae* was primarily driven by 3-methylbutyl acetate (isoamyl acetate, banana oil) and repulsion by acetic acid, a known attractant to *D. melanogaster* (also known as vinegar fly). Using T-maze choice tests and synthetic compounds we show that these responses were strongly influenced by compound concentration. Moreover, the behavioral response is further impacted by the chemical context of the environment. Thus, chemical communication between yeasts and flies is complex, and is not simply driven by the presence of single volatiles, but modulated by compound interactions. This demonstrates the ecological context of chemical communication needs to be taken into consideration when testing for ecologically realistic responses.

# Chemically armed ants pillage and protect fungus-farming societies

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Like human societies, ant colonies build protected fortresses, manufacture weaponry used by soldiers, maintain mutualisms that maximize food availability (i.e., farm), and live socially to enhance offspring survival. However, the concentrated resources attract organisms specialized in breaking through the fortifications and defenses of these highly organized societies. My work encompasses a dynamic symbiotic network that includes three ant species, a fungus garden, and bacterial communities. Selective pressures from these different organisms with shared or competing interests shape how these species interact and evolve. We show that guest ant social parasites act as mercenary warriors (www.megalomyrmex.com/videos) as their venom is much more effective in nest defense than the biting strategy of the host ants. Cooperation between these two ant species is enforced by external dangers according to the principle that the enemy of my enemy is my friend. We demonstrate that the alkaloid-based venom of the guest ants play an influential role in this complex species network and serves as a toxic chemical weapon that protects the host ants, farm and ultimately their shared home. The mere presence of the guest ant parasite discourages a more virulent raider ant species from attempting a raid. Counter to expectation, the symbiotic relationship between the *Megalomyrmex symmetochus* guest ant and their fungus farming host, *Sericomyrmex amabilis*, may be beneficial rather than costly under certain ecological conditions.

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# Chemical communication in a mutualistic system – The myrmecophilous Australian butterfly *Jalmenus evagoras* (Lepidoptera: Lycaenidae)

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Communication is critical to the maintenance of the often extraordinary levels of cooperation that may occur between individuals of the same and different species. Ants are frequent partners in interspecific cooperative relationships, including with the larvae of butterflies of the family Lycaenidae (Lepidoptera). Although the costs and benefits of lycaenid-ant associations have been extensively documented, recognition and communication mechanisms are still poorly understood. It is widely thought that cuticular hydrocarbons, important for mediating nestmate recognition in ants, may also play a significant role in initiating and maintaining cooperative behaviour in lycaenid caterpillars and their tending ants. To unravel the role of chemical signals as recognition cues in lycaenid-ant associations, we examine the larval cuticular hydrocarbons of the Australian butterfly *Jalmenus evagoras* and its attendant ants by targeting a variety of *J. evagoras* populations associated with different ant species and on different *Acacia* host plants. We examine the initial acceptance of early instars by the associating ant colony by documenting ontogenetic changes in the larval chemical profile. We discuss how these patterns of cuticular chemical profiles maintain the mutualistic association between larvae and ants.

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# The fungus, Raffaelea lauricola, manipulates release of host plant odors causing initial repellency and subsequent attraction of trees to its symbiont and vector, the redbay ambrosia beetle (*Xyleborus glabratus*).

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The redbay ambrosia beetle, *Xyleborus glabratus*, is an important pest of redbay (*Persea borbonia*) and swamp bay (*P. palustris*) trees in forests of the southeastern USA. *X. glabratus* is the vector of a symbiotic fungus, *Raffaelea lauricola*, that causes laurel wilt, a highly lethal disease of the Lauraceae. The beetle is attracted to host wood volatiles, particularly sesquiterpenes. Contrary to other ambrosia beetles that attack stressed, possibly pathogen-infected, and dying trees, *X. glabratus* readily attacks healthy trees. An olfactometer bioassay was developed to test the behavioral response of *X. glabratus* to plant volatiles. We first found that *X. glabratus* was attracted to the leaf odors of their hosts, redbay and swamp bay, with no attraction to a non-host tree tested. Gas chromatography-mass spectrometry (GS-MS) analysis of leaves revealed the absence of sesquiterpenes known to be attractive to *X. glabratus* and present in host wood. An artificial blend of chemicals was developed based on GC-MS analyses of leaf volatiles and this blend was attractive to *X. glabratus* under laboratory and field conditions. A possible change in the leaf odor profile of *P. palustris* following infection with *R. lauricola* was investigated. Volatile collections and behavioral tests were performed -1, 3, 10, 20 days after infection (DAI). At 3 DAI, we found a significant repellency of *X. glabratus* by the odor of infected swamp bay as compared with non-infected controls. However, at 10 and 20 DAI, *X. glabratus* was more attracted to infected than non-infected trees. GC-MS analyzes revealed an increase in methyl salicylate at 3 DAI, whereas an increase of sesquiterpenes in leaf volatiles was observed at 10 and 20 DAI. Methyl salicylate is a repellent of *X. glabratus* in laboratory bioassays. Overall our findings provide a better understanding of how a fungal pathogen manipulates plant response to recruit its symbiont and vector.

# Volatile predator cues drive non-consumptive effects

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Predators can affect prey in two ways—by reducing their density (consumptive effects) or by changing their behavior, physiology or other phenotypic traits (non-consumptive effects). Understanding the cues and sensory modalities prey use to detect predators is critical for predicting the strength of non-consumptive effects and the outcome of predator–prey encounters. While predator-associated cues have been well studied in aquatic systems, less is known about how terrestrial prey detect their predators. We evaluated how Colorado potato beetle, *Leptinotarsa decemlineata*, larvae perceive predation risk by isolating cues from its stink bug predator, the spined soldier bug, *Podisus maculiventris* and in field trials where predators where confined on plants. Field experiments showed reduced damage to plants where predators where contained nearby. Volatile odor cues from predators reduced beetle feeding overall, although male predators caused a stronger reduction than females. Headspace volatile collections from the predators allowed us to pinpoint three major chemical players in this interaction. In laboratory assays, beetle larvae avoid these chemicals and eat less in their presence. The augmentative use of these chemicals in the field may lead to reduced damage in potato fields.

# MICROBIAL CHEMICAL ECOLOGY

# *Trichoderma* volatile reduces fungal phytopathogenic symptoms in *Arabidopsis*

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*Trichoderma* is a fungal genus present in many ecosystems. Some strains have the ability to reduce the severity of plant diseases via various mechanisms, including the action of biologically active compounds. Volatiles bioactive compounds were so far little studied in this regard. *Trichoderma*/*Arabidopsis* dual cultures were used to investigate the effects of low molecular weight volatile compounds on *Arabidopsis thaliana*. We observed reduced but robust plants with increased anthocyanin content. Other defence compounds such as $H_2O_2$, camalexin and glucosinolates were also investigated to learn more about the physiological reactions of *A. thaliana* upon fungal volatile exposure. Furthermore, the expression of pathogenesis relevant genes were measured via gus-promoter studies.

To unravel the nature of the bioactive compound(s) *Trichoderma* headspace volatiles were analysed via GC-MS. Prominant volatiles were solely applied to *A. thaliana* to study the growth and defence reactions in the plant under normal and challenged conditions.

# Identification and biosynthesis of new bicyclic alkaloids from the entomopathogenic bacteria *Xenorhabdus*

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Entomopathogenic nematodes in the families Heterorhabditidae and Steinernematidae form mutualistic-symbiotic associations with *Xenorhabdus* or *Photorhabdus* bacteria, respectively, that increase their virulence toward insect prey. The bacteria reside in the gut of the nematode and are released upon infection of insect larvae, where they produce a range of toxic metabolites. The interaction between the nematode host and its bacterial partner is mediated by a complex network of chemical signalling molecules, most of which are still unidentified. As part of our ongoing investigations into the molecular basis of this tritrophic interaction between insects, nematodes, and bacteria, we have identified a new two-gene cluster (otpAB) that is highly conserved across almost all *Xenorhabdus* and *Photorhabdus* strains. Given the conservation of this biosynthetic capability, the metabolites resulting from expression of otpAB are likely to occupy an important role in mediation of symbiosis or pathogenesis. We aimed to identify metabolites arising from expression of otpAB, which encodes both a bimodular non-ribosomal peptide synthetase (NRPS, OtpA) and a hydroxylase (OtpB). This was accomplished via a nuclear magnetic resonance (NMR) metabolomics approach, making use of differential analysis by 2D NMR spectroscopy (DANS) to reveal the bicyclic alkaloid scaffold and saturated sidechain of the major biosynthetic product of heterologous otpAB expression. In particular, DANS using correlation spectroscopy (COSY) provided a high sensitivity method of accessing detailed structural information about otpAB dependent metabolites without prior purification of the culture extracts. Finally, a suite of new, related metabolites were isolated following large scale cultivation and purification, and their structures and stereochemistry elucidated via a combination of mass spectrometry (MS), 1D and 2D NMR spectroscopy, chemical synthesis, and chiral chromatography. The biosynthesis, including a complex rearrangement, will be presented as well as initial results towards the biological function of these compounds.

# Not visible, but highly effective: plant volatile manipulation by endophytic fungi and responses of herbivores

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Plants are regularly colonized by endophytic fungi; however, the resulting multitrophic interactions are poorly understood. We analyzed the effect of inoculations with two different species of endophytic fungi (*Beauveria bassiana* and *Trichoderma koningiopsis*), differing in their mode of action (insect vs. plant pathogens), on tomato volatiles organic compounds (VOCs) and resulting responses of herbivores with regard to host plant selection behaviour. We hypothesized that endophytically colonized plants should be more attractive for herbivores, mediated by the VOC profiles, but should not exhibit differences when colonized by a plant pathogen antagonist.

Tomato roots were inoculated with *B. bassiana* and *T. koningiopsis* and analyzed for volatile emissions and compared with non-inoculated plants. A total of 10 treatments were evaluated: *Beauveria bassiana* strains Bb1, Bb2, and Bb3 and *Trichoderma koningiopsis* strain Th003; each endophyte treatment combined with an aphid attack. Headspace volatiles of each plant were collected using TDS tubes filled with a porous polymer based on 2.6-diphenyleneoxide (Tenax ® TA). Samples were analyzed using a GC-MS with a non-polar column.

*B. bassiana* and *T. koningiopsis* inoculated plants modified tomato VOCs, with clear differences between different isolates. When combined with an aphid attack (*Myzus persicae*) VOC profiles differed with regard to the amount of volatiles produced. Entomopathogenic fungal colonization of plants reduced the amount of those compounds known as repellent to herbivores, while *T. koningiopsis* increased specifically sesquiterpene emissions. In a multiple choice experiment, we offered five different odor sources (i.e. plants) to winged *M. persicae*. Aphids were significantly more attracted to endophyte inoculated plants compared to untreated plants after 24 h. Also, an additional non-choice oviposition experiment using *Helicoverpa armigera* laid significantly more eggs on endophytic inoculated plants compared to control plants under laboratory conditions. These results support our hypothesis that i) endophytic fungi manipulate plant metabolism specific to each fungal isolate, that ii ) insect herbivores are able to distinguish between VOC profiles and iii) that VOC changes may be to the detriment of the herbivores.

# Sniffing out the enemy: Fungus-growing termites can differentiate between mutualistic and parasitic fungi using volatiles

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Fungus-growing termites *Odontotermes obesus* farm a mutualistic fungus *Termitomyces* inside their nests for food and keep the fungal gardens free from parasitic fungi such as *Pseudoxylaria*. Nest microclimate, antifungal compounds of termite origin, antibiotic-producing bacteria, and weeding by termites have been proposed as mechanisms to control the growth of *Pseudoxylaria* inside the nest and thus to maintain the obligate mutualism between termites and *Termitomyces*. Whether termites use antibiotic compounds or weeding, it is important that they are able to differentiate between the two fungi so that they can selectively use the antifungal mechanism—whether chemical or behavioural—against *Pseudoxylaria*. Therefore, in the present study we examined whether termites demonstrate a differential response towards the two fungi. We designed a novel laboratory-based dual choice assay for this purpose. Additionally, when termites are allowed to smell but not to physically access the fungi, we investigated if they can still differentiate between the mutualistic and parasitic fungi. Our results show that termites can use olfactory cues for this task. We also present results on the volatile profiles of the two fungi.

# Volatile affairs in microbial interactions

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The importance of bacterial interactions in microbial ecology is increasingly recognized. However, little attention is paid to the role of bacterial volatiles in microbial interactions. Due to their physical properties, volatiles can act on a wide scale in soil and consequently play a key role in "long distance" inter-specific microbial interactions and in the development of microbial soil communities.

The major aim of our study was to obtain more insight in the role of volatiles in microbial interactions. For this we performed several experiments in glass plates, which were designed as such that the growth of different soil microorganisms occurred in physically separated areas within a common atmosphere. In this way we studied the role of volatiles in fungal-bacterial and bacterial-bacterial interactions.

The obtained results revealed that volatiles play important role in the interaction between soil microorganisms. Here we will report on microbial volatiles acting as info-chemicals affecting the behaviour, growth, antibiotic production and gene expression in responding bacteria. Furthermore we will report on the volatiles produced by active microbial community and their effect on the growth and behaviour of non-active (starving) bacteria as well as on the importance of interspecific interactions on the volatile production.

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## Terpene arms race in the *Seiridium cardinale – Cupressus sempervirens* pathosystem

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*Seiridium cardinale*, the main fungal pathogen responsible for cypress bark canker, is the largest threat for cypresses worldwide. We investigated the production of terpenes by canker-resistant and non canker-resistant (susceptible) *Cupressus sempervirens* artificially infected with *S.cardinale*. Next, we tested the effect of those terpenes on fungal growth, and the biotransformation of those terpenes by the fungus. We used Gas-Chromatography Mass-Spectrometry (GC-MS) to analyze phloem terpene concentrations, performed in vitro tests to determine fungal growth inhibition by applying in planta and arbitrary concentrations of 15 terpenes, and analyzed samples of the in vitro tests with GC-MS to look for fungal biotransformations. Results show that trees produced de novo terpenes and underwent strong terpene inductions under infection, especially of minor mono- and diterpenes, but canker-resistant trees had stronger terpene profiles and responses than susceptible trees. The results of the fungal growth inhibition tests suggest that the concentrations of the resistant trees could be more inhibitory than those of the susceptible trees (in planta concentration tests). In the arbitrary concentration tests, the highly-induced and the de novo terpenes produced strong inhibitions (sometimes more than a reference fungicide) and appeared to have remarkably concentration-dependent inhibitions. Contrarily, the major terpenes were distinctive for their little concentration-dependent inhibition. Finally, we found that, in its turn, *S. cardinale* biotransformed three tree terpenes, with results suggesting a possible transformation even before hyphae contact. Thus, our results indicate terpenes are key *C. sempervirens* defences that trees efficiently use, and that on the other side, *S. cardinale* is ready for the battle.

# Elucidation of the secondary metabolite of the rhizobacterium *S. plymuthica* 4Rx13

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Microorganisms appear universal in nature and are important factors shaping our environment by producing a large diversity of secondary metabolites (e.g. antibiotics and toxins). Overlooked for a long time was the ability of microorganisms to release volatile compounds, compiled in the database mVOC (1). They play important roles in inter- and intraspecies signaling and communication. Regarding volatile emission and due to their growth promoting effects on plants especially rhizobacteria were studied over the last decade (2). It is our aim to investigate the volatile-based communication belowground between bacteria, plants and fungi.

Our study focused on the rhizobacterium *Serratia plymuthica* 4Rx13, which was found to emit a rich blend of volatiles, including one dominant compound that was not hitherto found in other bacteria. The analysis with NMR uncovered an unusual bicyclic structure, which only consists of carbon and hydrogen atoms and is fully methylated ($C_{16}H_{26}$); it was named *sodorifen* (3). It is hypothesized that the emission of sodorifen in such high quantities is cost intensive but provides advantages for the producer in its habitat (ecosystem). Exploring the potential of this extraordinary compound is the center of ongoing studies, for example unraveling the involved biosynthetic pathways. Thus, a systematic search for genes and enzymes involved in the biosynthesis of sodorifen was applied including i) a comparative genome analysis of related *Serratia* species, ii) a differential proteome analysis under high and low producing conditions was examined and iii) the transcriptomes of sodorifen producer and non-producers strains were compared. Together, these strategies pointed towards a cluster of four genes, which are presently under intensive investigations.

1) Lemfack et al.; 2013, Nucl. Acid Res., 1-5
2) Kai et al.; 2010, Appl. Microbiol. Biotechnol. 88, 965-976.
3) Reuss et al., 2010, Angew. Chem. Int. Ed. 49, 2009-2010

# APPLIED CHEMICAL ECOLOGY

# Insect pheromones for insect management: Promise versus reality

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Even before the identification of the first insect pheromone from *Bombyx mori* in 1959, scientists had recognized the enormous potential for applications of pheromones. The powerful attraction of numerous species to their pheromones suggested that pheromones might be a “magic bullet”, that could provide effective control of pest insects with no deleterious effects on human health or the environment. This hope and optimism drove most pheromone research during the 1960s and 1970s, and to some extent it continues today. However, it has become clear that the development of pheromone-based strategies as a general method of controlling pest insects is much more difficult than anyone had anticipated, for a variety of biological, logistical, technical, and economic reasons. Thus, we are faced with a conundrum: the pheromones of many insects are clearly powerful attractants, but many efforts to exploit that biological activity for managing insects have had limited or no success, despite years of effort. Conversely, in some cases, pheromone-based methods have been spectacularly successful, with the target insect virtually eradicated in a short period of time. Thus, why is it that pheromone-based techniques of insect control can sometimes be highly successful, whereas in other cases, satisfactory control is not achieved? What are the important characteristics of successful systems, and how do they differ from systems in which only partial or no control is achieved? In developing new systems, where should we direct our efforts to have the maximum chances of success? Conversely, which systems are likely to be so difficult or so costly that pheromone-based control should not be attempted?

To illustrate these issues, examples will be chosen from a variety of insects, including Lepidoptera, Heteroptera, Homoptera, and Coleoptera. Examples of successes and failures in the development of pheromones for detection and monitoring of insects also will be briefly described.

# Pheromone monitoring as a game changer in insect biodiversity and conservation research

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One major obstacle for evidence-based insect conservation is the lack of reliable data regarding present distribution, population status and population trends of most rare and threatened species. Assessing these parameters in relation to conservation management efforts is a formidable task with traditional sampling methods. We have exploited the legendary attractiveness of insect sex pheromones to monitor rare and threatened insects. Identifying pheromones for individual species remains a severe bottleneck. However, for several species for which efficient pheromones are available, we can now perform population monitoring with unprescedented spatial and temporal accuracy over large geographic regions. For these species, and associated species affected by similar landscape processes, access to pheromone monitoring systems truly constitutes a game changer for biodiversity and conservation work. We have mapped species distributions in relation to habitat and linked to the distribution of other saproxylic species, and studied their dispersal biology in fragmented landscapes. Within a few seasons we have been able to fast-forward decades of work corresponding to traditional methods and provide regional, accurate distribution maps highlighting the most important hotspots in the landscape. We have demonstrated that individual species may constitute excellent indicators for saproxylic insect biodiversity and pin-point the most valuable and prioritized hotspots in the landscape. We have also provided robust models for habitat managment regimes for individual species that incorporate habitat thresholds on different spatial scales, and the likely limits for dispersal and colonization among patchily distributed habitat resources.

# Monitoring the common pine sawfly populations with pheromone traps in managed boreal forests

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Boreal forests will experience increasing problems with insect pests, caused by climatic warming. Efficient and accurate methods are needed for monitoring and predicting changes in pest status and defoliation. Monitoring with species specific pheromones, integrated by forest stand characteristics may give a cost efficient survey method for forest protection.

We outline an approach to forecast population density and forthcoming defoliation intensity of Scots pine (*Pinus sylvestris* L.) by the common pine sawfly (*Diprion pini* L.) with pheromone traps. Three traps per site were baited with 2.5 mg of the *D. pini* sex pheromone, threo-1, 2, 6 –trimethyldodecyl propionate, in mature Scots pine stands of managed forests in 2008-2012. The Palokangas area in eastern Finland (62°53´N, 30°54´E) provided a field laboratory for our test due to fluctuating outbreak density of the pine sawfly. The interpretation was based on 15 georeferenced permanent field plots with trap groups, stand characteristics, forest floor vegetation, and visually assessed annual changes in foliage biomass caused by the defoliator.

A mild decreasing trend of mean defoliation, but increasing trend of mean trap catch was observed in 2008-2012. There were no statistically significant differences in mean trap catch between the years. Coverage of heather on forest floor, number of trees per hectare, and basal area correlated moderately with mean trap catch. According to Random Forest test, the most important variables predicting mean trap catch were plot-wise mean defoliation, mean diameter, mean tree height and coverages of blueberry and lingonberry on forest floor. More research needs to be done before *D. pini* pheromone monitoring can be incorporated into forest health monitoring systems. Furthermore, there should be another monitoring method conducted simultaneously with the pheromone monitoring of the pine sawflies.

# Detection, monitoring, and forecast using pheromone traps for three spruce seed feeding moths

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Lepidopteran seed predators may consume a large proportion of the spruce seed yield in seed orchards. Together with collaborators we have earlier identified the sex pheromones of the three most harmful moths in Europe (Wang et al. 2010, 2015, Löfstedt et al. 2012). We now report the results of monitoring these species with pheromone traps in six seed orchards in southern Sweden during up to five years. *Cydia strobilella* and *Eupithecia abietaria* show a relatively concentrated flight period, with peaks in late May and early summer, respectively, whereas *Dioryctria abietella* is caught throughout the summer and early autumn. Here we also present preliminary results from analyses of trap catches in relation to the degree of crop infestations and estimated population densities. Such analyses become complicated because of cone harvesting and in some cases treatment of the orchards with *Bacillus thuringiensis* against *D. abietella* in years of expected high yield.

Wang et al. (2010) J. Chem. Ecol. doi: 10.1007/s10886-010-9754-x
Löfstedt et al. (2012) J. Appl. Entomol. doi: 10.1111/j.1439-0418.2011.01619.x
Wang et al. (2015) J. Appl. Entomol. doi: 10.1111/jen.12167

# Use of a vertebrate's semiochemical for long-term protection of horses' ears against black flies (Diptera: Simuliidae)

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Horses are commonly harassed by various species of haematophagous arthropods, which bites constitutes major causes of stress. One of these insects, Blackflies (Diptera, Simulidae), bites horses inside their ears and generates acute stress, lesions and allergic reactions. Despite various chemical products or protection material, no really effective protection is currently available. The aim of this study was to assess the efficacy of a semiochemical, derived from a Mustelid/Viverrid secretion, to prevent the infestation of the horse ears by this diptera.

A randomized and blinded paired-samples study was carried out on 9 horses. For each horse, insects present in the ear were removed, the inside of the ears was washed and a treatment applied. Each horse received on its ears either a the control or the semiochemical treatment (randomization stratified especially concerning laterality). Eight, nine and ten hours after treatment, 2 independent observers counted the number of simuliidae present in each horse’s ears.

During the 3 observation period a total of 2 insects were observed on semiochemical treated ears against 411 observations on the control ear. A significant difference between treatments was observed during each observation period and for the whole assay ($p = 0.0228$; $p < 0.0001$; Mixed model for two within-subject factors). The tested semiochemical allows over 98% of protection against the infestation of horses’ ears by simuliidae up to 10 hours.

Additional studies need to be performed to further investigate efficacy duration of this semiochemical. These preliminary data highlight the potential interest of the semiochemical approach as a new strategy against hematophagous insects without the use of toxic or banned xenobiotic substances to improve the welfare of horse.

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

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

# Innovative control strategies for phytoplasma vectoring insects by infochemicals

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Phytoplasmas are worldwide responsible for more than 700 different plant diseases and have an important economic impact. *Phytoplasma* species belonging to the apple proliferation group are the economically most important fruit tree phytoplasmas and are widespread in the temperate regions of Europe. Phloem feeding insects (*Psyllids*) were identified as vectors, often one species transmitting a specific phytoplasma. We studied in different systems the role of semiochemicals for vector behavior and the influence of phytoplasma infections on volatile production of host plants.

Volatile compounds from host plants under field and laboratory conditions were collected and analyzed by gas chromatography coupled with mass spectrometry. The olfactory preferences of psyllids for certain plants and volatile chemical compounds were investigated in Y-shaped olfactometer bioassays. Several compounds were tested in field traps in apples, stone fruit and pears in various locations. Host plants were selected based on their attractiveness to psyllids under field conditions.

We found that all investigated psyllid species use chemical cues for the identification of their host plants during migration between different host plants. The production of plant volatiles could be in some cases influenced by phytoplasma infections which indirectly influenced the behavior of vector insects. For some species we have identified species specific attractive compounds and also repellent chemicals.

Attractive compounds will be used in traps as lures for monitoring and mass trapping purposes. By combination of attractive compounds in traps and repellent compounds in dispensers these chemicals may be used in push-and-pull strategies. We already started constructing traps and repellent dispensers and tested them in field experiments. These results are presented and discussed.

# Studies on interference between different lacewing attractants: new perspectives for Central European species (Neuroptera: Chrysopidae)

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As predators of many soft-bodied pest species, green lacewings (Chrysopidae) are of special importance in biological control. In Europe the common green lacewings (*Chrysoperla carnea* species-complex) are possibly of the highest importance, due to their tolerance to several factors, their wide distribution and their greater abundance in various agroecosystems. However since adults of *Chrysoperla* spp. lacewings are not predatory, baits attracting multiple species with different life history traits (e.g. being predators as adults of *Chrysopa* spp.) could yield benefits for agricultural practice. However, in our previous studies serious interference between baits attracting different green lacewings was found, that is, with the use of nepetalactol-based baits, attraction of common green lacewings to a ternary floral lure decreased markedly. When behavioural response to different stimuli comes into question, overlap between the chemical ecology of species in different geographic regions provides an interesting issue.

In our present study we tested squalene, a compound previously found attractive for the Nearctic *Chrysopa nigricornis* species. Both field experiments and electroantennographic screenings were performed with some species native to Europe, in order to test the perception and potential behavioural response to this compound.

In our experiments males of *Chrysopa formosa* were found to be attracted to squalene. When tested in combination with the floral lure highly attractive to common green lacewings, no interference was experienced.

In conclusion, these preliminary results suggest that squalene may provide new perspectives in the development of baits attracting multiple lacewing species with different life history traits.

# Insecticide resistance mutation (Kdr) in *Anopheles gambiae* modulates host choice and olfaction in presence of pyrethroid-treated net

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Major mean of malaria vector control are based on use of insecticides. Their efficiency is threatened by widespread resistance mechanisms. In addition to the physiological resistance mechanisms already well studied, the issue of the behavioral modulation as cause or consequence of the resistance is largely overlooked. Nevertheless there are evidences that insecticide-based control tools alter mosquito behavior before any contact, suggesting that the mosquitoes can detect the presence of the insecticide. In the present study, we tested this hypothesis by investigating the behavioral and olfactory responses of different resistant genotypes (differing by presence of L1014F (Kdr) mutation) of *Anopheles gambiae* to host odors and permethrin treated net. Behavior experiments showed that heterozygous were more actives than two other genotypes. Moreover, homozygous resistant preferred host behind the permethrin treated net than host behind untreated net. Electrophysiology studies showed that three genotypes expressed different sensitivity to known attractant, but only homozygous sensitive responded to permethrin at tested concentration.

These results confirm that mosquitoes can perceive insecticide on net and adapt their behavior in response of it. Moreover, resistance mutation modulates this response. These results must be taken in account for future insecticide-based control tool development. Our original study highlighted the urgent need for further investigation of chemical ecology of malaria vector in a vector control pressure context.

# Can herbivore induced plant volatile compounds be used to enhance attraction of entomopathogenic nematodes?

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Recent work has shown the potential for enhanced efficacy of entomopathogenic nematodes through their attraction to herbivore induced plant volatiles. However, there has been little investigation into the utilization of these attractants in systems other than in those in which the compounds were identified. We compared (*E*)-β-caryophyllene (EβC) (maize) and pregeijerene (PG) (citrus) in the highbush blueberry (*Vaccinium corymbosum*) system in their ability to enhance the attractiveness and efficacy of entomopathogenic nematodes (EPN) against the system's herbivore, oriental beetle (*Anomala orientalis*). Using an endemic strain of the EPN Steinernema glaseri in a six-arm olfactometer, the relative attractiveness of EβC and PG was tested in the lab to gather baseline values of attraction to the chemicals alone in sand substrate. Before the field study, the soil of 30 plants was sampled and baited with *Galleria mellonella* larvae to select the 10 plants with the highest *S. glaseri* activity. An arrangement similar to that of the 6-arm olfactometer was used in the field. Six gasket traps containing third-instar oriental beetle larvae or *G. mellonella* larvae with and without compound were placed into the soil around the base of the 10 plants. The gaskets were removed after 72 hours and insect baits retrieved and assessed for EPN infection. After removal of the insect bait from the gasket, the remaining clean sand packed in the gasket was exhaustively baited with *G. mellonella* larvae to assess EPN density. The lab results suggest that in sand alone EβC is significantly more attractive than PG to endemic *S. glaseri*. Conversely, there was no difference in attractiveness or efficacy between the chemicals in the field study. However, endemic *S. glaseri* were more attracted to gasket traps without oriental beetle larvae than traps with oriental beetle larvae.

# Observations from a bluebell and bracken climax vegetation and approaches for bluebell conservation

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Native British bluebells (*Hyacinthoides non-scripta* (L.) Chouard ex Rothm.) and bracken (*Pteridium aquilinum* (L.) Kuhn) form a climax vegetation on mineral poor substrate on exposed hillsides in Wales. The growth of both plants was assessed during two growth periods. While bluebells predominantly grow throughout the winter – spring period, with dormancy during summer, bracken actively grows throughout spring to autumn. The chemistry of both plants was investigated and the transfer of metabolites from plant to soil assessed. As the biomass relationship is between 20 to 50 (bracken); 1 (bluebells), the main metabolites detected in soil were bracken derived with quercetin and pterosin B.

While the initial aim was to establish whether any bluebell or bracken metabolites may exert an allelopathic effect, our findings to date suggest that mineral supply has a stronger influence on plant performance. Of particular interest was the sequestration of phosphorus in the form of phytate in bluebell bulbs. We hypothesise that the avaibalibity of phosphate restricts bracken growth during bluebell flowering. This is supported by an increase in the concentration of pterosin B, C and N in bracken roots by a factor 5, 20 and 10, respectively, of during this time. In addition the concentration of pterosin B, C and N has also increased during seed formation. We hence hypothesise that these findings concur with the carbon nutrient balance hypothesis in relation to phosphorus availability.

In the context of conserving biodiversity, British bluebells have protected plant status in Great Britain. Bluebells usually form carpets in woodlands but can also be found on exposed cliffs and hillsides. They form dense population once established, which are maintained for centuries, hence bluebells are taken as indicators of ancient woodlands. For conservation purposes, the seed oil composition was explored and found suitable for commercial applications.

# Bed bugs-down the trap

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Bed bugs, for long a forgotten nuisance, have reappeared and detection and eradication of them have become a million dollar industry. Bed bugs are well shielded from watery solutions of pesticides by a waxy outer cuticula and they easily become resistant to pesticides. Approximately ten weeks after infestation a bed bug population change from linear to exponential growth. To detect an infestation before this point is a and o for successful treatment. Our aim is to develop a monitoring system that detect bed bugs at low densities partly substituting costly ocular inspections and that can evaluate the outcome of a treatment.

We collected and analyzed emissions from bed bug colonies with headspace, enfleurage and GC-MS methods. Bed bug attraction in test arenas to the pheromones resulted in a solution of five chemicals, which, applied in traps, were presented together with control traps and tested in Mesocosms. Pheroemission decreased with time, but was easily detectable after six days. Ten bed bugs (4 females, 6 males) were released at start, after 24 and 72 hrs and retrieved after 24, 72 and 144 hrs, respectively. Pherotraps caught 44, 35 and 37 % of the introduced bed bugs during the first, second and third period, respectively, which is a higher efficiency than any other products we know of. Pherotraps caught significantly more bb than control traps (p=*****). The catch of both sex were highest in the first period and lowest for females in the third and for males in the second period. The efficiency is concentration dependent and currently we work to optimize the strength and life length of the bait and to tests trap efficiency in the field.

# Chemical tools for training dogs to find bed bugs

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Dogs (*Canis lupus familiaris*) have a very sensitive olfactory system and canine scent detection has so far been used, amongst other search tasks, to locate insects such as termites, bark beetles, gypsy moths and individuals of endangered species. During the last decade there has been a drastic increase in reported cases of bed bug infestations in private homes, schools, student dorms, hotels and hostels in Sweden. In order to efficiently screen for bed bugs, dogs that by nature rely on olfaction rather than vision, can been trained to detect bed bugs. Methods for training sniffer dogs has so far included the use of live bed bugs or bed bug scent extraction. In this paper we present a method based on natural substances ((*E*)-2-hexenal and (*E*)-2-octenal), emitted by bed bugs, which can be applied as a "bed bug scent". These compounds are used in the bed bugs chemical communication and are therefore present in relative high amount when bed bugs are present. Our results indicate that our method is equally good as using live bed bugs for training.

## Semiochemical-based technologies for fly management

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Filth flies are important insect pests that have caused over billions of dollars damage in animal production, food contamination and disease transmitting. The present presentation reports our recent findings on the development of filth fly control using semiochemical-based technologies to reduce their negative impacts. Several host and host environmental associated attractants were identified and implemented to increase captures of attractant-based mass trapping devices. We have further studied the effects of fly vision preference towards the enhancement of trap catches. We also explored plant-based antimicrobial agents being used to manipulate microbial community in the fly larval development. Novel botanical-based antibacterial agents were identified from several plant species, with demonstrated as larval growth inhibitors as well as oviposition deterrents. We have explored which bacterial species or their complex community that are important for the larval development, as well as how they affect infochemical interactions between filth flies and their host location. The further development using these findings in novel fly control strategy will be discussed.

# Prospects of managing a social parasite using pheromone supplements

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Social parasitism is one of the unique characteristics traits of the Cape honeybee *Apis mellifera capensis*. These sub-species of honeybees that are endemic to the Cape region of South Africa do usurp colonies of other sister sub-species such as the Savannah honeybees *A. m. scutellata*. Upon successfully infesting a host colony, they take over reproductive activities through thelytokous parthenogenesis and by mimicking the queen's mandibular pheromone (QMP) secretions. Since these capensis workers do not contribute towards colony chores, the host colony dwindles and gradually dies out. A phenomenon known as the 'capensis calamity' (CC). CC is one of the major problems affecting beekeepers in the Northern region of South Africa who lost an average of 100,000 colonies annually. The only available control strategy against capensis infestation is by killing infested hives in order to prevent the spread of the parasites within the apiary. Using behavioural and analytical techniques, we explored the use of synthetic QMPs as a potential management strategy by transforming honeybee workers into mobile pheromone careers (PCs). In laboratory cage experiments, PCs elevated their pheromone production from those of workers which are characterised by C10 fatty acids to those of queens with C9 fatty acids. Retinues where also formed around the PCs for both sub-species eliciting similar behavioural response as queens. Thus, pheromone supplements has prospects in the management of *A. m. capensis* social parasites.

# Commercial applications of semiochemicals – Current status and future prospects

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Pheromones and other semiochemicals are commonly used in Integrated Pest Management programs for insect pests. The two primary uses of pheromones in IPM are for detection / monitoring and for mating disruption. It is estimated that the sale of traps and semiochemical-based lures account for over $55million worldwide at the manufacturer's level. Witzgall et al. (2010) estimated that there were over 20 million monitoring lures sold worldwide covering over 10 million hectares. Sex pheromone-based lures account for the majority of attractant dispensers sold although, increasingly, attractants from other sources are also being used, such as food and oviposition attractants. Major insect species are being monitored in forestry, top fruit, grapes and cotton. It is undoubtedly the case that such use has led to a much more rational and cost-effective use of conventional insecticides over the last 25 years. As legislation in the future will demand the adoption of IPM practices within the European Union and elsewhere, the use of such diagnostic devices will continue to increase over the next decade. It is estimated that over one million hectares of crops and forestry are now treated per annum with sex pheromones for control of insect pests by mating disruption. This has a market value of nearly $300 million at the manufacturer's level. Many moth species are controlled using this technique. Market demand, legislative pressures and significant technological advances in pheromone manufacture and controlled release have all contributed to a substantial increase in the use of sex pheromones for mating disruption in recent years. Current application strategies involving insect pheromones are discussed in this paper together with a look at future developments that may enhance their use further in insect pest management.

# CHEMICAL ECOLOGY OF BLOOD SUCKING ANIMALS

# Chemical Ecology of Bed Bugs

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Bed bugs, *Cimex lectularius*, are well adapted to find their hosts efficiently and then return to nearby, hidden refuges. In aggregations within the refuges, mating, oviposition, defecation, and molting occur. Both the movement to and from the refuges, and the interactions amongst individuals within these discreet places have been found to be influenced by chemical signals and cues. Here we review what is known about the chemical ecology of bed bugs, and present results of ongoing studies that suggest novel ways that host cues and chemical signals may be involved. Carbon dioxide, host odors, and body heat all have been documented to play a role in host finding. Volatile and non-volatile pheromones influence aggregation responses to harborages. While sex pheromones have not been clearly implicated in the interactions between males and females, emitted volatile chemicals do reduce the frequency of potentially damaging male-male and male-nymph copulations.

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

# Grass volatiles partially explain oviposition site selection by *Anopheles* mosquitoes

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Oviposition site selection by gravid malaria mosquitoes is a key moment in determining the reproductive success of the individual, and thus the population dynamics of the species. Consequently, the search for an oviposition site has important implications with regard to the control of malaria vectors. To date, there has been little research investigating how gravid *Anopheles gambiae sensu lato* females locate and select oviposition sites. Here, we show that gravid An. gambiae and An. arabiensis females are differentially attracted to odour extracts collected from wild grasses. In two-choice and multi-array oviposition assays we show that female mosquitoes select and discriminate between water conditioned with the different odor extracts. Work on domesticated grasses shows similar results. Here, we have used linked gas chromatography and electroantennodetection analyses to identify the bioactive compounds in two cultivars each of three important crop grasses. Synthetic blends of these compounds attract and stimulate oviposition in *An. arabiensis* in laboratory and semi-field experiments. These findings provide a breakthrough needed for developing novel monitoring and control strategies for malaria vector control.

# Identification of Host Volatiles and their Role in the Behavioural Modulation of Host-Seeking Culicoides Biting Midges

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Culicoides biting midges are vectors of Bluetongue (BTV) and Schmallenberg (SBV) viruses. The emergence of BTV and SBV in northern Europe caused substantial economical losses to animal production, and highlighted the need to develop tools to monitor and manage these disease vectors. Biting midges, as most haematophagous insects, rely on host volatiles to locate their hosts. By identifying these volatiles, novel methods can be envisioned for the manipulation of insect-host interactions.

Vectors of BTV and SBV have a preference for large vertebrate hosts, including cattle, and locate these primarily through olfaction. Through linked gas chromatograph and electroantennographic detection (GC-EAD) analysis of air entrainments from cattle hair and urine we identified 23 bioactive compounds. When combined with carbon dioxide, these compounds elicited either attraction or behavioural inhibition in *Culicoides nubeculosus*, in a dose dependent manner. Field evaluation of a selection of these compounds showed similar responses in *C. impunctatus*. Both laboratory and field assays emphasized the importance of release rate of the tested compound to obtain optimal behavioural responses.

UV light traps are to date the most commonly used method for monitoring biting midges. However, based on laboratory and field data, host volatile baited traps provide a more efficient tool for monitoring host-seeking midges. Future challenges include e.g. optimizing synthetic blends of bioactive compounds to increase the efficacy of traps.

# Discovery of an odor bait for gravid malaria vector mosquitoes of the *Anopheles gambiae* species complex

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New strategies are needed to manage malaria vector populations that resist insecticides and bite outdoors. This study describes a breakthrough in developing 'attract and kill' strategies targeting gravid females by identifying and evaluating an oviposition attractant for *Anopheles gambiae s.l.*.

Previously, the authors found that gravid *An. gambiae s.s.* females were two times more likely to lay eggs in lake water infused for six days with soil from a natural oviposition site in western Kenya compared to lake water alone or to the same but autoclaved infusion. Here, the volatile chemicals released from these substrates were analysed with a gas-chromatograph coupled to a mass-spectrometer (GC-MS). Furthermore, the behavioural responses of gravid females to one of the compounds identified were evaluated in dual choice egg-count bioassays, in dual-choice semi-field experiments with odour-baited traps and in field bioassays.

One of the soil infusion volatiles was readily identified as the sesquiterpene alcohol cedrol. Its widespread presence in natural aquatic habitats in the study area was confirmed by analysing the chemical headspace of 116 water samples collected from different aquatic sites in the field and was therefore selected for evaluation in oviposition bioassays. Twice as many gravid females were attracted to cedrol-treated water than to water alone in two choice cage bioassays (odds ratio (OR) 1.84; 95% confidence interval (CI) 1.16-2.91) and in experiments conducted in large-screened cages with free-flying mosquitoes (OR 1.92; 95% CI 1.63-2.27). When tested in the field, wild malaria vector females were three times more likely to be collected in the traps baited with cedrol than in the traps containing water alone (OR 3.3; 95% CI 1.4-7.9).

Cedrol is the first compound confirmed as an oviposition attractant for gravid *An. gambiae s.l.*. This finding paves the way for developing new 'attract and kill strategies' for malaria vector control.

# Parasite-induced olfactory cues influence mosquito attraction to malaria-infected hosts

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Vector-borne pathogens can alter the traits of their primary hosts in ways that influence the frequency and nature of interactions between hosts and vectors. Increasing evidence suggests that malaria parasites can alter the attractiveness of infected hosts to mosquito vectors. Working with the rodent malaria pathogen *Plasmodium chabaudii*, we recently documented changes in the attractiveness of infected hosts versus uninfected controls over the course of infection, along with associated changes in host volatile profiles. We observed enhanced mosquito attraction to infected mice during a key period after the subsidence of acute malaria symptoms, but during which mice remained highly infectious. This attraction corresponded to an overall elevation in the volatile emissions of infected mice observed during this period. Furthermore, experimental manipulation of several individual compounds that exhibited altered emission levels during the period when differential vector attraction was observed (compared to later timepoints and to healthy individuals) also elicited enhanced mosquito attraction, indicating that compounds being influenced by malaria infection status also mediate vector host-seeking behavior. These findings provide important insights into the cues that mediate vector attraction to hosts infected with transmissible stages of malaria parasites, as well as documenting characteristic changes in the odors of infected individuals that may have potential value as diagnostic biomarkers of infection. Building on this work, we are currently investigating the effects of Plasmodium infection on the odors of human hosts and associated changes in mosquito attraction.

# Behavioral responses of *Culex tarsalis* to fish-associated semiochemicals in wind tunnel bioassays

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The Western encephalitis mosquito, *Culex tarsalis*, has been shown to respond to the presence of fish-associated semiochemicals in oviposition sites by decreasing the number of egg rafts laid, in both laboratory and field bioassays. A decrease in oviposition rate on water that contained fish semiochemicals, has been attributed to the presence of different classes of chemical compounds which cause the mosquitoes to alter their behavior. Whether or not the compounds present act solely as long or short-range deterrents has not been determined. In wind tunnel bioassays, we evaluated the resulting behavioral sequences of female *C. tarsalis* to chemicals associated with the Western mosquitofish, *Gambusia affinis*, and evaluated their potential role on mosquito oviposition behavior.

# The Malaria whistle for immediate transfer

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Mosquitoes have more intense desire for malaria-infected blood [1]. This makes sense since it is an essential step for its transmission, which may quantify as vectorial-capacity. We tested the vector-parasite response to a parasite-associated-molecule, known to give rise to an innate immune response in humans (E)-hydroxy-3-methyl-but-2-enyl pyrophosphate (HMBPP) and found activation of selected immune genes and pathways in the vector [2].

*Anopheles gambiae s.s.* were allowed to feed on blood with/without HMBPP. RNA-sequencing analyses (Illumina) at four different time points (1, 3, 6 1nd 24 hpf) and mosquito fitness studies were performed and evaluated. Notably, increased transcription was recorded from genes of neural-receptors. Mosquitoes were offered *Plasmodium falciparum* infectious-blood for investigating the effect of HMBPP on parasite infectiousness. While HMBPP had no influence on mosquito survival, still enhanced their blood ingestion and feeding rate. Moreover, the correlation between blood-meal size and fecundity decreased. Dual choice and feeding rate experiments were conducted for comparing the attractable and stimulatory behaviour of mosquitoes towards blood-meal with/without HMBPP. We found that females were attracted by a volatile. When serum with/without HMBPP were tested, there was no difference in attraction, while in feeding rate experiments females fed much faster on HMBPP serum than on serum alone.

This study provides the first evidence that HMBPP causes volatile attraction and soluble signals. Our results are in line with the notion that mosquitoes are more attracted to and feed more aggressively on malaria-infected than on healthy-humans [3] and demonstrate that HMBPP is a parasite-molecule that can cause this difference.

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# Chemical ecology of *Rhodnius prolixus*

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Background: NGS techniques facilitate understanding the molecular bases of chemically mediated behavior. *Rhodnius prolixus* is the second most important vector of Chagas disease and its genome sequence has been made available.

Aims and objectives: The mass sequencing of the transcripts produced in the antennae of 5th instar larvae, male and female adults of *Rhodnius prolixus* will be presented, as well as data on the relative abundance of selected target genes.

Methods: Three RNAseq libraries from antennae of 5th instar larvae, males and females were obtained using Illumina's 'TruSeq Stranded RNA Sample Prep kit. A single lane generating paired-end reads (350 million) was used to mass sequence the libraries (Illumina HiSeq technology). We generated two transcriptome assemblies (Trinity and SoapDenovo), which were used to correct sequences of sensory genes partially assembled in the *Rhodnius* genome. Finally, gene expression levels were quantified (by means of TopHat-Cufflinks-Cuffdiff) and compared between libraries.

Findings: A total of 17,190 genes were predicted by Cufflinks-Cuffmerge. The overall comparison of expression data from the three libraries suggests that antennae of 5th instar larvae differ the most from those of adult males and females. Most ORs (111), as well as IRs (33) showed consistent expression in all libraries and a trend of increased expression in the antennae of adult bugs. As expected, most OBPs and CSPs showed very high expression levels in all instars studied. Diverse gustatory receptors found in the genome of this species (28) had expression in antennae.

Conclusions: The molecular bases of sensory processes (e.g., chemical and thermal) have been characterized. As chemoreception seems to be enriched for triatomine adult life, ORs and IRs showing increased expression in adults deserve functional studies. We hypothesize that they relate to the detection of sexual signals recently reported for these vector insects.

# Heritability of Attractiveness to Mosquitoes

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Female mosquitoes display preferences for certain individuals over others, which is determined by differences in volatile chemicals produced by the human body and detected by mosquitoes. Body odour can be controlled genetically (Roberts et al., 2005) but the existence of a genetic basis for differential attraction to insects has never been formally demonstrated. We aimed to identify if attractiveness to mosquitoes was heritable by evaluating the response of *Aedes aegypti* (=*Stegomyia aegypti*) mosquitoes to odours from the hands of identical and non-identical twins in a Y-tube dual-choice assay. Volatiles from individuals in an identical twin pair showed a high correlation in attractiveness to mosquitoes, while non-identical twin pairs showed a significantly lower correlation. Overall, there was a strong narrow-sense heritability of 0.62 (SE 0.124) for relative attraction and 0.67 (0.354) for flight activity based on the average of ten measurements per treatment per twin pair. The results demonstrate an underlying genetic component to our odour which is detectable by mosquitoes. This pilot study provides the basis for future work exploring the genes association with greater attraction or repellence in individuals. The development of this work could better improve formulation of specific repellents, improve mathematical modelling, refine odour-based disease diagnostics and potentially further our understanding of human odour communication.

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# Utilization of flowers and their volatiles for surveillance and management of mosquitoes and other biting flies.

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Throughout their life cycle mosquitoes and other biting flies require nectar for flight and to sustain other life functions. The overall objective of our current research objective is discover flowering plants which are utilized by these blood sucking insects in order to isolate and identify the volatiles emanating from these flowering plants in order to use them in the development of novel surveillance and/or management strategies. Initial emphasis has been placed on determining the phenology of flowering plants in natural ecosystems and those used as landscape plants in suburban neighborhoods in Florida. Laboratory olfactometer and field studies have identified several flowering plants that attract both sexes and all physiological states of female mosquitoes and ceratopogonidae biting midges. Preliminary studies have shown great promise in manipulating the behaviors of our target species with either the volatiles or the intact plants. The challenge of utilizing these volatiles in "attract-and-kill" strategies is to discover chemicals which attract mosquitoes and biting flies, but not pollinators.

# Gustatory synergy between sugars and amino acids in the yellow fever mosquito, *Aedes aegypti*

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Many insects including mosquitoes, butterflies, honeybees, flesh flies and ants have been shown to prefer diets that contain both sugars and amino acids. Fitness gains associated such as enhanced longevity, flight capacity and fecundity, suggest an ultimate evolutionary mechanism for the preference of amino acid-rich sugar diets. However, the proximate mechanism(s) behind this preference has not yet been explored. In a recent study, the yellow fever mosquito, Aedes aegypti, was unable to detect individual amino acids unless combined with a behaviorally sub-threshold concentration of a sugar. This behavioral response to combination of two nutrients at individual sub-threshold concentrations indicates a synergistic proximate mechanism underlying the mosquito gustatory preference. To investigate this further, we conducted feeding bioassays and single-sensillum electrophysiological recordings using a combination of trehalose and various concentrations of either one of seven ecologically relevant amino acids (alanine, arginine, glycine, leucine, phenylalanine, threonine and valine). In feeding assays, females prefer and imbibe mixed diets in significantly higher volumes in a dose-dependent manner than trehalose alone for all amino acids except glycine & arginine. The single-sensillum recordings allowed us to generate a chemotopic map of the dorsal labellar sensilla that describes five of the seven test mixed diets as eliciting responses in different sensilla, either increasing (leucine, valine & alanine) or decreasing (glycine & arginine) gustatory neuron firing rates. Our results indicate that the synergistic behavioral response of female *Ae. aegypti* to amino acid and sugar mixed diets is a result, at least in part, of combinatorial coding of the gustatory neuron input from the dorsal sensilla on the labellar lobes.

# Difference in mosquito attraction; between humans, within humans and between humans and great apes

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Humans vary in their attractiveness to mosquitoes because of the odours they emit. To investigate the mechanisms determining the differential attractiveness of humans to mosquitoes, we tested the attractiveness of 48 volunteers to the malaria mosquito *Anopheles gambiae*. In addition, we determined the odour and bacterial profiles of these volunteers. Groups of individuals that was highly attractive and poorly attractive were identified and degree of attractiveness correlated with their bacterial profiles.

To determine intra-individual differences in attraction, odour samples were taken from three different body parts of eight individuals and tested for attractiveness to mosquitoes. Volatiles from the armpit were less attractive than volatiles from hand and foot, however, contamination by deodorant compounds were identified in the odour profiles. When the individuals were asked not to use perfumes and deodorants for 5 days before sampling, no contamination and no differences in attractiveness between body parts were found. Subsequent tests revealed that one of the deodorant compounds was repellent, which indicates that some deodorants may influence a person's attractiveness to mosquitoes.

Little is known about mosquitoes that could transmit diseases between great apes and humans. A preliminary study in an olfactometer showed that anthropophilic mosquitoes are also attracted to chimpanzee and gorilla odours to the same extend as to human odour. Future experiments will investigate how similar the bacterial and volatile profile is between humans and great apes and how this may facilitate disease transmission between different species.

# Plant volatiles in perspective of mosquito disease vector control

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The control of mosquito populations remains one of the most effective ways of decreasing the incidence of the diseases they transmit. The widely used vector control strategies, insecticide-treated bednets and indoor residual spraying (IRS), are faced with increasing resistance in mosquito populations, and hence the need to explore novel approaches. Promising complementary approaches include the use of traps baited with attractants to reduce vector populations. Current traps make use of human/animal-based lures which mainly target blood seeking females and require $CO_2$ as an essential ingredient. The high cost of commercial $CO_2$ and logistical challenges for its use in remote poor resource communities, is a major setback in the use of these lures which are highly dependent on $CO_2$ for their efficacy. One area that is understudied is the chemical ecology of plant feeding in mosquito disease vectors, which offers a promising new target for vector control and possibly requiring minimal use of $CO_2$. Besides the potential to trap mosquitoes of varying physiological status and sexes, plant-odor attraction can serve as an effective target against outdoor populations of mosquito vectors thereby reducing human-vector contact. We present advances in the identification of plant volatiles and evaluation of their attractiveness to mosquito vectors of three diseases including malaria, Rift Valley fever and dengue. We use a range of techniques varying from laboratory behavioral assays, GC-EAD and GC-MS analyses and field assays and show that plant volatiles can be exploited in traps to reduce mosquito disease vector population.

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# Disruption of Varroa-honeybee association by chemosensory inhibition

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In recent years many honey bee colonies are lost due to infection with the ectoparasitic mite Varroa destructor. The mite has two life stages: the phoretic stage, in which it preferentially attaches to adult bees and feeds on their haemolymph, and reproductive phase, in which the mites reproduce within brood cells. Chemical cues play a crucial role in behavior of both mites and honey bees. The mites detect their hosts by the special chemosensory organ located on the forelegs, while colonial activities of honeybees are coordinated mainly by chemical cues detected by the antennae. In view of limited success in mite control and differences in olfactory system of insects and mites we investigated the possibility to hinder the Varroa-honeybee association by use of volatile chemosensory disruptive compounds. The effect of the compounds was evaluated by monitoring electrophysiological response of the Varroa foreleg and honeybee antennae and by behavioral assays on Varroa and honeybees. Using this approach, several potent chemosensory disrupting compounds for Varroa were identified so far. Two types of electrophysiological effects were detected on isolated Varroa foreleg: short term inhibition and long term inhibition of response to nurse bee head space. The compounds that elicited long term inhibition were found to have a profound effect on Varroa behavior but not on that of the honeybee. The effect varied between the tested compounds: some caused switch in host preference from nurse to forager while other inhibited host selection (1; 2). The specificity of the tested compounds for Varroa chemosensing reflects profound differences in the chemosensory machinery of the parasite and its host thus opening new venues for Varroa management.

# Optimizing pheromone aerosol emitters for codling moth mating disruption

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High levels of orientational disruption have been achieved with deployment of as few as two aerosol-emitting devices per hectare. Although encouraging, making the approach robust or consistently effective will arise through sorting out the mechanism by which disruption is achieved and improving the economics. Aerosol emitters were deployed at various densities in commercial apple and walnut orchards to generate dosage-response profiles in order to elucidate the behavioral mechanism of disruption. The resulting set of profiles matched the predictions for competitive rather than non-competitive disruption. Aerosol emitters disrupt CM principally by inducing false-plume following. Males move toward the aerosol emitters, bypassing females and traps along the way. From a practical standpoint, growers are limited to deploying only one or two aerosol emitters per acre because of the high cost of the unit and especially the pheromone. The amount currently released per day was essentially chosen to equate the release rate from one emitter to that of 400 hand-applied dispensers. In an effort to refine this technology and increase its cost-competitiveness, we conducted a series of studies evaluating the effectiveness of emitters designed to release smaller than standard amounts of pheromone active ingredient. Field experiments demonstrated that excellent codling moth suppression could be achieved using aerosol dispensers filled with half the current amount of pheromone. Additional trials revealed that further reductions are possible by limiting emissions to the first few hours of peak evening flight activity and reducing the number of emissions per hour to one or two compared to the current four per hour. Combined, these reductions in the pheromone requirement could significantly reduce the cost of aerosol emitters. Furthermore, the control achieved using aerosol emitters may be improved if less costly units that release lower rates of pheromone were deployed at densities of 3-6 per acre

# Unifying the field of floral chemical ecology

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Studies of floral chemical ecology have mainly focused on one of two subfields: either floral volatiles or nectar chemistry (e.g. 'toxic nectar'). Despite a shared interest in understanding the chemical ecology of flowers, these aspects of floral chemistry have generally been studied as somewhat distinct fields. Here we summarize the current status of floral chemical ecology and argue that understanding the chemistry of flowers will benefit from a more unified approach. We highlight examples from our own work that demonstrate how floral compounds can have multiple roles in plant interactions, both as signalling compounds and as components of reward. We demonstrate how adopting and unifying theory and approaches developed for understanding either floral volatiles or nectar chemistry could provide a deeper understanding of the ecology and evolution of floral chemistry as a whole.

CONCEPTS IN CHEMICAL ECOLOGY

# Insect Olfaction - Quo vadis?

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Since Schneider’s pioneering work, allowing us to record directly from insect olfactory sensory neurons, we have seen about 55 years of results from our field. One more event can be singled out as a game changer; the identification of olfactory receptors around 2000 and the molecular revolution ensuing. Which are the upcoming challenges? As always the main challenge is to somehow understand the data we are collecting. How does the insect olfactory system make sense of the chaotic chemical world surrounding it? Despite the advances made since Schneider’s days we lack a direct link between input (antenna, palp, antennal lobe) and output (wings, legs). To form this link and to do it in an ecological context is the major challenge facing us in the coming 55 years. I will provide some examples where we might direct our research to approach this goal.

# Insect cuticular hydrocarbons: exciting functions of boring chemicals?

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Insect cuticular hydrocarbons (CHCs) are undoubtedly among the most intensively studied semiochemicals. CHCs typically occur in low-volatile multicomponent mixtures of compounds differing widely in chain lengths as well as in the number and positions of methyl-branches and double bonds. Thus, CHCs have a great potential for encoding chemical information, and indeed, numerous fascinating interactions of insects with con- and heterospecifics have been shown to be mediated by CHCs. However, the fact that CHCs are easily detectable in virtually any insect extract, and that their profiles can be distinguished by humans using statistical methods, does not necessarily mean that they are used by insects for chemical communication. Hence, biological activity has to be demonstrated in each particular system rather than extrapolating results from related taxa or relying merely on correlational evidence, as is done much too often. The gold standard for the demonstration of bioactivity is the use of synthetic analogues of the natural products. It is thus becoming clear that we are not as far as we thought with CHC research, since many synthetic methyl are still unavailable and have an as-yet unknown stereochemistry . The interplay of genetic and extrinsic factors influencing the composition of CHC profiles, as well as the mechanisms that control their perception, are also only poorly understood. In my talk I will highlight some recent advances concerning the biological functions, evolution, chemistry and perception of CHCs, and point to some old yet unsolved problems in the study of CHC mediated interactions.

CON03

## Estimating rates of spread of invasive insects with pheromone-baited traps

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**Andrew Liebhold**, US Forest Service Northern Research Station, Morgantown, West Virginia, USA

For many years, pheromone-baited traps have been used worldwide to detect and map the presence of various invasive insect species. Their ability to detect the presence of very low densities is a common attribute. Data from numbers captured in pheromone-baited traps have been used to estimate the rate of spread of invasive species. Various mathematical models of spread have been used for this purpose. I will illustrate this process with examples from the spread of winter moth, Operophtera brumata, and gypsy moth, Lymantria dispar in the eastern United States

# Mating system and induction shape the volatile emissions of wild potatoes

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**Stuart Campbell**, University of Toronto, Toronto, Canada; **Andre Kessler**, Cornell University, Ithaca, USA

The diversity of floral volatiles observed in flowering plants has been attributed to diverse interactions with mutualists (e.g. pollinators) and antagonists (e.g. herbivores). Hypotheses about the function of floral volatiles in pollinator attraction assume that floral volatile production is dependent on the plant mating system and therefore should correlate with the dependence on pollinator visitation. In the nightshades (Solanaceae) self-incompatibility (i.e. total dependence on pollinators) has been lost repeatedly and we have demonstrated interactions between herbivore defense and pollinator attraction in several wild tomato species (Kessler & Halitschke, 2009; Kessler et al., 2011). Here we characterize volatile emissions of wild potato plants with different mating systems. Constitutive emissions and wound hormone-induced changes in leaf and flower volatile emissions show different patterns in self-compatible and self-incompatible potato plants. Self-incompatible plants emit stronger and more diverse floral volatile bouquets whereas elicitation by methyl jasmonate application had a stronger effect on self-compatible plants. Furthermore, volatiles of several compound classes (monoterpenes, sesquiterpenes, benzenoids) show differential responses to the induction treatment and plant mating system. The results of the potato study will be discussed in the context of the recently developed framework of ecological consequences of plant mating system-dependent interactions between herbivore resistance and pollinator attraction.

Kessler A & Halitschke R (2009) Testing the potential for conflicting selection on floral chemical traits by pollinators and herbivores: predictions and case study. Functional Ecology 23: 901-912.

Kessler A, Halitschke R & Poveda K (2011) Herbivory-mediated pollinator limitation: negative impacts of induced volatiles on plant-pollinator interactions. Ecology 92: 1769-1780.

CON05

# Advances in material science allow a fully integrative study of visual and chemical ecology

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**Thomas Baker**, Penn State University, University Park, PA, USA; **Akhlesh Lakhtakia**, Penn State University, University Park, PA, USA

For decades, it has been possible to replicate and reductively analyze chemical cues that affect insect behavior. This approach has led to intensive research in a wide array of fields including applied entomology, olfactory perception, and the evolution of mating systems. Recent advances in material science now also allow high-fidelity replication of complex visual cues that trigger behavioral responses. For organisms in which both visual and chemical signals interact, a more rigorous examination of the ecological basis for insect communication is now possible. We created nanoscale replicas of the surface structure of the emerald ash borer, an invasive wood-boring buprestid beetle. The visual appearance of these beetles resting on leaves elicits stereotypical male mating flights from up to 1 m away. We presented decoys with and without these carefully replicated nanoscale cuticle surface features to wild males. Only the high fidelity bioreplicated decoys, which generate the same distinctive light-scattering pattern as real resting females, promoted the stereotypical behavior. A trap was successfully created by electrifying these bioreplicated decoys. Host odors and beetle-produced compounds, which were also incorporated into trap designs, attract these beetles and interact with the visual signal. Furthermore, there are upwards of 15,000 buprestid species, most of which have distinctive metallic coloration patterns. We have documented color-based mating preferences in another species, Agrilus angustulus. Thus an integrative understanding of buprestid chemical and visual communication can now be achieved for topics ranging from trapping applications to speciation theory.

Domingue MJ, Lakhtakia A, Pulsifer DP, Hall LP, Badding JV, Bischof JL, Martín-Palma RJ, Imrei Z, Janik G, Mastro VC, Hazen M, Baker TC. 2014. Bioreplicated visual features of nanofabricated buprestid beetle decoys evoke stereotypical male mating flights. Proc. Nat. Acad. Sci. USA 111, 14106–14111.

# Critical Themes in Research on Tritrophic Interactions

**J. Daniel Hare**
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There has been more than 30 years' worth of research on the interactions between plants, their arthropod herbivores, and the natural enemies of those herbivores. Several themes have emerged, with the most important is that the plant does indeed influence the interactions between herbivorous arthropods and the natural enemies of those herbivores. Yet, it has proven difficult to develop more specific generalizations about how such interactions might have evolved and are maintained. Focusing upon the induced production of volatile organic compounds (VOCs) by plants, I review some of the critical and vexing themes in the study of tritrophic interactions. Does the production of such compounds improve plant fitness, and, if so, then how? How might better evidence for such fitness benefits be obtained? Do blends produced by plants attacked by different combinations of herbivore species vary in composition, and is such variation adaptive? Why does the ability of plants to produce VOCs vary with plant development, and how is such variation also adaptive? What other roles might these induced VOCs play? How might such roles themselves vary in simple vs. complex plant communities? Although we have a huge body of evidence now showing how plant traits can influence herbivore-natural-enemy interactions, there remains a critical need to better understand the mechanisms of natural selection that resulted in the establishment and development of such traits.

CON07

## Stabilizing Selection on Moth Pheromone Blends: Fact of Fiction?

**Jeremy Allison**
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Although asexual reproduction has evolved independently several times in the Lepidoptera, sexual reproduction is an almost universal condition among moths. It is typically preceded by long-distance attraction of males and short-range courtship of females mediated by pheromones. Chemical specificity in moth mate recognition systems is hypothesized to be a product of qualitative (e.g., presence/absence of components and differences in component double bond configuration or position, enantiomer configurations, chain length, and functional moieties) and often quantitative (ratios of components) differences in the pheromone blend. Although there is little debate about whether these differences can confer reproductive isolation, there is considerable debate how these differences evolve and whether these differences are involved in driving the speciation process or whether they are sequelae that follow divergence. Several models of sex pheromone evolution in moths have been proposed. These models can be differentiated on the basis of the predicted shape of the receiver preference function relative to the distribution of female pheromone signals (i.e., the ratio of variance in male response and female signaling traits). The Wallflower, Asymmetric Tracking, and Stasis models predict that male preference functions will be stabilizing while the Competitive Signal Evolution hypothesis predicts directional preference functions, with receiver preferences for increasingly exaggerated signals. This talk will review the existing literature on male moth preference function shape. The primary conclusions are that: 1) few studies have characterized male preference function shape over the range of signals actually produced by females; 2) those that have provide mixed support for the Stasis and Asymmetric Tracking models; and 3) that additional empirical work is needed.

# Functional, evolutionary and teleological perspectives on finding a source of odor

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Finding a distant, resource-linked source of odor is a crucial behavior for many organisms. The first task is finding the odor plume, and, although foraging strategies favoring either upwind, downwind, or crosswind trajectories have been proposed, field observations and simulation modeling suggest that random orientation may be optimal. Once the plume is contacted, the compounds inducing orientation along the plume commonly are labeled "attractants," but, as John Kennedy pointed out in 1972, this is a teleological concept, in this case describing only the endpoint of orientation. Attractants simply do not themselves "attract," but rather these stimulate upwind flight. Studies with moths and mosquitoes have shown that upwind flight requires visual feedback to determine upwind direction and that the fine-scale structure of odor plumes guides displacement upwind. But moths and mosquitoes also can navigate along plumes, at least briefly, in still air, guided in part by the distribution of odor and perhaps using flow of the visual field to continue along a path set in wind. Finally, with moths contact with the odor's source may be contingent on a few compounds constrained by stabilizing selection. With mosquitoes, a disquieting number of compounds may guide navigation. Behavioral assays aimed at unraveling the interplay of these sensory inputs have relied on many kinds of bioassays. For moths, the wind tunnel has played a pivotal role in defining the natural pheromone blend. For mosquitoes, several kinds of bioassays are used, but many current studies rely on suction field traps baited with carbon dioxide augmented with host odors. Such "end-point" assays do not permit expression of all of the orientation maneuvers used in nature, particularly landing on a prospective host. This raises the intriguing question of whether the bioassays that are used in chemical ecology narrowly circumscribe the question posed and therefore dictate the answer.

# POSTER PRESENTATIONS

| | Surname | Name | University | Title of abstract | Session |
|---|---|---|---|---|---|
| 1 | Bergström | Gunnar | University of Gothenburg | A chemical ecology OLPHABET | I |
| 2 | Rahmani | Rizan | Mid Sweden University | Applications of preparative GC for purification of diterpenes | III |
| 3 | Schlyter | Fredrik | Swedish University of Agricultural Sciences | Three fallacies in understanding evolution of insect-habitat odour cue responses | I |
| 4 | Suckling | Maxwell | University of Auckland | How can chemical ecology contribute to biosecurity against invasive Arthropods? | I |
| 5 | Wang | Yi | Swedish University of Agricultural Sciences | Neurophysiological mechanisms underlying the experience dependent behavioral adaptations | II |
| 6 | Zarbin | Paulo | UFPR | Recent advances in the pheromone chemistry of stink bugs | III |
| 7 | Cardenas | Paco | Uppsala University | Sponge taxonomy 2.0 meets pharmacognosy and chemical ecology | I |
| 8 | Cheseto | Xavier | International Centre of Insect Physiology and Ecology | Two African fruit flies (Diptera: Tephritidae) produce identical host marking pheromones | II |
| 9 | Dobson | Heidi | Whitman College | Floral volatiles in host-flower recognition in a pollen-specialist bee | III |
| 10 | Ilag | Leopold | Stockholm University | Biomolecular MS of amino acids and their role in chemical ecology | I |
| 11 | Keller | Matthieu | CNRS | Neuroendocrine and behavioral consequences of peripubertal exposition to male odor in female mice | II |
| 12 | Lee | Dae-Weon | Kyungsung University | Comparative transcriptome analysis of pheromone glands on sex pheromone biosynthesis in Plutella xylostella | III |
| 13 | Morkunas | Iwona | Poznan University of Life Sciences | A differential induction of defence signaling molecules in pea response to Acyrthosiphon pisum and the effect of exogenous nitric oxide donor on its infestation | I |
| 14 | Pavia | Henrik | University of Gothenburg | Chemistry releases an invasive seaweed from native enemies | II |
| 15 | Escobar-Bravo | Rocío | University of Leiden | Exploring inducible trichome-mediated resistance to the Western flower thrips in tomato | I |
| 16 | Lei | Hong | University of Arizona | Intrinsic and network mechanisms constrain neural synchrony in the moth antennal lobe | II |
| 17 | Quero | Carmen | IQAC-CSIC | Friend or foe? Mutualism and aggressive mimicry in an aphid | II |
| 18 | Godschalx | Adrienne | Portland State University | Does extrafloral nectar release a volatile scent? | I |
| 19 | Grabarczyk | Malgorzata | Maria Curie-Sklodowska University | Intermolecular interactions in systems containing Bi(III) – ClO – HO – homocysteine in the aspect of catalysis of Bi(III) electroreduction | II |
| 20 | Klutsch | Jennifer | University of Alberta | Impact of interactions among native biotic disturbances on range expansion of mountain pine beetle into novel jack pine forests | II |

This symbol marks contestants for the Best Student Presentation Award

| | Surname | Name | University | Title of abstract | Session |
|---|---|---|---|---|---|
| 21 | Antony | Binu | King Saud University | Genes involved in sex pheromone biosynthesis of Ephestia cautella, an important food storage pest, are determined by transcriptome sequencing | III |
| 22 | Aragón | Sandra | Georg-August University | Not visible, but highly effective: Plant volatile manipulation by endophytic fungi and responses of herbivores | I |
| 23 | Kofronová | Edita | Institute of Organic Chemistry and Biochemistry | Do commercial bumblebees Rrepresent a threat for local subspecies? | I |
| 24 | Jirošová | Anna | IOCB AS CR | Disentangling the biosynthesis of termite-produced nitro compounds | II |
| 25 | Asproni | Pietro | Research Institute in Semiochemistry and Applied Ethology | Does spontaneous vomeronasalitis influence chemical communication in domestic animals? A new approach linking pathology to behavior. | III |
| 26 | Goulart Santana | Antonio Euzebio | Federal University of Alagoas | Identification of the Volatile Organic Compounds Profile in a Resistant Cultivar of Cassava (Manihot esculenta) | I |
| 27 | Goulart Santana | Antonio Euzebio | Federal University of Alagoas | Does the association between sugarcane (Saccharum spp.) and diazotrophic microorganisms provide pest resistance? | II |
| 28 | Yon | Felipe | Max Planck Institute for Chemical Ecology | Floral scents to the touch: Scents inform moth's proboscis for probing and foraging, thus determining the plant fitness | I |
| 29 | Nieberding | Caroline | University of Louvain-la-Neuve | Food-stressed males display compensation in fitness-associated physiological and behavioural traits and females get fooled if they cannot assess the male odour in the butterfly Bicyclus anynana. | II |
| 30 | Utherdyany Bicalho | Keylla | Federal University of São Carlos | Acetogenins from Annona mucosa for leaf-cutting ants control | I |
| 31 | Laumann | Raul Alberto | Embrapa Genetic Resources and Biotecnhology | The responses of egg parasitoids Telenomus podisi and Trissolcus basalis (Hymenoptera: Platygastridae) to chemical footprints of stink bugs are related to host preference. | II |
| 32 | Perlatti | Bruno | Federal University of São Carlos | Identification of symbiont bacteria of Diabrotica speciosa using MALDI-TOF MS | III |
| 33 | Boeckler | Andreas | Max-Planck Institute for Chemical Ecology | Processing of salicin-derived poplar phenolics in the gypsy moth | I |
| 34 | Dreisewerd | Klaus | University of Münster | Laser MS imaging of cuticular lipids using etched silver substrates | II |
| 35 | Azeem | Muhammad | COMSATS Institute of Information Technology | Antagonistic activity of Bacillus subtilis A18–A19 strains against Heterobasidion species | III |
| 36 | Wedin | Cecilia | KTH Royal Institute of Technology | Chemodiversity in Begonia flower fragrances | I |
| 37 | Colazza | Stefano | University of Palermo | Attraction of egg parasitoids toward oviposition induced plant volatiles in a multi-herbivore perspective | II |
| 38 | Guerrero | Angel | IQAC-CSIC | Antagonists of the sex pheromone of the tomato leafminer Tuta absoluta. Synthesis, functional assays, and electrophysiological activity | III |

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| | Surname | Name | University | Title of abstract | Session |
|---|---|---|---|---|---|
| 39 | Quero | Carmen | IQAC-CSIC | Mating status affects pheromone production and EAG responses in the tomato leafminer Tuta absoluta | I |
| 40 | Butkiene | Rita | Center for Physical Science and Technology | Antifungicidal and antioxidant activities of Rhododendron tomentosum essential oils | II |
| 41 | Heath | Jeremy | North Carolina State University | Male preference imposes stabilizing selection on female pheromone phenotype | III |
| 42 | Sobhy | Islam | Okayama University | The diurnal emission pattern of constitutive and induced rice volatiles | I |
| 43 | Lazebnik | Jenny | Wageningen University | Potato blight, aphids and Colorado potato beetles: How do potatoes cope? | II |
| 45 | Antony | Binu | King Saud University | Two fatty acyl-CoA reductase gene involved in moth pheromone biosynthesis | III |
| 46 | Koschier | Elisabeth | University of Natural Resources and Life Sciences | Behavioural responses of Frankliniella occidentalis Pergande to cis-jasmone | I |
| 47 | Kordan | Bożena | University of Warmia and Mazury | Natural and altered tterpenoids as modifiers of aphid probing | II |
| 48 | Proffit | Magali | Centre d'Ecologie Fonctionnelle et Evolutive | Are plant volatiles mediating highly specialized plant-insect interactions affected by environmental conditions? | III |
| 49 | Verheggen | François | University of Liege | First evidence of a volatile sex pheromone in lady beetles | I |
| 50 | Timbilla | James | QCC-City University of New York | Prospect for field management of Zonocerus variegatus with Pyrrolizidine alkaloid based-attracticide bait in sub Saharan Africa | II |
| 51 | Vidkjær | Nanna Hjort | Aarhus University | Ecological chemistry of weaver ants – nutrient cycling and search for novel chemical characteristics | III |
| 52 | Wickham | Jacob | Institute of Chemistry | Surface hydrocarbon layers affect attractiveness of dispersing female Monochamus alternatus, the vector stage of the pinewood nematode | III |
| 53 | Clausen | Mette | University of Copenhagen | Possible dual role of cytochromes P450 from Eucalyptus | I |
| 54 | Moghbel | Nahid | University of Queensland | Chemical and molecular characterization of nicotine to nornicotine conversion phenotype in Australian Nicotiana species used as chewing tobacco | III |
| 55 | Adams | Rachelle | University of Copenhagen | Evolutionary transitions in alarm systems of fungus-farming ants | II |
| 56 | Salamanca | Jordano | Universidade Federal de Lavras | Testing herbivore-induced plant volatiles for natural enemy conservation in agricultural systems | III |
| 57 | Girling | Robbie | University of Reading | Trajectories of pheromone plumes in an orchard canopy at night | I |
| 58 | Niemeyer | Hermann | University of Chile | Role of cuticular compounds in defense by soldiers of the one-piece termite Neotermes chilensis | II |
| 59 | Dam | Marie Inger | Technical University of Denmark | Yeast cell factory for production of insect pheromones | III |

This symbol marks contestants for the Best Student Presentation Award

| | Surname | Name | University | Title of abstract | Session |
|---|---|---|---|---|---|
| 60 | Cuervo Martínez | Mónica Adriana | University of Ulm | How do long-horned bee pollinated sexually deceptive orchids attract their pollinators? | I |
| 61 | Gabirot | Marianne | Cardiff University | How blue Petrels find their scented burrow? | I |
| 62 | Porciani | Angélique | IRD | Insecticide resistance mutation (Kdr) in Anopheles gambiae modulates host choice and olfaction in presence of pyrethroid-treated net | II |
| 63 | Fernandez | Patricia | INTA. EEA Delta del Parana | Both volatiles and epicuticular plant compounds determine oviposition of the willow sawfly nematus oligospilus on leaves of Salix spp. (Salicaceae) | II |
| 64 | Williams | Livy | USDA-ARS | Methyl jasmonate induction of cotton: A field test of the "attract and reward" strategy of conservation biological control | III |
| 65 | Hall | David | University of Greenwich | Sex pheromone of the saddle midge, Haplodiplosis marginata: Effects of chirality and minor components | I |
| 66 | Tolasch | Till | University of Hohenheim | Decrypting a cryptic click beetle species (Coleoptera: Elateridae) using chemical ecology | II |
| 67 | Günther | Catrin | University of Auckland | The chemical context of interepecific communication: Ecological chemistry as driver for mutualism of yeasts and flies | III |
| 68 | Fürstenau | Benjamin | Free University of Berlin | How background odor affects the host searching behavior of the ectoparasitoid Holepyris sylvanidis. | I |
| 69 | Nedveckytė | Irena | Nature Research Centre | Oviposition responses of Plodia interpunctella (Hübner) (Lepidoptera: Pyralidae) to cereal contaminated by fungi | II |
| 70 | Lhomme | Patrick | Swedish University of Agricultural Sciences | Effect of larval experience to plant olfactory cues on host plant choice in a polyphagous moth | III |
| 71 | Nyabuga | Franklin N. | Lund University | Clover seed weevils: Flowers for the females | I |
| 72 | Castano-Duque | Lina | Pennsylvania State University | Caterpillar and rootworm feeding differentially affects defense protein accumulation in corn | III |
| 73 | Filgueiras | Camila C | Universidade Federal de Lavras | Aboveground application of elicitors recruits an entomopathogenic nematode belowground | I |
| 74 | Schott | Matthias | Justus Liebig University | MALDI-MS imaging of insects – metabolite landscapes of the internal anatomy | II |
| 75 | Mann | Florian | TU Braunschweig | New insights into the chemical composition of androconial organs of Ithomiines | III |
| 76 | Webb | Owen | Queensland University of Technology | Fermentation volatiles associated with larval infestation act as innate deterrents for a polyphagous pest species. | I |
| 77 | Jelvez Serra | Nadia | Federal University of Alagoas | Sex attractant of the annona fruit borer, Cerconota anonella Sepp. (Lepidoptera: Oecophoridae) | II |
| 78 | Milonas | Panagiotis | Benaki Phytopathological Institute | Use of oviposition induced volatiles in tomato plants by Trichogramma parasitoids | III |
| 79 | Schorkopf | Dirk Louis P. | Swedish University of Agricultural Sciences | The scent of African malaria mosquito natural oviposition sites: Odours influence but do not dictate oviposition decision making in Anopheles gambiae s.l. | I |

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| | Surname | Name | University | Title of abstract | Session |
|---|---|---|---|---|---|
| 80 | Ljunggren | Joel | Mid Sweden University | Combinatorial retention-activity relationship of fractionated turpentine on fungal growth | II |
| 81 | Andersson | Håkan S | Linnaeus University | Discovery of peptide toxins in ribbon worms: Challenging claims of tetrodotoxin production | III |
| 82 | Kubanek | Julia | Georgia Institute of Technology | The chemistry of induced resistance in marine plankton: Tackling the challenges of identifying waterborne cues | I |
| 83 | Hefetz | Abraham | Tel Aviv University | Functional exocrinology of social bees - comparative analyses of two bumble bee | II |
| 84 | Guarino | Salvatore | CNR | Role of olfaction in Odonata sex recognition | III |
| 85 | Üveges | Bálint | Hungarian Academy of Sciences | Resource-dependent changes in the production of chemical defences during early ontogeny in the common toad (Bufo bufo) | I |
| 86 | Mathur | Vartika | University of Delhi | Does better growth imply better immunity? Effect of biofertilizers on induced responses of Brassica juncea | II |
| 87 | Etxebeste | Iñaki | Swedish University of Agricultural Sciences | The kairomonal response of sibling Tomicus species reflects the differences in their breeding hosts | II |
| 88 | Colazza | Stefano | University of Palermo | Drought stress affect host-induced volatile organic compounds emission from plants and parasitoid response | III |
| 89 | Jakobsson | Johan | Lund University | Olfactory and visual cues affecting the atraction of Cydia strobilella L. to oviposition sites | I |
| 90 | Santos | Isabel | University of São Paulo | Flower scents of Campomanesia phaea (Myrtaceae) as attract for nocturnal bee pollinators | II |
| 91 | Wicker-Thomas | Claude | CNRS | Regulation of Drosophila pheromones by sex peptide | III |
| 92 | Carlsson | Mikael | Stockholm University | Interaction of aphrodisiac pheromone components in the butterfly Pieris napi | I |
| 93 | Dreyer | Bastian | Leibniz University | Piezoelectric-active polymer coatings and their influence on bacterial cell-cell interaction | II |
| 94 | González | Andrés | Universidad de la República | Studies on the chemical communication of the bronze bug, Thaumastocoris peregrinus (Heteroptera: Thaumastocoridae), a pest of Eucalyptus | I |
| 95 | Ziesche | Lisa | TU Braunschweig | N-acylhomoserine lactones of macroalgae associated Roseobacter bacteria | III |
| 96 | Murungi | Lucy | International Centre of Insect Physiology and Ecology | Plant volatiles signal the host seeking process in root-knot nematodes | I |
| 97 | Ozawa | Rika | Kyoto University | Genetic variation of Tetranychus kanzawai confer different abilities on acaricide tolerance | II |
| 98 | Suzuki | Yuri | Yamagata University | Growth inhibition activity of Sugi (Cryptomeria japonica) components against Microcystis aeruginosa | III |
| 99 | Zhang | Dan-Dan | Lund University | Functional characterization of a receptor for a Type II sex pheromone in the winter moth, Operophtera brumata (Lepidoptera: Geometridae) | III |

○ This symbol marks contestants for the Best Student Presentation Award

| | Surname | Name | University | Title of abstract | Session |
|---|---|---|---|---|---|
| 100 | Schubert | Fredrik | KTH Royal Institute of Technology | Repelling Aedes aegypti – a sustainable plant based solution | I |
| 101 | Ortiz | Antonio | University of Jaen | Electrophysiological (EAG) responses of Simulium spp to human sweat volatiles | II |
| 102 | Pankoke | Helga | Bielefeld University | The impact of abiotic and biotic challenges and their interactions on plant morphology and the phytometabolome of Plantago lanceolata | III |
| 103 | Tóth | Miklós | Plant Protection Institute CAR HAS | Non-sticky trap for Meligethes (Coleoptera, Nitidulidae) combining visual and chemical stimuli | I |
| 104 | Li | Tao | University of Eastern Finland | Ozone pollution compromises within-plant signalling via volatiles | II |
| 105 | Hughes | Gabriel | Purdue University | Determination of the absolute configurations of the contact sex pheromone components of the longhorned beetle, Neoclytus acuminatus acuminatus (F.) (Coleoptera: Cerambycidae) | II |
| 106 | Roese | Ursula | University of New England | Antimicrobial properties of inducible compounds in the brown macroalgae Fucus vesiculosus | III |
| 107 | Austel | Nadine | German Federal Institute for Risk Assessment | Influence of fumigants on sunflower seeds: Characteristics of fumigant desorption and alterations of volatile profiles | I |
| 108 | Rehermann del Rio | Guillermo | Universidad de la República | Pheromone autodetection in a noctuid moth: GC-EAD response of female Pseudaletia adultera to its own sex pheromone components | II |
| 109 | Kim | Hyun Kyung | Chungbuk National University | Effects of sesquiterpene from Perilla frutescens against the diamondback moth, Plutella xylostella L. | III |
| 110 | Kim | Hyun Kyung | Chungbuk National University | Repellency of 33 plant materials and Curcuma longa L. against Culex pipiens and Aedes albopictus | I |
| 111 | Adams | Rachelle | University of Copenhagen | Chemically armed ants pillage and protect fungus-farming societies | III |
| 112 | Wardak | Cecylia | Maria Curie-Skłodowska University | Application of ion-selective electrodes to determination of naproxen and diclofenac in various samples. | II |
| 113 | Wardak | Cecylia | Maria Curie-Skłodowska University | Ion-selective electrode with solid contact for monitoring of nitrate content in surface waters and food | III |
| 114 | Grabarczyk | Malgorzata | Maria Curie-Skłodowska University | Analysis of the Cr(VI) concentrations in cement materials | I |
| 115 | Jacksén | Johan | KTH Royal Institute of Technology | Simple fabrication of hydrophobic AKD coated MALDI concentration plates for increased sensitivity | II |
| 116 | Holighaus | Gerrit | University of Göttingen | Eight-carbon volatiles as infochemicals for fungivores | III |
| 117 | Stökl | Johannes | University of Regensburg | Flexibility of chemical defense in the parasitoid wasp Leptopilina heterotoma | I |
| 118 | Hammerbacher | Almuth | Max Planck Institute for Chemical Ecology | Biochemical characterization of lignan biosynthesis in Picea abies in response to infection by the bark beetle associate Enodconidiophora polonica | II |

This symbol marks contestants for the Best Student Presentation Award

| | Surname | Name | University | Title of abstract | Session |
|---|---|---|---|---|---|
| 119 | Ray | Ann | Xavier University | Evaluation of generic attractants for trapping the velvet longhorned beetle, Trichoferus campestris | III |
| 120 | Morkunas | Iwona | Poznań University of Life Sciences | Surface microlayer of lobelia lake: 48-hour dynamics of heavy metals and chlorophyll a variability | I |
| 121 | Hall | David | University of Greenwich | Polyunsaturated hydrocarbons as synergists in moth pheromones: The female sexpheromone of Dioryctria mendacella | II |
| 122 | Nagnan-Le Meillour | Patricia | CNRS | Localization of OBP post-translational modifications by high-resolution MS | III |
| 123 | Takabayashi | Junji | Kyoto University | Exposure of soybean to weeding-related volatiles reduces damage and increases defensive compounds in their seeds | I |
| 124 | Gabryś | Beata | University of Zielona Góra | Alteration of peach potato aphid Myzus persicae (Sulz.) probing behavior by natural and modified jasmonates | II |
| 125 | Pulido | Hannier | Pennsylvania State University | Effects of beneficial rhizobacteria and a pathogenic virus on soybean leaf metabolites and interactions with a beetle vector | III |
| 126 | Elfstrand | Malin | Swedish University of Agricultural Sciences | Norway spruce ATAF1-like NAC transcription factors modulate stress | III |
| 127 | Steidle | Johannes | University of Hohenheim | Early learning of chemical host cues drives ecological divergence during speciation processes in a parasitoid wasp | I |
| 128 | Wei | Xianqin | Leiden University | Testing the generalist-specialist dilemma: The role of pyrrolizidine alkaloids in resistance to invertebrate herbivores in Jacobaea species | II |
| 129 | Jirošová | Anna | IOCB AS CR | Smells like home: Chemically mediated co-habitation of two termite species in a single nest | III |
| 130 | Kyjaková | Pavlína | IOCB AS CR | Cavitene a new diterpene with original skeleton identified in soldiers of the Neotropical termite Cavitermes tuberosus | I |
| 131 | Chattington | Sophie | Swedish University of Agricultural Sciences | Testing the effect of larval population density and host plant on larval immune response and adult oviposition choice in a noctuid moth, Spodoptera littoralis | II |
| 132 | Apšegaitė | Violeta | Nature Research Centre | Olfactory responses of Indian meal moth, Plodia interpunctella, to volatiles of stored-grain contaminated by fungi | III |
| 133 | Willett | Denis | University of Florida Citrus Research and Education Center | Aggregation in entomopathogenic nematodes | I |
| 134 | Arce | Carla | Federal University of Viçosa | Why does the leaf miner Tuta absoluta avoid oviposition on Meloidogyne incognita-infested tomato plants? | I |
| 135 | Imrei | Zoltán | Plant Protection Institute CAR HAS | Identification of an aggregation pheromone component of Sitona humeralis Stephens based on increased pheromone production induced by synthetic juvenile hormone III | II |

This symbol marks contestants for the Best Student Presentation Award

| | Surname | Name | University | Title of abstract | Session |
|---|---|---|---|---|---|
| 136 | Martine | Hossaert-McKey | CNRS | Insights into diversification of floral chemical signaling in the Ficus-mutualistic pollinator interaction: The case of Ficus septica in South East Asia | III |
| 137 | Ban | Liping | China Agricultural University | Unusual Chemoreceptors in Aphids | I |
| 138 | López-Goldar | Xosé | Misión Biológica de Galicia | Diterpene-based induced defences in pine trees in response to a bark chewing weevil and a folivorous caterpillar | II |
| 139 | Jelvez Serra | Nadia | Federal University of Alagoas | Intraspecific variation of cuticular hydrocarbon profiles in the Anastrepha fraterculus (Dipteria: Tephritidae) species complex | III |
| 140 | Fischer | Izabela | University of Gdańsk | Chemical composition of preen waxes in Herring Gull (Larus argentatus) during winter and in the breeding season | I |
| 141 | Levi-Zada | Anat | ARO, Volcani center | Putative male pheromones: Release of specific components in a circadian rhythm from males of the greater date moth, Aphomia (Arenipses) sabella | II |
| 142 | Carrasco | David | Swedish University of Agricultural Sciences | Interaction between exotic and native noctuid moth species: Differential oviposition in the presence of conspecific and heterospecific larvae | III |
| 143 | Knudsen | Jette | Nattaro Labs | Bed bugs-down the trap | II |
| 144 | Herfurth | Anna-Maria | TU Braunschweig | Cyanide detoxification by β-cyanoalanine synthase in the glucosinolate specialist Pieris rapae | I |
| 145 | Peters | Birte | Paris Lodron University | The influence of bacterial communities colonizing lettuce (Lactuca sativa) on grazing activity of slugs (Arion vulgaris) | II |
| 146 | d'Ettorre | Patrizia | University of Paris | The scent of mixtures: Rules of odour processing in ants | III |
| 147 | Borges | Miguel | Embrapa Genetic Resources and Biotecnhology | Identification and field evaluation of the sex pheromone in a Brazilian population of Hypsipyla grandella (Zeller) (Lepidoptera:Pyralidae) | I |
| 148 | Elia | Marta | University of Turin | Social parasites change host-nest odors in social wasps | II |
| 149 | Yuvaraj | Jothi Kumar | Lund University | Early evolution of pheromone receptors in Lepidoptera | III |
| 150 | Kandasamy | Dineshkumar | Max Planck Institute for Chemical Ecology | Attraction and attachment of Norway spruce pathogen Endoconidiophora polonica to its vector Ips typographus | I |
| 151 | Caspers | Jana | TU Braunschweig | The chemical basis of flehmen behaviour in domestic cats | II |
| 152 | Bento | José Mauricio S. | University of São Paulo | Response of entomopathogenic nematodes to sugarcane root volatiles under herbivory by sugarcane spittlebug nymphs | III |
| 153 | Hidalgo Bucheli | William Fernando | Max Planck Institute for Chemical Ecology | Unraveling the role of phenylphenalenones as key metabolites in Musa plants against the attack by the pathogen Mycosphaerella fijiensis | I |

This symbol marks contestants for the Best Student Presentation Award

| | Surname | Name | University | Title of abstract | Session |
|---|---|---|---|---|---|
| 154 | Laumann | Raul Alberto | Embrapa Genetic Resources and Biotecnhology | Differential responses of egg and adult stink bug parasitoids in relation with host sex pheromones | II |
| 155 | Michereff | Mirian Fernandes Furtado | Embrapa Cenargen | The influence of volatiles from stink bugs eggs extracts and oviposition damage plants on the foraging behavior of the egg parasitoid Telenomus podisi | III |
| 156 | de Castro | Érika | University of Copenhagen | Cyanogenic glucosides in Heliconius butterflies: Sequestration, de novo biosynthesis and specialization | I |
| 157 | Schäpers | Alexander | Stockholm University | Leaf quality discrimination in butterflies | II |
| 158 | Jósvai | Júlia Katalin | Plant Protection Institute CAR HAS | Pear ester-based lure attracting a butterfly Pearly Heath (Coenonympha arcania L.) | III |
| 159 | Blassioli-Moraes | Maria Carolina | Embrapa Genetic Resources and Biotecnhology | The influence of chemical and vibrational communication in the mating behaviour of Dichelops melacanthus (Heteroptera: Pentatomidae) | I |
| 160 | Nagahama | Kazuhiro | Sojo University | Ecological role of Ewingella sp. KTH G3-2 with pine weevil | II |
| 161 | Trapp | Marilia | Federal University of São Carlos | MS in chemical ecology: From characterization of oxylipins to their role in plant-microbes interactions | II |
| 162 | Orlova | Margarita | Tel Aviv University | Quality control: Honeybee workers assess queens by pheromonal and genetic correlates of quality | III |
| 163 | Abdullah | Fauziah | University Malaya | Antifeedant and toxicity properties of selected Malaysian plants towards termite and bioactives compounds | III |
| 164 | Nieberding | Caroline | University of Louvain-la-Neuve | Selection on male sex pheromone composition contributes to butterfly reproductive isolation | I |
| 165 | Verschut | Thomas | Stockholm University | Searching for food in complex environments: Using laboratory approaches to test for associational effects | II |
| 166 | Hall | David | University of Greenwich | Components of the sex pheromone of blackcurrant sawfly, Nematus olfaciens (Diptera: Tenthredinidae): Novel isopropyl esters and the role of hydrocarbons | III |
| 167 | Conchou | Lucie | Swedish University of Agricultural Sciences | Do odours mediate innate host plant preferences in adult female Spodoptera littoralis? | I |
| 168 | Nemesio Gorriz | Miguel | Swedish University of Agricultural Sciences | Transcriptional regulation of the flavonoid biosynthesis in Picea abies | II |
| 169 | Burse | Antje | Max Planck Institute for Chemical Ecology | Functional adaptation of secretory proteins in the detoxification of sequestered phytochemicals during Chrysomelina leaf beetle evolution | III |
| 170 | Bursztyka | Piotr | Research Institute in Semiochemistry and Applied Ethology | Predation threat cues as adjustment variables for the expression of self-maintenance behaviours in terrestrial gastropods | I |
| 171 | Chauvet | Caroline | Research Institute in Semiochemistry and Applied Ethology | Tick Attack Inhibition Semiochemical (TAIS): An innovative and promising approach against Rhipicephalus sanguineus | II |

This symbol marks contestants for the Best Student Presentation Award

|  | Surname | Name | University | Title of abstract | Session |
|---|---|---|---|---|---|
| 172 | Katariya | Lakshy | Indian Institute of Science | Sniffing out the enemy: Fungus-growing termites can differentiate between mutualistic and parasitic fungi using volatiles | I |
| 173 | Grimm | Christopher | TU Braunschweig | Characterization of sex pheromones in the digger wasp genus Cerceris | III |
| 174 | Aldrich | Jeffrey | University of California | Why don't Chrysopa green lacewings make pheromone in the lab? | I |
| 175 | Kalinová | Blanka | IOCB AS CR | Male sex pheromones in Ceratitis FAR complex | II |
| 176 | Kalinová | Blanka | Institute of Organic Chemistry and Biochemistry | Male sex pheromones in Ceratitis FAR complex (Diptera: Tephritidae) | III |
| 177 | Borg-Karlson | Anna-Karin | KTH Royal Institute of Technology | Utilization of pheromone precursors for the adult butterfly Pieris napi in flower odors and nectar | I |
| 178 | Chiriboga M. | Xavier | University of Neuchâtel | Diffusion of (E)-β-caryophyllene in different soil textures and its effects on the foraging behavior of the entomopathogenic nematode Heterorhabditis megidis | II |
| 179 | DeLeon | Sara | Justus Liebig University | Do immune-challenged Tribolium castaneum change their volatile profile? | III |
| 180 | Schott | Matthias | Justus Liebig University | Aversive trained insects as detectors | I |
| 181 | Hillier | Kirk | Acadia University | Decoding evolution of pheromone communication in Heliothine moths | I |
| 182 | Jakobsson | Stina | University of Gothenburg | Chemical defense in Zostera marina (eelgrass) against Labyrinthula zosterae | II |
| 183 | Yadav | Pratibha | Indian Institute of Science | Knock, knock. Who is there? Host location mechanisms in non-pollinating fig wasps | III |
| 184 | Zhou | Jing-Jiang | Rothamsted Research | The Lepidoptera odorant binding protein gene family: Gene gain and loss within the GOBP/PBP complex of moths and butterflies | III |
| 185 | Peri | Ezio | University of Palermo | Consequences of dual biotic stresses on plant volatile emission and recruitment of egg parasitoids | I |
| 186 | Schiebe | Christian | Linnaeus University | Bark beetles and their fungal associates: A marriage of convenience or true love? | II |
| 187 | Binyameen | Muhammad | Bahauddin Zakariya University | Effect of different animal manures on attraction and reproductive behaviors of common house fly, Musca domestica L | III |
| 188 | Martin | Kyle | Cornell University | Spatial and temporal variation in volatile composition suggests olfactory division of labor within the trap flowers of Aristolochia gigantea | I |
| 189 | Svensson | Glenn | Lund University | Consequences of pollinator-mediated gene-flow on trait expression versus genotype in varieties of Joshua tree (Agavaceae) | II |
| 190 | Gimenes | Leila | Federal University of São Carlos | Evaluation of fungicidal activity from Picramnia bahiensis extracts for leaf-cutting ants control | III |
| 191 | Hopkins | David | University of Sheffield | The chemical ecology of divergent host plant acceptance behaviour in the speciation in the pea aphid (Acyrthosiphon pisum) | I |

This symbol marks contestants for the Best Student Presentation Award

| | Surname | Name | University | Title of abstract | Session |
|---|---|---|---|---|---|
| 192 | De Backer | Lara | University of Liège | Macrolophus pygmaeus perception of HIPV's from Tuta absoluta infested tomato plants | II |
| 193 | Schmitt | Thomas | University of Würzburg | Evidence for a chemical arms race: Chemical mimicry in the cuckoo wasp genus Hedychrum | III |
| 194 | Evanden | Maya | University of Alberta | The importance of plasticity in response to semiochemicals in the development of monitoring tools for the pea leaf weevil, Sitona lineatus L. (Coleoptera: Curculionidae) | II |
| 195 | Lohr | Jennifer | University of Hamburg | Biological role of triplicated Na, K-ATPase1α genes in the large milkweed bug, with regard to target-site insensitivity against cardiac glycosides | III |
| 196 | Karlsson Green | Kristina | Swedish University of Agricultural Sciences | Experience-based predator avoidance in response to waterborne chemical cues in the freshwater isopod Asellus aquaticus | II |
| 197 | Kimball | Bruce | USDA-APHIS-WS-NWRC | Spamalot: A quest for the holy grail of brown treesnake oral baits | III |
| 198 | Zemeitat | Dany | University of Melbourne | Chemical communication in a mutualistic system – The myrmecophilous Australian butterfly Jalmenus evagoras (Lepidoptera: Lycaenidae) | III |
| 199 | Quero | Carmen | IQAC-CSIC | Evidence of short-term peripheral sensitization by briefly exposure to pheromone components in Spodoptera littoralis | I |
| 200 | Choi | Kyung San | Rural Development Administration | Carbon dioxide receptor involved in the perception and production of the sex pheromone | II |
| 201 | Doddala | Prasad | Swedish University of Agricultural Sciences | Gustatory synergy between sugars and amino acids in the yellow fever mosquito, Aedes aegypti | III |
| 202 | Noge | Koji | Akita Prefectural University | Identification of the foraging stimulant of the brown marmorated stink bug, Halyomorpha halys | II |
| 203 | Cravcenco | Alexei | KTH Royal Institute of Technology | An efficient approach for synthesis of trun-call 1 and trun-call 2, the aggregation pheromone components of larger grain borer (Prostephanus truncatus) | III |
| 204 | Tebayashi | Shinichi | Kochi University | Browning mechanism on the roots of rice plant attacked by rice root aphid, Rhopalosiphum rufiabdominalis | I |
| 205 | de Araújo | Clara Vitória | Georg-August University | HIP-VOC released by the buffalo bur (Solanum rostratum), the former host plant of the Colorado beetle (Leptinotarsa decemlineata) and their detection by electroantennography | II |
| 206 | Holighaus | Gerrit | University of Göttingen | Host volatiles VS non-host volatiles: Host odor of a deadwood colonising beetle is no matter of tree species | III |
| 207 | Adams | Rachelle | University of Copenhagen | How fungus-growing ants are fooled by their parasites…or are they? | I |
| 208 | Khaling | Eliezer | University of Eastern Finland | Defense chemistry and community ecology of Brassica nigra | II |

This symbol marks contestants for the Best Student Presentation Award

| | Surname | Name | University | Title of abstract | Session |
|---|---|---|---|---|---|
| 209 | Greff | Stephane | Aix Marseille University | A chemical ecology approach to assess the proliferation of the red alga Asparagopsis taxiformis: Metabolomics, natural toxicity and biological effects | II |
| 210 | Mieles | Alejandro | SUNY - ESF | Chemical attractants of Philornis downsi an invasive avian parasite on the Galapagos Islands | III |
| 211 | Halitschke | Rayko | Justus Liebig University | Mating system and induction shape the volatile emissions of wild potatoes | I |
| 212 | Romey-Glüsing | Renja | University of Hamburg | Become a specialist with an enzyme - The senecionine-monooxygenase of Longitarsus jacobaeae | II |
| 213 | Ullah | Chhana | Max Planck Institute for Chemical Ecology | Flavan-3-ols are a general chemical defense in poplar against fungal pathogen attack | I |
| 214 | Giron-Calva | Patricia Sarai | University of Eastern Finland | Effects of plant-plant signaling on the oviposition preference of Pieris brassicae and Plutella xylostella females under elevated ozone conditions | II |
| 215 | Lundborg | Lina | KTH Royal Institute of Technology | Protection of Norway spruce against blue stain fungus | III |
| 216 | Kusumoto | Norihisa | Forestry and Forest Products Research Institute | Identification of fomannoxin in Picea abies naturally infected by Heterobasidion parviporum and the effects of terpene constituents on its production | I |
| 217 | Schubert | Fredrik | KTH Royal Institute of Technology | The plant Hyptis suaveolens (Lamiaceae) repellency on Ixodes ricinus (Acari: Ixodidae): Structure activity studies of sesquiterpene oxides and sulfides | II |
| 218 | Fomsgaard | Inge Sindbjerg | Aarhus University | The benzoxazinoid 2,4-dihydroxy-7-methoxy-1,4-benzoxazin-3-one (DIMBOA) inhibits trichothecene accumulation in liquid culture by suppression of tri gene expression | III |
| 219 | Mutyambay | Daniel | International Centre of Insect Physiology and Ecology | Oviposition-induced indirect defence trait in maize against herbivorous insects: Effects of domestication and breeding | I |
| 220 | Weber | Donald C. | U.S. Department of Agriculture | Brown marmorated stink bug pheromone: Interplay of purity, cross-attraction and synergy for an invasive insect species (Hemiptera: Pentatomidae) | II |
| 221 | Unelius | Rikard | Linnaeus University | Morganella morganii bacterium producing phenol in the colleterial gland of females of the New Zealand grass grub, Costelytra zealandica, attracts males | III |
| 222 | Markovic | Dimitrije | Swedish University of Agricultural Sciences | Brief mechanical stimuli between plants affect herbivore insects | I |
| 223 | Eneh | Lynda | KTH Royal Institute of Technology | Cues associated with malaria mosquito egg laying site | II |
| 224 | de Cássia Domingues | Vanessa | Federal University of São Carlos | Bioguided study of Lithraea molleoides against Atta sexdens rubropilosa and Leucoagaricus gongylophorus. | III |
| 225 | Raheem | Dotsha | Bangor University | The chemistry of bluebell (Hyacinthoides non-scripta) | I |

This symbol marks contestants for the Best Student Presentation Award

| | Surname | Name | University | Title of abstract | Session |
|---|---|---|---|---|---|
| 226 | Becker | Tobias | Max Planck Institute for Chemical Ecology | Synthesis and Analysis of Leaf Beetle Defensive Compounds | II |
| 227 | Gonzalez | Francisco | Swedish University of Agricultural Sciences | Differences and commonalities between pheromone receptors of tortricid moths | III |
| 228 | Revadi | Santosh | Swedish University of Agricultural Sciences | Host plant preferences in Spodoptera littoralis larvae | I |
| 229 | Blažytė-Čereškienė | Laima | Nature Research Centre | Norway spruce volatile and bark beetle Ips typographus: Role of trans-4-thujanol | II |
| 230 | Zas | Rafael | Consejo Superior de Investigaciones Cientificas | Integration of defensive strategies and defensive investment with other life history traits in a Pinus pinaster population | III |
| 231 | Shelomi | Matan | Max Planck Institute for Chemical Ecology | Activity and evolution of Phasmatodea beta-1,4-endoglucanases and their implications for polyneopteran herbivory | I |
| 232 | Lassance | Jean-Marc | Harvard University | Vomeronasal receptor families in the deer mouse Peromyscus maniculatus: Towards an evolutionary analysis | II |
| 233 | Park | Youngjin | Andong National University | Genes associated with glycerol biosynthesis in the red-spotted Apollo butterfly, Parnassius bremeri in Korea | III |
| 234 | Shan | Yu | Nanjing Botanical Garden | Chemical and bioactive investigation of Kosteletzkya virginica (Malvaceae) | I |
| 235 | Feng | Xu | Nanjing Botanical Garden | Chemical and antifungal investigation of Salicornia biggelovii and S. europaea | II |
| 236 | Ashitani | Tatsuya | Yamagata University | Chemical characterization of branch galls on Cryptomeria japonica trees in Japanese forest | III |
| 237 | Ômura | Hisashi | Hiroshima University | Mating enhances behavioral and antennal responses of female small white butterfly Pieris rapae (Lepidoptera: Pieridae) to host plant volatiles | I |
| 238 | Shinya | Tomonori | Okayama University | Isolation of novel plant-defense-inducing elicitors from rice herbivores | II |
| 239 | Ohlsson | Anna B | KTH Royal Institute of Technology | Epigenetic effect of juglone - important in allelopathy? | III |
| 240 | Peram | Pardha Saradhi | TU Braunschweig | Synthesis of a macrolide library of putative pheromones used by mantellid frogs from Madagaskar | I |

This symbol marks contestants for the Best Student Presentation Award

Poster number: *1*

A Chemical Ecology

# OLPHABET

*by Gunnar Bergström*

ANALYSES are ways to know
of what and when, of quantity and how

BIOLOGY is study of the living
of eating, mating and evolving

CHEMISTRY studies molecules and their
functions, interactions, structures and
formations

DIVERSITY of molecules and species
a richness greater than French cheeses

ECOLOGY is how creatures live together
in one house, built for any weather

FIELD EXPERIMENTS mark the
beginning and the end
of any study aimed to find the active blend

GC-MS is a foremost tool
sensitive, informative and real cool

HORMONE, PHEROMONE,
TRANSMITTER
help survival of the fitter

INTERDISCIPLINARY synergy
comes through Chemical Ecology!

JEAN-HENRI FABRE – un chercheur
spirituel
with an idea of a “radiograph” of smell

KNOWLEDGE is built up with all
amused
but how can it at best be used?

LABORATORY can be a little room
where good ideas breed and bloom

MOLECULAR BIOLOGY and
GENETICS
are a kind of chemical athletics and
aesthetics

NONTOXIC methods of control
use pheromones in a selective role

OLFACTORY SIGNALS: ODOURS and
PERFUMES
as markings, trails and scent-plumes

PHYSICS: the largest and the smallest
things unseen
- and also everything between

QUANTUM of evolutionary change
volatiles are minimally modified or
rearrange

R with S is chiral twin
one is out and one is in

SIGNAL SYSTEMS help convey
messages of ay and nay

THEORY, PRINCIPLE and LAW
to summarize and predict from what we
saw

UBIQUITOUS is chemical
communication
a challenge for continued exploration

VARIATION in plants by their scent
repellent, defense and attrahent

WATER also carries odour signals
of fish and shrimps, of whales and other
navals

X, Y, Z - Chemical Ecology’s three dimensions
Evolution, Structures and Functions

Poster number: *2*

## Applications of preparative GC for purification of diterpenes

**Rizan Rahmani**

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Diterpenes are one of the most diverse groups of natural products, a type of terpene, is an organic compound composed of four isoprene units. They derive from geranylgeranyl pyrophosphate (GGPP). Diterpenes form the basis for biologically important compounds such as retinol, retinal, and phytol. They are known to be antimicrobial and anti-inflammatory

In plants, the biosynthesis of the universal precursor molecule geranylgeranyl pyrophosphate (GGPP) into the diterpene core structure is catalyzes by a group of enzymes denoted diterpene synthases (diTPS).

For purification of natural products, preparative chromatography (Prep-GC), typically liquid chromatography (LC) is performed, however this requires establishment of a sensitive detection method connected to the LC system. According to our Danish collaborators work on the detection of several diterpenes by Mass Spectrometry (MS) coupled to LC, their attempts were thwarted by difficulties to ionize the diterpenes, which is needed for MS detection. Diode array detection (DAD) as detection method was also tested, but was found of limited use for detection of the target diterpenes due to the lack of UV absorbing chromophores, such as aromatic rings or double bond systems.

So far, the only possible way to reliably separate and detect target diterpenes is by GC-MS analysis. We believe that the optimal way of purifying the molecules for NMR analysis is through preparative GC. NMR is used for structure elucidation of compounds that are unknown.

Poster number: *3*

## Three fallacies in understanding evolution of insect-habitat odour cue responses

**Fredrik Schlyter**

Plant Protection Biology, Swedish University of Agricultural Sciences, Alnarp, Sweden
fredrik.schlyter@slu.se

1."Except for pheromones, volatiles are unspecific cues received by generalist neurons, perceived by cross-fibre patterns."

The face value of responses in bioassays with major hosts (plants, fungi, blood) has been taken as the true reflection of reception. This has gradually become called in question. First, the vapour concentrations (true dose of stimulation) of these often highly volatile compounds are dramatically higher than 'dose on filter paper'. (When you find a smaller molecule most active, its activity is often inflated.) Hence, response to stimuli like GLVs often appears broad at high screening doses. Recent work on *Drosophila* labelled-lines and on *Spodoptera* plant volatiles ORs are examples of the anti-thesis.

Second, in contrast to pheromone components, volatiles of hosts have been less known and the few candidates tested were poor proxies. 'When you find the correct ligand, olfactory sensory neurons show a specific response.'

2. "Cues from host organisms are the one thing we need care about."

Considering the ambience of an insect in a natural habitat, the volatiles from non-hosts are clearly dominant. Somewhat surprising, in spite of the many examples of co-evolution of insect-host, examples abound of sensory investment in non-host detection and of behavioural responses where non-host volatiles modify responses to host odours.

3."Present-day landscapes and habitats form the backdrop against which we should explain extant adaptations of the olfactory system."

Again a straw man is erected, which when made explicit, stands stark naked in its fallacious simplicity. Considering the very short duration of pure monocultures in agriculture and production forestry (200 -300 yr) compared to plausible time scales of evolution of olfactory systems (10 000 yr), this is the apex of fallacies.

Anerdsson, Schlyter, Hill, Dekker. 2012: What reaches the antenna? How to calibrate odor flux and ligand-receptor affinities. Chem. Senses 37, 403-420.

Poster number: *4*

## How can chemical ecology contribute to biosecurity against invasive Arthropods?

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Biosecurity includes responses to new invasive species which have recently expanded their geographic range (by means of delimitation, containment or eradication), as well as long-term management tactics against established arthropod pests which cannot be eradicated. A sound knowledge of the chemical ecology of invasive species is fundamental to both areas and can contribute greatly to improving outcomes by minimising the long term costs of control, including economic, social and environmental costs of pests. Identification of the nature and role of many hundreds of semiochemicals involved in attraction has led to the largest number of practical applications. This has occurred through the delimitation of the distribution of new pests and assessment of the results of interventions for their containment or eradication, as well as the development of monitoring and decision support systems for established pests. Semiochemicals have also been developed into direct control tactics ! such as mating disruption, mass trapping and lure and kill systems against a wide range of insects including fruit flies, moths, beetles and some other groups. Straight chained lepidopteran sex pheromones have emerged as a source of market advantage in orchard pest management, with trapping systems and residue-free multiple species disruption systems being increasingly adopted to reduce insecticide applications and consequently residues on fruit. Despite some successes, for the majority of invasive arthropods pests, there are few tools that can meet current needs for practical and cost-effective solutions. Worldwide, there is a rising rate of incursions of alien invasive species and this has been matched by a rise in costly official eradication programs which strongly supports the need for further investment in the areas of discovery and development of surveillance and eradication technologies, from the science of chemical ecology.

Suckling DM (2015) Can we replace toxicants, achieve biosecurity, and generate market position with semiochemicals? Frontiers in Ecology and Evolution 3(17):1-7. doi: 10.3389/fevo.2015.00017

Poster number: *5*

## Neurophysiological mechanisms underlying the experience dependent behavioral adaptations

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Although understanding the processing of odour mixtures is a focus in olfaction research previous studies through a neuroethological approach, have demonstrated that different odour types, sex and habitat cues are coded together in an insect herbivore. Stronger flight attraction of codling moth males, *Cydia pomonella*, to blends of female sex pheromone and plant odour, compared with single compounds, was corroborated by functional imaging of the olfactory centres in the insect brain, the antennal lobes (ALs). The macroglomerular complex (MGC) in the AL, which is dedicated to pheromone perception, showed an enhanced response to blends of pheromone and plant signals, whereas the response in glomeruli surrounding the MGC was suppressed. These findings underscore that, in nature, sex pheromone and plant odours are perceived as an ensemble. That mating and habitat cues are coded as blends in the MGC of the AL highlights the dual role of plant signals in habitat selection and in premating sexual communication. It suggests that the MGC is a common target for sexual and natural selection in moths, facilitating ecological speciation (Trona et al. 2013).

So in this proposed project we will compare the response of MGC in naïve and experienced males to blends of pheromone and experienced plant signals. We will use the functional imaging to study the response of MGC in antennal lobes to sex pheromone and plant odour. In certain herbivorous insects, such as moths and flies, the important role of chemosensory information for behavioural decisions, together with very good knowledge of the structure and physiology of the chemosensory parts of the brain, makes this system a favourable model to study experience based changes with multi-modal approaches. With their relatively short generation time, some insect herbivores such as *Spodoptera littoralis*, is thus an ideal model not only for studying experience-based adaptation to changing natural environments within but also across generations and thus might provide us with important tools to investigate also evolutionary questions (Anderson and Anton, 2014). So this project will focus on males reared from artificial diet and cotton plants, previous studies reported that males are more attracted to female sex pheromone in combination with volatiles from the experienced host plant when compared to pheromone and volatiles from a host plant they had not experienced (Thöming et al. 2013).

Poster number: *6*

# Recent advances in the pheromone chemistry of stink bugs

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

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

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

Poster number: 7

## Sponge taxonomy 2.0 meets pharmacognosy and chemical ecology

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Proper species identification and classification is crucial to any scientific study. Naming a species and using a proper classification is the only way to make sure that all the data linked to conspecific specimens but produced by different researchers can be understood, associated and compared. Linking biological data (molecular, morphological, biochemical, ecological) to an incorrect species name or to no species name will result in these data losing tremendous value. This is an important issue in the field of sponge natural products or sponge chemical ecology since 1) the biodiversity of sponges is still poorly known and since 2) sponge taxonomy has been very unstable in the last decades. In fact, in the past 13 years, the classical taxonomy has been considerably overturned by an increasing number of molecular phylogenetic studies, with numerous polyphyletic groups revealed or confirmed and new clades discovered. Based on these results, Morrow and Cárdenas (2015) now propose a revised classification of the Demospongiae, hoping to convince end-users to 1) abandon the use of artificial groups (=polyphyletic), and to 2) use the new/resurrected names when referring to the new Demospongiae clades. I will illustrate with examples from previous studies and from my current research how this revised classification can facilitate communication between end-users, reduce taxonomically biased results, and shed a new light in the fields of sponge natural product and chemical ecology.

Morrow C. and Cárdenas P. (2015) Proposal for a revised classification of the Demospongiae (Porifera). Frontiers in Zoology.

Poster number: *8*

## Two African fruit flies (Diptera: Tephritidae) produce identical host marking pheromones

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Complementary control tools are required to combat infestation by a myriad of fruit flies which pose a major constraint to large scale production of fruits in Africa. One of such is the application of host marking pheromones (HMPs) which some gravid female insects use to mark oviposition substrates serving to deter conspecifics and at times heterospecifics from utilizing the same resource for egg laying. We investigated whether HMPs are used by the fruit flies, *Ceratitis rosa* and *C. fasciventris*, by monitoring their responses on mango slices treated with solutions of crude fecal and the female specific synthetic compound in relation to control. Our findings confirmed the use of HMPs in these fruit flies. We then used liquid chromatography coupled to quadruple time of flight mass spectrometry (LC-Qtof-MS) to identify the nature of the HMPs. Further behavioral assays in the laboratory using synthetic standard of the identified HMP at varying doses are ongoing and appear to reveal a similar trend as the crude extracts. We discuss the findings in terms of the potential of deploying HMP as a management tool for these pest species.

Poster number: *9*

## Floral volatiles in host-flower recognition in a pollen-specialist bee

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Solitary bee species that restrict pollen collecting to specific plant taxa (oligolectic bees) may rely on different combinations of visual and olfactory cues to recognize their host flowers. In *Chelostoma florisomne*, which is oligolectic on *Ranunculus* spp, newly emerged (foraging-naïve) female bees have been shown to require the color yellow and the main pollen volatile protoanemonin to recognize their host-plant. However, after bees have foraged on flowers, the olfactory (but not visual) component of their search image changes: protoanemonin is no longer sufficient to elicit attraction in two-way choice behavioral experiments. To determine which volatiles in the floral scent of the common host plant *R. acris* are used by experienced bees to recognize the flowers, bees were offered a choice of two odor samples, with and without the tested volatiles; bee preferences were evaluated by the number of landings (= measure of attraction) and number of landings accompanied by feeding attempts (= measure of recognition). When offered the dominant whole-flower volatile *trans*-β-ocimene, bees showed strong attraction but not recognition. When *trans*-β-ocimene was combined with the pollen volatiles protoanemonin and (*cis, trans*)-α-farnesene (second most abundant volatile in pollen, third most abundant in whole flowers), bees significantly preferred samples with the scent mixture in terms of their landings as well as feeding attempts. The findings indicate that experienced bees differ from foraging-naïve bees in requiring the main petal volatile to locate host flowers, but that they still require pollen volatiles for host-flower recognition, emphasizing the importance of olfactory pollen cues for this bee species.

Poster number: *10*

## Biomolecular MS of amino acids and their role in chemical ecology

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Mass spectrometry (MS) is a powerful selective and sensitive tool for the analyses of biomolecules. We are especially interested in using liquid chromatography (LC)-MS for the analysis of proteinogenic and non-proteinogenic amino acids. We study the non-proteinogenic and neurotoxic amino acid β-N-methylamino-L-alanine or BMAA which is believed to be potentially bio-incorporated into proteins although the actual mechanism(s) by which this is accomplished remains unresolved. Our lab has made a significant contribution for detecting and identifying BMAA 1 that was used to show transfer of this toxin within the food chain in the Baltic Sea 2. We have elucidated how to distinguish BMAA from its isomers 3; quantify trace level amounts4 and have unraveled eukaryotic sources: diatoms and dinoflagellates 5,6 increasing the risk for exposure.

Significant amounts of free proteinogenic amino acids are present in flower nectar. They are an energy source of fundamental ecological importance and might correlate with the kind of pollinators the flowers attract. In order to fully establish the ecological role of different amino acids in floral nectar, a proper determination of their occurrence and distribution is fundamentally important 7.

In both studies the implementation of LC-MS/MS in multiple reaction monitoring mode as a selective, sensitive and very reliable tool will be described.

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3 Jiang, L., Aigret, B., De Borggraeve, W.; Spacil, Z.; Ilag, L.L. (2012) Selective LC-MS/MS method for the identification of BMAA from its isomers in biological samples Anal Bioanal Chem. Anal Bioanal Chem. 403(6):1719-30

4 Jiang L, Johnston E, Åberg KM, Nilsson U, Ilag LL. (2013) Strategy for quantifying trace levels of BMAA in cyanobacteria by LC/MS/MS. Anal Bioanal Chem.;405 (4):1283-92

5 Jiang, L., Eriksson, J., Lage, S., Jonasson, S., Shams, S., Mehine, M., Ilag, L.L., and Rasmussen, U. (2014) Diatoms: A Novel Source for the Neurotoxin BMAA in Aquatic Environments. PlosONE

6 Jiang, L and Ilag, L.L. (2014) Detection of endogenous BMAA in dinoflagellate (Heterocapsa triquetra) hints at evolutionary conservation and environmental concern. PubRaw Science 2:1-8

7 Zurita, J.,Mozuraitis, R, Borg-Karlson, A.-K. and Ilag, L.L. (2015) Profiling Nectar Amino Acids Using a Sensitive and Selective Mass Spectrometry Based Approach. In preparation

Poster number: *11*

## Neuroendocrine and behavioral consequences of peripubertal exposition to male odor in female mice

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Reproductive physiology in female mice is profoundly affected by male odor. A well-known effect induced by male odor is the acceleration of puberty onset observed in prepubertal female mice following exposure to male urine. The chemosignals involved are known to be androgen-dependent as this effect disappears when using chemosignals derived from castrated males. In our study, we showed that exposure to male chemosignals promote the maturation of the kisspeptin system, a key neuropeptide involved in pubertal transition. This effect is not found in unexposed females or females exposed to chemosignals derived from castrated males. We also confirm the olfactory nature of the stimulation because this effect on the kisspeptin system is lost following removal of the vomeronasal organ. In addition,to this effect at the neuroendocrine level, we showed that the peripubertal exposure to male odor has also long-term behavioral consequences as female mice exposed to male-soiled bedding showed advanced vaginal opening associated with early expression of male-directed odor preference in adulthood. This effect can be reproduced by exposure to a blend of chemosignals already known to advance puberty onset and found in male soiled bedding, namely (1*R*, 5*S*, 7R)-3,4-dehydro-exo-brevicomin (DHB), 6-hydroxy-6-methyl-3-heptanone (HMH) and (*S*)-2-sec-butyl-4,5-dihydrothiazole (SBT).As a whole, our results show that peripubertal exposition to male odor not only promote neuroendocrine and reproductive changes but also influence the latter expression of adult behavior.

Jouhanneau et al., Horm. Behav., 2014; Jouhanneau et al., Frontiers Chem. Ecol., 2015

Poster number: *12*

# Comparative transcriptome analysis of pheromone glands on sex pheromone biosynthesis in *Plutella xylostella*

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The pheromone biosynthesis in *Plutella xylostella* is more active in the scotophase than in the photophase, which suggests that there may be changes of gene expression in the pheromone glands. To identify genes contributing to change in pheromone production, we analyzed transcriptomes from pheromone glands of both decapitated females in the photophase and normal ones in the scotophase. Comparative analysis were performed with transcriptomes of pheromone glands from non-decapitated (PG) females and decapitated ones for identification and expression of putative genes associated with pheromone biosynthesis pathway. Deep sequencing for mRNAs in the pheromone gland yielded approximately 7.5Gb and totally 17,265 transcript were constructed under a homology cutoff of 10-6 E value. Genes putatively involving in pheromone biosynthesis were identified such as acetyl-CoA carboxylase, acetyl-CoA dehydrogenase, fatty acid synthase (FAS), desaturases (Δ9 and Δ 11) and fatty acid reductases (FAR) including pgFAR, alcohol oxidase, aldehyde oxidase and aldehyde reductase, etc. Expression of 6 signal genes involving in pheromone biosynthesis such as acyl-CoA desaturase, FAR, PBAN receptor, fatty acid transporter, acyl-CoA binding protein did not exhibited ant significant different in both transcriptomes. Quantitative RT-PCR revealed that expressions of FAS, Δ11 desaturase and pgFAR were higher in PG than that in ΔPG. Based on results, Δ11 desaturase and pgFAR may have a crucial role in sex pheromone biosynthesis of *P. xylostella*.

Poster number: *13*

# A differential induction of defence signaling molecules in pea response to *Acyrthosiphon pisum* and the effect of exogenous nitric oxide donor on its infestation

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The first aim of this study was to verify whether the enhanced generation of signaling molecules occurs in the leaves of *P. sativum* L.cv. Cysterski seedlings in response to *Acyrthosiphon pisum* (Harris) infestation. It was also significant to determine the generation time of these molecules and the time-dependent aspect of defense response induction to pea aphid. The second objective of this study was to investigate the effect of exogenous nitric oxide donors on *A. pisum* infestation using electronic recording of penetration behavior by aphid (EPG, Electrical Penetration Graph). In time from 0 to 96 h after *A. pisum* infestation these signaling molecules accumulated transiently. Moreover, the convergence of these signaling pathways occurred. Among the signaling molecules, NO is an extremely interesting molecule, and has been demonstrated to be an essential regulator of several physiological processes in plants. In the plant-insect interactions, NO plays a defensive role in plant cells after herbivore attack. Relative generation of NO in pea seedling leaves was detected by staining leaves with the specific fluorescent dye, 4,5-diamino fluorescein diacetate (DAF-2DA) examined under a confocal laser microscope. The strongest emission of green fluorescence originating from the DAF-2DA covering the largest area of leaves was observed at 48 h of infestation. In turn, analysis of EPG parameters during 24 hours monitoring revealed a strong decrease in the duration of the penetration by *A. pisum* of the leaves of pea seedlings treated with NO donors, i.e. S-nitrosoglutathione (GSNO) or sodium nitroprusside (SNP) as well as a significant reduction in the duration of phloem phase.

These results contribute to a better understanding of the regulatory mechanisms during the plant-aphid interactions bringing new knowledge to the contemporary plant biology.

The study was supported by the Polish National Science Centre (NCN, grant no. 2011/01/B/NZ9/00074)

Poster number: *14*

## Chemistry releases an invasive seaweed from native enemies

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The filamentous red alga *Bonnemaisonia hamifera*, which has its origin in Asian waters, has invaded large parts of European and North American coasts. It has rapidly become one of the most abundant subtidal macroalgae in Scandinavian coastal waters. We show that *B. hamifera* contains a previously described poly-halogentated 2-heptanone that is effective against multiple native enemies, including herbivores, microbes and competitors. The production of the defense chemical entails a cost for *B. hamifera* but this seems to be outweighed by its ecological benefits. The provision of shelter from fish predation to consumers of native algae gives *B. hamifera* an additional advantage over native competitors and facilitates its spread in the new area. In conclusion, the results suggest that the costly but highly effective chemical defense produced by *B. hamifera*, which is novel to native enemies, is important in explaining the invasion success of this red alga.

Poster number: *15*

# Exploring inducible trichome-mediated resistance to the Western flower thrips in tomato

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The western flower thrips *Frankliniella occidentalis* is one of the major pests of cultivated tomatoes. Natural host resistance in wild tomato species is often correlated with glandular trichomes and their associated exudates. Here we describe resistance to thrips in a cultivated tomato based on the presence of type-VI glandular trichomes. Thrips resistant plants showed high type-VI trichome densities which were negatively correlated with thrips damage as well as plant age. Upon contact with thrips the trichome glands rupture whereupon a sticky thread is formed. Thrips are glued to the leaf leading to mortality. LC-MS analysis of type-VI gland contents revealed the flavonoid rutin as the main component. It is known that trichome densities as well as flavonoids can be induced by ultraviolet-B (UV-B) radiation. Moreover, UV-B can augment sensitivity of jasmonic acid (JA)-mediated plant responses against herbivores, which positively affects insect-mediated induction of defensive secondary metabolites. Here we explored how non-ecologically (UV-B and C short-term irradiation) and ecologically (UV-B long-term irradiation) UV light treatments affect tomato resistance to thrips and trichome-mediated defenses. In addition, the interactive effects of short-term UV light treatment and thrips on induction of JA-dependent trichome-based defenses have been investigated. Exposition to long-term UV-B irradiation induced type-VI trichome production on the adaxial leaf surface, leading to an increased rutin accumulation per leaf, while no effect was observed on the abaxial surface. Short-term UV-B treatment did not have any effect on trichome production. Accordingly, short-term treatment did not amplify JA-associated trichome induction observed after thrips infestation. Both UV-B treatments did not significantly affect thrips damage in relation to the whole plant. Implications of our results for plant protection against thrips in tomato are discussed.

Poster number: *16*

# Intrinsic and network mechanisms constrain neural synchrony in the moth antennal lobe

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It has long been understood that intrinsic cellular properties as well as recurrent connectivity both play a role in the dynamics of the moth antennal lobe (AL). Nevertheless, it is still unclear how these features interact, and to what extent they influence the functional properties of the AL. Following a series of computational modeling studies, we were lead to hypothesize that the after-hyperpolarization (AHP) phase comprises both an intracellular component – likely due to small conductance Calcium-dependent potassium (SK) channels, as well as a synaptic component – likely due to strong recurrent connectivity within and across glomeruli. Our computational network model predicted that this strong recurrent connectivity allows the PNs to respond vigorously and synchronously to odor stimulus, whereas the inhibitory AHP phase serves to keep the AL in check; muting the after-hyerpolarization activity of each PN and readying the system for its next odor-triggered response. Mathematically disabling the AHP in our model gave rise to a 'runaway' phenomenon wherein the strong recurrent connections take over: one vigorous synchronized PN burst triggered another after ~50ms, then another, and so forth. This kind of runaway phenomenon would be undesirable from a behavioral standpoint; runaway synchronization would entrain the AL on timescales of ~50ms, preventing a sensitive response to further filamentous odor stimuli. Thus we propose that the AHP plays an important role in the AL: the AHP constrains the system after each odor-triggered synchronous burst, preventing the strong recurrent connectivity from taking over and maintaining a 'high-gain' state appropriate for a sensory response. Finally, as a step towards future work, we performed further experiments by blocking the GABA-A receptors with picrotoxin (PTX). Although the PTX presumably blocks inhibitory currents, our results show that PTX-application actually reduces PN activity while strengthening the AHP-phase. These unexpected PTX results prompted us to fine tune our network model to include a disinhibitory circuit that involves a heterogeneous network of inhibitory local neurons.

Poster number: *17*

## Friend or foe? Mutualism and aggressive mimicry in an aphid

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The best known relationship between aphids and ants is regarded as mutualistic as both partners seem to benefit from the association: aphids provide ants with honeydew while receiving hygienic services and protection in return. In this study, we report an unprecedented aggressive aphid-ant interaction in which one of the two clonally produced root-dwelling morphs of the aphid *Paracletus cimiciformis* imitates the cuticular hydrocarbons of *Tetramorium* ant larvae, inducing ants to transport the aphids to their brood chamber, where they suck on ant larva hemolymph.

The aphid *P. cimiciformis* presents two morphs, one of them exhibits the conventional trophobiotic (mutualistic) relationship with ants of the genus *Tetramorium*, and the alternative morph is transported by the ants to their brood chamber and cared for as if it were true ant larvae. Gas chromatography-mass spectrometry analyses reveal that the innate cuticular hydrocarbon profile of the mimic morph resembles the profile of ant larvae more than that of the alternative, genetically identical nonmimic morph. Of 31 identified compounds, six provoked coupled gas chromatography-electroantennographic detection responses on antennae of adult ants, and three of these compounds correspond to peaks that were found only in ant larvae and mimic aphids, whereas the other three correspond to peaks present in both morphs and ant larvae. Furthermore, ants failed to discriminate dummies impregnated with cuticular extracts of the mimic aphids from those impregnated with extracts of ant larvae, adopting equally both of them. Finally, we observed that once in the brood chamber mimic aphids suck on ant larva hemolymph. This work describes for the first time a new dimension to the classical paradigm aphid–ant relationships revealing a complex system at the evolutionary interface between cooperation and exploitation.

Poster number: *18*

## Does extrafloral nectar release a volatile scent?

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Extrafloral nectar (EFN) is a widely distributed and well-characterized indirect plant defense, which acts by attracting carnivorous invertebrates. However, the mechanism by which these predators locate EFN is not known. While some carnivores such as predatory wasps and spiders may rely on visual cues, others may follow chemical signals. In particular ants, the most important visitors of EFN and plant defensive arthropods, commonly utilize olfactory cues for orientation. However, no study has explored the question of whether EFN emits cues as volatile organic compounds (VOCs). In this study we collected the headspace VOCs released from lima bean (*Phaseolus lunatus*) EFN using solid-phase microextraction (SPME) and analyzed VOC composition with gas chromatography- mass spectroscopy for time-of-flight (GC-TOFMS). We found a diverse blend of EFN-derived compounds including 2-butanone, benzophenone, and propanenitrile, 3-methoxy-, all of which have been shown in other studies to be plant-produced VOCs. We suggest that these EFN derived VOCs may serve as efficient semiochemicals attracting ants and other arthropods to extrafloral nectar for indirect plant defense.

Poster number: *19*

# Intermolecular interactions in systems containing Bi(III) – ClO – HO – homocysteine in the aspect of catalysis of Bi(III) electroreduction


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Water is a critical component of many chemical processes. In chemical, biological, ecological and other systems, water frequently occurs in very crowded situations: it interacts with a variety of interfaces and molecular groups. Water's behavior in different environments is an important factor in chemical systems. In biology, water is found in cells, where it hydrates membranes and large biomolecules. In geology, interfacial water molecules can control ion adsorption and mineral dissolution. In chemistry, water is an important polar solvent that is often in contact with various interfaces, for example, in ion-exchange resin systems.

Bismuth is a very interesting element, due to its characteristics and application in many branches of industry. In recent years, bismuth has been promoted as a "green element" and has been used as a "non-toxic" substitute for lead in various applications. Due to the broad range of bismuth applications, it is necessary to seek and develop new methods of its determination.

Complex studies on bismuth properties in aqueous environment gave insight into the states of equilibrium of different chemical and electrochemical processes and the thermodynamic stability of different forms of bismuth.

It has been found that homocysteine catalyzes the process of Bi(III) ion electroreduction in chlorates(VII), thus meeting the requirements of the "cap-pair" rule. Studies conducted at different water activity levels suggest that the composition of active complexes changes following the transfer of consecutive electrons, influencing the kinetics and mechanism of Bi(III) ion electroreduction.

Poster number: *20*

# Impact of interactions among native biotic disturbances on range expansion of mountain pine beetle into novel jack pine forests

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With recent range expansions of species due to climate change, it is important to understand the impact of endemic biotic disturbances on exotic species. Our research investigated the expansion of mountain pine beetle (*Dendroctonus ponderosae*) into the novel host jack pine (*Pinus banksiana*), which is an ecologically and economically important component of the Canadian boreal forest. We focused on plant secondary compounds to understand interspecies interactions and identified the impact of induced host defense compounds due to the infection of a widespread native parasitic plant (dwarf mistletoe, *Arceuthobium americanum*) on the success of mountain pine beetle. Although mistletoe-induced changes to host physical characteristics negatively affected mountain pine beetle, the chemical changes due to mistletoe infection reduced the competitive effect of the subcortical insect community on mountain pine beetle performance. Furthermore, the host susceptibility to mountain pine beetle-associated fungi was affected by the mistletoe-induced changes in chemical defense concentrations. We show that native plant pathogen-induced defense chemicals can influence interspecific interactions, which have important implications for community dynamics and the maintenance of mountain pine beetle in a novel host.

Poster number: *21*

# Genes involved in sex pheromone biosynthesis of *Ephestia cautella*, an important food storage pest, are determined by transcriptome sequencing

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Insects use pheromones, the chemical signals that underlie the behaviors of all animals, for communication and attracting mates. The chemical structures of thousands of pheromone molecules are known, the biosynthetic pathways leading to pheromone production have been shown and many pheromone biosynthesis enzymes (PBEs) have been characterized. We used an Illumina next-generation sequencing approach to characterize the pheromone gland transcriptome of the Pyralid moth, *Ephestia cautella*, a destructive storage pest, to reveal putative candidate genes involved in pheromone biosynthesis, release, transport, detection and degradation.

We identified the *E. cautella* major pheromone precursors, 14: acyl, 16: acyl, E14-16: acyl, Z9-14: acyl, E12-14: acyl and Z9E12-14: acyl. Based on the abundance of methylated precursors, two possible pheromone biosynthesis pathways are proposed. Illumina sequencing yielded a total of 83,792 transcripts, and we obtained a PG transcriptome of ~49.5 Mb. A total of 191 PBE transcripts were selected from the dataset, which included pheromone biosynthesis activating neuropeptides, fatty acid transport proteins, acetyl-CoA carboxylases, fatty acid synthases, desaturases, several β-oxidation enzymes, fatty acyl-CoA reductases (FARs) and fatty acetyltransferases (FATs). The PBE transcripts, which are highly abundant in the PG, were selected and putative functions in pheromone biosynthesis were proposed. A comparison of the *E. cautella* transcriptome data with three other Lepidoptera PG datasets revealed that 45% of the sequences were shared. PBAN receptor isoforms C and A were identified. Phylogenetic trees were constructed for desaturases, FARs and FATs, and transcripts that clustered with Δ14, Δ12 and Δ9 desaturases, PG-specific FARs and potential candidate FATs, respectively, were identified. Transcripts encoding putative pheromone degrading enzymes, and candidate pheromone carrier and receptor proteins expressed in *E. cautella* PG were also identified.

The study provides robust background information on the pheromone biosynthesis pathway and PBEs, which will be useful for the in vitro production of *E. cautella* sex pheromones.

Poster number: *22*

# Not visible, but highly effective: Plant volatile manipulation by endophytic fungi and responses of herbivores

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Plants are regularly colonized by endophytic fungi; however, the resulting multitrophic interactions are poorly understood. We analyzed the effect of inoculations with two different species of endophytic fungi (*Beauveria bassiana* and *Trichoderma koningiopsis*), differing in their mode of action (insect vs. plant pathogens), on tomato volatiles organic compounds (VOCs) and resulting responses of herbivores with regard to host plant selection behaviour. We hypothesized that endophytically colonized plants should be more attractive for herbivores, mediated by the VOC profiles, but should not exhibit differences when colonized by a plant pathogen antagonist.

Tomato roots were inoculated with *B. bassiana* and *T. koningiopsis* and analyzed for volatile emissions and compared with non-inoculated plants. A total of 10 treatments were evaluated: *Beauveria bassiana* strains Bb1, Bb2, and Bb3 and *Trichoderma koningiopsis* strain Th003; each endophyte treatment combined with an aphid attack. Headspace volatiles of each plant were collected using TDS tubes filled with a porous polymer based on 2.6-diphenyleneoxide (Tenax ® TA). Samples were analyzed using a GC-MS with a non-polar column.

*B. bassiana* and *T. koningiopsis* inoculated plants modified tomato VOCs, with clear differences between different isolates. When combined with an aphid attack (*Myzus persicae*) VOC profiles differed with regard to the amount of volatiles produced. Entomopathogenic fungal colonization of plants reduced the amount of those compounds known as repellent to herbivores, while *T. koningiopsis* increased specifically sesquiterpene emissions. In a multiple choice experiment, we offered five different odor sources (i.e. plants) to winged *M. persicae*. Aphids were significantly more attracted to endophyte inoculated plants compared to untreated plants after 24 h. Also, an additional non-choice oviposition experiment using *Helicoverpa armigera* laid significantly more eggs on endophytic inoculated plants compared to control plants under laboratory conditions. These results support our hypothesis that i) endophytic fungi manipulate plant metabolism specific to each fungal isolate, that ii ) insect herbivores are able to distinguish between VOC profiles and iii) that VOC changes may be to the detriment of the herbivores.

Poster number: *23*

## Do commercial bumblebees represent a threat for local subspecies?

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The bees (Apidae) are undoubtedly the most important pollinators; bumblebees represent their most diversified subfamily in Europe. Bumblebees are increasingly used to pollinate various agricultural plants, both in the field and in greenhouses. Currently, the Eurasian *Bombus terrestris* (*B. t.*) is the most frequent species in laboratory rear, which diversified into a number of subspecies. The dominant rearing subspecies is the South European *B. t. dalmatinus* because of its prevailing success rate as well as the large colony size appeared as the most suitable for commercial use.

Escaping of workers and reproductive individuals from greenhouses represents ecological risks for local subspecies and populations [1]. In Middle Europe, fertilized young queens survive winter in the diapausing stage, but South European *Bombus terrestris* subspecies (*B. t. dalmatinus*) pass rather through a summer aestivation. The accumulation of the nutrients (lipids and glycogen) in fat body (FB) before the diapausing period is crucial for their survival and successful establishment of a new colony. Our research aims at bringing an insight into the adaptation of *B. t. dalmatinus* subspecies in a colder climate. The approach is based on nutrient comparison of two subspecies – *B. t. terrestris* (local subspecies in the Czech Republic) and *B. t. dalmatinus* during hibernation and aestivation. The glycogen concentration in the FB of *B. t. dalmatinus* was determined by a coupled enzyme assay, which produces a colorimetric product, proportional to the glycogen present. The current results show that the FB weight in aestivating queens falls down rapidly compared to the same length of hibernation, but the glycogen content on milligram of FB remains unchanged.

The authors thank the Czech Science Foundation (grant no. 14-04291S) for financial support of this work.

1. Ornosa C., 1995. Una nota de atención sobre la introducción artificial de subespecies foráneas de abejorros polinizadores en la Península Ibérica (Hymenoptera: Apidae, Bombinae). Boln. Asoc. Esp. Entomol. 20, 259–260.

Poster number: *24*

## Disentangling the biosynthesis of termite-produced nitro compounds

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In 1974, (E)-1-nitropentadec-1-ene (NPD) was the first-described insect-produced nitro compound, identified in the defensive secretion of termite soldiers of the genus *Prorhinotermes*. However, its biosynthesis has long remained unknown. We reported previously the first steps of NPD biogenesis and proposed a biosynthetic scenario based on a modified sphingolipid biosynthetic pathway(1).

Here, we show a more detailed dissection of the hypothesized scenario using a combination of three complementary methods, i.e. (i) mass spectrometric search for putative intermediates of the proposed pathway, (ii) in vivo incorporation studies of individual labelled intermediates and (iii) comparative RNA-Seq analysis of NPD-producing frontal glands with control tissues of soldiers and workers. We detected the presence of several expected intermediates as well as the incorporation of their deuterium labelled forms. In addition, in the soldier frontal glands we identified highly upregulated transcripts corresponding to two enzymes involved in the first two steps of sphingolipid biosynthetic pathway. These results provide a strong support to the initial hypothesis and add to our understanding of the origin of NPD.

(1) Jirošová A. et al. 2014, ChemBioChem 15 : 533–6.

Poster number: *25*

## Does spontaneous vomeronasalitis influence chemical communication in domestic animals? A new approach linking pathology to behavior.

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The vomeronasal organ (VNO) is a peripheral chemosensory structure strongly involved in semiochemicals detection in vertebrates. It is composed by a non-sensorial epithelium (NSE) and a vomeronasal sensory epithelium (VNSE), which is directly responsible for pheromones detection. It has been widely demonstrated that VNO experimental lesions produce deficits in social and reproductive behavior in various species, while no reports described spontaneous VNO pathologies as cause of animal behavioral disorders. The aim of this communication is to present a new approach that investigates the vomeronasalitis as factor in animal social life alterations. VNOs from 20 cats and 18 pigs were submitted to routine tissue processing and stained in haematoxylin and eosin for histological examinations. VNO lesions were classified depending on the duration of the process in acute or chronic inflammation in both VNSE and NSE. Of the same animals, data about the presence of social life disorders were collected. In cats, VNSE and NSE were affected by chronic inflammation in 11/20 (55%) and 13/20 (65%) subjects, respectively, and the Fisher exact test showed that VNSE inflammation was correlated to intercat aggressiveness (P=0.038). In pigs, VNSE and NSE were inflamed in 16/18 (89%) and 18/18 (100%) animals. Even if no statistical significant correlation was observed, Multiple Correspondence Analysis showed that pigs bearing bilateral VNSE inflammation presented more skin lesions due to intraspecific biting than unilaterally affected or healthy subjects. We describe for the first time the relationship between spontaneous VNO alterations and behavioral disorders in animals. Social life is strongly controlled by chemical messages and the VNO is the structure involved in their detection. Our results suggest that the inflammation of its sensorial part affects chemical communication leading to intraspecific social alterations. These new and promising data need to be firmer confirmed investigating other species and a wider number of animals.

Poster number: *26*

# Identification of the volatile organic compounds profile in a resistant cultivar of Cassava (*Manihot esculenta*)


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Brazil is the second largest cassava producer worldwide. Cassava cultivars are of great economical importance as they are used as one of the main food sources both for humans and animals in tropical countries. In addition, cassava has been used as raw material for many other agro-industrial products. This study aimed to identify and characterize the volatile organic compound (VOCs) released from the resistant cassava cultivar EQUADOR 72. This cultivar has shown a resistance towards several pests in the field including mites, whitefly, and the bug *Vatiga illudens*, (Drake, 1922) (Hemiptera:Tingidae). VOCs were collected from plants prior- and post infestation with *V. illudens* and identified using gas chromatography coupled mass spectrometry (GC-MS). The cultivar BRA JARI, previously classified as susceptible, was used as control. Our results show that the EQUADOR 72 cultivar has a constitutive emission of high levels of the monoterpene β-ocimene. In addition, the sesquiterpenes β-caryophellene and farnesol were also constitutively released by the resistant cultivar in comparison to the undamaged susceptible cultivar. These compounds have been reported amongst various plant species and are associated with pest repellence, attraction of natural enemies, and plant-plant interactions. Interestingly, the analysis of the VOCs profile of the susceptible BRA JARI cultivar, showed an increase in the release of β-ocimene and the presence of β-caryophellene and farnesol only after herbivore damage. This VOC profile is similar to that of the resistant cultivar. These results suggest that the volatile semiochemistry of the cassava cultivar EQUADOR 72 may play a crucial role in resistance against the mentioned pests. In addition, the results also can provide a new approach in which organic volatile compounds can be used as a possible marker applied to cassava breeding projects.

Poster number: *27*

# Does the association between sugarcane (*Saccharum* spp.) and diazotrophic microorganisms provide pest resistance?

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*Gluconacetobacter* sp. and *Azospirillum* sp. are beneficial endophytic diazotrophic bacteria that can live in association with sugarcane. These bacteria can provide fixed-nitrogen and plant growth hormones to host plants, therefore promoting increase in plant biomass. Other benefits, as insect-control, have been reported to some endophytic microorganisms, however this activity have not been described to diazotrophs. Here we aimed to study the effects of the association between both *Gluconacetobacter* sp. and *Azospirillum* sp. with sugarcane plants in the sugarcane borer (*Diatraea saccharalis*) host preference and performance. Sugarcane plants cv SP-79-1011 were inoculated with a pool of diazotrophic bacteria and non-inoculated plants were used as control. The host preference behaviour was investigated using a two-choices arena. In order to assess the caterpillar relative growth rate a non-choice feeding bioassay was performed. The volatile organic compounds (VOCs) were collected from both plant treatments and identified using gas chromatography coupled mass spectrometry (GC-MS). The sugarcane borer showed a preference for the control plants avoiding the inoculated plants. There was no difference in caterpillars feeding between treated and control plants. When analysed the VOCs there were no pronounced qualitative differences between treatments, nevertheless a general suppression of the VOCs was identified in plants associated with bacteria. The results suggest that *Gluconacetobacter* sp. and *Azospirillum* sp. in addition to nitrogen fixation and plant growth promotion, can make the sugarcane less attractive to *Diatraea saccharalis*. The change in the VOCs profile may be one of the mechanisms involved. Therefore, the association between sugarcane (*Saccharum* spp.) and diazotrophic microorganisms can increase resistance to *Diatraea saccharalis*. In the future an overall understanding of the mechanisms can provide tools to maximize the benefits of this association.

Poster number: *28*

# Floral scents to the touch: Scents inform moth's proboscis for probing and foraging, thus determining the plant fitness


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It is assumed that floral scent functions as long distance attractants, whereas flower fine handling is guided by visual and tactile cues (Goyret et al. 2007, Goyret 2010), but it is known that flowers emit volatiles from specific tissue areas. Field observations of *Nicotiana attenuata* show that visitation rates of scenting (EV) and non-scenting (CHAL) flowers are similar while nectar removal is significantly higher in plants producing floral scent, and a lack of floral scent leads to reduced fitness (Kessler et al. 2008). If visitation rate is the same then how differential fitness is explained? And will the scents work only at long distance?

We conducted tent and wind tunnel experiments to observe the behavior of moth *Manduca sexta*, in both scenarios visitation rate was the same but not the foraging rate. The moth spends more time in probing CHAL flowers, by inspecting the outer corolla limb, but similar foraging time compare to EV flowers. We assessed the fitness in the wind tunnel by manually adding pollen to the proboscis and letting the moth visit either EV or CHAL flower sets at a time. Pollinated flowers were allowed to set seeds and results showed a differential pollen transfer, with higher number of capsules and seeds in EV flowers compare to CHAL. We hypothesize that *M. sexta*'s proboscis works as a chemo-tactile extension while probing the corolla limb surface for signals such as benzyl acetone (BA), the major bouquet component. Electron microscopy imaging revealed several sensilla in the proboscis surface that can potentially fill this role. BA emission is very important for the flower handling efficiency of *M. sexta* and is essential to ensure pollination (maternal fitness). BA thus plays a large role as a short range cue, besides navigational cue.


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Kessler, D., Gase, K. and Baldwin, I.T. (2008) Field experiments with transformed plants reveal the sense of floral scents. Science, 321, 1200–2.

Poster number: *29*

# Food-stressed males display compensation in fitness-associated physiological and behavioural traits and females get fooled if they cannot assess the male odour in the butterfly *Bicyclus anynana*.


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Sexually-selected traits often display large phenotypic variation that affects fitness, and this variation is due to the genotype, the environment (i.e. phenotypic plasticity) or their interaction. We tested whether male sexual signals showed adaptive plasticity and signalled developmental and adult environments in the model lab-reared butterfly *Bicyclus anynana*. In *B. anynana*, two sexually-selected signals are known to affect mate choice: the UV-reflectance of forewing eyespot centers (UV-EC) and the male sex pheromone (MSP) composition (1,2). While UV-EC is fixed at adulthood, MSP composition varies with age and we hypothesized that this signal may be sufficiently plastic to track lifetime environmental heterogeneity. Using a full-factorial family design, we tested whether a food stress at larval or adult stages affects the expression of the male sexually-selected traits, the mating success of males and their fitness. Both larval-stressed adult stressed-males induced heightened condition-dependent expression of the MSP composition, suggesting that a sex pheromone can act as a short- and a long-term signal of male condition. UV-EC analyses are now running. Adult-stressed males invested also more in courtship. Compensation by stressed males at both physiological (SP) and behavioural (courtship) traits let them maintain their mating success despite poorer condition, perhaps as a terminal investment (male survival analysis now running). On the female side, wild-type females resisted mating attempts of stressed males significantly more than that of control males. In contrast, smell-blocked females mated randomly and more rapidly, which induced fitness costs when mating stressed males. Thus olfaction matters to allow females identify the cheating, food-stressed, males from the control ones.



1. Nieberding et al (2012) Ecology Letters 15:415;

2. van Bergen et al (2013) Proc Roy Soc B 280:1471.

Poster number: *30*

# Acetogenins from *Annona mucosa* for leaf-cutting ants control

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Leaf-cutting ants from the species *Atta sexdens rubropilosa* are dominant herbivores in the tropical regions and are known by their strong power of destruction of plantations, being responsible for significant losses in agriculture. The control of this pest is actually done by employing synthetic commercial insecticides that are usually non-specific, toxic to the environment and promote the appearance of resistance [1].

In this perspective, natural products from plants have become a great source of new insecticide and fungicide compounds. *Annona mucosa*, a tropical fruit tree common found in Brazil was submitted to guided phytochemical studies by bioassays.

Initially, crude ethanolic extracts from leaves (MUL), branches (MUB) and seeds (MUS) were tested against workers of leaf-cutting ants and it was verified that all extracts were toxic, with the seed extract (MUS) causing 100% of mortality of ants at day 7. All extracts were fractionated and the hexanic and dichlorometanic subfractions from leaves (MULHe and MULD) and branches (MUBHe and MUBD) showed great insecticide potential with more than 90% of mortality for the leaves subfractions and more than 84% for the branches subfractions. The two subfractions from seed extracts, hexanic (MUSHe) and hidroalcoholic (MUSHi) showed high toxicity causing 100% and 80% of mortality, respectively.

The subfractions MULHe, MULD and MUSHi were studied trough chomatographic methods resulting in the isolation of lignans and acetogenins. Acetogenins is a class of compounds known by the several biological activities displayed that act as inhibitors of complex I in the mitochondrial electron transport chain promoting cellular apoptosis [2]. In this work were isolated and identified 4 acetogenins, rolliniastatin-1 and 2, jimenezin and a novel acetogenin, standing out the potential of these compounds as insecticides and the possibility of employing them in ecological control of leaf-cutting ants or as lead structures for the development of new insecticides.

1. Della Lucia, T M C; Gandra, L C; Guedes, R N C. Pest Manag. Sci., 70, 14-23, 2014.

2. Bermejo, A; Figadére, B; Zafra-Polo, M C; Barrachina, I; Estornell, E; Cortes, D. Nat. Prod. Rep., 22, 269-303, 2005.

Poster number: *31*

## The responses of egg parasitoids *Telenomus podisi* and *Trissolcus basalis* (Hymenoptera: Platygastridae) to chemical footprints of stink bugs are related to host preference.


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Chemical trails from the footprints of their hosts are important for host finding in stink bug egg parasitoids. The objective of this work was to verify whether the egg-parasitoids *Trissolcus basalis* and *Telenomus podisi* are able to recognize chemical trails from different species of Pentatomidae, and also if they show any preference for traces of their preferential hosts (*Nezara viridula* for *T. basalis* and *Euschistus heros* for *T. podisi*). To accomplish this, the stink bugs *Nezara viridula*, *Euschistus heros* and *Dichelops melacanthus* were released in arenas, made of glass plates, physically divided into two parts, to determine the preference of the parasitoids by traces of different stink bugs. Three different treatment combinations were prepared contrasting pairs of footprints of the three stink bug species. The parasitoids were released separately in the arenas and their behavior monitoring with a software that record and  process insect movements, SACAM. To compare the responses toward different stink bugs footprint total time, total trajectories, tortuosity and turn rates in areas treated with footprint of each species were calculated. It was found that the parasitoids *T. podisi* and *T. basalis* identified and differentiated their host chemical trails. *T. basalis* showed preference to chemical trails of *N. viridula* where they stay for longer time and showed higher trajectories and more tortuous movements, while *T. podisi* preferred the chemical trails of *E. heros* with similar alteration of its behaviour. Thus, it was concluded that footprints are cues that could be used in both searching and selection behaviour when foraging for hosts by the egg parasitoids. To identify the chemical components of footprints and to evaluate the influence of these components on the egg parasitoid behaviour, chemical analyses are under execution.



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Poster number: *32*

## Identification of symbiont bacteria of *Diabrotica speciosa* using MALDI-TOF MS

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The pressure for more sustainable methods for integrated crop management pushes the development of cleaner and safer strategies for pest control. At the same time, there are an increasing number of insects acquiring resistance to established crop protection methods in a relatively small amount of time. In an interesting example, Chu et al. (2013) attributed the crop rotation resistance of western corn rootworm (*Diabrotica virgifera*) to changes in gut microbiota composition. As such, a clever and promising strategy for pest control could be through manipulation of insect's bacterial symbionts. *Diabrotica speciosa* is a highly polyphagous plague insect in Brazil causing severe damages to several crops, and in order to start the evaluation of this possible strategy for pest control *D. speciosa* larva and adults were reared under different conditions and their culturable gut bacteria were identified using MALDI-TOF and 16S DNA sequencing. It was possible to isolate 73 strains, from 17 different genera, including some bacteria described for the first time in association with insects. MALDI-TOF was able to correctly identify 95% of the isolates at least to genus level, and the ones that were not identified were because of lack of specific database according to results given by sequencing. MALDI-TOF was an interesting tool for initial identification of symbiont bacteria providing faster, cheaper and more reliable results than partial 16S sequencing, which could be used to greatly reduce the number of samples for molecular methods.

Chu et al., (2013), PNAS, 110(29), 11917-11922

Poster number: *33*

## Processing of salicin-derived poplar phenolics in the gypsy moth

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The ingestion of plant defense metabolites can be harmful to insect herbivores, but investigations of the underlying molecular mechanisms for toxicity have rarely been carried out. Consequently our knowledge of the metabolic conversion of defense compounds in insect herbivores is very limited and we do not often understand why they are toxic to some species, or broadly tolerated by others. This is especially true for generalists that feed on a wide spectrum of host plants and may not have adapted to specific types of plant defense metabolites. We studied the metabolic conversion of salicinoids, a group of phenolic defense compounds produced by the Salicaceae, in gypsy moth (*Lymantria dispar*) caterpillars, generalist feeders whose diets include salicaceous tree species such as poplars. A fecal metabolite screening revealed several conjugates that originate from post-ingestive salicinoid degradation. Subsequent bioassays indicate that the gypsy moth can conjugate and excrete some degradation products better than others, and that the processing rates are negatively correlated to toxicity. Based on empirical evidence we argue that the electrophilic nature of some salicinoid metabolites may be an explanation for their toxicity.

Poster number: *34*

# Laser MS imaging of cuticular lipids using etched silver substrates

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Ultraviolet laser desorption/ionization mass spectrometry (UV-LDI-MS) can be used to analyze cuticular lipids (including unsaturated hydrocarbons, alcohols, acetates, fatty acids, and triacylglycerols) directly from insect cuticles [1-3]. However, due to the lack of functional groups for enabling their ionization, saturated long chain hydrocarbons (HC) are not detected by UV-LDI-MS. Previous studies have shown that long-chain alkanes can be analyzed by UV-LDI-MS if AgNO3 or silver nanoparticles are added to cuticular extracts, giving rise to [M+Ag]+ ions [4]. Here we present a modified approach in which etched silver substrates are used. We show that this method provides high analytical sensitivity for saturated HCs as well as numerous other lipid species when standards or whole cuticular extracts are spotted onto the surfaces. Also, MS imaging of flat insect surfaces (such as wings) is possible after blotting cuticular compounds by physical contact. The technology could enable chemical imaging of small-scale features such as glands with a lateral resolution in the low ten micrometer range.

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Poster number: *35*

# Antagonistic activity of Bacillus subtilis A18–A19 strains against *Heterobasidion species*

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*Heterobasidion annosum* and *H. parviporum* are severe pathogens of conifers causing butt and root rot and reducing the economic value of timber. Here the antifungal activity of *Bacillus subtilis* strains A18 and A19 against *Heterobasidion* species was investigated by co-culturing method. Anti-fungal activity of bacterial culture filtrate (CF) and solvent extracts was also tested by disc diffusion method. *B. subtilis* isolates showed strong antagonistic activity for both *Heterobasidion* species on potato dextrose agar medium. The growth inhibition of *H. parviporum* by *B. subtilis* isolate A19 was greater compared with isolate A18. Both bacterial isolate showed similar inhibitory activity for *H. annosum*. Culture filtrate extract of both bacterial isolates significantly inhibited the growth of both test fungi. The spore germination test indicated the impairment of germ tubes when treated with CF extracts. The presence of lipopeptide and biosurfactant compounds like Plipastatin, esperin, fengycin and surfactin were the main compounds found in bacterial CF extracts. These compounds might have been the possible putative compounds for antifungal activity against *Heterobasidion* spp. The results emphasize the potential application of *B. subtilis* isolates as a good alternative to the existing biocontrol agent.

Poster number: *36*

## Chemodiversity in *Begonia* flower fragrances

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The purpose of the study was to determine the chemical diversity of flower volatiles of a number of *Begonia* species. The *Begonia* species were selected to be representatives for bird, insects and wind pollination.

Dynamic fragrance collection using PorapakQ and organic solvent extraction of pollen and pistils were made for the respective species. In addition, the numbers of male and female flowers during the flowering season were counted. A Finningan SSQ 7000/Varian 3400 GC-MS instrument with a DBwax column was used for the analyses.

Most of the *Begonia* species that are either pink or white release complex mixtures of aliphatics, terpenoids and aromatics, while the red ones have no detectable odor. Male and female flowers have in certain species like *B. egregia* different scents, males producing a series of aliphatic 2 ketones and females a number of monoaromatics. *B. undulata* shows a circadian rhythm induced by light in the emission of (-)-linalool and corresponding furan oxides,.

The chemodiversity found in *Begonia* species with different pollination systems give us a good background for further pollinator studies.

Poster number: *37*

# Attraction of egg parasitoids toward oviposition induced plant volatiles in a multi-herbivore perspective

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In response to insect herbivory, plants emit volatile organic compounds which may act as indirect plant defenses by attracting natural enemies of the attacking herbivore. In nature, plants are often attacked by multiple herbivore species, but so far most of the studies investigating indirect plant defenses have not been conducted under a multiple herbivore scenario. Here, we investigated attraction of two egg parasitoids of lepidopteran hosts (*Trichogramma brassicae* and *T. evanescens*) toward plant volatiles induced by alien and native insect herbivores in olfactometer bioassays. We used a system consisting of a wild crucifer, *Brassica nigra*, two of its naturally associated herbivores (eggs and caterpillars of the butterfly *Pieris brassicae* and the aphid *Brevicoryne brassicae*), and an alien invasive herbivore (eggs and caterpillars of the moth *Spodoptera exigua*). We found that *Trichogramma* wasps are attracted by *P. brassicae*-egg induced plant volatiles but not by *S. exigua*-egg induced plant volatiles indicating specificity of plant responses toward lepidopteran species. Chemical analyses show significant differences between volatiles emitted by *P. brassicae*- and *S. exigua*- egg induced plants, in agreement with the behavioral observations. We also investigated attraction of the *Trichogramma* species toward *P. brassicae*-egg induced volatiles in plants simultaneously attacked by native or alien non-hosts. Results indicated that native and alien chewing caterpillars, but not phloem-feeding aphids, can disrupt *Trichogramma* species attraction toward *P. brassicae*-egg induced volatiles. Results are discussed in the context of indirect plant defenses in a multiple herbivore perspective.

Poster number: *38*

# Antagonists of the sex pheromone of the tomato leafminer *Tuta absoluta*. Synthesis, functional assays, and electrophysiological activity

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The tomato leafminer *Tuta absoluta* (Lepidoptera: Gelechiidae) is a major pest of tomato crops in many countries from Europe, Asia and Africa. Since control of this insect essentially relies on multiple insecticide treatments, serious problems of resistance have arisen in the last years. Therefore, more specific and environmentally acceptable approaches for an effective integrated management of the pest are urgently needed.

Trifluoromethyl ketones are a family of compounds able to inhibit the antennal esterases present in insect olfactory tissues. These are key degrading enzymes of pheromone esters, thus maintaining a low stimulus noise level in sensory hairs, which is crucial for a successful attractant and mating behavior. Possibly as a consequence of their esterase inhibition effect, these chemicals also display a remarkable antagonistic activity of the pheromone responses in a variety of Lepidoptera. In the same line, methyl ketone analogues structurally close to the pheromone have been reported by us and others as remarkable inhibitors of the male responses to the pheromone. In this context, we present herein the synthesis, electrophysiological activity and functional assays of new methyl and trifluoromethyl ketone analogues of both components of the pheromone of *T. absoluta*, (E,Z,Z)-3,8,11-tetradecatrienyl acetate and (*E,Z*)-3,8-tetradecadienyl acetate, as antagonists of the pheromone response and their prospects as potential control agents of the pest. The methyl and trifluoromethyl ketone analogues result from the structural replacement of the acetate group of the pheromone components by the acyl and trifluoroacyl group, respectively

Poster number: *39*

# Mating status affects pheromone production and EAG responses in the tomato leafminer *Tuta absoluta*

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The tomato leafminer *Tuta absoluta* (Lepidoptera: Gelechiidae) is one of the most devastating pest of tomato, which has rapidly spread from South America into Europe, Africa and Asia. Although several integrated management approaches have been implemented, including natural enemies, in most cases application of insecticides are still one of the most effective strategies. However, the continuous use of insecticides involves development of resistance in addition to serious negative effects to the environment and other living organisms. Therefore, development of environmentally-friendly strategies for control of the pest is of utmost importance and urgency.

The sex pheromone of *T. absoluta* was identified as a mixture of (*E*,*Z*,*Z*)-3,8,11-tetradecatrienyl acetate and (*E*,*Z*)-3,8-tetradecadienyl acetate in 90:10 ratio (Attygalle et al., 1995). In this work we present a new and convenient synthesis of both pheromone components in approaches which compare favorably with other previously reported. We have compared the activity of both compounds, both in isolated form and in the 9:1 natural mixture, in virgin and mated males.

It is well known the important role that plant volatiles (VOCs) play in females attraction and egg-laying. We describe here that the production of sex pheromone is also a function of the females mating status, with pheromone glands of virgin females containing higher amounts of both pheromone components than those of mated females. In addition, production of pheromone is higher in the presence of the plant than in its absence. Electrophysiological activity of the 4 major VOCs of the plant, i.e. β-phellandrene, limonene, 2-carene, and (*E*)-β-caryophyllene, which represent about 70% of the total tomato leaf volatiles, indicate that only 2-carene provoked different EAG responses in mated and unmated females.

Poster number: *40*

# Antifungicidal and antioxidant activities of *Rhododendron tomentosum* essential oils

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It has long been recognized that naturally occuring substances in plants including essential oils have biological activity 1. and for this reason have a widespread application.

Aim of this work is to reveal antioxidant and antifungal activity of *Rhododendron tomentosum* Harmaja essential oils against *Penicillium cyclopium* Thom and *Trichoderma harzianum* Rifai.

Plant material was collected near Juodupė (shoots; Rokiškis district) and Šilėnai (shoots; Vilnius district) villages and Samanis marsh (shoots and flowers; Utena district) in 2007. The essential oils were obtained by hydrodistilation (2h) of air dried material in Clevenger type apparatus. Oils were analysed using GC/MS and components were identified comparing mass spectrums with computer mass spectra libraries and literature data 2. Antifungal screening was conducted by agar-diffusion method. Antioxidant activities of volatile oils were analysed using ABTS+ assay.

Predominant constituents were found to be palustrol (26.9 – 42.8%), ledol (23.1 – 30.8%), myrcene (0.5 – 11.4%) and cyclocolorenones (2.7 – 9.3%) in all samples also limonene (3.7 – 11.0%) in sample from Šilėnai and iso-ascaridol (12.9 – 14.2%) in two samples from Samanis marsh. Growth of *T. harzianum* was inhibited by all oils under investigation. The totally suppressed development of this fungus after seven days was achieved by the oils richest in myrcene and limonene. Weaker antifungal activity was observed against *P. cyclopium* using shoots (Šilėnai) and flower (Samanis) essential oils. Only two oils completely suppressed growth of this fungus. In the ABTS+ assay *R. tomentosum* essential oils indicated significant antioxidant activities.

This study has confirmed that *R. tomentosum* essential oils can be used as growth inhibitors of some micromycetes and as natural antioxidants.

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Poster number: *41*

## Male preference imposes stabilizing selection on female pheromone phenotype

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Changes in female pheromone quality are supposed to be under strong stabilizing selection imposed by male preference functions whose shape is influenced by the cost of interspecific mating. This poses a major hurdle to understanding how pheromone blends diverge and ultimately how speciation occurs in groups that rely heavily on sex pheromone communication. However, to our knowledge, sexual selection on female pheromone phenotype (FPP) has never been measured, but rather assumed from male trap-catch data. Trapping data do not actually measure the probability of mating and therefore provide only a proxy of the strength of sexual selection on females. Furthermore, because female pheromone components are strongly correlated biosynthetically, it is difficult to deduce from trapping data which pheromone components are the major targets of selection.

We conducted mating studies with tobacco budworm (*Heliothis virescens*, Noctuidae) female moths under field conditions to obtain selection gradients on FPP. Surprisingly, we found significant stabilizing selection on the secondary pheromone component, Z9-14:Ald, as well as on a biosynthetically correlated component, Z9-16:Ald. This latter compound is the secondary pheromone component of two related and sympatric moth species, *Heliothis subflexa* and *Helicoverpa zea*. Interestingly, we found no evidence of stabilizing selection on the major pheromone component, Z11-16:Ald. This is the major component of all three species and may be indirectly stabilized via its biosynthetic correlation with Z9-14:Ald or Z9-16:Ald.

Stabilizing selection is inherently difficult to detect because it tends to “erase its traces”. Our field assays were designed to artificially broaden the phenotypic variation of the female pheromone blend to effectively detect even the slightest level of stabilizing selection. Although these assays were conducted in the field with a natural *H. virescens* population, they also imposed some unnatural conditions, such as restricting female mobility by clipping their wings. Nevertheless, our results offer new hypotheses and field designs for assessing the strength and evolutionary consequences of stabilizing sexual selection on FPP.

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## The diurnal emission pattern of constitutive and induced rice volatiles

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Plants have evolved highly effective defense mechanisms to resist attacks by herbivores. Insect resistance traits include 'indirect defense' which is the ability to respond to herbivore attack by synthesizing a complex bouquet of herbivore-induced volatile organic compounds (HI-VOCs) that attract natural enemies of herbivores. Using the trophic model of rice plant and the generalist Loreyi armyworm, *Mythimna loreyi* Duponchel (MYL), we characterized the inducibility of rice HI-VOCs upon MYL mimicked herbivory. Our results show that simulated MYL feeding significantly increases the emission of a number of key HI-VOCs, including linalool and MeSA. Given that many abiotic factors affect VOCs emissions, such as the photoperiod (Gouinguené and Turlings 2002), the diurnal emission patterns of HI-VOCs were determined in control rice plants and those subjected to MYL simulated feeding on a previous day. Our findings stress that both control and induced rice plants followed a diurnal pattern in VOCs emission; however, induced plants emitted remarkably higher amounts of HI-VOCs, 2-10 folds in some compounds, compared with control plants. It suggests that such enhanced HI-VOCs blend and its composition fully depends on the photoperiod of the environment when the natural enemies are actively foraging during the day.

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Poster number: *43*

## Potato blight, aphids and Colorado potato beetles: How do potatoes cope?

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Aphids, Colorado potato beetles and the late blight pathogen are among the potato plant's most important stressors. How do potato plants deal with multiple attacks, and how do previous attacks on potatoes affect the newcomers thereafter? Understanding how plants integrate their defence responses can provide valuable insights into ecological interactions in an agroecosystem. In the current research we are investigating how herbivorous insects of different feeding guilds (the leaf chewing beetle *Leptinotarsa decemlineata*, and the phloem-sucking aphid, *Myzus persicae*) affect potato defence responses; and also how the oomycete, late blight (*Phytophthora infestans*) affects these responses in the two phases of its hemibiotrophic life cycle. We measured plant responses by quantifying expression of genes involved in two important defence pathways. These are the salicylic acid signalling pathway, which can be triggered by stress from phloem feeders or biotrophic pathogens; and the jasmonic acid pathway, normally triggered by stress from chewing herbivores and necrotrophic pathogens. We also observed how each of these single stressors can affect a second attacker by measuring insect body mass and monitoring development; or quantifying *Phytophthora* biomass in leaf tissue with or without previous stress. Results demonstrate that plant responses to insects with different feeding strategies induce different phytohormonal pathways. The potato blight pathogen induces hormonal cascades at different rates than the insect stressors, and the pathogen infection can have opposite effects on insects with different feeding strategies

Poster number: *45*

# Two fatty acyl-CoA reductase gene involved in moth pheromone biosynthesis

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Pheromone gland specific fatty acyl reductase (pgFAR) is an evolutionary conserved category of enzyme involved in sex pheromone biosynthesis, which catalyzes the conversion of fatty acyl-CoA precursor to the corresponding alcohol with distinct stereo-specificities, and contribute the pheromone based reproductive isolation in moth. Highly selective and semi-selective single pgFAR could produce mono and multicomponent pheromone signals had been reported from silk-moth, pyralid, yponomeutid and noctuid moth. Here we reported two pgFARs (named SexpgFAR A and B) simultaneously expressing in the pheromone gland (PG) of beet armyworm moth, *Spodoptera exigua*. The expression of both pgFARs was restricted to the female PG. SexpgFAR A and B transcript abundance in the PG was almost same in 2-3 day old female moth. The deduced amino acid sequence of SexpgFAR A and B showing overall identity of 42.73%. Both pgFARs encoded proteins comes under the lepidopteran-specific pgFAR gene subfamily, suggesting their role in pheromone biosynthesis. Heterologous gene expression in recombinant yeast (*Saccharomyces cerevisiae*) system supplemented with C12- or C14- or C16-fatty acyl pheromone precursors (as reported in 17 *Spodoptera species*, http://www.pherobase.com/database/genus/genus-Spodoptera.php) confirmed that SexpgFAR A showing high substrate preference for C16 fatty acyl derivatives and unable to convert the natural pheromone precursors of diunsaturated fatty acids to corresponding alcohol. Whereas SexpgFAR B is able to produce the intermediate fatty alcohol from C14 and C16 mono and diunsaturated pheromone precursors and showing high preference to C14 fatty acyl derivatives, especially (*Z*)-9-tertradecenoic acid. When presenting the ten precursors in equal ratios to recombinant yeast expressing SexpgFAR B, all reduced to corresponding alcohol, however, 14: acid, Z9-14:acid, Z11-14:acid, E11-14:acid and Z11-16: acid preferentially over Z9E12-14:acid. Similar functional assay with SexpgFAR B preferentially reduced C16:acid and Z11-16:acid and unable to convert Z9-14:acid and Z11-14:acid to corresponding alcohol. Most Spodopteran moths use, (Z)-9-tetradecenyl acetate and (*Z*,*E*)-9,12-tetradecadienyl acetate as major sex pheromone component and (*Z*)-11- hexadecenyl acetate as minor pheromone. We conclude that SexpgFAR B with broad specificity and SexpgFAR A with narrow specificity are involved in spodopteran moth pheromone biosynthesis.

Poster number: *46*

## Behavioural responses of *Frankliniella occidentalis* Pergande to *cis*-jasmone

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*Frankliniella occidentalis* Pergande (Thysanoptera: Thripidae), the western flower thrips, is a major pest in agricultural and horticultural crops worldwide. The plants are damaged directly by feeding of adults as well as immature stages on epidermal and sub-epidermal plant tissues and indirectly by the transmission of various plant viruses. Approaches to improve thrips control include the potential use of secondary plant compounds for behavioural manipulation. Previous findings have indicated that western flower thrips adults respond negatively to jasmonic acid derivatives. *Cis*-jasmone, an essential oil constituent, is known to play a major role in plant defence mechanisms against herbivores.

The aim of our study was therefore to investigate the deterrent and behavioural effects of an artificial application of *cis*-jasmone to bean leaf discs and potted bean plants on *F. occidentalis* adults and second instar larvae.

Comparing the FDC50, i.e. the concentration of *cis*-jasmone required to produce 50 % feeding deterrence determined in choice bioassays, showed that thrips larvae respond to a 3-fold lower concentration of *cis*-jasmone than adult females. Significantly more thrips larvae left *cis*-jasmone-treated whole potted bean plants by migrating to the soil compared with control plants. In no-choice leaf disc assays conducted over four consecutive days *cis*-jasmone applied at a low concentration (FDC15) was deterrent to *F. occidentalis* females only during the first two days of the testing period. While thrips showed a tendency to habituate to *cis*-jasmone at the low concentration, they were deterred by the FDC50 over a longer period and repeated exposures to a high concentration (FDC95) did not result in habituation effects over all four days of exposure.

Bioactive plant compounds that modify the behaviour of different life stages of *F. occidentalis* might be used as synergists for various biological or chemical control measures in conventional, integrated or organic farming systems.

Poster number: *47*

## Natural and altered terpenoids as modifiers of aphid probing

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Aphids transmit nearly 30% of all known plant virus species and 50% of insect-borne viruses. Plant diseases caused by these viruses may reduce yields by up to 80%. Aphids acquire and inoculate viruses during various stages of plant penetration with sucking-piercing mouthparts: during brief intracellular probes in epidermis and parenchyma (mesophyll in leaves) that precede feeding in phloem vessels, aphids may transmit non-persistent and semi-persistent viruses and when aphid stylets reach sieve elements, persistent viruses may be transmitted. Reducing or eliminating penetration of plant tissues by aphids could reduce infection by pathogens.

Considering the selectivity and behavior-modifying potential of plant-derived chemicals, especially the terpenoids, several attempts have been made to apply these compounds as alternatives to conventional neurotoxic pesticides. Following the biopesticide-related approach to aphid control and reduction of virus transmission, we present results of our multi-year research on aphid probing behavior-modifying activity of several natural and chemically-modified terpenoids. Research included innovative application of electrical penetration graph (EPG) technique for monitoring feeding deterrent activity against aphids. Aphid probing was impeded at pre-ingestive (pre-phloem) and/or ingestive (phloem) phases, which revealed that compounds passed through the plant surface and were distributed systemically within plant tissues. Chemical modification of naturally occurring terpenoids, e.g., incorporation of functional groups, epoxidation, or lactonization, produced significant changes in their activity profiles. Modified terpenoids varied in potency and persistence of behavioural effects on aphid probing, and certain modifications caused a shift from attractant to deterrent properties, or vice versa.

Poster number: *48*

# Are plant volatiles mediating highly specialized plant-insect interactions affected by environmental conditions?


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Plant volatile organic compounds (VOCs) play key roles in the interactions between plants and their abiotic and biotic environments. For instance, pollinators often rely on floral scents to locate plants. Environmental conditions can have an important impact on plant chemical signals by affecting differentially the quality and/or quantity of VOCs emitted. These variations may disturb the recognition of flowers by the pollinators. The aim of the present study is to evaluate the impact of several environmental factors (temperature, precipitation and ozone concentration) on the VOCs emitted by the dioecious Mediterranean fig, *Ficus carica*, in order to attract its pollinator. This species is engaged in an obligatory mutualism with a specific pollinating wasp, *Blastophaga psenes*, which reproduces only within figs. Using behavioral test, we observed that *B. psenes* is attracted by scents of male and female receptive figs. In addition, using gas chromatography coupled with electroantennographic detection recordings, we revealed that only 4 out of 27 VOCs emitted by receptive figs were detected by the pollinator. Currently, we are sampling in situ the scent emitted by receptive figs of *F. carica* in 7 localities in the French Mediterranean region and analyzed these samples using gas chromatography coupled with mass spectrometry. The impact of several environmental factors (temperature, precipitation and ozone concentration) on the VOCs emitted by *F. carica* to attract its pollinator will be evaluated.

Poster number: *49*

## First evidence of a volatile sex pheromone in lady beetles

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To date, volatile sex pheromones have not been identified in the Coccinellidae family; yet, various studies have suggested that such semiochemicals exist. Here, we collected volatile chemicals released by virgin females of the multicolored Asian lady beetle, *Harmonia axyridis* (Pallas), which were either allowed or not allowed to feed on aphids. Virgin females in the presence of aphids, exhibited « calling behavior », which is commonly associated with the emission of a sex pheromone in several Coleoptera species. These calling females were found to release a blend of volatile compounds that is involved in the remote attraction (i.e., from a distance) of males. Gas Chromatography-Mass Spectrometry (GC-MS) analyses revealed that (–)-β-caryophyllene was the major constituent of the volatile blend (ranging from 80 to 86%), with four other chemical components also being present; β-elemene, methyl-eugenol, α-humulene, and α-bulnesene. In a second set of experiments, the emission of the five constituents identified from the blend was quantified daily over a 9-day period after exposure to aphids. We found that the quantity of all five chemicals significantly increased across the experimental period. Finally, we evaluated the activity of a synthetic blend of these chemicals by performing bioassays which demonstrated the same attractive effect in males only. The results confirm that female *H. axyridis* produce a volatile sex pheromone. These findings have potential in the development of more specific and efficient biological pestcontrol management methods aimed at manipulating the behavior of this invasive lady beetle.

Poster number: *50*

# Prospect for field management of *Zonocerus variegatus* with Pyrrolizidine alkaloid based-attracticide bait in sub Saharan Africa

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The native *Zonocerus variegatus* assumed pest status in sub Saharan Africa in the 1970s following the introduction and establishment of the noxious invasive weed, *Chromolaena odorata*. Subsequently, both the insect and the weed have impacted negatively on agriculture, forestry and conservation. A pilot study was conducted in Ghana to validate the efficacy of a novel pyrrolizidine alkaloid (PA) based attracticide for the field management of *Zonocerus variegatus*. The attracticide comprised poisoned roots of *C. odorata* with 1.0% w/v Carbofuran® 3G. The results though preliminary showed that PA based attracticide was most efficient when placed at the same level as the field vegetation. Also, the developed attracitice had an efficiency of about 0.3% knock down per trap of field population of *Z. variegatus*. The possibility of prolonging the shelf life of the PA-based attracticide is discussed.

Poster number: *51*

# Ecological chemistry of weaver ants – nutrient cycling and search for novel chemical characteristics

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The tropical weaver ants nest in the canopies of trees and have for centuries been used in pest control in tropical orchards where they protect several cropsagainst more than 50 insect pests. Experiments have suggested that these ants not merely control insect pests by preying on them, but produce repellent semiochemicals (Offenberg 2014). The chemistry potentially underlying the repellence has hitherto not been investigated. In the literature limited knowledge on the chemistry and semiochemicals of the weaver ants was found. In ongoing experiments we therefore not only perform targeted investigations of potentially repellent semiochemicals, but also an extensive exploration of the chemistry of the weaver ants, which could potentially uncover important new chemical characteristics of these ants.

Field studies of weaver ant-hosting trees have also illustrated that the feces they generously deposit onto the leaves of their host trees supply nutrients. Nutrient cycling of especially nitrogen from other ant species to plants have previously been demonstrated. Our chemical analyses established that the feces from weaver ants contain valuable nitrogen nutrients such as amino acids and the known foliar nutrient urea. In an untargeted GC-TOF-MS metabolomics experiment greenhouse *C. arabica* plants hosting the Asian weaver ant *Oecophylla smaragdina* displayed metabolic changes analogous to plants supplied with increased levels of nitrogen (Vidkjær et al. 2015). The results additionally suggested that nitrogen nutrients were not only assimilated, but were also translocated, thereby instigating systemic effects in the plants. This was later confirmed by uptake and translocation of urea in biologically relevant concentrations in *C. arabica* plants.

It would greatly enhance the benefits of using weaver ants in pest control if these ants through their dietary intake of pests can transform these from a threat to crop health into high value nutrients and repellent semiochemicals via their metabolic machinery.

Offenberg, J. (2014). Pest repelling properties of ant pheromones. Pheromones and other semiochemicals, IOBC-WPRS Bulletin, 99, 173-176.

Vidkjær NH, Wollenweber B, Gislum R, Jensen KMV, Fomsgaard IS (2015) Are ant feces nutrients for plants? A metabolomics approach to elucidate the nutritional effects on plants hosting weaver ants. Metabolomics DOI 10.1007/s11306-014-0757-4.

Poster number: *52*

## Surface hydrocarbon layers affect attractiveness of dispersing female *Monochamus alternatus*, the vector stage of the pinewood nematode

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Monochamus alternatus is an important forest pest in China. Cuticular hydrocarbons of beetles encode information regarding mate recognition, and in some cases, function as precursors to volatile long-range pheromones (Wickham et al. 2012, Insect Science). My research investigated the surface chemistry of *M. alternatus* for contact pheromones and pheromones precursors. As an alternative hypothesis, all volatiles collected from beetles were examined for pheromone activity in case pheromones are produced by another mechanism, which indeed was the case with male- produced aggregation pheromone monochamol (Teale, Wickham et al. 2011, Journal of Econ. Entomol.). Although the pheromone was discovered, the results of hydrocarbon analyses were nonetheless interesting. Results show surface hydrocarbon profiles change discreetly as females complete maturation feeding , in fact immature females go through an unusually long mandatory 1-month long period of maturation feeding before they become sexually mature. The immature, supplementary feeding stages are dispersing beetles, whereas the sexually mature beetles are ovipositioning beetles. This dispersal stage is a very important vector stage for the pinewood nematode, which causes pine-wilt disease. Furthermore, there appears to be a layering effect of cuticular hydrocarbons: subsurface compounds in immature beetles (as shown in solvent extract analyses, absent from SPME) are found on the surface of mature females (SPME analysis). Male mating behavior assays show these subsurface hydrocarbons to be biologically active. The most striking results show the number of trials with positive mating responses of male *M. alternatus* increased from 12/20 (60%) for freeze-killed 13-d old beetles to 18/20 (90%) following solvent reapplication, rendering them equally attractive as sexually mature females. While most mating behavior bioassay studies show the sequence of activity - no activity - restored activity using freeze-killed, solvent-washed, and extract-reapplied females, this study is unique in showing enhanced attractiveness of a group of beetles following redistribution of hydrocarbons on its surface.

Poster number: *53*

# Possible dual role of cytochromes P450 from *Eucalyptus*

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The important Australian genus *Eucalyptus* is grown globally for uses including essential oils, pulp (for paper production) and timber. *Eucalyptus* species produce a large amount of different specialized metabolites for chemical defense such as terpenoids, phenolics and cyanogenic glucosides. The phenylalanine-derived cyanogenic glucoside, prunasin, occurs in approximately 4% of *Eucalyptus* species. Upon tissue disruption, for example by herbivory, prunasin is hydrolyzed and toxic cyanide is released.

*Eucalyptus* species exhibit different patterns of prunasin synthesis throughout ontogeny. For example, *E. cladocalyx* seedling leaves contain high levels of prunasin compared to adult leaves, whilst *E. yarraensis* and *E. camphora* seedling leaves possess lower levels of prunasin compared to adult leaves. To understand the regulation of prunasin biosynthesis through ontogeny, foliar prunasin content in *E. cladocalyx, E. yarraensis*, *E. camphora* and acyanogenic *E. grandis* was continually tracked for a period of 6 months. Specific time points, where prunasin levels were found to be significantly different between the species, have been targeted to identify candidate regulatory and biosynthetic genes by transcriptomics.

The first step of cyanogenic glucoside biosynthesis, the conversion of an amino acid to its corresponding oxime, is catalyzed by a cytochrome P450 from the CYP79 family. We have identified several CYP79s from both cyanogenic and acyanogenic *Eucalyptus* species. The presence of CYP79s in acyanogenic species suggests that these genes may be involved in functions other than the synthesis of cyanogenic glucosides, such as the production of volatile oximes and nitriles.

Characterization of the several *Eucalyptus* CYP79s has been achieved by transient expression in tobacco, revealing differential substrate specificities. Future experiments will characterize CYP79 expression patterns in leaves and flowers and measure their volatile profiles as it has been shown in other species that oximes and nitriles are emitted from flowers to attract pollinating moths, and from leaves in response to herbivore attack.

Poster number: *54*

# Chemical and molecular characterization of nicotine to nornicotine conversion phenotype in Australian *Nicotiana* species used as chewing tobacco

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A range of *Nicotiana* species endemic to Australia are chewed by Aboriginal populations of Australia. Among the preferred species are *N. gossei, N. excelsior, N. goodspeedii, N. benthamiana, N. cavicola* and *N. velutina*. However alkaloids levels and nicotine to nornicotine conversion trait vary with species, environmental, and preparation factors. In tobacco research, nicotine to nornicotine conversion has a vital importance because nornicotine affects tobacco quality and has hazardous health outcomes. The molecular identity of the conversion factor in *Nicotiana* is of great importance. A group of cytochrome P450 genes is reported to be involved in the conversion process.

To determine the alkaloid chemical phenotype of chewed species of Australian *Nicotiana* spp. from different regions and the responsible locus for the conversion of nicotine to nornicotine in them.

A HPLC technique was validated to quantitate the alkaloids. Leaves were extracted in aqueous MeOH, followed by separation of the alkaloids on a C18 column with a mobile phase of acetonitrile and ethylammonium formate.

Total DNA was extracted from the leaves and the conversion locus was PCR amplified. Agarose gel electrophoresis was performed subsequently. Regions of the gel containing the amplified DNA fragments of interest were excised and purified for sequencing.

The quantification results were used for determining the conversion trait of species. The studied species were classified into non-converters (no nornicotine detected), medium converters (<50% conversion) and high converters (>50% conversion). The results indicated that *N. gossei* the most important chewed species is a non-converter, while *N. benthamiana* and *N. excelsior* are medium converters and *N. goodspeedii, N. cavicola* and *N. velutina* are high converters of nicotine to nornicotine. Gel electrophoresis results confirmed the presence of the conversion locus in all of the studied species. Therefore, the expression level of the genes is responsible for determination of different conversion chemotypes in them.

1. Pakdeechanuan P, Teoh S, Shoji T,Hashimoto T. Non-Functionalization of Two CYP82E Nicotine N-Demethylase Genes Abolishes Nornicotine Formation in *Nicotiana langsdorffii*. Plant and Cell Physiology. 2012 DEc, 2012;53(12):2038-46.

2. Gorrod, J.W.W.J., Nicotine and related alkaloids : absorption, distribution, metabolism and excretion. 1993, London [etc.]: Chapman and Hall.

Poster number: *55*

## Evolutionary transitions in alarm systems of fungus-farming ants

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Social organization of ant societies, from nestmate recognition to foraging, is governed by complex chemical cues. This is especially true in the fungus-farming ants of the tribe Attini who must not only regulate behavior of other ants, but also fungal cultivars they have domesticated over millions of years and depend upon for food. Recent phylogenetic advances indicate several major evolutionary transitions in farming ant societies, from colony-farms with less than 100 workers, to industrial-scale leaf-cutter ants whose massive fungus gardens sustain millions of specialized workers. Fungal cultivars provide attines access to a stable food source, but large standing resources (e.g., fungal crops and ant brood) also make the ant farmers vulnerable to predators and thieves. We thus predicted that attine alarm systems have been under intense selection. We first identified attine alarm systems, pheromones that elicit defensive responses in nestmates, from aggressive resistance to passive tolerance. We then tested for gradual or abrupt transitions across attine genera with diverse farming systems spanning millions of years of evolutionary time. According to the transitions hypothesis, alarm systems are conserved within attine lineages using similar farming systems, but differ across major farming transitions. Alternatively, the ecological hypothesis predicts that evolutionary changes in alarm systems are more strongly associated with other ecological factors such as behavior, morphology, colony size, or presence of soldier castes. Specifically, we measure exocrine gland secretions in workers and map them on an attine phylogeny. Moreover, we use behavioral assays to match alarm pheromones and alarm behavior in these species. The results shed light on how attine ants cope with one of the central costs of their farming lifestyle, and link chemistry, behavior and symbiotic coevolution.

Adams, R. M. M., Jones, T. H., Jeter, A. W., De Fine Licht, H. H., Schultz, T. R., & Nash, D. R. (2012). A comparative study of exocrine gland chemistry in *Trachymyrmex* and *Sericomyrmex* fungus-growing ants. Biochemical Systematics and Ecology, 40, 91–97.

Poster number: *56*

# Testing herbivore-induced plant volatiles for natural enemy conservation in agricultural systems

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Herbivore-induced plant volatiles (HIPVs) have the potential to attract natural enemies of insect pests, such as predatory arthropods, and thus conserve them in agroecosystems. However, only a few studies have shown whether this attraction leads to greater predation. Methyl salicylate (MeSA) is a commonly-emitted HIPV, induced by herbivorous arthropods from different feeding guilds, and known to attract natural enemies of agricultural pests. In this study, we tested the hypothesis that predator attraction to MeSA leads to higher predation. To test this hypothesis, we conducted laboratory, greenhouse, and field studies to: (1) determine if males and females of the convergent lady beetle, *Hippodamia convergens* (Coleoptera: Coccinellidae), respond physiologically to different concentrations of MeSA (0.02, 0.2, 2.0 and 20.0 mg) using electro-antennography (EAG); (2) determine whether *H. convergens* is attracted to MeSA in cage studies, and if this attraction increases predation of "sentinel" eggs of the European corn borer, *Ostrinia nubilalis*; and, (3) investigate if *H. convergens* is attracted to MeSA over various distances in commercial cranberry (*Vaccinium macrocarpon*) fields, using a mark-release-recapture technique with egg albumin protein as a marker. In the laboratory, male and female *H. convergens* antennae responded to MeSA concentrations of 0.2, 2.0., and 20.0 mg. In greenhouse cages, *H. convergens* adults were attracted to artificial plants baited with MeSA, which resulted in 90% increase in egg predation at 2.0 mg. In mark-release-recapture field experiments, more *H. convergens* beetles were caught near (0 m) traps baited with MeSA than on MeSA-baited traps placed at 5, 10, 15 and 20 m from the release site. Most (greater than 80%) of all beetles caught on traps were marked, indicating that our mark-release-recapture methodology was successful. In conclusion, lady beetle *H. convergens* adults are capable of detecting and responding positively to MeSA both physiologically and behaviorally, which led to increased predation; however, attraction to MeSA acted only at short distances. Our results indicate that HIPVs such as MeSA could increase number of predators and their function in agroecosystems.

Poster number: *57*

## Trajectories of pheromone plumes in an orchard canopy at night

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The trajectories of pheromone plumes in canopied habitats, such as orchards, have been little studied. We documented the capture of male navel orangeworm moths, *Amyelois transitella*, in female-baited traps positioned at 5 levels, from ground level to the canopy top, at approximately 6 m above ground, in almond orchards. Males were captured in similar proportions at all levels, suggesting that they do not favor a particular height during ranging flight. A 3-D sonic anemometer was used to establish patterns of wind flow and temperature at 6 heights from 2.08 to 6.65 m in an almond orchard with a 5 m high canopy, every 3 h over 72 h. The horizontal velocity of wind flow was highest above the canopy, where its directionality also was the most consistent. During the time of *A. transitella* mating (0300–0600), there was a net vertical displacement upward. Vertical buoyancy combined with only minor reductions in the distance that plumes will travel in the lower compared to the upper canopy suggest that the optimal height for release of pheromone from high-release rate sources, such as aerosol dispensers (“puffers”), that are deployed at low densities (e.g., 3 per ha.) would be at mid or low in the canopy, thereby facilitating dispersion of disruptant throughout the canopy. Optimal placement of aerosol dispensers will vary with the behavioral ecology of the target pest; however, our results suggest that current protocols, which generally propose dispenser placement in the upper third of the canopy, should be reevaluated.

Poster number: *58*

# Role of cuticular compounds in defense by soldiers of the one-piece termite *Neotermes chilensis*

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Nestmate recognition is important to support the social structure of a colony. Termites are eusocial insects whose colonies are mainly composed by three castes: primary reproductor pair (de-alate pair which reproduces after a swarming event), pseudo-workers (nest maintenance) and soldiers (defense). While one-piece nesters nest and forage in a single piece of wood, separate-piece nesters nest and forage in different substrates. The presence of soldiers in colonies of separate-piece nesters seems justifiable given the flux of individuals between the colony and the outside; the role of soldiers seems less intuitive in one-piece nesters because individuals do not have contact with the outside. However, soldiers may act defensively during swarms, when de-alates searching for a place to found a new colony represent potential invaders of occupied nests and also when colonies of conspecifics are present in the same substrate piece whose individuals may become invaders when digging galleries for colony expansion. Hence, we hypothesized that soldiers show nestmate recognition towards all castes and, based on work on other social insects, nestmate recognition is based on cuticular compounds (CC). We tested the first hypothesis through bioassays in which a soldier was confronted with a nestmate or non-nestmate de-alate, primary reproductor, pseudo-worker, or another soldier, and the second hypothesis through chemical bioassays in which a soldier was confronted with untreated, CC-deprived, and chemically-disguised nestmate or non-nestmate dead primary reproductors. The results show that soldiers are more intolerant towards non-nestmates than nestmates for all castes and that this nestmate recognition is mediated by CC.

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Poster number: *59*

# Yeast cell factory for production of insect pheromones

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Using sex pheromones for mating disruption of pest insects is an environmentally and health safe alternative to conventional insecticides. One barrier for wider application of pheromone-based pest management is high costs of synthetic pheromones. Presently, pheromones are produced by chemical synthesis, which commonly involves multiple reaction steps, expensive precursors, catalyzers and purification. Alternatively, pheromones could be produced by fermentation of sugar feedstocks with microbial cell factories expressing pheromone biosynthetic genes.

Metabolically engineered yeast *Saccharomyces cerevisiae* is an attractive host for production of chemicals and recombinant proteins, currently used for making ethanol, succinic acid, resveratrol, insulin, etc. The genetic tools for *S. cerevisiae* are well developed, which makes it possible to rapidly engineer a competent cell factory. Efficient heterologous gene expression can be achieved using integrative EasyClone system (Jensen et al., 2013), and gene knock-outs can be performed with CRISPR/Cas9 tools (Jakočiūnas et al., 2015; Stovicek et al., 2015).

We metabolically engineered *S. cerevisiae* for production of (*Z*)-11-hexadecenol, an active sex pheromone component of more than 20 moth species, including many pests (El-Sayed, 2014). Furthermore, we optimized fermentation conditions, and product titer was increased several-fold in comparison to the original strain described in Hagström et al., 2013.

El-Sayed AM 2014. The Pherobase: Database of Insect Pheromones and Semiochemicals. <http://www.pherobase.com>.

Hagström et al. (2013) A moth pheromone brewery: production of (*Z*)-11-hexadecenol by heterologous co-expression of two biosynthetic genes from a noctuid moth in a yeast cell factory. Microb Cell Fact 12: 125.

Jakočiūnas et al. (2015) Multiplex metabolic pathway engineering using CRISPR/Cas9 in *Saccharomyces cerevisiae*. Metab Eng 28: 213-222

Jensen et al. (2014) EasyClone: method for iterative chromosomal integration of multiple genes in *Saccharomyces cerevisiae*. FEMS Yeast Res 14: 238-248-11.

Stovicek et al. (2015) CRISPR-Cas system enables fast and simple genome editing of industrial Saccharomyces *cerevisiae* strains. Metab Eng Commun in press.

Poster number: *60*

# How do long-horned bee pollinated sexually deceptive orchids attract their pollinators?

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*Ophrys* orchids mimic the sex-pheromones of virgin females of their pollinators and thereby attract males that attempt to copulate with the flower labellum, for pollination. Previous studies on *Andrena* pollinated *Ophrys* species revealed that virgin females and orchid flowers use the same blend of hydrocarbons for eliciting mating behavior in the males. To gain further insight on sexually deceptive orchids the aim of our investigation was to identify chemical compounds of *O. leochroma* that play a role in male attraction of their pollinator *Eucera kullenbergii* (Anthophoridae).

Using coupled gas chromatography-electroantennographic detection (GC-EAD), gas chromatography-mass spectrometry (GC-MS) and behavioral field tests performed in Neapoli (Crete, Greece), we identified behavior-mediating compounds in the orchid and its pollinator females.

In electroantennographic analyses we found 9 GC-EAD active compounds in female surface extracts and 19 GC-EAD active compounds in labellum extracts that released reactions in the male antennae. They were identified as aldehydes, furthermore benzyl alcohol, hexadecyl acetate, octanol and dodecanoic acid. In addition we identified GC-EAD active hydrocarbons. Some of the compounds occurred in common in orchids and female bees.

In bioassays that we performed with surface extracts of virgin females, labellum extracts, polar and unpolar fractions of female and labellum extracts and synthetic mixtures of GC-EAD active compounds we found that polar fractions of female surface extract and labellum extracts elicited a significantly higher number of male reactions, amongst them pseudocopulatory attempts with the dummy bee than the solvent control. Furthermore, our results show that polar compounds such as aldehydes do play an important role in pollinator attraction in *Eucera* pollinated *Ophrys* species. Additional research is presently performed in order to understand if and how these compounds contribute to premating isolation in these orchid species.

Poster number: *61*

# How blue Petrels find their scented burrow?


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Sensory ecology, bird olfaction and emissions of volatile organic compounds (VOCs) are understudied. The contribution of olfaction to avian behaviour has been largely ignored by ornithologists and emphasis has generally been placed on vocal and visual signals. However, recent studies provided evidence that olfaction plays a fundamental role in the avian ecology, especially in hypogean petrels.

Petrels are known to return by night to the same nest with the same partner in the same colony each year. This nocturnal behaviour is probably driven by predation pressure from skuas and gulls. Latest investigations strongly suggested that birds use at least olfactory cues in nest and partner recognition with emission of specific and complex chemical labels. Olfactory cues that lead petrels towards the burrow entrance, might have a variety of sources such as owner's feathers and glandular excretions and plants. The ultimate carrier of these secretions is the plumage and the characteristic musky scent of petrels emanates only from it. The chemical composition of nest odour, the nature of components that facilitate recognition even after a yearlong absence, as well as the extent and nature of variation between nests, years, seasons, etc. remain unknown. Surprisingly, no study to date has focused on these scents. We proposed in this work to characterize chemically the composition and identity of VOCs emanating from plumage and nests to assess presence of such scent profiles. Samples of plumage and VOCs from nest were collected from breeding blue Petrels in the Kerguelen Islands, Southern Indian Ocean and analysed using GC-MS methods. Results suggested that nests carry a specific bouquet, but differ from bird scents. However, this is not excluding that some components of individual scents stay in the nest and help to the recognition of burrow each year.

Poster number: *62*

## Insecticide resistance mutation (Kdr) in *Anopheles gambiae* modulates host choice and olfaction in presence of pyrethroid-treated net

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Major mean of malaria vector control are based on use of insecticides. Their efficiency is threatened by widespread resistance mechanisms. In addition to the physiological resistance mechanisms already well studied, the issue of the behavioral modulation as cause or consequence of the resistance is largely overlooked. Nevertheless there are evidences that insecticide-based control tools alter mosquito behavior before any contact, suggesting that the mosquitoes can detect the presence of the insecticide. In the present study, we tested this hypothesis by investigating the behavioral and olfactory responses of different resistant genotypes (differing by presence of L1014F (Kdr) mutation) of *Anopheles gambiae* to host odors and permethrin treated net. Behavior experiments showed that heterozygous were more actives than two other genotypes. Moreover, homozygous resistant preferred host behind the permethrin treated net than host behind untreated net. Electrophysiology studies showed that three genotypes expressed different sensitivity to known attractant, but only homozygous sensitive responded to permethrin at tested concentration.

These results confirm that mosquitoes can perceive insecticide on net and adapt their behavior in response of it. Moreover, resistance mutation modulates this response. These results must be taken in account for future insecticide-based control tool development. Our original study highlighted the urgent need for further investigation of chemical ecology of malaria vector in a vector control pressure context.

Poster number: *63*

## Both volatiles and epicuticular plant compounds determine oviposition of the willow sawfly *Nematus oligospilus* on leaves of *Salix* spp. (Salicaceae)

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Plant volatiles and contact cues play a role in selection and acceptance of host plants by herbivorous insects. Here we studied volatile and contact cues used by the willow sawfly *Nematus oligospilus* (Hymenoptera: Tenthredinidae) to seek and accept its host plant. First, we recorded behavioral orientation in a Y-tube olfactometer of willow sawfly females to volatiles of the highly preferred genotype *Salix nigra* and the non-preferred genotype *S. viminalis*. The volatiles released by undamaged plants were analyzed by coupled gas chromatography-mass spectrometry. Afterwards, we recorded oviposition preference between intact leaves, and leaves in which their cuticular wax layer was removed by means of Arabic gum treatment. Contact cues were evaluated by studying the micromorphology and chemical composition of abaxial and adaxial leaf surfaces. Willow sawfly females oriented preferentially to *S. nigra* volatiles, which contained significantly higher amounts of (*Z*) and (*E*)-β-ocimene, undecane, decanal, and β-caryophyllene. Once on the plant, sawflies laid fewer eggs on *S. nigra* leaves after Arabic gum treatment, showing the importance of cuticular wax layer. No differences were found among the micromorphology of the leaf surfaces between preferred and non-preferred genotypes. Chemical composition of the cuticular waxes showed higher quantity and diversity of long chain alcohols in the preferred genotype that might be related to oviposition. Our studies suggest that several cues act in concert to provide oviposition cues for *N. oligospilus*: females are attracted to volatiles from a distance and, once alighting on the plant, they seek specific chemical contact cues in order to lay eggs.

Poster number: *64*

# Methyl jasmonate induction of cotton: A field test of the "attract and reward" strategy of conservation biological control


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Natural or synthetic elicitors can affect plant physiology by stimulating direct and indirect defense responses to herbivores. For example, increased production of plant secondary metabolites, a direct response, can negatively impact herbivore survival, development, and fecundity. Indirect responses include increased emission of plant volatiles that influence herbivore and natural enemy behavior, and production of extrafloral nectar (EFN) that serves as a food source for natural enemies after their arrival on induced plants. Therefore, use of elicitors has potential for the study of basic aspects of tritrophic interactions, as well as application in biorational pest control, i.e., an "attract and reward" strategy. We conducted a field study to investigate the effects of methyl jasmonate (MeJA), an elicitor of plant defense responses, on three trophic levels: the plant, herbivores, and natural enemies. We made exogenous applications of MeJA to transgenic cotton and measured volatile emission, EFN production, and plant performance (yield). We also assessed insect abundance, insect performance, and parasitism and predation of brown stink bug, *Euschistus servus* (Say), eggs in MeJA-treated and untreated control plots. Application of MeJA increased emission of several volatiles and production of EFN, but not yield, compared to the control treatment. Despite increased volatile and EFN production, MeJA application did not affect abundance of the insects that were monitored, plant bug performance, or mortality of *E. servus* egg masses. Mortality of *E. servus* eggs varied over the course of the study. Overall, MeJA treatment affected cotton plants, but not the insects that inhabit the plants. Factors that may have influenced our results include spatially and temporally variable patterns of volatile diffusion and the subsequent effect on natural enemy behavior; and density dependent interactions between natural enemies and their hosts. Much remains to be learned before crop physiology can be manipulated to enhance pest control.

Poster number: *65*

# Sex pheromone of the saddle midge, *Haplodiplosis marginata*: Effects of chirality and minor components

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The saddle midge, *Haplodiplosis marginata* (von Roser) (Diptera: Cecidomyiidae) is a sporadic pest of cereals in North and Central Europe and is becoming increasingly important in the UK. Recently the major component of the sex pheromone produced by female saddle midge was reported to be 2-nonyl butyrate, but the importance of chirality on attractiveness and the behavioural effects of minor pheromone components were not investigated.

We confirmed the identity and enantiomeric composition of the major pheromone component produced by virgin female saddle midge. In analyses of volatile collections by gas chromatography coupled to electroantennographic recording from antennae of males at least one minor component caused a strong EAG response. In field tests we found that the chirality of the major component was important for attractiveness which is unusual for midge pheromones with one chiral centre. We investigated the effects of minor components produced by the female and also developed practical dispensers to provide an optimised lure for use in monitoring traps.

Poster number: *66*

# Decrypting a cryptic click beetle species (Coleoptera: Elateridae) using chemical ecology

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The click beetle *Idolus picipennis* (BACH, 1852) represents the only species of its genus in Europe. Although it is widely distributed, it only occurs locally near belays and stone runs in low mountainous regions. The species is regarded as rare, threatened and quite variable in colour and size (4.5 - 6.5 mm).

In order to identify its sex pheromone, primarily for structure comparison with other genera, we investigated gland extracts of female I. picipennis from populations in southern Germany.

GC/MS analyses revealed the presence of merely two compounds, neryl hexanoate (~10 %) and neryl octanoate (~ 90 %). A synthetic blend of both, as well as the main compound alone, proved to be highly attractive to swarming males in the field.

Surprisingly, when tested in other locations, we found some *Idolus* populations where those lures completely failed to work. Subsequent analyses of pheromone gland extracts from these populations showed different compounds, namely several geranyl- and/or farnesyl esters, while neryl esters were absent. As expected, synthetic mixtures of these esters in the ratios found in the glands were attractive in the respective populations.

Detailed morphological investigation of beetles from the different populations confirmed our assumption, that *Idolus picipennis* is not a single highly variable species, but a complex of several different species which show, taken individually, an almost homogenous morphology. Further confirmation was obtained by subsequent molecular biological studies.

Finally, the discovery of locations where two of the species coexist (different pairs of species in different locations), and where males only react to synthetic pheromones simulating their own females, respectively, proved the complete reproductive isolation.

This study highlights the role of sex pheromones as useful tool in integrative taxonomy as well as their potential for monitoring rare and threatened insects.

Poster number: *67*

## The chemical context of interepecific communication: Ecological chemistry as driver for mutualism of yeasts and flies


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Mutualism of *Drosophila* flies and the yeast *Saccharomyces cerevisiae* is driven by chemical communication (Buser et al. 2014). While single volatiles have been implicated in the attraction of *D. melanogaster*, the semiochemicals affecting the behaviour of the sibling species *D. simulans* are less well characterised.

Here, we comprehensively scrutinize a broad range of GC-MS identified fermentation volatiles of attractive and repulsive yeasts to experimentally evaluate the chemical nature of communication between *S. cerevisiae* and *D. simulans* inhabiting the same ephemeral food source.

When grown in liquid or on agar-solidified grape juice, attraction to *S. cerevisiae* was primarily driven by 3-methylbutyl acetate (isoamyl acetate, banana oil) and repulsion by acetic acid, a known attractant to the vinegar fly *D. melanogaster*. These responses were, however, strongly influenced by compound concentration. Using T-maze choice tests and synthetic compounds we show that the behavioural response (attraction or repulsion) is further impacted by the chemical context of the environment.

Therefore, chemical communication between yeasts and flies is complex, being modulated by compound interactions and not simply driven by the presence of single volatiles. The chemical context of interspecific communication therefore needs to be taken into consideration when testing for ecologically realistic responses.


Buser CC, Newcomb RD, Gaskett AC, Goddard MR (2014) Niche construction initiates the evolution of mutualistic interactions. Ecol Lett 17: 1257-1264.

Poster number: *68*

## How background odor affects the host searching behavior of the ectoparasitoid *Holepyris sylvanidis*.


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Habitat background odor has been shown to affect an insect´s response to resource-indicating cues in either negative (i.e. masking of relevant odors), irrelevant, or positive (i.e. enhanced responsiveness) ways*. However, little information is available on how habitat volatiles influence a parasitoid´s response to host odors in static environments.

In the present study we investigated the odor-mediated foraging behavior of the ectoparasitoid *Holepyris sylvanidis* (Hymenoptera, Bethylidae) which attacks larvae of the confused-flour beetle *Tribolium confusum* (Coleoptera, Tenebrionidae), an important stored product pest. Special emphasis was paid on the question whether background odor affects the parasitoid´s olfactory orientation to volatile cues released by the host. Olfactometer bioassays revealed that *H. sylvanidis* females were attracted to odor released from host larval feces and from wheat grist infested by *T. confusum*. In contrast, volatiles released from host larvae (without feces) or from non-infested wheat grist (= habitat background odor) do not elicit any behavioral responses. Headspace volatile collections and GC-MS analyses revealed quantitative and qualitative differences in the composition of attractive and non-attractive odors. Two compounds that were specific for the attractive resources elicited electrophysiological and behavioral responses. Habitat background odor from non-infested wheat grist enhanced the parasitoid’s response to these cues which are suggested to act as key components for the attraction of *H. sylvanidis*. Our results may support the development of new measures for integrated control of *T. confusum* without adversely affecting stored products.


*Hilker, M. and McNeil, J. 2008. Chemical and behavioral ecology in insect parasitoids: How to behave optimally in a complex odorous environment.

: Wajnberg, E., Bernstein, C., van Alphen, J. (eds.). Behavioural ecology of insect parasitoids. Blackwell Publishing, pp. 99–112.

Poster number: *69*

# Oviposition responses of *Plodia interpunctella* (Hübner) (Lepidoptera: Pyralidae) to cereal contaminated by fungi

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The aim of the study was to examine whether *Plodia interpunctella* are capable to identify substrate for egg laying contaminated by fungus *Aspergillus flavus*. For this purpose two-choice oviposition test was performed (cereal versus cereal contaminated by fungus). The total number of eggs layed by females was approximately the same in both treatments (control and fungus infested). However, difference in egg-laying behaviour was noted. It was revealed that females prefer lay eggs directly on fungus free grain (control) but not on that contaminated by the fungus (77,30 ± 4,98 % and 17,37 ± 3.14 % correspondingly). The results suggests that some of fungus contaminated grain volatiles are important for oviposition, namely for egg distribution depending on presence/absence of fungi. Analysis which VOCs are involved in egg laying behaviour modification is in progess.

Poster number: *70*

# Effect of larval experience to plant olfactory cues on host plant choice in a polyphagous moth

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In the polyphagous moth *Spodoptera littoralis*, host plant choice is based on a stable plant preference hierarchy that can be modulated by larval host experience. This plant preference plasticity could facilitate fast adaptation to changing environments. However, larval experience of a suboptimal host plant does not affect subsequent female host plant choice. This plasticity thus appears to be selective and associated with an ability of larvae to evaluate host plant quality.

In this context, the aim of this work was to explore the mechanisms underlying this experienced-based behavioral plasticity. We first tested if experience to plant olfactory cues during larval stage mediates behavioral decision-making in subsequent adults. Our results showed that female plant preference was affected by preimaginal olfactory experience. We then tested if food quality could mitigate the transfer of larval olfactory experience. For that we exposed groups of larvae to different plant odors and fed them simultaneously with different qualities of artificial diet (normal diet, low protein diet or aversive diet). Our results show that larvae are able to associate plant odors with the diet quality. They are attracted to plant odors associated with good quality diet and avoid the same odors when associated with bad quality diet. As expected, the positive larval experiences affect female plant preference but interestingly the larval negative experiences do not. Females reared as larvae on bad quality diet do not avoid the associated plants but instead seem to rely on their innate plant preference.

These results shed new light on the mechanisms associated with the phenotypic plasticity involved in oviposition decisions.

Poster number: *71*

## Clover seed weevils: Flowers for the females

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The clover seed weevils *Apion fulvipes* Geoffroy and *A. trifolii* L. (Coleoptera: Apionidae) cause major losses to seed production of white clover (*Trifolium repens*) and red clover (*T. pratense*), respectively. Clover is an important nitrogen-fixating crop and a green manure legume, particularly in organic farming where inorganic fertilizers are prohibited, and the use of insecticides in management of these weevils is discouraged because clover is mainly pollinated by bees. We sought to empirically collect baseline information to be able to develop a semiochemicals-based seed weevil pest management strategy for clover seed production. Field trap catches revealed that *A. fulvipes* and *A. trifolii* were abundant in white and red clover, respectively, even though both weevil species co-occurred in either species of clover. For both weevil species, female catches were positively correlated to the number of clover buds and flowers in the field. In feeding and olfactory bioassays, females of *A. fulvipes* and *A. trifolii* showed a preference for white and red clover, respectively. The feeding preference was however lost when the antennae were removed. These results demonstrate that olfaction is important in finding and/or selecting host plants by females of the two weevil species. Headspace volatile organic compound (VOCs) analyses show subtle differences among white and red clover. Are these differences in VOCs key to host choice and preference by *A. fulvipes* for white clover and *A. trifolii* for red clover?

Nyabuga et al. (2015). Journal of Economic Entomology. doi: 10.1093/jee/tou099

Andersson et al. (2012). Journal of Insect Physiology. 58: 1325-1333. doi: 10.1016/j.jinsphys.2012.07.006.

Poster number: *72*

## Caterpillar and rootworm feeding differentially affects defense protein accumulation in corn

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When corn is attacked by insect herbivores it responds by accumulating a suite of defense proteins. Two defense proteins that are produced in response to foliar feeding by fall armyworm (FAW) are the insecticidal cysteine protease Mir1-CP and ribosome inactivating protein-2 (RIP2). However, there is little information regarding the accumulation of these defense proteins in response to belowground feeding by the western corn rootworm (WCR). Furthermore, the effects of jasmonic acid (JA) and ethylene (ET) on the regulation of abundance of these defensive proteins and their gene expression is unknown. In this study, we show that the kinetics and levels of mir1 and rip2 transcript accumulation in whorls and roots is different depending on the herbivore attacking the plant. Immunoblot analysis indicated that foliar FAW feeding increased Mir1-CP abundance in both whorls and roots, and root feeding by WCR increased Mir1-CP abundance in these two organ suggesting a systemic response. On the other hand, RIP2 protein abundance increased only in the tissues immediately attacked by FAW or WCR. The effects of blocking JA synthesis or ET perception on Mir1-CP and RIP2 accumulation during FAW or WCR infestation suggests that these defense proteins could have a different hormonal regulation process in whorl and roots depending on the insect attacking the corn plant.

Poster number: *73*

# Aboveground application of elicitors recruits an entomopathogenic nematode belowground


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Plant hormones play important roles in regulating developmental processes and signaling networks that mediate plant responses to a wide range of abiotic and biotic stresses. In particular, stimulation of the salicylic acid and jasmonic acid pathways are important plant responses to infection and herbivory. Here we investigate the effects of aboveground foliar application of the elicitors methyl salicylate (MeSA) and methyl jasmonate (MeJA) on belowground recruitment of the entomopathogenic nematode *Steinernema diaprepesi*. In four arm olfactometers, citrus plants treated with foliar applications of MeSA recruited *S. diaprepesi*. Citrus plants treated with foliar applications of MeJA did not recruit significantly more *S. diaprepesi*. Analysis of root volatile profiles of citrus plants receiving foliar application of MeSA revealed production of D-limonene that was absent in controls. Two choice olfactometer trials with D-limonene suggest that this compound may be responsible for recruiting *S. diaprepesi*. These results suggest that aboveground stimulation of citrus plant defenses may have ramifications for belowground multitrophic interactions.

Poster number: *74*

## MALDI-MS imaging of insects – metabolite landscapes of the internal anatomy


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High-resolution atmospheric-pressure scanning microprobe matrix-assisted laser desorption/ionization mass spectrometry imaging (HR-AP-SMALDI MSI) is a promising tool for insect physiologists (Römpp and Spengler, 2013). With this method a nitrogen laser scans a matrix-coated (2,5-dihydroxybenzoic acid) tissue sample, obtained by cryo-sectioning. The laser ablates material from the surface of the tissue section spot by spot and ionizes its components. Mass spectra for each pixel of the resulting image are obtained using a high-resolution and high-mass-accuracy orbital trapping mass spectrometer. In the experiment, tissue sections were scanned with a step size of 10 to 20 µm. Ion images were generated using the MIRION software package.

Sagittal and transversal tissue sections, as well as dissected organs from *Paederus riparius* and *Harmonia axyridis* were analyzed in order to understand the distribution of their defense compounds pederin and harmonine, respectively. Ion images generated from the mass spectral data were compared with optical images of histologically stained tissues and dissected organs. This method not only helps to explain the distribution of defensive compounds and other metabolites, but also allows the identification of tissue-specific marker molecules. The distinct metabolite distributions give new insights in insects biochemistry and provide new clues for molecular pathways.


Römpp A., Spengler B. (2013) Mass spectrometry imaging with high resolution in mass and space. Histochem Cell Biol 139:759–783

Poster number: *75*

## New insights into the chemical composition of androconial organs of Ithomiines

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The clearwing butterflies Ithomiinae are widespread butterflies occurring in Central and South America. During mating, males fly in front of female antennae exposing their androconial fringes at the costal edges of their hindwings. Then the female decides whether it will accept their mate. Similarly to danaines, ithomiines use derivatives of pyrrolizidine alkaloids as pheromones [1]. For example, *Prittwitzia hymenaea* produces the hydroxylated γ-lactone ithomiolide A and hydroxydanaidal [2]. No attention has been given to other compounds occurring in the androconia, although they constitute in several cases the major constituents of the secretion. Extracts of androconia of *Ithomia salapia* were investigated by GC-MS. While pyrrolizidine alkaloid derivatives were present, different esters were identified as the main components in high abundance. Microderivatization procedures led to the unequivocal assignment of modified long-chain fatty acid esters with methylbutenols and additional acetoxy groups. The esters were stereoselectively synthesized to prove the structural assignment and enable biological testing. These tests are needed to determine the biological role of these esters.

1. Schulz, S.; Beccaloni, G.; Brown jr., K. S.; Boppré, M.; Freitas, A. V. L.; Ockenfels, P.; Trigo, J. R., Biochem. Syst. Ecol., 2004, 32, 699-713.

2. S. Schulz Eur. J. Org. Chem., 1998, 13-20.

Poster number: *76*

# Fermentation volatiles associated with larval infestation act as innate deterrents for a polyphagous pest species.

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Fruit odours are complex structures, made up of multiple volatiles, which are necessary for host detection and orientation in herbivorous insects. While different host fruits produce species-specific odours, their composition at any time is determined by a combination of factors such as ripeness and damage. Therefore the structure of fruit odours not only indicates host presence but also host suitability. This research aims to identify naturally occurring chemical compounds, which represent non-suitable hosts and act as oviposition deterrents in the Queensland fruit fly (*Bactrocera tryoni*). These deterrents could then be deployed alongside attractants in push-pull management strategies in order to prevent crop damage by this pest species.

We investigated whether characteristic fermentation volatiles associated with larval infestation act as signals to ovipositing adult flies. Trials measuring proportional oviposition showed avoidance of oviposition on tomato agar pots when infested with larvae. Volatile analysis of tomato agar pots as well as whole tomato, guava, and nectarine fruits identified acetoin as a common fermentation volatile associated with larval infestation across multiple host species. Aversion to acetoin was then tested and results show that acetoin induces oviposition avoidance at a range of concentrations. It is proposed that acetoin represents a reliable signal for the presence of larval infestations in host fruit. Following this, ecological reasons for avoiding infested fruits were explored. These include tests investigating larval competition and degradation of host quality. Additional results will be presented from a comparative study to test the potential of these volatiles to act as a niche separating mechanism between *B. tryoni* and *Drosophila melanogaster*, two polyphagous species with broadly overlapping host ranges.

Poster number: *77*

# Sex attractant of the annona fruit borer, *Cerconota anonella* Sepp. (Lepidoptera: Oecophoridae)


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The Annona fruit borer, *Cerconota anonella* Sepp., is a serious agricultural pest in many tropical areas of the world. The identification of an attractant for male *C. anonella* could offer new methods for detection and control. Female pheromone gland hexane extracts were analyzed by one and two-dimesional chromatography with mass spectrometric detection and by gas chromatography coupled to electroantennographic detection. The identified compounds were synthetized and their behavioral activity was evaluated in laboratory bioassays and field trails. A mixture of compounds extracted from female *C. anonella* elicited antennal depolarization in the male borer. These compounds were identified as octadecanal, 1-octadecanol, octadecyl acetate, (*Z*)-octadec-9-enal (Z9-18:Ald), (*Z*)-octadec-9-en-1-ol (Z9-18:OH), and (*Z*)-octadec-9-enyl]acetate (Z9-18:Ac) by one- and two-dimensional gas chromatography-mass spectrometry. In laboratory bioassays synthetic individual compounds as well as synthetic mixtures were found to be attractive to males. In addition, field tests using Delta traps with 1 mg of the ternary mixture composed of Z9-18:Ac, Z9-18:Ald and Z9-18:OH in the ratio of 1:3:5 caught as many males as traps containing virgin females. The ternary mixture of Z9-18:Ac, Z9-18:Ald, Z9-18:OH was identified as attractant to *C. anonella* males and can be used to detect and control populations of this insect in Annonaceae plantations.

Poster number: *78*

## Use of oviposition induced volatiles in tomato plants by *Trichogramma* parasitoids

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The tomato leafminer *Tuta absoluta* (Lepidoptera: Gelechiidae) is a devastating pest of cultivated tomato *Solanum lycopersicum* throughout South and Central America and Europe. However, the specificity and role of tomato plant volatiles induced during the early phase of attack, especially on egg deposition by *T. absoluta*, and their consequences on insects and their natural enemies remain poorly explored. The main aim of this study is to identify oviposition-induced plant volatiles of tomato leaf by the deposition of eggs from tomato leaf miner, *T. absoluta* and to test the behavior of two Trichogramma species (*T. cordubensis* and *T. achaea*) towards tomato volatiles.

Static and dynamic headspace combined with gas chromatography-mass spectrometry (GC-MS) was employed for the identification of the emitted volatiles at 24, 36, 48, 72 and 96h after oviposition.

The chemical profile in the static headspace of both oviposited and control plants show that β-phellandrene was the predominant compound followed by 2-δ-carene, α-phellandrene, (*E*)-β-caryophyllene and α-pinene. Verbene, β-myrcene α-terpinene, β-elemene, δ-elemene (*E*)-β-ocimene, γ-elemene and α-humullene were detected in lower quantities. Hexanal and (*Z*)-3-Hexen-1-ol were found only in oviposited plants. The dymamic headspace profile of plants was enriched in decanal, nonalal, camphor, xylene (o-, p-, m-), benzenoid hydrocarbons and unidentified esters. Behavioral and electrophysiological studies are on the way to test the response of the two *Trichogramma* species to volatile compounds of tomato. Crude extracts of trapped volatiles as well as identified individual compounds are tested both electrophysiologial and behavioral. The electrophysilogical activity is evaluated with electroantenography and the behavioral response is evaluated in Y-tube and static olfactometer. Bioassays are going and detailed results will be presented during the conference.

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Poster number: *79*

# The scent of African malaria mosquito natural oviposition sites: Odours influence but do not dictate oviposition decision making in *Anopheles gambiae* s.l.

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We show that natural breeding sites from Ethiopia, Kenya and Tanzania emit odours which can substantially influence the decision making of gravid females for oviposition sites in the Sub-Saharan main malaria vectors, *Anopheles gambiae* s.s. and *Anopheles arabiensis*. Focusing on North-eastern Tanzania, we screened productive *Anopheles* breeding sites typical for the studied regions, for the importance of the emitted odours in gravid mosquito decision making. Odours from breeding sites always elicited electroantennographic responses in both tested species and thus likely to possess some characterizing scent to gravid females in search of an adequate breeding site. Importantly however, odours from productive breeding sites were not always “attractive”, some did not significantly influence gravid females’ choice and some breeding site scents were even observed to slightly “repel” gravid mosquitoes when offering them distilled water as alternative.

Poster number: *80*

# Combinatorial retention-activity relationship of fractionated turpentine on fungal growth

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Today, the pharmaceutical industry is struggling to find new drugs against multi-resistant bacteria and even though combinatorial chemistry and high throughput methods for testing bioactivity are readily available, the time from lead to a commercial drug is steadily increasing. An often overlooked source of diversity is essential oils from nature, complex mixtures with congenic substances and known for their fragrance and in some cases their use as an antiseptic agent in traditional medicine and food preservatives. Using turpentine, essential oil from spruce (*Picea abies*), we developed a versatile analytical method that can, with high probability, discern the most active component in a complex mixture without testing the compounds one at a time. As a model species, we used the wood-decaying fungus *Coniophora puteana* which was tested against D-optimal mixtures of fractioned turpentine. 38 different mixtures of turpentine were run on a gas-chromatography mass-spectrometry instrument. Integrated areas were used as x-variables and growth rate of *C. puteana* as y-variable in a partial least squares model. Results show ($R2X = 0.988$, $R2Y(cum) = 0.88$, $Q2(cum) = 0.825$) and CV-ANOVA ($p << 0.05$) that this is a viable method to account for the different growth rates of *C.puteana*. We therefore propose that this method may be applied to other systems such as multi-resistant bacteria, parasites, insects, moulds etc.

Poster number: *81*

# Discovery of peptide toxins in ribbon worms: Challenging claims of tetrodotoxin production

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Ribbon worms (nemerteans) are marine predators which capture their prey using a proboscis containing a mixture of toxins which brings on rapid paralysis [1]. In addition, the epidermis of ribbon worms contains a thick mucus of similar constitution. The highly potent neurotoxin tetrodotoxin (TTX) has been identified as one of the toxins of ribbon worms [2-5]. The toxicity of TTX (lethal by ingestion of 0.5-2 mg [6]) stems from its ability to block voltage-gated sodium channels [7]. Although several bacterial species, among these *Vibrio* sp., have been linked to its synthesis, the biogenic origin and biosynthesis is unclear [8]. One hypothesis is that TTX production occurs in a symbiotic relationship with its host, which in this case would be a nemertean [5].

We tested this hypothesis in a setup for TTX production through the cultivation of *Vibrio alginolyticus* in nutrient broth infused with mucus from the ribbon worm *Lineus longissimus*. Toxicity was demonstrated by fraction injections into shore crabs, but it could be shown conclusively that toxicity was unrelated to TTX and the *Vibrio* culture itself, and rather a constituent of the ribbon worm mucus. A compound of near identical molecular weight was observed. This obscured the analysis, but LC retention and MS fragment ions differed from those of TTX. Further LC-MS analyses indicated the presence of peptides in the toxic mucus fractions, which could be linked to the toxic effect.

We suggest that the paralytic effect of *L. longissimus* mucus can be explained by the presence of toxic peptides, and that this observation may warrant reinvestigation of some published claims of TTX production.

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# The chemistry of induced resistance in marine plankton: Tackling the challenges of identifying waterborne cues

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We sought to decipher the chemical basis for a critical question in ocean science: How do single-celled algae, which are responsible for almost half of Earth's photosynthesis, sense their environment in order to respond to the lethal threat of predation? The increasing frequency of toxic algal blooms with worldwide consequences to human health, fisheries, and marine ecosystem functioning has garnered much attention in recent years, but it has remained unclear how algal toxicity is regulated. Chemical cues that induce resistance against grazers in the marine plankton have presented chemists with daunting technical challenges hindering our understanding of predator-prey dynamics. In particular, chemical ecologists have struggled to characterize compounds that are: 1) produced in low yields by tiny organisms that are not readily cultured; 2) effective as blends of multiple molecules in specific ratios; 3) released into seawater (not just a vast medium, but a salty one); 4) polar (compounding the problem of the salty medium); 5) detected using a bioassay requiring days of phytoplankton exposure followed by complex toxin analysis; and 6) unstable. Thankfully, chemical cues that cause algae to up-regulate their toxicity threw just 4 of these 6 challenges at our interdisciplinary group of chemists and biologists. We show that substantial (20X) induction of algal toxicity occurs when algae are exposed to a family of previously unknown chemical cues produced by predatory copepods. This represents the first discovery of chemical cues mediating interactions between marine zooplankton and their prey.

Poster number: *83*

# Functional exocrinology of social bees - comparative analyses of two bumble bee

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Bumblebees are typified in that the queens/workers conflict over reproduction is overtly manifested as mutual aggression and oophagy. Pheromones certainly play a role in social regulation and the transition from cooperative to competitive behavior. Two exocrine glands are implicated in conflict resolution, the labial and Dufour's glands. While each appears to regulate specific physiology and behavior, they seem to exhibit a considerable functional overlap, emphasizing strong selection for tight regulation of potential social conflicts. Chemical analyses of the two glandular secretions in *Bombus terrestris* and *Bombus impatiens* revealed underlying similarities but also specific differences. In both species and both glands two classes of compounds dominate the secretion: long chain hydrocarbons and long chain fatty esters. Among these, only the esters show considerable variation, according to caste and physio-social status. In Dufour's gland workers of both species possess an extra set of long chain esters that are, however, species specific, octyl esters in *B. terrestris* and dodecyl esters in *B. impatiens* predominate. In the labial glands, although both queens and workers of both species possess fatty esters, their amount varies with context. The types of esters and their distribution indicate that while different bumblebee species may share common biosynthesis pathways, expression of these esters is highly flexible, consistent with their postulated role as social regulators.

Poster number: *84*

# Role of olfaction in Odonata sex recognition


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To date there is no evidence that damselflies use modes of communication other than vision (and tactile stimuli) in mate searching and sex recognition and it is still controversial which precise cues are relevant to males for discriminating among potential mates. In polymorphic damselflies discrimination of females is complex owing to the presence of andromorph and gynomorph females. The behavioural (one-way olfactometer) and electrophysiological (EAG) investigations performed in the present study on *Ischnura elegans*, a polymorphic damselfly, prove for the first time the involvement of chemical cues in Odonata sex recognition. The bioassays demonstrate that males prefer female to male odour, while no significant difference was present in the male behavior between the stimuli from males and the control. The biossays suggest also some ability of males to distinguish between the two female morphs on the basis of chemical stimuli. The ability of males antennae to perceive odours from females has been confirmed by our electrophysiological recordings. These findings are important to get insight into the chemical ecology of Odonata, a field so far uninvestigated owing to the remarkable power of vision of these insects. The present data can also shed light into the problem of olfaction in Odonata insects that, together with Ephemeroptera, were considered anosmic for a long time. Moreover, investigating the role of olfaction in Odonata reproduction could be useful to clarify the controversial aspects of the mating behavior of polymorphic coenagrionids.

Poster number: *85*

# Resource-dependent changes in the production of chemical defences during early ontogeny in the common toad (*Bufo bufo*)


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Many anuran Amphibians contain toxins that effectively defend them against predators. Some taxa sequester toxins from their prey, others produce noxious substances de novo. The ontogenetic pattern of toxin production in the latter group and the ecological factors influencing this pattern have remained largely unknown, despite their obvious relevance for survival. Members of the family Bufonidae produce cardiotonic steroids, called bufadienolides. In the present study we scrutinized ontogenetic changes in toxin production of the common toad (*Bufo bufo*) during early life-history, and investigated the influence of predation threat and food availability on this pattern. We reared tadpoles in a laboratory-based experiment with four treatment combinations: with or without a mixture of cues from tadpole-fed adult smooth newts (*Lissotriton vulgaris*) and southern hawker larvae (*Aeshna cyanea*), combined with ad libitum or reduced food levels. On five occasions (Gosner developmental stages 19, 26, 34, 40 and 46) we sampled tadpoles and conserved them in 70 % methanol for subsequent HPLC-DAD-MS analysis. In general, toxin components showed a marked pattern of increasing quantities during ontogeny with only minute amounts after hatching and peak quantities immediately before the onset of metamorphosis (developmental stage 40). Exposure to chemical cues on predation threat did not affect toxin production. Food level manipulation, however, had significant effects, with tadpoles receiving ad libitum food producing more bufadienolides. Contrary to the previously described importance of maternal provisioning and no documented toxin production by tadpoles in another bufonid, we provide evidence that *B. bufo* hatchlings do not obtain significant quantities of toxins from their mothers, but start to produce bufadienolides already at intermediate larval stages. Finally, toxin production in *B. bufo* tadpoles does not appear to be inducible by predation threat, but is highly resource-dependent and, thus, likely to incur high production costs.

Poster number: *86*

# Does better growth imply better immunity? Effect of biofertilizers on induced responses of *Brassica juncea*

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Biofertilizers are emerging as promising plant growth promoters for farmers. However, when plants are stressed, they undergo several morphological and chemical changes, known as induced responses, which may utilize a substantial share of plant nutrition.

We determined whether biofertilizers boost both plant growth and immunity or is biofertilizer-facilitated growth compromised as a tradeoff for immunity. For this, we studied various systemically induced responses occurring due to insect herbivory in Indian/Brown mustard (*Brassica juncea*), which was grown in soil supplemented with phosphate solubilizing bacteria (PSB) and vesicular-arbuscular mycorrhiza (VAM), separately and in combination. Each treatment was analyzed for morphological and chemical induced responses after damage by Tobacco cutworm (*Spodoptera litura*). We divided our experiments into quick (3 to 72 hours) and delayed responses (4th and 9th day). Antioxidants were found to increase significantly all through the experimental period in PSB supplemented induced plants, whereas they increased only after 24 hour in VAM supplemented induced plants. Overall increase in antioxidants was better in induced plants supplemented with the biofertilizers separately, as compared to induced plants supplemented with PSB-VAM combination. Trichome number and density increased more in induced plants supplemented with VAM than in other treatments.

We tested the effects of induced changes on orientation and feeding preferences of *S. litura*. Although plants supplemented with PSB-VAM combination attracted most larvae when intact, this attraction was significantly reduced when plants were induced. Conversely, orientation and feeding preference of *S. litura* increased significantly when the plants supplemented with PSB were induced.

Our study suggests that biofertilizers enhance induced plant responses along with their growth, but they do not necessarily elevate induced resistance. Therefore, fitness of induced and uninduced plants supplemented with biofertilizers should be compared in an environment that includes herbivores, before establishing a positive correlation between growth and immunity.

Poster number: *87*

## The kairomonal response of sibling *Tomicus* species reflects the differences in their breeding hosts


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The sibling bark beetle (Col., Scolytinae) species *Tomicus destruens* (Wollaston) and *T. piniperda* (L.) are among the main conifer forest pests in the Mediterranean region, but also through Europe. Even if sympatric populations of both species can be found through the Iberian Peninsula, *T. destruens* is more frequently found at lower and warmer areas. Traditionally, both species have been distinguished by differential flight periods, which have been further supported by potential differences in response patterns to host kairomones. Five experiments carried out between 2008 and 2012 studied the response of both species to different blends of ethanol and α-pinene, in pine stands composed *Pinus pinaster*, *P. pinea* and *P. sylvestris*. Stands were affected by moderate to high *Tomicus* spp. infestation levels reflected by a high number of attacked shoots. Our results confirm the different responses of sibling *Tomicus* species to host kairomones, reflecting species specific adaptations to predominant host availabilities. Intraspecific *T.piniperda* differences with results from other European experiments were observed too. A very strong synergism between ethanol and α-pinene was found even at high α-pinene release rates for *T. piniperda*, while *T. destruens* showed a preference towards high levels of ethanol, the terpene reduced the response when emitted together with ethanol, although not significantly. Increased ethanol production in stressed pine trees suffering water deficit, has been recently linked to the induction of *T. destruens* attacks. Similarly, α-pinene emission increases in fire- or mechanically-damaged trees. Thus, each sibling *Tomicus* species apparently react to signals simulating those emitted by the most common sources of breeding material present in their habitats. Improving the knowledge of the chemical ecology of these important conifer pests will help us designing better monitoring and control management programs.

Poster number: *88*

# Drought stress affect host-induced volatile organic compounds emission from plants and parasitoid response


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Drought stress is the main abiotic stresses strongly affecting plant productivity and survival. Plants cope with drought through acclimatory and adaptive responses that involve molecular, biochemical, and physiological changes. How these responses to drought affect trophic interactions in the food chain is largely unknown. When plants are damaged by herbivore insects, blends of volatile organic compounds (VOCs) are induced, and then released. Plant VOCs are used by carnivore insects (parasitoids) to locate and attack their preys. We assessed the impact of drought on VOC-mediated information in the trophic system *Vicia faba* (plant), *Nezara viridula* (herbivore), and *Trissolcus basalis* (carnivore parasitoid). In particular, the impact of drought stress combined with *N. viridula* feeding and oviposition on the blend of plant VOCs, and on the behavioural response of the parasitoid was assessed. Three drought stress levels were considered, on the base of water stress kinetics as expressed by the fraction of the fraction of transpirable soil water (FTSW): mild (FTSW=0.8), medium (FTSW=0.5) and severe (FTSW=0.1). Analysis of the VOC blend revealed emission differences among well watered (WW) and drought stressed (DS) plants, before and after the herbiovore attack. A Projection to Latent Structures Discriminant Analysis (PLS-DA) of the VOCs separated the different treatments according to the parasitoid response. Indeed we demonstrated that, under drought stress alone, *T. basalis* females responded to plants subjected to severe drought stress compared to WW plants. Under biotic and abiotic stress interaction, the parasitoid preferred plants subjected to severe and medium drought stress to WW plants. In conclusion, drought stress alone or in combination with a biotic stress induced changes in the VOC emissions of *V. faba* that favoured attraction of parasitoids, and that can potentially be exploited in sustainable protection practices.

Poster number: *89*

# Olfactory and visual cues affecting the atraction of *Cydia strobilella* L. to oviposition sites

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*Cydia strobilella* (Spruce Seed Moth) is one of the most damaging seed predators on *Picea abies* (Norway spruce) that together with other seed predators can reduce the yield of seed orchards by up to 85% (Seifert et al., 2000). We investigated olfactory and visual cues, which could be used by *C. strobilella* during host search. Volatiles emitted from *P. abies* flowers and cones were identified. Five volatiles were found to trigger consistent electroantennographic responses: (–)-α-pinene, (+)-α-pinene, (–)-β-pinene, (+)-β-pinene, and myrcene. The emission of these volatiles were quantified and followed across the development from flowers to cones. Field experiments using these five volatiles in natural amounts and proportions in traps resulted in small but consistent attraction of *C. strobilella* males. The measured emission of volatiles peaked at the same time as the observed maximum activity of *C. strobilella*. To examine if the colour of *P. abies* flowers could be a cue for *C. strobilella* during host search, we measured the seasonal variation in spectral reflectance of flower and cones and compared these data with the visual sensitivity of male and female moths obtained from electroretinographic measurements. The results from the measurements were used for the construction of traps of different spectral properties used in field experiments. Visual modification of traps did not lead to increased catches, but black traps had a repellent effect on *C. strobilella*.

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Poster number: *90*

# Flower scents of *Campomanesia phaea* (Myrtaceae) as attract for nocturnal bee pollinators

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Some plants are pollinated by nocturnal bees, however nothing is known about the signals these plants use to attract their pollinators. *Campomanesia phaea* (O.Berg) Landrum (Myrtaceae) is a fruit tree of economic interest and endemic of Brazilian Atlantic Forest. Anthesis starts before the sunrise (5 am) and flowers depend on nocturnal Halictidae (Megalopta sodalis and Megommation insigne) and Colletidae (*Ptiloglossa latecalcarata* and *Zikanapis seabrai*) bees for pollination. To understand how these bees find the flowers of *C. phaea* in darkness, flower volatiles were collected at night and day by dynamic headspace and analyzed by gas chromatography-mass spectrometry. Field bioassays were performed with single or mixtures of synthetic compounds to test if the identified components are able to attract the nocturnal bee pollinators. Fourteen volatile compounds were identified in the scent samples, the same during day and night. When considering all samples collected, six compounds represented 96% of the total amount of volatiles: 1-hexanol (40%), 1-octanol (30%), 2-phenylethanol (13%), benzyl alcohol (6%), (Z)-3-hexen-1-ol (4%), and hexanal (3%). The volatile emission was higher during the activity time of nocturnal bees. Further, the scent pattern differed between the day and night samples. Some compounds were more eminent during the night (e.g., 1-octanol, night: 38 ng/flower/hour versus day: 15 ng/flower/hour) and some during the day (e.g., 2-phenylethanol, night: 6 ng/flower/hour versus day: 25 ng/flower/hour). In the bioassays, *M. insigne* and *P. latecalcarata* bees were attracted by a mixture of the six main compounds and by the main nocturnal compound, 1-octanol. We conclude that volatiles emitted at night by *C. phaea* flowers are attractants for nocturnal bee pollinators with 1-octanol being most attractive.

Poster number: *91*

## Regulation of *Drosophila* pheromones by sex peptide

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Sex peptide (ACP70A) is a component of the male ejaculate, which is transmitted, along with sperm, into females during mating. It is responsible for decreased mating receptivity, enhanced ovogenesis, egg-laying and activation of juvenile hormone (JH) production in mated females. The latter effect is due to the activation of the *corpora allata* through the N-terminal part of the peptide whereas the other effects are due to the binding of the C-terminal part to the sex peptide receptor (SPR).

Here we demonstrate that ACP70A is also involved in the down-regulation of female sex pheromones and hydrocarbon (CHC) production. This down-regulation also occurs in ACP70A injected flies RNAi knocked-down for SPR and in flies which overexpress ACP70A N-terminal domain, suggesting a role of JH activation in this regulation. Female flies exposed to a JH analog had reduced amounts of pheromones, whereas genetic ablation of the *corpora allata* or knock-down of the JH receptor Met, resulted in higher amounts of both CHCs and pheromonal dienes.

The inhibition of pheromone synthesis, exerted by ACP70A might be a side-effect of JH induction which plays a crucial role in physiological processes including development, reproduction and sexual maturation.

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Poster number: *92*

## Interaction of aphrodisiac pheromone components in the butterfly *Pieris napi*

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Males of the Green-veined White, *Pieris napi*, emit an aphrodisiac pheromone from wing glands. This pheromone is requisite for females to accept mating with a courting male. The pheromone has been identified as citral[1], which has a lemonish smell to humans. Citral is a racemic mixture of the two isomers geranial (*E*-isomer) and neral (*Z*-isomer) in an approximate 1:1 ratio. We managed to purify these two components and tested female acceptance behaviour and physiological responses to citral and the two components.

A male dummy consisting of frozen wings washed in hexane to remove natural odours was impregnated with citral, geranial, neral or solvent. The dummy was waived in front of an alighted female and the females' acceptance behaviour was recorded.

A male dummy coated with geranial or neral solution evoked significantly less female acceptance behavior than the model treated with citral solution. In fact, the models treated with geranial or neral did not differ from that of the control (hexane) even if we doubled the doses. This indicates that both neral and geranial are necessary to evoke female acceptance behavior and a strong additive interaction effect is evident between the components of citral.

Using $Ca^{2+}$ imaging of odour evoked activity in the antennal lobe we showed that geranial and neral activated different, though partially overlapping, subsets of glomeruli, despite their similar structure. These glomeruli also overlapped with glomeruli responding to plant-related compounds. We could, however, not detect any significant synergistic effect at the level of the antennal lobe, implicating that mixture interactions occur at a higher processing region of the brain.

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Poster number: *93*

# Piezoelectric-active polymer coatings and their influence on bacterial cell-cell interaction

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Since several years, a steadily increasing number of studies have shown that electrical stimulation promotes bone fracture healing and can induce antimicrobial effects through direct damaging of bacteria or strongly increasing their antibiotic susceptibility. Therefore, the functionalization of implant surfaces with piezoelectric coatings is a promising approach to promote tissue integration also as rendering these surfaces antibacterial. The underlying bases of eukaryotic and prokaryotic cell-cell interaction, induced by electrical stimulation, are yet not clearly understood. Therefore, the aim of this study was a) identification of appropriate implant coating materials and b) determination of piezoelectric parameters.

As coating material, polyvinylidene fluoride (PVDF) was evaluated and the oral commensal *Streptococcus gordonii* was chosen as model organism. Appropriate test specimens were produced by crystallization, electrospinning, or mechanical drawing depending on the appropriate medical application. For the determination of piezoelectric parameters, an electrical stimulation lid was fitted on 6-well multiwall plates and the influence on microbial adherence and biofilm formation of *S. gordonii* was assessed under different stimulation conditions. Microbial growth on PVDF surfaces was quantified by live/dead staining in combination with confocal laser scanning microscopy and scanning electron microscopy.

Microbial adherence was abolished when *S. gordonii* cells were stimulated on PVDF membranes with 2mA, 60µS, 10Hz over 24h (rectangular, alternating current). Below an output current of 2mA, biofilm formation was observed under the given experimental settings.

Further studies will focus on the manufacturing of piezoelectric PVDF with desired electrical properties also as on the molecular biological characterization of cell-cell interaction in terms of intercellular communication (quorum sensing) and the involved messenger molecules.

Poster number: *94*

# Studies on the chemical communication of the bronze bug, *Thaumastocoris peregrinus* (Heteroptera: Thaumastocoridae), a pest of *Eucalyptus*

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The bronze bug, *Thaumastocoris peregrinus* (Heteroptera: Thaumastocoridae), is an exotic emerging pest in *Eucalyptus* commercial forests in South America, Africa and southern Europe. Information on the chemical communication system and reproductive ecology of this insect is scant, and it may be relevant for designing management strategies for eucalypt plantations. Adults and nymphs usually aggregate in the field, possibly by means of chemical signals. We will summarize our recent findings on these chemical signals, as part of an ongoing effort to further understand the role of pheromones in the mating behavior of *T. peregrinus*, and the eventual practical applications that may be derived for the management of the insect in commercial Eucalyptus forests. Males emit large amounts of 3-methyl-2-butenyl butyrate, which attracts conspecific adult males but not females. Males emit this compound following a circadian rhythm, but the presence of females results in a decrease in the amount emitted by the males. Nymphs do not emit the compound, but late-instar male nymphs are attracted both to adult male volatiles and to synthetic 3-methyl-2-butenyl butyrate, whereas female nymphs show no attraction. The ecological role of this putative male aggregation pheromone remains unknown. The compound appears to play a role in the formation of nymph/adult aggregations, but the relevance of the specific intra-gender inter-stage attraction remains to be explained. Possible explanations are the exploitation of food resources, or a reproductive strategy for newly-emerged males. Also remains unknown why males emit 3-methyl-2-butenyl butyrate following a circadian rhythm, and why the presence of females decrease the amount emitted by the males, while they themselves show no attraction towards the compound.

Poster number: *95*

## N-acylhomoserine lactones of macroalgae associated *Roseobacter* bacteria

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The *Roseobacter* clade is one of the most important and dominant groups of marine bacteria. Many strains produce N-acylhomoserine lactones (AHLs) as signaling compounds, regulating various traits as antibiotic production, cell-differentiation and biofilm formation. These autoinducers are able to move through the cell wall and regulate physiological changes in a concentration dependent manner. [1,2] This so called "quorum-sensing" system is thought to be strain specific, in the sense that different AHLs are used by different bacteria. AHLs contain a γ-lactone ring with an N-acylated side chain, mostly derived from fatty acids. The side chain may exhibit different length, double bonds or functional groups. We became interested to investigate whether *Roseobacter* isolates specifically associated with macroalgae are using structural similar or identical AHLs or whether wide structural variation occurs. In several *Roseobacter* compounds structurally closely related to AHLs, N-acylalanine methyl esters (NAMEs) were identified by GC/MS. AHLs and NAMEs were synthesized for structural proof and evaluation of their biological properties. In bioassays against bacteria and algae originating from the natural habitat of the bacteria antialgal activity in the nMol range was observed. This might explain some of the ecological functions of the macroalgae associated *Roseobacter* strains investigated here.

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Poster number: *96*

## Plant volatiles signal the host seeking process in root-knot nematodes


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Root-knot nematodes (RKNs), mainly from the genus *Meloidogyne* are the major constraints to production of high value vegetables in East Africa. Losses associated with RKNs in smallholder farms are not known but could range between 30-100%, depending upon the cropping system. The second stage juvenile (J2), which is the infective stage, hatches from an egg in the soil and infects roots causing the development of root-knot galls and drain the plant of photosynthates and nutrients. However, little is known about the olfactory cues that attract RKNs to hosts. We hypothesized that RKNs use plant specific volatile signals for host seeking and location. We explored this scenario using soil olfactometer bioassays and chemical analysis in tomato and spinach as model plants with a view to understanding the underlying mechanisms mediating infestation. Our results showed that RKNs are attracted to tomato and spinach roots 2-3-fold more when paired with a control. However, when the two plants were paired, tomato was more preferred (>50%) than spinach. Chemical analysis revealed distinct volatile chemistry between the roots of the two plants. In bioassays, a tomato-specific compound elicited a dose-dependent RKN attractive response compared to either a control or roots of the tomato plant. Results are discussed in relation to the management of RKNs.

Poster number: *97*

# Genetic variation of *Tetranychus kanzawai* confer different abilities on acaricide tolerance

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Kanzawa spider mites (KSMs) (*Tetranycus kanzawai*) are polyphagous herbivores that leave two colors of scars (red or white) on leaves of several bean plant species. In one KSM population, we identified two genetically distinct strains that left either red scars or white scars (hereafter called Red strain and White strain). We already showed that each strain induced different defense responses and the emission of a different blend of volatiles in lima bean leaves (*Phaseolus lunatus*). In this study, we carried out RNAseq-based transcriptome analysis for mRNA of Red and White strains on kidney bean plants (*P. vurgalis*) to identify KSM genes expressed specifically in either Red or White strain. Of particular interest is the fact that some genes involved in the detoxification system in KSMs were expressed higher in Red strains than in White strains, but not in vice versa. Further, some of detoxification enzyme activities were higher in Red strains than in White strains. We hypothesized that the differences in detoxification would affect the tolerance of acaricides. We will show the data on the differences in the tolerance in two strains. Implications of distinct physiologies between two strains are discussed.

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Poster number: *98*

# Growth inhibition activity of Sugi (*Cryptomeria japonica*) components against Microcystis aeruginosa

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Sugi (*Cryptomeria japonica* D. Don) has been planted as one of the major species in Japanese forest. Sugi has several bio-active allelochemical components in the bark, leaves and heartwood [1-3]. Especially, the bark components had growth inhibition activity against red tide plankton in our previous study [4]. In this study, we examined the growth inhibition activities of extracts obtained from Sugi against *Microcystis aeruginosa* known as a cyanobacteria sp. causing harmful algal bloom in fresh water ecosystem. Each sample of inner bark, outer bark, heartwood, and leaves of Sugi was extracted successively with hexane, ethyl acetate, and methanol. The inhibition activity was observed in the inner bark ethyl acetate and methanol, outer bark hexane, heartwood hexane, and leaves ethyl acetate extracts. The inner bark ethyl acetate and methanol extracts showed stronger activity than other extracts. The active extracts were fractionated by column chromatography and then tested inhibition activity. From analysis for active fractions, several flavonoids and terpenoids were suggested as main active components in polor (ethyl acetate and methanol) and non-polar extracts respectively. Thus, the Sugi bark extracts could be used as inhibition reagents against harmful algae

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Poster number: *99*

# Functional characterization of a receptor for a Type II sex pheromone in the winter moth, *Operophtera brumata* (Lepidoptera: Geometridae)

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Female moths produce two major types of species-specific sex pheromones along distinct biosynthetic pathways. Type I pheromones consist of C10-C18 straight-chain alcohols, acetates and aldehydes, whereas Type II pheromones are C17-C23 straight-chain polyenes and corresponding epoxymonoenes or epoxydienes (Millar, 2000; Ando et al., 2004). Female released pheromone signals are detected by the distinct receptors hosted in the antennal olfactory sensory neurons of conspecific males. To date, all functionally characterized pheromone receptors are tuned to Type I pheromones, and it is unknown how receptors matching Type II pheromones evolved. Here we report the identification of the first receptor, namely ObruOR1 tuned to a Type II pheromone, (*1,3Z,6Z,9Z*)-nonadecatetraene from the winter moth, *Operophtera brumata*. Similar to previously identified receptors for Type I pheromones, ObruOR1 is found in neurons housed in antennal sensilla trichodea and is expressed in a male-biased pattern. Phylogenetic analysis shows that ObruOR1 nests within the subfamily of receptors for Type I pheromones, however in a particular cluster containing a number of ligand-unknown orthologues. The strong purifying selection on this cluster suggests a conserved polyene-responding profile, which is supported in the present study by the response of a noctuid orthologous gene to a selection of polyenes. Our results suggest that moths did not evolve an entirely new type of receptors, but recruited a cluster from the existing pheromone receptor gene tree to match the novel Type II pheromone compounds.

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Poster number: *100*

## Repelling *Aedes aegypti* – a sustainable plant based solution

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The mosquito *Aedes aegypti* is the main vector of the estimated average of 3 million dengue fever episodes in Asia each year. Due to insecticide resistance and mosquito behavioral changes, among other factors, it could be argued that the most effective measure would be to decrease mosquito-human contact. Plant-based topical repellents, using locally sourced plants and manufactured at village level would overcome the cost complication and alleviate insecticide resistance.

Ongoing ethnobotanical surveys in Lao PDR are yielding an ever-growing list of candidate species, for the use of as topical repellents. The findings are examined both by in vitro and in vivo studies.

Evaluation and screening of possible plant candidates resulted in a handful of candidates for further testing. Combined formulations of *Cympobogon citratus* and *Zingiber officinale* gave over one hour of full protection, but showed less efficacy when tested separately. The main constituents of *C. citratus* are neral (34.8%) and geranial (56.4%) while in *Z. officinale* the main components are linalool (9.8%), geranial (14.4%) and zingiberene (14.4%).

An additive effect was observed from the combination of the two plants. Additive effects, in which it is more difficult for mosquitoes to overcome the volatile repellent barrier, can be due to the higher complexity of the mixture, and can impede development of repellent resistance. The mixture of ginger and lemongrass and other possible formulations could be further improved by stabilizing the volatiles through oxidation or sulfidation, thus extending the effect.

Herbaceous plants are an inexpensive and readily available source of raw material for the production of topical insect repellents.

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Poster number: *101*

## Electrophysiological (EAG) responses of *Simulium* spp to human sweat volatiles

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Black flies as a pest are found in some areas of Spain, mainly in Aragon, Valencia and Catalonia regions, but until now, their populations were not present in enough quantities to be considered a public and animal health threat in Andalucía. In recent years, from early summer to late October, olive farmers in the Baeza area (Andalucía, Southern Spain) located close to the Guadalquivir river, have been bitten by black flies.

How black flies females locate and choose their host for blood meals it is not very well understood yet. Recent research (Young et al., 2015)) shows that odours emitted by humans are used as olfactory cues. Females of most *Simulium* spp., (Diptera: Simuiidae) present in this region feed during the day and they usually bite on the upper part of the body.

The role of volatile semiochemicals in mediating the location and selection of human hosts by black flies was investigated using gas chromatography linked to electrophysiology (GC-EAD) detection. Contact solid-phase micro-extraction (SPME) technique was used to collect volatiles from humans neck, chest, back, and axillae sweat. Electroantennography (EAG) response was recorded from adults *Simulium* spp. to a broad range of human skin volatile constituents, plus two general hematophagous insect attractants such as ammonium salts and carbon dioxide. It was confirmed that female *Simulium* spp. responded strongly to ammonia and CO2 by EAG. Additionally, in this study only 8 out of the 34 tested common human skin volatiles produced consistent EAG activity. The strongest EAG responses from these compounds were obtained to aliphatic straight-chain saturated C6-C10 aldehydes: hexanal, (*E*)-2-nonenal, nonanal and decanal. At certain concentrations, the skin volatile constituent's 6-methyl-5-hepten-2-one, 1-octen-3-ol, hexanoic and octanoic acids elicited antennal responses.

Key words: Simuliidae, black fly, sweat human volatiles, electroantennogram.

Ryan M. Young et al. 2015. Identification of Human Semiochemical Attractive to the Major Vectors of Onchocerciasis. PLOS Neglected Tropical Diseases. 9:1 e3450.

Poster number: *102*

# The impact of abiotic and biotic challenges and their interactions on plant morphology and the phytometabolome of *Plantago lanceolata*

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During development, plants suffer from multiple challenges such as abiotic stress or biotic interactions with antagonists, competitors, and symbionts, which might influence their performance and their eco-physiological and biochemical responses in complex ways. In particular, the combination of different stressors and their impact on biomass production and the ability to metabolically adjust to these challenges are less well understood. To study the effects of mineral nitrogen (N) availability, interspecific competition and the association with arbuscular mycorrhizal fungi (AMF) on biomass production, biomass allocation patterns and metabolic responses, we performed a full-factorial experiment with the model organism *Plantago lanceolata* L. (Plantaginaceae). Treatment effects and their interactions on the phytometabolome were analyzed using a metabolic fingerprinting approach. Limited supply of mineral N most severely affected plant biomass production and biomass allocation patterns, and altered the concentrations of more than one third of the polar phytometabolome. Competition also impaired plant biomass production, yet affected the phytometabolome to a much lesser extent than limited mineral N supply. The association with AMF did not enhance biomass production, but altered biomass allocation patterns such as the root/shoot ratio and the specific leaf area. Interestingly, we did not find significant AMF-related changes in the phytometabolome. In general, the interaction of limited mineral N supply and competition led to additive changes, while the association with AMF in any case alleviated the observed stress responses of the plant. Our results indicate that the analyses of biomass production, biomass allocation and metabolic traits in response to the combination of different abiotic and biotic challenges enhance our understanding of complex interactions in a more global way.

Poster number: *103*

## Non-sticky trap for Meligethes (Coleoptera, Nitidulidae) combining visual and chemical stimuli

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The *Meligethes* spp. (Coleoptera, Nitidulidae) are regarded as important pests of rape, in most cases the most abundant among them being *M. aeneus* Fabricius. It is general knowledge that *M. aeneus* responds to yellow, however, there are some reports on its trapping with white or even blue traps. In a preliminary test comparing colours, we recorded most *Meligethes* specimens in fluorescent yellow traps in April, while in the later period of the test highest catches were observed in white or blue traps. The bright yellow or transparent traps caught significantly lower numbers. However, in this test the species identity of the *Meligethes* specimens caught was not determined.

In further tests aimed at studying the reason for this discrepancy, when the species identity of all specimens caught was determined, we found that while *M. aeneus* and *M. viridescens* Fabricius strongly responded to fluorescent yellow, *M. coracinus* Sturm and *M. nigrescens* Stephens. preferred white or blue. Few specimens of *M. atratus* Olivier caught in one of the tests also came to white and blue traps.

In the literature it is not mentioned frequently that species belonging to the same genus and having similar host plants would show differing colour preference. Our results suggest that previous literature data on *M. aeneus* trappings with white or blue traps may have been a result of other species coming to the traps, which remained unidentified in the respective studies.

When comparing attraction to synthetic floral compounds, strongest responses were observed to a blend of (*E*)-anethol, (*E*)-cinnamyl alcohol and (*E*)-cinnamaldehyde, much stronger than to isothiocyanates described previously as attractants for *Meligethes*. There was no difference among the species in their chemical preference. A combination of the optimal visual and chemical attractive stimuli resulted in a surprisingly selective, non-sticky trap device for *Meligethes*.

Poster number: *104*

## Ozone pollution compromises within-plant signalling via volatiles

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In response to attack by herbivores, plants release a complex bouquet of volatile organic compounds (VOC), both locally and systemically. In addition to mediating a wide array of interactions between plants and their associated organisms, plant VOCs released from damaged parts have recently been shown to trigger a systemic defense response in as yet undamaged parts of a plant, which has long been documented to be initiated by internal vascular signals. However, until now, the phenomenon of within-plant signalling by VOCs has been described for only five plant species – sagebrush, lima bean, poplar, blueberry and birch, and among them only studies with sagebrush and lima bean were conducted in the field. Therefore, there is still ongoing debate on the commonness of VOC-mediated within-plant signalling in nature. Furthermore, the co-existence of abiotic factors may affect the likelihood of occurrence of this process. One such abiotic factor – tropospheric ozone – has been shown to not only alter VOC emission patterns, but also react rapidly with many VOCs in the atmosphere. As a consequence, VOC-mediated interactions between plants and other organisms are compromised in the ozone-polluted atmosphere. For example, several studies have found that elevated ozone concentrations reduce the foraging efficiency of arthropod herbivores and their natural enemies, as well as the distance over which airborne plant-plant communication occurs. Yet, no attempts have been made to assess the impacts of ozone pollution on VOC-mediated within-plant signalling. Here, we used a system consisting of hybrid aspen (*Populus tremula* × *tremuloides*) and a specialist leaf beetle (*Phratora laticollis*) to demonstrate that VOC-mediated within-plant signalling occurs in both laboratory and field conditions. More importantly, we will present both field and laboratory data to address the role that moderate ozone levels can play in influencing this process.

Poster number: *105*

# Determination of the absolute configurations of the contact sex pheromone components of the longhorned beetle, *Neoclytus acuminatus acuminatus* (F.) (Coleoptera: Cerambycidae)


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Cuticular hydrocarbons play important roles in the biology of insects. Many of these compounds, such as methyl-branched hydrocarbons, have one or more chiral centers and can exist in various stereoisomeric forms. Chirality is important in the long-range chemical communication of insects, but technological limitations have hampered investigations on the role of chirality in insect contact chemoreception. Here, we used reverse phase high performance liquid chromatography (RP-HPLC) and digital polarimetry to isolate and determine the absolute configuration of a female-produced contact sex pheromone component of the cerambycid beetle, Neoclytus acuminatus acuminatus (F.). The pheromone consists of 7-methylpentacosane (7-MeC25), 7-methylheptacosane (7-MeC27) and 9-methylheptacosane (9-MeC27), though the chirality of these compounds is unknown. We found that the absolute configuration of the most abundant pheromone component, 7-MeC25, was (*R*). We then used enantiomerically pure synthetic pheromone components to test the hypothesis that males respond more strongly to (*R*)-enantiomers than to (*S*)-enantiomers of the three pheromone components. We also tested blends of (*R*)-7-MeC27 (the major component) with the (*S*)-enantiomers of the minor components and vice versa to determine whether unnatural stereoisomers might inhibit mating behavior. Only solvent-washed females treated with (*R*)-7-MeC27, alone or in combination with minor components, elicited mating behaviors from males stronger than those exhibited towards solvent-washed females, suggesting chirality of the major component plays an important role in contact chemoreception and mate recognition in *N. a. acuminatus*. We also found that males responded more strongly to females treated with the blend of (*R*)-enantiomers than to (*R*)-7MeC27 alone or in combination with (*S*)-minor components, suggesting that chirality of the minor pheromone components is also an important factor in the mating behavior of this species.

Poster number: *106*

# **Antimicrobial properties of inducible compounds in the brown macroalgae *Fucus vesiculosus***

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*Fucus vesiculosus* are abundant in the intertidal zones of the Gulf of Maine despite considerable wave action and herbivore pressure. This implies that algae may contain defense mechanisms that protect them against herbivore and microbial attack. We investigated the inducebility of compounds in the alga *F. vesiculosus* in response to directly applied stressors including mechanical injury and methyl jasmonate in field experiments. To determine the antimicrobial properties of algae extracts, we prepared methanol and pentane extracts from ground algae tissue samples. Extracts were pipetted onto sterile disks and added to bacterial strains grown on agar plates. Plates were incubated overnight and the zone of inhibition was measured. We found that methanol extracts of algae had a greater effect on the inhibition of bacterial strains than pentane extracts. Crude extracts from algae treated with methyl jasmonate had an overall greater effect on bacterial inhibition compared to mechanically injured or uninjured controls, but were generally lower than the antibiotics. Overall, methanol extracts inhibited the growth of gram positive bacteria more than the gram negative bacteria. Compounds extracted with methanol were mostly polar whereas compounds extracted with pentane were mostly non-polar.

Poster number: *107*

# Influence of fumigants on sunflower seeds: Characteristics of fumigant desorption and alterations of volatile profiles

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The main pest insects of stored sunflower seeds are *Tribolium casteneum* and *Ephestia cautella* (Kumaranag et al. 2007). Fumigation of transport containers is common practice to protect stored products from pests in the shipping industry or to avoid the spread of alien species (ISPM 15). However, little is known on the effects caused by fumigation on the volatile profiles of respective goods and how this could affect recolonization of sored products by pest insects.

To investigate the interaction of the fumigant with ingredients of stored products, we fumigated peeled sunflower (*Helianthus annuus*) seeds with phosphine (PH3), the mostly used fumigant against pest insects in stored products. Fumigations were conducted at 100 ppm for 72 h in 4 L fumigation chambers. After complete ventilation, the fumigated seeds were transferred to a desorption chamber (53 L). To characterize desorption behaviour of PH3 from the seeds, chamber air was sampled on consecutive days, until the fumigant concentration attained the detection limit (outgassed seeds). The volatile pattern of non-fumigated, fumigated, and outgassed sunflower seed were sampled via solid-phase micro-extraction (SPME) headspace sampling and analyzed by gas chromatography connected to mass spectrometry (GC-MS).

Within several days, PH3 was completely desorbed from sunflower seeds. The volatile pattern of seeds upon fumigation with PH3 and outgassing was significantly different from non-fumigated seeds. Fumigated seeds and outgassed seeds released significantly more terpenoids than non-fumigated seeds. This altered volatile pattern may influence the orientation of the recolonizing pest-insects of stored sunflower seeds and its natural enemies.

Kumaranag, K.M., Jagadish, K.S., & Shadakshari, Y.G. (2007), Insect-pest fauna of stored sunflower, Helianthus annuus L., in southern Karnataka. Pest Management and Economic Zoology 15(2):217-220

Poster number: *108*

# Pheromone autodetection in a noctuid moth: GC-EAD response of female *Pseudaletia adultera* to its own sex pheromone components

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Pheromone autodetection, the detection of conspecific sex pheromones by females, has been reported in a small number of moth species, and it has been suggested that this allows a female to modulate her emission of sex pheromones in response to the presence of other individuals. We have shown that when in the presence of sexually mature conspecifics, females of the noctuid *Pseudaletia adultera* become sexually mature at a younger age, and spend more time calling than isolated individuals. In this study, we used GC-EAD to examine the electrophysiological response of both virgin female (N = 13) and male (N = 16) antennae to the three main sex pheromone components: (*Z*)-11-hexadecen-1-ol, (*Z*)-11-hexadecen-1-yl acetate, and (*Z*)-11-hexadecenal (10 ng each). Both sexes clearly responded to all three pheromone components, with female EAD signals being significantly lower in amplitude than those of males ($P < 0.001$, mixed linear model). Furthermore, while the strongest EAD responses of male antennae were elicited by the major component of the natural female sex pheromone, (*Z*)-11-hexadecen-1-ol, female antennae showed higher signal intensities to the minor pheromone components that to the major one. This may reflect differences in the relative abundance of different sensillae types. Our results support our hypothesis that the differences observed in various aspects of calling behaviour between grouped and isolated females is the result of pheromone autodetection. The behavioral effects and adaptive function of pheromone autodetection in *P. adultera* are subjects of current investigation.

Poster number: *109*

# Effects of sesquiterpene from *Perilla frutescens* against the diamondback moth, *Plutella xylostella* L.


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Insecticidal and antifeedant activities were observed when *Plutella xylostella* L. was treated with whole plant extracts from *Perilla frutescens* L. Feeding deterrent response was determined with electrophysiological method.

The active ingredient in P. frutescens was identified as the sesquiterpenoid α-farnesene by spectroscopic analysis. In a leaf-dipping bioassay based on 24 h LD50 values, α-farnesene (LD50 = 53.7 ppm) was 3.9-fold more toxic than β-farnesene (LD50 = 206.9 ppm) against third instar larva of *P. xylostella*. The feeding inhibition rate against *P. xylostella* at 10 ppm of α-farnesene was 82.98%. The antifeeding responses were determined by using an oscilloscope to detect electrophysiological responses. The electrophysiological responses of the medial styloconic sensillum (MSS) were approximately 7-fold more sensitive at 100 ppm than the lateral styloconic sensillum (LSS).

These results indicated that the insecticidal effect of the sesquiterpenoid was largely a result of antifeedant properties. The naturally occurring α-farnesene described in this study merits further study as a potential control agent for the diamondback moth.



Keywords: *Plutella xylostella*, *Perilla frutescens*, Insecticidal activity, Antifeedant effect, Farnesene, Electrophysiological response

Poster number: *110*

# Repellency of 33 plant materials and *Curcuma longa* L. against *Culex pipiens* and *Aedes albopictus*

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The repellent activities of 33 plant extracts against *Culex pipiens* and *Aedes albopictus* were examined using a patch test for adult. Most plant extracts showed 100% repellency effect to *C. pipiens*. But, The six plant extracts (*Magnolia denudate*, *Dryopteris crassirhizoma*, *Cnidium officinale*, *Lindera obtusiloba*, *Magnolia kobus*, and *Houttuynia cordata*) showed over 70% repellency activities to *Ae. albopictus*. The insecticidal activity of *Curcuma longa* against *C. pipiens* and *Ae. albopictus* was also determined. *C. longa* hexane extraction showed 100% larvicidal activity at 1,000 ppm after treated 24 h. Purification of the biologically active constituents from the hexane extraction with larvicidal activity was done using silica gel column chromatography. H1 fraction gave 100% mortality to *C. pipiens* and *Ae. albopictus* at 100 ppm. H12 fraction was determined 100% and 87.8% larvicidal activity to *C. pipiens* and *Ae. albopictus* at 50 ppm respectively. Active constituent was analysed as the sesquiterpene, ar-turmerone (C15H20O) by GC and GC-MS. *C. longa* extract gave highly protection against mosquito bites. *C. longa* induced a protection time, repellency, landing, and biting mosquitoes against *Ae. albopictus*.

These results suggest that the some plant extracts and *C. longa* have the potential to be used an eco-friendly materials for the control of mosquitoes.

Key words: *Curcuma longa* L., *Aedes albopictus*, ar-turmerone, Plant extracts

Poster number: *111*

## Chemically armed ants pillage and protect fungus-farming societies

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Like human societies, ant colonies build protected fortresses, manufacture weaponry used by soldiers, maintain mutualisms that maximize food availability (i.e., farm), and live socially to enhance offspring survival. However, the concentrated resources attract organisms specialized in breaking through the fortifications and defenses of these highly organized societies. My work encompasses a dynamic symbiotic network that includes three ant species, a fungus garden, and bacterial communities. Selective pressures from these different organisms with shared or competing interests shape how these species interact and evolve. We show that guest ant social parasites act as mercenary warriors (www.megalomyrmex.com/videos) as their venom is much more effective in nest defense than the biting strategy of the host ants. Cooperation between these two ant species is enforced by external dangers according to the principle that the enemy of my enemy is my friend. We demonstrate that the alkaloid-based venom of the guest ants play an influential role in this complex species network and serves as a toxic chemical weapon that protects the host ants, farm and ultimately their shared home. The mere presence of the guest ant parasite discourages a more virulent raider ant species from attempting a raid. Counter to expectation, the symbiotic relationship between the *Megalomyrmex symmetochus* guest ant and their fungus farming host, *Sericomyrmex amabilis*, may be beneficial rather than costly under certain ecological conditions.

Adams, R. M. M., Liberti, J., Illum, A. A., Jones, T. H., Nash, D. R., & Boomsma, J. J. (2013). Chemically armed mercenary ants protect fungus-farming societies. Proceedings of the National Academy of Sciences USA, 110(39), 15752–15757.

Poster number: *112*

# Application of ion-selective electrodes to determination of naproxen and diclofenac in various samples.

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Diclofenac [2-(2,6-dichlorophenyl)amino]benzeneacetic acid and naproxen (S)-6-methoxy-α-methyl-2-naphthaleneacetic acid are non-steroidal anti-inflammatory drugs belonging to the group of heterocyclic and arylic derivatives of acetic acid or propionic acid respectively. Non-steroidal antiinflammatory drugs are are widely applied thanks to their anti-inflammatory action, but also as analgesics and antipyretics. These drugs are used more and more frequently. From the investigations it results that during one day 30 million people worldwide take the NSAIDs, of whom 40% are over 65 years of age. Because of wide applications of the NSAIDs the number of new preparations, based on these components, is constantly growing [1]. As a result new analytical problems arise as concerns: the environmental pollution (determination of drugs or metabolites in wastewater, surface water), the production and quality control of these drugs, (determination of drugs in pharmaceuticals ) health of humans (determination of drugs or metabolites in biological samples - blood, serum, urine) food chemistry (determination of drugs or metabolites in bovine milk, drinking water). From veterinarian reports follows that NSAIDs were an important group of compounds that were routinely used for the treatment of food-producing animal.

The aim of the presented work was to the application of selected naproxen and diclofenak cyclodextrin based electrodes to determination of drugs in various samples. Diclofenak was successfully applied for determining in pure samples (Sigma), samples of pharmaceutical preparations, and urine samples of different concentrations. Naproxen electrode was used for determining in pure samples (Sigma), samples of pharmaceutical preparations, urine and bovine milk. The obtained results are characterized by exactness typical of the determination methods employed

[1] J. Lenik, Materials Science and Engineering C 33 (2013) 311-316.

Poster number: *113*

# Ion-selective electrode with solid contact for monitoring of nitrate content in surface waters and food

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Nitrates are wide used in inorganic fertilizers, in production of explosives and for glass making. Nitrates also occur naturally in plants. They are present both surface waters and groundwater as a consequence of agricultural activity, from waste water treatment and from oxidation of nitrogenous waste products in human and animal excreta. High level of nitrate content in surface waters causes eutrophication of the natural waterbodies what leads to frog spit and devastation of local ecological systems. The main source of nitrate exposure for humans other than drinking water is food including vegetables, cured meat, fish and dairy products. The nitrate concentration in vegetables is depend on the use of fertilizers and growing conditions. They are often present in high concentrations above 2.5 g/kg in vegetables such as lettuce, spinach and radish especially those cultivated in greenhouses.

The toxicity of nitrate to human is connected with its reduction to nitrite. Nitrites can cause the oxidation of normal haemoglobin (Hb) to metHb which is unable to transport of oxygen to tissues. Moreover the interaction between nitrite and secondary amines led to formation of N-nitroso compounds associated with gastric cancer. Taking into account above mentioned information analytical control of nitrate concentration in natural waters and foodstuffs is important.

In presented research analytical application of recently developed nitrate-selective electrode [1] for monitoring of nitrate content in tap water, river water, waste water and vegetables is presented. Determination of nitrate was performed by application of direct potentiometry and multiple standard additions methods. The obtained results were comparable with those obtained by reference method.

C. Wardak , Electroanalysis 26 (2014) 864-872.

Poster number: *114*

## Analysis of the Cr(VI) concentrations in cement materials

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Protection of the environment, and moreover, protection of the health and safety of people, is a vital national interest. Cement, as a basic material for building, influences the environment both during its production and in the process of its consumption causing a negative impact on human health. It is connected mainly with the toxic property of water soluble compounds of hexavalent chromium, which are present in the cement. Chromium is an indelible non-volatile trace element of raw materials used in cement clinker production. Naturally occurring chromium(III) is not initially harmful, but hexavalent form of chromium is very harmful and allergenic. The cement process, specifically kiln conditions, oxidizing atmosphere, alkali concentration, finish mill can influence the amount of Cr (VI) formed. For that reason Europe Directive (2003/53/EC) limits the use of cement so that it contains no more than 2 ppm of water soluble Cr (VI). Nowadays reducing agents (e.g. iron(II) sulphate), which convert the hexavalent chromium into the trivalent chromium, in order to meet the EU Directive, are added during production of cement.

The main purpose of this work was determination of Cr(VI) in Portland cements eluted by water and with the use of three different extraction procedures. The quantification of Cr (VI) released from cement was performed by spectrophotometric and adsorptive stripping voltammetric method. Two certified reference materials: BCS-CRM No. 372/1 Ordinary Portland Cement and BCS-CRN No. 353 Sulphate Resisiting Portland Cement were used in experiments.

Poster number: *115*

# Simple fabrication of hydrophobic AKD coated MALDI concentration plates for increased sensitivity

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The need for detection of biological samples of low abundance is of vital importance and one of the hindrances for screening of e.g. biological disease markers. Even after enrichment, the abundance can be too low for straight forward MALDI-MS detection. For enhanced MALDI-MS sensitivity, different targets have been presented based on hydrophobic/hydrophilic interactions e.g. using coatings such as silicone and Teflon. Commercial MALDI concentration plates are expensive, restricted in the configuration/dimensions of the spots and have a limited lifetime, thus simple manufacturing of cheap plates with high degree of freedom regarding the architecture of the plate spots is desired.

Here we present a method to manufacture concentration MALDI-plates with alkyl ketene dimer (AKD) as a new hydrophobic coating material with potential for superhydrophobicity. The fabrication of the superhydrophobic AKD layer, on the MALDI plate involved dip coating and subsequent application by airbrush in layers, following dissolving in ethanol at $80^{0}$C. The contact angle for water droplets was measured to validate the surface property. To generate the hydrophilic anchor spots negative contact printing was used. The dimension of the spots was determined by the diameter of the printing substrate and the depth the substrate was lowered into the AKD layer. Sample deposition on to the anchors was performed in open air, either by a deposition capillary mounted in a robotic system for sample volume control, or manually by standard protocol deposition using a pipette. The plates were simple to clean and recoat after use. To achieve a sufficiently hydrophobic surface, the number of layers applied, distance and the drying time was evaluated on a regular stainless steel MALDI plate. The AKD compound itself is not enough to achieve high hydrophobicity, but the morphology of the surface also has significant impact. Contact angles on the tested plate were 132˚ with a relative standard deviation of 4.0%. Superhydrophobicity can technically be achieved and is expected to increase the concentrating effect. The diameters of fabricated spots were between 150 and 600 µm, optically measured showing steel surface at the spots. The reproducibility of the anchor diameter was studied for 400 µm (average 412 µm), resulting in a relative standard deviation of 3.1%, compared to 4.2% for a commercial concentration plate. Robotic system was therefore preferred, providing high target to target reproducibility. On commercial anchor MALDI-MS plates, the sample was observed to spread outside the target area in a first stage, subsequently retreating to the target during the solvent evaporation. This generated a larger contact surface area for the sample than only the target area, which could lead to sample loss due to adsorption. With the AKD coated plate the deposited sample droplets were contained within the anchor spot to a higher degree, and were also self-adjusted onto the spot if slightly misplaced. Using the AKD MALDI concentration plate there were no observed contamination MS-peaks from the AKD, and there were higher amounts of “sweet spots” than when using a regular MALDI plate. Increased S/N values could be seen when utilizing the AKD surface plate. Overall, S/N values were 90-150% higher compared to a standard steel plate, and detection of peptides not seen on the standard plate was achieved with fully automated acquisition of 0,5 µl sample on 400 µm anchors. The AKD-coated plate outperformed the commercial concentration plate in all tests performed.

Poster number: *116*

## Eight-carbon volatiles as infochemicals for fungivores

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Volatile substances released by fungi are of high chemical diversity. Just like plant volatiles are host cues to herbivores, fungivores may acquire valuable information by use of fungal volatiles. Eight-carbon volatiles dominate among many fungal species, most notable example is the mushroom alcohol 1-octen-3-ol. Analogous to the green leaf volatiles of plants they typically appear upon wounding as products of lipid oxidation. While oxylipins in plants are multifunctional agents against herbivores and pathogens, such functions are unknown in fungi. Studying obligate fungivorous beetles of the families Tenebrionidae (*Bolitophagus reticulatus*), Ciidae (*Cis boleti*) and Elateridae (*Ampedus pomorum*), we got insight into eight-carbon volatile release of their basidiomycete host substrates - bracket fungi as fruiting bodies, or rotten wood digested by them. Overall, eight-carbon volatiles are dominant and widespread and seem to be suitable cues separating preferred substrates. We studied the sensitivity of the insect olfactoric sense to eight-carbon volatiles that ultimately led to discriminative behavior. Some of them seem to repell fungivores in a wound-activated response, while others attract them in a dose-dependent manner. First studies on Collembolans as fungal feeding soil arthropods (*Folsomia, Sinella, Tomocerus*) show similar responses. However, eight-carbon volatiles are only a part of the volatile chemical profile of fungi, further infochemicals involved in host selection are not excluded. Eight-carbon volatiles are oxylipins yet seem to emerge at the heart of fungal biology and their informational content is advantageously versatile to fungivores that should be studied further.

Poster number: *117*

## Flexibility of chemical defense in the parasitoid wasp *Leptopilina heterotoma*

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Chemical defense mechanisms against predators are widespread among arthropods. The biosynthesis of the defensive compounds is costly and emptying a gland's reservoir on first contact with a predator can leave an insect unprotected during subsequent assaults. Insects should thus use their defensive secretion economically and adapt their defensive behavior to the magnitude of the threat. Females of the parasitoid wasp *Leptopilina heterotoma* produce (-)-iridomyrmecin as a defensive allomone against predators, like e.g. ants (Stökl et al. 2012). Here we show that the females of *L. heterotoma* can adapt their defensive mechanism to the size of the predator. Using headspace analyses and behavioral observations, we tested whether the amount of (-)-iridomyrmecin released by the females depends on the duration of aggressive interaction with a predator or the size of the predator. The data showed, that only the size of the predator influences the amount of (-)-iridomyrmecin released by the wasps. The duration of the aggressive interaction only had a slight, but not significant, effect on the released amount of the defensive secretion. We don't know yet which sensory modality the wasps use to sense the size of the predators, but our data show that an apparently simple defensive behavior still can be fairly complex and finely tuned.

Stökl J, Hofferberth J, Pritschet M, Brummer M and Ruther J (2012) Stereoselective chemical defense in the Drosophila parasitoid Leptopilina heterotoma is mediated by (−)-iridomyrmecin and (+)-isoiridomyrmecin. J Chem Ecol 38:331–339

Poster number: *118*

# Biochemical characterization of lignan biosynthesis in *Picea abies* in response to infection by the bark beetle associate *Enodconidiophora polonica*

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Norway spruce (*Picea abies*) forests suffer periodic fatal attacks by the bark beetle *Ips typographus* and its fungal associate, *Endoconidiophora polonica*. *P. abies* protects itself against fungal and bark beetle invasion by production of terpenoid resins and polyphenols. We investigated lignans, a group of phenolic compounds found in *P. abies* bark and wood which are modified dimeric coniferyl alcohol derivatives. During *E. polonica* infection, lignan biosynthesis was up-regulated as evidenced by elevated levels of pinoresinol, lariciresinol, secoisolariciresinol, matairesinol, α-conidendrin and nortrachelogenin. Genes coding for enzymes involved in the sequential reductions of pinoresinol to lariciresinol and to secoisolariciresinol (pinoresinol-lariciresinol reductases) were highly expressed after fungal infection. Furthermore, transcript levels of enzymes coding for further sequential reductions of secoisolariciresinol to lactol and to matairesinol (short-chain dehydogenases) also increased during infection of Norway spruce by *E. polonica*. In vitro bioassays where pure lignans where fed to *E. polonica* resulted in fungal growth inhibition, demonstrating that these compounds are highly bioactive defense chemicals against microbial attack.

Poster number: *119*

## Evaluation of generic attractants for trapping the velvet longhorned beetle, *Trichoferus campestris*

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The velvet longhorned beetle, *Trichoferus campestris* (Faldermann)(VLB; Cerambycidae: Cerambycinae: Hesperophanini), is native to east Asia where it feeds on a wide range of tree species, including orchard and timber trees. Larvae of VLB can be transported in wood packing material, and individuals are frequently intercepted in quarantine facilities. Populations of VLB have established outside of the native range of the species, including near Salt Lake City, UT USA. Adults are non-descript and nocturnal, and nothing is known of their pheromone-mediated biology, which hinders monitoring and control efforts. We conducted field bioassays testing the response of adult VLB to traps baited with “generic” cerambycid attractants and ethanol. These attractants included compounds conforming to the conserved 2,3-alkanediol/ hydroxyketone chemical structure. Compounds of similar chemical structure are male-produced pheromone components of multiple species in the subfamily Cerambycinae. A total of 104 adult VLB were captured in bioassays. Significantly more adult VLB were captured in traps baited with racemic 3-hydroxybutan-2-one or ethanol than in traps baited with other attractants. In addition, histological sectioning revealed subcuticular metathoracic glands present in males, but not females. Glands were connected to pits in the cuticle. The glands and pits are diagnostic for production of volatile pheromones of the 2,3-alkanediol/hydroxyketone structure by males of other cerambycine species.

Poster number: *120*

# Surface microlayer of lobelia lake: 48-hour dynamics of heavy metals and chlorophyll a variability

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The air-water interface named as surface microlayer is a very thin film between the water and the atmosphere. This is an important area of exchange of matter and energy that both affects and is affected by global change. This important form of air-water exchange ecotone, which constitutes the surface microlayer of water, is a specific environment as to its chemical and physical characteristics and is different from subsurface waters. It can accumulate chemical substances like as heavy metals, nutrients, organic substances, organisms like as phytoneuston and bacterianeuston in greater quantities in comparison to subsurface water. This characteristic feature, among others, from the processes of transport at the contact of hydrosphere-atmosphere and physico-chemical and biological features of this microlayer. This ecoton between different environmental compartments represent critical interfaces for physical processes, chemical and biological components. The surface microlayer is a very dynamic ecoton.

In presented study we examined by 48 - hours (intervals 4 hours) dynamics of heavy metals and chlorophyll a content in the surface microlayer and the subsurface waters of Gubisz lake and we analyze enrichment factor. Samples of surface microlayer was collected by the Garrett net method (thickness of 250 – 300 μm). Subsurface water was collected at a depth of 15 cm from the water surface by immersing the container. Concentrations of 20 heavy metals was measured by ICP-MS spectrometer Perkin Elmer and chlorophyll a by Jeffrey and Humphrey spectrophotometric method.

The surface microlayers of analyzed lobelia lake Gubisz show higher ability to accumulate heavy metals compounds than in subsurface water. Concentrations of analyzed biological and many chemical components depends mutually. Daily variability of concentrations of chemical and biological compounds in surface microlayer is more dynamic than in subsurface water.

Poster number: *121*

# Polyunsaturated hydrocarbons as synergists in moth pheromones: The female sexpheromone of *Dioryctria mendacella*

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Polyunsaturated hydrocarbons (Type II pheromone components) have been reported to be synergists for unsaturated acetates, alcohols or aldehydes (Type I components) in several species of Lepidoptera. However, there is some debate over whether the active components are the hydrocarbons themselves or more volatile degradation products. We found females of the cone moth, *Dioryctria mendacella* (Lepidoptera: Pyralidae), produce (*Z,E*)-9,11-tetradecadienyl acetate (ZE9,11-14:Ac) and (*Z,Z,Z,Z,Z*)-3,6,9,12,15-pentacosapentaene (ZZZZZ3,6,9,12,15-25:H). The former elicits a strong EAG response from males while no response could be recorded to the latter. In field trapping tests, both compounds were individually unattractive to males, but blends of the two compounds were highly attractive. It was demonstrated that the relatively involatile hydrocarbon is actually released from the dispensers used and no significant degradation could be detected. Furthermore, use of analogues with fewer carbons and/or double bonds that might be expected to produce similar degradation products to the ZZZZZ3,6,9,12,15-25:H were inactive. This indicates a specific response to the hydrocarbon itself, further substantiated by the observation that related hydrocarbons did not interfere with the activity of ZZZZZ3,6,9,12,15-25:H. Thus a two-step conversion of cod liver oil was used to produce a blend of unsaturated hydrocarbons containing ZZZZZ3,6,9,12,15-25:H as the major single component, albeit only 30% of the total, and this was as attractive to male *D. mendacella* as an equivalent amount of the pure material in combination with ZE9,11-14:Ac.

Poster number: *122*

# Localization of OBP post-translational modifications by high-resolution MS


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Recently, we have characterized the olfactory secretome of the pig *Sus scrofa* by proteomics (Nagnan-Le Meillour et al., 2014). It is mainly composed of OBP (Odorant-Binding Proteins) isoforms coming from post-translational modifications (PTM), phosphorylations and O-N-acetylglucosaminylation (O-GlcNAc). The presence of these PTM were shown by immunoreactivity to specific antibodies. To go further in their characterization, identification, and localization on the polypeptidic chain, we analyzed spots cut from 2D-E gels (two-dimensional electrophoresis) by Nano-LC-MS-MS (Orbitrap) after trypsin digestion. By using the PEAKS 8 software and home-made database, we localized the phosphorylation and O-GlcNAc sites on OBP sequences. The results confirmed data previously obtained by BEMAD method. More than 30 isoforms are generated by PTM, suggesting that OBP are important players in odor discrimination.


Proteomic analysis of pig (Sus scrofa) olfactory soluble proteome reveals O-linked-N-acetylglucosaminylation of secreted odorant-binding proteins. Nagnan-Meillour P, Vercoutter-Edouart AS, Hilliou F, Le Danvic C, Lévy F (2014) Frontiers in Endocrinology, doi: 10.3389/fendo.2014.00202

Poster number: *123*

# Exposure of soybean to weeding-related volatiles reduces damage and increases defensive compounds in their seeds


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In plant-plant communication, an undamaged plant that had been exposed to volatiles from an artificially-damaged or herbivore-infested plant became more resistant to herbivores. In agroecosystems, such interactions may occur between weeds and crops while weeding. In this poster, we show that weeding-related volatiles enhanced defensive responses in soybean plants under field conditions. Further, the volatiles enhanced some of the secondary metabolites in their seeds. Our results demonstrate a novel ecological function of weeding-related volatiles in the context of plant-plant communications.

Poster number: *124*

# Alteration of peach potato aphid *Myzus persicae* (Sulz.) probing behavior by natural and modified jasmonates

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Herbivore attack is one of the main stress factors that initiate plant defense responses regulated by endogenous phytohormones: jasmonic acid, salicylic acid, and ethylene. Jasmonic acid participates also in many life processes in plants, such as growth regulation, germination, morphogenesis, ageing, and adaptive reactions to stress factors. Exogenous application of jasmonic acid and its derivatives may enhance plant stress responses and cause changes in herbivore behavior and feeding.

The aim of the study was to assess the effect of jasmonic acid derivatives on probing and feeding behavior of the polyphagous herbivore, the peach potato aphid *Myzus persicae* (Sulz.). Biological activity of ten compounds was examined: *cis*-jasmone and dihydrojasmone, which are natural compounds, and eight derivatives that were obtained by chemical laboratory synthesis and biotransformation using fungal strains.  The effect of structural modifications on biological activity and capacity for limitation of virus transmission were analyzed as well. The following aspects of aphid behavior were studied: responses related to initial stages of plant exploration by freely moving aphids in no-choice situation, responses related to stylet penetration in plant tissues in no-choice situation (innovative application of electrical penetration graph (EPG) technique for monitoring behavioral effects of jasmonate application), and long-term responses, i.e. settling on plants in choice situation.

The application of compounds studied caused various modifications in different phases of aphid probing, mainly the pathway and phloem phases. The most spectacular modifications included the impeding of phloem sap ingestion by two jasmonate derivatives.

Poster number: *125*

## Effects of beneficial rhizobacteria and a pathogenic virus on soybean leaf metabolites and interactions with a beetle vector

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Soil-borne microorganisms can have significant effects on aboveground interactions between plants and other organisms. For example, association with some non-pathogenic soil microbes stimulates plants to exhibit induced systemic resistance (ISR) against pathogens and herbivores. We examined how two species of beneficial rhizobacteria—the PGPR species *Delftia acidovorans*, which lives in the rhizosphere in close association with root surfaces, and the nitrogen-fixing *Bradyrhizobium japonicum*—influence the interactions of soybean plants (Glycine max cultivar Williams82) with the herbivorous beetle *Epilachna varivestis*, which vectors an economically important pathogen of cultivated soybean, Bean pod mottle virus (BPMV). In a multi-factorial experiment, we characterized the primary metabolites produced by soybean plants in the presence/absence of the two rhizobacteria and with or without infection by BPMV. We then assessed how the observed changes in metabolite profiles correlate with the performance of beetle larvae. Using GC-MS-based metabolite profiling and data-mining techniques, we documented significant changes in leaf metabolite profiles associated with virus infection and the presence of rhizobacteria. These changes are correlated with significant differences in *E. varivestis* performance on the different treatments. Beetle larvae that fed on virus-infected plants gained more weight and also exhibited lower mortality rates than those that fed on healthy plants regardless of rhizobacterial treatment. A BPMV-rhizobacteria interaction was also observed to affect performance: larvae that fed on healthy, rhizobacteria-inoculated plants had higher mortality rates and lower weight than those fed on virus-infected, non-inoculated plant. Our results indicate that BPMV infection causes dramatic changes in metabolites related to plant nutrition and defense, with significant consequences for soybean interactions with an important herbivore and virus vector. Beneficial rhizobacteria also induce changes in metabolite concentration in soybean, but the observed differences in beetle performance are more significant for the virus-infected plants than for the rhizobacteria-inoculated plants when compared with controls. Our ongoing research in this system aims to understand how the observed microbial effects on larval performance (and other aspects of the plant-beetle interaction) are influenced by microbe-induced changes in plant nutritional and defense chemistry. Metabolomics data will be integrated within a larger project that correlates upstream gene expression signatures of the different rhizobacteria and BPMV treatments.

Poster number: *126*

## Norway spruce ATAF1-like NAC transcription factors modulate stress

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Norway spruce (*Picea abies*) is an economically very important production tree species in Europe. Stem and root rot of Norway spruce caused by *Heterobasidion annosum* s.l. create significant economic losses, in Sweden alone the costs are estimated up to 2 million SEK every day! Thus breeding for resistance has a very high potential for economic and biodiversity gains.

We have previously identified metabolite and gene induction patterns that show correlation to the level of resistance to *H. annosum* in four genotypes of Norway spruce [1]. In the current study we focused on NAC transcription factors Norway spruce differentially regulated in response to *H. annosum* in these four Norway spruce genotypes.

Our analyses identified a number of transcripts with significant similarity to *Arabidopsis* clade III-3 NACs which include the well-characterized repressors of plant defense responses ATAF1, ATAF2, ANAC019 and ANAC055 [2]. Expression analyses show that the candidates NAC01210 and NAC02452 were induced in response to *H. annosum* as predicted by the transcriptome analysis. NAC01210 were selected for overexpression in Norway spruce. Results from the overexpression study in Norway spruce will be presented and discussed in the context of regulation of specialized metabolism and host defence responses.

1.Danielsson, M., et al., Chemical and transcriptional responses of Norway spruce genotypes with different susceptibility to Heterobasidion spp. infection. BMC Plant Biology, 2011. 11:154(154).

2.Jensen, M.K., et al., NAC genes: time-specific regulators of hormonal signaling in Arabidopsis. Plant Signaling and Behavior, 2010. 5(7): p. 907-910.

Poster number: *127*

# Early learning of chemical host cues drives ecological divergence during speciation processes in a parasitoid wasp

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Ecological speciation, i.e. speciation via the evolution of ecotypes that are separated into different ecological niches, is considered an important mode of speciation in herbivouros and parasitoid insects. However, the mechanism behind the first step of separation, the switch of individuals into new niches, is unclear. One longstanding hypothesis, which was never tested, is that early learning of chemical host cues causes new ecological preferences, leading to a switch into a new niche within one generation. Here we show that a host switch occurred within a parasitoid wasp, associated the splitting into separate lineages during speciation. This host switch was most likely enabled by the ability for early learning (i.e. learning during larval development and after emergence from the host substrate) of chemical host cues in one of the lineages. The parasitoid wasp *Lariophagus distinguendus* (Chalcidoidea) consists of two genetically distinct lineages, which are reproductively isolated and have to be considered different species. One species has an innate preference for chemical cues from drugstore beetle larvae, which can not be altered by experience. Our data indicate that this was also true for the common ancestor of both species. In contrast, the second species is mostly found on Sitophilus weevils as hosts and changes its preference for chemical host cues by early experience. Therefore, a host switch must have occurred in the second species, which must have been enabled by early learning of host cues. Because early learning is a widespread mechanism in insects, it might have facilitated ecological divergence and associated speciation in this megadiverse group.

Poster number: *128*

# Testing the generalist-specialist dilemma: The role of pyrrolizidine alkaloids in resistance to invertebrate herbivores in *Jacobaea* species

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Plants produce a diversity of secondary metabolites (SMs) to protect them from generalist herbivores. On the other hand, specialist herbivores use SMs for host plant recognition, feeding and oviposition cues, and even sequester SMs for their own defense. Therefore plants are assumed to face an evolutionary dilemma stemming from the contrasting effects of generalist and specialist herbivores on SMs. To test this hypothesis, bioassays were performed with F2 hybrids from *Jacobaea* species segregating for their pyrrolizidine alkaloids (PAs), using a specialist flea beetle (*Longitarsus jacobaeae*) and a generalist slug (*Deroceras invadens*). Our study demonstrated that while slug feeding damage was negatively correlated with the concentration of total PAs and that of senecionine-like PAs, flea beetle feeding damage was not affected by PAs. It was positively correlated though, with leaf fresh weight. The generalist slug was deterred by senecionine-like PAs but the specialist flea beetle was adapted to PAs in its host plant. Testing other herbivores in the same plant system it was observed that the egg number of the specialist cinnabar moth (*Tyria jacobaeae*) was positively correlated with jacobine-like PAs, while the silver damage of generalist thrips (*Frankliniella occidentalis*) was negatively correlated with senecionine- and jacobine-like PAs, and the pupae number of generalist leaf miner (*Liriomyza trifolii*) was negatively correlated with otosenine-like PAs. Therefore while the specialist herbivores showed no correlation whatsoever with PA concentration, the generalist herbivores all showed a negative correlation with at least one type of PA. We concluded that the generalist herbivores were deterred by different structural groups of PAs while the specialist herbivores were attracted or adapted to PAs in its host plants.

Poster number: *129*

## Smells like home: Chemically mediated co-habitation of two termite species in a single nest

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Termites mounds have long be inspiring the naturalists by their size, structure and complexity, making them the most elaborate buildings made by animals. However, some termite species do not build any nest at all and found their colonies inside the nests built and inhabited by other termite species. These so-called termite inquilines are particularly frequent in the competitive environment of tropics and must have evolved strategies how to circumvent the conflicts with their hosts, the nest builders. Spatial segregation of the two colonies appears as the most obvious strategy.

Here, we show that soldiers of the South-American termite *Inquilinitermes inquilinus* (Termitinae), obligatory inquilines in the nests of *Constrictotermes cavifrons* (Nasutitermitinae), produce in their frontal glands large amounts of wax esters consisting of fatty acids of various chain lengths and unsaturated C12 alcohols (*Z*)-dodec-3-en-1-ol and (3*Z*,6*Z*)-dodeca-3,6-dien-1-ol. These alcohols are known to be used by various termite species as trail-following pheromones with very high biological activities. Our investigations revealed that the C12 alcohols are gradually released from the wax esters and occur as free alcohols in the headspace of soldiers. Both the soldiers as well as the synthetic standards of the C12 alcohols are highly attractive to all colony members and elicit an arresting behavior. In addition, we detected significant quantities of the C12 alcohols also in the walls of the nest parts inhabited by the inquiline. These results suggest that *Inquilinitermes inquilinus* produce and release the C12 alcohols as a home odor and add to the list of functions of the exocrine secretions of termite soldiers.

# Cavitene a new diterpene with original skeleton identified in soldiers of the Neotropical termite *Cavitermes tuberosus*

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"Termite soldier chemical defenses are chemically more novel and variable than in any other insect taxon of comparable species number". This statement by G.D. Prestwich, dating back to early eighties, proves to be true even thirty years later: over four hundred defensive chemicals have been identified in termite soldiers as yet, and new and original compounds are still being discovered in the defensive frontal gland of soldiers. Here, we report on the defensive chemistry of the Neotropical higher termite *Cavitermes tuberosus* (Termitidae: Termitinae), a member of an understudied group with "snapping" soldiers.

*Cavitermes tuberosus* soldiers possess a well-developed frontal gland situated in the frontal projection on their heads. The gland produces a blend of hydrocarbons: unbranched alkenes, alkadienes, and three diterpene hydrocarbons. We isolated these diterpenes from the total extract by means of preparative gas chromatography and characterized the molecular structure of the most abundant of them by NMR experiments using micro-cryo-probe. Cavitene, a new diterpene hydrocarbon with previously undescribed bicyclic prenyl-himachalene structure, is a new contribution to the diversity of carbon skeletons in Termitidae terpenic defensive compounds. The quantities of the two minor diterpenes have not allowed for full structural identification. Nevertheless, the high similarity of their mass spectra fragmentation patterns with that of cavitene suggests that they are derived from the same bicyclic skeleton.

Poster number: *131*

# Testing the effect of larval population density and host plant on larval immune response and adult oviposition choice in a noctuid moth, *Spodoptera littoralis*

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Polyphagy presents difficulties to an insect, especially in terms of host choice. The adult insect must choose from a large number of possible host plant species, something which requires extensive neural capacity to process (Bernays, 2001). In order to decrease the amount of information to process, it has been suggested that polyphagous insects could rely on previous larval experience to make a choice (Anderson et al., 2013).

In the polyphagous noctuid moth *Spodoptera littoralis* it has frequently been proven that female oviposition choice is driven by ‘larval memory’, indicating that the adult chooses to oviposit upon the same host upon which it fed as a larva (Anderson et al. 2013). However, is there a larval density effect on how the host plant is perceived, and does this effect adult behaviour?

In this on-going project we study the effect of larval memory modulation on adult host choice when the larvae have been bred in different population densities. A high density might lead to competition between larvae. In addition, populations of *S. littoralis* experiencing conditions of high population density commonly invest more in prophylactic immune functions, which is seen as melanised phenotypes (Cotter et al. 2008).

In this feeding bioassay we breed larvae on cotton, cabbage, and maize, in high and low population densities. The effect of diet and density on individual larval development until pupation is studied, and Y-tube assays assess larval host plant choice. Oviposition preference of adult females is being studied to assess whether or not there is a change in oviposition preference caused by its overall larval experience.

Furthermore, the immune response of the larvae in the different treatments is being addressed, whereby artificial encapsulation is used to determine the extent to which the insects are allocating energy to immune response.

Anderson, P., Sadek, M. M., Larsson, M., Hansson, B. S., Thöming, G., 2013. Larval host plant experience modulates both mate finding and oviposition choice in a moth. Animal Behaviour 85:1169-1175

Bernays, E. A. 2001 Neural limitaions in phytophagous insects: Implications for diet breadth and evolution of host affiliation. Annual Review of Entomology 46: 703-727

Cotter, S. C., Myatt, J. P., Benskin, C. M. H., Wilson, K. 2008. Selection for cuticular melanism reveals immune function and life-history trade-offs in *Spodoptera littoralis*. Journal of Evolutionary Biology 21:1744-1754

Poster number: *132*

# **Olfactory responses of Indian meal moth, *Plodia interpunctella*, to volatiles of stored-grain contaminated by fungi**

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*Plodia interpunctella* (Hübner) (Lepidoptera: Pyralidae) females use volatiles of wheat to locate substrate suitable for oviposition (Sambaraju and Phillips, 2008). Contamination by fungi is critical for larvae development, thus females should be choosy for substrate quality.

Our aim was: 1) to identify VOCs perceived by *P. interpunctella* emitted by wheat grain free of fungi and that contaminated by fungus *Aspergillus flavus*; 2) examine the compound emission dynamics following contamination.

The solid phase micro-extraction (SPME) technique was used to collect compounds and the coupled gas-chromatography with two detectors (GC-FID/EAD and GC-MS) was used to isolate and identify EAG active VOCs.

It was revealed that *P. interpunctella* females responded at least to four compounds (hexanol, nonanal, phenylacetaldehyde and still unidentified compound) emitted by fungi-free grain as well as to extra compound (3-methylbutanol) emitted by fungi contaminated grain. Emission of the latter compound peaked on 5th day following contamination and tended slightly decrease on 10th day. The active VOCs emission by fungi-free grain dropped down on 3rd day following contamination. Behavioural tests of synthetic compounds as well as of their blends are in progress.

Sambaraju K.R. and Phillips T.W. 2008 Effects of physical and chemical factors on oviposition by Plodia interpunctella (Lepidoptera: Pyralidae). Annals of the entomological society of America. 101(5): 955-963

Poster number: *133*

## Aggregation in entomopathogenic nematodes

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Self-organized aggregation, observed across trophic levels and environments, is facilitated by conspecific recognition and communication that can be mediated by chemical cues. We investigate the potential chemical basis for aggregation in the entomopathogenic nematodes *Steinernema diaprepesi* and *Heterorhabditis indica*. In sand-filled six-arm olfactometers, *S. diaprepesi* aggregates in the presence of pregeijerene, an herbivore-induced plant volatile that is a known attractant, but not in its absence. In trials in the presence of pregeijerene, nematodes responded in greater numbers to and aggregated more in sand moistened with water taken from nematode cultures (aqueous culture media) than in sand moistened with plain water. In the absence of pregeijerene, nematodes responded in slightly greater numbers to but did not aggregate to the same degree in sand moistened with aqueous culture media. Additionally when added sequentially to olfactometers over the course of 48 hours, *H. indica* presence was highly correlated with *S. diaprepesi* aggregation; *H. indica* tend to follow *S. diaprepesi*. These results suggest that aggregation in entomopathogenic nematodes is both chemically mediated and that the chemicals mediating such aggregation may be shared across species.

Poster number: *134*

# Why does the leaf miner *Tuta absoluta* avoid oviposition on *Meloidogyne incognita*-infested tomato plants?

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Plant systemic induced responses mediate the indirect interaction between aboveground and belowground herbivores. The aim of this work was to test the “preference-performance” hypothesis, which predicts that insects choose the best host plant for the development of their offspring. We investigated whether root herbivory by *Meloidogyne incognita* affected the acceptance of *Solanum lycopersicum* leaves for oviposition by *Tuta absoluta*, and whether the development of the resulting offspring was compromised. To link oviposition preference and the offspring development with plant systemic induced responses, we measured photosynthetic rates, leaf trypsin protease inhibitor activities and analyzed the profile of volatiles emitted by the leaves of root-infested and non-infested plants. *Tuta absoluta* females preferred to lay eggs on non-infested plants. Moreover, the aboveground volatile profiles emitted by control and infested plants differed, which could partially explain the oviposition preference observed. Larvae of *T. absoluta* feeding on control plants developed faster than their counterparts’ larvae feeding on root-infested plants. Similarly, root herbivory negatively affected the pupation process; although we did not find changes in the pupal weight, we observed that a larger proportion of deformed pupae were originated from root-infested plants. Finally, the longevity and fecundity of females that emerged from control and root-infested plants did not differ. *M. incognita* herbivory decreased photosynthetic rates and increased leaf trypsin protease inhibitor activity in tomato leaves, which may explain the developmental delay and deformed pupae of *T. absoluta* on root-infested plants. In conclusion, our results showed that *T.* absoluta is able to distinguish and prefer to oviposit on healthy plants benefiting the development of its offspring. Our results provide conclusive evidence for “preference-performance” hypothesis.

Poster number: *135*

# Identification of an aggregation pheromone component of *Sitona humeralis* Stephens based on increased pheromone production induced by synthetic juvenile hormone III

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At present, the only known aggregation pheromone with field activity for the genus *Sitona* is 4-methyl-3,5-heptanedione (MHD), originally identified from male *S. lineatus* specimens. In earlier field experiments in Hungary we found that in addition to *S. lineatus*, other *Sitona* species, particularly *S. crinitus* and *S. humeralis*, were also attracted to synthetic MHD. Catches of *S. crinitus* were always significantly higher in treated than in untreated traps, while *S. humeralis* catches in treated traps were usually numerically higher, but significant differences were not detected in all cases, suggesting a much weaker activity of the compound towards this species (although quantitative correlations with actual population densities of the spp. were not assessed).

We collected volatiles from the headspace of male and female *S. humeralis*. The male extracts showed biological activity in EAG and GC-EAG experiments, although only small quantities of the EAG-active MHD were found in the male extracts. As juvenile hormone (JH III) treatment can often induce pheromone production in insects, we treated male and female beetles with synthetic JH III, which caused at least a 20-fold increase in the amount of MHD in males. Also, *S. humeralis* males and females gave clear EAG responses to synthetic MHD.

We showed that males of *S. humeralis* produce MHD and both sexes can perceive the compound. Thus, MHD might be an aggregation pheromone component of the species.

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Poster number: *136*

# Insights into diversification of floral chemical signaling in the *Ficus*-mutualistic pollinator interaction: The case of *Ficus septica* in South East Asia

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Each *Ficus* species is associated with one or some mutualistic, host specific wasps that breed within their closed inflorescence (called fig). Receptive figs ready to be entered and pollinated emit floral odors that attract the short lived insects. The odor is species specific. *Ficus* and fig pollinating wasps constitute the most extreme case of plant insect co-diversification documented to date. However, little is known about how receptive fig odors may diversify and whether this diversification is concomitant with wasp diversification. We document here genetic differentiation in *Ficus septica* between Taiwan, Luzon, Negros Occidental and Mindanao and variation in receptive fig odors among locations. We also document the subdivision of the wasps into a species complex with co-occurring species within the region. Finally we add some data on odor perception by the wasps using GC-EAD.

Our first set of data suggested that wasps and fig tree had co-diversified within the system of islands, and that receptive fig odors diverged even faster that wasp and plant gene pools. More systematic sampling suggests 1) a more complex situation of intricate diversification and 2) that the pollinators are probably more constrained by ecological conditions than by geographical variation in receptive fig odor.

Poster number: *137*

## Unusual Chemoreceptors in Aphids

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The sensilla on the antennae of peach aphid *Myzus persicae* were mapped using scanning and transmission electron microscopy. Three placoid sensilla (one big and two small ones) and four coeloconic sensilla were found on the 6th segments, while a single sensillum placoideum was located on the 5th segment. The ultrastructure of big placoid sensillum reveals the presence of 3 groups of neurons, with 2-3 dendrites in each neuron group, while both small placoid sensilla are equipped with a single group of neurons, consisting of three dendrites. Coeloconic sensilla in primary rhinaria are of two morphological types, both equipped with two dendrites. The ultrastructure data also indicated the difference between two types of coeloconic sensilla. two types of trichoid sensilla were present all through the length of the antenna. Trichoid sensilla of type I are innervated by single neurons, ending in a tubular body beneath the hair, while type II hair, mainly located on the tip of the antennae, are innervated by two to four neurons, with some outer dendrites reaching the distal end of the hair.

Immunocytochemical localization of odorant binding proteins (OBPs) was performed on ultrathin sections of antennal chemosensilla. The antiserum against OBP7 intensively labeled all placoid sensilla from both primary and secondary rhinaria, with gold granules concentrated in the lymph surrounding the dendrite. OBP6 and OBP3 could also be detected in placoid sensilla, but less strongly than OBP7.

Poster number: *138*

# Diterpene-based induced defences in pine trees in response to a bark chewing weevil and a folivorous caterpillar

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Pine trees are long-lived, widespread organisms that have to cope with multiple biotic stressors during its life cycle. To face against biological aggressions pine trees harbour a wide variety of secondary compounds that may avoid or deter the attack providing resistance. Terpenoids and phenolics are the major pine chemical defences. Mono and sesquiterpenes, the volatile fraction of oleoresin, have been found to be adaptive resistance traits and to differ between and within species. However much less attention has been paid to sources of phenotypic variation in diterpenes, the non-volatile fraction of oleoresin that seals the stem wounds after oxidization.

The aim of this study is i) to examine the induced defensive responses of the diterpene fraction in two pine species, one native (Maritime pine) and one exotic (Monterey pine) to Europe, against two native insect herbivores, the bark chewing pine weevil and the pine processionary caterpillar; and ii) to explore the association between the induced chemical profile of diterpenes in each species and the subsequent damage. We found that the two pine species showed similar constitutive total diterpene concentrations, whereas they differed in their diterpene chemical profile. In addition, only the native *P. pinaster* produced an induced diterpene response against the two herbivores involving an increased concentration of the preexisting diterpene chemical species, with no new compounds produced in response to damage.

Poster number: *139*

# Intraspecific variation of cuticular hydrocarbon profiles in the *Anastrepha fraterculus* (Dipteria: Tephritidae) species complex

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The goal of the present study was to define whether cuticular hydrocarbons could be used for taxonomic determination of putative species hidden in the *Anastrepha fraterculus* cryptic species complex, widespread from Argentina to Mexico. Recently, increasing evidence of phenotypic and genetic variability has resulted in the characterization of eight morphotypes within this complex. The cuticular hydrocarbon (CH) profiles of six *A. fraterculus* populations from Argentina, Brazil, Peru, Colombia and Mexico were analyzed in the present study by two-dimensional gas chromatography coupled with mass spectrometry. In parallel, multiple factorial analyses were used to elucidate population structures. Vector populations segregated into four distinct groups. The analysis demonstrated that the studied populations from Peru, Argentina and southern Brazil (Vacaria) might be classified in accordance with the earlier division of the *A. fraterculus* complex into Peruvian, and Brazilian-1 cryptic species, using the specific CH profiles. Population from southeastern Brazil (Piracicaba) formed separated group. Mexican and Andean (Colombian) putative species had similar CH signatures, when compared to each other.

Poster number: *140*

## Chemical composition of preen waxes in Herring Gull (*Larus argentatus*) during winter and in the breeding season


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Preen gland is the only skin gland in most birds and the main function of its secretion is feather maintenance, but can also influence the colour or smell of the individual. The main components of preen gland secretion are waxes (esters of fatty acids and long chain alcohols) and its composition often changes during the year. The aim of this study was to determine difference in chemical composition of preen waxes in adult herring gulls from winter and breeding season. Preen gland secretion was collected from 17 herring gulls (8 females and 9 males) during winter and 17 herring gulls (8 females and 9 males) captured in breeding season during eggs incubation. Preen waxes were hydrolyzed to fatty acid and alcohol moieties, which were then analyzed using gas chromatography-mass spectrometry (GC-MS). Chemical composition of preen waxes in both males and females varied between winter and breeding season with much higher content of unbranched compounds and lower content of 2,6- and 2,8-dimethyl fatty acids during breeding season. These changes where significantly larger in breeding males than breeding females. No changes were found between sexes during winter. In herring gulls both male and female incubates and time of incubation is shared equally between partners, with male tendency to incubate more often at night and female during the day. Changes in preen gland secretion during breeding season may be beneficial for clutch protection and greater changes in breeding males may be especially important for protection against mammalian predators which use olfaction to detect their prey and which are more active at night. This is first time when seasonal differences in chemical composition of preen waxes in gulls were noted. Project was financed from European Social Fund.

Poster number: *141*

# Putative male pheromones: Release of specific components in a circadian rhythm from males of the greater date moth, *Aphomia* (*Arenipses*) *sabella*

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Mating in moths typically involves long range sex pheromones emitted by females. Males of various moths release male-specific compounds that are involved in the sexual communication at close range. The biological roles of these compounds are diverse and may act as courtship pheromones, aphrodisiacs and enhancing female acceptance of copulation. In several species, particularly of Pyralidae: subfamily Gallerinae, the role of searching and signaling may be reversed between the sexes involving male pheromones and/or visual and acoustic signals.

The release of pheromonal components from both males and females of the Greater date moth (GDM), *Aphomia sabella* Hampson (Lepidoptera: Pyralidae: Galleriinae), has been investigated using the sequential SPME/GCME analysis, developed recently by us (1). Males emit a complex mixture of compounds in a circadian rhythm during the night between 3:00AM to 5:00AM. Six compounds were identified: benzaldehyde, sulcatol, geranyl acetone, phenylacetaldehyde, 2-phenylpropenal and (*R*)-fuscumol. Benzaldehyde, sulcatol and geranyl acetone were found only in trace amounts. The putative male pheromone components were found in glands located in the forewing. This is the first finding of a circadian release of male-specific compounds in moths. GC-EAD analysis of male and female antennae with synthetic compounds released by males shows that the female antenna is stimulated by all six compounds while the male antenna responds only to phenyl acetaldehyde.  Exposure of the main male components in field tests or in screen cages did not reveal any significant response from females.

(1) Levi-Zada, A. et al.,  (2014). Naturwissenschaften 101: 671–678; and references therein.

Poster number: *142*

# Interaction between exotic and native noctuid moth species: Differential oviposition in the presence of conspecific and heterospecific larvae

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Plant volatiles play the important role of transferring information between plants and insects. For instance, herbivore induced plant volatiles (HIPV) deliver the information about the presence of herbivores on a particular plant. Such information can be used by other species to make decisions accordingly to their needs (1). However, the arrival of an exotic species to a new community opens room for the creation of novel interactions. At the same time, it may disrupt the already established fine-tuned interactions among native species.

We investigated the outcome of intra- and interspecific interactions at concrete life-time situations (ovisposition and larval development) between two noctuid moth species exotic and native of Europe: the Egyptian cotton leafworm (*Spodoptera littoralis*) and the cabbage moth (*Mamestra brassicae*), respectively.

Firstly, we assessed female egg-laying preferences in two-choice oviposition assays combining non-infested, conspecific or heterospecific-infested plants. Secondly, we measured the developmental rate of larvae of either species in direct interaction but using an ad libitum food regime.

The results from this study show that *M. brassicae* only recognises plants attacked by conspecific larvae and oviposits on the alternative plant. However, when given the choice to oviposit between non-infested and heterospecific-infested plants, they showed no preference. On the other hand, *S. littoralis* also recognises heterospecific larvae and avoids egg-laying in both cases. Moreover, if both species meet on the same plant as larvae, *S. littoralis* seem to perform better in the presence of heterospecifics. However, whether the latter is caused by an increased larval feeding rate or heterospecific predation remains to be elucidated.

Being able to decrease offspring competition regardless of the species present may be a good strategy to maximise fitness, and perhaps one explanation for why the presence of *S. littoralis* is expanding rapidly throughout Europe.

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Poster number: *143*

# Bed bugs-down the trap

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Bed bugs, for long a forgotten nuisance, have reappeared and detection and eradication of them have become a million dollar industry. Bed bugs are well shielded from watery solutions of pesticides by a waxy outer cuticula and they easily become resistant to pesticides. Approximately ten weeks after infestation a bed bug population change from linear to exponential growth. To detect an infestation before this point is a and o for successful treatment. Our aim is to develop a monitoring system that detect bed bugs at low densities partly substituting costly ocular inspections and that can evaluate the outcome of a treatment.

We collected and analyzed emissions from bed bug colonies with headspace, enfleurage and GC-MS methods. Bed bug attraction in test arenas to the pheromones resulted in a solution of five chemicals, which, applied in traps, were presented together with control traps and tested in Mesocosms. Pheroemission decreased with time, but was easily detectable after six days. Ten bed bugs (4 females, 6 males) were released at start, after 24 and 72 hrs and retrieved after 24, 72 and 144 hrs, respectively. Pherotraps caught 44, 35 and 37 % of the introduced bed bugs during the first, second and third period, respectively, which is a higher efficiency than any other products we know of. Pherotraps caught significantly more bb than control traps (p=*****). The catch of both sex were highest in the first period and lowest for females in the third and for males in the second period. The efficiency is concentration dependent and currently we work to optimize the strength and life length of the bait and to tests trap efficiency in the field.

Poster number: *144*

# Cyanide detoxification by β-cyanoalanine synthase in the glucosinolate specialist *Pieris rapae*

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*Pieris rapae*, the Small Cabbage White (Lepidoptera: Pieridae), is a specialised herbivore that feeds on plants defended by the glucosinolate-myrosinase system. Upon tissue disruption, glucosinolates are hydrolysed by myrosinases and toxic isothiocyanates are formed. Larvae of *P. rapae* have adapted to their food plants by expressing a gut nitrile-specifier protein (NSP), which redirects glucosinolate breakdown to form nitriles. Aliphatic nitriles are excreted with the faeces. Nitriles of phenylalanine-derived glucosinolates are further metabolised in the larvae leading to the formation of cyanide. We found that *P. rapae* larvae are exceptionally tolerant to cyanide and detoxify cyanide to SCN- and β-cyanoalanine [1]. The aim of our study is to test the relevance of cyanide detoxification enzymes for host plant adaptation in Pieridae.

Commonly known enzymes of cyanide detoxification, β-cyanoalanine synthase and rhodanese, are believed to play a role in cyanide detoxification in insects, but have not been identified at a molecular level in insects. We have used a PCR approach with degenerate primers to isolate three cDNAs encoding proteins with 32-34% identity to known β-cyanoalanine synthases from *Caenorhabditis elegans* and 59-65% identity to the recently identified β-cyanoalanine synthase from the two-spotted spider mite *Tetranychus urticae* [2]. Enzyme characterisation after heterologeous expression in *E. coli* and purification showed that all three cDNAs encode β-cyanoalanine synthases. Together with metabolite and enzymatic analyses of *P. rapae* this shows that β-cyanoalanine synthases play a role in cyanide detoxification in P. rapae.

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2. Wybouw, N., Dermauw, W., Tirry, L., Stevens. C., Grbic, M., Feyereisen, R., Van Leeuwen, T. (2014) A gene horizontally transferred from bacteria protects arthropods from host plant cyanide poisoning. eLife 2014;3;e02365

Poster number: *145*

# The influence of bacterial communities colonizing lettuce (*Lactuca sativa*) on grazing activity of slugs (*Arion vulgaris*)

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Various factors are known to influence the attractiveness of plants to generalist herbivores. While all plant parts are commonly colonized by diverse bacterial communities, their impact on the interactions with higher trophic levels such as feeding choices by herbivores is largely unknown. However, from other systems, such as plant-pollinator interactions, it is known that bacteria affect the behaviour of invertebrates. To test whether herbivory depends on the bacterial community on foliage, we offered leaves of butterhead lettuce *Lactuca sativa* to slugs (*Arion vulgaris*), generalist herbivores known as considerable pests in cultivations of various horticultural plants and farm crops. We are tested if and how bacteria influence feeding choice and grazing activity of slugs on lettuce. Slugs were fed with an artificial diet containing communities or single strains of bacteria, isolated from lettuce leaves, to identify their preferences or avoidance behaviour. To study in vivo effects, sterile lettuce plants were inoculated with communities or single strains of bacteria, which were used in choice tests with slugs and to compare the volatiles emitted by sterile plants and those treated with defined bacterial communities.

Poster number: *146*

## The scent of mixtures: Rules of odour processing in ants

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Natural odours are complex blends of numerous components. Understanding how animals perceive odour mixtures is central to chemical ecology and related disciplines. A phenomenon particularly useful for studying how animals perceive stimulus mixtures is overshadowing, which occurs when (a) an individual trained with a binary mixture responds less to one component (overshadowed component) than to the other (overshadowing component), and (b) the response to the overshadowed component is lower than that obtained when this component is trained alone. We investigated olfactory overshadowing in carpenter ants, for which odour cues play a fundamental role in various behavioural contexts. Ants were trained individually with alcohols and aldehydes varying in carbon-chain length, either as single odours or binary mixtures. They were then tested with the mixture and the components. We found numerous cases of overshadowing among the binary mixtures tested, suggesting that this phenomenon is widespread in ants. Overshadowing resulted from the interaction between chain length and functional group: alcohols overshadowed aldehydes, and longer chain lengths overshadowed shorter ones; yet, combinations of these factors could cancel each other and suppress overshadowing. Our results show how carpenter ants treat binary olfactory mixtures and set the basis for predictive analyses of odour perception in social insects.

Poster number: *147*

# Identification and field evaluation of the sex pheromone in a Brazilian population of *Hypsipyla grandella* (Zeller) (Lepidoptera:Pyralidae)

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The composition of the sex pheromone gland of the mahogany shoot borer, *Hypsipyla grandella* (Zeller) was re-investigated using insects from a Brazilian population. In addition to the two previously identified compounds (*9Z,12E*)-tetradeca-9,12-dien-1-ol and (*9Z,12E*)-tetradeca-9,12-dienyl acetate, two additional compounds were identified in the blend: (*9Z*)-tetradec-9-en-1-ol and (*9Z*)-tetradec-9-enyl acetate. Gas chromatography-electroantennographic analysis showed that these four components elicited antennal response from conspecific males. Wind tunnel bioassays with binary, ternary and quaternary mixtures using different amounts of the components elicited male response similar to the response to conspecific calling females, but a quaternary mixture containing the four components, Z9 14:OH, Z9,E12 14:OH, Z9 14:OAC and Z9,E12 14:OAC at 0.05 µg elicited a higher proportion of male responses. When a binary mixture and a quaternary mixture were tested in field conditions males were attracted only to the traps containing the quaternary mixture. The results obtained in this work provide a new pheromone blend for *H. grandella* that might be used as a monitoring tool or control measure.

Poster number: *148*

# Social parasites change host-nest odors in social wasps

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In *Polistes* social wasps, nest odor is important for colony odor learning by young wasps. Usually, foundresses mark their paper nests with their own chemical signature. When obligate social parasites invade social wasp colonies, they become chemically indistinguishable from host colonies, but how they contribute to nest odor is unclear. In the present work we examine nest odor in the mountain *P. biglumis* social wasp and we compare changes occurring in free-living colonies with those occurring in colonies parasitized by the social parasite *Polistes atrimandibularis*, to identify the selective pressures imposed by social parasites on the chemical signature of their hosts. We analyzed the compounds of 150 nest surfaces from two populations in the Alps by collecting small pieces of nests all along the nesting season. Colony odor changes largely in quality and even more in quantity, with heavier compounds increasing in all nests through the summer. We also tracked for the first time the effect of the chemical strategy of the parasite on host nest odor and showed that, within very few days from invasion, parasite-specific unsaturated compounds appear and then disappear from the surfaces of the host nests, contributing to the integration of the parasites in host colonies and to their take over.

Poster number: *149*

## Early evolution of pheromone receptors in Lepidoptera

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The olfactory system is essential for mate finding, host location and reproductive isolation in insects. Sex pheromones play an important role in moth sexual communication, involving the production of species-specific sex pheromones in females and the corresponding selective detection by the pheromone receptors (PRs) in conspecific male antennae. Based on the carbon chain length and biosynthetic origin, sex pheromones are classified in three categories: Type 0, I, and II. Typically, Type I compounds are C10-C18 acetates, alcohols, or aldehydes, Type II are C17-C23 hydrocarbons and epoxides, and Type 0 are short-chain (C7-C9) alcohols and ketones. The primitive moth *Eriocrania semipurpurella* (Lepidoptera: Eriocraniidae) as well as the caddisfly *Rhyacophila nubila* (Rhyacophilidae), belonging to the Trichoptera, the sister order of Lepidoptera, have Type 0 pheromones1. Pheromone receptors have been identified from an increasing number of more advanced moths but not in primitive moths or in the Trichoptera. Thus, it remains largely unknown how the PRs of Lepidoptera evolve along with the changes in female-produced signals. The sex pheromone compounds of caddisflies and primitive moths are similar to general plant volatiles. Thus, their PRs might have evolved from general odorant receptors (ORs) responding to such compounds. Since Trichoptera and Lepidoptera are sister groups, out-group comparison should reveal the ancestral type of PRs. As a starting point, we sequenced antennal transcriptomes and identified the ORs of *E. semipurpurella* and *R. nubila*. A phylogenetic analysis including these ORs and the PRs from more advanced moths suggests that the PR clade of advanced Lepidoptera is rooted by a subfamily of ORs from *E. semipurpurella* and *R. nubila*. Some of these ORs have male-biased expression, making them candidate receptors for the female-produced pheromone compounds. The responsiveness of these receptors to pheromone compounds is currently being assessed using an HEK293cell-basedfunctional assay2.

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Poster number: *150*

# Attraction and attachment of Norway spruce pathogen *Endoconidiophora polonica* to its vector Ips typographus

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The blue stain fungus *Endoconidiophora polonica* is always seen in a symbiotic relationship with its vector, the European bark beetle (*Ips typographus*). The fungus detoxifies host defense compounds and the beetle in turn aids *E. polonica* endoconidia transmission from tree to tree. Successful attack depends on the number of viable spores that are carried by the invading beetles. *E. polonica* releases several volatile metabolites like terpenoids, aliphatic and aromatic esters as well as a lactone when grown in laboratory growth media which might act as a sensory cue for its vector. Some of these volatiles especially some terpene derivatives and esters have been shown previously to attract similar beetles to some extent. However, *I. typographus* has no recognizable "mycangia", a specialized structure on the surface of symbiotic insects to harvest and transport fungal spores. The scanning electron microscopy of *I. typographus* exoskeleton shows that *E. polonica* spores are scattered all over the surface and attached to microscopic hairs but the biophysics of this attachment is not understood. *E. polonica* produces a large amount of Cerato-platanin (Cer) - like protein which might mediate the reversible interaction between endoconidia with the insect surface. The members of Cer protein family are chitin binding proteins and shown to increase the polarity effects of surfaces i.e. in our case wettability of *E. polonica* spores. From the heterologous expression of Cer from *E. polonica* in *Aspergillus nidulans*, we have shown that Cer is a chitin binding protein and secreted into liquid medium. Moreover, *A. nidulans* mutants over-expressing Cer binds to both live and inactivated *I. typographus* more effectively than the wild type *A. nidulans*.

Poster number: *151*

# The chemical basis of flehmen behaviour in domestic cats

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The flehmen response is a native instinctive behaviour only performed by mammals with the vomeronasal organ (VNO), e.g. cats, horses, or elephants. It is characterized by intensive sniffing on a scent, e.g. urine mark, followed by drawing back the lips and lifting of the head. Presumably, this serves the transport of pheromones and chemoreceptor signals to the VNO. The compounds responsible for the flehmen behaviour in cats are still unknown. Cats are using urine for chemocommunication with other cats. Our study is the first approach to identify volatile and semi-volatile compounds from male cat urine, which induce the flehmen response.

Cat urine was extracted with chloroform/methanol and fractionated by HPLC. Two flehmen active fractions were isolated, containing exclusively carboxylic acids. These fractions were derivatized to methyl esters and analyzed by GC/EI-MS. A complex mixture of methyl- and ethyl-branched, saturated and unsaturated fatty acids were detected. The ethyl-branched fatty acids are of particular interest because of their rare occurrence in nature. Acids occurring in both fractions were synthesized to confirm structures and for testing of their biological activities in g-scale. Testing of their flehmen inducing activity is currently in progress.

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Poster number: *152*

## Response of entomopathogenic nematodes to sugarcane root volatiles under herbivory by sugarcane spittlebug nymphs

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So far only few systems have been described in which herbivore-induced root volatiles mediate attraction of EPNs and little is known about the nature of these interactions. EPNs are potential biological control agents of sugarcane spittlebug (*Mahanarva fimbriolata*) populations, especially ***Heterorhabditis indica*** and *Steinernema carpocapsae*. Here, we investigated the response of these two species of EPNs to sugarcane root volatiles damaged by *M. fimbriolata* nymphs in a belowground six-arm olfactometer. We also examined changes on root volatile profile in response to herbivory of sugarcane spittlebug nymphs. Each olfactometer replicate consisted on the response of 10,000 entomopathogenic nematodes released in the central chamber of the olfactometer. After 24h, the number of EPNs found in each side arm was estimated. Both EPN species equally chose arms containing undamaged sugarcane and moisten sand (blank). However, when EPNs were exposed to odors of nymph-damaged and undamaged sugarcane roots, both species significantly preferred odors of nymph-damaged root. Headspace collection followed by GC-MS analyses showed no qualitative difference (total of 11 compounds) between volatile profiles of nymph-damaged and undamaged sugarcane root. Nevertheless, we observed quantitative differences in the emission of dihydro myrcenol and β-iso-methionene, which were both suppressed in nymph-damaged roots. Different from the systems described in the literature, in which the up-regulation of a single or few compounds are responsible for the attraction of EPNs, our results indicate that EPN attraction to host-damaged roots is mediated by the suppression of volatile compounds. Future studies testing the effect of single compounds, isolated and in mixture, might reveal their role in attraction of *H. indica* and *S. carpocapsae* to *M. fimbriolata*-damaged roots.

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Poster number: *153*

# Unraveling the role of phenylphenalenones as key metabolites in *Musa* plants against the attack by the pathogen *Mycosphaerella fijiensis*

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Bananas are among the most important crops worldwide since they not only represent a staple food but also the major economical income for many producing countries. However, the production of commercial cultivars of *Musa* (banana) belonging to the Cavendish subgroup is enormously affected by insects, nematodes and microbial infections. The Black Sigatoka Disease (BSD), caused by the ascomycete fungus *Mycosphaerella fijiensis*, is considered the most detrimental disease of this crop [1, 2]. Despite the attempts of rearing genetically modified *Musa* plants resistant to this pathogen, the use of fungicides still remains the only available method to control this disease. Understanding how the pathogen recognizes its host and how the plant responds to the pathogen attack constitute the major challenge in order to address this problem in a more ecological friendly way. Previous studies have shown that the expression of pathogenesis related proteins, morphological modifications and the production of a class of secondary metabolites named phenylphenalenones (PP's) constitute the mean defense mechanisms in the *Musa* genus [3, 4]. Indeed, due to the antimicrobial and nematicidal properties of PP'-type- metabolites, they have been considered for playing an important role in the resistance displayed by some *Musa* plants against pathogens and herbivores [4, 5]. Hereby, a chemical phenotyping approach based on NMR and HPLC analysis was conducted in two *Musa* varieties, Khai Thong Ruang (resistant) and Williams (susceptible), for exploring the differential, spatial and temporal expression of the major phenylphenalenones triggered during the BSD. The results show clearly that the resistant variety is able to recognize the pathogen in an early stage of the disease whose chemical response involves a large pool of PP's; instead, the susceptible variety had a late response and a weak chemical profile highlighting the relevance of PP's as chemical weapons from *Musa*.

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Poster number: *154*

# Differential responses of egg and adult stink bug parasitoids in relation with host sex pheromones

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Stink bug parasitoids that attack egg and adult stages deal with very different host characteristics suggesting that they use different foraging strategies. In this work we evaluate and compare the foraging behaviour of an adult parasitoid *Hexacladia* sp. (Hymenoptera: Encyrtidae) and of two egg parasitoids, *Telenomus podisi* and *Trissolcus basalis* (Hymenoptera; Platygastridae), in relation to the sex pheromone of three stink bug species (*Euschistus heros*, *Nezara viridula* and *Piezodorus guildinii*). Although sex pheromone are released, in general, in very tiny amounts they are a reliable identity cue directly related to the presence of the host on the habitat. Considering the host stage used by each parasitoid and the preference for host observed in the field (*Hexcladia* and *T. podisi, E. heros* and *T. basalis, N. viridula*) our hypothesis was: as adult egg parasitoid searching for the stage that produce the pheromone its responses to sex pheromone may be stronger and related with the host preferences. Y-olfactometer bioassays were performed using synthetic solutions of each sex pheromone as treatment and solvent (n-hexane) as control. The initial choice and residence time in each arm of the olfactometer were analyzed. Females of *Hexacladia* sp. were attracted preferentially to the pheromone of their preferential host *E. heros*. Egg parasitoids showed non selective responses toward host sex pheromone. *T. podisi* showed attraction to *E. heros* ad *P. guildinii* and *T. basalis* to *E. heros* and *N. viridula* sex pheromones. Results show that host sex pheromones are reliable cues for parasitoids of adults and they use this cues for both searching and selection behaviours. In contrast egg parasitoid may use sex pheromone as an alternative or complementary cue in relation to the use of others cues during foraging behaviour and their responses are not related with host preference.

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Poster number: *155*

# The influence of volatiles from stink bugs eggs extracts and oviposition damage plants on the foraging behavior of the egg parasitoid *Telenomus podisi*


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At each stage of host selection, physical and chemical stimuli can act as important cues to change search behavior. The aim of this study was to evaluate on *Telenomus podisi* foraging behavior the influence of volatiles released by soybean plants treated with egg extracts of the stink bug *Euschistus heros* and also to the egg extracts individually. In Y-olfactometer, the response of *T.podisi* to volatiles of different treatments were evaluated: 1) plants treated with egg extracts (hexanic and acetonic), 2) plants with eggs laid naturally by the stink bug, 3) plants with eggs laid artificially, all tested in periods of 24, 48 and 72h after treatments, 4) extracts of 1 gram (~2000 eggs) and from an egg mass (~10 eggs) and 5) one egg mass (~10 eggs) with 24 h. *Telenomus podisi* was attracted to volatiles emitted by one egg mass contrasted with air, to acetonic extract of one egg mass compared with acetonic extract with one gram of eggs and also to the acetonic extract of one egg mass compared with air. The results suggest that the oviposition itself and also the acetonic and hexanic extracts when applied on the plants, did not cause changes of plant odor that are attractive to the egg parasitoids, but one cluster of eggs and the acetonic extract of this cluster have volatile compounds that change *T. podisi* foraging behavior.The chemical analysis of the extract from one cluster of eggs using GC-FID and GC-MS did not show the presence of quantifiable compounds, but several volatile compounds were identified from one gram of *E. heros* eggs. These compounds are mainly monoterpenes, short chain carboxylic acids and methyl and ethyl esters with 16 to 18 carbons. The function of these compounds in the foraging behavior of the egg parasitoid is been elucidated.



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Poster number: *156*

# Cyanogenic glucosides in *Heliconius* butterflies: Sequestration, de novo biosynthesis and specialization


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*Heliconius* larvae feed exclusively on *Passiflora* leaves and both plants and insects are cyanogenic, due to the presence of cyanogenic glucosides in their tissues. It has been suggested that the ability to synthesize the aliphatic cyanogenic glucosides, linamarin and lotaustralin from the amino acids valine and isoleucine, is a common trait of all *Heliconius* species. In contrast, only *H. sara*, *H. sapho* and *H. hewitsoni* were described to be able to sequester from their food plant, epivolkenin, a cyanogenic glucoside derived from non-proteic amino acid cyclopentenylglycine, (Engler et al., 2000). That study analysed the cyanogenic profile of eight different *Heliconius* species. Most of the species contained linamarin and lotaustralin, but not *H. sapho* and *H. hewitsoni*. Instead, high amounts of epivolkenin was detected in these species, suggesting that both may be losing the ability to synthesize linamarin and lotaustralin and becoming more specialized in the sequestration of epivolkenin from their host plants. Epivolkenin was also found in *H. erato* and *H. charithonia*, indicating that other *Heliconius* species are also able to sequester this cyanogenic glucoside. Another cyanogenic glucoside, dihydrogynocardin which is also cyclopentenylglycine-derived, was found in species where epivolkenin was not present (*H. melpomene*, *H. hecale* and *H. cydno*). Since the sequestration of gynocardin from Passifloraceae by *Acraea* larvae has been proposed by Raubenheimer (1989)), it is possible that the dihydrogynocardin present in *Heliconius* was sequestered from the host plant. Our results suggest that sequestration of cyanogenic glucosides is just as widely distributed in *Heliconius* species as synthesis, contrary to earlier suggestions. Apparently, *Heliconius* larvae may obtain cyclopentenyl cyanogenic glucosides produced by plants from non-proteic aminoacids, whereas aliphatic ones are synthesized from proteic aminoacids, indicating that the insects have evolved elaborate abilities to handle cyanogenic glucosides.



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Poster number: *157*

# Leaf quality discrimination in butterflies

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It is generally known that insects use visual, olfactory and gustatory cues to find and evaluate targets such as food sources or host plants, but the particular role of each of the sensory modalities in the search process is less clear. One trait affecting the efficiency and accuracy of the host search process is host plant breadth. For example, species with a broader diet breadth seem to pay a cost through decreased oviposition accuracy. In an assay of behavioral choice in five butterflies with different host range - which all include *Urtica dioica* in their diet – we found variation in preference for plants that differ in quality. Larvae of all species grew slower and to smaller size on poor quality leaves. In female choice experiments, the generalists, among them *Polygonia c-album*, did not show any preference for high quality leaves, whereas two of the specialists *Aglais io* and *A. urticae*, preferred high quality leaves. However, in a behavioral assay of the olfactory abilities of *A. urticae* and *P. c-album* females we did not find any preference for the odour of high or low quality host plant leaves. This was especially surprising, since females of the specialized species made such fine-tuned differentiation when they were allowed to come in contact with the plant. These results suggest that there may be a limit to how much information these butterfly species are able to extract from volatile olfactory cues and that host plant choice at a fine-tuned level requires more than olfactory information, even for adapted specialized species.

Poster number: *158*

## Pear ester-based lure attracting a butterfly pearly heath (*Coenonympha arcania* L.)

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During field testing of pear ester [ethyl (*E,Z*)-2,4-decadienoate] and acetic acid in Hungary, significant catches of the pearly heath (*Coenonympha arcania* L.; Lepidoptera: Nymphalidae) were recorded.

In consecutive years it was confirmed that the presence of both pear ester and acetic acid are needed for full field activity in contrast to some other satyrid butterflies which responded only to acetic acid. Consecutive EAG tests revealed that high amplitude responses were evoked from antennae of male and female *C. arcania* by pear ester, further confirming the field results.

Trapping nymphalid butterflies with fermented sugar solutions has been documented (Platt 1969) and the attraction of *C. arcania* to acetic acid - which is a microbial fermentation product in sweet baits - could be explained on this basis. However, it is an interesting question why pear ester - which is the volatile compound from ripe pear and apple - could be detected by a butterfly, which is not known to feed on fruits and flies in spring, when ripe fruits are scarce in nature.

As *C. arcania* is a popular model insect in ecological research (Binzenhofer et al. 2005), an effective synthetic bait could become a useful tool for these studies.

Binzenhofer B., et al. (2005) Biol. Cons. 126: 247-259.

Platt A.P. (1969) J. Lepid. Soc. 23: 97-101.

Poster number: *159*

# The influence of chemical and vibrational communication in the mating behaviour of *Dichelops melacanthus* (Heteroptera: Pentatomidae)

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Y-tube olfactometer bioassays using live *Dichelops melacanthus* as odour source showed that females responded to males (p=0.006), but they did not respond to other females, and males did not respond to odour emitted by either males or females. Chemical analysis of the air-entrainment extracts from both genders of *D. melacanthus* showed the presence of 18 compounds in both sexes, and in a few samples from males, five male-specific compounds were identified. Females were attracted to male air-entrainment extracts, without or containing traces of the male specific compounds (p=0.003), but they were not attracted to the male air-entrainment extracts containing quantifiable amounts of these compounds (p=0.02). The male crude extract that attracted females was fractionated, and four different fractions were obtained and tested in Y-tube olfactometer bioassays: fraction A containing hydrocarbons, fraction B containing as major compound (*E*)-2-octenyl acetate, fraction C containing as major compound (*E*)-2-octenal and fraction D containing as major compound linalool. *D. melacanthus* adults did not respond to any of these fractions, but a marginal response was obtained to the fraction containing linalool. Therefore, solutions containing a racemic linalool, and the *R* and *S* isomers were evaluated. Males (p=0.01) and females (p=0.01) of *D. melacanthus* responded to (*R*)-linalool when tested in a similar amount released by one insect. In arena bioassays, females were attracted to males (p=0.01) and males were attracted to females (p<0.001). In these bioassays, vibrational signals were recorded from females and these signals are propagated through the arena surface. To evaluate the influence of the signals on male responses to females, bioassays were repeated with males without antenna, and these males were attracted to females (p < 0.001). Studies are been conducted to find out the importance of the vibrational and chemical signals to the mating behaviour of *D. melacanthus*.

Poster number: *160*

# Ecological role of Ewingella sp. KTH G3-2 with pine weevil

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The pine weevil, *Hylobius abietis* L., is one of the economically most important pests in reforestation areas in large part of Europe and Asia. The female weevil excavates a cavity in the root bark with her snout and places an egg, adds her feces and closes the cavity with chewed bark. We hypotezise that the microbial community in frass and feces, comprised of yeast and filamentous fungus, function as tools to protect the eggs from feeding conspecifics as fungal metabolites has been found to mask the host plant odor for the pine weevil (1). In addition, an isolated bacteria from gut of the pine weevil, was identified as *Ewingella* sp. KTH G3-2 (NBRC109474, G3-2) based on a rRNA sequence, and converted ferrulic acid to to 4-vinyl guaiacol with antifeedant properties and produced ammonia on a medium included 0.5% glucose and yeast extract, pH7.0, G3-2. Whether this ammonia production is as a result of nitrogen fixation is unknown.

This presentation focus on confirming the nitrogen fixing ability of G3-2. For this aim we used primers prepared based on nifH gene from *Rahnella aquatilis*, a related species that has been reported present in the gut of various beetles. Also, acetylene reduction assay was performed to determine the nitrogen fixation capacity. G3-2 could grow well on nitrogen less medium, WAT4, but acetylene reduction activity of G3-2 on WAT4 medium with 10% acetylene concentration of head space of culture was very low. Elucidation of nitrogen fixation system of G3-2 is currently in progress.

A fungal metabolite masks the host plant odor for the pine weevil (Hylobius abietis) Fungal Ecology, 13, 103-111, 2015

Poster number: *161*

# MS in chemical ecology: From characterization of oxylipins to their role in plant-microbes interactions

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Mass spectrometry is a powerful technique for both identification and quantification of bioactive molecules, particularly when they are present in small amounts and in complex samples. These features make mass spectrometry a useful tool in chemical ecology studies. In the present work, we used high throughput screening analysis in order to characterize and identify the antibiotic oxylipins present in the Brazilian medicinal plant *Alternanthera brasiliana*. A bioguided-assay fractionation and HPLC-HRMS analysis leaded to the identification of 17 antibiotic oxylipins (oxidized octadecanoic acids).1 These compounds are mainly identified in the *A. brasiliana* stem, which is also colonized by a large number of endophytic bacteria. These oxidized fatty acids are well known as stress response compounds to both physical damage and infection. Moreover, they have also been reported as bacterial metabolites.2 Therefore, in order to understand the role of these oxylipins in plant-microbes interaction we developed and validated an HPLC-MS/MS method to accurately quantify oxylipins in different plant tissues. The quantification of both fatty acids and bacterial cells strongly suggest that the higher is the concentration of bacterial cells higher is the amount of some oxidized fatty acids present in these tissues. We are now studying bacterial metabolism and performing re-inoculation experiments to understand the biosynthetic origin of such oxylipins. These results show the great potential of mass spectrometry to identify new compounds involved in ecological interactions and understand their ecological function.

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# Quality control: Honeybee workers assess queens by pheromonal and genetic correlates of quality

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One of the theories explaining worker sterility in social insects is worker self-restraint, which supposedly must be advantageous in terms of inclusive fitness. Therefore the most important condition underlying a worker's decision to forego reproduction is her ability to assess the quality of the queen – the individual to whom reproduction is conceded. Our study intended to test such ability in honeybee workers. We exposed groups of worker to pairs of queens, separated by a queen excluder, which differed in reproductive status and examined their attitude to each queen and measured presumed correlates of each queen quality, i.e., reproductive, pheromonal and genetic properties. Our results indicate that workers show attention preference toward queens with higher reproductive potential, and that display pheromonal and genetic parameters indicative of higher quality. These included queens that secreted higher amounts of QMS and of esters in the Dufour's gland (conveying information about quality), queens exhibiting higher expression of vitellogenin – a protein correlated with higher fertility and longevity, as well as higher expression of alcohol dehydrogenase – an enzyme participating in the QMP biosynthesis. Summarily, our findings suggest that worker possess an ability to distinguish between queens of different qualities in terms of reproductive potential, health and longevity, and that pheromonal composition plays an important role in such distinction.

Poster number: *163*

## **Antifeedant and toxicity properties of selected Malaysian plants towards termite and bioactives compounds**

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Dual choice bioassays were used to evaluate the antifeedant property of essential oil and methanolic extract of *Alpinia galanga* against two species of termites, *Coptotermes gestroi* and *Coptotermes curvignathus* (Isoptera: Rhinotermitidae). A four centimeter diameter paper disc treated with *A. galanga* essential oil and another treated with either methanol or hexane as control were placed in a petri dish with ten termites. Mean consumption of paper discs (mg) treated with 2000 ppm of essential oil by *C. gestroi* was 3.30 ± 0.24 mg and by *C. curvignathus* was 3.32 ± 0.24 mg. *A. galanga* essential oil showed significant difference in antifeedant effect, 2000 ppm of *A. galanga* essential oil was considered to be the optimum concentration that gave maximum antifeedant effect. The essential oil composition was determined using Gas Chromatography-Mass Spectrometry (GC-MS). The major component of the essential oil was 1,8-cineol (61.9%). Antifeedant bioassay using 500 ppm of 1,8-cineol showed significant reduction in paper consumption by both termite species. Thus the bioactive agent in *A. galanga* essential oil causing antifeeding activity was identified as 1,8-cineol. Hence 1,8-cineol may be developed as an alternative control against termite in sustainable agriculture practices.

# Selection on male sex pheromone composition contributes to butterfly reproductive isolation

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Selection can facilitate diversification by inducing character displacement in mate choice traits that reduces the probability of maladaptive mating between lineages. Although reproductive character displacement (RCD) has been demonstrated in two-taxa case studies, the frequency of this process in nature is still debated. Moreover, studies have focused primarily on visual and acoustic traits, despite the fact that chemical communication is probably the most common means of species recognition. Here we showed in a large, mostly sympatric, butterfly genus, a strong pattern of recurrent RCD for predicted male sex pheromone composition but not for visual mate choice traits. Our results suggest that RCD is not anecdotal, and that selection for divergence in male sex pheromone composition contributed to reproductive isolation within the *Bicyclus* genus. We propose that selection may target olfactory mate choice traits as a more common sensory modality to ensure reproductive isolation among diverging lineages than previously envisaged.

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Poster number: *165*

## Searching for food in complex environments: Using laboratory approaches to test for associational effects

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Interactions between consumer and resource organisms are fundamental components of ecological communities. Quite frequently neighboring organisms are able to influence consumer-resource interactions through associational effects. For example, the presence of a specific plant neighbor can decrease or increase insect attraction to a focal plant. Within plant-insect studies it is well known that neighboring plants often affect the strength of interactions between individual plants and their herbivores. However, how processes at between- and within-patch scales interact and determine attack rates on different plant species is not fully understood yet. By conducting laboratory experiments with *Drosophila melanogaster* we try to determine how spatial arrangements of qualitative and quantitative different resources can lead to associational effects between focal and neighboring resources. Firstly, we tested whether *D. melanogaster* showed different attraction rates to odor sources released from three distances. We found that the relative detection distance decreased with increasing dilutions of a quantitative odor source. Using this information we will create patches with different odor point sources to test for associational effects between the different resources. We expect that the outcome of these associational effects depend on traits of both the consumer and the resource organism.

Poster number: *166*

# Components of the sex pheromone of blackcurrant sawfly, *Nematus olfaciens* (Diptera: Tenthredinidae): Novel isopropyl esters and the role of hydrocarbons

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Blackcurrant sawfly, *Nematus olfaciens* (Diptera: Tenthredinidae), is a common and frequently damaging pest of blackcurrant, probably present to varying degrees in all UK blackcurrant plantations. Infestation is sporadic and localised, and damage can occur rapidly. No practical, systematic sampling methods or attendant crop damage thresholds have been developed, and pheromone traps could provide such a tool.

Previous work on related sawfly species has suggested that breakdown products of cuticular hydrocarbons act as components of the female sex pheromone. We found that male and female blackcurrant sawfly produce large quantities of identical suites of long-chain saturated and unsaturated hydrocarbons, of which (*Z*)-9-tricosene is the most abundant. Analysis of collections of volatiles from virgin females by gas chromatography linked to electroantennographic recording from the antennae of males showed no EAG responses to the hydrocarbons but very strong responses to at least three compounds present in trace quantities. These were identified as mono-unsaturated isopropyl esters, and the synthetic compounds also elicited strong EAG responses from male blackcurrant sawfly. No EAG responses were observed to likely breakdown products of the unsaturated hydrocarbons. In field tests, blends of the isopropyl esters attracted male blackcurrant sawfly and addition of (*Z*)-9-tricosene further increased the attractiveness.

These results suggest a role for cuticular hydrocarbons that is somewhat different from that proposed for related sawfly species.

Poster number: *167*

# Do odours mediate innate host plant preferences in adult female *Spodoptera* littoralis?

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Host plant preferences by herbivorous insects are of great ecological and evolutionary importance. In the generalist herbivore *Spodoptera littoralis*, adults display an innate preference hierarchy among a series of host plant species (Thöming 2013), where cowpea is most preferred, cotton intermediate and cabbage the least preferred. However, the sensory mechanisms behind these preferences are still barely understood. What is the relative importance of different sensory modalities (olfaction, vision, taste, touch), how do they interact?

Here, we investigate the role of olfaction in mediating host-plant preferences by naïve *S. littoralis* females: Can plant odours alone influence oviposition behavior? Are they enough to reproduce the host species preference hierarchy observed when females are offered real plants to oviposit on?

We perform dual choice cage oviposition experiments where mated *S. littoralis* females are separated from the stimulus plant(s) by an opaque cloth screen – they can smell them but neither see nor touch them. When females are offered a cowpea plant against a control, they lay more eggs close to the plant than close to the control, suggesting that odours on their own are enough to influence oviposition by *S. littoralis*. We expect cotton versus control, and cabbage versus control, respectively, to confirm this trend. Results from dual choices between two different plant species will be compared to the results previously obtained when giving females access to the plants (Thöming 2013). Any difference would indicate that odours are not enough to explain innate host plant preferences. In parallel, electrophysiologically active compounds from these three plant species, which could potentially mediate host plant preferences, are being identified.

Thöming, G., Larsson M.C., Hansson B.S., Anderson P. (2013) Comparison of plant preference hierarchies of male and female moths and the impact of larval rearing hosts. Ecology, 94(8) 1744–1752

Poster number: *168*

## Transcriptional regulation of the flavonoid biosynthesis in *Picea abies*

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Flavonoids are specialized metabolites that fulfill multiple functions in plants including defense against pathogens. In *Picea abies* flavonoids play a central role in constitutive and induced defense against economically important pathogens like the root- and stem-rot fungus *Heterobasidion annosum* s.l. Flavonoids such as catechin are synthesized in response to fungal infection limiting the spread of the pathogen. In the model plant *Arabidopsis thaliana*, but also in woody species like poplar and grape, gene expression in the flavonoid biosynthetic pathway is known to be regulated by specific transcription factors (TFs), often acting in complexes, such as the MYB/bHLH/WD40 TF complex.

Our aim was to identify TFs that control flavonoid biosynthesis in *P. abies* in response to *H. annosum* s.l. infection. RNAseq databases and the *P. abies* genome were searched for potential members of this TF complex. Eleven candidate TF genes (one WD40, three bHLH and seven MYB genes) were identified in silico and cloned from *P. abies* cDNA. Candidates were characterized by protein interaction studies using yeast-two-hybrid assays, expression analyses, chemical profiling and genetic transformation of *P. abies* cell lines. The results from these experiments will be discussed.

Poster number: *169*

## Functional adaptation of secretory proteins in the detoxification of sequestered phytochemicals during Chrysomelina leaf beetle evolution

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The ubiquitous consumption of plants by insect herbivores requires in many cases detoxification of noxious phytochemicals. One of the most ingenious detoxification strategies widespread in insects is sequestration. Frequently, sequestered compounds are not only stored securely by the insects, but further; insects evolved the ability to use exogenous compounds for their own benefit within the competitive interactions in natural ecosystems. Very often these compounds are further enzymatically converted to adapt their biological activity. For example, the larvae of the leaf beetle subtribe Chrysomelina, transform plant derived glucosides into deterrents in defensive secretions. Phyologenetic analyses of selected species of this subtribe revealed that the phenomenon of sequestration is predated by the de novo synthesis of iridoids. Here we ask the question, how did the protein function in the defensive secretions of the different biosynthetic strategies adapt during the Chrysomelina evolution? The transformation involves the hydrolysis of glucosides and an oxidation reaction in both, iridoid de novo synthesis and sequestration. The kinetic parameters of the glucosidases demonstrated substrate selectivity which reflects the adaptation of Chrysomelina to the chemistry of their host plants during the course of evolution. But at the same time it allowed building a chemical defense which is dependent on the host, but did not lead to an “evolutionary dead end”. Unlike the glandular ß-glucosidases, the GMC oxidoreductases have a narrow substrate spectrum. The evolution of host independent chemical defence followed by a shift to host dependent chemical defence in Chrysomelina beetles coincided with independent recruitments of genes from different GMC subfamilies. It seems that the substrate diversity in redox reactions supplied by this multi-gene family equips insects with a toolbox that allows them to adjust to the particular biotic and abiotic conditions that may result, for example, when shifting host plants.

Poster number: *170*

## Predation threat cues as adjustment variables for the expression of self-maintenance behaviours in terrestrial gastropods


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Predation is long been recognized as a major selective force that drives evolution of organisms, shaping their life history traits, morphology, ecology and behaviours (Lima & Dill, 1990). Thus, cues that allow preys to avoid predators without direct contact with them appear particularly suitable to enhance their survivorship (Kats & Dill, 1998). In this regard, chemical cues detection that betrays the presence of predators have been recognized efficient and are well documented in numerous taxa. However, data are still lacking for many organisms, even among those whose main activities and biological interactions are chemically mediated, as in terrestrial gastropods.

Over a series of trial choice experiments, we assessed the modification of self-maintenance behaviours in one pest slug and one pest snail species to cuticular compounds from predatory ground beetles species. In a first experiment, the slug *Deroceras reticulatum* spent significantly more time in the control shelter than in the shelter treated either with *Carabus nemoralis* or *Carabus coriaceus* cuticular extracts (Z=2,43 ; p=0,0151 and Z=3,31 ; p<0,01 respectively, Wilcoxon matched-pairs signed-ranks test). In a second experiment, cues from *Carabus nemoralis* significantly impaired food intake of *D. reticulatum* compared to a control (Z=2,19 ; p= 0,028 Wilcoxon matched-pairs signed-ranks test). In a third two-choice test, *Xeropicta derbentina* avoided to climb on the vertical area treated with chemical cues from the ground beetle *C. morbillosus* (p=0,019 ; Student t-test). These results are supported by eco-evolutionary concepts of predator-prey relationships (Cox & Lima, 2006) and open up new perspectives for the management of pests' species.



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Kats, L. B., & Dill, L. M. (1998). The scent of death: chemosensory assessment of predation risk by prey animals. Ecoscience, 5(3), 361–394.

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Poster number: *171*

# Tick Attack Inhibition Semiochemical (TAIS): An innovative and promising approach against *Rhipicephalus sanguineus*

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Rhipicephalus sanguineus is one of the main urban tick pest spread worldwide, able to transmit several diseases affecting both animals and humans (Gray et al 2013; Uspensky, 2008). The indiscriminate use of acaricides over time has resulted in the selection of resistant ticks and increased environmental pollution (Dantas-Torres, 2008). New approaches to fight this pest should be developed. The aim of this study was to assess, using an attractive device, the Tick Attack Inhibition Semiochemical (TAIS) from a non-attacked host, regarding the host-seeking behaviour of Rhipicephalus sanguineus.

A controlled and blinded study on parallel groups was carried out. In order to perform the test, a warm artificial attractive host was developed, increasing the tick's motivation associated with the host-seeking behaviour. The device's attachment surface received 2 treatments: a 5% TAIS ethyl solution or a control (pure ethanol). 30 ticks per treatment successively passed the test for a maximum of 2 minutes. We compared the number of ticks climbing the cylinder between the 2 groups and we assessed the attraction inhibitory effect (AIE) with the formula AIE=100
non-inhibited ticks; t: treatment; c: control).

Regarding the number of climbing ticks, a statistically significant difference was observed (chi-square test df=1 $\chi^2$=32.4 p<0.0001). The TAIS' attraction inhibitory effect was of 81.5%.

These results show that the tested semiochemical disturbs the host-seeking behaviour in *Rhipicephalus sanguineus* and so confirm the possibility to use semiochemistry as a promising strategy in the fight against this tick but also in controlling pest species.

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Uspensky, I. (2008). Ticks (Acari: Ixodoidea) as urban pests and vectors with special emphasis on ticks outside their geographical range. In Proc. 6th Int. Conf. Urban Pests, Hungary (pp. 333–348).

Poster number: *172*

## Sniffing out the enemy: Fungus-growing termites can differentiate between mutualistic and parasitic fungi using volatiles

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Fungus-growing termites *Odontotermes obesus* farm a mutualistic fungus *Termitomyces* inside their nests for food and keep the fungal gardens free from parasitic fungi such as *Pseudoxylaria*. Nest microclimate, antifungal compounds of termite origin, antibiotic-producing bacteria, and weeding by termites have been proposed as mechanisms to control the growth of *Pseudoxylaria* inside the nest and thus to maintain the obligate mutualism between termites and *Termitomyces*. Whether termites use antibiotic compounds or weeding, it is important that they are able to differentiate between the two fungi so that they can selectively use the antifungal mechanism—whether chemical or behavioural—against *Pseudoxylaria*. Therefore, in the present study we examined whether termites demonstrate a differential response towards the two fungi. We designed a novel laboratory-based dual choice assay for this purpose. Additionally, when termites are allowed to smell but not to physically access the fungi, we investigated if they can still differentiate between the mutualistic and parasitic fungi. Our results show that termites can use olfactory cues for this task. We also present results on the volatile profiles of the two fungi.

Poster number: *173*

## Characterization of sex pheromones in the digger wasp genus *Cerceris*

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Males of the digger wasp genera *Cerceris* are known to use long-range sex pheromones to attract conspecific females. Male wasps establish small territories by applying a cephalic gland secretion on the vegetation or soil. Female wasps approach these territories and mate in or in the near vicinity of these territories. To function as a potential barrier against hybridization these sex pheromones should be species specific. In order to test this prediction we plan to identify the composition of the cephalic gland secretions of several sympatric *Cerceris* species. Therefore, secretions were collected from male heads of *C. arenaria*, *C. interrupta*, *C. quadrifasciata*, *C. quinquefasciata*, *C. rybyensis*, and *C. sabulosa* and analyzed using gas chromatography coupled with mass spectrometry. Qualitative and quantitative differences in the composition of these secretions could be detected and several terpenes were identified. Unknown components are likely to be dioxygenated bishomomonoterpenes, based on derivatizations and characteristic mass-spectrometric fragmentation. To prove the structural assignments, a synthetic library of various analogs was generated and compared with the natural compounds. A first major component could be identified using this method.

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Poster number: *174*

## Why don't *Chrysopa* green lacewings make pheromone in the lab?

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Field-collected male goldeneyed lacewings, *Chrysopa oculata*, release (*1R,2S,5R,8R*)-iridodial (1) with comparable amounts of nonanal, nonanol and nonanoic acid (Zhang et al. 2004); 1-baited traps attract males into traps, and females to the vicinity (Chauhan et al. 2007). Laboratory-reared *C. oculata* males do not produce 1, despite their healthy appearance, normal fertility, and usual amounts of C9 compounds. Observation that males caught in 1-baited live-traps tried to eat the lure (Q-HZ), combined with previous reports of *Chrysopa* spp. eating *Actinidia polygama* (Actinidiaceae), prompted one of us (JRA) to further investigate this phenomenon. Males fed synthetic aphid pheromone component, (*4aS,7S,7aR*)-nepetalactone (2), converted ~75% to dihydronepetalactone (3), but did not produce 1. (Wild *C. oculata* males collected in May often contained traces of 3.) Only males fed the second common aphid pheromone component, (*1R,4aS,7S,7aR*)-nepetalactol (4), totally converted this to 1. In California the peak late-season attraction of green lacewings to 4 (2 is unattractive) occurs at least a month earlier than the peak in aphid oviparae (Symmes 2012), consistent with the hypothesis that *Chrysopa* males feed on oviparae to obtain 4 as a precursor to 1. Adult males from laboratory-reared *C. oculata* larvae fed 4 failed to produce 1, and wild *C. oculata* males collected early in the spring produce less 1 than males collected later in the season. Therefore, we further hypothesize that Asian Chrysopa eat *A. polygama* to obtain iridoid precursors in order to make their pheromone, and that other iridoid-producing plants elsewhere in the world are similarly usurped by male *Chrysopa* species to sequester iridoid pheromone precursors, analogous to pharmacophagous feeding of male *Bactrocera* fruit flies (Tephritidae). Whether or not sequestration of precursors to 1 from oviparae and/or iridoid-containing plants is truly the explanation for lack of pheromone in laboratory-reared *Chrysopa* awaits further research.

Chauhan, K. R., V. Levi, Q.-H. Zhang, and J. R. Aldrich. 2007. Female goldeneyed lacewings (Neuroptera: Chrysopidae: *Chrysopa oculata*) approach but seldom enter traps baited with the male-produced compound, iridodial. J. Econ. Entomol. 100: 1751-1755.

Symmes, E. J. 2012. Improving Management of Mealy Plum Aphids (*Hyalopterus pruni*) and Leaf-Curl Plum Aphids (*Brachycaudus helichrysi*) in Dried Plum Orchards Using Sex Pheromones. Ph.D., Ph D thesis, Univ. Calif., Davis ProQuest LLC, Ann Arbor, MI.

Zhang, Q.-H., K. R. Chauhan, E. F. Erbe, A. R. Vellore, and J. R. Aldrich. 2004. Semiochemistry of the goldeneyed lacewing *Chrysopa oculata* (Neuroptera: Chrysopidae): Attraction of males to a male-produced pheromone. J. Chem. Ecol. 30: 1849-1870.

Poster number: *175*

# Male sex pheromones in *Ceratitis* FAR complex

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Fruit fly males attract females to mate by means of multimodal signals including sex pheromones. To assess a possible role of pheromone communication in pre-mating isolation and speciation, we studied the composition of male sex pheromones and female antennal sensitivity to pheromone components in African fruit flies referred to as *Ceratitis* FAR complex (*Ceratitis fasciventris*, *Ceratitis anonae* and *Ceratitis rosa*).

The male emanations were collected on Super-Q absorbent from adult males using dynamic head space collection technique. After the collection, volatiles were rinsed out by hexane and samples were analyzed by means of two-dimensional gas chromatography with time-of-flight mass spectrometry (GC×GC-TOFMS) followed by multivariate analyses. Female sensitivity and specificity was assessed by means gas chromatography coupled with electroantennographic detection (GC-EAD).

The GC×GC-TOFMS data revealed that male sex pheromones are highly specific complex mixtures that significantly differed in the compared populations both qualitatively and quantitatively. The GC-EAD data showed that only small portions of compounds present in male pheromone emanations are important for pre-mating communication.

High specificity of both pheromone composition and female perception suggest that pheromones play important role in the development of pre-mating isolation in fruit flies. Our data do not exclude a possible presence of more than three taxonomic entities within *Ceratitis* FAR complex as suggested by recent large scale genetic analysis of many *Ceratitis* FAR complex populations [1].

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1.Virgilio M, Delatte H, Quilici S, Backeljau T, De Meyer M (2013) Molecular Ecology 22: 2526-2539.

Poster number: *176*

# Male sex pheromones in Ceratitis FAR complex (Diptera: Tephritidae)

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Insect pheromones are species-specific and are considered an important part of pre-mating isolation associated with speciation. In fruit flies, the pre-mating behavior is initiated by male sex pheromones that attract females to mate. *Ceratitis fasciventris, C. anonae* and *C. rosa* are Afrotropical tephritid fruit flies considered to form a cryptic species complex, called the *Ceratitis* FAR complex. To determine the taxonomical relationships within the *Ceratitis* FAR complex, we studied male sex pheromones of four available genetically different populations from Kenya [1]. Specifically we investigated two populations of C. rosa, and one population each of C. anonae and C. fasciventris.

Pheromones were collected by dynamic headspace technique from adult males (24 hrs, Super-Q absorbent, hexane elution). Samples were analyzed by two-dimensional gas chromatography with time-of-flight mass spectrometry (GC×GC-TOFMS) followed by multivariate analyses. Female sensitivity and specificity were assessed by gas chromatography coupled with electroantennographic detection (GC-EAD).

The GC×GC-TOFMS analysis followed by multivariate analysis revealed that male sex pheromones are complex mixtures that significantly differ both quantitatively and qualitatively in the compared populations. The GC-EAD analysis showed that only a small set of identified compounds are perceived by female antennae, which are specifically tuned to conspecific pheromones.

Our data suggest that sex pheromone composition and perception in the studied fruit fly populations are specific and thus can contribute to pre-mating isolation.

Virgilio M, Delatte H, Quilici S, Backeljau T, De Meyer M (2013) Molecular Ecology 22: 2526-2539

Poster number: *177*

## Butterfly pheromone precursors present in flower odors and nectar

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Flower nectar is the main source of nutrition for adult butterflies. Here we investigate the importance of flower volatiles and nectar constituents for the biosynthesis of pheromone components in adult butterflies. Chemical analyses of volatiles released by flowers and nectar as well as identification of aromatic amino acids in nectar were made on two crucifer species *Alliaria petiolata* and *Bunias orientalis*. Floral volatiles were collected by solid phase microextraction (SPME) and identified by gas chromatography mass spectrometry. The identification of amino acids in nectar were made after dervatisation and subsequent analyses by LC/MSMS.

*A. petiolata* flowers mainly released benzaldehyde, methyl benzoate, methyl salicylate, benzyl cyanide, and formotoluidide whereas floral volatiles of *B. orientalis* comprised mainly of 2-phenylethanol, phenylacetaldehyde, and benzylalcohol. Incorporation of stable isotopes from $^{13}$C6-labelled [L-ring-$^{13}$C6] phenylalanine in the flower volatiles of *A. petiolata* and *B. orientalis* showed that 7 and 6 compounds respectively, contained labelled atoms. Male *Pieris napi* butterflies fed on these flowers incorporated the $^{13}$C labelled aromatic ring into methyl salicylate, the antiaphrodisiac pheromone.

Our results show that this method could be used ifor detailed studies of resource allocation and biosynthesis of pheromones in butterflies when feeding on flower nectar before and after mating.

Poster number: *178*

# Diffusion of (*E*)-β-caryophyllene in different soil textures and its effects on the foraging behavior of the entomopathogenic nematode *Heterorhabditis megidis*

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Maize roots respond to herbivore feeding by releasing (*E*)-β-caryophyllene (Eβc), which attracts entomopathogenic nematodes (EPNs). Previous studies showed that Eβc is a suitable belowground signal in sandy systems, but its diffusion in typical agricultural soils remains largely unknown. Because soil texture drives the pore-size we hypothesized that both Eβc diffusion and EPN attraction will be reduced when sand content decreases. To test this we evaluated Eβc diffusion and EPN foraging success in three soil types.

Using gas chromatography-mass spectrometry analyses diffusion of two doses of synthetic Eβc (200 ng and 20000 ng) injected in sand was compared with diffusion in clay, clay-loam and sandy-loam soils, at 3 moisture levels (5, 10 and 20% water) 5 or 10 cm from an Eβc source. In subsequent experiments we used glass-tray mesocosms to assess migration of the EPN Heterorhabditis megidis toward 1) an Eβc-releasing capillary near larvae of *Galleria mellonella* and 2) insect-damaged maize plants that produce Eβc (var. Graf) or not (var. Pactol).

In agricultural soils, Eβc was detected at the high dosage, but only at 5 cm from the injection point. Best detection was at 20% moisture. Higher larval mortality due to EPN infection was found in sand and sandy-loam soil, and in sand more EPNs reached the rhizosphere of maize var. Graf than the one of var. Pactol. However, Eβc presence/production did not affect EPN movement in any of the soils.

The results imply that in real soils diffusion of Eβc is enhanced by moisture, which is in sharp contrast with what was previously found for sand. In agricultural soils, the distance at which EPN are attracted by Eβc might strongly depend on soil type. This knowledge may help in efforts to establish new rational methods for the control of soil dwelling pests.

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Poster number: *179*

## Do immune-challenged *Tribolium castaneum* change their volatile profile?

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External immunity, as defined as chemicals secreted by an organism onto its surface and/or into the environment (1), is an additional defense to the internal immune system. This chemical secretion has the potential to not only protect the organism but also affect conspecifics. Additionally, effective signaling from an immune compromised individual to a conspecific indicating immune status is of central importance to the health of the group as a whole. In one such group-living species, the red flour beetle (*Tribolium castaneum*), individuals upregulate their immune response when immune-challenged. Furthermore, it has been shown in other group-living species, such as bees, that individuals can sense their counterpart's health status and respond, i.e. by removing it from the colony or upregulating their own immune defense. Here we investigate the volatile profile of *T. castaneum*, exploring whether secreted chemicals might be an effective signaling method of immune status between group members. Using age-controlled adult male and female beetles, we sampled the headspace of individual immune-challenged and control beetles using a needle trap device. Samples were analyzed by gas chromatography-mass spectrometry (GC-MS). Results show sex-specific differences, as well as variation between immune-challenged and control individuals. We discuss these results in the context of external immunity and explore the potential functions of chemical secretions as a signal of the individual's immune status to group members.

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Poster number: *180*

# Aversive trained insects as detectors

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The detection of specific, low concentrated volatiles is desirable for various applications and the sensing capabilities of insect antennae greatly out-perform those of current analytical instruments. As the molecular replication of the antennal sensing steps for utilization in portable artificial sensors is still in its early stages and far from being applicable, different methods have to be utilized. Portable electroantennography is one method that addresses the need for sensitive and selective sensors, but with it comes the requirement for trained personnel to dissect and mount insect antenna, as well as the extreme sensitivity of the measuring devices to outside influences. However, trained insects provide the opportunity of using their sensitive odour receptors and their fast and reliable pattern recognition capabilities.

Positive conditioning is widely applied in entomological cognition research but rarely applied in field sensor systems. Reasons for the limited utilization might be that the ‘detector insects’ must be in a specific state (e.g. hungry) or manually restrained to record the indicator behaviour. Aversive conditioning has the advantage that free moving insects can be used and that the conditioning is less dependent on the insects internal state, since avoidance behaviour is evolutionary important.

Here we present our studies on aversively trained insects by means of an automated training and read out chamber (APIS, Kirkerud et al. 2013). This behaviour recording system represents a reliable and easy to use method, utilizes a rugged apparatus, and is highly sensitive since it uses the natural sensory capacity of insects. The system has been applied to detect anthropogenic volatiles, as well as plant-derived volatiles which indicate plant pathogen infections. We will discuss our findings in context of the infochemical effect, plant health monitoring, security, and human health.

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Poster number: *181*

## *Decoding evolution of pheromone communication in Heliothine moths*

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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 genomic tools to characterize odorant receptor shifts associated with communication, we are developing a comprehensive comparative database of olfaction for this group. The long term goal of this study will be the establishment of a global network of collaborators using heliothines as a model system to correlate shifts in communication manifested by differential olfactory receptor (OR) expression with 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 behavioral pheromone preference and physiological processing of these odorants as these species diverge.

Poster number: *182*

# Chemical defense in *Zostera marina* (eelgrass) against *Labyrinthula zosterae*

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Costal ecosystems provide important services worth trillions of dollars each year (Costanza et al. 2014). One of these systems is formed by seagrasses, marine plants inhabiting shallow soft bottom areas around the globe. They form a three dimensional mosaic, known as seagrass meadows, that harbors a wide variety of organisms, many of which are commercially important (Beck et al. 2001).

Unfortunately, seagrass meadows have been disappearing over the past decades (Waycott et al. 2009). The underlying causes of this decline vary, with degradation by pathogens being one suggested explanation. In the 1930's the temperate seagrass Zostera marina was attacked by the protist *Labyrinthula zosterae*, ultimately killing 90 % of all populations along the Atlantic coast (Muehlstein et al. 1988). These outbreaks, known as wasting disease, are a reoccurring threat to seagrass meadows.

It has been suggested that *Z. marina* might withstand the infection by producing secondary defense metabolites (Vergeer et al. 1995). A well known group of defense compounds in vascular plants is phenolic acids (Levin 1971). Many studies have therefore investigated *Z. marina* derived phenolic acids and their inhibitory properties towards *L. zosterae* (Vergeer et al. 1995, Vergeer and Develi 1997, McKone and Tanner 2009).

In this study, we instead used bioassay-guided fractionation approach to investigate if *Z. marina* is chemically defended against *L. zosterae* and to identify defense metabolites. The results show that some fractions of *Z. marina* extracts can inhibit pathogen growth, and that this is due to other compounds than the already known phenolic acids. These findings suggest that *Z. marina* is chemically defended against pathogen attacks, and that variation in these defenses may explain variation in pathogen susceptibility among *Z. marina* populations.

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Poster number: *183*

# Knock, knock. Who is there? Host location mechanisms in non-pollinating fig wasps

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In the fig–fig wasp pollination system, low volatility compounds and surface chemistry of globular enclosed fig inflorescences (syconia) can serve as cues in short range host location by non-pollinating fig wasps such as gallers and parasitoids that parasitise the mutualism by ovipositing into syconia from the exterior. In behavioral assays, non-pollinating fig wasps exhibited the interesting dual behaviors of joining and avoidance in that they preferred to congregate on fig syconia previously exposed to oviposition by conspecifics but avoided prior oviposition sites on a syconium. This indicated the possible role of chemical signatures such as chemcial footprints and oviposition marking pheromone left by wasps on the surface of a syconium. We, therefore, analysed surface hydrocarbon profiles and changes in local volatile profiles of syconia with different oviposition histories. We also analysed footprint extracts of these wasps and recorded the response of wasps to these compounds. Surface hydrocarbon profiles and volatile profiles of these syconia with different oviposition histories were found to differ and a differential response of wasps to these profiles was recorded. Additionally, we also investigated the role of CO2 and surface hydrocarbons of single galls as cues to assist parasitoids in host recognition via sensilla on their ovipositors. Our results show that change in surface chemistry and local volatile profiles of the host syconium as a result of the act of oviposition by wasps informs fig wasps about the contents of the syconium and induces them to avoid or engage in oviposition.

Poster number: *184*

# The Lepidoptera odorant binding protein gene family: Gene gain and loss within the GOBP/PBP complex of moths and butterflies

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Butterflies and moths differ significantly in their daily activities: butterflies are diurnal while moths are largely nocturnal or crepuscular. This life history difference is presumably reflected in their sensory biology, and especially the balance between the use of chemical versus visual signals. Odorant Binding Proteins (OBP) are a class of insect proteins, at least some of which are thought to orchestrate the transfer of odor molecules within an olfactory sensillum (olfactory organ), between the air and odor receptor proteins (ORs) on the olfactory neurons. A Lepidoptera specific subclass of OBPs are the GOBPs and PBPs; these were the first OBPs studied and have well documented associations with olfactory sensilla. We have used the available genomes of two moths, *Manduca sexta* and *Bombyx mori*, and two butterflies *Danaus plexippus* and *Heliconius melpomene*, to characterize the GOBP/PBP genes, attempting to identify gene orthologs and document specific gene gain and loss. First, we identified the full repertoire of OBPs in the *M. sexta* genome, and compared these with the full repertoire of OBPs from the other three lepidopteran genomes, the OBPs of *Drosophila melanogaster* and select OBPs from other Lepidoptera. We also evaluated the tissue specific expression of the *M. sexta* OBPs using an available RNAseq databases. In the four lepidopteran species, GOBP2 and all PBPs reside in single gene clusters; in two species GOBP1 is documented to be nearby, about 100 kb from the cluster; all GOBP/PBP genes share a common gene structure indicating a common origin. As such, the GOBP/PBP genes form a gene complex. Our findings suggest that (1) the lepidopteran GOBP/PBP complex is a monophyletic lineage with origins deep within Lepidoptera phylogeny, (2) within this lineage PBP gene evolution is much more dynamic than GOBP gene evolution, and (3) butterflies may have lost a PBP gene that plays an important role in moth pheromone detection, correlating with a shift from olfactory (moth) to visual (butterfly) communication, at least regarding long distance mate recognition. These findings will be clarified by additional lepidopteran genomic data, but the observation that moths and butterflies share most of the PBP/GOBP genes suggests that they also share common chemosensory-based behavioral pathways.

Poster number: *185*

## Consequences of dual biotic stresses on plant volatile emission and recruitment of egg parasitoids


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Plants respond to insect oviposition by emission of oviposition-induced plant volatiles (OIPVs), which can recruit egg parasitoids of the attacking herbivore. To date, studies demonstrating egg parasitoid attraction to OIPVs have been carried out in tritrophic systems. Less attention has been given to plants experiencing multiple attacks by host and non-host herbivores that potentially could interfere with the recruitment of egg parasitoids as a result of modifications to the OIPV blend. Egg parasitoid attraction could also be influenced by the temporal dynamics of multiple infestations, when the same non-host herbivore damages different organs of the same plant species. In this scenario we investigated the responses of egg parasitoids to feeding and oviposition damage using a model system consisting of *Vicia faba*, an above-ground insect herbivore, the pentatomid bug *Nezara viridula*, an above-and below-ground insect herbivore, the curculionid beetle *Sitona lineatus*, and an egg parasitoid of *N. viridula*, the platygastrid wasp *Trissolcus basalis*,. The response of wasp females to *V. faba* volatiles was investigated in a Y-tube olfactometer testing plants that were infested with above-ground, below-ground, and both above- and below-ground insects. The emission of plant volatiles in response to above- and below-ground attacks were also chemically analyzed by Gas chromatography-Mass Spectrometry (GC-MS). The results showed that the non-host *S. lineatus* disrupts wasp attraction toward plant volatiles induced by the host *N. viridula*. Chemical analysis indicated differences between volatiles emitted by *V. faba* plants in response to *N. viridula* feeding and oviposition and volatile emitted as consequence of dual insect infestation.

Poster number: *186*

## Bark beetles and their fungal associates: A marriage of convenience or true love?

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Tree - killing bark beetles vector a diversity of ophiostomatoid fungi to their mutual hosts. For the fungi, some of which are strong tree pathogens, this is an obligate symbiosis. To which extent this relationship is advantageous for bark beetles is still a matter of debate. The overall objective of the presented study is to assess olfactory responses of the European spruce bark beetle *Ips typographus* to the interaction between associated fungi and their mutual host *Picea abies*. It would be a strong indicator for a mutualistic symbiosis within this system if shown that tree- killing bark beetles had evolved specific olfactory traits to receive markers for that fungus – tree interaction. Living trees, partly treated with a wound-signaling plant hormone in order to obtain bark with different resistance, were inoculated with three associated fungi and a non-associated fungus. We collected headspace samples continuously from inoculations for testing olfactory perception on beetle antennae by GC-EAD and GC-SSR. For chemical analysis of differences between fungi and their interaction in trees we also collected phloem samples over one month after inoculation. We are cultivating fungi on different agars to identify specific fungal metabolic products. Preliminary results show very strong responses to compounds related to tree defence, which was strongly triggered by the two most aggressive fungal species. Some of these compounds eliciting the strongest responses are present only in minute amounts below detection limit of GC-MS. In the inoculation volatiles from trees we also identified compounds eliciting GC-EAD responses that were in common with fungal agar cultivations. We thus hypothesize that beetles have evolved a specific ability to perceive markers for their host’s defence status and for the establishment of their associates. The recognition of tree defence and its breakdown should be of uttermost importance for beetles inhabiting a strongly defended ecological niche.

Poster number: *187*

# Effect of different animal manures on attraction and reproductive behaviors of common house fly, *Musca domestica* L


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Insects mainly rely on their well-developed and highly sophisticated olfactory system to discriminate volatiles cues released from host and non-host substances, mates, oviposition substrates, and food sources. Onset of 1st mating, mating duration, Onset of 1st oviposition, oviposition period, fecundity (number of eggs laid by a female), and longevity of freshly emerged *Musca domestica* L (Diptera: Muscidae) adults were observed in presence of volatiles released from four different animal manures: cow, horse, donkey, poultry, and an artificial diet (control). The *M. domestica* adults exposed to volatiles released from horse manure showed significant delay in onset of mating, prolonged mating duration and reduced fecundity compared to the control. Likewise, the fecundity was reduced in the presence of donkey manure volatiles as compared to control. While the mating duration in the presence of cow manure volatiles was significantly shortened as compared to control and no oviposition was observed until the end of experiment. However, the reproductive behaviors and all fitness measures in adults exposed to poultry manure volatiles were better or comparable to the control. Surprisingly, in a free-choice attraction assay, the highest numbers of adult flies were attracted towards the cow manure as compared to all other manures as well asnd the control. However, the numbers of flies captured in all other types of manures were not different than control. Furthermore, chemical analysis of headspace volatiles revealed both qualitative and quantitative differences in odor profiles of all manures and artificial diet indicating that behavioral differences could be due to the difference in chemistry of adult oviposition substrate and larval growth medium. This study may contribute in understanding of linking ecological adaptations and host selection mechanisms to develop pest management strategies against this serious medical pest.

Poster number: *188*

# Spatial and temporal variation in volatile composition suggests olfactory division of labor within the trap flowers of *Aristolochia gigantea*

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The olfactory components of floral advertisement can be highly complex, often showing dynamic patterns of emission and chemical composition that may reflect diverse functions regarding pollinator behavior. In this study we investigated the spatial and temporal variation of volatile production in the distinctive kettle-trap flowers of the Neotropical pipevine *Aristolochia gigantea*, which show unusual complexity in scent chemistry and floral morphology in addition to human perceptible odor changes at distinct stages during the ontogeny of the flowers. Specifically, volatiles were collected from separate ontogenic stages in development (bud, female, male, wilted flower), and within each stage from different functional units (limb, collar, utricle). Our results document high chemical complexity and diversity of scent composition across all known biosynthetic classes, which changes markedly during floral ontogeny and between flower parts. Female stage flowers are dominated, in a seeming paradox, between sweet smelling (to the human nose) citronella-like compounds including *E*-citral, *Z*-citral, citronellol, and citronellal while at the same time emitting brood-site associated volatiles such as dimethyl disulfide, 2-heptanone, and 3-methyl-1-butanol. Volatile emission is dramatically reduced in male stage flowers approximately 24 hours later, except for the novel and intense production of linalool from the flower's trapping chamber. Volatiles emitted from wilted flowers resemble the vegetative background as soon as 48 hours post anthesis. Multidimensional scaling analysis has revealed unexpected patterns of volatile emissions across morphological units of the complex flower (e.g. within vs. outside of the trap), as well as at different stages of sex expression during the dichogamous maturation of the flower. This raises the question of whether kettle-trap flowers or inflorescences with temporal segregation of male and female functions utilize a chemical division of labor, in concert with visual and tactile cues, to choreograph pollinator behavior such that male and female floral function is optimized. These results suggest several avenues for further study, including behavioral assays and phylogenetic/comparative initiatives.

Poster number: *189*

## Consequences of pollinator-mediated gene-flow on trait expression versus genotype in varieties of Joshua tree (Agavaceae)

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The obligate mutualism between *Yucca* and their associated moth pollinators represents a classic example of coevolution. Recent research has revealed that pollinator attraction in this insect-plant interaction is mediated by floral scent [1]. The Joshua tree, *Yucca brevifolia*, is divided into two varieties based on morphology: the western *Y. b. brevifolia*, and the eastern *Y. b. jaegeriana*. Two sister species of *Tegeticula* moths are the exclusive pollinators of these yuccas: *T. synthetica* on western plants, and *T. antithetica* on eastern plants. The two moth-plant pairs have a parapatric distribution, and only coexist in a 4 km wide contact zone in Tikaboo Valley, Nevada. Here many intermediate plants are found, including those with floral characters of *Y. b. jaegeriana*, but vegetative features of *Y. b. brevifolia*, whereas trees with Y. b. brevifolia floral characters and *Y. b. jaegeriana* vegetative features never occur, indicating extensive asymmetric hybridization between hosts and difference in host discrimination between pollinators [2]. We used estimates of vegetative and floral morphological traits, microsatellite genotyping, and identified floral volatiles from headspace collections to analyse how the floral scent phenotype, which differs between the ancestral hosts, is manifested in hybrids. The floral scent profile was a good predictor of floral morphology, but correlated poorly with genetic background and vegetative morphology. Thus, a coherent floral phenotype is maintained in hybrids in spite of substantial introgression, whereas such plants otherwise constitute a mosaic of intermediate traits. These results are discussed in relation to the host specificity of the pollinators in this mutualism.

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Poster number: *190*

# Evaluation of fungicidal activity from *Picramnia bahiensis* extracts for leaf-cutting ants control

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The agriculture plays a key role in food production and it moves the economy of a country. For the eradication of various pests, synthetic pesticides are used indiscriminately in order to obtain quick profits, leading to a gradual and irreversible contamination of the environment and the human health [1]. The leaf-cutting ants are considered pests of difficult control and living in symbiosis with the fungus *Leucoagaricus gongylophorus,* in a total dependency between them. So, the control can be done using fungus inhibitors [2]. In this context, the use of natural products for agricultural pest control becomes a valuable tool with regard to ecological and sustainable awareness, to develop products with low environmental impact and specific action.

The genus *Picramnia* shows high diversity of secondary metabolites, such as anthrones, oxanthrones, anthraquinones, coumarins, and triterpenes, classes of compounds with insecticidal and fungicide activities [3].

Therefore, this study investigated the toxic effect of the hexane, dichloromethane and methanol extracts from stem and leaves of *P. bahiensis* against the symbiont fungus *L. gongylophorus*.

The fungus was isolated from a leaf-cutting ants nest of *Atta sexdens rubropilosa* and maintained under laboratory conditions. The samples were added in the culture medium at a final concentration of 1 mg/ml and the mycelial growth inhibition for each extract was evaluated in a 30 days interval. The methanol extract of the leaves (PBFMe) was the most active, inhibiting 64% of mycelial growth, followed by dichloromethane extracts of leaves (PBFD) and stem (PBCD) presented both an inhibition of 57%. Furthermore, it was also observed a minor inhibition of 34% for hexane leaves extract (PBFHe) and 28% to hexane (PBCHe) and methanol (PBCMe) stem extracts.

These results were very promising, thus the study of most active extract is in progress in order to isolate and identify the compounds responsible for this activity.

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Poster number: *191*

# The chemical ecology of divergent host plant acceptance behaviour in the speciation in the pea aphid (*Acyrthosiphon pisum*)

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Understanding divergent specialisation to host plants by phytophagus insects is a key to explaining the high level of speciation seen in many diverse taxa. Population genetic methods show host specialisation in *A. pisum* is associated with the divergence of a few specific genes. This includes genes involved with chemoreception and chemical metabolism. Consequently, in order to investigate speciation fully in *A. pisum* we need to understand the system at a physiological level.

In this study I have used untargeted metabolomic profiling of host plants and random forest modelling to study divergence of host plant acceptance by two *A. pisum* races. This is with the aim of teasing out the chemical compounds in plants that might elicit discriminate host plant choice and so act as selection pressures in *A. pisum* host-plant speciation. These findings not only offer a plant metabolomic perspective to *A. pisum* speciation but also offer a pipeline to link questions from disparate biological fields to their chemical ecology.

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Poster number: *192*

# *Macrolophus pygmaeus* perception of HIPV's from *Tuta absoluta* infested tomato plants

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*Tuta absoluta* Meyrick (Lepidoptera, Gelechiidae) is one of the most damaging pest of tomato crops in South America and in Europe, since its accidental introduction. Integrated Pest Management strategies against this pest are mainly based on the release of its natural enemies. One of the advantages to use predators lies in their ability to find the pest by using volatile compounds emitted by infested plants (HIPV's). *Macrolophus pygmaeus* Rambur (Heteroptera: Miridae) is a biological agent currently used to control *T. absoluta* in southern European greenhouses. In our previous works, we have shown the ability of *M. pygmaeus* to discriminate a *T. absoluta* infested tomato plant from a non-infested plant, and we have characterized the HIPV's associated with *T. absoluta* infestatrions. Here, we investigated the olfactory responses of *M. pygmaeus* to the previously identified HIPV's and the behavioural responses each HIPV induces on *M. pygmaeus* males and females. Among the 21 compounds tested in electroantennography (EAG), 17 induced electrical depolarization from *M. pygmaeus* antennae. Adult *M. pygmaeus* were exposed to each EAG active chemical individually in double choice bioassays to evaluate their attraction potential. Natural extracts of infested tomato volatiles and synthetic HIPVs blends were also tested. Some of the EAG active chemicals significantly attracted *M. pygmaeus* in the olfactometer, but none of the behavioural responses were comparable to those observed with natural or synthetic blend.

Poster number: *193*

## Evidence for a chemical arms race: Chemical mimicry in the cuckoo wasp genus *Hedychrum*

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Mimicry of cuticular hydrocarbons (CHC) is a strategy of social parasites, myrmecophiles and brood parasitoids to exploit resources of their hosts inconspicuously. There are basically two alternative sub-strategies to mimic the hosts' CHC profile. Either the parasite acquires the CHCs directly from their host or alternatively the parasite evolves the ability to produce a CHC profile similar to the host by altering the usage its own biosynthetic pathways. In the latter case, an arms race is expected to take place between host and parasite. Although there have been several studies on the adjustment of the CHC profile by the parasite, evidence for an evasion by the host is scarce. To investigate a chemical arms race between hosts and parasites, we compared the CHC profiles of a group of several brood parasitic cuckoo wasp species of the genus *Hedychrum* and their hosts (digger wasps of the subfamily Philanthinae) which in turn can be divided into two groups. One group of hosts uses a highly specific hydrocarbon composition with very little interspecific variation for prey preservation and exhibits this specific CHC composition also on their cuticle. Strong stabilizing selection is expected to act on the composition of the hosts' CHC profile and in these cases the cuckoo wasps indeed achieve an almost perfect match. In a second group of hosts hydrocarbons are not used for prey preservation. In contrast to the first group the CHC profiles are not under stabilizing selection and show highly species specific patterns. The chemical mimicry of the cuckoo wasps is far less precise in these cases. Consequentially, we conclude that the large interspecific variation of the hosts which can vary their CHC profile is a result of evading the chemical mimicry of their cuckoo wasp species.

Poster number: *194*

# The importance of plasticity in response to semiochemicals in the development of monitoring tools for the pea leaf weevil, *Sitona lineatus* L. (Coleoptera: Curculionidae)

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The pea leaf weevil, *Sitona lineatus* L. (Coleoptera: Curculionidae) is an invasive pest of many pea-producing regions. It is established in North America including in the Prairie Provinces, where most of Canada's pulse production occurs. Pea leaf weevils have one generation per year, with two periods of adult activity. In the spring, adults emerge from overwintering sites and utilize host plant volatiles and a male-produced aggregation pheromone (4-methyl-3,5-heptanedione) (Blight et al 1984; Blight & Wadhams 1987), to aggregate for mating at oviposition sites. It is not known if pea leaf weevils use semiochemicals in the fall when they leave pea fields to find overwintering habitat. Semiochemical-baited traps have been developed to monitor spring activity of pea leaf weevil in Europe (Blight & Wadhams 1987; Nielsen & Jensen 1993). The objective of this work is to develop a semiochemical-based monitoring system that can monitor weevil activity in spring and fall and detect range expansion. We compared the attractiveness of aggregation pheromone with and without bean volatiles (linalool, *Z*-(3)-hexanol, and *Z*-(3)-hexenyl acetate) at low and high release rates to weevils in the spring and fall. Weevils captured in semiochemical-baited pitfall traps were counted and separated by sex. Pea leaf weevils responded to aggregation pheromone during both seasons but the presence of host plant volatiles was only important to orientation in the fall. More males than females were attracted to semiochemical-baited traps in the spring whereas an equal number of both sexes were trapped in the fall.

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Poster number: *195*

## Biological role of triplicated Na, K-ATPase1α genes in the large milkweed bug, with regard to target-site insensitivity against cardiac glycosides

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Plants produce a wide variety of secondary metabolites, including alkaloids, terpenoids and glycosides, many of which serve as antiherbivore or antimicrobial defense compounds. In response, a substantial number of insect species have evolved adaptations specific to the particular defensive compounds of their host plants. The large milkweed bug, *Oncopeltus fasciatus*, feeds on cardiac glycoside containing plants, which might potentially block their Na, K-ATPase´s. As an adaptation to counter the toxic effects of these cardiac glycosides, milkweed bugs contain three copies of the Na, K-ATPα subunit coding gene (copies A-C). Molecular docking simulations, as well as genetically engineered Na, K-ATPase constructs, suggest that the three copies differ greatly in their sensitivity to cardiac glycosides. Moreover, a preliminary gene-expression study indicates that the two putatively less sensitive copies (A and B) are expressed in the gut where the cardiac glycosides are processed, whereas the putatively more sensitive copy (copy C) is localized to the brain, where the glial sheath likely acts as a barrier against cardiac glycosides. Here we systematically categorize the function and expression patterns of these gene copies using RNAi. We designed copy-specific dsRNA probes, approximately 350-400bp in length, and injected them into adult bugs. Differences in expression of the gene copies were quantified using qPCR. The copy-specific knockdowns were highly effective; there was an over 80% reduction in expression for each copy. Interestingly, the B copy was expressed at a much lower level than the A and C copies. Knockdowns of the A, B and C copies resulted in cross compensation, and knocking out of the C copy was lethal at 3 days post-injection.

Poster number: *196*

# Experience-based predator avoidance in response to waterborne chemical cues in the freshwater isopod *Asellus aquaticus*

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Waterborne chemical cues may be an important source of information for aquatic organisms, especially when assessing the risk of predation. We studied the response to predator cues in the freshwater isopod *Asellus aquaticus*. In many Swedish lakes, this isopod is present in two distinct ecotypes, which diverged following colonisation of a novel habitat. The ancestral ecotype inhabits reed stands along the shorelines while the novel ecotype lives in stonewort habitats in the lake centre. These ecotypes encounter different predator communities in their respective habitats and, here, we studied the behavioural response to chemical cues of predators typical for the two different habitats: larval dragonflies and perch. This laboratory-based choice experiment was performed with both wild-caught and lab-reared isopods of the two ecotypes. Our results show that individuals with prior predator experiences elicited strong avoidance to cues from both predators and while both ecotypes had similar antipredator behaviour, the sexes differed since males in general had a stronger response than females.

Poster number: *197*

## Spamalot: A quest for the holy grail of brown treesnake oral baits

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The brown treesnake (BTS; *Boiga irregularis*) was introduced to Guam approximately 60 years ago, ultimately causing extensive economic and ecological damage. Because transportation represents a pathway for BTS to negatively impact other Pacific islands, interdiction measures have been in place at ports and cargo staging areas for nearly two decades. Underpinning these measures, large-scale BTS suppression throughout Guam remains a primary goal. Efficient suppression rests on delivery of a highly effective toxicant (i.e. acetaminophen). BTS attraction to carrion is exploited for this purpose by using dead neonatal mice (DNM) as toxicant delivery devices. While use of DNM baits can be effective, alternatives have been actively pursued for the past 15 years. Among the undesirable characteristics of DNM are: cost (as much as $0.60 each), handling requirements (must be stored frozen), and poor longevity (effective field life of only 4 or 5 days). We conducted a series of chemical and behavioral assays to identify materials with similar sensory qualities as DNM toward developing baits that are less expensive, can be stored at room temperature, and have longer field life. Among the many items tested in a series of field experiments with free-ranging BTS in Guam was an artificial mouse fat mixture developed from our analyses of DNM skin. When the mixture was applied to Spam® (processed meat product), the test bait was highly preferred by BTS and demonstrated excellent durability under field conditions. This bait offers great potential to satisfy many desirable attributes for BTS baiting operations and island-wide suppression.

Poster number: *198*

# Chemical communication in a mutualistic system – The myrmecophilous Australian butterfly *Jalmenus evagoras* (Lepidoptera: Lycaenidae)

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Communication is critical to the maintenance of the often extraordinary levels of cooperation that may occur between individuals of the same and different species. Ants are frequent partners in interspecific cooperative relationships, including with the larvae of butterflies of the family Lycaenidae (Lepidoptera). Although the costs and benefits of lycaenid-ant associations have been extensively documented, recognition and communication mechanisms are still poorly understood. It is widely thought that cuticular hydrocarbons, important for mediating nestmate recognition in ants, may also play a significant role in initiating and maintaining cooperative behaviour in lycaenid caterpillars and their tending ants. To unravel the role of chemical signals as recognition cues in lycaenid-ant associations, we examine the larval cuticular hydrocarbons of the Australian butterfly *Jalmenus evagoras* and its attendant ants by targeting a variety of *J. evagoras* populations associated with different ant species and on different *Acacia* host plants. We examine the initial acceptance of early instars by the associating ant colony by documenting ontogenetic changes in the larval chemical profile. We discuss how these patterns of cuticular chemical profiles maintain the mutualistic association between larvae and ants.

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Poster number: *199*

# Evidence of short-term peripheral sensitization by briefly exposure to pheromone components in *Spodoptera littoralis*

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It has been widely demonstrated that Lepidoptera exhibit a certain degree of plasticity, displayed as either habituation (reduction of the response to a repeated stimulus) [1] or sensitization, which involves an increase in the response [2]. This plasticity may lead to long-term structural changes at central nervous system and to an increase in sensitivity of olfactory receptors neurons at both central and peripheral levels. This effect has been unnoticed when measured by electroantennography. However, in contrast to this fact, it has been reported that stimulation of the male antennae of *Spodoptera littoralis* with 1 µg of the major pheromone component (*Z,E*)-9,11-tetradecadienyl acetate at different times from the initial stimulation induced subsequent higher responses to the same dose [3]. In this presentation and to deepen into the mechanism of this sensitization effect, we try to disclose whether this effect is dose-dependent and elicited by other pheromone components. To this aim, two different set of electroantennographic trials were conducted on virgin males: i) a puffed pre-exposure to a dose (1 or 10 µg) of (*Z,E*)-9,11-tetradecadienyl acetate or the minor components (*Z,E*)-9,12-tetradecadienyl acetate and (*Z*)-9-tetradecenyl acetate, and evaluation of the subsequent responses to the same dosage of the corresponding compound in successive five-minute intervals, and ii) an initial brief exposure to low amounts (from 0.1 to 10 ng) of the main pheromone component and determination of the posterior response to 1 µg of this compound at 5 and 15 min. Significant higher responses to (*Z,E*)-9,11-tetradecadienyl acetate and (*Z,E*)-9,12-tetradecadienyl acetate were found in pre-exposed antennae when compared to näive antennae, whereas (*Z*)-9-tetradecenyl acetate did not trigger any increase. In addition, we observed that low stimuli may induce in pre-exposed antennae a short-term increase on sensitivity, with a prevalence in time positively correlated with the amount of the pre-stimulus.

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Poster number: *200*

## Carbon dioxide receptor involved in the perception and production of the sex pheromone

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The effect of increased carbon dioxide ($CO_2$) concentration in atmosphere was examined on the pheromone system of *Helicoverpa armigera*. $CO_2$ gas was treated at three identical rearing room (2×2×2 m) where the $CO_2$ concentration was 450 ppm (Control), 601 ppm (600 ppm), 971 ppm (1000 ppm), respectively.

Elevated $CO_2$ affected the EAG response decreased and lowered attractancy to sex pheromone lure at 1000 ppm in adult males. While the sex pheromone production increased in female adult. We hypothesized that $CO_2$ receptor in labial palp is involved in those processes and examined on the EAG response and attractancy of the male and the sex pheromone production of the female with/without $CO_2$ receptor. EAG responses of normal head were coincided with the antennal responses at all different $CO_2$ levels, while the responses of head without $CO_2$ receptor were apart from the antennal response. The attractancy of the males without $CO_2$ receptor was lowered than that of the normal males in wind tunnel. Therefore, $CO_2$ receptor is involved in the process in the perception of sex pheromone by tuning the signal from antenna at different $CO_2$ environments. Sex pheromone production of the female without $CO_2$ receptor decreased and showed an altered diel rhythm particularly in the low $CO_2$ levels. we speculate that $CO_2$ receptor is involved in controling a valve for the emission of PBAN from SOG.

Poster number: *201*

# **Gustatory synergy between sugars and amino acids in the yellow fever mosquito, *Aedes aegypti***

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Many insects including mosquitoes, butterflies, honeybees, flesh flies and ants have been shown to prefer diets that contain both sugars and amino acids. Fitness gains associated such as enhanced longevity, flight capacity and fecundity, suggest an ultimate evolutionary mechanism for the preference of amino acid-rich sugar diets. However, the proximate mechanism(s) behind this preference has not yet been explored. In a recent study, the yellow fever mosquito, *Aedes aegypti*, was unable to detect individual amino acids unless combined with a behaviorally sub-threshold concentration of a sugar. This behavioral response to combination of two nutrients at individual sub-threshold concentrations indicates a synergistic proximate mechanism underlying the mosquito gustatory preference. To investigate this further, we conducted feeding bioassays and single-sensillum electrophysiological recordings using a combination of trehalose and various concentrations of either one of seven ecologically relevant amino acids (alanine, arginine, glycine, leucine, phenylalanine, threonine and valine). In feeding assays, females prefer and imbibe mixed diets in significantly higher volumes in a dose-dependent manner than trehalose alone for all amino acids except glycine & arginine. The single-sensillum recordings allowed us to generate a chemotopic map of the dorsal labellar sensilla that describes five of the seven test mixed diets as eliciting responses in different sensilla, either increasing (leucine, valine & alanine) or decreasing (glycine & arginine) gustatory neuron firing rates. Our results indicate that the synergistic behavioral response of female *Ae. aegypti* to amino acid and sugar mixed diets is a result, at least in part, of combinatorial coding of the gustatory neuron input from the dorsal sensilla on the labellar lobes.

Poster number: *202*

# Identification of the foraging stimulant of the brown marmorated stink bug, *Halyomorpha halys*

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The brown marmorated stink bug, Halyomorpha halys (Heteroptera: Pentatomidae) is one of the well-known invasive insects. This plant-sucking bug infests various crops, for example, soybean, orange and apple. This bug is also known to emit an unpleasant odor from its scent gland and it is a nuisance when it invades houses, hotels and school buildings. The bug is easy to maintain with peanut as a food source in the laboratory. We often observe that the bug prefers fresh peanuts, but tend to avoid already infested peanuts. This observation indicates that there must be a stimulant(s) involved in the recognition of preferable food sources. Thus, we planned to investigate the foraging stimulant(s) from peanuts that might be useful as a potential chemical lure to manage this harmful stink bug. First, we evaluated the feeding preference of *H. halys*. An individual bug was put into a petri dish and then either a fresh or infested peanut with its inner skin was introduced into the petri dish. The bugs found fresh peanuts more quickly than infested ones, suggesting that there must be an olfactory cue to recognize the preferable food source. Then, we analyzed the volatiles from peanuts using gas chromatography/mass spectrometry. Hexanal was identified as a characteristic volatile from fresh peanuts, while this compound was not detected from infested peanuts. A T-junction choice assay revealed that *H. halys* adults were attracted to hexanal. Hexanal also induced proboscis-protruding behavior of *H. halys,* suggesting that hexanal is a key compound for finding a preferable food source. Hexanal is widely distributed as a component of fruit flavor such as apples which is one of the preferable food sources of *H. halys*. The responses of *H. halys* to hexanal, a general plant odor, can explain its polyphagy.

Poster number: *203*

# An efficient approach for synthesis of trun-call 1 and trun-call 2, the aggregation pheromone components of larger grain borer (*Prostephanus truncatus*)


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The larger grain borer, *Prostephanus truncatus* (Horn) (Coleoptera: Bostrichidae) is an important pest of farm stored maize and dried cassava in Africa and Central America [1]. After a male has reached a suitable food source, an aggregation pheromone is released that is attractive to both females and males. 1-Methylethyl-*E*-2-methyl-2-pentenoate and 1-methylethyl-(*E*,*E*)-2,4-dimethyl-2,4-heptadienoate also known as trun-call 1 and trun-call 2, respectively, were reported as aggregation pheromone major components of *P. truncatus* [2]. Synthetic aggregation pheromone is used to survey the distribution of this pest and to determine the effectiveness of control measures against it [3]. We report a novel and efficient approach to synthesis of both components with an overall yield of 65% considering 2-bromopropionic acid as a starting material. Isopropyl 2-bromopropionate was obtained by means of Fischer esterification of 2-bromopropionic acid with isopropyl alcohol. Ester was converted to the corresponding phosphonate employing Arbuzov reaction and further on treated with propanal and 2-methylpent-2-enal in Horner-Wadsworth-Emmons reaction to obtain trun-call 1 and trun-call 2 respectively.


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Poster number: *204*

# Browning mechanism on the roots of rice plant attacked by rice root aphid, *Rhopalosiphum rufiabdominalis*

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A browning caused by attacking pathogens is one of important defensive systems. Our previous study revealed that the browning on a rice root was also induced by an infestation of aphids and the aphids avoided the deep browning area on the roots. At this time, serotonin, which could inhibit survival of nymph aphids, was accumulated at high concentration in the root. Therefore, it was concluded that rice plants could induce both of physical and chemical defense system against insect pests. However the mechanism of this resistance is unclear.

Rice seeds were sown on a paper towel, and cultivated at 27℃. A root of rice seeding at 4 days old was infected by a winged aphid and they were kept under the same condition. The rice roots were used for chemical, biochemical,and molecular biological analyses. When a root was attacked by aphids, the transcript of serotonin biosynthesis-related genes (Anthranil synthase, Tryptophan synthase, Tryptophan decarboxylase, and Tryptamine-5-hydroxylase) was enhanced at 3 – 5 days after infection. On the other hand, an activation of peroxidase (POX) activity, which could metabolize serotonin, was delayed than the expression of Tryptamine-5-hydroxylase, which could synthesize of serotonin. Thus, this deviation of the expression timings would induce the accumulation of serotonin. Furthermore, the substrate property of the induced POX was different from that of the control POX. It was thought that a major part of the defense mechanism was caused by an activation of a normal biosynthesis pathway, but the final part of the mechanism was caused by the induction of the special POX(s).

Poster number: *205*

# HIP-VOC released by the buffalo bur (*Solanum rostratum*), the former host plant of the Colorado beetle (*Leptinotarsa decemlineata*) and their detection by electroantennography

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The potato plant (*Solanum tuberosum* L. 1753) was introduced from the Andes by European settlers into the West of North America, were it met the Colorado beetle (*Leptinotarsa decemlineata* Say 1824) around 1850. Up to that time the Colorado beetle fed primarily at the Buffalo Bur (*Solanum rostratum* Dun) but quickly extended its host plant range to potato plants. Later on the beetle followed the potato to Europe and caused widespread devastations in potato cultures in the beginning 20st century. The question arises, how did the Colorado beetle manage to extend its host range from the chemo-ecological point of view? In order to address this question, pattern of volatile organic compounds released by both plants were compared and, despite a high similarity in pattern, consistent differences could be identified. Especially differences induced by beetle feeding as the release of methyl benzoate can be detected by the Colorado beetle with high sensitivity as was shown utilizing GC-MS/EAD and EAG-technique. Thus, despite feeding on the potato plant in Europe for more than 100 years, the Colorado beetles are still able to detect host volatiles of their old host plant.

Poster number: *206*

## Host volatiles VS non-host volatiles: Host odor of a deadwood colonising beetle is no matter of tree species

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Host recognition in insects is to a large extent achieved by the olfactory sense and mediated by host volatiles of a certain host–specificity. To give an example, host–volatiles for a bark beetle are those released by colonised trees, while non–host volatiles are unique to avoided tree species. This concept has been generalised and transferred to other feeding habits. Things become more complicate with complexity of feeding habits and hosts might be only the substrate for the actual diet. The ship timber beetle *Elateroides dermestoides* (Lymexyloidea) cultivates a yeastlike fungal symbiont in galleries of many soft– and hardwood species. We studied host recognition in this generalist, by analysing volatile chemistry of the major host trees beech, oak, larch and spruce. Electroantennographic recordings of *E. dermestoides* female with a GC/MS–EAD setup identified disparate and species specific chemical profiles among infested host trees. Surprisingly, major antennal responses were recorded in equal measure to compounds present in all four host trees: Alcohols and acetates that are volatile products of general yeast metabolism. We demonstrate that they are released by many fungal species found in the galleries. However, EAG dose–response experiments confirmed strong antennal responses and high sensitivity for a compound that is produced by the fungal symbiont alone. This corroborates the assignment of *E. dermestoides* as a fungal specialist. We conclude that host recognition of *E. dermestoides* barely includes host–tree specific volatiles and is primarily dependent on metabolites of fungi present in dying trees. This probably accounts for the indiscriminative host tree selection. However, an ability to distinguish fungal species and assess their activity by specific and/or general fungal volatiles also transfers the hypothesis of species–specific odour recognition versus ratio–specific odour recognition from the plant into the fungal kingdom.

Poster number: *207*

## How fungus-growing ants are fooled by their parasites…or are they?

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Ants typically react aggressively to protect their colony's resources. They can discriminate between members from their own colony (nestmates) and individuals from a foreign colony (non-nestmates). This discrimination is based on odors that consist of different low-volatile compounds on the ants' body surface (cuticular hydrocarbons: CHCs). During allogrooming and food sharing, CHCs are exchanged between nestmates which homogenizes the colony odor. The components are species-specific and differ slightly between colonies. Due to their highly sensitive olfactory system, ants are remarkably fast and accurate in recognizing non-nestmates and protecting their home from invaders. Social parasites fool the ants to gain access to the host colony's resources. They achieve this by expressing host-specific CHCs ('chemical mimicry'), having few CHCs ('chemical insignificance'), and/or by using chemical weaponry to confuse the host species. Our chemical analysis revealed that the parasites comprise of large quantities of volatile venom alkaloids and very few CHCs. We compared host and parasite chemical profiles and found that the parasite CHCs are not only fewer but are of higher molecular weight than those of the host. In addition, we performed behavioral aggression tests to investigate how the presence of the parasites influences the aggression levels of the host ants. We discuss our results in the context of host defense strategies where in some systems, tolerance to parasites, may result in less fitness loss than resistance.

Poster number: *208*

## Defense chemistry and community ecology of *Brassica nigra*


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A range of environmental stresses may act in synergy or antagonistically to influence the concentrations and induction of plant secondary metabolites 1. One such stress, the ground-level ozone is phytotoxic, it can potentially affect the expression of secondary metabolites 2. Ozone is an irritant or toxin to animals with potential direct effects on insect performance and it degrades biogenic volatile organic compounds (BVOCs) in the atmosphere with potential effects on the direct or indirect defences conveyed by those chemicals 2,3. This study (part of a multidisciplinary European collaborative project) used a system comprising *Brassica nigra*, *Pieris brassicae* and *Cotesia glomerata* to investigate several questions related to the impact of ozone on plant-insect interactions and the impact of combined biotic and abiotic stresses on plant defence by adopting an approach combining transcriptomics and metabolomics with chemical ecology. We have gained knowledge on the effects of the stress combinations on the transcriptomes and metabolome of the plants through microarray experiments and use of ultra-high performance liquid chromatography electrospray ionization/ time of flight mass spectrometry (UHPLC-ESI/TOF-MS). We have identified the profiles of volatiles emitted by *B. nigra* under combinations of ozone and herbivore-feeding stresses [dynamic headspace sampling technique and analyzed by gas chromatograph–mass spectrometry (GC–MS)]. Parasitoid behavior has been observed in a novel wind-tunnel capable of maintaining stable ozone concentration. The change in plant chemistry correlated with the behavior/performance of herbivores and orientation of parasitoids. Further work is in progress to find the use of the data collected in optimizing biological control methods in polluted environments and ecological risk assessments of fragile environments.



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Poster number: *209*

# A chemical ecology approach to assess the proliferation of the red alga *Asparagopsis taxiformis*: Metabolomics, natural toxicity and biological effects

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In marine sub-tropical and tropical ecosystems, algal proliferations are impacting several species. The competition between algae and corals modify the functioning and the structure of the reefs. The genus *Asparagopsis* (Rhodophyta) is known to be cosmopolite, and may even be invasive in some locations. The lack of knowledge on these algae and their real impacts on the reefs require a global and multi-disciplinary study to better understand, and manage some invasion events (ERA-NET BIOME project www.seaprolif.ird). The main goals of this franco portuguese consortium are: i) to assess the worldwide distribution of *A. taxiformis*, and its dynamics in tropical/temperate sites; ii) to evaluate the link between the genotypes and their metabolic contents, in association with their microbial diversity; iii) to study mechanisms involved in the interaction between *A. taxiformis* and indigenous tropical/temperate corals. In this context, our work will focus on two particular points: i) previous studies highlighted five different lineages for *A. taxiformis* [1]. Using UHPLC-QqToF, metabolomic fingerprints of the different lineages were recorded to determine the link between specialized metabolism, genotypes and their bioactivities in order to explain their possible invasiveness; ii) the major specialized metabolites of this species were isolated and characterized in order to determine their contribution to algal toxicities. We also studied their implication in tropical and temperate coral interactions. The first studies allow the isolation and characterization of two new highly brominated cyclopentenones [2], while only highly halogenated compounds (C1 to C4) were identified previously. Algal extracts tested in situ on four tropical corals show only low effects on coral bleaching, while no change in the algal metabolism was highlighted in contact with the massive coral Porites. However, the alga in direct interaction with the Mediterranean orange coral *Astroides calycularis* develop a specific metabolism with a toxicity increase after 15 days in contact.

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Journal of Natural Products, 77 (5), 1150-1155, DOI: 10.1021/np401094h

Poster number: *210*

# Chemical attractants of *Philornis downsi* an invasive avian parasite on the Galapagos Islands

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Since it was introduced in the Galapagos Islands, the parasitic fly hematophagous *Philornis downsi* has caused high levels of mortality in several species of endemic birds like the Floreana mockingbird, the medium ground finch, and the mangrove finch which are critically endangered (Dudaniec and Kleindorfer 2006 Fessl et al. 2006, Fessl et al. 2010, O'Connor et al., 2010). Mortality is caused by blood loss in chicks, which often cause the death of into 100% of the offspring. The proposed research is based on the years of research conducted at SUNY-ESF with the primary goal of identifying chemical attractants that can serve as the cornerstone of an effort to control this pest in the future. A subclass of semiochemicals, pheromones (sex and aggregation) are secreted substances to the outside by an individual and received by a second individual of the same species in which a specific reaction are released for communication with the same species (intraspecific chemical signals) (Wyatt 2003). In this sense it is possible to consider the chemical manipulation as a means of deception to attract and control aggressive introduced species. The chemical dilution is one of the most striking in the field of chemical ecology, interacting in diverse biochemical and physiological levels of the animal and plant kingdoms covering a wide range of organisms (Bagneres and Lorenzi. 2010). This study attempts to use chemical communication, field observation, capture, sampling and chemical analysis to determine the most effective and attractive chemistry combination for *P. downsi*. The potential uses of specific chemical attractants against *P. downsi* including population monitoring, detection, mass trapping and mating disruption.

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# Mating system and induction shape the volatile emissions of wild potatoes

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The diversity of floral volatiles observed in flowering plants has been attributed to diverse interactions with mutualists (e.g. pollinators) and antagonists (e.g. herbivores). Hypotheses about the function of floral volatiles in pollinator attraction assume that floral volatile production is dependent on the plant mating system and therefore should correlate with the dependence on pollinator visitation. In the nightshades (Solanaceae) self-incompatibility (i.e. total dependence on pollinators) has been lost repeatedly and we have demonstrated interactions between herbivore defense and pollinator attraction in several wild tomato species (Kessler & Halitschke, 2009; Kessler et al., 2011). Here we characterize volatile emissions of wild potato plants with different mating systems. Constitutive emissions and wound hormone-induced changes in leaf and flower volatile emissions show different patterns in self-compatible and self-incompatible potato plants. Self-incompatible plants emit stronger and more diverse floral volatile bouquets whereas elicitation by methyl jasmonate application had a stronger effect on self-compatible plants. Furthermore, volatiles of several compound classes (monoterpenes, sesquiterpenes, benzenoids) show differential responses to the induction treatment and plant mating system. The results of the potato study will be discussed in the context of the recently developed framework of ecological consequences of plant mating system-dependent interactions between herbivore resistance and pollinator attraction.

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Poster number: *212*

# Become a specialist with an enzyme - The senecionine-monooxygenase of *Longitarsus jacobaeae*

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The flea beetle *Longitarsus jacobaeae* is one of only a few animals specialized to feed on the tansy ragwort (*Senecio jacobaea*). This common wild flower is highly toxic, containing pyrrolizidine alkaloids, mainly senecionine-N-oxide, which function as a defense mechanism against herbivores. Ingestion of these compounds causes hepatotoxic or even lethal effects not only in insects, but in farm animals as well.

In this study we investigated which physiological adaptions allow *Longitarsus jacobaeae* to feed unharmed on *Senecio jacobaea*. The challenge in dealing with pyrrolizidine alkaloids lies in their conversion from N-oxides into tertiary alkaloids in the herbivores' gut, as this is where the toxicity normally originates. Once reduced to the lipophilic tertiary alkaloids, pyrrolizidine alkaloids can passively cross the gut membrane. One possible solution to this problem would be to immediately reconvert the tertiary alkaloids after intake. Flavin-monooxygenases are enzymes known to perform such conversions, and thus we search for and identified two sequences in the beetle's transcriptome that are similar to known flavin-monooxygenases. Based on this information we preformed tissue-specific gene expression analyses. We found clear tissue-specific expression for both genes. In addition we expressed and harvested the two genes as recombinant proteins in Sf9 cells. Activity tests with tertiary senecione and the co-factor NADPH +H+ demonstrated that the flea beetle has two senecionine-monooxygenases which are able to specifically convert tertiary senecionine into harmless N-oxides.

Poster number: *213*

# Flavan-3-ols are a general chemical defense in poplar against fungal pathogen attack

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Poplars (*Populus* spp.) are important forest tree species known for their fast growth, phytoremediation ability and use for biofuels and timber. The availability of a complete genome for one species as well as genetic transformation platforms has quickly established *Populus* as the best model system for woody plant research. Under natural conditions, poplar trees are attacked by diverse biotrophic and necrotrophic fungi. As potential anti-fungal defenses, poplars synthesize substantial amounts of phenolic secondary metabolites constitutively, including salicinoids, flavonoids and condensed tannins via the phenylpropanoid pathway. Previous studies on poplar revealed that many genes involved in the phenylpropanoid pathway are transcriptionally upregulated after infection by the rust fungus *Melampsora medusae*. However, it is still unknown which chemical compounds are produced as a defense response upon pathogen attack. In this study, two fungal pathogens, *M. larici-populina* causing leaf-rust and a newly-described fungus *Plectosphaerella populi* causing canker-like symptoms in stems, were used to explore the phenolic defense responses of black poplar (*P. nigra*) during compatible interactions. Analysis of the chemical changes in poplar saplings upon separate controlled inoculation trials with each fungus revealed that accumulation of the flavan-3-ols, catechin and epicatechin, and the flavan-3-ol dimer proanthocyanidin is highly induced following infection. Genes involved in the last step of flavan-3-ol biosynthesis were identified in black poplar and their catalytic function was verified in assays using heterologously expressed proteins. Transcript abundance of three leucoanthocyanidin reductase (LAR) and two anthocyanidin reductase (ANR) genes, responsible for catechin and epicatechin biosynthesis, respectively, increased after infection indicating that biosynthesis was up-regulated. Research is also focused on studying fungal fitness in transgenic poplar clones with reduced proanthocyanidin biosynthesis. Artificial supplementation of flavan-3-ols in vitro to quantify the inhibition of fungal spore germination and mycelial growth is being carried out to investigate the role of these substances as direct anti-fungal defense compounds.

Poster number: *214*

# Effects of plant-plant signaling on the oviposition preference of *Pieris brassicae* and *Plutella xylostella* females under elevated ozone conditions

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Plant-plant signaling mediated by volatile organic compounds (VOCs) is a process typically known to enhance the resistance of the receiver plants or, more recently shown, to increase the susceptibility to herbivore attack (Li & Blande 2015). Once released in the atmosphere, VOCs are prompt to be degraded by air pollutants such as ozone altering sensitive ecological interactions such as plant-plant signaling (Blande et al. 2010). The specific effects of VOCs on a receiver plant and its interactions with herbivores as well as air pollutants have received little attention. We conducted a series of plant-plant signaling experiments in field and climate-controlled chamber conditions under ambient-ozone and elevated-ozone concentrations in order to investigate and compare the host-plant (cabbage (*Brassica oleracea* cv. Lennox)) selection behavior of females of *Pieris brassicae* and *Plutella xylostella*. In dual-choice tests, cabbage receiver plants in ambient-ozone concentrations in field exposed to herbivore-infested cabbage plants (Amb-irVOC) were slightly less attractive to mated females of *P. brassicae* than cabbage receiver plants exposed to undamaged cabbage neighbors (Amb-crVOC, 36.8% vs. 63.17%; P = 0,164, Paired t-test). This effect was not observed under elevated-ozone concentrations (Ozo-crVOC vs. Ozo-irVOC; 55.4% vs. 44.6%; P = 0,661, Paired t-test). *P. xylostella* females did not show a clear preference towards any of the previous treatments under either ambient-ozone or elevated-ozone concentrations. Multiple-choice test were performed under laboratory conditions, shifting among cabbage and broccoli (*Brassica oleracea var. italica*) as the emitter plants. Four treatments were offered simultaneously to mated adults of *P. xylostella* (Amb-crVOC vs. Amb-irVOC vs. Ozo-crVOC vs Ozo-irVOC). Preliminary results showed that Amb-irVOC receiver plants are more susceptible to oviposition when broccoli and not cabbage is the emitter. Most of the interactions were impaired by ozone.

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Poster number: *215*

## Protection of Norway spruce against blue stain fungus

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Induced defence systems of trees are of importance to withstand abiotic and biotic threats. In Europe, blue stain fungus (*Ceratocystis polonica*) associated with the spruce bark beetle (*Ips typographus*) make severe damage to mature trees. In plant metabolism the jasmonic acid (JA) pathway can be manipulated with chemical elicitors to evoke chemical defences prior to fungi attack. Through this pathway volatile and semi-volatile constituents (mono and sesquiterpenes) are produced as well as the heavier diterpene acids. Triggering of defences, can serve as a vaccination of the spruce against pathogens and biotic aggressors. The ecological incentive is to synthetically provide a naturally occurring plant hormone instead of insecticides, to evoke the natural defence system of the tree.

For the purpose of relating defence response of clones to their resistance to fungi growth, young seedlings of Norway spruce (*Picea abies*) were used as model plants. These were treated in summer 2014 with methyl jasmonate (MeJA) to prime resistance and thereafter inoculated with blue stain fungus (*Ceratocystis polonica*). Tissue samples were solvent extracted for chemical analysis of defence components and analysed by gas chromatography-mass spectrometry (GC-MS). The research aim was to measure plant responses qualitatively and quantitatively. In preliminary runs, an increase of geranyl linalool has been observed in the vicinity of the inoculation zone. It is unclear whether this substance is produced by tree or by fungus.

Poster number: *216*

# Identification of fomannoxin in *Picea abies* naturally infected by *Heterobasidion parviporum* and the effects of terpene constituents on its production

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The aim of this study was to estimate further relationships between *H. parviporum* and *P. abies* focusing on the fungal metabolite and terpene constituents. In our previous studies, we investigated the terpenoid compositions around the reaction zone of ca. 50 years old *Picea abies* naturally infected by the *Heterobasidion parviporum* 1, and antifungal properties as well as biotransformation of the major hydrophobic constituents of *P. abies* by *H. parviporum* 2. As a part of the first study, the components not presented in the healthy tree such as oxidized monoterpenes and considerable compound A (M+ = m/z 188) were observed at vertical reaction zone. The second study revealed that some oxygenated monoterpenes such as bornyl acetate and α-terpineol showed antifungal activities against *H. parviporum*. When medium surface re-extraction method conducted after the antifungal test, most α-terpineol remained unchanged on the surface while bornyl acetate was biotransformed into camphor and borneol. As a part of this second study, compound A was also found on the medium surface as a metabolite of *H. parviporum*; however, it seemed that the amounts of this compound were different among each constituent applied. First of all, this compound was isolated from the growth medium culture and identified as the phytotoxic fungal metabolite, fomannoxin. Re-considering the data of second study, some oxygenated monoterpenes apparently inhibited fomannoxin production of *H. parviporum*.

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Poster number: *217*

# The plant *Hyptis suaveolens* (Lamiaceae) repellency on *Ixodes ricinus* (Acari: Ixodidae): Structure activity studies of sesquiterpene oxides and sulfides

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*Ixodes* ticks are among the most prominent arthropod vectors of pathogenic infections affecting humans and domesticated animals. *Hyptis suaveolens*, a plant traditionally used as a mosquito repellent, originating from Laos and from Guinnea-Bissau for investigation of the repellent properties against nymphs of the tick *Ixodes ricinus*.

Compounds from essential oils and fresh and dried leaves were identified by GC-MS and tested in a tick repellency bioassay. Structure activity studies of oxidation or sulfidation products of germacrene D, α-humulene and β-caryophyllene were performed in order to test possible enhancement of repellency.

(+) and(-)-Sabinene were present in high amounts in all preparations, and dominated the emission from dry and fresh leaves together with 1,8-cineol and α-phellandrene. 1,8-Cineol and sabinene were major compounds in the essential oils from *H. suaveolens* from Laos. Main compounds in *H. suaveolens* from Guinea-Bissau were (-)-sabinene, limonene and terpinolene. Among the sesquiterpene hydrocarbons identified, α-humulene exhibited strong tick repellency (96.8%). The structure activity studies of oxidation or sulfidation products of germacrene D, α-humulene and β-caryophyllene, showed strong tick repellency: mint sulfide (59.4 %), humulene-6,7-oxide (94.5%) and caryophyllene-6,7-oxide (96.9%).

Despite the difference in the proportions of the main constituents, the oils of *Hyptis* from both Guinea-Bissau and Laos showed strong tick repellent activity. Compared with previously known tick repellents, such as the oxygentated monoterpenes the caryophyllene and humulene-based sulfur-and oxygen-derivatives are more promising for practical applications since they have a lower volatility but still exhibit strong repellency.

Adding sulfur to the terpene hydrocarbons is a simple and inexpensive synthesis resulting in compounds with lower volatility, thus giving a longer-lasting preparation. This may increase the practical use of essential oils, for developing plant based insect repellents.

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Poster number: *218*

# The benzoxazinoid 2,4-dihydroxy-7-methoxy-1,4-benzoxazin-3-one (DIMBOA) inhibits trichothecene accumulation in liquid culture by suppression of tri gene expression

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Fusarium head blight (FHB) is a severe disease in wheat, caused mainly by *Fusarium graminearum*, resulting in significant yield losses and accumulation of trichothecene mycotoxins in grain. Susceptibility to *Fusarium* and mycotoxin accumulation in grain is cultivar dependent. In order to reduce the use of pesticides to control FHB in wheat, various plant secondary metabolites have been studied for their ability to inhibit trichothecene biosynthesis, e.g. ferulic acid and 4-acetylbenzoxazolin-2-one.

Seven secondary metabolites (belonging to benzoxazinoids, phenolics and flavonoids) naturally occurring in wheat were tested for their inhibitory activity against trichothecene production in liquid cultures with a *F. graminearum* lineage producing 15-ADON. The benzoxazinoid 2,4-dihydroxy-7-methoxy-1,4-benzoxazin-3-one (DIMBOA) suppressed toxin production in a concentration dependent manner with complete inhibition of 15-ADON accumulation at 250 μM DIMBOA.

Employing gene expression studies, we showed that DIMBOA suppressed expression of tri6, a key transcriptional regulator of the majority of genes involved in the biosynthesis of 15-ADON. In cultures supplemented with DIMBOA the expression pattern of tri5 followed that of tri6, consistent with tri5 gene expression being regulated by tri6.

In agreement with Ponts et al (2011), the phenolics ferulic acid and coumaric acid stimulated 15-ADON production in the liquid cultures. Ponts et al. (2011) showed that the toxin stimulation was a result of an upregulation of trichothecene gene expression, specifically that of tri5.

To our knowledge this is the first time that the benzoxazinoid DIMBOA has been tested for its suppression on trichothecene accumulation in liquid culture.

Our results indicate that DIMBOA plays an important role in the accumulation of toxic trichothecenes in wheat grain by targeting gene trichothecene expression. We are currently finalizing data from a large field study correlating the content of secondary metabolites and trichothecene accumulation in wheat heads. The study will shed light on the importance of compounds like DIMBOA in the combat against FHB.

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Poster number: *219*

## Oviposition-induced indirect defence trait in maize against herbivorous insects: Effects of domestication and breeding

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Maize, *Zea mays* L., is one of the most important crops worldwide. It is a genetically diverse crop having been domesticated from its wild ancestor, teosinte. Lepidopteran pests are a major constraint to maize production especially in sub-Saharan Africa causing yield loss up to 80%. Maize, like most other Graminae, naturally defends itself against herbivore attack through a number of mechanisms, including the production of herbivore-induced plant volatiles (HIPVs) following larval feeding. These HIPVs act, both, to deter further colonisation by adult moths and attract insects antagonistic to these pests. However, these HIPVs are produced long after damage has been inflicted to the plant by the feeding larvae. Recently we showed that there are landraces whose defense systems are inducible by egg oviposition leading to production of HIPVs that deter further oviposition and attract both egg and larval parasitoids. These tritrophic interactions have rarely been targeted in crop breeding programmes. Crop plants were domesticated from wild ancestor species which had evolved adaptations that allowed them to survive in their natural habitats where they were exposed to attacking organisms and were selected for yield and quality by humans over thousands of years. Current plant breeding trials are usually carried out in the background of a robust pesticide programme. This could have affected the plants' defences. For better understanding of the ecological relevance and evolutionary history of oviposition-induced plant signaling it was prudent to study these signals in wild, landrace and hybrid systems. HIPVs were collected from plants exposed to egg deposition by *Chilo partellus* through headspace sampling. Four-arm olfactometer bioassay was carried out using parasitic wasps *Trichogramma bournieri* and *Cotesia sesamiae*. Coupled GC-MS and GC-EAG were used for chemical analysis. We found genotypic variation in the indirect plant defence trait: it is more prevalent in wild and landrace maize lines and very rare in elite hybrid maize lines suggesting breeding for yield may have affected this defence trait. Certain improved maize lines with the trait have now been identified and links between this phenotype and the genotype are being explored. Introgression of this indirect defence trait into improved maize lines can be used to increase indirect defense against stemborers in agroecosystems.

Poster number: *220*

# Brown marmorated stink bug pheromone: Interplay of purity, cross-attraction and synergy for an invasive insect species (Hemiptera: Pentatomidae)

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Brown marmorated stink bug, *Halyomorpha halys* (Stål)(Hemiptera: Pentatomidae), is a polyphagous invasive pest of fruits, vegetables, field crops and ornamental plants recently introduced to North America and to Europe from east Asia. The male-produced aggregation pheromone of *H. halys* consists of (*3S,6S,7R,10S*)- and (*3R,6S,7R,10S*)-10,11-epoxy-1-bisabolen-3-ol in approximate 3.5:1 ratio. Harlequin bug (*Murgantia histrionica* (Hahn)) is a North American native pest of Brassicaceae which has closely-related pheromone chemistry consisting of a common main component (*SSRS* isomer) and a divergent minor component (*SSRR*) in ~1.4:1 ratio.

Both species are attracted to off-ratio and mixed-isomer preparations of 10,11-epoxy-1-bisabolen-3-ols which contain their respective pheromone components. Isomer mixtures arising from racemic reactants (yielding all 16 possible isomers) are much less expensive to make, and appear to be much more attractive per unit expense; details of the tradeoffs between cost and attractancy are not yet completely clear. However, there clearly are contrasts in semiochemical response of these two species, in spite of the close relateness of their pheromones.

*H. halys* (but not *M. histrionica*) responds to methyl (*E,E,Z*)-2,4,6-decatrienoate, the pheromone of another stink bug species, *Plautia stali* Scott, with which it is sympatric in its native Asia, and surprisingly, this putative kairomone synergizes *H. halys* attraction by its own pheromone. *M. histrionica* responds also to host-specific mustard plant kairomones, additive in attraction to its own pheromone, but, so far, possible plant-produced attractants for *H. halys* are not known.

Pheromone discovery is key to its use, but the many considerations of purity, cross-attractancy, potential repellency, as well as combination with other insect- and plant-produced semiochemicals, makes for a complicated and fascinating “balancing act” necessary to achieve practical application for pest management.

Poster number: *221*

# *Morganella morganii* bacterium producing phenol in the colleterial gland of females of the New Zealand grass grub, *Costelytra zealandica*, attracts males

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*Costelytra zealandica* (Coleoptera: Scarabeidae) is a univoltine New Zealand endemic species that has colonised introduced clover and ryegrass pastures, that form about half of the land area of the country. Damage from larval stadia cause significant losses in pastures. This insect was previously shown to use phenol as the sex pheromone, males being attracted to females. The phenol was reported to be produced by symbiotic bacteria in the accessory gland in the oviduct. In this study, bacterial strains have been isolated from this oviduct and tested for attractiveness towards grass grub males in traps in the field. The active (phenol-producing) bacterial strain has been identified by partial sequencing of the 16SrRNA gene as *Morganella morganii*. We tested the hypothesis that the phenol is biosynthesized from the amino acid tyrosine from the bacteria. This has been proven to be correct, by addition of isotopically labelled tyrosine (deuterium) to the bacterial broth and then detection of the deuterolabelled phenol by SPME-GCMS.

Poster number: *222*

# Brief mechanical stimuli between plants affect herbivore insects

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Many plant species are able to perceive mechanical stimuli and respond with physiological, morphological or biochemical adjustments. Such changes in plant status may be detected by herbivory insects providing them with reliable cues about host plant quality. The broader ecological significance of induced plant responses to mechanical stimuli on herbivore insect behavior has not been explicitly investigated. In this study examined whether plant response to 1 min daily touching treatment over a period of 6 days affects olfactory response and host plant acceptance by the bird cherry-oat aphid *Rhopalosiphum padi* L. on maize and by the black bean aphid *Aphis fabae* Scop. on bean. Both tested aphid species showed significantly reduced acceptance of touched plants compared with untouched plants. Volatiles released by touched and untouched plants were collected and identified by gas chromatography-mass spectrometry (GC/MS). Stepwise discriminant analyses identified (*E*)-nerolidol and (*E*)-β-caryophyllene in maize and 6-methyl-5-hepten-2-one and an unidentified sesquiterpene in bean as the best discriminating compounds in the volatile profiles of touched plants. Olfactory bioassay demonstrated aphid avoidance of volatiles emitted by touched plants. Our study suggests that even brief mechanical stimuli can induce changes in plants that have potential to affect host plant selection and acceptance by aphids. The link between plant response to mechanical stimuli and insect behaviour identified in our study represents a new phenomenon that contributes to the broader ecological significance of induced plant responses to mechanical stress.

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Poster number: *223*

## Cues associated with malaria mosquito egg laying site


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*Anopheles gambiae* sensu lato mosquito is the principal vector of malaria in sub-Sahara Africa. In order to develop an effective control method, it is necessary to understand its life important behavior including egg laying - oviposition and blood feeding behavior. While the blood-feeding behavior of this species have been well studied, very few studies have been performed on the oviposition behavior. We thus studied the colonization by wild *An. gambiae* s.l. of artificial oviposition sites (ponds) in an open field setting in Mbita, Western Kenya. The oviposition pond consists of soil mixed with water that had aged for 4, 8 or 21 days (Ages 1, 2 and 3 respectively) and opened for colonization. Chemical and physical parameters were measured in each pond and water samples were collected for bacterial and volatile profile analysis. Chemical profile of water samples were determined with gas chromatography-mass spectrometry (GC-MS) and bacterial community analysis was performed using denaturing gradient gel electrophoresis (DGGE). The physical parameters studied include pH, temperature, nitrite, turbidity, phosphorus, nitrate and oxygen content of the ponds. The number of early instar larva in each age group was also counted as a proxy for pond attractiveness. Results were visualized using multivariate analysis.

Age 1 ponds had on average two times more larvae than the older age groups (Mean ± 95% confidence interval is 49 ±25, 24 ±12 and 20 ±12 for Ages 1, 2 and 3 respectively). Furthermore, the ponds of different age differed in their physical, bacterial and chemical profiles as visualized using multivariate analysis. Turbidity was one of the physical parameter associated with high larva density age 1 ponds. Identification of bacteria species and compounds associated with this age group is ongoing. Further analysis will be needed to determine if these differences can be used to identify oviposition attractant/semiochemical that can be utilized in 'attract and kill' strategies of malaria mosquito control.

Poster number: *224*

# Bioguided study of Lithraea molleoides against Atta sexdens rubropilosa and Leucoagaricus gongylophorus.

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Leaf-cutting ants, *Atta sexdens rubropilosa*, dominant herbivores in the tropics, are considered a serious pest for agriculture, especially when they attack cultivated plants [1]. They cut vegetal matter to feed its symbiotic fungus, *Leucoagaricus gongylophorus*, and this symbiotic relationship is essential to their survival [2].

In this context, the diversity of secondary metabolites present in plants with a wide range of biological activities have justified the interest in studying *Lithraea molleoides* (Anacardiaceae) searching for insecticides and fungicides compounds to control *A. sexdens rubropilosa* and its symbiotic fungus.

The ethanolic extracts of leaves (EL), branches (EB) and stem (ES) of *L. molleoide* were incorporated into artificial diet at a concentration of 2.0 mg.mL-1 for the toxicity bioassay against the ant workers and also incorporated into culture medium of the *L. gongylophorus* at a concentration of 1000 ug.mL-1 to evaluate the growth inhibition of the fungus.

The EL extract showed significant activity against the ants and the fungus. The statistical results indicated significant difference for the median survival (Md) of ant workers in relation to the control (Md = >25days), where were observed Md = 12 days and 94% inhibition of the symbiotic fungus growth, when compared with controls.

The EL extract was submitted to a liquid-liquid partition and the hexanic, dichloromethanic, ethyl acetate and hydroalcoholic fractions were tested again in both bioassays. For the assays with ants, the fractions (1.0 mg.mL-1) with higher activity were the hexanic and dichloromethanic with Md-EL-hex = 10 days and Md- EL-dichloro = 15 days in relation to the control (Md = >25 days). However, the bioassays with these same fractions against the fungus didn't show significant activity.

The extracts and fractions with higher activity are been subjected to chromatographic processes bioguided by bioassays searching for compounds with insecticide potential.

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Poster number: *225*

## The chemistry of bluebell (*Hyacinthoides non-scripta*)

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The life cycle of a bluebell as a perennial bulbous plant is characterised with distinctive stages including growth of vegetation, flowering and seed formation followed by aging and death of the above ground organs in preparation for dormancy. The transition between these stages is accompanied by changes in the chemistry of the plant. Both primary and secondary metabolites change in different patterns and proportions in response to changes in a variety of internal and external factors. Climatic influence (temperature, sunshine hours, rainfall, etc), competition and availability of nutrient, and defence against herbivores or attraction of pollinators are some of the factors that influence the onset and duration of the different stages.

Carbohydrates are plant's source of energy for growth and development besides providing intermediates for synthesising a range of cellular components. Seasonal variation of mono- and disaccharides represented by glucose, fructose and sucrose, and of polysaccharides in the form of fructans showed a significant change in the type and quantity of these molecules. Preparation for dormancy is characterised with refilling the fructans in the bulbs with very low concentrations of mono and disaccharides whereas early growth is characterised with minimum values of storage polysaccharides and high quantities of simple sugars.

Phenolics, as a class of secondary metabolites with contribution to plant defence, attraction, alleopathy and resistance to oxidation show a different pattern over the season. The main flavonoid in bluebells is apigenin. Other phenolic acids (e.g. p-coumaric acid) are also found. These compounds are mainly located in the above ground organs reaching their seasonal high values during flowering time which might refer to the involvement of these compounds in attracting pollinators at times when they are most needed.

Poster number: *226*

## Synthesis and analysis of leaf beetle defensive compounds

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Diverse genera within the Chrysomelinae (Chrysomelidae) subfamily produce and sequester a spectrum of different defensive compounds.1,2 Isoxazolin-5-one glucosides form an important class of such secondary metabolites.3 These de novo biosynthesized compounds are widespread in the Chrysomelinae subfamily and present in different life stages of the beetles. Adult leaf beetles produce a defensive secretion that contains mM concentrations of these glucosides, mainly consisting of two compounds: 2-(β-D-glucopyranosyl)-3-isoxazolin-5-one (ISO1) and 2-[6´-(3´´-nitropropanoyl)-β-D-glucopyranosyl]-3-isoxazolin-5-one (ISO2).3 The second compound (ISO2) contains an ester moiety of the neurotoxin 3-nitropropanoic acid (3-NPA), which is an inhibitor of the mitochondrial respiration.4 Up to now it is not known that these compounds occur in the larval stage of the leaf beetles. Recently we identified ISO1 and ISO2 in the hemolymph of leaf beetle larvae using synthetic references by HPLC/MS and NMR analysis. Furthermore we determined the amount of these metabolites in a number of different *Chrysomelina* species. Novel synthetic routes for compounds ISO1 and ISO2 are described allowing a simple production of significant amounts of the substances.5,6 Due to the widespread occurrence of these glucosides we started investigations concerning the biosynthesis and detoxification of 3-NPA in the leaf beetle larvae. The chemical synthesis, biosynthesis and transport phenomena of isoxazolin-5-one glucosides in leaf beetles are discussed.

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Poster number: *227*

## Differences and commonalities between pheromone receptors of tortricid moths

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The Tortricidae family includes serious pests in a range of important crops such as apple, pear, walnut, plums, pea and chestnut. Although the ecology, pheromones and behavior of many of the species considered as pests have been characterized, there are still many unanswered questions regarding the evolutionary divergence across species within this group. In this sense, characterizing pheromone-detecting receptors (PRs) represents an enlightening source of information that might lead to novel control strategies. In an attempt to compare the divergence of pheromone receptors in members of the Tortricidae family we identified new candidate PRs from the species *Hedya nubiferana*, *Cydia fagiglandana* and *C. nigricana* by means of antennal transcriptome analysis revealed by Illumina –based RNA-sequencing. By comparison with *C. pomonella* and other tortricid olfactomes identified to date, we found that, just as observed in other orders of insects, the odorant receptor gene family is highly diverse across members of the same family. However, when it comes to PRs this divergence is much more conspicuous. Although some degree of conservation is observed for receptors with similar functions under similar ecological conditions, most of the pheromone receptors seem to be under low sequence conservation, leading to specialized receptors in each species. Our results demonstrate that receptors specialized in detecting common odors for host finding might be conserved, while sex-specific receptors are much more subject to change due to both physiological response to the environment and sexual selection.

Poster number: *228*

# Host plant preferences in *Spodoptera littoralis* larvae

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The cotton leafworm *Spodoptera littoralis* is a highly polyphagous and devastating pest on many economically important crops worldwide. Adult moths use their olfactory sense to find their suitable host plants. This is primarily achieved through the insect "nose", the antenna, which is densely packed with chemosensory hair-like sensilla that filter odorants to neuronal input channels. Caterpillars, on the other hand, have relatively fewer sensilla on their antenna to effectively discriminate complex host volatiles compared to adults. Larvae, which experience tremendous physiological changes during metamorphosis, have an overlap with the adult in olfactory preferences, but to which extent has remained unknown. With this hypothesis, we are examining larval olfaction at different levels, namely the responses to complex plant odors at molecular, electrophysiological and behavioral levels. At the molecular level, we are examining the larval transcriptome for the odorant receptors and other proteins in the antenna in comparison with the repertoire of olfactory proteins of adults. Using antennal electrophysiology recordings, we are comparing responses of larva to different odors from selected host plants like *Gossypium herbaceum* (cotton), *Trifolium alexandrinum* (clover), *Vigna unguiculata* (cowpea), *Brassica oleracea* (cabbage) and *Zea mays* (corn). Behaviorally, we are testing synthetic blends of volatiles from these host plants to compare the responses of larva on the Tracksphere Locomotion Compensator (Syntech). The aim of the project is to combine our transcriptomic, electrophysiological and behavioral analyses to understand the ecological relevance of larval olfaction and behavior in responses to host plant volatiles.

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Poster number: *229*

# Norway spruce volatile and bark beetle *Ips typographus*: Role of trans-4-thujanol


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*Ips typographus* is one of the most important pests of the Norway spruce (*Picea abies*) which prefers to infest old trees. We looked for spruce volatiles that were perceived by *I. typographus*, and which differed as the host tree aged.

The GC-EAD analysis of spruce tree bark extract revealed the presence of several EAD-active compounds of which *trans*-4-thujanol was consistently active and was unequivocally identified. The analysis of dose-EAG response to synthetic *trans*-4-thujanol demonstrated that *I. typographus* females responded to lower concentration of the compound than males. Behavioural assay in Y-tube olfactometer revealed that this compound is repellent to both sexes of the beetle.

*I. typographus* beetles prefer to attack older trees; therefore the presence of trans-4-thujanol in spruce trees depending on their age was checked. The bark of 10-year-old trees contained three times higher amount of *trans*-4-thujanol than that of 35–40-year-old trees, 27 times and even 200 times higher amount compare to that of 70–80-year-old and 120-year-old trees, correspondingly.

In conclusion, *trans*-4-thujanol is active compound of Norway spruce that varies in amount with tree age and affects spruce bark beetle behaviour under laboratory conditions. It is suggested that it might have a role both in spruce tree defence and suitable tree choice by the beetles.

Poster number: *230*

# Integration of defensive strategies and defensive investment with other life history traits in a *Pinus pinaster* population

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As any other living entity, forest trees must optimize resource investment to growth, reproduction and maintenance, but investment in one function may occur at the expense of other functions, resulting in trade-offs that limit maximization of all processes. Global change is imposing new challenges to Mediterranean forests, in particular combined abiotic and biotic stressors such as drought and epidemic outbreaks of pests and pathogens that may require increased defensive investment. Mediterranean pines are expected to harbour a valuable adaptive genetic diversity, already shaping the populations since long ago, for facing these challenges. Little is known, however, about how populations will respond to multiple challenges, and also about the correlated responses to a single stressor in other life history traits. Here we used as a model a Maritime pine (*Pinus pinaster* Ait.) population historically exploited by tapping for oleoresin extraction and artificially selected for increased turpentine and rosin production. In a common garden with 60 open-pollinated half-sib families (selected for resin production plus a control seedlot) we evaluated the constitutive and induced resin flux as a proxy of defensive investment and concentration of major groups of chemical defences in phloem-cortex and needles. We also measured a wide set of traits related to growth and maintenance (height, diameter and bark thickness), reproduction (cone counts and serotiny level) and drought-resistance ($^{13}C$-based water-use efficiency). Our objectives were to determine the intrapopulation genetic variation in these key phenotypic traits and to explore the existence of tradeoffs among adaptive life history traits. Preliminary results showed the existence of significant genetic variation among families for most assessed variables, and distinct trade-offs between growth and maintenance, reproduction and defense. We will discuss to what extent variations in the carbon-water balance at the intra-specific level may mediate the conflicts between investment in growth and defence in a Mediterranean climate.

Poster number: *231*

# Activity and evolution of Phasmatodea beta-1,4-endoglucanases and their implications for polyneopteran herbivory

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Recent transcriptomic data found endogenously-produced plant cell wall degrading enzymes in the Phasmatodea: beta-1,4-endoglucanases (cellulases) and polygalacturonases (pectinases), the latter homologous to bacterial genes despite being encoded in the insect's own genome, suggesting a horizontal gene transfer. Each phasmid species has several genes of each enzyme class, with the possibility that some lost or changed their function. This study examines the activity of the cellulases and traces their evolutionary history.

Phasmatodea gut extract activity was tested against several cell wall polysaccharide substrates. RACE PCR was used to obtain full-length sequences for each enzyme from seven species' transcriptomes. These were aligned and maximum likelihood trees made to identify groups of orthologs. Enzyme genes from representative species were heterologously expressed in insect SF9 cells and their activity against different substrates tested via plate assays and thin layer chromatography.

Phasmid cellulases are GH9 endoglucanases found in many Metazoans, but with distinct gene duplication events occurring within the order. Some phasmid GH9 enzymes can digest xylan or xyloglucan, an unusual finding that may explain why Phasmatodea have maintained so many cellulase gene copies: some have been modified for xylolytic activity. The significance of these findings for Phasmid evolution and physiology, in particular their leaf-based diet, is discussed.

Poster number: *232*

# Vomeronasal receptor families in the deer mouse *Peromyscus maniculatus*: Towards an evolutionary analysis

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Chemoreceptors are the first element in a complex pathway that can ultimately affect behavior -- changes in their biochemical properties or expression pattern have the potential to modulate specific behavioral responses. In particular, the activation of sensory neurons in the VNO is directly associated with changes in behavior, such as aggression, avoidance or mating. To understand the diversity and evolution of pheromone systems in wild populations, we are studying deer mice (genus *Peromyscus*), which diverged from *Mus* approximately 25 MYA. Here, we report on the characterization of VNO receptors from the most common and widespread species in North America, *P. maniculatus*. In total, we identified 150 and 90 putative V1R and V2R genes, respectively, in the *Peromyscus* genome, fewer than in *M. musculus* (239 and 120, respectively). While clades previously identified in *Mus* have representatives in *Peromyscus*, our phylogenetic reconstructions indicate that most gene duplications took place after the split between the two lineages; several clades show sign of lineage-specific expansion or contraction. These differences in the chemosensory receptor repertoires likely reflect the difference in habitats as well as social and mating behavior of deer mice. The establishment of a functional map of receptor-ligand interactions will be discussed.

Poster number: *233*

# Genes associated with glycerol biosynthesis in the red-spotted Apollo butterfly, *Parnassius bremeri* in Korea

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The red-spotted apollo butterfly, *Parnassius bremeri*, immatures grow during winter and spring. Supercooling point of larvae during January goes much below -20℃. Morphologically, the larvae appear to be adapted to cold temperatures. Dark-colored body surface is useful to absorb solar energy and spiny integument may prevent any external ice formation on the body surface. Biochemically, *P. bremeri* larvae elevate glycerol as a cryoprotectant. This study reports two genes associated with glycerol biosynthesis in *P. bremeri*. Larval transcripts were analyzed using RNA-Seq technique. A total of 14 Gb transcripts were read by Illumina HiSeq and assembled to be 127,279 contigs. To specify the genes associated with glycerol biosynthesis, a biosynthetic pathway to synthesize glycerol from dihydroxyacetone-3-phosphate was predicted with two genes of glycerol-3-phosphate dehydrogenase (GPDH) and glycerol kinase (GK). Both genes were annotated in the transcriptome of *P. bremeri*. Pb-GPDH encodes 166 amino acid residues containing NAD+-binding region, catalytic site, and calcium binding region. The predicted amino acid sequence was clustered with other lepidpopteran GPDH genes. Three Pb-GK genes were annotated from the transcriptome. Pb-GK1 encodes a full open reading frame of 514 amino acid residues. A ohylogenetic analysis showed that these three GKs were separately clustered. Interestingly, Pb-GK1 was clustered with other GKs that were known to be associated with rapid cold hardiness.

Poster number: *234*

## Chemical and bioactive investigation of *Kosteletzkya virginica* (Malvaceae)

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The evolved chemical defensive strategy provided us a new approach in searching for potential bioactive molecules from natural sources. *Kosteletzkya virginica* (L.) Presl., was a perennial plant native to the saline tidal marshes and belonged to the genus of *Kosteletzkya* in the family Malvaceae. As an excellent economic halophyte, *K. virginica* has been served as a candidate species of the current development and utilization of saline flats in the east of China. According to chemical and bioactive investigation, we found that extreme environment induced this species to biosynthesize the necessary substances to establish their chemical defensive systems and these compounds possessed various bioactive properties. In our study, 39 compounds were isolated and identified from the ethanol extract of *K. virginica* tuberous roots by a variety of modern chromatography and spectroscopic methods, including a new naphthalene, 8-methoxy-2,7-dihydroxyl-4-(1'-hydroxyl-isopropyl)-6-methyl-1-naphthaldehyde named as virginicin. Their structures were elucidated by spectroscopic methods, including 1D-, 2D-nuclear magnetic resonance (NMR) and high-resolution time of flight electrospray ionization mass spectrometry (HRTOFESIMS). All of these compounds were obtained from the genus *Kosteletzkya* for the first time and evaluated for their potential in scavenging diphenyl-picryl hydrazyl radical (DPPH•), inhibition of nitric oxide (NO) induced by lipopolysaccharide (LPS), and cytotoxic activity. The new compound showed activities against DPPH•, NO, human acute promyelocytic leukemia (HL-60) and human colorectal adenocarcinoma (LOVO), with IC50 of 34.6, 12.5, 40.5, 31.7 μmol/L respectively.

Poster number: *235*

## Chemical and antifungal investigation of *Salicornia biggelovii* and *S. europaea*

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The terrestrial halophytes, *Salicornia* L., which were evaluated as one of the strongest salt-tolerant higher plant genus in the word, grew in an extreme coastal desert environment, including saline land, salt lake and intertidal zone. Extreme environment induced this genus to biosynthesize the necessary substances to establish their chemical defensive systems. The evolved chemical defensive strategy provided us a new approach in searching for potential bioactive molecules from natural sources. In order to exploit new molecules with agricultural bioactivity, *S. biggelovii* and *S. europaea* were selected for isolation procedures based on bioassay-guided assay against Colletotrichum gloesporioides. 68 compounds were isolated from the ethanol extracts of these two whole plants. The structures were identified on the basis of spectral analysis, physical and chemical properties, including 6 nortriterpene saponins, Salibege A [3-O-β-D-glucuropyranosyl-30-norolean-12,20(29)-dien-23-oxo-28-oic acid], Bigelovii A [3-O-(6-butyl ester)-β-D-glucuropyranosyl-30-norolean-12,20(29)-dien-28-oic acid-28-O-β-D-glucopyranoside], Bigelovii B [3-O-β-D-glucuropyranosyl-30-norolean-12,20(29)-dien-23-aldehyde-28-oic acid-28-O-β-D-glucoside], Pfaffine B [3-O-β-D-glucuronopyranosyl-30-norolean-12,20(29)- dien-23-aldehyde-28-O-β-D-glucopyr anosyl ester], 3-O-[(6'-methyl-ester)-β-D-glucuronopyranosyl]-30-norolean-12,20(29)-dien-28-oic-28-O-β-D-glucuronopyranosyl ester, Boussingoside A2 [3-O-β-D-glucuronopyranosyl-30-norolean-12,20(29)-dien-28-oic-28-O-β-D-glucuronopranosyl ester]. Some of 68 compounds showed potential antifungal activities in vitro. Among these compounds, Salibege A, a novel nortriterpene saponin, showed a significant antifungal activity against *C. gloeosporioides*, with 69.21% inhibition at 100μg/ml after 72h. Moreover, it displayed broad-spectrum inhibitory activity against *Alternaria alternate*, *A. solani*, *Botrytis cinerea*, C. gloesporioides, Fusarium graminearum, F. verticillioide, Thanatephorus cucumeris and *Sclerotinia sclerotiorum*, with EC50 values ranging from 13.6 to 36.3μg/ml. It may be promising leads for the development of potential antifungal agents, especially against *B. cinerea* and *T. cucumeris*.

Poster number: *236*

# Chemical characterization of branch galls on *Cryptomeria japonica* trees in Japanese forest

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*Cryptomeria japonica* (Japanese name: Sugi) is a main plantation tree in Japanese forest. Galls on the stem and branch of the *C. japonica* tree are known as a disease caused by fungal attack [1-3]. However, chemical components of the branch gall have not been reported. In this study we investigated the chemical components of the galls. The galls of 1-11 years old were collected from Yamagata university forest and classified according to its size. Hollocellulose and Klason lignin contents of the sample were determined by standard method after alcohol-benzene (ethanol-benzene) extraction. Analysis of flavanol contents was performed by vanillin-HCl method. The yield of alcohol-benzene extracts of small size galls (younger galls: 1-3 years old) showed higher than that of big size galls (older galls: over 6 years old). The small size galls had higher flavanol contents value than the big size galls. The hollocellulose and Klason lignin contents had tendency to increase with gall size increases. Thus, it was indicated that the low polar components and flavonoids were produced on infected parts in early stage of galls germination; however, the produced components were disappeared with growth of the gall.

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Poster number: *237*

# Mating enhances behavioral and antennal responses of female small white butterfly *Pieris rapae* (Lepidoptera: Pieridae) to host plant volatiles

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Mating may significantly affect the host selection behaviors of phytophagous insects. Here, we investigated the post-mating changes in behavioral and antennal responses of *Pieris rapae* (Lepidoptera: Pieridae) females to host plant volatiles. Gas-chromatography mass-spectrometry analysis revealed that 15 compounds identified in intact cabbage seedlings were general plant volatiles, of which 7 compounds were also found in the volatiles of intact lettuce seedlings. The results of two-choice bioassays between scented and unscented (control) artificial plant models showed that mated females visited the model scented with a synthetic blend of cabbage volatiles consisting of 15 compounds more frequently than the unscented control, whereas virgin females did not show such preference. Moreover, mated females (but not virgin females) visited the plant model scented with the cabbage blend more frequently than the model scented with a synthetic lettuce blend consisting of 7 compounds. These results indicate that *P. rapae* females became more sensitive to plant volatiles after mating. Gas chromatography–electroantennographic detector analysis revealed that 9 out of 15 compounds in the cabbage blend elicited antennal responses in mated females. Mated females visited the model scented with the blend of these 9 compounds more frequently than the unscented control, suggesting that these compounds served as olfactory cues for host finding and selection. Out of the 9 volatile compounds that induced antennal responses in mated females, 4 compounds (3-octanone, octanal, linalool, and acetophenone) caused little response in the antennae of virgin females. Interestingly, the antennae became responsive to octanal and acetophenone 1–3 h after mating, and to 3-octanone and linalool 3 days after mating, although the increase in the median EAG responses to the 4 compounds was not statistically significant. These results demonstrate that post-mating changes in *P. rapae* occurred at the antennal sensory level.

Poster number: *238*

# Isolation of novel plant-defense-inducing elicitors from rice herbivores

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Plants are able to induce specific defense responses against various types of herbivores. These responses are simultaneously triggered by the wound-derived signals and/or perception of specific elicitors contained in the oral secretions (OS) and/or saliva of herbivorous insects. However, only a limited number of insect elicitors have been identified so far, compared to a relatively large number of known microbe-derived plant elicitors. In this study, we analyzed potential elicitors in OS of *Mythimna loreyi* and *Parnara guttata*, two typical chewing herbivores found in the rice paddy fields in Japan. Both types of crude insect OS showed high elicitor activity assayed as induced reactive oxygen species (ROS), defense-related gene expression and secondary metabolite levels in cultured rice cell system. Fatty acid-amino acid conjugates (FACs) are well-characterized elicitors previously isolated from various Lepidopteran insect larvae. While OS from *M. loreyi* contained large amounts of conventional FACs, *P. guttata*'s OS did not contain any typical FAC constituents detectable by highly sensitive LC-MS method. Apart from FACs, we examined *M. loreyi* OS for the presence of other elicitor-active compounds. Here we provide the first evidence for the existence of other, possibly novel, elicitor compound(s) in the OS of this insect. We show that a partly purified (FAC-deprived) elicitor fraction from *M. loreyi* OS can induce ROS production and secondary metabolite accumulation in the cultured rice cell system.

Poster number: *239*

# Epigenetic effect of juglone - important in allelopathy?

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The naphthoquinone juglone is responsible for the allelopathic effects of the Black walnut tree (*Juglans nigra* L.). Exposure to juglone leads to oxidative damage and cell death in many plant species. However, knowledge about the mechanisms of action is still sparse. Epigenetic mechanisms, for example DNA methylation, play important roles in regulation of gene expression, and our objective was to investigate a possible involvement of epigenetics in plant cells in response to juglone exposure.

Tobacco BY-2 cell culture was used as a model plant system. Cultures in the exponential growth phase were exposed to juglone for 24 h or 48 h. Lipid peroxidation in the cells was analyzed as formation of thiobarbituric acid reactive substances [1]. Changes in DNA methylation level were analyzed by a modified LUMA assay [2].

Exposure of cells to juglone caused decreased growth, increased lipid peroxidation and decreased DNA methylation (hypomethylation). The effects were seen for 60 µM and higher juglone concentrations and were more pronounced with increased exposure time. For more details, see [3].

Exposure of tobacco BY-2 cells to juglone resulted in DNA hypomethylation. The effect may be important for activation of defense genes, and introduces epigenetics as a possible mechanism in defense signaling in response to the allelopathic substance juglone.

Juglone-exposed cells respond with the formation of reactive oxygen species (ROS) and oxidative stress [4], which in turn causes lipid peroxidation. The DNA hypomethylation observed may be a result of these oxidative effects and lead to a more open and available chromatin structure, ready for activation of defense genes.

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Poster number: *240*

## Synthesis of a macrolide library of putative pheromones used by mantellid frogs from Madagaskar

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Anuran amphibians (frogs) use mostly acoustic, visual, and tactile signals for their communication. Besides non-volatile peptides, some frogs produce volatile compounds, currently under investigation by our group. Males of mantellid frogs from Madagaskar possess variable femoral glands that can disseminate volatile compounds. These compounds can acts as attractants to females [1].The femoral glands of *Mantidactylus betsileanus* were excised and extracted with dichloromethane. The extracts were analyzed by GCMS. Macrolides and fatty acid ethyl esters are the major compounds. Interestingly, some unknown compounds with the mass 212 with different mass spectral fragmentations were present. Moreover, these are likely the positional isomers of methyl-11-dodecanolide based on our experience of fragmentations with macrolides of lower molecular mass [1, 2]. Fatty acid biosynthesis allows us to propose five isomers as likely candidates of these macrolides. A synthetic library of macrolides is synthesized to prove the structural proposals. The detailed synthetic schemes and analytical results as well as their occurrence of macrolides in different mantellid frogs will be presented on the poster.

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*Antifeedant and toxicity properties of selected Malaysian plants towards termite and bioactives compounds* (P-*163*)

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***MIC06*: *Terpene arms race in the Seiridium cardinale – Cupressus sempervirens pathosystem***

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***MUT12*:** *Chemically armed ants pillage and protect fungus-farming societies* (P-*111*)
Evolutionary transitions in alarm systems of fungus-farming ants (P-55)
How fungus-growing ants are fooled by their parasites…or are they? (P-207)

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*Why don't Chrysopa green lacewings make pheromone in the lab?* (P-*174*)

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***CON07*:** *Stabilizing Selection on Moth Pheromone Blends: Fact of Fiction?*

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***APP04*:** *Detection, monitoring, and forecast using pheromone traps for three spruce seed feeding moths*

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***INS01*:** *Experience to plant cues affects host plant choice behaviours in a moth*

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*Discovery of peptide toxins in ribbon worms: Challenging claims of tetrodotoxin production* (P-*81*)

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***NEU09*:** *The physiological and ecological significance of olfactory sensory neuron co-localization*

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***UNR04*:** *Isolation of a behaviorally active sex pheromone component of the mushroom fly Lycoriella ingenua, using GC/EAG and an unusual, coupled GC/Behavior technique*

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*Two fatty acyl-CoA reductase gene involved in moth pheromone biosynthesis* (P-*45*)
Genes involved in sex pheromone biosynthesis of Ephestia cautella, an important food storage pest, are determined by transcriptome sequencing (P-21)

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*Olfactory responses of Indian meal moth, Plodia interpunctella, to volatiles of stored-grain contaminated by fungi* (P-*132*)

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***MIC03*:** *Not visible, but highly effective: Plant volatile manipulation by endophytic fungi and responses of herbivores* (P-*22*)

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*Why does the leaf miner Tuta absoluta avoid oviposition on Meloidogyne incognita-infested tomato plants?* (P-*134*)

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*Chemical characterization of branch galls on Cryptomeria japonica trees in Japanese forest* (P-*236*)

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*Does spontaneous vomeronasalitis influence chemical communication in domestic animals? A new approach linking pathology to behavior.* (P-*25*)

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*Influence of fumigants on sunflower seeds: Characteristics of fumigant desorption and alterations of volatile profiles* (P-*107*)

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***MUT09*: *Antennal sensitivity to floral scents of Campanula: A comparative study of polylectic and oligolectic bees***

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*Antagonistic activity of Bacillus subtilis A18–A19 strains against Heterobasidion species* (P-*35*)

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***NEU08***: *Atomic Force Microscopy of Moth Trichoid Sensilla Across Species Reveals Common Themes and Specializations for Odorant Capture*

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***RES14***: *Herbivore damage induces a transgenerational increase of cyanogenesis in wild lima bean (Phaseolus lunatus)*

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*Unusual Chemoreceptors in Aphids* (P-*137*)

**Becker**, Tobias, *Max Planck Institute for Chemical Ecology*, Germany; tbecker@ice.mpg.de
*Synthesis and Analysis of Leaf Beetle Defensive Compounds* (P-*226*)

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*Response of entomopathogenic nematodes to sugarcane root volatiles under herbivory by sugarcane spittlebug nymphs* (P-*152*)

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*A chemical ecology OLPHABET* (P-*1*)

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*Effect of different animal manures on attraction and reproductive behaviors of common house fly, Musca domestica L* (P-*187*)

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***UNR08***: *CATCH AND RELEASE – HOW TO EFFICIENTLY TRAP, ANALYZE AND FORMULATE PLANT ODORS*

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***RES04***: *Inducible Plant Signalling: Opportunities for Real Time Management of Pests*

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***FOR05***: *Improved forest regeneration by triggering the induced defense of conifer seedlings against bark-feeding insects*

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***RES05***: *Volatile-mediated plant-plant interactions: requirements for induced resistance*

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*The influence of chemical and vibrational communication in the mating behaviour of Dichelops melacanthus (Heteroptera: Pentatomidae)* (P-*159*)

**Blažytė-Čereškienė**, Laima, *Nature Research Centre*, Lithuania; blazyte@ekoi.lt
*Norway spruce volatile and bark beetle Ips typographus: Role of trans-4-thujanol* (P-*229*)

**Boeckler**, Andreas, *Max-Planck Institute for Chemical Ecology*, Germany; aboeckler@ice.mpg.de
*Processing of salicin-derived poplar phenolics in the gypsy moth* (P-*33*)

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***MUT07*: *Choosy wasp cheated by copycat orchid chemistry***

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*Identification and field evaluation of the sex pheromone in a Brazilian population of Hypsipyla grandella (Zeller) (Lepidoptera:Pyralidae)* (P-*147*)

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*Utilization of pheromone precursors for the adult butterfly Pieris napi in flower odors and nectar* (P-*177*)

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***VER02***: *Of mice and amines: genetic analysis of odor perception*

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***ISP05***: *Chemical cues of the interaction Gonipterus platensis – Eucalyptus globulus*

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***MUT03***: *Functional role of the floral volatile, S-(+)-linalool, in Penstemon digitalis*

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*Functional adaptation of secretory proteins in the detoxification of sequestered phytochemicals during Chrysomelina leaf beetle evolution* (P-*169*)

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*Predation threat cues as adjustment variables for the expression of self-maintenance behaviours in terrestrial gastropods* (P-*170*)

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*Antifungicidal and antioxidant activities of Rhododendron tomentosum essential oils* (P-*40*)

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***PHE08***: *Chemical profiling and temporal biochemical polymorphism in Lasius ants*

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***CON08***: *Functional, evolutionary and teleological perspectives on finding a source of odor*

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***PCY05***: *Sponge taxonomy 2.0 meets pharmacognosy and chemical ecology* (P-*7*)

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*Interaction of aphrodisiac pheromone components in the butterfly Pieris napi* (P-*92*)

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*Interaction between exotic and native noctuid moth species: Differential oviposition in the presence of conspecific and heterospecific larvae* (P-*142*)

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*The chemical basis of flehmen behaviour in domestic cats* (P-*151*)

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***RES08*: *Caterpillar and rootworm feeding differentially affects defense protein accumulation in corn*** (P-*72*)

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***ISP07*: *Are invasive plants more toxic than native plants? An example of rapid evolution after invasion***

**Challinor**, Victoria, *Goethe University*, Germany; Challinor@bio.uni-frankfurt.de
***MIC02*: *Identification and biosynthesis of new bicyclic alkaloids from the entomopathogenic bacteria Xenorhabdus***

**Chattington**, Sophie, *Swedish University of Agricultural Sciences*, Sweden; sophie.chattington@gmail.com
*Testing the effect of larval population density and host plant on larval immune response and adult oviposition choice in a noctuid moth, Spodoptera littoralis* (P-*131*)

**Chauvet**, Caroline, *Research Institute in Semiochemistry and Applied Ethology*, France; c.chauvet@group-irsea.com
*Tick Attack Inhibition Semiochemical (TAIS): An innovative and promising approach against Rhipicephalus sanguineus* (P-*171*)

**Cheseto**, Xavier, *International Centre of Insect Physiology and Ecology*, Kenya; xcheseto@icipe.org
*Two African fruit flies (Diptera: Tephritidae) produce identical host marking pheromones* (P-*8*)

**Chiriboga**, Xavier, *University of Neuchâtel*, Switzerland; xavier.chiriboga@unine.ch
*Diffusion of (E)-β-caryophyllene in different soil textures and its effects on the foraging behavior of the entomopathogenic nematode Heterorhabditis megidis* (P-*178*)

**Choi**, Kyung San, *Rural Development Administration*, Korea; mutant8@korea.kr
***NEU16*: *Carbon dioxide receptor involved in the perception and production of the sex pheromone*** (P-*200*)

**Clardy**, Jon, *Harvard Medical School*, USA; jon_clardy@hms.harvard.edu
***UNR01*: *Keeping it lonely at the top: competition between Pseudonocardia strains in fungus-growing ant systems***

**Clarke**, Anthony, *Queensland University of Technology*, Australia; a.clarke@qut.edu.au
***RES12*: *The 'Red Bull'® effect: an additional explanation for response to phenylpropanoids by male Bactrocera fruit flies***

**Clausen**, Mette, *University of Copenhagen*, Denmark; mettecl@plen.ku.dk
*Possible dual role of cytochromes P450 from Eucalyptus* (P-*53*)

**Colazza**, Stefano, *University of Palermo*, Italy; stefano.colazza@unipa.it
*Attraction of egg parasitoids toward oviposition induced plant volatiles in a multi-herbivore perspective* (P-*37*)
Drought stress affect host-induced volatile organic compounds emission from plants and parasitoid response (P-88)

**Conchou**, Lucie, *Swedish University of Agricultural Sciences*, Sweden; lucie.conchou@slu.se
*Do odours mediate innate host plant preferences in adult female Spodoptera littoralis?* (P-*167*)

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***NEU12*: *Pheromone reception in the lightbrown apple moth, Epiphyas postvittana***

**Crava**, Cristina, *Fondazione Edmund Mach*, Italy; maria.crava@fmach.it
***INS07*: *Keeping your food fresh: active manipulation of cytokinin-metabolism by a cell content feeder***

**Cravcenco**, Alexei, *KTH Royal Institute of Technology*, Sweden; alexeic@kth.se
*An efficient approach for synthesis of trun-call 1 and trun-call 2, the aggregation pheromone components of larger grain borer (Prostephanus truncatus)* (P-*203*)

**Creton**, Benjamin, *Research Institute in Semiochemistry and Applied Ethology*, France; b.creton@group-irsea.com
***APP05*: *Use of a vertebrate's semiochemical for long-term protection of horses' ears against Black flies (Diptera : Simuliidae)***

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*How do long-horned bee pollinated sexually deceptive orchids attract their pollinators?* (P-*60*)

**Cunningham**, Paul, *Queensland University of Technology*, Australia; paul.cunningham@qut.edu.au
***APP06*: *Fruit ripening volatiles act synergistically as host cues in a pest tephritid fruit fly.***

**Dam**, Marie Inger, *Technical University of Denmark*, Denmark; madam@biosustain.dtu.dk
*Yeast cell factory for production of insect pheromones* (P-*59*)

**Danielsson**, Marie, *Vetenskapens Hus*, Sweden; mariepe@kth.se

**de Araújo**, Clara, *Georg-August University*, Germany; klaravitoria.lima@gmail.com
*HIP-VOC released by the buffalo bur (Solanum rostratum), the former host plant of the Colorado beetle (Leptinotarsa decemlineata) and their detection by electroantennography* (P-*205*)

**De Backer**, Lara, *University of Liège*, Belgium; ldebacker@doct.ulg.ac.be
*Macrolophus pygmaeus perception of HIPV's from Tuta absoluta infested tomato plants* (P-*192*)

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*Bioguided study of Lithraea molleoides against Atta sexdens rubropilosa and Leucoagaricus gongylophorus.* (P-*224*)

**de Castro**, Érika, *University of Copenhagen*, Denmark; erca@plen.ku.dk
*Cyanogenic glucosides in Heliconius butterflies: Sequestration, de novo biosynthesis and specialization* (P-*156*)

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*Do immune-challenged Tribolium castaneum change their volatile profile?* (P-*179*)

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***ISP09*: *The true cost of exotic perfumes: impact of invasive herbivores on native infochemical networks.***

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*The scent of mixtures: Rules of odour processing in ants* (P-*146*)

**Dobson**, Heidi, *Whitman College*, USA; dobsonhe@whitman.edu
*Floral volatiles in host-flower recognition in a pollen-specialist bee* (P-*9*)

**Doddala**, Prasad, *Swedish University of Agricultural Sciences*, Sweden; Prasad.Doddala@slu.se
***BLO11*: *Gustatory synergy between sugars and amino acids in the yellow fever mosquito, Aedes aegypti*** (P-*201*)

**Domik**, Dajana, *University of Rostock*, Germany; dajana.domik@uni-rostock.de
***MIC07*: *Elucidation of the secondary metabolite of the rhizobacterium S. plymuthica 4Rx13***

**Domingue**, Michael, *Pennsylvania State University*, USA; mjd29@psu.edu
***CON05*: *Advances in material science allow a fully integrative study of visual and chemical ecology***

**Dreisewerd**, Klaus, *University of Münster*, Germany; dreisew@uni-muenster.de
*Laser MS imaging of cuticular lipids using etched silver substrates* (P-*34*)

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*Piezoelectric-active polymer coatings and their influence on bacterial cell-cell interaction* (P-*93*)

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***FOR06*: *Insect egg deposition - a warning signal enhancing sex-biased anti-herbivore defence in a tree***

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***FOR14*: *Norway spruce ATAF1-like NAC transcription factors modulate stress*** (P-*126*)

**Elia**, Marta, *University of Turin*, Italy; marta.elia@outlook.com
*Social parasites change host-nest odors in social wasps* (P-*148*)

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***CON03*: *Estimating rates of spread of invasive insects with pheromone-baited traps***

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***BLO07*: *The Malaria whistle for immediate transfer***

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***UNR15*: *High performance separation and mass spectrometry for chemical ecology research***

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*Cues associated with malaria mosquito egg laying site* (P-*223*)

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***M2*: *Plant toxins induce defensive signals- Evolutionary explanations for a functional paradox***

**Escobar-Bravo**, Rocío, *University of Leiden*, The Netherlands; r.bravo@biology.leidenuniv.nl
*Exploring inducible trichome-mediated resistance to the Western flower thrips in tomato* (P-*15*)

**Etxebeste**, Iñaki, *Swedish University of Agricultural Sciences*, Sweden; inaki.echeveste@slu.se
*The kairomonal response of sibling Tomicus species reflects the differences in their breeding hosts* (P-*87*)

**Evenden**, Maya, *University of Alberta*, Canada; mevenden@ualberta.ca
*The importance of plasticity in response to semiochemicals in the development of monitoring tools for the pea leaf weevil, Sitona lineatus L. (Coleoptera: Curculionidae)* (P-*194*)

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**Fernandez**, Patricia, *INTA. EEA Delta del Parana*, Argentina; pcfernan@agro.uba.ar
***INS05:**Both volatiles and epicuticular plant compounds determine oviposition of the willow sawfly nematus oligospilus on leaves of Salix spp. (Salicaceae)* (P-*63*)

**Fernandez-Grandon**, Mandela, *University of Greenwich*, UK; fg26@gre.ac.uk
***BLO09*: *Heritability of Attractiveness to Mosquitoes***

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***INS04*: *Aboveground application of elicitors recruits an entomopathogenic nematode belowground*** (P-*73*)

**Fischer**, Izabela, *University of Gdańsk*, Poland; izabela.fischer@phdstud.ug.edu.pl
*Chemical composition of preen waxes in Herring Gull (Larus argentatus) during winter and in the breeding season* (P-*140*)

**Fomsgaard**, Inge Sindbjerg, *Aarhus University*, Denmark; Inge.Fomsgaard@agro.au.dk
*The benzoxazinoid 2,4-dihydroxy-7-methoxy-1,4-benzoxazin-3-one (DIMBOA) inhibits trichothecene accumulation in liquid culture by suppression of tri gene expression* (P-*218*)

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***UNR09*: *Spiros are Everywhere***

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***MUT08*:** *Floral scent in a geographic mosaic of coevolution*

**Fürstenau**, Benjamin, *Free University of Berlin*, Germany; fuerstenau@zedat.fu-berlin.de
*How background odor affects the host searching behavior of the ectoparasitoid Holepyris sylvanidis.* (P-*68*)

**Gabirot**, Marianne, *Cardiff University*, UK; marianne.gabirot@gmail.com
***VER05*:** *How blue Petrels find their scented burrow?* (P-*61*)

**Gabryś**, Beata, *University of Zielona Góra*, Poland; b.gabrys@wnb.uz.zgora.pl
*Alteration of peach potato aphid Myzus persicae (Sulz.) probing behavior by natural and modified jasmonates* (P-*124*)

**Galis**, Ivan, *Okayama University*, Japan; igalis@rib.okayama-u.ac.jp
***UNR14*:** *Emerging role of phenolamides as universal plant defense metabolites*

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***MIC05*:** *Volatile affairs in microbial interactions*

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***FOR01*:** *Terpenoid resin defenses in Norway spruce: Herbivore induction and insensitivity*

**Gimenes**, Leila, *Federal University of São Carlos*, Brazil; leilagimenes@gmail.com
*Evaluation of fungicidal activity from Picramnia bahiensis extracts for leaf-cutting ants control* (P-*190*)

**Girling**, Robbie, *University of Reading*, UK; r.girling@reading.ac.uk
*Trajectories of pheromone plumes in an orchard canopy at night* (P-*57*)

**Giron-Calva**, Patricia Sarai, *University of Eastern Finland*, Finland; sarai.gironcalva@uef.fi
*Effects of plant-plant signaling on the oviposition preference of Pieris brassicae and Plutella xylostella females under elevated ozone conditions* (P-*214*)

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**Goddard**, Matthew R., *University of Auckland*, New Zealand; mgoddard@lincoln.ac.uk
***MUT11*:** *The Chemical Context of Interspecific Communication: Ecological Chemistry as a Driver of Mutualisms*

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*Does extrafloral nectar release a volatile scent?* (P-*18*)

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*Differences and commonalities between pheromone receptors of tortricid moths* (P-*227*)

**González**, Andrés, *Universidad de la República*, Uruguay; agonzal@fq.edu.uy
***FOR13*:** *Studies on the chemical communication of the bronze bug, Thaumastocoris peregrinus (Heteroptera: Thaumastocoridae), a pest of Eucalyptus* (P-*94*)

**Goulart Santana**, Antonio Euzebio, *Federal University of Alagoas*, Brazil; aegsal@gmail.com
*Identification of the Volatile Organic Compounds Profile in a Resistant Cultivar of Cassava (Manihot esculenta)* (P-*26*)
Does the association between sugarcane (Saccharum spp.) and diazotrophic microorganisms provide pest resistance? (P-27)

**Grabarczyk**, Malgorzata, *Maria Curie-Skłodowska University*, Poland; mgrabarc@poczta.umcs.lublin.pl
*Intermolecular interactions in systems containing Bi(III) – ClO – HO – homocysteine in the aspect of catalysis of Bi(III) electroreduction* (P-*19*)
Analysis of the Cr(VI) concentrations in cement materials (P-114)

**Grau Paricio**, Carlos, *Research Institute in Semiochemistry and Applied Ethology*, France; c.grau@group-irsea.com
***VER04*****:** *Does Fel d 1, the main cat's allergen, have a kairomone role?*

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***ISP13*****:** *A chemical ecology approach to assess the proliferation of the red alga Asparagopsis taxiformis: Metabolomics, natural toxicity and biological effects* (P-*209*)

**Grimm**, Christopher, *TU Braunschweig*, Germany; ch.grimm@tu-bs.de
*Characterization of sex pheromones in the digger wasp genus Cerceris* (P-*173*)

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***PHE04*****:** *The genetic basis underlying sex pheromone variation in moths*

**Gross**, Jürgen, *Julius Kühn-Institut, Federal Research Centre for Cultivated Plants*, Germany; juergen.gross@jki.bund.de
***APP07*****:** *Innovative control strategies for phytoplasma vectoring insects by infochemicals*

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***NEU01*****:** *Chemosensation between environment and internal state*

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***ISP03*****:** *Impact of an invasive pentatomid on ecophysiology and volatile organic compounds emission in Brassica oleracea L. var Botrytis*
Role of olfaction in Odonata sex recognition (P-84)

**Guerrero**, Angel, *IQAC-CSIC*, Spain; angel.guerrero@iqac.csic.es
*Antagonists of the sex pheromone of the tomato leafminer Tuta absoluta. Synthesis, functional assays, and electrophysiological activity* (P-*38*)

**Gut**, Larry, *Michigan State University*, USA; gut@msu.edu
***BLO15*****:** *Optimizing pheromone aerosol emitters for codling moth mating disruption*

**Günther**, Catrin, *University of Auckland*, New Zealand; catrins.guenther@gmail.com
*The chemical context of interepecific communication: Ecological chemistry as driver for mutualism of yeasts and flies* (P-*67*)

**Göransson**, Ulf, *Uppsala University*, Sweden; ulf.goransson@fkog.uu.se
***PCY03*****:** *Peptides from plants and animals: ecology connected to drug discovery*

**Haavisto**, Fiia, *University of Turku*, Finland; fiia.haavisto@utu.fi
***RES03*****:** *Induced and constitutive phlorotannins as defenses in a perennial brown alga*

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***CON04*****:** *Mating system and induction shape the volatile emissions of wild potatoes* (P-*211*)

**Hall**, David, *University of Greenwich*, UK; d.r.hall@gre.ac.uk
***ISP11*****:** *Chemical Ecology of Monochamus galloprovincialis, Vector of Pine Wood Nematode in Europe*
Sex pheromone of the saddle midge, Haplodiplosis marginata: Effects of chirality and minor components (P-65)
Polyunsaturated hydrocarbons as synergists in moth pheromones: The female sexpheromone of Dioryctria mendacella (P-121)
Components of the sex pheromone of blackcurrant sawfly, Nematus olfaciens (Diptera: Tenthredinidae): Novel isopropyl esters and the role of hydrocarbons (P-166)

**Hammerbacher**, Almuth, *Max Planck Institute for Chemical Ecology*, Germany; ahammerbacher@ice.mpg.de
***FOR04*****:** *A common fungal associate of the spruce bark beetle metabolizes the stilbene defenses of Norway spruce*
Biochemical characterization of lignan biosynthesis in Picea abies in response to infection by the bark beetle associate Enodconidiophora polonica (P-118)

**Hansson**, Bill, *Max Planck Institute for Chemical Ecology*, Germany; Hansson@ice.mpg.de
***CON01*****:** *Insect Olfaction - Quo vadis?*

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***CON06*****:** *Critical Themes in Research on Tritrophic Interactions*

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***NEU06*****:** *A herbivore-induced volatile interferes with host plant and mate location in moths through jamming signaling pathways in the brain*

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***RES01*****:** *The dynamics of offense and defense: Induced defenses, intraspecific variance, and the ecological important of plasticity*

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***BLO01*****:** *Chemical Ecology of Bed Bugs*

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*Male preference imposes stabilizing selection on female pheromone phenotype* (P-*41*)

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***PHE03*****:** *Chemical Ecology of Fruit Flies Provided Primary Impetus in Successful Synonymization of Four Major Pest Species in the Bactrocera dorsalis complex*

**Hefetz**, Abraham, *Tel Aviv University*, Israel; hefetz@post.tau.ac.il
*Functional exocrinology of social bees - comparative analyses of two bumble bee* (P-*83*)

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***RES07*****:** *Eavesdropping plants: Insect odors prime plant defenses*

**Herfurth**, Anna-Maria, *TU Braunschweig*, Germany; a.herfurth@tu-bs.de
*Cyanide detoxification by β-cyanoalanine synthase in the glucosinolate specialist Pieris rapae* (P-*144*)

**Hermann**, Sara, *Cornell University*, USA; slh275@cornell.edu
***MUT15*****:** *Volatile Predator Cues Drive Non-consumptive Effects*

**Hidalgo Bucheli**, William Fernando, *Max Planck Institute for Chemical Ecology*, Germany; whidalgo@ice.mpg.de
*Unraveling the role of phenylphenalenones as key metabolites in Musa plants against the attack by the pathogen Mycosphaerella fijiensis* (P-*153*)

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***PHE01*****:** *Decoding evolution of pheromone communication in Heliothine moths* (P-*181*)

**Holighaus**, Gerrit, *University of Göttingen*, Germany;
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*Eight-carbon volatiles as infochemicals for fungivores* (P-*116*)
Host volatiles VS non-host volatiles: Host odor of a deadwood colonising beetle is no matter of tree species (P-206)

**Hopkins**, David, *University of Sheffield*, UK;
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*The chemical ecology of divergent host plant acceptance behaviour in the speciation in the pea aphid (Acyrthosiphon pisum)* (P-*191*)

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*Insights into diversification of floral chemical signaling in the Ficus-mutualistic pollinator interaction: The case of Ficus septica in South East Asia* (P-*136*)

**Hughes**, Gabriel, *Purdue University*, USA;
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*Determination of the absolute configurations of the contact sex pheromone components of the longhorned beetle, Neoclytus acuminatus acuminatus (F.) (Coleoptera: Cerambycidae)* (P-*105*)

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***BLO02*****:** *Grass volatiles partially explain oviposition site selection by Anopheles mosquitoes*

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*Biomolecular MS of amino acids and their role in chemical ecology* (P-*10*)

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*Identification of an aggregation pheromone component of Sitona humeralis Stephens based on increased pheromone production induced by synthetic juvenile hormone III* (P-*135*)

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***BLO03*****:** *Identification of Host Volatiles and their Role in the Behavioural Modulation of Host-Seeking Culicoides Biting Midges*

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*Simple fabrication of hydrophobic AKD coated MALDI concentration plates for increased sensitivity* (P-*115*)

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*Olfactory and visual cues affecting the atraction of Cydia strobilella L. to oviposition sites* (P-*89*)

**Jakobsson**, Stina, *University of Gothenburg*, Sweden;
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*Chemical defense in Zostera marina (eelgrass) against Labyrinthula zosterae* (P-*182*)

**Jelvez Serra**, Nadia, *Federal University of Alagoas*, Brazil; nadiajelvez@gmail.com
*Intraspecific variation of cuticular hydrocarbon profiles in the Anastrepha fraterculus (Dipteria: Tephritidae) species complex* (P-*139*)
Sex attractant of the annona fruit borer, Cerconota anonella Sepp. (Lepidoptera: Oecophoridae) (P-77)

**Jimenz-Alemán**, Guillermo, *Max Planck Institute for Chemical Ecology*, Germany; gjimenez-aleman@ice.mpg.de
***RES10*****:** *JA-Ile-Macrolactones induce nicotine accumulation in Nicotiana attenuata leaves and reduce Manduca sexta mass gain and survivorship*

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*Disentangling the biosynthesis of termite-produced nitro compounds* (P-*24*)
Smells like home: Chemically mediated co-habitation of two termite species in a single nest (P-129)

**Johnson**, Steven, *University of KwaZulu-Natal*, South Africa; johnsonsd@ukzn.ac.za
***MUT01*****:** *Concepts and Challenges in Mimicry Research*

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***APP15*****:** *Commercial Applications of Semiochemicals – Current status and future prospects*

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*Pear ester-based lure attracting a butterfly Pearly Heath (Coenonympha arcania L.)* (P-*158*)

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***MUT06*****:** *Floral microbial ecology – patterns, mechanisms, consequences and functions of bacterial colonization on flowers*

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***ISP10*****:** *The effects of Tree tobacco nectar alkaloids on Palestine sunbird foraging behavior and performance*

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*Male sex pheromones in Ceratitis FAR complex (Diptera: Tephritidae)* (P-*176*)

**Kandasamy**, Dineshkumar, *Max Planck Institute for Chemical Ecology*, Germany; dkandasamy@ice.mpg.de
*Attraction and attachment of Norway spruce pathogen Endoconidiophora polonica to its vector Ips typographus* (P-*150*)

**Karlsson Green**, Kristina, *Swedish University of Agricultural Sciences*, Sweden; kristina.karlsson.green@slu.se
*Experience-based predator avoidance in response to waterborne chemical cues in the freshwater isopod Asellus aquaticus* (P-*196*)

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***MIC04*****:** *Sniffing out the enemy: Fungus-growing termites can differentiate between mutualistic and parasitic fungi using volatiles* (P-*172*)

**Keller**, Matthieu, *CNRS*, France; mkeller@tours.inra.fr
*Neuroendocrine and behavioral consequences of peripubertal exposition to male odor in female mice* (P-*11*)

**Khaling**, Eliezer, *University of Eastern Finland*, Finland; eliezer.khaling@uef.fi
*Defense chemistry and community ecology of Brassica nigra* (P-*208*)

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***UNR16*****:** *Stereoisomeric Libraries for Pheromone Identifications: 1-Bisabolen-3-ols*

**Kim**, Hyun Kyung, *Chungbuk National University*, Korea; nshk0917@gmail.com
*Effects of sesquiterpene from Perilla frutescens against the diamondback moth, Plutella xylostella L.* (P-*109*)
Repellency of 33 plant materials and Curcuma longa L. against Culex pipiens and Aedes albopictus (P-110)

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***VER03*****:** *Host Immune Function and the Volatile Metabolome*
Spamalot: A quest for the holy grail of brown treesnake oral baits (P-197)

**Kline**, Daniel, *USDA-ARS*, USA; dan.kline@ars.usda.gov
***BLO10*****:** *Utilization of flowers and their volatiles for surveillance and management of mosquitoes and other biting flies.*

**Klutsch**, Jennifer, *University of Alberta*, Canada; klutsch@ualberta.ca
***FOR07*: *Impact of interactions among native biotic disturbances on range expansion of mountain pine beetle into novel jack pine forests*** (P-*20*)

**Knaden**, Markus, *Max Planck Institute for Chemical Ecology*, Germany; mknaden@ice.mpg.de
***NEU04*: *Escaping death by sensing your enemy's sex odor***

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***APP12*: *Bed bugs-down the trap*** (P-*143*)

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***APP08*: *Studies on interference between different lacewing attractants: new perspectives for Central European species (Neuroptera: Chrysopidae)***

**Kofroňová**, Edita, *Institute of Organic Chemistry and Biochemistry*, Czech Republic; kofronova@uochb.cas.cz
*Do commercial bumblebees Rrepresent a threat for local subspecies?* (P-*23*)

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*Natural and altered tterpenoids as modifiers of aphid probing* (P-*47*)

**Koschier**, Elisabeth, *University of Natural Resources and Life Sciences*, Austria; elisabeth.koschier@boku.ac.at
*Behavioural responses of Frankliniella occidentalis Pergande to cis-jasmone* (P-*46*)

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***UNR13*: *The perfume of termite queens: quest for termite queen pheromones***

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***FOR02*: *Defense priming in Norway spruce: chemical and molecular evidence***

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**Kubanek**, Julia, *Georgia Institute of Technology*, USA; julia.kubanek@biology.gatech.edu
*The chemistry of induced resistance in marine plankton: Tackling the challenges of identifying waterborne cues* (P-*82*)

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***FOR11a*: *Chemical and transcriptome analysis of resistant and susceptible Eucalyptus genotypes to the insect pest Leptocybe invasa***

**Kusumoto**, Norihisa, *Forestry and Forest Products Research Institute*, Japan; norihisakusumoto@gmail.com
*Identification of fomannoxin in Picea abies naturally infected by Heterobasidion parviporum and the effects of terpene constituents on its production* (P-*216*)

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*Cavitene a new diterpene with original skeleton identified in soldiers of the Neotropical termite Cavitermes tuberosus* (P-*130*)

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***PHE06*: *Male butterflies use an anti-aphrodisiac pheromone to tailor ejaculates***

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***APP02*: *Pheromone monitoring as a game changer in insect biodiversity and conservation research***

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*Vomeronasal receptor families in the deer mouse Peromyscus maniculatus: Towards an evolutionary analysis* (P-*232*)

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***ISP02*****:** *Admixture in invasive plants: effects on herbivory and chemistry*

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***RES09*****:** *Jasmonate-dependent depletion of plant carbohydrates constrains resistance and tolerance against herbivores*

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***PLE05*****:** *Exploring the white spruce giga-genome for biosynthetic pathways of novel insect defense metabolites*

**Mann**, Florian, *TU Braunschweig*, Germany; f.mann@tu-bs.de
*New insights into the chemical composition of androconial organs of Ithomiines* (P-*75*)

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**Markovic**, Dimitrije, *Swedish University of Agricultural Sciences*, Sweden; dimitrije.markovic@slu.se
*Brief mechanical stimuli between plants affect herbivore insects* (P-*222*)

**Martin**, Kyle, *Cornell University*, USA; krm243@cornell.edu
*Spatial and temporal variation in volatile composition suggests olfactory division of labor within the trap flowers of Aristolochia gigantea* (P-*188*)

**Martini**, Xavier, *University of Florida Citrus Research and Education Center*, USA; xmartini@ufl.edu
***MUT14*****:** *The fungus, Raffaelea lauricola, manipulates release of host plant odors causing initial repellency and subsequent attraction of trees to its symbiont and vector, the redbay ambrosia beetle (Xyleborus glabratus).*

**Mathew**, Dennis, *University of Nevada, Reno*, USA; dennismathew@unr.edu
***NEU02*****:** *Functional Contributions of Olfactory Receptor Neurons in Drosophila melanogaster larva*

**Mathur**, Vartika, *University of Delhi*, India; vmathur@svc.ac.in
***RES15*****:** *Does better growth imply better immunity? Effect of biofertilizers on induced responses of Brassica juncea* (P-*86*)

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***BLO05*****:** *Parasite-induced olfactory cues influence mosquito attraction to malaria-infected hosts*

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**Michereff**, Mirian F F, *Embrapa Cenargen*, Brasil; mirianfm@terra.com.br
*The influence of volatiles from stink bugs eggs extracts and oviposition damage plants on the foraging behavior of the egg parasitoid Telenomus podisi* (P-*155*)

**Mieles**, Alejandro, *SUNY - ESF*, USA; aemieles@syr.edu
*Chemical attractants of Philornis downsi an invasive avian parasite on the Galapagos Islands* (P-*210*)

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***APP01*****:** *Insect Pheromones for Insect Management: Promise versus Reality*

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*Use of oviposition induced volatiles in tomato plants by Trichogramma parasitoids* (P-*78*)

**Mitchell**, Robert, *University of Arizona*, USA; rfmitchell@email.arizona.edu
***FOR09*****:** *The mesquite borer Megacyllene antennata produces an aggregation-sex pheromone composed of floral and green leaf volatiles*

**Moghbel**, Nahid, *University of Queensland*, Australia; n.moghbel@uq.edu.au
***PCY07*: *Chemical and molecular characterization of nicotine to nornicotine conversion phenotype in Australian Nicotiana species used as chewing tobacco* (P-*54*)

**Mori**, Boyd, *Swedish University of Agricultural Sciences*, Sweden; boyd.mori@slu.se
***ISP04*: *Untangling the role of yeasts in attraction and oviposition site selection in an invasive frugivorous insect, Drosophila suzukii*

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*A differential induction of defence signaling molecules in pea response to Acyrthosiphon pisum and the effect of exogenous nitric oxide donor on its infestation* (P-*13*)
Surface microlayer of lobelia lake: 48-hour dynamics of heavy metals and chlorophyll a variability (P-120)

**Mozūraitis**, Raimondas, *KTH Royal Institute of Technology*, Sweden; raimis@kth.se
***VER06*: *Sex pheromone dynamics during oestrous cycle in dairy cows*

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*Plant volatiles signal the host seeking process in root-knot nematodes* (P-*96*)

**Mutyambay**, Daniel, *International Centre of Insect Physiology and Ecology*, Kenya; dmunyao@icipe.org
*Oviposition-induced indirect defence trait in maize against herbivorous insects: Effects of domestication and breeding* (P-*219*)

**Nagahama**, Kazuhiro, *Sojo University*, Japan; kazuhiro@bio.sojo-u.ac.jp
*Ecological role of Ewingella sp. KTH G3-2 with pine weevil* (P-*160*)

**Nagel**, Nadja, *Max Planck Institute for Chemical Ecology*, Germany; nnagel@ice.mpg.de
***UNR03*: *Harmonia axyridis' Defense Alkaloid Harmonine – Synthesis and Bioactivity*

**Nagnan-Le Meillour**, Patricia, *CNRS*, France; patricia.le-meillour@univ-lille1.fr
*Localization of OBP post-translational modifications by high-resolution MS* (P-*122*)

**Nedveckytė**, Irena, *Nature Research Centre*, Lithuania; inedveckyte@gmail.com
*Oviposition responses of Plodia interpunctella (Hübner) (Lepidoptera: Pyralidae) to cereal contaminated by fungi* (P-*69*)

**Nemesio Gorriz**, Miguel, *Swedish University of Agricultural Sciences*, Sweden; miguel.nemesiogorriz@slu.se
*Transcriptional regulation of the flavonoid biosynthesis in Picea abies* (P-*168*)

**Newcomb**, Richard, *Plant & Food Research*, New Zealand; Richard.Newcomb@plantandfood.co.nz
***NEU03*: *Insect odorant receptors: The state of play on how these novel integral membrane proteins mediate odour detection in insects*

**Nieberding**, Caroline, *University of Louvain-la-Neuve*, Belgium; caroline.nieberding@uclouvain.be
***NEU13*: *Transcriptomics of olfactory communication mediated by male sex pheromone in a butterfly*
Selection on male sex pheromone composition contributes to butterfly reproductive isolation (P-164)
Food-stressed males display compensation in fitness-associated physiological and behavioural traits and females get fooled if they cannot assess the male odour in the butterfly Bicyclus anynana. (P-29)

**Niemeyer**, Hermann, *University of Chile*, Chile; niemeyer@abulafia.ciencias.uchile.cl
*Role of cuticular compounds in defense by soldiers of the one-piece termite Neotermes chilensis* (P-*58*)

**Ninkovic**, Velemir, *Swedish University of Agricultural Sciences*, Sweden; velemir.ninkovic@slu.se
***RES13*: *Volatile interactions between undamaged plants affect herbivore insects and their natural enemies*

**Nishida**, Ritsuo, *Kyoto University*, Japan; ritz@kais.kyoto-u.ac.jp
***M1*: *Ecological significance of plant secondary metabolites in insect–plant interactions*

**Noge**, Koji, *Akita Prefectural University*, Japan;
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*Identification of the foraging stimulant of the brown marmorated stink bug, Halyomorpha halys* (P-*202*)

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*Clover seed weevils: Flowers for the females* (P-*71*)

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***MUT02*: *Flowers of Aristolochia rotunda mimic pheromones of true bugs to attract and trap fly pollinators***

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*Epigenetic effect of juglone - important in allelopathy?* (P-*239*)

**Ômura**, Hisashi, *Hiroshima University*, Japan;
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*Mating enhances behavioral and antennal responses of female small white butterfly Pieris rapae (Lepidoptera: Pieridae) to host plant volatiles* (P-*237*)

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***PHE07*: *Quality control: Honeybee workers assess queens by pheromonal and genetic correlates of quality*** (P-*162*)

**Ortiz**, Antonio, *University of Jaen*, Spain;
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*Electrophysiological (EAG) responses of Simulium spp to human sweat volatiles* (P-*101*)

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*Genetic variation of Tetranychus kanzawai confer different abilities on acaricide tolerance* (P-*97*)

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***ISP06*: *Polar secondary plant metabolites provide a high resistance against natural enemies to native and invasive populations of Buddleia davidii in the invasive range***
The impact of abiotic and biotic challenges and their interactions on plant morphology and the phytometabolome of Plantago lanceolata (P-102)

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***BLO16*: *Unifying the field of floral chemical ecology***

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*Genes associated with glycerol biosynthesis in the red-spotted Apollo butterfly, Parnassius bremeri in Korea* (P-*233*)

**Pavia**, Henrik, *University of Gothenburg*, Sweden;
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*Chemistry releases an invasive seaweed from native enemies* (P-*14*)

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***PLE01*: *Sex pheromone chemistry of thynnine wasps and the evolution of its exploitation by sexually deceptive orchids***

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*Synthesis of a macrolide library of putative pheromones used by mantellid frogs from Madagaskar* (P-*240*)

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*Consequences of dual biotic stresses on plant volatile emission and recruitment of egg parasitoids* (P-*185*)

**Perlatti**, Bruno, *Federal University of São Carlos*, Brazil; bperlatti@gmail.com
*Identification of symbiont bacteria of Diabrotica speciosa using MALDI-TOF MS* (P-*32*)

**Peters**, Birte, *Paris Lodron University*, Austria; birte.peters@stud.sbg.ac.at
*The influence of bacterial communities colonizing lettuce (Lactuca sativa) on grazing activity of slugs (Arion vulgaris)* (P-*145*)

**Petschenka**, Georg, *Cornell University*, USA; Georg.Petschenka@googlemail.com
***PCY04*****:** *Phytochemical diversity: structure-activity-relationships in the toxin-receptor interaction between milkweeds and monarchs*

**Piechulla**, Birgit, *University of Rostock*, Germany; birgit.piechulla@uni-rostock.de
***MIC01*****:** *Trichoderma volatile reduces fungal phytopathogenic symptoms in Arabidopsis*

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***APP09*****:** *Insecticide resistance mutation (Kdr) in Anopheles gambiae modulates host choice and olfaction in presence of pyrethroid-treated net* (P-*62*)

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***UNR10*****:** *Chemical components of urine mediate predator-prey interactions*

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*Are plant volatiles mediating highly specialized plant-insect interactions affected by environmental conditions?* (P-*48*)

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*Effects of beneficial rhizobacteria and a pathogenic virus on soybean leaf metabolites and interactions with a beetle vector* (P-*125*)

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*Friend or foe? Mutualism and aggressive mimicry in an aphid* (P-*17*)
Mating status affects pheromone production and EAG responses in the tomato leafminer Tuta absoluta (P-39)
Evidence of short-term peripheral sensitization by briefly exposure to pheromone components in Spodoptera littoralis (P-199)

**Raffa**, Kenneth F., *University of Wisconsin–Madison*, USA; raffa@entomology.wisc.edu
***PLE04*****:** *Chemical ecology of bark beetles in expanding ranges made assessible by climate change*

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*The chemistry of bluebell (Hyacinthoides non-scripta)* (P-*225*)

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*Applications of preparative GC for purification of diterpenes* (P-*2*)

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*Evaluation of generic attractants for trapping the velvet longhorned beetle, Trichoferus campestris* (P-*119*)

**Rehermann del Rio**, Guillermo, *Universidad de la República*, Uruguay; grehermann@fq.edu.uy
*Pheromone autodetection in a noctuid moth: GC-EAD response of female Pseudaletia adultera to its own sex pheromone components* (P-*108*)

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*Host plant preferences in Spodoptera littoralis larvae* (P-*228*)

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***APP10*****:** *Can herbivore induced plant volatile compounds be used to enhance attraction of entomopathogenic nematodes?*

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***VER01*****:** *The sick sense is in the nose*

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*Antimicrobial properties of inducible compounds in the brown macroalgae Fucus vesiculosus* (P-*106*)

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***INS06*****:** *Become a specialist with an enzyme - The senecionine-monooxygenase of Longitarsus jacobaeae* (P-*212*)

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***CON02*****:** *Insect cuticular hydrocarbons: exciting functions of boring chemicals?*

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***NEU05*****:** *Elucidating the neuronal architecture of olfactory glomeruli in the Drosophila antennal lobe*

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*Testing herbivore-induced plant volatiles for natural enemy conservation in agricultural systems* (P-*56*)

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***FOR08*****:** *Intraespecific variation in constitutive and inducible defensive allocation in Maritime pine, a model Mediterranean species*

**Santos**, Isabel, *University of São Paulo*, Brazil; isabelha@usp.br
*Flower scents of Campomanesia phaea (Myrtaceae) as attract for nocturnal bee pollinators* (P-*90*)

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*Bark beetles and their fungal associates: A marriage of convenience or true love?* (P-*186*)

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*Three fallacies in understanding evolution of insect-habitat odour cue responses* (P-*3*)

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***MUT05*****:** *Odour genes and pollinator-driven speciation in sexually deceptive Ophrys orchids*

**Schmitt**, Thomas, *University of Würzburg*, Germany; thomas.schmitt@uni-wuerzburg.de
*Evidence for a chemical arms race: Chemical mimicry in the cuckoo wasp genus Hedychrum* (P-*193*)

**Schorkopf**, Dirk Louis, *Swedish University of Agricultural Sciences*, Sweden; dirk.louis.research@outlook.com
*The scent of African malaria mosquito natural oviposition sites: Odours influence but do not dictate oviposition decision making in Anopheles gambiae s.l.* (P-*79*)

**Schott**, Matthias, *Justus Liebig University*, Germany; matthias.schott@agrar.uni-giessen.de
*Aversive trained insects as detectors* (P-*180*)
MALDI-MS imaging of insects – metabolite landscapes of the internal anatomy (P-74)

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*Repelling Aedes aegypti – a sustainable plant based solution* (P-*100*)
The plant Hyptis suaveolens (Lamiaceae) repellency on Ixodes ricinus (Acari: Ixodidae): Structure activity studies of sesquiterpene oxides and sulfides (P-217)

**Schulz**, Stefan, *TU Braunschweig*, Germany; stefan.schulz@tu-bs.de
***UNR07*****:** *Synthesis and mass spectra of macrolides used in chemical communication systems*

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*Leaf quality discrimination in butterflies* (P-*157*)

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***RES02*****:** *Lipid signaling in marine plankton*

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***ISP08*****:** *Chemical Ecology of Subcortical Insects in New Contexts: The Roles of Climate Change and Invasion of Naïve Habitats*

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*Activity and evolution of Phasmatodea beta-1,4-endoglucanases and their implications for polyneopteran herbivory* (P-*231*)

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*Isolation of novel plant-defense-inducing elicitors from rice herbivores* (P-*238*)

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*The diurnal emission pattern of constitutive and induced rice volatiles* (P-*42*)

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***NEU14*****:** *Molecular cloning and RNAi demonstration of olfactory co-receptor gene from two palm weevils species*

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***BLO14*****:** *Disruption of Varroa-honeybee association by chemosensory inhibition*

**Steidle**, Johannes, *University of Hohenheim*, Germany; jsteidle@uni-hohenheim.de
*Early learning of chemical host cues drives ecological divergence during speciation processes in a parasitoid wasp* (P-*127*)

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***PLE02*****:** *A molecular rationale for the unpredictable nature of female behavior*

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***PCY01b*****:** *Diptera attracted to the truffle Tuber aestivum and dimethylsulfide in Sweden.*

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*Flexibility of chemical defense in the parasitoid wasp Leptopilina heterotoma* (P-*117*)

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***ISP01*****:** *How can chemical ecology contribute to biosecurity against invasive Arthropods?* (P-*4*)

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*Growth inhibition activity of Sugi (Cryptomeria japonica) components against Microcystis aeruginosa* (P-*98*)

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*Consequences of pollinator-mediated gene-flow on trait expression versus genotype in varieties of Joshua tree (Agavaceae)* (P-*189*)

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**Takabayashi**, Junji, *Kyoto University*, Japan; junji@ecology.kyoto-u.ac.jp
*Exposure of soybean to weeding-related volatiles reduces damage and increases defensive compounds in their seeds* (P-*123*)

**Tebayashi**, Shinichi, *Kochi University*, Japan; tebayasi@kochi-u.ac.jp
*Browning mechanism on the roots of rice plant attacked by rice root aphid, Rhopalosiphum rufiabdominalis* (P-*204*)

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***VER07*****:** *Major urinary protein (MUP) profiles show dynamic changes rather than individual 'barcode' signatures*

**Thoss**, Vera, *Bangor University*, UK; vera.thoss@bangor.ac.uk
***APP11*****:** *Observations from a Bluebell and Bracken Climax Vegetation and Approaches for Bluebell Conservation*

**Timbilla**, James, *QCC-City University of New York*, USA; jtimbilla@qcc.cuny.edu
*Prospect for field management of Zonocerus variegatus with Pyrrolizidine alkaloid based-attracticide bait in sub Saharan Africa* (P-*50*)

**Tolasch**, Till, *University of Hohenheim*, Germany; tolasch@uni-hohenheim.de
*Decrypting a cryptic click beetle species (Coleoptera: Elateridae) using chemical ecology* (P-*66*)

**Torto**, Baldwyn, *International Centre of Insect Physiology and Ecology*, Kenya; btorto@icipe.org
***BLO13*****:** *Plant volatiles in perspective of mosquito disease vector control*

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*Non-sticky trap for Meligethes (Coleoptera, Nitidulidae) combining visual and chemical stimuli* (P-*103*)

**Trapp**, Marilia, *Federal University of São Carlos*, Brazil; mariliatrapp@gmail.com
***UNR05*****:** *MS in chemical ecology: From characterization of oxylipins to their role in plant-microbes interactions* (P-*161*)

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***M3*****:** *Exploiting plant distress signals for crop protection*

**Ullah**, Chhana, *Max Planck Institute for Chemical Ecology*, Germany; cullah@ice.mpg.de
*Flavan-3-ols are a general chemical defense in poplar against fungal pathogen attack* (P-*213*)

**Unelius**, Rikard, *Linnaeus University*, Sweden; rikard.unelius@lnu.se
*Morganella morganii bacterium producing phenol in the colleterial gland of females of the New Zealand grass grub, Costelytra zealandica, attracts males* (P-*221*)

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***FOR10*****:** *Black poplar volatiles in biotic interactions*

**Utherdyany Bicalho**, Keylla, *Federal University of São Carlos*, Brazil; keyllabicalho@yahoo.com.br
*Acetogenins from Annona mucosa for leaf-cutting ants control* (P-*30*)

**Walker**, William, *Swedish University of Agricultural Sciences*, Sweden; william.b.walker.iii@slu.se
***NEU11*****:** *Whole Transcriptome Analysis of Chemosensory Receptor Expression in the Codling Moth, Cydia pomonella.*

**Wallin**, Erika, *Mid Sweden University*, Sweden; erika.wallin@miun.se
***APP12*****:** *Chemical tools for training dogs to find bed bugs*

**Valterova**, Irena, *IOCB AS CR*, Czech Republic; irena@uochb.cas.cz
***PHE05*****:** *Biosynthesis of isoprenoids in the male marking pheromone of bumblebees: Through regulation of gene expression to speciation?*

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**Vander Meer**, Robert, *USDA*, USA; bob.vandermeer@ars.usda.gov
***ISP14*****:** *Biochemical evidence for cryptic fire ant species in Argentina*

**Wang**, Honglei, *Lund University*, Sweden; hong-lei.wang@biol.lu.se
***UNR11*****:** *Identification of the sex pheromone in three heliozeliid leafminer species infesting grapevine in South Africa and Italy*

**Wang**, Yi, *Swedish University of Agricultural Sciences*, Sweden; yi.wang@slu.se
*Neurophysiological mechanisms underlying the experience dependent behavioral adaptations* (P-5)

**Wardak**, Cecylia, *Maria Curie-Skłodowska University*, Poland; cecylia.wardak@poczta.umcs.lublin.pl
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**Webb**, Owen, *Queensland University of Technology*, Australia; owen.webb@qut.edu.au
*Fermentation volatiles associated with larval infestation act as innate deterrents for a polyphagous pest species.* (P-*76*)

**Weber**, Donald, *U.S. Department of Agriculture*, USA; Don.Weber@ars.usda.gov
*Brown marmorated stink bug pheromone: Interplay of purity, cross-attraction and synergy for an invasive insect species (Hemiptera: Pentatomidae)* (P-*220*)

**Wedén**, Christina, *Uppsala University*, Sweden; Christina.Weden@fkog.uu.se
***PCY01a*: *Diptera attracted to the truffle Tuber aestivum and dimethylsulfide in Sweden.***

**Wedin**, Cecilia, *KTH Royal Institute of Technology*, Sweden; ceciliawedin@gmail.com
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**Wei**, Xianqin, *Leiden University*, The Netherlands; weix@biology.leidenuniv.nl
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**Weinberger**, Florian, *Helmholtz Center for Ocean Science*, Germany; fweinberger@geomar.de
***ISP12*:** *Selection for increased production of Prostaglandin E2 and related deterrents during the invasion history of a seaweed, Gracilaria vermiculophylla*

**Verheggen**, François, *University of Liege*, Belgium; fverheggen@ulg.ac.be
*First evidence of a volatile sex pheromone in lady beetles* (P-*49*)

**Verhulst**, Niels, *Wageningen University*, The Netherlands; niels.verhulst@wur.nl
***BLO12*:** *Difference in mosquito attraction; between humans, within humans and between humans and great apes*

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*Searching for food in complex environments: Using laboratory approaches to test for associational effects* (P-*165*)

**Why**, Adena, *University of California*, USA; awhy001@ucr.edu
***BLO06*:** *Behavioral responses of Culex tarsalis to fish-associated semiochemicals in wind tunnel bioassays*

**Wicker-Thomas**, Claude, *CNRS*, France; claude.wicker-thomas@egce.cnrs-gif.fr
*Regulation of Drosophila pheromones by sex peptide* (P-*91*)

**Wickham**, Jacob, *Institute of Chemistry*, China; wickham@iccas.ac.cn
*Surface hydrocarbon layers affect attractiveness of dispersing female Monochamus alternatus, the vector stage of the pinewood nematode* (P-*52*)

**Vidal**, Diogo, *Federal University of Paraná*, Brazil; diogomvidal@gmail.com

**Vidkjær**, Nanna Hjort, *Aarhus University*, Denmark; nanna.vidkjaer@agro.au.dk
***UNR06*:** *Ecological chemistry of weaver ants – nutrient cycling and search for novel chemical characteristics* (P-*51*)

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**Williams**, Livy, *USDA-ARS*, France; lwilliams@ars-ebcl.org
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***UNR02*: *Chemical ecology of the dried bean beetle: first results and future prospects***

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***MUT04***: *Knock, knock. Who is there? Host location mechanisms in non-pollinating fig wasps* (P-*183*)

**Yew**, Joanne, *University of Hawaii at Manoa*, USA; jyew@hawaii.edu
***NEU10***: *Neuropeptide control of pheromone detection in a gustatory neural circuit*

**Yon**, Felipe, *Max Planck Institute for Chemical Ecology*, Germany; fyon@ice.mpg.de
***INS02***: *Floral scents to the touch: Scents inform moth's proboscis for probing and foraging, thus determining the plant fitness* (P-*28*)

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**Younginger**, Brett, *Portland State University*, USA; b.younginger@pdx.edu
***RES06***: *Fungal endophytes prime pathogen resistance in wild lima bean (Phaseolus lunatus)*

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***APP14***: *Prospects of managing a social parasite using pheromone supplements*

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*Early evolution of pheromone receptors in Lepidoptera* (P-*149*)

**Üveges**, Bálint, *Hungarian Academy of Sciences*, Hungary; uveges.balint@yahoo.de
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**Zagrobelny**, Mika, *University of Copenhagen*, Denmark; miz@plen.ku.dk
***PCY06***: *Volatiles from the burnet moth Zygaena filipendulae (Lepidoptera) and associated flowers, and their role in mating communication*

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*Recent advances in the pheromone chemistry of stink bugs* (P-*6*)

**Zas**, Rafael, *Consejo Superior de Investigaciones Cientificas*, Spain; RZAS@MBG.CSIC.ES
*Integration of defensive strategies and defensive investment with other life history traits in a Pinus pinaster population* (P-*230*)

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**Zhang**, Dan-Dan, *Lund University*, Sweden; dan-dan.zhang@biol.lu.se
***NEU15***: *Functional characterization of a receptor for a Type II sex pheromone in the winter moth, Operophtera brumata (Lepidoptera: Geometridae)* (P-*99*)

**Zhang**, Zhen, *Chinese Academy of Forestry*, China; zhangzhen@caf.ac.cn
***FOR12***: *Semiochemicals regulating intraspecific and interspecific relationships of three Tomicus species in Pinus yunnanensis Franch*

**Zhao**, Tao, *KTH Royal Institute of Technology*, Sweden; taozhao@kth.se
***FOR03***: *Fungal symbionts of the spruce bark beetle synthesize aggregation pheromone and reduce tree defense monoterpenes*

**Zhou**, Jing-Jiang, *Rothamsted Research*, UK; jing-jiang-zhou@rothamsted.ac.uk
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**Zhu**, Junwei, *USDA-ARS*, USA; jerry.zhu@ars.usda.gov
***APP13***: *Semiochemical-based technologies for fly management*

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