

International Society of Chemical Ecology



# ISCE

**international society of chemical ecology**



## 26<sup>th</sup> Annual Meeting Tours, France

**July 31<sup>st</sup> - August 4<sup>th</sup>, 2010**

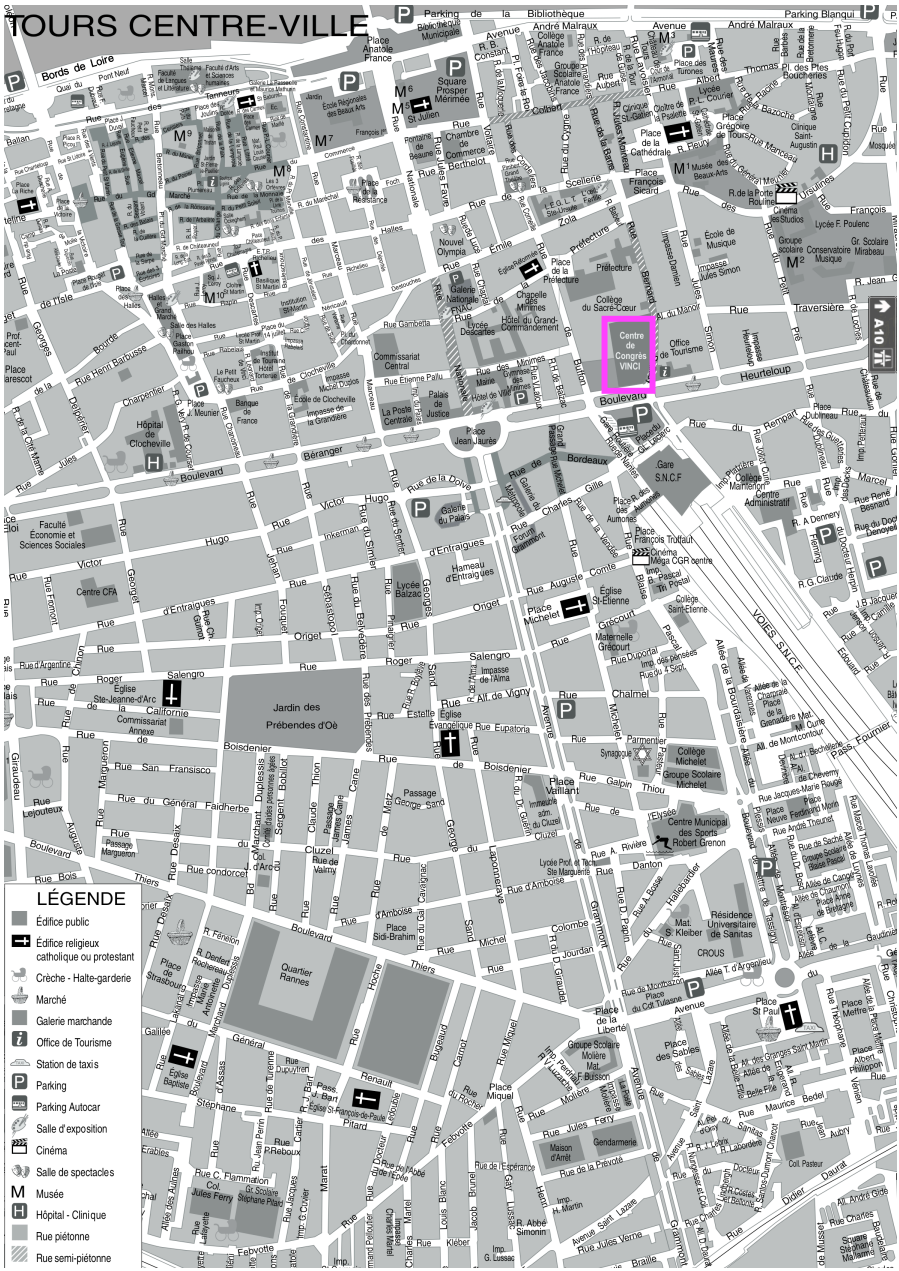




Where to go?

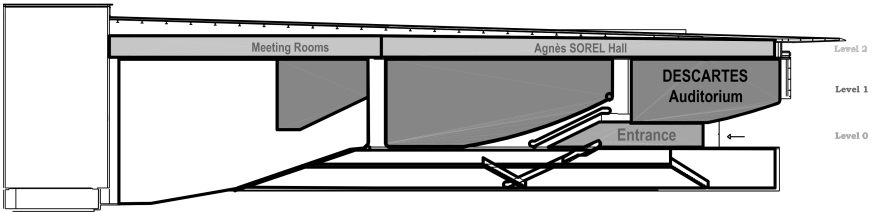
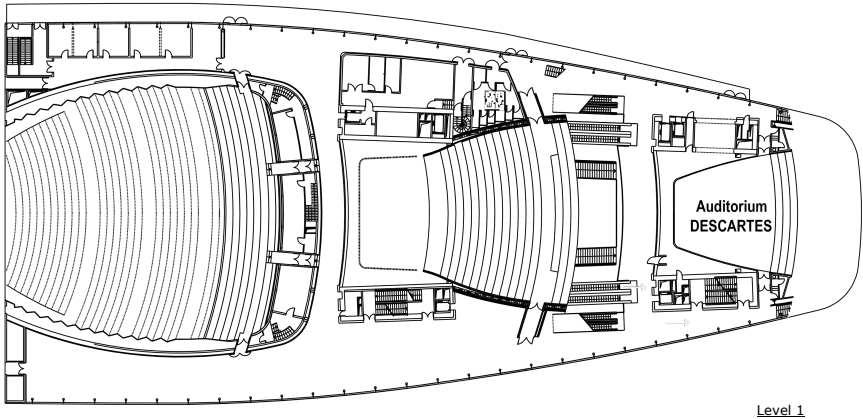
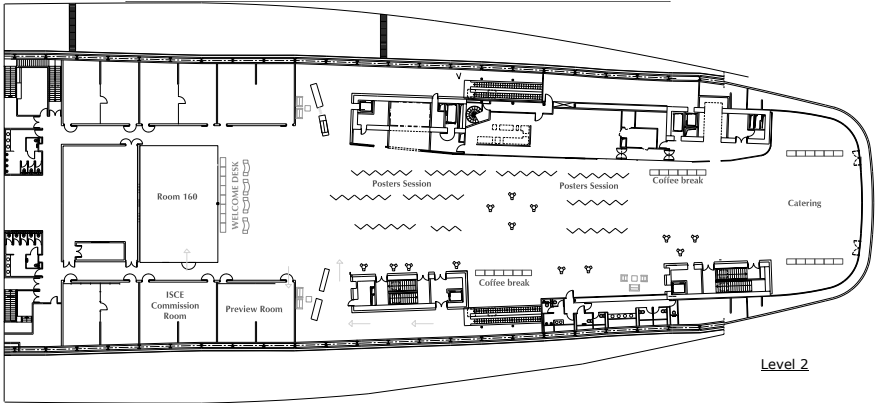


# TOURS CENTRE-VILLE



- LEGENDE**
- Édifice public
  - ✙ Édifice religieux catholique ou protestant
  - 🛒 Crèche - Halle-garèrie
  - 🏪 Marché
  - 🛍️ Galerie marchande
  - 🏠 Office de Tourisme
  - 🚏 Station de taxis
  - P Parking
  - 🚗 Parking Autocar
  - 🎨 Salle d'exposition
  - 🎬 Cinéma
  - 🎪 Salle de spectacles
  - M Musée
  - H Hôpital - Clinique
  - 🚶 Rue piétonne
  - 🚶 Rue semi-piétonne









## Meeting Overview





	Saturday 31st July	Sunday 1st August	Monday 2nd August	Tuesday 3rd August	Wednesday 4th August
09:00-09:00		<b>Welcome - Registration</b>	<b>Welcome - Registration</b>	<b>Welcome - Registration</b>	<b>Welcome - Registration</b>
09:00-09:30		Formal welcome-talks 45min	S3: Chemical communication within, among and around plants	S4: The diverse roles of non volatiles compounds	S6: Chemical ecology of multitrophic interactions
09:30-10:00		Silver Medal 45min 9:00-9:45	S3: Chemical communication within, among and around plants	S5: Evolutionary aspects of chemical communication	S6: Chemical ecology of multitrophic interactions
10:30-11:00		9:45-10:30	S4: The diverse roles of non volatiles compounds	S5: Evolutionary aspects of chemical communication	Sheridan Simpson Medal 30 min
11:00-11:30		<b>Coffee Break</b>	<b>Coffee Break</b>	<b>Coffee Break</b>	<b>Coffee Break</b>
11:30-12:00		S1: New technologies in chemical ecology	S3: Chemical communication within, among and around plants	Guest: I. BARKER, Lyon, FR	S5: Evolutionary aspects of chemical communication
12:00-12:30		S1: New technologies in chemical ecology	S4: The diverse roles of non volatiles compounds	Outside session Talks	S6: Chemical ecology of multitrophic interactions
12:30-13:00		S2: Mechanisms of intraspecific communication in animals			
13:00-13:30		<b>Lunch</b>	<b>Lunch</b>	<b>Sandwiches</b>	<b>Lunch</b>
13:30-14:00					
14:00-14:30		S2: Mechanisms of intraspecific communication in animals	Odd numbered posters session and coffee		Even numbered posters session and coffee
14:30-15:00					
15:00-15:30		Society Committee	S3		S5
15:30-16:00	Registration	S1: New technologies in chemical ecology	S4		S6
16:00-16:30		S2: Mechanisms of intraspecific communication in animals			
16:30-17:00	Workshop		Student session talks	<b>Excursion or free afternoon or visit IRBI</b>	Extra session talks
17:00-17:30		<b>Visit to winery</b>	Guest: M.C. GRASSE, Musée du parfum, Grasse, FR		Business Meeting and closure
17:30-18:00			17:30-18:15		
18:00-18:30	<b>Welcome cocktail</b>		JOCE meeting (for editorial board members only)		<b>Barquet</b>
18:30-19:00					
19:00-.....					





## Main Program



Saturday 31st July

<b>Registration</b> 14h00 to 18h00 Agnès Sorel Hall		<b>ISCE Executive meeting</b> (Committee members only) 14h00 to 18h00 Commission Room
	<b>Workshop</b> 16h00 to 18h00 Organizer: <i>Frank Shroeder</i> Room 160	

18h00 – 20h00	<b>Welcome Reception</b> Agnès Sorel Hall
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## Sunday 1st August

08h00  
–  
09h00 **Welcome – Registration**  
Agnès Sorel Hall

09h00  
–  
09h45 **Opening talks**  
Descartes Auditorium

**Silver Medal lecture**  
Descartes Auditorium

09h45  
–  
10h30 **BORDEN JH**  
Lessons learned in the life of an ancient chemical ecologist **31**

10h30  
–  
11h00 **Coffee Break**  
Agnès Sorel Hall

**S1 : New technologies in chemical ecology**  
Descartes Auditorium

*Plenary talks*

11h00 **SCHAL C** **47**  
–  
11h30 Simple, but efficient offline integration of preparative GC and NMR for analysis of mass-limited small volatile compounds

11h30 **SRINIVASAN J** **49**  
–  
12h00 A modular library of small-molecule signals regulates social behaviors in the nematode *Caenorhabditis elegans*

## Sunday 1st August

<b>S2 : Mechanisms of intraspecific communication in animals</b>		<i>Plenary talk</i>
Descartes Auditorium		
12h00	<b>SCHAAL B</b>	<b>61</b>
–	Mammary odor cues and pheromones: Mammalian infant-directed communication	
12h30	about maternal state, mammae, and milk	

12h30	Lunch	
–		
14h00	Agnès Sorel Hall	

<b>S2 : Mechanisms of intraspecific communication in animals</b>		<i>Plenary talk</i>
Descartes Auditorium		
14h00	<b>STROHM E</b>	<b>63</b>
–	Communication between the sexes and between mothers and their progeny in a solitary digger wasp, the European beewolf <i>Philanthus triangulum</i> (Hymenoptera, Crabronidae)	
14h30		

<b>S1 : New technologies in chemical ecology</b>		<b>S2 : Mechanisms of intraspecific communication in animals</b>		
Descartes Auditorium		Room 160		
14h30	<b>SVATOS A</b>	<b>51</b>	<b>RUTHER J</b>	<b>65</b>
–	MALDI-Mass spectrometry imaging of semiochemicals: from bacterial antibiotics to fly hydrocarbons		Sex pheromone communication in the model organism <i>Nasonia vitripennis</i>	
14h45				
14h45	<b>YEW J</b>	<b>52</b>	<b>RICHARD FJ</b>	<b>66</b>
–	Spatial and chemical profiling of <i>Drosophila mojavensis</i> and <i>Drosophila arizonae</i> cuticular hydrocarbons using ultraviolet laser desorption/ionization mass spectrometry		Odor and sexual selection in terrestrial isopods	
15h00				



Sunday 1st August

<b>S1 : New technologies in chemical ecology</b> Descartes Auditorium		<b>S2 : Mechanisms of intraspecific communication in animals</b> Room 160		
15h00 – 15h15	<b>GRONQUIST M</b> Arthropod natural products research: Advances in instrumentation afford new opportunities	53	<b>HANUS R</b> Chemical ecology of the termite genus <i>Prorhinotermes</i> : from anatomy to chemistry and function	67
15h15 – 15h30	<b>KEELING CI</b> the tria project: mountain pine beetle system genomics	54	<b>JARAU S</b> Geraniol from labial glands of nurse workers triggers queen development in larvae of <i>Melipona</i> stingless bees	68
15h30 – 15h45	<b>LUCAS C</b> Genomics in chemical communication	55	<b>LIM H</b> Ovulated female common carp ( <i>Cyprinus carpio</i> ) release a F prostaglandin-based species-specific pheromone complex that attracts conspecific males	69
15h45 – 16h00	<b>LIEBIG J</b> Fertility signalling, cuticular hydrocarbon synthesis and differential gene expression in the ant <i>Harpegnathos saltator</i>	56	<b>SCHULZ S</b> The first volatile pheromone from amphibians	70
16h00 – 16h15	<b>JASSBI AR</b> Virus-induced gene-silencing as a high-throughput bioassay to determine the ecological function of natural products	57	<b>LEAL W</b> Of moths and flies: Bombykol receptors	71
16h15 – 16h30	<b>ANDO T</b> Chiral HPLC analysis of Lepidopteran sex pheromones	58	<b>NAGNAN-LE MEILLOUR P</b> Odorant-Binding Proteins as an example of convergent evolution: but do they have really the same function?	72
16h30 – 18h00	<b>Visit to winery</b>			

## Monday 2nd August

08h00  
–  
09h00 **Welcome – Registration**  
Agnès Sorel Hall

### **S3: Chemical communication within, among and around plants**

Descartes Auditorium

*Plenary talks*

09h00–  
09h30 **TITTIGER C** **79**  
Pheromone-biosynthetic and resin-detoxifying enzymes from the mountain pine beetle, *Dendroctonus ponderosae*

09h30  
–  
10h00 **HALITSCHKE R** **81**  
Changes in floral and vegetative volatile emissions mediate herbivore-induced pollinator limitation in wild tomato plants

### **S4: The diverse roles of non volatile compounds**

Descartes Auditorium

*Plenary talk*

10h00  
–  
10h30 **OZAKI M** **97**  
Tolerance and aggression switching regulated by cuticular hydrocarbons in ants

10h30  
–  
11h00 **Coffee Break**  
Agnès Sorel Hall

<b>S3: Chemical communication within, among and around plants</b> Descartes Auditorium		<b>S4: The diverse roles of non volatile compounds</b> Room 160	
11h00 – 11h15	<b>POTIN P</b> <b>83</b> Waterborne signaling primes the expression of defense genes and buffers the elicitor-induced oxidative responses in the brown alga <i>Laminaria digitata</i>	<b>GREENE M</b> <b>101</b> Relative abundance of <i>n</i> -alkane cuticular hydrocarbons codes for a harvester ant task recognition cue.	
11h15 – 11h30	<b>EL-SAYED A</b> <b>84</b> Do carnivorous plants actively separate reproduction and feeding?	<b>PERDEREAU E</b> <b>102</b> Variations in the chemical compounds between native (USA) and invasive (French) populations of the termite <i>Reticulitermes flavipes</i> and the indigenous European termite <i>R. grassei</i>	
11h30 – 11h45	<b>MEINERS T</b> <b>85</b> Orientation in complex odorous environments: Does plant species diversity affect complexity of vegetation odor and arthropod orientation?	<b>GEISELHARDT S</b> <b>103</b> Mate choice is a matter of “taste”: Host plant shifts induce changes of contact pheromones and affect mate and species recognition in herbivorous insects	
11h45 – 12h00	<b>ERB M</b> <b>86</b> Mechanisms and ecological implications of leaf herbivore-induced root resistance	<b>BOS N</b> <b>104</b> Significance of chemical recognition cues is context dependent in ants	
12h00 – 12h15	<b>WENKE K</b> <b>87</b> Effects of rhizobacterial volatiles on <i>Arabidopsis thaliana</i>	<b>HURST J</b> <b>105</b> Darcin: a male pheromone that stimulates female memory and sexual attraction in the house mouse	
12h15 – 12h30	<b>BOSAK L</b> <b>88</b> When caterpillars don’t elicit the typical volatile profile: maize developmental stage affects indirect and direct defense expression	<b>SAVARIT F</b> <b>106</b> Social integration of the inquiline ant, <i>Ectatomma parasiticum</i> : a chemical camouflage or mimicry?	

## Monday 2nd August

12h30 – 14h00	<b>Lunch</b>  Agnès Sorel Hall
14h00 – 15h30	<b>Posters sessions and coffee</b>  Agnès Sorel Hall

	<b>S3: Chemical communication within, among and around plants</b> Descartes Auditorium		<b>S4: The diverse roles of non volatile compounds</b> Room 160
15h30 – 15h45	<b>THÖMING G</b> Host plant hierarchy and female <i>Synchlora betularis</i> – a comparison in reproductive host choice behaviour	<b>89</b>	<b>CARLSON J</b> The cellular and molecular basis of bitter taste in <i>Drosophila</i>
15h45 – 16h00	<b>BERAN F</b> Identification of the male-produced aggregation pheromone from <i>Phyllotreta striolata</i> (Coleoptera: Chrysomelidae) and its interaction with host plant volatiles	<b>90</b>	<b>SILK P</b> Short-Range Attraction of <i>Agrilus planipennis</i> Fairmaire (Coleoptera: Buprestidae) to a Macrocylic Lactone

	<b>Student sessions 1</b> Descartes Auditorium		<b>Student sessions 2</b> Room 160
16h00 – 16h15	<b>SOLER C</b> Phylogenetic conservatism in floral odours functioning as pollinator-attractant signals: a case study of Ficus / Agaonidae mutualisms	<b>163</b>	<b>IVANISEVIC J</b> Metabolic fingerprinting as an indicator of biodiversity: towards understanding inter-specific relationships among <i>Homoscleromorpha</i> sponges
16h15 – 16h30	<b>VON ARX M</b> Something about water – Do moths use humidity gradients as an indicator for a nectar-rich flower?	<b>164</b>	<b>STEENHUISEN SL</b> The importance of floral scent in the evolutionary shift from bird to beetle pollination in <i>Protea</i>

## Monday 2nd August

<b>Student sessions 1</b> Descartes Auditorium		<b>Student sessions 2</b> Room 160		
16h30 – 16h45	<b>MEWIS I</b> Effects of UV-B radiation on non-volatile secondary metabolites and subsequent changes in plant defense against different insect species	<b>165</b>	<b>NURINGTYAS TR</b> Epidermal metabolomic comparison of <i>Senecio jacobaea</i> , <i>Senecio aquaticus</i> and their hybrids	<b>173</b>
16h45 – 17h00	<b>AZEEM M</b> Styrene, produced by a fungus isolated from pine weevil frass, is repellent to the large pine weevil, <i>Hylobius abietis</i>	<b>166</b>	<b>ROMERO R</b> Tomato's best thrips shield: acylsugars, methylketones, phenolics or sesquiterpenes?	<b>174</b>
17h00 – 17h15	<b>AMSALEM E</b> Sterility-linked quantitative differences in <i>Bombus terrestris</i> cephalic-labial glands secretions	<b>167</b>	<b>FÉLIX AE</b> Phylogeny of twelve species of <i>Geotrupidae</i> (Coleoptera) inferred from analysis of cuticular compound blends and cytochrome oxidase I and 18s ribosomal DNA data sets	<b>175</b>
17h15 – 17h30	<b>PIERRE P</b> Aboveground herbivory affects orientation and parasitism by belowground parasitoid: laboratory and field evidence	<b>168</b>	<b>TOLASCH T</b> Sex pheromone of a twisted-wing parasite	<b>176</b>

<b>Guest plenary talk</b> Descartes Auditorium		
17h30 – 18h15	<b>GRASSE MC</b> The history of perfumery in Grasse: centuries of know-how	<b>43</b>

18h15 – 20h00	<b>JOCE meeting</b> (for Editorial Board Members only)
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## Tuesday 3rd August

### Tuesday 3rd August

08h00  
–  
09h00 **Welcome – Registration**  
Agnès Sorel Hall

#### **S4: The diverse roles of non volatiles compounds**

Descartes Auditorium

*Plenary talk*

09h00  
–  
09h30 **VANDER MEER R**  
Fire ant colony defense: detection, recognition, and response

**99**

#### **S5: Evolutionary aspects of chemical communication**

Descartes Auditorium

*Plenary talks*

09h30  
–  
10h00 **BAUDINO S**  
Biosynthesis and secretion of rose scent compounds

**113**

10h00  
–  
10h30 **GROOT AT**  
Identifying sources of intraspecific variation in moth sexual communication

**115**

10h30  
–  
11h00 **Coffee Break**  
Agnès Sorel Hall

#### **Guest plenary talk**

Descartes Auditorium

11h00  
–  
11h30 **LEMAIRE M**  
Sustainable chemistry: a challenge and opportunity

**39**

Tuesday 3rd August

<b>Outside session talks 1</b> Descartes Auditorium		<b>Outside session talks 2</b> Room 160		
11h30 – 11h45	<b>ANDERSSON M</b> Unsuitable host compound cineole inhibits response to pheromone in spruce bark beetle, <i>Ips typographus</i> , at olfactory receptor neuron and behavioral levels: Raison d'être of receptor cell co-localization?	<b>147</b>	<b>FOSTER S</b> A twin metabolite reservoir for pheromone production in moths	<b>155</b>
11h45 – 12h00	<b>PÉNEZ N</b> Variability in the production of alkaloids by the mediterranean red gorgonian <i>Paramuricea clavata</i>	<b>148</b>	<b>RYTZ R</b> Molecular evolution of the <i>Drosophila</i> olfactory system	<b>156</b>
12h00 – 12h15	<b>BLOMQUIST G</b> An ipsdienol oxidoreductase is critical in determining the final pheromone stereochemistry in <i>Ips</i> spp.	<b>149</b>	<b>HACKBART DE MEDEIROS A</b> Characterization of sugarwin, an insect-induced gene of sugarcane	<b>157</b>
12h15 – 12h30	<b>BORDEN JH</b> Development of a new product based on the honey bee brood pheromone	<b>150</b>	<b>BECHER PG</b> Associations between <i>Drosophila</i> and yeast	<b>158</b>
12h30 – 12h45	<b>SCHATZ B</b> Colour-scent associations in a tropical orchid: three colours but two odours	<b>151</b>	<b>UNELIUS R</b> Identification of compounds emitted by kissing bugs (Triatomines), new structures in pheromone chemistry	<b>159</b>
12h45 – 13h00	<b>MILLAR J</b> Recent advances in the chemistry and commercialization of mealybug pheromones	<b>152</b>	<b>BURSE A</b> The exploitation of plant derived glucosides by leaf beetle larvae	<b>160</b>

13h00 – 14h00	<b>Sandwiches</b> Agnès Sorel Hall
14h00 – 19h00	<b>Social Program or free afternoon</b>

## Wednesday 4th August

### Wednesday 4th August

08h00  
–  
09h00 **Welcome – Registration**  
Agnès Sorel Hall

**S6 : Chemical ecology of multitrophic interactions**  
Descartes Auditorium

*Plenary talks*

09h00 **RAGUSO R** **129**  
–  
09h30 Emerging patterns of volatile chemistry in the Yucca-Yucca moth mutualism

09h30 **SOLER R** **131**  
–  
10h00 Plant-mediated interactions between insects across and within trophic levels

**Silverstein-Simeone Lecture**  
Descartes Auditorium

10h00 **SHROEDER F** **35**  
–  
10h30 Worm chemical biology: lessons from small molecules

10h30  
–  
11h00 **Coffee Break**  
Agnès Sorel Hall

**S5: Evolutionary aspects of chemical communication**  
Descartes Auditorium

**S6 : Chemical ecology of multitrophic interactions**  
Room 160

11h00 **COPPÉE A** **117**  
–  
11h15 *Bombus terrestris* L.: a complex species or a species complex?

**GIRON D** **133**  
Leafminer insects trigger the host plant physiology through an unexpected association with endosymbiotic bacteria



Wednesday 4th August

<b>S5: Evolutionary aspects of chemical communication</b> Descartes Auditorium		<b>S6 : Chemical ecology of multitrophic interactions</b> Room 160	
11h15 – 11h30	<b>MAGRO A</b> Oviposition deterring infochemicals in ladybirds: the role of phylogeny	<b>118</b>	<b>VAN LOON J</b> <b>134</b> Pollinator visits to flowers of herbivore-induced black mustard plants: nectar chemistry and quantity
11h30 – 11h45	<b>BAKER T</b> Why most male ORNs are tuned to the most abundant sex pheromone components	<b>119</b>	<b>ALI JG</b> <b>135</b> Constant versus herbivore induced chemical signaling for recruitment of beneficial and parasitic nematodes by plant roots: effects of nematode life history and plant breeding
11h45 – 12h00	<b>HUIGENS T</b> <b>120</b> Chemical espionage on butterfly anti-aphrodisiac pheromones by tiny hitch-hiking parasitic wasps		<b>TAKEMOTO H</b> <b>136</b> Preimaginal olfactory experience of host-induced plant volatiles induces specific preferences in an aphid parasitoid
12h00 – 12h15	<b>WURDACK M</b> <b>121</b> The art of stealth - evolution of chemical mimicry in cuckoo wasps (Hymenoptera, Chrysididae)		<b>RANGANATHAN Y</b> <b>137</b> A coat of many scents: fig wasp cuticular compounds as potential cues for prey recognition by ants
12h15 – 12h30	<b>AYASSE M</b> <b>122</b> <b>The chemical disguise of the cuckoo bumblebee <i>Bombus vestalis</i> to sneak into power of reproduction</b>		<b>SIMPSON M</b> <b>138</b> <b>Herbivore-induced plant volatiles and floral resources increase natural enemy abundance and pest control in field crops</b>

12h30 – 14h00	<b>Lunch</b> Agnès Sorel Hall
14h00 – 15h30	<b>Posters sessions and coffee</b> Agnès Sorel Hall

Wednesday 4th August

<b>S5: Evolutionary aspects of chemical communication</b> Descartes Auditorium		<b>S6 : Chemical ecology of multitrophic interactions</b> Room 160	
15h30 – 15h45	<b>LÖFSTEDT C</b> A “reductionist approach” to the evolution of pheromone diversity in corn borer moths ( <i>Ostrinia</i> spp.)	123	<b>RYAN G</b> The effect of elevated atmospheric carbon dioxide on a grass-endophyte defensive mutualism: implications for aphid herbivory
15h45 – 16h00	<b>TRUMBLE J</b> Evolutionary and ecological implications of insect-plant interactions as impacted by pollution	124	<b>MATHUR V</b> Temporal patterns of induced plant responses and their effects on multitrophic interactions

<b>Extra talks 1</b> Descartes Auditorium		<b>Extra talks 2</b> Room 160	
16h00 – 16h15	<b>Topic S3</b> <b>WITZGALL P</b> Plant volatile signatures mediate host finding in insect herbivores - chemical analysis and behavioural physiology	91	<b>Topic S6</b> <b>CUSUMANO A</b> Chemicals released by the organ pipe mud dauber wasp <i>Trypoxylon politum</i> have a kairomonal effect on its parasitoid <i>Melittobia digitata</i>
16h15 – 16h30	<b>Topic S4</b> <b>DRIJFHOUT F</b> Chemical recognition in social insects - Solving a 100-year-old mystery including warnings for possible pitfalls	109	<b>Topic S3</b> <b>McNEIL J</b> Does fluctuating asymmetry in male sex pheromones affect female choice?

## Wednesday 4th August

<b>Extra talks 1</b> <i>Moderator: Sylvie Baudino</i> Descartes Auditorium		<b>Extra talks 2</b> <i>Moderator: Nicole Van-Dam</i> Room 160	
16h30 – 16h45	<b>Topic S6</b> <b>FATOUROS N</b> Male-derived anti-aphrodisiac compounds increase risk of egg parasitoid attack by inducing plant synomone production	<b>142</b>	<b>Topic S6</b> <b>KROISS J</b> Teaming up in defence: Symbiotic <i>Streptomyces</i> provide antimicrobial combination prophylaxis for wasp offspring
16h45 – 17h00	<b>Topic S2</b> <b>HARRACA V</b> Intraspecific communication in two human bed bug spp. with evidence of perception and behavioural effect	<b>73</b>	<b>Topic S2</b> <b>PLETTNER E</b> Insect odorant-binding proteins: ligand binding kinetics
17h00 – 17h15	<b>Topic S2</b> <b>VALTEROVA I</b> Chemical and acoustic communication in pre-mating behavior of <i>Aphomia sociella</i>	<b>74</b>	<b>Topic S6</b> <b>SCHOENIAN I</b> Chemical aspects of the synergism and antagonism in microbial communities of leaf cutting ants
17h15 – 17h30	<b>Topic S5</b> <b>PALACIO C. AM</b> Geographic variation of sex pheromone, mitochondrial DNA and PBP expression in <i>Diatraea saccharalis</i> (Fab., 1794) (Lepidoptera: Crambidae)	<b>125</b>	<b>Topic S3</b> <b>BOHMAN B</b> Orchids sexual deception: Towards the identification of semiochemicals that attract wasps for pollination of <i>Drakaea</i> orchids in South-Western Australia
17h30 – 18h30	<b>Business Meeting and closure</b> Descartes Auditorium		
19h30 – 23h00	<b>Banquet at the Villandy Castle and Gardens</b>		



# **Oral Presentation**

## Abstracts





# Silver Medal Lecture

*Moderator: Jeffrey Aldrich*



## Lessons learned in the life of an ancient chemical ecologist

**BORDEN JH<sup>a</sup>**

<sup>a</sup> *Contech Enterprises Inc., Delta, BC, CANADA*

*john.borden@contech-inc.com*

Over a 47-year lifetime in chemical ecology, mostly practiced in my role as a forest entomologist, I have learned many things. These can be encapsulated in 21 Lifetime Lessons, as enumerated below.

1. Older does not necessarily mean wiser.
2. Go where your heart lies. Things will turn out just fine.
3. The insects always have the right answer.
4. Economically important insects are as interesting as non-economically important insects.
5. Applied research is no different from basic research, except that a use has been identified for the data from the outset.
6. There is nothing like the joy of discovery.
7. The buzz one gets by taking research to the operational phase comes close to the joy of discovery.
8. It's easier to get an insect to do what it wants to do than it is to stop it from doing what it wants to do.
9. Treasure your anomalies.
10. Adversity can be flipped over into success.
11. Keep a place in your heart for inventors. They really are special.
12. My ability to predict the future sucks.
13. Surround yourself with the best and the brightest. They will enrich your life and make you look good.
14. Do not assume that other people are as smart as you are.
15. Expect the unexpected. View each unexpected occurrence as an opportunity.
16. Never underestimate the capacity for people to come up with "crackpot" solutions to a major problem.
17. Not all "crackpot" solutions are really crackpot.
18. Pick a good mentor. Honor and emulate him or her.
19. A career in research and teaching is excellent training for a job in business.
20. Pick a creed that fits you early in life and stick with it.
21. Work is a great retirement hobby.

Among the best and the brightest under Lesson No. 13 are 101 graduate students, four of whom (Jim Richerson, Ernie Lapis, Norm Alexander and Terry VanderSar) have predeceased me. This presentation is dedicated to them.







# Silverstein-Simeone Medal Lecture

*Moderator: Jocelyn Millar*



**Worm chemical biology: lessons from small molecules**SCHROEDER F<sup>a</sup><sup>a</sup> Boyce Thompson Institute - Cornell University, Ithaca, USA

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The *Caenorhabditis elegans* genome is one of the best annotated among animals, and many *C. elegans* physiological pathways show close analogies to corresponding pathways in higher animals. However, only recently the importance of small molecules in the biology of *C. elegans* – and other model organisms - has started to emerge. For example, small molecules play a crucial role in both exocrine and endocrine signaling that controls the “non-aging” dauer phase in *C. elegans* (Srinivasan 2008, Edison 2009). These examples suggest that a systematic characterization of structures and function of small molecules in model organisms will be critical for advancing our understanding of many biological processes. We have developed NMR spectroscopic methodology that enables linking small molecule metabolites directly with corresponding mutant phenotypes and probable biological functions. Application of this approach to identifying the *C. elegans* mating and dauer pheromones revealed a complex signaling system based on a multi-functional group of signaling molecules, the ascarosides (Pungaliya 2009). Low concentrations of ascarosides attract males and thus appear to be part of the *C. elegans* sex pheromone, whereas higher concentrations induce developmental arrest at the dauer stage. More detailed studies have shown that the originally identified dauer-inducing ascarosides are part of a much larger family of compounds that form a modular library of signaling compounds, mediating at least four different aspects of *C. elegans* biology: dauer formation, mate attraction, aggregation and ageing. Intriguingly, only mixtures of several ascarosides produce strong phenotypes at physiologically relevant concentrations, and individual ascarosides exhibit different though overlapping activity profiles. Cellular and genetic analyses of the ascarosides ascr#3 suggest the role of both core and sex-specific sensory neurons in regulating the response to this metabolite. Additional studies indicate that different ascarosides act through different neuronal and genetic pathways. These findings present a significant departure from the one-compound one-phenotype paradigm and emphasize the need to develop systemic approaches for correlating genome, phenotype, and metabolome. Lastly, the ascarosides provide an example for a family of signaling molecules that facilitate both inter- and intraorganismal signaling.

A. S. Edison, *Curr. Opin. Neurobiol.* **2009**, *19*, 378.

J. Srinivasan, F. Kaplan, R. Ajredini, C. Zachariah, H. Alborn, P. Teal, R. U. Malik, A. S. Edison, P. W. Sternberg\*, F. C. Schroeder, *Nature* **2008**, *454*, 1115.

C. Pungaliya, J. Srinivasan, B. W. Fox, R. U. Malik, A. H. Ludewig, P. W. Sternberg, F. C. Schroeder. *Proc. Natl. Acad. Sci. USA* **2009**, *106*, 7708.





## Guest Talk: Marc LEMAIRE

*Moderator: Anne-Geneviève Bagnères*



## Sustainable chemistry: challenge and opportunity

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The approaches and the methods underlying in the concept of «green chemistry» appeared about twenty years ago with the publication of articles of B. Trost (Science, 1991) and of R. Sheldon (Chemistry and Industry, 1992). These concepts, the hardening of the regulations and the societal request, inferred numerous researches in academic and industrial laboratories. The “twelve principles of green chemistry” proposed by P.T. Anastas and J.C. Warner. (Green Chemistry: Theory and Practice, 1998) can be considered as an outcome of these theoretical and experimental works. The presentation will show from concrete examples how these concepts were able to be implemented.

1- Replacement of methods of synthesis leading to the production of by-products by cleaner catalytic methods (principles 2, 5 and 9 of "Green Chemistry"). In this part we describe new methods for the synthesis of ether and a catalytic version of the Ullmann coupling

2- Creation of heterogeneous asymmetric catalysts as effective and selective as the homogeneous systems but easier to separate and to recycle than these last ones (principles 9 of the “green chemistry”). In this part we present our results on the modification of two types of ligand, the bisoxazoline and the Binap in order to ease their separation and their recycling.

3- Research for reagent little dangerous and environment-friendly reduction reagent useful for the reduction of phosphine oxide, nitrile and amide functions (principles 1, 4, 5),...

The “twelve principles of "Green Chemistry" do not describe all the areas of research concerning the sustainable chemistry development. In particular, none of these twelve principles takes into account stages of separation and a purification. These are however very expensive and time consuming and often the separations steps generate a large part of the effluents. The “twelve principles of the "Green Chemical Engineering" (P.T. Anastas and J.B. Zimmerman; Environmental science and technology 95, 2003) deal with these aspects. Within the framework of cooperation with the metallurgy, nuclear and petroleum industry, we created new systems of separations which use the liquid extraction / liquid or specific resins, or membranes.







## Guest Talk: Marie-Christine GRASSE

*Moderator: Martine Hossaert-Mckey*



## The history of perfumery in Grasse: centuries of know-how

### GRASSE M-C<sup>a</sup>

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From the Middle Ages onwards, Grasse developed two activities – the tannery and flower growing –, which would make it the perfume capital of France. Grasse already benefited from an exceptional microclimate and substantial livestock, which allowed the town to specialise in tanning from the XIVth century onwards.

The XVIIth century was a beneficial period for the city. The use of perfume developed and the demand for raw materials increased. This was the period when the cultivation of scented plants consolidated its position in Grasse. Jasmine, orange trees, roses, tuberose, acacia ... were cultivated here in the open fields.

In the early XVIIIth century, perfumers stood out from pharmacists and tanners. In 1729, they received their official status as glove makers-perfumers, which they retained up until the Revolution.

From the beginning of the industrial era, the perfumers in Grasse had to redefine their profession: finished products or raw materials? They choose the production and trading of raw materials.

The advent of industrial organic synthesis, using petroleum and its derivatives, provided perfumers with substances at extremely interesting prices. In addition, the palette was enriched with as many new essences as the expanded transport systems could import. Finally, one and the same plant was found to release several odours.

In less than 20 years, most of the molecules responsible for the odour or flavour of products used in perfumery were isolated, analysed and synthesised. The 1960s marked a turning point .





# S1. New technologies in chemical ecology

*Moderators: Matthew Gronquist & Claude Wicker-Thomas*



## PLENARY TALK

### **Simple, but efficient offline integration of preparative GC and NMR for analysis of mass-limited small volatile compounds**

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Semiochemicals are often produced in infinitesimally small quantities, so their isolation requires large amounts of starting material, not only requiring significant effort in sample preparation, but also resulting in a complex mixture of compounds from which bioactive compounds need to be purified and identified. Often, compounds cannot be unambiguously identified by their mass spectra alone, and NMR analysis is required for absolute chemical identification, further exacerbating the situation because NMR is relatively insensitive and requires large amounts of pure analyte. We developed an integrated approach for purification and NMR analysis of <1 µg of material. Collections from high performance preparative GC (1) are directly eluted with minimal NMR solvent into capillary NMR tubes. This simple off-line integration of preparative GC and NMR has facilitated the purification and chemical identification of novel volatile compounds, including a cockroach sex pheromone and a mosquito oviposition attractant kairomone, both of which occur in minute quantities within a complex volatile blend.

#### *References cited*

1. Nojima, S. et al. 2008. *Journal of Chemical Ecology* 34: 418–428.





## PLENARY TALK

### **A modular library of small-molecule signals regulates social behaviors in the nematode *Caenorhabditis elegans***

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Small-molecule signaling plays an important role in the biology of *Caenorhabditis elegans*. We have previously shown that ascarosides, glycosides of the dideoxysugar ascarylose, regulate both development and behavior in *C. elegans* [1]. Using differential analysis of NMR spectra (DANS), we identified additional ascarosides in the *C. elegans* metabolome [2]. We found that the mating signal consists of a synergistic blend of three dauer-inducing ascarosides, ascr#2, ascr#3, and ascr#8 [2]. The ascarosides ascr#2, ascr#3 and ascr#8 carry partially overlapping information, as ascr#3 is more potent as a male attractant than ascr#8 and ascr#2, whereas ascr#2 is slightly more potent than ascr#3 in promoting dauer formation. Two types of neurons, the ASK neurons and the male-specific CEM neurons, are required for male attraction by ascr#3, whereas only CEM neurons are required for response to ascr#8. We have now identified a number of novel variants of ascarosides, including several indole derivatives, representing a highly modular library of signaling molecules. Biological testing of synthetic samples of the recently identified compounds revealed that femtomolar concentrations of these ascarosides act as components of a potent aggregation pheromone for both *C. elegans* hermaphrodites and males. We are currently testing neuronal and genetic requirements for the response to the newly discovered ascarosides.

1. Srinivasan J, et al. (2008) A blend of small molecules regulates both mating and development in *Caenorhabditis elegans*. *Nature* 454:1115–1118.
2. Pungaliya et al. (2009). A shortcut to identifying small molecule signals that regulate behavior and development in *Caenorhabditis elegans*. *PNAS*, 106, 7708- 7713.



## MALDI-Mass spectrometry imaging of semiochemicals: from bacterial antibiotics to fly hydrocarbons3

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Matrix-assisted laser desorption/ionization (MALDI) mass spectrometric imaging (MSI) was recently extended to study semiochemicals and other natural products<sup>1,2</sup>. In MALDI-MSI molecules-of-interest are volatilized or desorbed/ionized from a well-defined area by irradiation with a laser. Mass analyzers and mass detectors are then used to determine the mass of the molecules (more exactly, their mass over charge  $m/z$  ratio), intensities, and possibly their structures through the use of tandem mass spectrometry. Mass spectra in the selected  $m/z$  range are obtained from hundreds to thousands of spots of predefined coordinates, similar to scanning electron microscopy. After individual ion peaks of desorbed ions are plotted on the coordinates, two- or three-dimensional representations of their intensities are obtained, typically as pseudo-color images.

Recently we were able to use MALDI MSI to localize diverse semiochemicals produced in bacteria<sup>3</sup>, plant<sup>4,5</sup> and insects<sup>6</sup>. Technical details including sample preparation and selection of MALDI matrices for particular application will be summarized. Novel application of MSI in addressing semiochemicals in *Drosophila melanogaster* will be reported.

### References:

1. Svatos, A. *Trends in Biotechnol.* (2010) *in press*.
2. Esquenazi, E., *et al.* (2009) *Natural Product Reports* 26, 1521-1534.
3. Kroiss, J., *et al.* (2010) *Nature Chemical Biology* 6, 261 – 263.
4. Hölscher, D., *et al.* (2009) *Plant J.* 60, 907-918.
5. Shroff R., *et al.* (2008) *PNAS* 105, 6196-6201.
6. Vrkoslav V., *et al.* (2010) *J. Amer. Soc. Mass Spectrom.* 21, 220-231.

## **Spatial and chemical profiling of *Drosophila mojavensis* and *Drosophila arizonae* cuticular hydrocarbons using ultraviolet laser desorption/ ionization mass spectrometry**

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Many hydrocarbons (HC) on the cuticle of *Drosophila* function as pheromones that can mediate mate choice. These compounds are analyzed primarily by gas chromatography mass spectrometry (GC-MS). Recently, we introduced a new method of HC analysis using ultraviolet laser desorption/ionization (UV-LDI) MS<sup>1</sup>. In contrast to GC-MS which analyzes cuticular extracts, UV-LDI MS allows rapid, direct chemical profiling of individual intact insects with a spatial resolution of ~100 µm. Here, we used UV-LDI MS to analyze the HC profiles of the sibling species *D. arizonae* and *D. mojavensis*. The legs, proboscis, and abdomen of both species showed qualitatively similar hydrocarbon profiles consisting mainly of long-chain monoenes, dienes, and trienes (alkanes are not detected by UV-LDI MS). However, quantitative differences were apparent between *D. mojavensis* and *D. arizonae* and between males and females of the same species. Importantly, the male anogenital region of both species showed distinct male-specific and species-specific chemical profiles. Several oxygen-containing hydrocarbon species in addition to high intensity signals corresponding to triglycerides were detected in this region. Some of these compounds were transferred to female cuticles after copulation. This is the first analysis showing that triglycerides may be a separate class of courtship-related signaling molecules in drosophilids. Our findings reinforce previous studies indicating that variation in HC profiles contribute to the formation of new *Drosophila* species<sup>2</sup>.

<sup>1</sup> Yew JY, Dreisewerd K, H, Luftmann H, Müthing J, Pohlentz G, Kravitz EA. 2009. *Curr Biol*. 19: 1245 – 1254.

<sup>2</sup> Etges WJ and Jackson LL. 2001. *J Chem Ecol*. 27: 2125-2149.

### **Arthropod Natural Products Research: Advances in Instrumentation Afford New Opportunities**

**GRONQUIST M<sup>a</sup>**

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Natural products play a central role in the ecology of arthropods. Studies aimed at the chemical ecology of arthropods are, however, often hindered due to the inherent nature of arthropod-derived samples. These samples frequently comprise complex mixtures of metabolites, and are often available in only miniscule amounts. In recent years, many of the challenges inherent in the study of arthropod natural products have been attenuated through advances in analytical instrumentation. In particular, advances in nuclear magnetic resonance spectroscopy, combined with novel approaches to data analysis, have served to offset many of the experimental limitations commonly associated with the analysis of arthropod-derived natural products mixtures. Recent examples taken from fireflies, spiders, and cercopids will be discussed.

## The Tria Project: Mountain Pine Beetle System Genomics

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The mountain pine beetle (MPB, *Dendroctonus ponderosae*) is having a devastating effect on the pines in Western North America, particularly in British Columbia, Canada. MPB is now entering into new habitats and onto new hosts on the Eastern side of the Rocky Mountains. To understand the interactions between the mountain pine beetle, its associated fungi, and the host pine trees, the Tria Project\* seeks to develop genomic resources and generate new information for the MPB, MPB-associated fungi, and host pine trees. This information will be used to produce integrated genetic landscape maps of the MPB-fungal-tree complex and the combined genomic and genetic information will be incorporated into ecological risk models. Within the larger project, we specifically have developed expressed sequence tag (EST) and genome sequence resources for the beetle, the fungi, and the pines. In the beetle, we are studying olfaction and pheromone biosynthesis using functional genomics approaches and have begun functionally characterizing P450 cytochromes and other enzymes and proteins in these processes.

\* The Tria Project Consortium ([www.thetriaproject.ca](http://www.thetriaproject.ca))

Project Leaders: Jörg Bohlmann<sup>1</sup> & Janice Cooke<sup>3</sup>,

Co-Investigators: Brian Aukema<sup>2,4</sup>, Colette Breuil<sup>1</sup>, David Coltman<sup>3</sup>, Barry Cooke<sup>4</sup>, Nadir Erbilgin<sup>3</sup>, Maya Evenden<sup>3</sup>, Richard Hamelin<sup>4</sup>, Grant Hauer<sup>3</sup>, Robert Holt<sup>5</sup>, Dezene Huber<sup>2</sup>, Steven Jones<sup>5</sup>, Christopher Keeling<sup>1</sup>, Marco Marra<sup>5</sup>, Brent Murray<sup>2</sup>, Felix Sperling<sup>3</sup>, & Tim Williamson<sup>4</sup>

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## Genomics in chemical communication

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We are in the privileged position to witness the rapid development of new genomic techniques such as microarrays, qPCR and deep sequencing. These powerful methodologies allow us to identify differentially expressed genes that may account for important phenotypes. These techniques start to be generalized to non-model species which could already be studied through the "gene candidate approach". For example, the *foraging* gene first discovered in flies could be studied in the context of defense behavior in ants. In the ant *Solenopsis invicta*, a gene (GP9) encoding an odorant binding protein is linked to the number of reproductives in a colony. With the use of the RNAi techniques, the "gene candidate approach" will open even more interesting and important possibilities to study chemical communication.



## **Fertility signaling, cuticular hydrocarbon synthesis and differential gene expression in the ant *Harpegnathos saltator***

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The main feature of eusocial insects is reproductive division of labor between one or a few fertile individuals and many infertile nestmates. Reproduction in these societies is assumed to be largely regulated by fertility signals. Several studies indicate that cuticular hydrocarbons represent these signals. How these signals are produced and how they relate to reproduction remains, however, poorly understood. We investigated the association between putative fertility signaling and the expression of enzymes involved in hydrocarbon synthesis in the ponerine ant *Harpegnathos saltator* utilizing the newly available annotated genome. This species shows several ancestral characters including the presence of workers with a high reproductive potential that allows them replacing the queen as functional reproductive upon the queen's death. Both, in queens and reproductive workers, increased ovarian activity is associated with a shift in their cuticular hydrocarbon profile to longer-chained components, indicative of the reproductive status. We identified genes producing enzymes with a putative elongase function in hydrocarbon synthesis and measured differences in expression levels between reproductive and infertile workers. We found that six fatty acid elongases are up-regulated in the abdomen of reproductive workers. Increased activity of these elongases is presumably responsible for the shift in the hydrocarbon profile in reproductive workers. The next step will be to lower the expression of these elongases using the RNAi approach. Using reverse genetics, we not only hope to provide strong support for the hypothesis of fertility signaling through cuticular hydrocarbons but this will also allow us to elucidate the underlying molecular mechanisms.

## **Virus-induced gene-silencing as a high-throughput bioassay to determine the ecological function of natural products**

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Plants employ bioactive secondary metabolites to defend themselves against herbivore, microorganisms and other plants. *In vitro* bioassay-guided purification followed by structural elucidation of secondary metabolites from plant extracts is the first step to determine the ecological function and biological activity of the metabolites. Alteration of the chemical structures of the isolated metabolites during the extraction and purification procedure, or in the process of bioassays may affect on the biological potential of the extracted compounds compared to that when the ecological or biological potential of the natural products is measured in the living organism tissues. One of the methods to evaluate the ecological function of natural products in the plants is virus-induced gene-silencing (VIGS). In this paper, I will review the ecological role of some bioactive natural products which were first isolated from their plant source by bioassay guided purification and identification and then a key gene in their biosynthetic pathway was silenced or over-expressed. The levels of bioactive compounds were measured in the VIGS plant and the ecological potential of the metabolites were correlated to the levels of the metabolite in the plant tissues by appropriate bioassay on the plants. Glycosides of geranylinalool with antifeeding potential against tobacco hornworm isolated from *Nicotiana attenuata* and *Nicotiana obtusifolia*, nicotine and flavonoids glycosides are among the studied compounds.

### **References:**

1. Jassbi, A. R. et al. 2006. Z. Naturforsch. 61b, 1138-1142.
2. Jassbi, A R. et al. 2008. Plant Physiology 146, 974-986.
3. Heiling, S. et al. 2010. Plant Cell, 22(1), 273-292


## Chiral HPLC Analysis of Lepidopteran Sex Pheromones

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Since the pheromone content in insects is very low, the absolute configuration of natural pheromones has been estimated by the biological activity of the synthetic stereoisomers, such as field attraction. However, it is important to understand the stereochemistry of the pheromones actually produced by the insects. In the case of lepidopteran female sex pheromones, epoxy compounds include chiral centers. A chiral GC column seems to have the possibility to separate enantiomers, but the applications are still very limited. We examined the resolution abilities of commercialized chiral HPLC columns in detail and observed desirable separation under a normal-phase condition. By applying the chiral columns, we successfully determined the absolute configuration of several epoxy pheromones. Recently, we identified methyl-branched ketones from a lichen moth as sex pheromone components; 6-methyl-2-octadecanone (**1**) and 14-methyl-2-octadecanone (**2**). These components include chiral centers. Male moths were attracted by a mixture of (*S*)-**1** and (*S*)-**2** and also by a mixture of racemic **1** and **2**. While the chiral GC did not achieve the resolution of the methyl-branched compounds, a normal-phase chiral HPLC column interestingly succeeded in the enantiometric separation of **1** and **2**. The chiral HPLC analysis of the pheromone extract revealed that the females did not produce optically pure compounds but dominantly (*S*)-**1** and (*S*)-**2**. This study is the first successful application of chiral HPLC to methyl-branched lepidopteran sex pheromones, and it is noteworthy that enantiomers of methyl-branched compounds showed different chromatographic behaviors on a chiral HPLC column.



## S2. Mechanisms of intraspecific communication in animals

*Moderators: Stefan Jarau & Manfred Ayasse*



### PLENARY TALK

## **Mammary odor cues and pheromones: Mammalian infant-directed communication about maternal state, mammae, and milk**

**SCHAAL B<sup>a</sup>**

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Neonatal mammals are exposed to an outstandingly powerful selective pressure at birth, and any mean to alleviate their localization effort and accelerate acceptance to orally grasp a nipple and ingest milk should have had advantageous consequences over evolutionary time. Thus, it is essential for mammalian females to display a biological interface structure that is sensorily conspicuous and executively easy for their newborns. Female strategy to increase the conspicuousness of nipples could only exploit the newborn most advanced sensory systems, touch and olfaction, and selection has accordingly shaped tactilely and olfactorily conspicuous mammary structures. This evolutionary modification has worked either by affecting structural features of mammaries or indirectly by affecting maternal behavioral propensities to create olfactory traces on them. These predictions will be considered in mammalian cases that have received empirical attention among marsupials, rodents, lagomorphs, ungulates, carnivores and primates. It appears that broadcasting chemical cues and/or signals from the mammae is a pan-mammalian reproductive strategy to pilot neonatal arousal, motivation and attraction to the mother, provide assistance in localizing and orally grasping the mammae, and boost up rapid learning. But the ways by which these chemical cues are produced and assembled on the mammae are both diverse between species and complex within species, offering an outstanding opportunity for comparative analyses in chemical communication.



### PLENARY TALK

## **Communication between the sexes and between mothers and their progeny in a solitary digger wasp, the European beewolf *Philanthus triangulum* (Hymenoptera, Crabronidae)**

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One important aspect of communication is the question, how signal evolution is influenced by the receiver's sensory properties. In the European beewolf, males scent mark territories to attract receptive females. We tested the hypothesis that the composition of the pheromone and in particular the occurrence of the major component, (Z)-11-eicosen-1-ol, is the result of sensory exploitation of the female sensitivity for certain compounds that are relevant for hunting. Using chemical analyses and behavioural assays we show that honeybees, the prey of female beewolves, bear (Z)-11-eicosen-1-ol on their cuticle, that this substance is in the headspace of foraging honeybees and that this alcohol is essential for prey recognition by beewolf females. GC-EAD analyses emphasized the high sensitivity of females for (Z)-11-eicosen-1-ol.

Beewolf females have also to communicate with their progeny. Their well closed brood cells are often constructed deep in the soil with the consequence that newly eclosed progeny has problems making its way to the surface. Females provide a secretion from antennal glands in the brood cells that indicate the location of the main burrow of the nest. Larvae orientate their cocoon-spinning according to this cue. After eclosion the young beewolves direct their digging activity towards the main burrow and by this leave the nest on the easiest way. The cue might consist of either hydrocarbons or bacteria that are both contained in the antennal secretion and have to be perceived by the larvae prior to cocoon spinning. How this unique form of communication has evolved is not clear yet.





### **Sex pheromone communication in the model organism *Nasonia vitripennis***

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Understanding the mechanisms controlling the reproduction of parasitic Hymenoptera is an important prerequisite to exploit these beneficial insects in pest control. We studied sexual communication in the model organism *Nasonia vitripennis*. Males of this parasitoid attract females by releasing a three-component sex pheromone consisting of a mixture of (4*R*,5*R*)- and (4*R*,5*S*)-5-hydroxy-4-decanolides and the synergizing trace component 4-methylquinazoline [1-2]. The substrate-borne pheromone is biosynthesized in the rectal papillae and released via the anal orifice by dabbing movements of the abdominal tip [3]. Interestingly, the sex pheromone attracts only virgin females. After mating, females no longer respond to the pheromone and prefer host odors instead [4]. By experimentally preventing females from experiencing particular elements of the mating and courtship sequence, we show that the postcopulatory change of the female sex pheromone response is independent of the transfer of a male ejaculate. Rather, our data suggest a fascinating pheromone interaction in that prior exposure of a female to one pheromone modulates her subsequent response to a second one. We show that the behavioral switch in *N. vitripennis* females is linked to the receptivity signal shown by females in response to a male oral aphrodisiac during pre-copulatory courtship.

#### *References cited:*

1. Ruther, J. et al. 2007. *J. Exp. Biol.* 210: 2163-2169.
2. Ruther, J. et al. 2009 *J. Chem. Ecol.* 34 : 99-102.
3. Abdel-latif, M et al. 2008. *Proc. Natl. Acad. Sci. USA* 105: 8914-8919.
4. Steiner, S. & Ruther, J.. 2009. *Behav. Ecol.* 20: 570-574.

### Odor and sexual selection in terrestrial isopods

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From prokaryotes to vertebrates, the use of chemical signals is widespread. Gregariousness and mate recognition have been found in some terrestrial isopods (Crustaceans). However, the underlying mechanisms that have led to these behaviors are poorly understood. *Armadillidium vulgare*, was used for this study and the presence of *Wolbachia*, an intracellular bacteria symbiont, results in feminization of genetic males into physiological and functional females. Previous results revealed that males interact more with uninfected females than feminized males. We focused on the ability of individuals to perceive other conspecifics at short-distance. To investigate biological parameters involved in individual attractions we tested individuals according to gender, moulting stage and *Wolbachia* infection status. Tested individuals were placed in a choice chamber separated by a mesh covered by a perforated opaque paper preventing visual and physical interactions. Males and females spent significantly more time close to a conspecific of the opposite gender than with those of the same gender. Moreover, males spent significantly more time close to females at the early moult stage than advanced moult or inter-moult stage. Finally, males were significantly more attracted by asymbiotic females compared to symbiotic females. Tested individuals perceived and used chemical cues for distance mate-finding as well as to discriminate infected female status. Our results provide clear evidence for chemical sex-recognition and preference to detect a female's reproductive status. The chemical signal of target individuals inform on the individual chemical pattern and led us to specific compounds involved in *A. vulgare* recognition cues.

### **Chemical ecology of the termite genus *Prorhinotermes*: from anatomy to chemistry and function**

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The preponderant role of chemical communication and defence in the life history of social insects is obvious and well documented by the variety of described exocrine glands and their products. Societies of termites are an excellent example in this respect. First, devoid of visual orientation, they have reached a social and functional complexity comparable to that of the most advanced social hymenopterans, with chemical signals being involved in nestmate and caste recognition, trail and food marking, alarm propagation, sexual attraction, and, last but not least, in caste regulation. Second, the richness of defensive chemicals identified in the unique termite defensive gland, the frontal gland, is unprecedented.

In the past four years, we have been devoting our time to an extensive quest for chemicals involved in communication and defence in the termite genus *Prorhinotermes* (Rhinotermitidae), namely *P. simplex*, *P. canalifrons* and *P. inopinatus*. To elucidate the chemical identity and function of these compounds, we used various approaches, from electron microscopy and analytical chemistry to electrophysiology and behavioural tests. This presentation should be a brief overview of our most important findings related to i) the role of the frontal gland of soldiers and imagoes in alarm communication and defence, ii) the sexual attraction by means of semiochemicals from the tergal glands of female imagoes, iii) the trail marking by means of pheromones from the sternal gland of pseudergates and soldiers, and iv) the role of proteinaceous compounds in the signalling of reproductive status by kings and queens.

### **Geraniol from labial glands of nurse workers triggers queen development in larvae of *Melipona* stingless bees**

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Stingless bees of the genus *Melipona* are unique among eusocial bees because queens and workers develop in brood cells of equal size and feed on similar amounts of food. Thus, trophic caste determination, as in honey bees and all other species of stingless bees, appears unlikely. The actual mechanism that triggers queen development in *Melipona* remained unresolved to this day. We now identified geraniol, the main compound in labial gland secretions of nurse workers, as an exogenous caste determination factor in *M. beecheii*. When 10µg of this terpene were added to the larval food in brood cells, 25% of the females developed into queens, which is a significantly higher proportion as compared to untreated brood cells (9% queens; Chi<sup>2</sup>-statistics on absolute numbers, p<0.001). This finding corroborates the two-locus, two-allele model of genetic caste determination in *Melipona* proposed by Kerr more than 50 years ago, in which only females that are heterozygous at both loci are capable of developing into queens (25% on average). Caste fate in *Melipona* apparently is controlled both genetically and trophically: Female larvae that are genetically predisposed towards being queens only follow this developmental pathway if they received sufficient amounts of a caste determining compound. In *M. beecheii* this compound is geraniol, which represents the first caste determination substance identified from the larval provision of a social insect. Furthermore, the identification of a nutritional factor triggering queen development clearly refutes a theoretical model, in which *Melipona* larvae are able to personally decide to become queens.

### **Ovulated female common carp (*Cyprinus carpio*) release a F prostaglandin-based species-specific pheromone complex that attracts conspecific males**

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Although it is well established that females of many fish species employ some type of a prostaglandin-derived sex pheromone when ovulated, the complexity of this cue and how it might be species-specific has yet to be examined. This study addressed this question in the common carp, a close relative of the goldfish and one of the better understood models of prostaglandin function. Previous electrophysiological studies of the carp have shown that this invasive species detects several prostaglandins at picomolar concentrations. Using a two-choice behavioral assay we first determined that sexually-active male carp strongly select the odor of recently ovulated female carp over blank water ( $P < 0.01$ ), non-ovulated female carp ( $P < 0.01$ ), and ovulated female goldfish ( $P < 0.01$ ). Sexually-inactive male carp did not show these preferences ( $P > 0.05$ ). Next, to determine the complete identity of the female sex pheromone, we fractionated ovulated female carp odor and examined the non-polar and polar fractions. We found that both fractions had behavioral activity and that they synergized each other's activity. The non-polar fraction was found to contain high concentrations of prostaglandin $F_{2\alpha}$ , 15-keto-prostaglandin $F_{2\alpha}$  and 13,14-dihydro-15-keto-prostaglandin $F_{2\alpha}$ , but in different ratios than the goldfish. Behavioral tests showed that these prostaglandins could explain all activity in the non-polar fraction, but that specific ratios were surprisingly unimportant. We conclude that ovulated female carp release a multi-component sex pheromone complex that is comprised of F prostaglandins and unknown polar species-specific compounds. We speculate that this characteristic may be common to many fish hormonal sex pheromones. (Funded by Invasive Animals Cooperative Research Centre).

### The first volatile pheromone from amphibians

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Frogs mainly communicate by acoustic, visual, and tactile signals but are known to also use pheromones during courtship. In some cases the pheromones used by amphibians have been chemically identified; in frogs as well as salamanders they proved to be peptides, readily dissolving in water or spreading on the water surface. An example is the peptide splendipherin, used as pheromone by the Australian tree frog, *Litoria splendida*. Here we report on the surprising finding of non-peptidic volatile compounds in distinct male-specific femoral glands from frogs in the endemic family Mantellidae from Madagascar, being structurally similar to volatile insect secretions and acting as pheromones.

The mantellinae are very species-rich and occur in the rain forest of Madagascar. The glands of the males were extracted and the extracts analyzed by GC-MS. The glandular profiles proved to be species-specific and to contain aliphatic compounds of medium to low volatility. The compounds proved to be alcohols, esters, and especially macrolides with a molecular weight between 150 and 300 amu, a similar range as found in many insect pheromones. While some of the frog compounds are known from beetles, others are not known from nature and are currently under structure elucidation. The structural identity of several compounds was proven by stereoselective synthesis and enantioselective GC. An orientating behavioral bioassay showed that male glandular components are indeed able to change the behavior of females, but the exact function of the pheromone is unknown. Work is in progress to identify more components, clarify the specificity of the compound occurrence in over 30 species, and to synthesize compounds for structural verification.

### Of moths and flies: Bombykol receptors

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Male moths are endowed with odorant receptors (ORs) to detect species-specific sex pheromones with remarkable sensitivity and selectivity. We serendipitously identified for the first time that an odorant receptor from the fruit fly is more sensitive to bombykol than the moth's endogenous receptor for the sex pheromone. By using an elegant heterologous expression system, the "empty neuron" of the fruit fly developed by the Carlson group at Yale University, we were able to identify the bombykol-sensitive OR in *Drosophila melanogaster* as DmelOR7a. The profiles of this receptor in response to bombykol in the native sensilla of the fly (ab4) or expressed in the empty neuron system (ab3 sensilla) were indistinguishable. Both wild type and transgenic flies responded with high sensitivity, in a dose-dependent manner, and with rapid signal termination. By contrast, the same empty neuron expressing the moth bombykol receptor, BmorOR1, showed low sensitivity and slow signal inactivation. However, when expressed in the trichoid sensilla T1 of the fruit fly, the neuron housing BmorOR1 responded with sensitivity comparable to that of the native trichoid sensilla in the silkworm moth. As opposed to the empty neuron system in the basiconic sensilla, the structural, biochemical, and/or biophysical features of the sensilla make the T1 trichoid system of the fly a better surrogate for the moth receptor. Interestingly, the receptors from the moth and the fruit fly have only 17% amino acid identity thus indicating that unrelated receptors can be sensitive to the same ligand.



### **Odorant-Binding Proteins as an example of convergent evolution: but do they have really the same function?**

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Evolutionary convergence is the use of one niche by organisms of different adaptive radiations. An interesting example is the olfactory system that is conserved among vertebrates and invertebrates. If the sensory organs differ, they house a similar molecular organization. In particular, Odorant-binding proteins (OBP) are the result of molecular convergent evolution, as they belong to very different families in Insects and Vertebrates, with no common ancestral gene. In mammals, OBP belong to the lipocalin family, which members bind hydrophobic molecules. Lipocalins exist in Arthropodes, but insect OBPs did not arise from this family of proteins. If insect OBP share even high sequence similarity between species (over 90% for GOBP sub-class), mammalian OBPs display unusually low levels of overall sequence conservation (below 20%). But do they share the same physiological function?

We will compare porcine and bovine OBP overall structures and demonstrate that they are involved in different functions submitted to high selective pressure in each species.

### **Intraspecific communication in two human bed bug spp. with evidence of perception and behavioural effect**

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Both the common bed bug *Cimex lectularius* and the tropical bed bug *C. hemipterus* have humans as preferred host. Whereas *C. lectularius* begins to be well studied due to its resurgence in developed country (1), little is still known of the tropical counterpart *C. hemipterus*. We found an extreme homogeneity in antennal structure as well as in the blend of alarm pheromones released by the two species (2). Alarm pheromones perceived by specific sensilla on the antenna (3,4), play an important role in bed bug communication, acting as dispersal-, aggregation- as well as identification- agents. Single sensillum recordings on *C. lectularius*'s antenna showed that (*E*)-2-hexenal and (*E*)-2-octenal released by all individuals (3) are perceived by specific pairs of smooth peg sensilla (4) whereas 4-oxo-(*E*)-2-hexenal and 4-oxo-(*E*)-2-octenal only emitted by the nymphs of both species (3,5) are perceived by grooved peg sensilla (*in prep*). The ratio of the two C6 and C8 aldehydes and the presence of oxo-aldehydes allow identification of sex and stage to a partner in order to avoid a mistaken costly traumatic insemination which is compulsory in *Cimex* spp. (1).

#### *References cited*

1. Reinhardt, K. and Siva-Jothy, M.T. 2007. *Annu. Rev. Entomol.* 52:351-374.
2. Liedtke, C.H. *et al.* *J. Chem. Ecol.* submitted 22/01/2010.
3. Levinson, H.Z. *et al.* 1974. *J. Insect. Physiol.* 20:1231-1248.
4. Harraca, V. *et al.* 2010. *Chem. Senses.* 35:195-204.
5. Feldaufer, M.F. *et al.* 2010. *J. Med. Entomol.* 47:140-143.

### **Chemical and acoustic communication in pre-mating behavior of *Aphomia sociella***

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*Aphomia sociella* L. is a parasite of bumblebees. Reproductive behavior of *A. sociella* is initiated by males and includes both ultrasonic and pheromone signals. The pheromone is produced in male wing glands. Components of their secretion were identified as 1-hexanol, 2-phenylethanol, (*R*)-(Z,Z)-nona-2,6-dien-4-olide, (*S*)-(Z)-non-6-en-4-olide, mellein, phytone, and an unseparable mixture of C-18 acids [1,2]. Calling males disperse the pheromone by wing fanning. Females attracted from a distance fly toward calling males and eventually land nearby. The female presence triggers male ultrasound production and courting behavior. Behavioral observation showed that ultrasound arrests females lured by male sex pheromone and triggers their pre-copulatory behavior. Evidence exists that male ultrasound production is triggered by close-range female sex pheromone produced by so far unidentified gland(s) on female body.

Males often call in groups, but they defend their territories when other male approaches. Rival behavior includes walking, wing fanning, ultrasound production, and effort to push away the intruder(s). Sound analysis showed that both mating and rival ultrasound songs share similar parameters. Replay behavioral experiments revealed that both rival and courting songs elicit wing fan response in females.

It is generally accepted that rival and courting songs provide a basis for sexual competition (both intra-sexual and inter-sexual). Our data suggest that in *A. sociella* both male competition and female choice may act in the same direction.

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1. Kunesch G. et al. 1987. Z. Naturforsch. C: Biosci. 42:657-659.
2. Kalinová B. et al. 2009. J. Nat. Prod. 72:8-13.

### **Insect odorant-binding proteins: ligand binding kinetics**

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Olfaction in insects is mediated through sensory hairs, hollow cuticular structures that are innervated by selective sensory neurons. The neurons in the hollow hairs are bathed by sensillar lymph, an electrolyte solution that also contains secreted proteins. The most abundant proteins found in the lymph are insect odorant-binding proteins (OBPs). In my group we have studied two members of this family of proteins, each tuned to one enantiomer of the gypsy moth pheromone, disparlure. Recent kinetic studies have shown that the pheromone and other ligands bind to the protein in three stages: a first collision step, a second rapid binding state and a third step in which the ligand becomes more stably bound. It appears that ligands of the protein differ in the rates of the second and third step, as well as in the final conformational regime accessed by the protein. The latter appears to be important in the transmission of the odorant signal to the neuron.

Blends of odorants often elicit a very different response than one would expect from the sum of individual components. We have investigated collections of compounds that modulate the responses of male gypsy moth antennae to the pheromone and other odorants. A subset of these compounds significantly lengthens the moth's response to the pheromone, thereby causing some sensory adaptation. These compounds appear to stabilize a particular conformer of the pheromone-binding protein/pheromone complex.





## S3. Chemical communication within, among and around plants

*Moderators: Arnaud Ameline, Sébastien Dugravot & Brigitte Frérot*



## S3. Chemical communication within, among and around plants

Monday 2<sup>nd</sup> August, 09h00-09h30

### PLENARY TALK

#### **Pheromone-biosynthetic and resin-detoxifying enzymes from the mountain pine beetle, *Dendroctonus ponderosae***

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The mountain pine beetle (*Dendroctonus ponderosae*) uses aggregation pheromones to coordinate colonization of host trees, where it is continuously exposed to host-produced defensive resin. Genes/enzymes responsible for terminal steps of pheromone-biosynthetic pathways and resin detoxification are good candidates for potential targeted regulation strategies. However, very little is known about the biochemistry and molecular biology of these processes. We therefore created a modest EST database representing 4034 tentative unique genes, and used custom oligonucleotide microarrays to survey their expression in eleven different biological states covering most of the beetles' life cycle. Combined sequence, microarray, and qRT-PCR data have tentatively identified several genes implicated in pheromone component production and resin detoxification. These include cytochromes P450, isoprenyldiphosphate synthases, and an oxidoreductase.

Three pheromone components: *trans*-verbenol, frontalin, and *exo*-brevicomin are each produced via different metabolic pathways. The isoprene-derived component, frontalin, is synthesized in male midguts. However, the fatty acid-derived component, *exo*-brevicomin, is produced in the fat body. Thus, the precedent of midgut-specific pheromone production set in *Ips pini* may be limited to *de novo*, isoprene-derived components.

The gene encoding cytochrome P450 CYP6DH2 is induced when adults are exposed to monoterpene vapors. Recombinant CYP6DH2 hydroxylates  $\alpha$ -pinene exclusively to myrtenol, suggesting its role in resin detoxification. CYP6DH1 is closely related and may also have a detoxification role.

These results represent early progress in our efforts to understand the biochemical mechanisms for pheromone production and resin detoxification.





## S3. Chemical communication within, among and around plants

Monday 2<sup>nd</sup> August, 09h30-10h00

### PLENARY TALK

#### **Changes in floral and vegetative volatile emissions mediate herbivore-induced pollinator limitation in wild tomato plants.**

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Herbivore-induced changes in plant secondary metabolism, their defensive role against herbivores, and fitness benefits and costs of their production have been characterized extensively. More recently, evidence for “ecological costs” of induced plant defenses suggests that ecological interactions of plants with organisms other than herbivores represent an important selective pressure in the evolution of chemical plant defenses. Compromised interactions with pollinators are cited as significant and probably underestimated costs of plant secondary metabolite production and evidence for the effects of herbivore damage on pollinator attraction is found frequently.

The emission of herbivore-induced leaf volatiles can function as attractive signals for predators and parasitoids, and thus as indirect defenses. Additionally, they may also have signaling function for pollinators, as they affect the composition of the odor plume emanating from a plant and the context in which flowers are perceived. Pollinators could use these volatile signals to gain information about the reward quality of the plant as well as risks associated with approaching a plant which is highly attractive to predators. Here we characterize the floral and vegetative volatiles emitted by the wild tomato plants *Solanum chmielewskii* and *S. neorickii*. We identify flower-specific compounds which mediate the attraction of pollinators and are specifically released by flowers of the outcrossing *S. chmielewskii* but not by the selfing *S. neorickii*. Furthermore, we demonstrate changes in leaf and flower volatile emission in *S. chmielewskii*. Both, an induced increase in vegetative monoterpene emissions and the reduction of flower-specific volatiles contribute to the herbivore-induced pollinator limitation in *S. chmielewskii*.



## S3. Chemical communication within, among and around plants

Monday 2<sup>nd</sup> August, 11h00-11h15

### **Waterborne signaling primes the expression of defense genes and buffers the elicitor-induced oxidative responses in the brown alga *Laminaria digitata*.**

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Until now, in marine species, little data has been available on the occurrence of inter-individual communication and the so-called priming effect known in terrestrial environments. This study explores the early steps of the defense responses of a large marine brown alga (kelp) and investigates its potential to transmit a warning message to neighboring conspecifics. We compared the early responses to elicitation with oligoguluronates in laboratory-grown and harvested wild sporophytes of *Laminaria digitata*. We followed the release of H<sub>2</sub>O<sub>2</sub> and volatile organic compounds. We used reverse transcription-quantitative polymerase chain reaction (RT-qPCR) to monitor the kinetics of ten defense genes following the oxidative burst. Laboratory-grown algae were transplanted in natural habitats to reacclimate them and to further evaluate their responses to elicitation. In addition, a novel conditioning procedure was established to mimic field conditions in the laboratory. Our experiments showed that *L. digitata* integrates waterborne cues released in the field and/or from elicited neighboring plants. The exposure to elicited conspecifics changes the patterns of oxidative burst and volatile emissions and potentiates this kelp for faster and stronger induction of specific defense genes in response to oligoguluronates. Thus, waterborne signals shape the defense responses of kelps through a priming effect.

## S3. Chemical communication within, among and around plants

Monday 2<sup>nd</sup> August, 11h15-11h30

### Do carnivorous plants actively separate reproduction and feeding?

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Carnivorous plant-insect relationships represent an extreme and complex example of diversity in plant-insect interactions. Insect-pollinated carnivorous plants have two different problems: attraction of pollinators for seed set and attraction of insect prey to digest. These two systems may be in conflict and could present a potential cost to the plant to manufacture two different scents for each purpose. The role of odour in resolving the pollinator-prey conflict (PPC) has been investigated in four sundew species, *Drosera arcturi*, *Drosera spatulata*, *Drosera auriculata*, *Drosera peltata*, and in the two pitcher plants, *Sarracenia alata* and *Sarracenia flava*. In both *D. arcturi* and *D. spatulata* where there is a spatial separation between flowers and traps, no odours were detected from either the flowers or traps, suggesting that these species utilize only spatial separation to resolve the prey-pollinator conflict. In both *D. auriculata*, *D. peltata* where flowers open in the close vicinity of traps, unique plant odours have been detected for either the flowers or the traps suggesting that odour plays a role in resolving PPC in these two species. In spite of the presence of temporal separation between flower and traps to resolve PPC in the pitcher plant, *S. alata*, and *S. flava*, unique floral and trap odour blends have been identified. This suggests that these species utilize both temporal separation and odour to resolve PPC. Our findings will be discussed with relation to the pollination system in these species.

## Orientation in complex odorous environments: Does plant species diversity affect complexity of vegetation odor and arthropod orientation?

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Olfactory orientation by arthropods requires the ability to navigate through complex odor blends. Odor complexity may vary with the composition of vegetation. Here, we tested the hypotheses that (a) complexity of odors is dependent on plant species diversity<sup>1</sup> and (b) olfactory orientation by arthropods is negatively affected by increasing plant species diversity. We used the monophagous weevil *Mecinus pascuorum*, its ubiquitous host plant plantain (*Plantago lanceolata*), and its larval parasitoid *Mesopolobus incultus* as a tritrophic model system. Odor blends of 27 plots with different plant species diversity were analyzed by GC-MS. The plots were located in three study areas (<http://www.biodiversity-exploratories.de/>) with different land use gradients. The number of volatile compounds detected and their quantities per plot were subjected to a Shannon-Wiener diversity analysis, which resulted in a complex pattern of relationships between plant species diversity and odor diversity. Surprisingly, incidence of the weevil *M. pascuorum* and the parasitoid *M. incultus* in these field plots corresponded positively with plant species diversity. Further laboratory olfactometer studies revealed that host plant finding by the weevil was not impaired by odor complexity in the surroundings of the host plant. By contrast, the beetle searching activity increased when the complexity of odor blends increased. Hence, analyses of relationships between plant species diversity, odor diversity, and olfactory orientation by arthropods can help elucidate the outcome of multitrophic interactions and improve knowledge important for successful biological control.

### References cited

1. Randlkofer, B., Obermaier, E., Hilker, M. & Meiners, T. (2010) Basic & Appl. Ecol. doi:10.1016/j.baee.2010.03.003

## Mechanisms and ecological implications of leaf herbivore-induced root resistance

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Insect attack profoundly changes plant physiology, even in distant tissues (1,2). Such systemic responses can have important consequences for other organisms that are associated with the plant (3). Many possible plant-mediated interactions remain unexplored. We investigated how leaf herbivory by caterpillars of *Spodoptera spp.* influences the metabolism of maize roots and their resistance against the root feeding larvae of the beetle *Diabrotica virgifera*. Our results demonstrate that leaf-herbivory reduces host-location, host acceptance, growth rates and survival of the root feeder, both in the field and the laboratory. The sequence of arrival appears to be an important factor shaping these effects. Targeted bioassays reveal that leaf-herbivory reduces the emission of volatile host-location cues and increases the production of feeding-deterrent compounds and/or toxins. Physiological measurements suggest that none of the classical phytohormones (jasmonic acid, abscisic acids, salicylic acid, auxin), common secondary metabolites (phenolics, hydroxamic acids), established defense markers (PR genes, proteinase inhibitors, etc.), or known feeding stimulants (sugars, long-chain fatty acids) can explain these effects. We therefore suggest that a novel, unknown mechanism is responsible for the observed changes. We are currently using an assay-guided fractionation and isolation approach as well as metabolic fingerprinting to find these elusive factors.

### *References cited*

1. Kaplan, I. et al. *Ecology*. 89 : 392-406
2. Erb, M. et al. 2009. *Trends in Plant Science*. 14: 653-659
3. Erb, M. et al. 2009. *The Plant Journal*. 59: 292-302

## Effects of rhizobacterial volatiles on *Arabidopsis thaliana*

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Volatile based communication does not only occur aboveground, but also in the rhizosphere. Especially bacteria release a great variety of volatile blends which is one mechanism how bacterial pathogens affect the growth of plants (1).

Microarray analysis was performed to compare the transcriptional responses of *Arabidopsis* to the inhibiting volatile blends of *Serratia odorifera* and *Stenotrophomonas maltophilia*. *Arabidopsis* seedlings co-cultivated with these rhizobacteria in dual cultures for 6, 12 and 24 hours showed a regulation of less than 1000 genes. Functional categorization of *S. odorifera* specifically regulated genes highlighted the involvement of the endomembrane system, lipid transport/binding processes, cell wall metabolism and lipid metabolism in response to the volatiles of *S. odorifera*. Furthermore the detoxification system and redox enzymes responded. In contrast, the *S. maltophilia* volatiles affected many plant genes related to ribosomes and protein machinery as well as to DNA structure. A group of just 162 genes were responsive to both bacterial volatiles. Functional categorization showed the regulation of WRKY transcription factors and genes involved in the trehalose metabolism. In vitro assays with plant mutants co-cultivated with *S. odorifera* and *S. maltophilia* have to confirm an essential role of WRKY proteins and the trehalose pathway in the volatile induced response. Furthermore, the response of *Arabidopsis* accessions (ecotypes) due to the presence of bacterial volatiles will be investigated.

1. Vespermann, A. et al. 2007. Applied and Environmental Microbiology 73:5639-5641.



## S3. Chemical communication within, among and around plants

Monday 2<sup>nd</sup> August, 12h15-12h30

### **When caterpillars don't elicit the typical volatile profile: maize developmental stage affects indirect and direct defense expression**

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Plants synthesize and emit complex blends of volatile organic compounds (VOCs) in response to herbivory. The VOC blend can function to attract parasitoids and predators, repel ovipositing moths, deter other herbivores, and signal within and between plants. Maize responds to fatty acid amino acid conjugates in caterpillar regurgitant by producing a VOC blend containing green leafy volatiles, terpenes, and aromatic compounds. Vegetative and reproductive stages of maize produce qualitatively and quantitatively different sesquiterpene profiles in response to herbivory. However, little is known about defense expression, indirect vs. direct, during maize seedling stages. In response to simulated and actual herbivory, v1 maize plants produce very low levels of volatiles (<100 ng/hr) after regurgitant application compared to v3 (1500 ng/hr). Terpene synthase transcript level, TPS10 and TPS23, will be assessed to confirm the low-level sesquiterpene induction in v1 stage plants. Not only do they differ quantitatively but the VOC blends differ qualitatively as well. The data suggest that very young seedlings may utilize alternative defense mechanisms. V1 plants are not undefended as evidenced by reduced caterpillar survival and growth on primed leaf tissue. V1 plants with a minimum of leaf area and seed energy stores probably trade-off volatile production, typically an indirect defense, and engage in a direct defense response. These results strongly suggest that direct defenses, such as proteinase inhibitors, are involved in the v1 defense profile. Several candidate proteinase inhibitors will be measured using quantitative PCR.

## S3. Chemical communication within, among and around plants

Monday 2<sup>nd</sup> August, 15h30-15h45

### Host plant hierarchy in male and female *Spodoptera littoralis* – a comparison in reproductive host choice behaviour

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The host plant preference of the Egyptian cotton leaf worm *Spodoptera littoralis* BOISDUVAL (Lepidoptera: Noctuidae) was studied to investigate the plasticity of the reproductive host selection behaviour of a polyphagous moth and the mechanisms behind the host plant choice. The comparison among the host plant hierarchies of male and female aims to provide an insight into the mechanisms behind polyphagy and its evolutionary implications.

For females, the host plant choice behaviour was tested in oviposition experiments and for males in mate finding experiments in the wind tunnel. For both sexes, respectively, a ranking among five different host plants was defined and the resulting host plant hierarchies for females and males were compared. All experiments were done with moths reared on neutral diet (artificial) as well as on different host plants to test the influence of larval experience on the host plant preference of the adults.

Males and females, respectively, followed consistently their specific host plant ranking during mate location and oviposition. Thus, the influence of both sexes on the specification of the reproductive host plant range can be assumed for *S. littoralis*. Additionally, it was demonstrated that the larval experience changed the reproductive host plant preference of both sexes towards the larval food plant. This plasticity in host choice behaviour of male and female provides an indication of the ability of *S. littoralis* to change its host plant preference fast and thus, to adapt rapidly to new or seasonal changing host plant availability.

## Identification of the male-produced aggregation pheromone from *Phyllotreta striolata* (Coleoptera: Chrysomelidae) and its interaction with host plant volatiles.

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The chrysomelid beetle *Phyllotreta striolata* is a pest of Brassicaceae particularly in North America and Southeast Asia. *P. striolata* aggregate on host plants, a behavior that enhances the damaging potential of this species. Here, we present the identification, synthesis, and field testing of the male-produced aggregation pheromone of a *P. striolata* population from Taiwan. In field bioassays, we assessed the attractiveness of volatile blends from intact and feeding-damaged host plants without beetles, and host plants infested with *P. striolata* males or females. Only host plant seedlings with actively feeding males were attractive to both sexes. The headspace from male and female *P. striolata* feeding on *Brassica juncea* leaves was compared and several male-specific sesquiterpenes were identified. In coupled gas chromatography-electroantennographic detection experiments, only one male-specific compound and host plant volatiles, e.g. 1-hexanol, cis-3-hexen-1-ol, and the glucosinolate hydrolysis products, allyl isothiocyanate, 3-butenyl isothiocyanate, and 4-pentenyl isothiocyanate, elicited physiological responses from male and female antennae. The male-specific compound was identified as (+)-6R,7S-himachala-(9,11)-diene by chiral gas-chromatography with coupled mass spectrometry and comparison with reference samples from *Abies nordmanniana* known to produce the corresponding enantiomer. The aggregation pheromone was synthesized from  $\alpha$ -himachalene isolated from *Cedrus atlantica*. The obtained product was tested in field trapping experiments in Taiwan alone and in combination with the known attractant allyl isothiocyanate. The synthetic pheromone alone was not active, but enhanced the attractiveness of allyl isothiocyanate.

### **Plant volatile signatures mediate host finding in insect herbivores - chemical analysis and behavioural physiology**

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Plant volatile compounds play multiple roles as communication signals and defense agents, mediating interactions with other plants, microorganisms, fungi and animals. These principle biological functions of plant volatiles are established, but the headspace of green plants contains hundred and more compounds and it is still unclear whether they all are essential and biologically active compounds or whether some of them are merely biosynthetic waste products. Assigning biological functions to plant volatiles is therefore a current research challenge.

Herbivorous insects exploit plant volatiles for host-finding, and recent studies in lepidopteran species support the concept that blends of only a few key compounds encode specific host attraction. The identification of the chemicals that guide gravid females to suitable egg-laying sites is therefore essential, both for the understanding of plant-insect interactions and for the development of safe plant protection strategies.

Many insect herbivores are specialist feeders and it is generally assumed that plant volatile signatures encode specificity. Yet, the plant volatiles blends known to attract moths cannot account for specific host finding and discrimination between host and non-host plants.

Attempts to identify plant volatile attractants are illustrated with ongoing studies. The experimental difficulties include the lack of correlation of production and response, difficulties to obtain synthetic standards, to purify and formulate baits for behavioural tests, to conduct field tests against a noisy chemical background and to correctly assign behavioural functions.

## S3. Chemical communication within, among and around plants

Wednesday 4<sup>th</sup> August, 16h15-16h30

### **Does fluctuating asymmetry in male sex pheromones affect female choice?**

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There are a number of documented cases where the degree of asymmetry in bilateral male traits significantly affected female mate choice in a wide variety of organisms including insects. However, while it has been postulated that the degree of fluctuating asymmetry (FA) in pheromone content of male hairpencils may influence mating success these has been hard evidence to support this claim.

We tested this hypothesis using the armyworm, *Pseudaletia unipuncta* by providing a receptive virgin female with two virgin males and then analysing the content of the left and right hairpencils of both the successful and unsuccessful male. When insects were reared under ideal conditions on artificial there was no difference in the level of pheromone FA in successful and unsuccessful males, nor did females mate with the more symmetrical male of the two. However, when reared in poor quality diet the successful males had lower FA than unsuccessful males, and females mated significantly more with the male in the pair that had more symmetrical pheromone content.

This is the first example showing that FA in male pheromone content affects mating success. It also underlines the importance of considering the ecological context when studying the importance of FA in mate choice.

## Orchids sexual deception: Towards the identification of semiochemicals that attract wasps for pollination of *Drakaea* orchids in South-Western Australia

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The Orchidaceae is the most threatened group of plants in Australia [1]. Orchids utilise diverse and specialised pollination systems, including sexual deception, where the flower attracts male pollinators through chemical mimicry of insect sex pheromones [2]. All known species of *Drakaea*, endemic to Western Australia, secure pollination by sexual deception of thynnine wasps. As part of a multidisciplinary program to better understand and conserve endangered *Drakaea* species, we are investigating the details of chemical communication between these orchids and their specific pollinators.

Our work so far has focused on three of the most abundant species: *D. glyptodon*, *D. livida* and *D. thynniphila*. The volatile compounds produced by these flowers have been sampled either by solvent extraction or solid phase microextraction (SPME) and analysed using GC/electroantennographic detection (GC/EAD) and GC/MS to determine the bioactive compounds. Further analysis by GC/high resolution mass spectrometry (GC/HRMS) supported by microderivatisation experiments has helped us to postulate the existence of several compounds possessing a pyrazine skeleton as semiochemicals from the *Drakaea* flowers.


At the meeting, we will present the latest results related to the elucidation of these compounds as well as synthetic routes to alkylpyrazines and novel bioactive oxygenated derivatives.

### References

[1] Data extracted from public threatened species list. EPBC Act 1999

[2] Peakall R, *Func. Ecol.*, **4**, 159 (1990)





## S4. The diverse roles of non volatiles compounds

*Moderators: Raphaël Boulay & Abraham Hefetz*





### PLENARY TALK

## Tolerance and aggression switching regulated by cuticular hydrocarbons in ants.

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It has been generally accepted that nestmate recognition relies on mixing pattern of the cuticular hydrocarbons in ants. Previously, we found a particular olfactory sensillum, which is sensitive to cuticular hydrocarbon (CHC) patterns different from own pattern, on the antennae of a Japanese carpenter ant, *Camponotus japonicus*. The workers of this species are tolerant toward the nestmates, whose CHCs would not stimulate the "CHC sensillum", but aggressive toward the non-nestmates, whose CHCs elicit vigorous impulses in the CHC sensillum. This sensillum houses 130 receptor neurons innervating the same number of glomeruli in the antennal lobe, the primary olfactory center in the brain. This system can theoretically discriminate more than  $2^{130}$  kinds of mixing pattern of CHCs. It is suggested that this system is used not only for discrimination between intraspecific nestmate and non-nestmate but also for rejection of heterospecific opponents. However, male ants have neither the CHC sensilla nor the corresponding glomeruli. Indeed, they are not aggressive at all, even when charged by different nest workers. Recently, we found that matching of the CHC profile is also concerned with parasitic interaction between the caterpillars of the lycaenid butterfly *Niphanda fusca*, and its host ants, *Camponotus japonicus*. The *N. fusca* caterpillars succeed to be accepted by workers and exploit their care by matching their CHC profile to that of the host males, since the males are fed by trophallaxis with workers in their natal nests.



### PLENARY TALK

#### Fire ant colony defense: detection, recognition, and response

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In social insects, maintenance of colony cohesiveness, sociality, and defense depends on sophisticated pheromone communication. The highly evolved fire ant, *Solenopsis invicta*, uses an alarm pheromone to “rally the troops” for colony defense against other ant species, as well as other con-specific fire ant colonies that compete for resources and territory. Alarm pheromones generally have no direct benefit to the emitter, but serve to put other colony members in a high state of alertness. What happens next depends on other stimuli. Behavioral and glandular source information regarding the fire ant alarm pheromone has been published for almost 50 years, but the chemistry of this pheromone system has only recently been elucidated. The ontogeny, species-specificity, and neurohormone relationships will be presented. Before a worker fire ant releases the alarm pheromone it must recognize another ant in its territory, hetero- or con-specific, as an intruder rather than a nestmate. Nestmate recognition has been the subject of investigation for decades and certain models for the recognition cues and detection have gained acceptance. Here the nestmate recognition cues used or not used by the fire ant will be discussed, as well as neuromodulators and the influence of the queen (primer pheromone) on colony worker acceptance or rejection of non-nestmate, con-specific workers, and other queens. The two *S. invicta* social forms, monogyne and polygyne, provide an interesting contrast. The mechanisms of colony defense are essential to maintain colony integrity, territoriality and functions to protect the reproductive queen(s) from predators and parasites, most of the time.



### **Relative abundance of *n*-alkane cuticular hydrocarbons codes for a harvester ant task recognition cue**

**GREENE M<sup>a</sup>**

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Social interactions provide local information cues that inform worker decisions in the regulation social insect colony behavior. Harvester ant (*Pogonomyrmex barbatus*) foragers are stimulated to leave the nest in search of seeds by the return of patrollers to the nest while other tasks such as nest maintenance workers do not stimulate foraging. Colonies are responsive to the rate of patroller return and when patrollers are prevented from returning to nest foraging will not begin. Foragers recognize patrollers returning to the nest using a cue present in patroller cuticular hydrocarbons. Cuticular hydrocarbons are a mixture of long-chain *n*-alkanes, methyl-branched alkanes, and *n*-alkenes which serve in communication, prevention of abrasion to the cuticle, and prevention of water loss. Harvester ant workers that work outside the nest, such as patrollers and foragers, have a higher amount of *n*-alkanes compared to workers that spend short amounts of time outside of the nest, such as nest maintenance workers. In this study, I tested the hypothesis that a high relative abundance of *n*-alkanes in cuticular hydrocarbons acts as a cue that allows foragers to identify patrollers during social interactions important in the regulation of colony foraging. I removed patrollers to prevent foraging and returned ant mimics - small glass beads coated with hydrocarbons - to the nest at an appropriate rate and then measured colony foraging levels. My data supported the hypothesis; when nest maintenance workers were supplemented with *n*-alkane hydrocarbons, colonies responded with foraging in a similar fashion as the return of patroller hydrocarbons.

### **Variations in the chemical compounds between native (USA) and invasive (French) populations of the termite *Reticulitermes flavipes* and the indigenous European termite *R. grassei***

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In social insects, cuticular hydrocarbons (CHCs) play a central role in nestmate recognition and have proved to be useful for identifying species and populations. The invasive termite *R. flavipes* has been introduced into several countries, recent analyses suggesting that this termite may have originated from southern USA populations. This study compared the degree of chemical variations within and between supposed native populations, and populations of *R. flavipes* introduced into France, some of which are in competition with an indigenous species *R. grassei*. The CHC profiles of workers were analyzed as well as soldier defensive secretions (SDSs) from colonies collected in nine *R. flavipes* populations in Louisiana, Florida and France and one *R. grassei* population. Principal component analyses of CHC profiles as well as the calculation of two distance parameters (Nei and Euclidean) revealed remarkable chemical homogeneity within and between introduced populations of *R. flavipes* that was much higher than in the indigenous *R. grassei* population and native (USA) *R. flavipes* populations. This homogeneity of recognition cues within introduced populations of *R. flavipes* could be the reason for the lack of intraspecific aggression that has been observed only within these populations. These analyses also showed that the CHC profiles of *R. flavipes* French populations were closer to those of populations in Louisiana. Of the six distinct SDS chemotypes, one was common to populations in France and Louisiana. The overall results revealed that Louisiana populations could be the origin of the French populations that were introduced into France several hundred years ago.

### **Mate choice is a matter of “taste”: Host plant shifts induce changes of contact pheromones and affect mate and species recognition in herbivorous insects**

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In herbivorous insects, a host shift may lead to reproductive isolation by habitat isolation, but little is known about the effects on sexual isolation. We used the sympatric mustard leaf beetles *Phaedon armoraciae* and *P. cochleariae* to investigate whether a host shift directly affects species and mate recognition which is known to be mediated by cuticular hydrocarbons (CHCs)[1]. In the laboratory, *P. armoraciae* and *P. cochleariae* were reared on their natural hosts, brooklime (Scrophulariaceae) and large bittercress (Brassicaceae), respectively, as well as on Chinese cabbage (Brassicaceae). Mating bioassays revealed that male mating behavior is significantly influenced by the host plant. In intraspecific mating trials, males of *P. armoraciae* mated more often with “same host” females than with “different host” females, while *P. cochleariae* males did not differentiate. In interspecific mating trials, *P. armoraciae* and *P. cochleariae* showed significant premating isolation when reared on their natural host plants, but were lacking premating isolation when reared on Chinese cabbage. Chemical analyses revealed that the host plants affected the beetles’ CHC profiles which serve as contact pheromones. A canonical discriminant analysis based on CHCs clearly separated the different groups according to sex, species, and host plant. The data indicate that shifts between different plant families have a stronger effect on CHC profiles than shifts within a plant family. Thus, our results elucidate the potential role of host shifts for premating isolation by changing species and mate recognition cues.

[1] Geiselhardt S, Otte T, Hilker M (2009) *J Chem Ecol* 35: 1162-71



### Significance of chemical recognition cues is context dependent in ants

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Recognition of group members is of fundamental importance in social animals, allowing individuals to protect resources against intruders and parasites, as well as insuring social cohesion within the group. In ants and other social insects, social recognition relies on multi-component chemical signatures, composed primarily by long-chain cuticular hydrocarbons. These signatures are colony-specific and allow discrimination between nestmates and non-nestmates. Nevertheless, the mechanisms underlying detection, perception and information processing of chemical signatures are poorly understood. Here, we investigate whether *Camponotus aethiops* ants can associate a complete cuticular hydrocarbon profile, consisting of about 40 compounds, with a food reward and whether the new association, developed in an appetitive context, may affect aggression against non-nestmates carrying the hydrocarbon profile associated with food. We show that individual ant workers are able to associate the non-nestmate chemical profile with food. However, conditioned ants are still aggressive when encountering a non-nestmate carrying the odour profile used as conditioned stimulus in our experiments. This suggests that ants, like some, but not all other insects, show interactions between different modalities (i.e. olfactory and visual), and can treat complex chemical cues differently, according to the context in which they are perceived. This plasticity ensures that learning in an appetitive context does not interfere with the crucial task of colony defence.

### **Darcin: a male pheromone that stimulates female memory and sexual attraction in the house mouse**

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Pheromones in scent play a critical role in mediating reproductive interactions and sexual attraction in many mammals. In mice, males deposit numerous urine scent marks around their territories, continually refreshing their scent and countermarking any scent marks from competitor males. These urine scents contain both sex- and individual-specific volatile and involatile components. Females spend more time near male than near female urine when able to contact the scents. However, they are only attracted to airborne urinary volatiles from individual males whose urine they have previously contacted. Even females with natural exposure to many males and females fail to develop generalised attraction to airborne male scents. This implies that information gained through contact with a specific male's urinary scent is essential to stimulate attraction. We separated male urine into different fractions using anion exchange chromatography, and analysed each fraction using mass spectrometry and bioassay of response. Female sexual attraction to male scent was due to a single fraction containing a male-specific protein pheromone that we named darcin. Heterologous expression of recombinant darcin and other major urinary proteins confirmed that darcin elicits attraction even when presented alone. Importantly, nasal contact with darcin also elicits the learned attraction of females to airborne volatiles from individual males. Darcin is thus a mammalian male sex pheromone that stimulates an adaptable response to individual-specific odours through associative learning and memory, allowing female sexual attraction to be inherent but selective towards particular males.

### **Social integration of the inquiline ant, *Ectatomma parasiticum*: a chemical camouflage or mimicry?**

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Inquilinism is an intriguing form of social parasitism because of the dependence of the parasite for its host. This lifestyle is widespread in Formicinae and Myrmicinae ants but rare in other subfamilies. *Ectatomma parasiticum*, the sole inquiline described in the poneromorph group, is known to usurp established colonies of the closely related species, *E. tuberculatum*, and to use their worker force to produce sexuals exclusively. The parasite is morphologically similar to the host queen, although smaller. During their life cycle, *E. parasiticum* queens need to enter into a host colony and to cohabit with the residents. However, some of them become the target of workers attack, suggesting that their social integration into the host colony is incomplete or unstable. We then aimed at investigating the recognition cues used in this species parasitism.

For this, we compared the chemical profiles of the parasite and host species from colonies collected in Apazapan (Veracruz, Mexico). Our analysis focused on cuticular hydrocarbons (CH) as mainly involved in ant recognition. Three groups of individuals, i.e. parasite queens, host queens and workers sampled either inside or outside the nest, were analysed using the SPME method. Both species and castes could be discriminated by CH, even if differences were slight. Parasites produced a fewer amount of chemicals than host queens but similar to host workers. Our results suggest that parasites have no chemical insignificance. We discuss on the possible chemical tactics (i.e. camouflage or mimicry) used by this inquiline ant.

### **The cellular and molecular basis of bitter taste in *Drosophila***

**CARLSON J<sup>a</sup>**

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We have carried out a systematic analysis of how bitter taste is encoded by the major taste organ of the head of *Drosophila melanogaster*, the labellum. Different bitter compounds elicit widely varying degrees of aversion in behavioral tests. Each of 16 bitter compounds has been tested physiologically against all 31 bitter-sensitive neurons of the organ. The responses of the neurons are surprisingly diverse in magnitude and dynamics. Five functional classes of bitter neurons are defined, and a functional map of the organ is established. The expression of all 68 members of the Gr family of taste receptors is examined. Expression analysis reveals five classes of bitter neurons, closely coinciding with the five functional classes. The results provide a receptor-to-neuron map of the organ. Misexpression of one receptor confers bitter responses as predicted by the map. These results reveal an unexpected degree of complexity that greatly expands the capacity of the system to encode bitter taste.

### **Short-range attraction of *Agrilus planipennis* Fairmaire (Coleoptera: Buprestidae) to a macrocyclic lactone**

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GC/MS analysis of volatiles from virgin female emerald ash borer, *Agrilus planipennis* Fairmaire (Coleoptera: Buprestidae) detected the presence of (3Z)-lactone [(3Z)-dodecen-12-olide], a putative sex-pheromone. Subsequent gas chromatographic/electroantennographic (GC/EAD) analysis of synthetic (3Z)-lactone, which contained 3% (3E)-lactone [(3E)-dodecen-12-olide], showed that the majority of male antennal responses were associated primarily with the minor (3E)-lactone component. Behavioral responses to the (3E)-lactone and host volatiles (Phoebe oil) differed depending on the assay used. Two-choice olfactometer laboratory assays clearly demonstrated strong short-range attraction of male *A. planipennis* (but not females) to the (3E)-lactone, but not to the (3Z)-lactone. However, in field trapping bioassays, neither lactone isomer significantly affected mean catch of males or females in purple prism traps, whether tested alone or in combination with green leaf volatiles or Phoebe oil. Conversely, Phoebe oil increased mean catch of both sexes in traps but was repellent in the olfactometer bioassays. These data suggest that Phoebe oil likely attracts *A. planipennis* to the vicinity of traps from long range, and that capture on sticky traps is not affected by close range attraction to the (3E)-lactone. The (3E)-lactone was below the detection limit in females and its presence could not be confirmed in the laboratory. However, exposure of (3Z)-lactone to UV light caused a significant isomerization to the (3E)-lactone. These data, along with previous behavioral observations lead us to hypothesize that female *A. planipennis* produce (3Z)-lactone when immature and then move into the sunlight upon sexual maturity to catalyze the production of the active (3E)-isomer.

### Chemical recognition in social insects - Solving a 100-year-old mystery including warnings for possible pitfalls

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Within social insect species colony signatures are required so that altruistic behaviour can be appropriately directed. It is widely accepted that within ant species nest-mate discrimination is down to a chemical signature determined by the cuticular hydrocarbons present. In recent years researchers have analysed the (complex) cuticular hydrocarbon profile using either GC-FID or GC-MS and with statistical analysis it was able to discriminate different species and/or colonies. However, assigning a nest-mate recognition role to an individual hydrocarbon or even to a class of hydrocarbons is much more difficult.

The ant species *Formica exsecta* is recognised as having one of the simplest chemical profiles consisting of various (Z9)-alkenes and alkanes, present in colony specific ratios. Therefore we investigated the role of each of the different hydrocarbon classes (alkenes and alkanes) in chemical nest-mate recognition in *Formica exsecta*. In subsequent studies we also looked at the role of respectively methyl branched hydrocarbons in *Formica lugubris* and alkenes in bumblebees.

Hydrocarbon analysis can be tricky if small differences within a complex profile are important and in this presentation I will also discuss some of the pitfalls (adequate detection, separation, temperature range and/or concentration) researchers may encounter in looking for chemical recognition systems or cues in social insects.





## S5. Evolutionary aspects of chemical communication

*Moderators: Thomas Schmitt & Carole Smadja*





### PLENARY TALK

#### Biosynthesis and secretion of rose scent compounds

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Roses are widely used as garden plants and for the cut flower market. They are also used for the production of essential oil for the cosmetic and perfume industries. More than 200 volatile molecules have been described in rose oil. A lot of botanical roses are fragrant but despite the efforts of the breeders, not all roses arriving on the market are heavily scented, specially the ones bred for the cut flower market. The cause of this lack of scent is not known.

In modern roses, scent is mostly emitted by petals, although stamens can make a significant contribution to the fragrance of the flower<sup>1</sup>. We recently showed that these scent compounds are concentrated in epidermal layers of the rose petal, putatively in lipid droplets<sup>2</sup>. Moreover, we are studying several genes involved in the biosynthesis of scent in rose. For example, we recently characterized the enzymes responsible for the so-called 'tea scent' emitted by Chinese roses<sup>3</sup>. We are also interested in genes involved in the scent of terpenes, which are mostly responsible for the typical rose scent.

#### References

**1 Dubois, A. et al.** "Tinkering with the C-Function: A Molecular Frame for the Selection of Double Flowers in Cultivated Roses. *PLoS ONE* 5, e9288.

**2 Bergougnoux, V. et al.** (2007). Both the adaxial and abaxial epidermal layers of the rose petal emit volatile scent compounds. *Planta* **226**, 853-866.

**3 Scalliet, G. et al.** (2008). From the Cover: Scent evolution in Chinese roses. *Proceedings of the National Academy of Sciences* **105**, 5927-5932.



### PLENARY TALK

## Identifying sources of intraspecific variation in moth sexual communication

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In night-flying moths, highly specific, long distance, pheromonal communication is crucial for mating success and reproductive isolation of species. Although there are thousands of moth species with unique pheromone blends, the evolutionary processes that resulted in this diversity are poorly understood. To gain insight into the evolution of premating isolation, we are quantifying the level of intraspecific variation in the pheromone signals of several moth species, on which selection may operate. Sources of variation in the habitat that likely affect chemical communication systems include the presence and abundance of species with similar chemical cues and different host plants. Environmental variables likely affect generalist moths differently from specialist moths that feed only on specific plant species. We are exploring the effect of these factors on the variation of sexual communication signals in different moth species. We are also assessing how much of the variation is genetic and how much is environmental, i.e. due to phenotypic plasticity. This seminar will give an overview of what we know, don't know and need to know of the genetic basis of, and environmental influence on, variation in moth prezygotic isolation signals to understand the evolution of these signals.



### ***Bombus terrestris* L. : a complex species or a species complex ?**

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*Bombus terrestris* L. is a widespread European bumblebee species. Its distribution is centered on the Mediterranean Sea extending from the Canary Islands in the West to the Altai on the East, and from the AntiAtlas Mountains of Morocco in the South to Southern Finland in the North. Nine subspecies are described showing morphological differentiation, particularly in isolated (e.g. insular) taxa. Males of this species have a patrolling pre-mating behavior, i.e. they lay sexual pheromones along a circuit they patrol searching for a conspecific virgin female. These secretions, produced by the cephalic labial glands (CLG) are known to be species-specific, and even subspecies-specific for 4 taxa of *B. terrestris*. Moreover slightly genetical divergences have already been shown in insular subspecies.

A complete analysis of CLG secretions coupled with ethological tests, as well as mtDNA analysis of the 9 subspecies all over Europe have been realised. Our results show that some taxa are poorly differentiated (*B. t. terrestris*, *B. t. dalmatinus*), while others show no genetical difference but well characterized CLG secretions (*B. t. audax*, *B. t. sassaricus*). Finally 3 taxa show a conspicuous genetical divergence as well as specific CLG secretions (*B. t. canariensis*, *B. t. africanus*, *B. t. xanthopus*) leading to questions about their taxonomic status.

### Oviposition deterring infochemicals in ladybirds: the role of phylogeny

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Faced with an ephemeral prey, aphidophagous ladybirds rely on the hydrocarbons present in the tracks of their larvae to choose an unoccupied patch for egg laying. Although both conspecific and heterospecific larval tracks might deter females from oviposition, the response to the later is often less striking. Several explanations have been suggested to account for this. In this work we tested the phylogeny hypothesis, which predicts that the chemical composition of the tracks of closely related species of ladybirds will be more similar to one another than to those of more distantly related species. Qualitative information on the chemical nature of the larval tracks and a molecular phylogeny of seven species belonging to 3 different genera are provided, and the congruence between these two sets of results assessed. The results confirm the phylogeny hypothesis and infer a gradual mode of evolution of these infochemicals.

### **Why most male ORNs are tuned to the most abundant sex pheromone components**

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One of the most puzzling and perplexing issues that has repeatedly popped up over the decades of research in insect sex pheromones has been the question of why, in nearly every species, are the majority of olfactory receptor neurons (ORNs) tuned to the most abundant pheromone component in a species' sex pheromone blend? A related question concerns ORNs co-compartmentalized in the same sensillum: why does the ORN apparently tuned to the most abundant pheromone component in the blend have a larger diameter dendrite than the ORN tuned to the minor component and produce a larger amplitude action potential than the ORN tuned to the minor component? It is seemingly counterintuitive that this is the case: the greatest sensitivity and the most ORNs should be tuned to the least abundant pheromone components, since these will be the components that are most difficult to detect in any blend. We have come up with a hypothesis for why these relationships between ORN number and dendrite diameter have been sculpted over evolutionary time in this fashion. The hypothesis applies nicely not only to the evolution of sex pheromone olfactory systems, but also to olfactory systems for general odorants involved in host-finding. Our argument is supported by abundant examples in the literature that we have examined from a different angle than usual, as well as by results from recent experiments from our laboratory.



### **Chemical espionage on butterfly anti-aphrodisiac pheromones by tiny hitch-hiking parasitic wasps**

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Parasitic wasps employ a wide range of chemical cues to find their hosts. Very recently, we discovered how two closely related parasitic wasps, *Trichogramma brassicae* and *Trichogramma evanescens*, exploit the anti-aphrodisiac pheromone benzyl cyanide of one of their hosts, the gregarious Large Cabbage White butterfly *Pieris brassicae*. The pheromone is transferred by male butterflies to females during mating to enforce female monogamy. Upon detecting the anti-aphrodisiac, the tiny parasitic wasps ride on a mated female butterfly to a host plant and then parasitize her freshly laid eggs<sup>(1,2)</sup>. Here, we demonstrate that both wasps similarly exploit the anti-aphrodisiac mixture of methyl salicylate and indole of another host, the more abundant solitary Small Cabbage White butterfly *Pieris rapae*. Interestingly, this behavior is innate in *T. brassicae*, whereas *T. evanescens* learns it after one successful ride on a mated female butterfly. Moreover, we show that the wasps only respond to the anti-aphrodisiacs of the two cabbage white butterflies when the ubiquitous compounds are part of a complete mated female odor blend<sup>(3)</sup>. Obviously, parasitic wasps use the sophisticated espionage-and-ride strategy to find eggs of different gregarious and solitary host species. From the wasp perspective there seems to be a trade-off between the abundance and egg-laying behavior of the butterflies. Our findings suggest that *Pieris* butterflies are under strong selective pressure to minimize the use of an anti-aphrodisiac.

#### References cited

1. Huigens, M.E. et. al. 2009. PNAS 106:820-825.
2. Fatouros, N.E. et. al. 2005. Nature 433:704.
3. Huigens, M.E. et. al. 2010. Behavioral Ecology 21:470-478.

### **The art of stealth - evolution of chemical mimicry in cuckoo wasps (Hymenoptera, Chrysididae)**

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In insects, brood parasites invade their host's nest to lay eggs where their larvae feed on the host's brood and the nutritional resources. The parasite needs to avoid detection by the host in order to neither be attacked while inside the nest nor risk the nest to be abandoned by the host afterwards. While visual recognition can be avoided by entering the nest in the host's absence, detection of chemical residue (e.g. cuticular hydrocarbons (CHC) adsorbed to nest material) is harder to avert. By mimicking the host's CHC profile, the parasite may manage to go unnoticed. In this study, solitary Hymenoptera and their – for the most part - highly specific Chrysidid parasitoids serve as a model to evaluate selection factors promoting chemical mimicry. We compare the CHC profiles of several parasitoid species with their respective hosts. Additionally, sex-specificity of CHC profiles is compared between different taxa of cuckoo wasps. This allows drawing conclusions about the influence of lifestyle, degree of specialization and intraspecific communication on the accuracy of chemical cloaking. We confront the CHC profiles with the parasitoid and host phylogenies to understand the evolutionary history and trajectories towards species- and/or sex-specific CHC profiles in this group of obligate parasitoids.

### **The chemical disguise of the cuckoo bumblebee *Bombus vestalis* to sneak into power of reproduction**

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During nest invasion, social parasitic bumblebees have to overcome the defensive system of their hosts and control worker reproduction. We performed behavioural experiments and chemical analyses to describe the process of *Bombus (Psithyrus) vestalis* parasite females conquering *Bombus terrestris* host colonies. We collected the scent of parasite females before and after nest invasion and compared it with the odour of host queens. Furthermore, we investigated whether there is a similar odour signal released by egg-laying queens and workers and cuckoo bumblebees that may have a function as a fertility signal. In behavioural experiments we found that the parasite upon recognition of a host nest displays a specific brushing behaviour thereby impregnating its body surface with odours. In chemical analyses we could show that immediately after perceiving a potential host nest, the parasite female increases production of semiochemicals<sup>1</sup>. Within the first day after nest invasion, the parasite adjusts its odour bouquet considerably to the existing colony scent by acquiring colony-specific odour from nest material or host workers. Breeding parasite females show an odour bouquet that totally resembles the odour of their host queen. In order to uphold the reproductive skew in a similar way as the host queen does in unparasitized colonies, they even produce a fertility signal, a mixture of wax-type esters that we also identified in egg-laying host queens and workers<sup>2</sup>.

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#### *References*

1. Sramkova, A. and Ayasse, M. 2009. Chemoecology 19:55–62
2. Sramkova, A. et al. 2008. Naturwissenschaften 95:515-522

### A “reductionist approach” to the evolution of pheromone diversity in corn borer moths (*Ostrinia* spp.)

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The European corn borer *Ostrinia nubilalis* and related *Ostrinia* species have become a model of choice for studying the evolution of pheromones and pheromone biosynthesis in particular. The recent identification and molecular characterization of the gene responsible for pheromone polymorphism in *O. nubilalis* constitutes a leap forward allowing a deeper understanding of moth pheromone evolution (1). In a comparative study we now examine the corresponding orthologous gene in a number of *Ostrinia* species. The results from our functional assays reveal that the gene in question, a fatty acyl reductase (FAR), act so as to determine the spectrum and ratio of components used by a given species. These findings substantiates the idea that mutations affecting the substrate specificity of the FAR enzyme has been a key to the divergence of pheromone signals not only within the genus *Ostrinia* but also in many other moth species using a similar pheromone theme (2). Understanding the genetic mechanisms involved in the diversification of pheromones is essential as pheromone specificity is closely associated with the evolution of reproductive isolation and the divergence of moth species.

1. Lassance, J.-M., Groot, A.T., Liénard, M.A., Antony, B., Borgwardt, C., Andersson, F., Hedenström, E., Heckel, D.G., and Löfstedt, C. 2010. *Nature (in press)*. DOI 10.1038/nature 09058
2. Liénard, M.A., Hagström, Å.K, Lassance, J.M., and Löfstedt, C. 2010. *PNAS (in press)*. DOI 10.1073/PNAS 10000823107

### **Evolutionary and ecological implications of insect-plant interactions as impacted by pollution**

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Anthropogenic pollution has at least two two major effects that affect insect- plant interactions. First, changes in plant nutrition and nitrogen utilization often follow elevated CO<sub>2</sub>, ozone, or acidic precipitation events. These responses can be either positive or negative for insect populations. Second, there are often substantial changes in plant defensive chemistry. Most recently, elevated levels of some heavy metals have been shown to provide some plants with exceptional defenses against insects (the elemental defense hypothesis). The implications these pollution-induced effects include the loss of effectiveness of some genetically engineered plants as the climate changes, altered success rates of herbivores used for biocontrol of weeds, a potential major impact on insect pollination at contaminated sites, and a variable pattern of increases or decreases in insect outbreaks (depending on the system).

### **Geographic variation of sex pheromone, mitochondrial DNA and PBP expression in *Diatraea saccharalis* (Fab., 1794) (Lepidoptera: Crambidae)**

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The main sex pheromone components of *Diatraea saccharalis*, (9Z,11E)-hexadecadienal and (Z11)-hexadecenal, were identified and quantified from four Brazilian and one Colombian populations using GC-EAD, GC-MS and GC analyses. Three different pheromone ratios were observed, 9:1, 3:1, and 6:1. The pheromone concentration for the major component, (9Z,11E)-hexadecadienal, varied from 21.9 to 6.8 ng/gland and from 6.5 to 1.7 ng/gland for the minor component, (Z11)-hexadecenal. Twenty-five *D. saccharalis* cytochrome oxidase II sequences were analyzed, they showed low intra-specific variation and represented only eleven haplotypes, with the most frequent being the one represented by specimens from São Paulo, Paraná, and Pernambuco states from Brazil. Specimens from Colombia showed the highest genetic divergence. Data on the genetic variability among specimens, more than their geographic proximity, were in agreement with data obtained from analyses of the pheromone extracts. These results suggest that the ratio of these two pheromone components used for monitoring or mating disruption experiments should account for the genetic variability and geographic location of this moth. The expression of a pheromone binding protein of *D. saccharalis* studied in *B.mori* PBP1 antibody yielded two immunoreactive bands with an apparent MW of approximately 15 and 18 kDa. These bands were observed in both males and females antennae. The level of PBP expression under a scotophase of L12:D12 photoregime was compared with a continuous light condition. The level of the PBP expression was significantly higher in male antennae. In addition, antennae tissues collected from males maintained under the scotophase showed a slightly higher PBP expression level.





## S6. Chemical ecology of multitrophic interactions

*Moderators: Martin Heil & Martine Hossaert-McKey*





## PLENARY TALK

**Emerging patterns of volatile chemistry in the Yucca-Yucca moth mutualism**

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The nursery pollination of *Yucca* plants by yucca moths (*Tegeticula* spp.) has been intensively studied as a model system for investigating the evolutionary dynamics of mutualism. Like figs, yuccas have obligate, specialized insect pollinators that oviposit into ovules of the flowers they have pollinated. Female yucca moths must gather pollen, pollinate and oviposit into flowers, whereas males seek females in flowers. Thus yucca odors could serve diverse fitness-related functions for female and male yucca moths, as revealed by bioassays with three species pairs. Delta traps baited with hidden flowers of *Y. filamentosa* attract primarily male *T. cassandra* moths. Furthermore, male and female *T. yuccasella* moths orient to *Y. glauca* floral odors significantly more than to humid air in Y-maze binary choice assays. Male and female *T. treculeanella* moths show agitated searching behavior when exposed to the odor of *Y. treculeana* flowers in 4-arm olfactometer assays, similar to the ambulatory behavior shown by both sexes within inflorescences. The waxy buds and stems of most *Yucca* species emit a series of 15-19C alkanes and N-alkenes, whereas open flowers emit nerolidol and larger amounts of the biosynthetically related homoterpene, *E*-4,8-dimethylnona-1,3,7-triene (DMNT), with a variable number of oxygenated derivatives of DMNT. GC-EAD analysis of the antennal responses of *T. cassandra* and *T. treculeanella* moths to floral headspace indicated an electrophysiologically active, novel dioxygenated compound (C<sub>11</sub>H<sub>14</sub>O<sub>2</sub>) common to most *Yucca* spp. and dominating the headspace of *Y. treculeana*. Finally, yucca odors show potential for character displacement in zones of contact between related species.



### PLENARY TALK

#### **Plant-mediated interactions between insects across and within trophic levels.**

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Feeding by insects results in significant changes in a plant's chemical phenotype, with consequences for insects at several trophic levels. Studies of multitrophic plant-insect interactions have long concentrated on the effects of single plant attackers on interactions of the plant with community members at different trophic levels, aboveground. However, plants are exposed to a variety of attackers, either simultaneously or sequentially, in shoots and roots, triggering much more complex interactions than have usually been investigated in the context of behavioral and chemical ecology of plant-insect interactions. I will present studies based on a cruciferous plant-insect model system that illustrate the magnitude of the impact/interference that belowground insects can exert on aboveground plant-insect interactions, at several trophic levels. Moreover, also the attack of plants by multiple aboveground attackers plays a significant role in shaping multitrophic plant-insect interactions. This is a research field that is rapidly developing in recent years. By taking a multi-plant-attacker approach, I will show examples where going from a single infestation to just a two-species infestation situation with insects with contrasting feeding guilds elucidates exciting indirect interactions that are displayed at least at three trophic levels.



### Leafminer insects trigger the host plant physiology through an unexpected association with endosymbiotic bacteria

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Gall-inducer arthropods are usually distinguished from other insect-generated shelters by the fact that they involve active differentiation of highly nutritive tissues. However, plant manipulation appears not to be restricted to gall-inducers only, as shown by the autumnal formation of 'green-islands' around mining caterpillars.

Our results on the *Malus domestica/Phyllonorycter blancardella* plant-leaf mining system show: (i) The ability of this leaf-miner caterpillar to manipulate its host plant in order to generate a microenvironment with all the nutrient supply needed for its survival. (ii) A decrease in plant defence compounds within the mined area. (iii) A large accumulation of cytokinins in the mined tissues which is responsible for the preservation of functional nutrient-rich green tissues at a time when leaves are otherwise turning yellow. (iv) The primary role played by endosymbiotic bacteria (*Wolbachia*) in the synthesis of these cytokinins and in the induction of nutrient-rich tissues. All together, these results clearly show the ability of leaf-miner insects to manipulate their host plant physiology and to create an "optimal" nutritional micro-environment through cytokinin production by their endosymbiotic partners. *Wolbachia* is suspected to play an essential role which, if this is the case, will be the first evidence of a *Wolbachia*-mediated effect on plant physiology.

### **Pollinator visits to flowers of herbivore-induced black mustard plants: nectar chemistry and quantity**

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In response to herbivore feeding plants synthesise defensive chemicals. Apart from allocation costs associated with this defence response, ecological costs may arise from modifying the interactions with mutualistic community members, in particular pollinators. We addressed the following questions: (1) Does feeding by *Pieris rapae* larvae on *Brassica nigra* leaves influence number and duration of flower visits by two common pollinator species? (2) Does nectar quantity and quality change upon herbivore feeding? We employed direct behavioural observations on two pollinator species, the hoverfly *Episyrphus balteatus* and the honeybee, *Apis mellifera*, in greenhouse and field studies and measured frequency and duration of flower visits. We determined sugar and glucosinolate concentrations in nectar and measured nectar volume per flower. The number of visits by syrphid flies did not differ between *P. rapae*-damaged and undamaged plants, but duration of visits was shorter, especially in the field. For the honeybees the number of visits was also not affected; however, we also observed reduced duration of flower visits, although only in the greenhouse. Concentrations of 5 sugars detected in nectar of flowers of herbivore-induced plants and control plants were similar. We report for the first time for a *Brassica* species that *B. nigra* nectar contained two glucosinolates, 2-propenylglucosinolate and 4-hydroxy-3-indolylmethyl-glucosinolate, their concentrations being similar for herbivore-induced and control plants. Contact cues, such as flower rewards or flower chemistry, could explain the reduced duration of the visits that we observed, in particular the reduction by 30% of nectar volume in individual flowers of herbivore-induced plants.

### **Constant versus herbivore induced chemical signaling for recruitment of beneficial and parasitic nematodes by plant roots: effects of nematode life history and plant breeding**

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Indirect plant defenses are well documented for the aboveground constituents of plants. Although less investigated to date, belowground signals that mediate multitrophic interactions are equally important. Entomopathogenic nematodes (*Stienernema diaprepesi*) are attracted to herbivore-induced volatiles released from Swingle rootstock, *Poncirus trifoliata* x *Citrus paradisi*, when fed upon by the root weevil, *Diaprepes abbreviatus*. Herein, we examined the extent to which belowground recruitment signals modify behavior of nematode species representing various foraging strategies (cruisers vs. ambushers), and trophic levels (plant parasitic vs. entomopathogenic). We compared attraction to extracts of weevil infested roots and non-infested roots from Swingle rootstock and a parent of the Swingle hybrid, *Poncirus trifoliata* (Pt) which are both resistant to plant parasitic nematodes. Swingle roots infested by weevils attracted more nematodes than non-infested roots irrespective of nematode foraging strategy and trophic status. The parental line of the swingle rootstock, Pt, attracted all nematode species irrespective of insect herbivory. Dynamic *in situ* collection and GC-MS analysis of volatiles from soil revealed that Pt roots release these recruitment signals constitutively regardless of weevil feeding. Dynamic volatile collections from above and belowground portions of citrus plants revealed that aboveground feeding by weevils does not induce recruitment of nematodes analogous to that induced by root damage. A separate citrus species (Sour Orange, *Citrus aurantium*), which is highly susceptible to plant parasitic nematodes, also exhibited herbivore-induced nematode recruitment chemicals. Constitutive release of nematode attractants by plant roots may vary depending on plant susceptibility to plant parasitic nematodes.



### **Preimaginal olfactory experience of host-induced plant volatiles induces specific preferences in an aphid parasitoid**

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When parasitic wasps search for their hosts, they use volatile organic compounds (VOCs) emitted by plants fed on by the hosts. The quality and/or quantity of those VOCs can be specific according to herbivore species, and this specificity may facilitate the host searching by parasitoids. If so, an intriguing question is how the wasps could respond to such a specific blend of VOCs from host-infested plants. Here, we show that preimaginal olfactory experience of VOCs induces host-specific responses in a parasitic wasp *Aphidius ervi* that attacks pea aphids *Acyrtosiphon pisum* in Japan. In a Y-tube olfactometer, *A. ervi* females showed significant preference for host-infested plant (broad bean plant, *Vicia faba*) volatiles over intact plant volatiles when they had previously experienced the host-infested plant volatiles during both mummification and emergence. However, when exposed to volatiles from plants fed on by *Aphis craccivora*, a nonhost aphid for *A. ervi*, during the mummification, the wasps showed olfactory preferences for neither host nor nonhost-induced plant volatiles. These results suggest that specific preimaginal learning of the particular blends of compounds enable wasps to specifically respond to the host-related VOCs.

### **A coat of many scents: fig wasp cuticular compounds as potential cues for prey recognition by ants**

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The interaction between figs (*Ficus* spp, Moraceae) and fig wasps (Hymenoptera, Agaonidae) — one of the classic examples of nursery pollination mutualisms — is largely mediated by chemical communication. *Ficus racemosa*, with its seven-member fig wasp community in India (which includes gallers, parasitoids and inquilines) and associated ants (predatory and trophobiont-tending) constitutes a complex multitrophic system. It was earlier demonstrated that predatory ants (*Oecophylla smaragdina* and *Technomyrmex albipes*) use volatile olfactory cues not only from fig wasps but also from figs to locate their fig wasp prey [1]. Using cuticular extracts of fig wasps, we investigated whether cuticular compounds could be used as prey recognition cues by ants. The predatory ants and the largely trophobiont-tending ant (*Myrmicaria brunnea*) were attracted to these cuticular extracts to varying degrees. These results in combination with differential predation pressure by ant species on the various fig wasps indicate varied prey recognition mechanisms. Contrary to earlier findings with volatiles [1], predatory ants resident on fig trees and on non-fig trees reacted similarly to cuticular compounds implying that responses to these compounds are not learned. Multivariate analysis demonstrated that each fig wasp species has a unique cuticular signature with galler- and parasitoid-specific compositions. Using the Random Forests algorithm [2] on the cuticular profiles of these fig wasps indicated potential compounds the ants may be using to discriminate between fig wasps.

#### References cited

1. Ranganathan, Y. & Borges, R. M. 2009. *Animal Behaviour* 77:1539–1545
2. Ranganathan, Y. & Borges, R. M. 2009. *Plant Biology* doi:10.1111/j.1438-8677.2009.00278.x

### **Herbivore-induced plant volatiles and floral resources increase natural enemy abundance and pest control in field crops**

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Evidence of enhanced natural enemy abundance, reduction in pests and crop damage using a novel approach to conservation biological control (CBC) 'Attract and Reward' is presented. This approach involves herbivore-induced plant volatiles (HIPVs) to attract beneficial taxa into crops; the 'reward' component integrates floral plants into crops, further improving residency and fitness of attracted natural enemies by providing food and shelter.

Six HIPV's (methyl salicylate, methyl anthranilate, methyl jasmonate, benzaldehyde, cis-3-hexenyl acetate, cis-hexen-1-ol) were evaluated in wine-grapes, broccoli and sweet-corn on their efficacy to attract natural enemies. The 'Attract and Reward' approach was tested in the same crops with the same HIPVs. Buckwheat (*Fagopyrum esculentum* Moench) was used as reward.

The 'Attract' experiments in the three crops revealed responses by parasitoids from several hymenopteran families; Trichogrammatidae, Encyrtidae, Bethyilidae, Scelionidae and Braconidae to the applied HIPVs. Parasitoids were attracted for up to six days after HIPV application suggesting that HIPVs directly attracted insects and that plants may have been induced to emit endogenous volatiles which attracted insects.

The 'Attract and Reward' experiments showed attraction of Scelionidae, Eulophidae, Encyrtidae and predatory insects to several HIPVs. Buckwheat as the reward increased abundance of several hymenopteran parasitoids in all three crops and predators in sweet-corn and broccoli. Significantly fewer *Helicoverpa* larvae were found on sweet-corn plants adjacent to reward plots; less *Helicoverpa* damage to cobs was evident for one of the HIPV treatments.

Results are discussed in relation to the potential use of 'Attract and Reward' as a strategy for improving CBC of pests.

### **The effect of elevated atmospheric carbon dioxide on a grass-endophyte defensive mutualism: implications for aphid herbivory.**

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Atmospheric carbon dioxide concentrations are predicted to rise to between 550 and 1000ppm from the current level of 390ppm by the year 2100. CO<sub>2</sub>-induced changes in plant resource allocation are expected to impact the ways in which plants interact with symbiotic partners which in turn may affect plant-herbivore dynamics. Endophytic fungi of the *Neotyphodium* genus form mutualistic associations with cool-season grasses and produce alkaloids that increase grass resistance to herbivory. Here we examined the effects of CO<sub>2</sub> enrichment on the interaction between tall fescue (*Schedonorus phoenix*) and its endophytic symbiont *Neotyphodium coenophialum* and the response of *Rhopalosiphum padi* – an aphid known to be negatively affected by the production of endophyte-derived alkaloids. Our results showed a significant interaction between CO<sub>2</sub> and endophyte infection whereby elevated CO<sub>2</sub> significantly decreased aphid abundance on endophyte-free plants but showed no effect on aphids feeding on endophyte-infected plants. The concentration of endophyte-derived loline alkaloids was reduced under elevated CO<sub>2</sub> possibly due to a dilution effect of increased carbohydrates, as both high-molecular weight (HMW) and low-molecular weight (LMW) carbohydrates were shown to increase under elevated CO<sub>2</sub>. Our study suggests that projected increases in atmospheric CO<sub>2</sub> may alter grass-endophyte interactions which in turn may have implications for plant productivity and insect herbivory.

### Temporal patterns of induced plant responses and their effects on multitrophic interactions

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Plants possess a wide range of morphological and chemical traits to reduce herbivory. Many of these traits may be induced. Since these responses usually peak only for a certain period of time as these responses are costly.

In this study we determined the temporal dynamics of various systemically induced responses occurring in *Brassica juncea* leaves after insect herbivory in India and NL. We analysed morphological responses, consisting of trichomes and leaf size, as well as chemical responses, including primary (sugars and amino acids) and secondary (glucosinolates and volatiles (VOC)) metabolites. Glucosinolates and trichomes were found to increase systematically between 4 and 13 days after herbivore damage, whereas amino acids, sugars and leaf size remained unaffected. The response at four and nine days after damage was most consistent and these time-points were used for insect performance and preference studies.

We tested the effects of induced changes on the preference of generalist *Spodoptera litura* and specialist *Plutella xylostella*. *S. litura* preferred to feed on leaves from undamaged plants, while *P. xylostella* preferred leaves from plants damaged nine days before.

To analyse the effects of temporal dynamics of herbivore-induced VOCs, orientation preference of these pests and their parasitoids *Cotesia marginiventris* and *C. plutellae* were examined between first three days of induction. *P. xylostella* and both parasitoids preferred induced plants, while *S. litura* preferred uninduced plants.

Our results suggest that temporal dynamics is an important and often overlooked factor for the effect of induced plant responses on herbivores and their parasitoids.

### **Chemicals released by the organ pipe mud dauber wasp *Trypoxylon politum* have a kairomonal effect on its parasitoid *Melittobia digitata***

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*Melittobia digitata* (Hymenoptera: Eulophidae) is a gregarious ectoparasitoid of the organ pipe mud dauber *Trypoxylon politum* (Hymenoptera: Crabronidae). To locate a host, *M. digitata* females discover a recently built nest and wait inside until the host larva has spun a cocoon, released the meconium and has become a prepupae, which is the suitable stage for parasitoid egg-laying and larval development. To date, no chemical compounds exploited by *M. digitata* in the host selection process are known. Hence, investigations were carried out to elucidate which semiochemicals were involved in this host-parasitoid relationship. Laboratory bioassays indicated that *M. digitata* females were surprisingly arrested by chemicals from host by-products (cocoon and meconium) while they did not react to cues coming from the prepupae. In particular, parasitoids reacted to both hexane and acetone extracts of the cocoon plus meconium, cocoon alone and meconium alone, but the stronger response was elicited by hexane extracts suggesting that non-polar compounds play a major role. Hexane extracts were analyzed in GS/MS identifying 12 linear fatty acids (C<sub>6</sub>-C<sub>18</sub>), 7 from the cocoon and 11 from meconium; despite qualitative and quantitative differences between cocoon and meconium fatty acid profiles, the main compounds were the same (octanoic acid; hexadecanoic acid; octadecanoic acid; cis-9-octadecenoic acid). Bioassays involving reconstructed blends of meconium and cocoon are in progress to identify the active compound(s) eliciting parasitoid's arresting behavior. The possible role of contact kairomones as recognition/suitability signals exploited in the host selection process of *M. digitata* are discussed from an ecological and evolutionary perspective.

### Male-derived anti-aphrodisiac compounds increase risk of egg parasitoid attack by inducing plant synomone production

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In many insects, males transfer anti-aphrodisiacs to females during mating that render them unattractive to other males. Males of cabbage white butterflies transfer species-specific anti-aphrodisiacs: benzyl cyanide (*Pieris brassicae*) or a mixture of indole and methyl salicylate (*P. rapae*).

Here, we assessed whether egg deposition by *Pieris* induces Brussels sprouts plants to arrest *Trichogramma* egg parasitoids, and whether the plant synomone is triggered by substances originating from the *Pieris* male seminal fluid. We showed that plants induced by *P. brassicae* egg clutches as well as by singly laid *P. rapae* eggs arrest the wasps three days after butterfly egg deposition. Furthermore, we revealed that eliciting activity was present in accessory gland secretion released by mated female butterflies during oviposition. In contrast, gland secretion from virgin female butterflies was inactive. We detected anti-aphrodisiac compounds in gland secretion from mated female butterflies of both *Pieris* species. When applied onto leaves, the synthetic anti-aphrodisiac compounds induced phytochemical changes that arrested the wasps. Thus, male-derived compounds can endanger the offspring of the butterfly by inducing plant defense<sup>1,2</sup>.

Recently, we showed that the same anti-aphrodisiacs play a role in host foraging of *Trichogramma*, by acting as a cue to facilitate phoretic transport by mated female butterflies to oviposition sites. Our results suggest that the anti-aphrodisiac pheromone incurs fitness costs for the butterfly by both mediating phoretic behavior and inducing plant defense.

#### Main references:

1. Fatouros, N. E. *et al.* PNAS USA **105**, 10033-10038 (2008)
2. Fatouros, N. E. *et al.* J.Chem.Ecol. **35**, 1373-1381 (2009)

### **Teaming up in defence: Symbiotic streptomycetes provide antimicrobial combination prophylaxis for wasp offspring**

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Mutualistic microorganisms are well-known to play a key role in providing nutrients for successful growth and reproduction in many insects. Recent studies indicate that they can be equally important for the protection of the host and its nutritional resources against pathogen attack. A highly specialized protective symbiosis has been described for the European beewolf (*Philanthus triangulum*, Hymenoptera, Crabronidae): Female beewolves cultivate specific *Streptomyces* bacteria in antennal gland reservoirs and apply them to their brood cells where they are later taken up by the larva. The incorporation of the symbionts into the cocoon silk has been shown to significantly improve the larval survival probability by providing protection against pathogenic fungi during the long phase of hibernation. Here, we report on the identification of the molecular basis of this protective symbiosis in the natural context and demonstrate that the bacteria produce an antibiotic 'cocktail' consisting of streptochlorin and eight piericidin derivatives. Using imaging mass spectrometry, we document the in situ spatial distribution of the most abundant antibiotics on beewolf cocoons and demonstrate that the abundance of the antibiotics on the outer surface of the cocoons is much higher than on the inside. This probably serves to enhance the protective activity against invading pathogens and reduces potential harmful side-effects on the beewolf larva itself. Using biological testing, we show that the complementary action of all symbiont-produced antibiotics confers a potent defense for the wasp larvae against a wide range of pathogens that parallels the 'combination prophylaxis' known from human medicine.



### Chemical aspects of the synergism and antagonism in microbial communities of leaf cutting ants

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Leaf cutting ants are members of a multitrophic system. They are cultivating the fungus *Leucoagaricus gongylophorus*, which serves them as major food source, in large chambers of their nests. This mutualistic symbiosis is threatened by pathogens such as the fungus *Escovopsis weberi*.<sup>1</sup> Therefore the ants defend their colony by removing any suspicious material from their fungus garden into waste chambers and by using chemical treatment. In addition to antimicrobial compounds from their own glands leaf cutting ants rely on antibiotic-producing symbiotic microorganisms.<sup>2,3</sup>

In contrast to the prevailing view there is a large diversity of microorganisms associated with leaf cutting ants. Considering this diversity a large variety of bioactive natural products can be expected that contributes to shaping the leaf cutting ants' ecosystem.

Beside their protective role for the leaf cutting ants and their fungus microbial symbionts use their chemical armory to pursue 'selfish' interests e.g. to compete with other microbial symbionts.

A new method to quickly identify the chemical basis of such diverse interactions will be presented.

<sup>1</sup>C.R. Currie, U.G. Mueller, D. Malloch. *Proc. Natl. Acad. Sci. U. S. A.* **1999**, *96*, 7998-8002.

<sup>2</sup>C.R. Currie, J.A. Scott, R.C. Summerbell, D. Malloch. *Nature* **1999**, *398*, 701-704.

<sup>3</sup>S. Haeder, R. Wirth, H. Herz, D. Spiteller. *Proc. Natl. Acad. Sci. U. S. A.* **2009**, *106*, 4742-4746.



# Outside Session Talks - 1

*Moderators: Jocelyn Milar & Anne-Geneviève Bagnères*



**Unsuitable host compound cineole inhibits response to pheromone in spruce bark beetle, *Ips typographus*, at olfactory receptor neuron and behavioral levels: Raison d'être of receptor cell co-localization?**

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Insects co-localize specific olfactory receptor neurons (ORN) in specific sensilla, possibly in order to improve compound ratio detection and/or the resolution of olfactory stimuli from different sources. Uncommon to most insects, the spruce bark beetle, *Ips typographus* (Coleoptera, Curculionidae, Scolytinae), group an ORN that responds to the host compound 1,8-cineole (cineole) together with a neuron that is tuned to the pheromone component *cis*-verbenol (cV). However, in other sensilla, this pheromone ORN is grouped with a non-responsive ORN.

Using single odorants and binary mixtures of cV and cineole, we show by means of single-sensillum recordings that when the cineole cell responds, the cV cell is inhibited, also when the cV cell is simultaneously stimulated with cV. This occurs almost exclusively in sensilla in which the two ORNs are co-localized. Thus, our results indicate that ORNs interact in the periphery, which suggests that ORNs do not act as independent response units as traditionally thought.

Interestingly, we also show that cineole is particularly abundant in attacked spruce trees and that the compound strongly reduces pheromone attraction of *I. typographus* in the field. Possibly, cineole is part of a multi-component 'unsuitable host signal' that regulates bark beetle density on attacked trees.

Our study is the first to systematically characterize interactions between co-localized ORNs. In addition, the relevance of such interaction is put into an ecological context.

## Variability in the production of alkaloids by the mediterranean red gorgonian *Paramuricea clavata*

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During the last decades the frequency of epizootic events and mass mortalities related to the warming of the Mediterranean Sea has appreciably increased. Sessile invertebrates, such as cnidarians, are particularly sensitive to such climatic events. In this context, the emblematic red gorgonian *Paramuricea clavata*, a species commonly found all along NW Mediterranean rocky bottoms, constitutes a model of choice for a chemical ecological study.

The purpose of the present work was to evaluate the variability in the production of secondary metabolites by *P. clavata* so as to understand how this gorgonian try to adapt to its environment.

As its chemical composition has been rarely studied, the first step of our work was to identify the major metabolites of *P. clavata*. In an unexpected way, alkaloids were isolated rather than isoprenoids or steroids, usually found in cnidarians. Some of these compounds were assayed for their toxicity against a marine bacterium (*Vibrio fisheri*) as well as for their capacity to inhibit the adhesion of a marine bacteria (*Pseudoalteromonas* sp.). Moreover, these compounds were quantified using a LC/DAD/ELSD/MS<sup>n</sup> method so as to determine qualitative and quantitative variations in the chemical composition of several samples of *P. clavata*. Thus, intracolony, micro- and macroscale variabilities were evaluated.

## An ipsdienol oxidoreductase is critical in determining the final pheromone stereochemistry in *Ips* spp.

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Ipsdienol and ipsenol are used extensively as aggregation pheromones among *Ips* spp. pine bark beetles, with additional specificity obtained by varying ratios of the R- and S-stereoisomers. In the final steps of pheromone production, which occurs in midguts of male beetles, a dual function geranyl diphosphate synthase/myrcene synthase produces myrcene, which is hydroxylated to a non-pheromonal ipsdienol blend. In both the western *I. pini* and in *I. confusus*, an approximate 85:15 ratio of R-(-)/S-(+) ipsdienol is produced by a midgut specific cytochrome P450, CYP9T2/1 that hydroxylates myrcene. A western *I. pini* male midgut specific ipsdienol dehydrogenase (IDOL DH) oxidizes R-(-) and S-(+) ipsdienol to the ketone, ipsdienone, and reduces the ipsdienone to 99 % R-(-) ipsdienol. Additionally, IDOL DH reduces ipsenone to 99% S-(-) ipsenol. The *I. confusus* homologue, IclIDOL DH, oxidizes R-(-) ipsdienol to ipdienone, which then is converted to ipsenone and ipsdienol (unknown enantiomer). We hypothesize that *Ips* species use CYP9T2/1, IDOL DH and an unknown ipsdienone to ipsenone reductase to make their pheromone components and that the final pheromone blends are determined by the ratio of activities of these three enzymes. The western *I. pini* obtains its final pheromonal ipsdienol blend (~95:5: -/+) by the IDOL DH catalyzed oxidation/reduction of the non-pheromonal (85:15 -/+) blend via the intermediate ipsdienone. The western *I. pini* and *I. confusus* make pheromonal ipsenol by the reduction of ipsdienone to ipsenone by an ipsdienone reductase followed by the reduction of ipsenone to 99% S-(-) ipsenol by IDOL DH.

### **Development of a new product based on the honey bee brood pheromone**

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Honey bee larvae emit a pheromone that tells workers “We’re here, and we’re hungry”. In response the workers forage more vigorously, enriched protein is produced in the mandibular and hypopharyngeal glands of nurse bees, the queen lays more eggs, and the vigor of the colony increases. Contech has been working with Dr. Tanya Pankiw of Texas A&M University since 2004 to develop a brood pheromone product that could be used effectively by beekeepers. The product, SuperBoost, is a blend of 10 fatty acid esters delivered in a small plastic pouch suspended in a holder between the frames in a hive. The non-volatile pheromone exudes through the polyethylene membrane for at least five weeks, and is picked up by contact with the bees. Two studies with SuperBoost have been completed at Contech. In the first, SuperBoost was administered for 10 weeks in a 12-replicate single-blind experiment to colonies during the late-winter feeding period. Compared to control colonies, treated colonies consumed 50% more BeePro<sup>®</sup> pollen substitute, had over double the area of brood comb, almost twice as many adults, and yielded four more daughter colonies. The second study utilized identical new colonies formed from 2 lb packages from New Zealand. By the end of summer, 59 treated colonies had produced well over double the honey harvest of 54 untreated control colonies. Inspection of all experimental colonies in October indicated no adverse effects of treatment with SuperBoost.

## Colour-scent associations in a tropical orchid: three colours but two odours

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Colour and scent are the major pollinator attractants to flowers, and their production may be linked by shared biosynthetic pathways. Species with polymorphic floral traits are particularly relevant to study the joint evolution of floral traits. We used in this study the tropical orchid *Calanthe sylvatica* from Réunion Island. Three distinct colour forms are observed, presenting, lilac, white or purple flowers. We investigated the composition of the floral scent produced by these colour varieties using the non invasive SPME technique in the wild. Scent emissions were dominated by aromatic compounds. Nevertheless, the presence of the terpenoid (E)-4,8-dimethylnona-1,3,7-triène (DMNT) is diagnostic of var. *purpurea*, with the VOCs produced by some individuals containing up to 60% of DMNT. We evidence specific colour/scent associations in *C. sylvatica* with two distinct scent profiles in the three colour varieties: the *lilacina*-like profile containing no or very little DMNT (< 2 %) and the *purpurea*-like profile containing DMNT (> 2 %). *Calanthe sylvatica* var. *alba* individuals group with one or the other scent profile independently of their population of origin. We suggest that white flowered individuals have evolved from var. *lilacina* and var. *purpurea* twice independently after island colonisation. White forms may have been favoured by the particular pollinator fauna characterising the island. These forms of *C. sylvatica* orchid which display three colours for two scents prove that color-scent associations may be complex according to the pollination ecology and the evolution of the considered species.



## Recent advances in the chemistry and commercialization of mealybug pheromones

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Over the past 5 years, we have identified the pheromones of the obscure (*Pseudococcus viburni*), longtailed (*P. longispinus*), and grape (*P. maritimus*) mealybugs, all of which are worldwide pests in vineyards and other crops. All three of these pheromones possess unique irregular monoterpenoid skeletons. The identification of each pheromone, including determination of relative and absolute configurations will be briefly described. All three of these pheromones are now commercially available, and are being widely used for detection and tracking mealybug population cycles. Economic and biological factors allowing their commercial development will be discussed, including the evolution of syntheses from the relatively inefficient, nonstereoselective syntheses used during proof of structures, to short and efficient syntheses on multigram scale.



## Outside Session Talks - 2

*Moderators: Eric Darrouzet & Didier Rochat*



## A twin metabolite reservoir for pheromone production in moths

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Virtually all species of moths use pheromones for communication between the sexes. The most common type of moth sex pheromone component is the fatty acid-derived compound, produced by females, de novo, in a specialized gland. The biosynthesis of these compounds involves production of saturated fatty acids, followed by limited metabolism, typically involving desaturation, cytosolic  $\beta$ -oxidation, reduction and/or oxidation and, often, esterification. We have demonstrated that hemolymph trehalose concentration influences the quantities of fatty acids and pheromone biosynthesized by female *Heliothis virescens*, probably by influencing levels of citrate and acetyl CoA in the pheromone gland. Although hemolymph trehalose levels decrease in moths with normal physiological activities (e.g., maturation of oocytes, extended flight or starvation), they can be replenished by feeding on plant nectar (sugars). Thus, nectar feeding in moths is important for maintaining production of pheromone to attract mates, as well as accelerating maturation of oocytes (i.e., increased fecundity). In this talk, we present a model for pheromone biosynthesis that involves twin metabolite reservoirs: trehalose in the hemolymph and glycerolipids in the pheromone gland. These connected reservoirs provide a buffered supply of metabolites for rapid and extended pheromone production. Importantly, they also function in complement with the reproductive state of the insect.

## Molecular Evolution of the *Drosophila* Olfactory System

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The olfactory system is an attractive model to study the genetic mechanisms underlying evolution and speciation. It mediates the detection and behavioural responses to an enormous diversity of volatile chemicals and displays rapid evolution, as species acquire, modify and discard olfactory receptors and circuits to adapt to new olfactory stimuli. *Drosophila melanogaster* makes use of two distinct families of olfactory receptors to perceive odours, the relatively well characterised Odorant Receptors (ORs) and the recently identified Ionotropic Receptors (IRs). We have performed a comparative genomic analysis of the IR repertoire in 12 sequenced *Drosophila* species, which has revealed that the olfactory IRs are well conserved across species, whereas the non-olfactory subset of this family appear much more variable. Two exceptions to the high conservation of olfactory IRs stand out: the duplication of one receptor, IR75d, in the cactophilic species *D. mojavensis* and the pseudogenisation of another receptor, IR75a, in the specialist *D. sechellia*. To identify ligands for IR expressing olfactory sensory neurons, we have performed an electrophysiological screen in *D. melanogaster* using a panel of 160 odours. We found that the IRs respond to a number of amines, aldehydes and acids, contrasting with the chemical specificity of the OR repertoire, which is mainly tuned to esters, alcohols and ketones. The identification of ligands for IRs in this species now allows us to study the physiological and behavioural significance of the receptor duplication in *D. mojavensis* and the loss in *D. sechellia*.

**Characterization of sugarwin, an insect-induced gene of sugarcane**

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Two isoforms of a sugarcane homologue of a barley wound inducible gene, *barwin*, were identified by *in silico* analysis and named *sugarwin* (sugarcane wound-inducible gene). Induction of *sugarwin* transcripts occurs in response to mechanical wounding, *D. saccharalis* feeding and methyl jasmonate treatment. Their expression is late-induced and restricted to the site of damage. The subcellular localization of the signal peptide fused to the *gfp* (green fluorescent protein) shows that these proteins are secreted. Multiple sequence alignment of BARWIN domain-containing sugarcane proteins and of mono and dicotyledoneous proteins reveals high similarity, suggesting that their function is conserved among species. Although the exact function of the barwin domain has not been completely elucidated, antipathogenic activities has been described for a number of homologues, and the same is true for sugarwins. This is the first report of a barwin-like protein induced by herbivory. The activity of this type of proteins against insects has never been studied. The ecological context of *D. saccharalis sugarwin* induction is interesting: in the field, *D. saccharalis* boring into the sugarcane stalk is followed by opportunistic fungi colonization which causes one of the main sugarcane diseases, the red rust. The exact function of induction of an antipathogenic protein by an insect remains to be elucidated.

### **Associations between *Drosophila* and yeast**

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Yeasts, like *Saccharomyces cerevisiae*, are closely associated with the fruit fly *Drosophila melanogaster*. They are of primary importance for *Drosophila*'s attraction to fruits, oviposition as well as for the development of the larvae. For example, females laid more eggs when yeast was present on the substrate. Wind tunnel experiments elucidated several yeast fermentation products as relevant cues for the mediation of the attraction behavior. *S. cerevisiae* was grown as batch culture under controlled conditions in a bioreactor and samples at different physiological stages were taken. Such odor samples were then tested for their impact on the fly attraction. Even if flies feed on yeast, the attraction of insects might be beneficial for the yeast because insect vectors most likely contribute to yeast distribution and colonization of new substrates. Indeed, we demonstrated that *Drosophila* flies trapped in a wine cellar carried diverse yeasts, which were then analyzed for their taxonomic relationship. The knowledge and a variety of molecular tools that are available for the model organisms, *Drosophila* and *Saccharomyces*, will allow us to understand their ecological, physiological and evolutionary relations in more detail.

## Identification of compounds emitted by kissing bugs (Triatomines): New structures in pheromone chemistry

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A compound released by the metasternal glands of at least two Triatomine vectors of the lethal parasite *Trypanosoma cruzi* (Chagas disease) has been reported as unknown.<sup>1</sup> Here we report on its structure elucidation. In addition, a group of analogs has been identified from *Triatoma* spp, forming a new class of semiochemicals with rather spherical structures. The compounds may be involved in chemical communication between sexes as indicated by Vitta et al. 2009.<sup>1</sup>

The synthesis of the main compound involved a novel key step in the preparation of secondary alcohols; the reduction of a carboxylic ester to the corresponding aldehyde by means of DIBAH and *in situ* alkylation by a Grignard reaction at low temperature. All four stereoisomers of the new compound were synthesized, and the absolute configuration of the natural product was determined by enantioselective gas chromatography and co-injection with the authentic reference samples on a modified cyclodextrin column.

[1] A.C.R. Vitta, et al. J. Chem. Ecol. **35**, (10) 1212-1221 (2009).



## The exploitation of plant derived glucosides by leaf beetle larvae

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Insects can have the capability to use plant derived secondary metabolites for their own chemical communication with other organisms. Especially the sequestration of glucosides represents a mechanism that is widely spread in the insect world. The leaf beetle larvae of the subtribe Chrysomelina developed specialized defensive glands in which such plant metabolites are converted, stored and released in case of disturbance. Analyzing the host plants of Chrysomelina beetles we identified several different glucosides. Due to their water solubility these compounds can pass membranes only under controlled conditions. Our detailed studies of the sequestration process revealed a functional network of transport processes guiding plant derived glucosides through the body of chrysomelid larvae. In order to identify transport proteins we have created reference sequences of the transcriptomes from Chrysomelina species. By annotation of the sequences we identified putative transport proteins. The detailed understanding of membrane transfer allows on one hand to draw parallels to other sequestering insects and on the other hand to discover factors that influence the adaptation of herbivorous insects to plants. Not only transport proteins have to deal with plant derived glucosides but also enzymes - especially in the gut and defensive secretion. In the secretion the glucosides are converted into their biological active form. Here we report the identification of a protein with glucosidase activity in the defensive secretion and gut lumen of Chrysomelina larvae.



# Student session - 1

*Moderator: David Giron*



**Phylogenetic conservatism in floral odours functioning as pollinator-attractant signals: a case study of Ficus / Agaonidae mutualisms**

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Mutualisms are interspecies interactions in which each participant gains net benefits from interacting with its partner. Like many other interspecies interactions, mutualisms are mediated by chemical signals. Understanding how mutualisms function, and how they might evolve, depends on deciphering the chemical information transmitted between mutualists, for example, those that guide one partner to the resources offered by the other in exchange for services. Most studies on chemical mediation between plants and pollinators stress the impact on the message of selection by pollinators. Nevertheless, constraints on the message emission might also be important. We studied whether phylogenetic history constrains the composition of plant chemical signals that mediate interactions with pollinators, i.e., we tested whether the evolution of floral scents exhibits phylogenetic conservatism. We studied the chemicals that mediate a group of interactions which has become a model system for understanding the evolution of mutualisms: the interactions between figs and their species-specific pollinating fig wasps. In this 'nursery pollination mutualism', the pollinators can breed only in receptive figs of their host tree, which depends in turn on the wasp as its sole pollinator. Encounter of the pollinator and the receptive fig is mediated by specific chemical signals. We used headspace adsorption/resorption methods to collect scents of several fig species in tropical forests from different tropical regions, and analyzed the composition of their volatile compounds using gas chromatography/mass spectrometry (GC-MS). We then built different chemograms, using both qualitative and quantitative traits of the bouquets of compounds, and compared them with phylogenies.

## Something about Water – Do Moths use Humidity Gradients as an Indicator for a Nectar-rich Flower?

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Nectar-feeding moths integrate sensory information provided by flowers to locate and feed from them. Quick, effective localization of profitable nectar sources is of major importance for the survival of the white-lined sphinx moth, *Hyles lineata*. Competition for nectar is high and moths visiting flowers right after anthesis will increase their chances to encounter nectar. Flower odors and colors do not change fast enough to track rapid changes in nectar abundance. In contrast, local gradients of relative humidity (RH) are more ephemeral and could more accurately indicate flower profitability to foraging moths. We characterized spatial and temporal RH gradients in the flower headspace of *Oenothera caespitosa* ssp. *marginata*. Furthermore, we tested whether *H. lineata* use changes in ambient RH as a cue to localize nectar sources while foraging. We found that RH in flower headspace is consistently increased over ambient levels for about 30min after anthesis. *Hyles lineata* prefer artificial flowers with increased RH levels over flowers with ambient RH levels in binary choice flight cage experiments. Our results underline the importance of sensing more general floral – environmental cues in the context of nectar foraging. Like carbon dioxide, floral RH gradients provide sufficient information to indicate freshly opened flowers and therefore profitable nectar sources to moths. Behavioral assays showed that increased levels of humidity play a role in the foraging behavior of *H. lineata*, documenting an additional sensory channel used by a nectar-feeding moth in the process of flower location.

## Effects of UV-B radiation on non-volatile secondary metabolites and subsequent changes in plant defense against different insect species

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Plant defense responses to herbivory are dependent on the plant evolutionary history as well as the physical environment affecting plant-insect associations. During the past 30 to 40 years potential effects of stratospheric ozone depletion and thus, enhanced UV-B radiation on plants became of major interest. UV-B effects on plant biochemistry have been usually linked with changes mostly increases in phenolic compounds. Contrary to this, little is known about UV-B mediated changes of other non-volatile secondary metabolites such as glucosinolates present in *Arabidopsis thaliana* L. and other members of the Brassicaceae family and the phytoalexin camalexin. Because UV-B induced plant responses overlap with responses to other stresses, which can significantly influence the plant-insect interaction, this was object of the study.

To study the impact of UV-B on plant defense against insect herbivores *A. thaliana* plants (Columbia) were exposed to short term UV-B radiation. Forced feeding experiments with UV-B treated plants compared to un-treated plants were conducted with the specialist herbivore *Pieris brassicae* L. and the generalist *Spodoptera exigua* (Hübner). The weight increase of *P. brassicae* larvae was significantly lower on UV-B treated plants, whereas *S. exigua* larval weight increase was higher on plants pre-exposed to UV-B. Insect performance was related to induction of specific glucosinolates and camalexin by UV-B due to a different plant defense response after pre-treatment with UV-B. Microarray analysis showed a decrease of most genes associated with photosynthesis while most plant defensive genes were activated. Combination treatments with insect and UV-B resulted in a generally stronger gene response.

**Styrene, produced by a fungus isolated from pine weevil frass, is repellent to the large pine weevil, *Hylobius abietis*.**

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A fungi strain 10D3 was isolated from pine weevil (*Hylobius abietis* L.) frass, that was identified as XXX by 16s RNA. The fungus was grown on sterilized *H. abietis* frass on agar media in 100ml Erlenmeyer flask, head space volatiles were collected by using SPME and analyzed by GC-MS. The major volatile compound of this fungus was styrene, 3-methylanisole was also present in small amount. These compounds were identified by comparing their retention time and mass spectra to that of standard compounds. Production of styrene and 3-methylanisole was studied by growing fungi on glucose, pine weevil natural frass and mechanically gnawed frass of pine (*Pinus sylvestris*) in liquid culturing media. Fungi grown on glucose media was producing more styrene as compared to natural frass and artificial frass media, where as fungi grown on natural frass proved better as compared to using artificial frass. In a multi choice arena test, styrene and 3-methylanisole showed good repellency towards pine weevil. These results showed that styrene compound could be a good repellent against pine weevil insect thereby protecting the pine forest.

**Sterility-linked quantitative differences in *Bombus terrestris* cephalic-labial glands secretions.**

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Worker reproduction in *Bombus terrestris* colonies is limited either to the competition phase in colony social development, or when made queenless. Reproductive skew in Hymenoptera is assumed to be pheromone-mediated, but the underlying mechanism in *B. terrestris* is yet clear. To elucidate the role of pheromones in reproductive conflicts in *B. terrestris* we have analyzed the cephalic-labial glands in 3 types of queens: virgin, fertile during PCP and fertile during CP, and in 3 types of workers: sterile during PCP; sterile during CP; and fertile, queenless workers. The glandular secretion was found to contain about 40 compounds, most of them straight-chain or branched hydrocarbons and esters with small amounts of fatty-acids. While the secretions of queens and workers, either fertile or sterile contain the full set of compounds, there were quantitative differences in the wax-esters, mainly in the dodecyl esters, being in greater quantities in sterile individuals. These findings, along previous finding of a sterile worker specific signal in Dufour's gland add another dimension to the pheromonal competition among queens and workers of *B. terrestris*. It further indicates that the transition from sterility to fertility is accompanied by major changes in multiple pheromone glands, suggesting a common regulatory mechanism. Pheromone signaling in *B. terrestris* thus constitutes an unusual case in which the sterile individuals, rather than the fertile ones, possess extra components (either quantitatively or qualitatively) raising an interesting evolutionary question of why should an individual advertise sterility as opposed to the logics in advertising fertility.



## **Aboveground herbivory affects orientation and parasitism by belowground parasitoid: laboratory and field evidence**

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Plants attacked by herbivorous insects are known to emit volatiles used by natural enemies to locate their hosts or prey. Yet, most studies have focused on simple interactions involving one species *per* trophic level and typically investigated the aboveground part of plants. The foraging behaviour of aboveground parasitoids can be influenced by root herbivory through changes in plant odours. The current study examines whether presence of the leaf herbivore *Pieris brassicae* on turnip plants influences the response of *Trybliographa rapae*, a specialist parasitoid of the root feeder *Delia radicum*. Our results show that the parasitoid is not attracted by volatiles emitted by plants under simultaneous attack. Also, parasitization levels are strongly reduced in the field on bi-infested plants. Analysis of plant volatiles revealed that volatiles emanating from bi-infested plants did not result in straightforward qualitative changes due root and/or leaf herbivory. Specific compounds were induced by root herbivory (i.e. alpha pinene and alkanes) or shoot herbivory (i.e. methylsalicylate), but were not higher after bi-herbivory.

A few lab and field studies have shown differences in the attractiveness of plants for parasitoids after multiple herbivory. The present study is the first so far confirming that the reduction in host attraction in the lab due to simultaneous attack by belowground and aboveground herbivores, also translates into lower levels of parasitism in the field. It highlights the importance of plant volatiles in structuring multitrophic systems, including at different plant compartments, and emphasizes the need to consider plant insect interactions at the community level.



## Student session - 2

*Moderator: Anne-Marie Cortesero*



## Metabolic fingerprinting as an indicator of biodiversity: towards understanding inter-specific relationships among Homoscleromorpha sponges

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Sponges are an important source of secondary metabolites showing a great diversity of structures and biological activities. Secondary metabolites can display specificity on different taxonomic levels, from species to phylum, which can make them good taxonomic characters. However, the knowledge available on the metabolome of non-model organisms is often poor. In this study, we demonstrate that sponge chemical diversity may be useful for fundamental issues in systematics or evolutionary biology, by using metabolic fingerprints as indicators of metabolomic diversity in order to assess interspecific relationships.

The sponge clade Homoscleromorpha is particularly challenging because its chemistry has been little studied and its phylogeny is still debated. Identification at species level is often troublesome, especially for the highly diversified *Oscarella* genus which lacks the fundamental characters of sponge taxonomy. An HPLC-DAD-ELSD-MS metabolic fingerprinting approach was developed and applied to 10 Mediterranean Homoscleromorpha species as a rapid assessment of their chemical diversity. A first validation of our approach was to measure intraspecific variability, which was found significantly lower than interspecific variability obtained between two *Oscarella* sister-species. Interspecific relationships among Homoscleromorpha species were then inferred from the alignment of their metabolic fingerprints. The resulting classification is congruent with phylogenetic trees obtained for a DNA marker (mitochondrial COI) and demonstrates the existence of two distinct groups within Homoscleromorpha. Metabolic fingerprinting proves a useful complementary tool in sponge systematics. Our case study calls for a revision of Homoscleromorpha with further phylogenetic studies and identification of additional chemical synapomorphic characters.

## The importance of floral scent in the evolutionary shift from bird to beetle pollination in *Protea*

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The majority of *Protea* species in South Africa occur in winter rainfall regions, produce large, colourful and unscented inflorescences pollinated by sunbirds and sugarbirds that feed on copious amounts of nectar, accessed by their long bills. Rodent pollination systems have been investigated in a few geoflorous species that produce yeasty scented inflorescences. In contrast, the role of insects as pollinators in this genus has been controversial. In this study, we present evidence that four *Protea* species in the summer rainfall regions of South Africa employ an insect pollination system. These species produce open bowl-shaped, colourful and fruity or sweetly scented inflorescences, most frequently visited by cetoniine beetles exploiting accessible nectar and pollen rewards. We analysed the volatile components of the scent of these and various Cape bird- and rodent-pollinated congeners using GC-MS. We investigated the source of scent production from dissected inflorescences, ontogeny of scent production and GC-EAD responses of the most common beetle visitor, *Atrichelaphinis tigrina*, to selected volatiles. The monoterpene linalool, a known attractant of cetoniine beetles, comprised more than 40% of all scent samples. Laboratory and field choice tests confirmed that *A. tigrina* shows a significant preference for the fruity scent of the flowers and volatile compounds such as linalool and methyl salicylate. We suggest that the evolution of a fruity scent has accompanied the shift from an ancestral bird pollination system to a cetoniine beetle pollination system in this clade.

## Epidermal metabolomic comparison of *Senecio jacobaea*, *Senecio aquaticus* and their hybrids

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Epidermis protects the inner-cell leaf from the external environment. Therefore, metabolome in the epidermis is important as the first barrier against abiotic and biotic factors. In this study we investigated the epidermis metabolome of *Senecio jacobaea*, *Senecio aquaticus* and their hybrids. For isolation of epidermis extracts, carborundum abrasion (CA) technique was applied. Subsequently, <sup>1</sup>H nuclear magnetic resonance (NMR) spectroscopy and multivariate data analyses were applied to compare the metabolome of the epidermal extracts with the abraded (mesophyll) extracts. Orthogonal partial least-squares-discriminant analysis (OPLS-DA) of the processed <sup>1</sup>H NMR showed a clear separation among the two different tissue extracts. The epidermal extracts contained significantly higher amounts of phenylpropanoids which were four times as much 5-O-caffeoyl quinic acid (CQA) and one and a half times as much 3-O-CQA and feruloyl quinic acid (FQA) compared to the mesophyll extracts. Both CQA and FQA are known for their inhibitory effect on herbivores and pathogens <sup>1</sup>. Also the defence compounds jacobine-type pyrrolizidine alkaloids (PAs) were identified. They were slightly increased in the mesophyll. Comparison of the epidermal extracts revealed a clear discrimination between parental species and hybrids. *S. jacobaea* contained higher amounts of proline, succinic acid and jacobine-like PAs while *S. aquaticus* contained higher amount of sucrose, fructose, and the PA senecionine. The hybrids metabolome contained compounds of both parents in intermediate amounts and no new compounds were detected. Our results emphasize the importance of studying the appropriate leaf tissue for chemical defences of herbivores and pathogens.

1) Leiss et al., Plant Physiology 150: 1567-1575

## **Tomato's best thrips shield: acylsugars, methylketones, phenolics or sesquiterpenes?**

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The tomato clade, especially its wild species, has a diverse trichomeborne arsenal proven to be effective against several classes of pests. The main resistance mechanisms are: a) Entanglement, caused by mucilaginous acylsugars and sesquiterpenes, b) Repellency/toxicity by methylketones and sesquiterpenes, and c) Immobilization, caused by the polymerization of phenolics upon gland rupture (present in cultivated tomatoes). Efforts in modern breeding have been undertaken in order to bring back into domesticated tomatoes these traits without having checked first their relative efficiencies. In an attempt to establish such ranking a set of wild species and cultivars, selected on the basis of their contrasting morphological and chemical features, will be confronted in terms of both the level of resistance to one of the most serious herbivores nowadays, the Western Flower Thrips, and the amount of biomass, as glandular trichome density (G) and exudate yield, that the plant devotes for each particular defence mechanism.

When compared throughout the set of tomatoes, G and exudate yield revealed that acylsugars and sesquiterpenes are effective deterrents to WFT only in rather large concentrations and in combination with dense trichome forests since only entanglement in between the glands and no acute toxicity effects were observed. Methylketones and rutin polymerization on the other hand showed strong comparable insecticidal activity with the lowest of G and exudate yield values. It is surprising that the so-long-pursued resistance trait is already within the domesticated germplasm.

Video footages on the interaction between WFT and key tomatoes provide complementary supporting evidence for the discussion.

**Phylogeny of twelve species of Geotrupidae (Coleoptera) inferred from analysis of cuticular compound blends and cytochrome oxidase I and 18s ribosomal DNA data sets**

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In our study, we used different approaches to generate and compare Geotrupidae phylogenies: chemotaxonomy and genetics. We analyzed the cuticular compounds for 12 species of Mediterranean Geotrupidae to create a chemical phylogeny. Moreover, we used mitochondrial and nuclear genes to elaborate molecular phylogenies. Among several compounds present in the cuticular extractions, we used 24 compounds which are interesting among the 12 species: common or specific compounds, common or specific ratio. All individuals belonging to the same species have the same chemical blend. Each species is associated with a different chemical pattern, even inside the same genus. Individuals are grouped in their respective species and also grouped in their respective genus with very low individual variability. Moreover, the most distant species are *Bolbelasmus gallicus* with an evident distinction from the others species with several compounds. Using these continuous data, we build a phylogenetic tree which isolate individuals from *B. gallicus* species and grouped Geotrupinae genus together more or less close depending on the genus or species. The maximum parsimony trees using in one hand a mitochondrial marker and in other hand a nuclear marker show the same major results: all the genus belonging to Geotrupinae subfamily are grouped in the same clade and *B. gallicus* species is isolated in another clade. These results are congruent between them and with those of classical classification and previous studies using morphological data. *B. gallicus* belongs to a specific group (*Bolbocerinae* subfamily) with a particular chemical profile and a strong genetic divergence with different markers.



## Sex pheromone of a twisted-wing parasite

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The Strepsiptera, commonly known as twisted-wing parasites, are a very small order of insects with about 600 known species, approximately 20 of them occurring in Central Europe. As endoparasites, they develop and spend the bigger part of their life time in other insects, mostly bees and wasps in Central Europe. The differences between males and females are extreme: Adult males are free-living small creatures (~ 2-6 mm) with large eyes, branched antennae, well developed hindwings and reduced forewings, resembling small flies at first glance. The females of most species never leave their hosts, and only the cephalothorax (fused head and thorax) is visible externally, neither showing eyes nor antennae. After mating, females release high numbers of free living first instar larvae through the brood canal opening in the cephalothorax, which actively search out new hosts.

Because adult males have a very short life span (usually ~ 5 h) and are quite weak fliers, there has to be an efficient way of mate finding in the Strepsiptera. This is believed to be mediated by chemical cues released by virgin females.

Here, we report the first identification and synthesis of a female produced strepsipteran sex pheromone, which proved to be highly attractive when offered in the field during the swarming period of the males.



**Poster Presentation**  
Abstracts





Poster Session  
S1. New technologies in chemical ecology



## Getting Buzzed on Sugar: Proof-of-Principle that the Brain of a Bee can be Imaged using 2-[<sup>18</sup>F]fluoro-2-deoxy-D-glucose

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Plants can synthesize thousands of compounds for defense, sexual reproduction and outcrossing. In the genus *Nicotiana*, nicotine, a powerful alkaloid, is produced in the plant's roots and delivered to the aboveground tissues where it acts as a defense toxin. Recent findings show that floral nectar can contain nicotine as well, and modify the feeding behavior of pollinators such as the honey bee, yet little is known about the effects it can have on the brain chemistry of the bee [1]. The purpose of this project was to determine whether brain activity of a bee could be imaged using 2-[<sup>18</sup>F]fluoro-2-deoxy-D-glucose (<sup>18</sup>FDG), a radioactive analog of glucose (half-life, 110 m) that was first developed at BNL in mid-70's to image sugar metabolism *in vivo* in humans using Positron Emission Tomography (PET). To render the tracer in a state suitable for insect ingestion, we developed a desalination method and tested the efficacy of using this tracer to image uptake of sugar into the brain region of a bee. In future research we will investigate the effect of nicotine additive in the feeding solution on total brain activity using <sup>18</sup>FDG uptake within the brain region as a measure of this function. This research was supported in part by the U.S. DOE, Office of Biological and Environmental Research, and in part by Deutscher Akademischer Austauschdienst.

[1] Afik, O.; Inbar, M.; Ne'eman, G.; Shafir, S.; Izhaki, I., *Nicotine in nectar: Consumption and perception by honey bees and its effect on their survival*, 2009 ISCE Meeting.

## **A new approach to studying defense-induced resource allocation and metabolic partitioning into anthocyanin biosynthesis using 2-[<sup>18</sup>F]fluoro-2-deoxy-D-glucose**

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The ability of plants to respond defensively may be facilitated or constrained by the flow of signals and/or resources between plant parts which is determined by vascular architecture. Sugars, transported between source and sink tissues, provide the necessary resources for growth of developing leaves and are hypothesized to serve as building blocks and additional energy sources for the production of carbon-based defenses in plants. Here we highlight the use of 2-[<sup>18</sup>F]fluoro-2-deoxy-D-glucose (<sup>18</sup>FDG) to measure glucose utilization in anthocyanin (AN) biosynthesis of Arabidopsis. <sup>18</sup>FDG is a radioactive analog of glucose that was first developed at Brookhaven National Laboratory in the mid-70's, and is used extensively to image sugar metabolism *in vivo* in humans by positron emission tomography. We combined autoradiography with radio HPLC analysis of tissue extracts to investigate the effects of defense induction through combined wounding and methyl jasmonate treatments, on <sup>18</sup>FDG allocation and its incorporation into AN biosynthesis of vegetative and bolted plants. Fundamental studies of this nature can further our understanding of plant vascular architecture and its role in resource allocation *in planta* sustaining growth and active defenses. This research was supported by the US Department of Energy through its Office of Biological and Environmental Research.

## Transcriptome Analysis of *Phaedon cochleariae* Using High-Throughput Sequencing

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During leaf beetle evolution, larvae of some species have developed the ability to use the secondary metabolites of their host plant to produce their own defensive compounds against predators. Up to now, a broad range of metabolites have been identified which are sequestered by the larvae. However, little is known about the molecular basis of the uptake mechanism and the sequestration process of these compounds.

Because of no available genomic data of these leaf beetles, the mRNA transcripts of *Phaedon cochleariae* larvae and adults were sequenced using the Roche-454 Genome Sequencer FLX System. The resulting 991,307 fragments and additional 4,747 Sanger sequences (ESTs) have been analyzed, assembled, and annotated to reconstruct the cDNA sequences.

Two different software tools have been applied to assemble the fragments into contiguous sequences: the newbler software (version 2.0.01.14) and PAVE – a program for assembling and viewing ESTs. The two contig sets have been compared, and the PAVE contigs were chosen for further studies, such as Gene Ontology annotation and binding motif search.

Using the PAVE transcriptome as reference, expression studies are planned. The transcripts of various tissues of *Phaedon cochleariae* are going to be sequenced using the Tag-seq method, and will be mapped onto the transcriptome. The transcript levels will be compared to identify putative transporters, as well as putative enzymatic proteins involved in the sequestration process and the *de novo* synthesis of the defensive compounds.



**Disrupting mating of the citrus leafminer with an unnatural blend.**LAPOINTE S<sup>a</sup>, STELINSKI L<sup>b</sup><sup>a</sup> USDA, ARS, Fort Pierce, FL, USA<sup>b</sup> University of Florida, Lake Alfred, FL, USA*stephen.lapointe@ars.usda.gov*

Attraction of male citrus leafminer, *Phyllocnistis citrella* (Gracillariidae), is achieved by a 3:1 blend of (Z,Z,E)-7,11,13-hexadecatrienal:(Z,Z)-7,11-hexadecadienal. In field trials, trap catch disruption was achieved (1, 2) by deployment of either pheromone component in a wax matrix (SPLAT™). Two new application methods are being developed for rapid ground application of pheromone products that include a mating disruptant (SPLAT-CLM, ISCA Technologies) and an attract-and-kill formulation (MaleEx™, Alpha Scents, Inc., Portland, OR). The first device consists of two disks connected by string loaded with SPLAT containing either (Z,Z,E)-7,11,13-hexadecatrienal or (Z,Z)-7,11-hexadecadienal. The devices were deployed using a two-dimensional multivariate design to determine the optimal rate of pheromone per unit area and degree of aggregation of the deployment devices (number of trees treated per unit area). The second device is a computer-controlled tractor-mounted

blower that delivers droplets of either SPLAT or MalEx to citrus foliage. Results of field tests of these devices will be presented.

*References cited*

1. Lapointe, S. L. et al. 2009. Journal of Chemical Ecology 35: 896-903.
2. Stelinski, L. L. et al. 2010. Journal of Applied Entomology 134: xxx-xxx (doi: 10.1111/j.1439-0418.2009.01453.x).

## **Proteomic analysis of the antennal proteins of the Moroccan locust *Dociopterus maroccanus***

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In the last years spread of locusts, particularly the Moroccan locust *Dociopterus maroccanus* (Orthoptera: Acrididae), has been observed in countries of the Mediterranean Basin. The high populations of these insects cause great losses in crops and social alarm. The development of environmentally-friendly new strategies to control these pests is required to reduce the indiscriminate use of pesticides, which are unselective and produce a considerable environmental contamination. As olfaction plays a major role in insect behaviour, a deep knowledge of the olfaction process including the proteins involved in it is required to control this pest by biorational methods. Among the proteins implicated in the reception and binding of odorant molecules, small soluble proteins such as odorant binding proteins (OBPs) and chemosensory proteins (CSPs) play an essential role. In this communication we present the first proteomic analysis of the proteins present in the antennae of males and females of *D. maroccanus*. For identification of the proteins involved in the detection of sex-specific signals, differential 2D gel electrophoresis (pH 3-10) followed by mass spectrometry (MALDI-TOF MS and nESI-ITMS/MS) were conducted on antennal extracts of both sexes. Among the more than one thousand spots detected, we have paid special attention to those located in the acidic region (4-6 pI) and of low molecular mass (14-20 KDa). After tryptic digestion, the obtained peptides have been analysed allowing the identification of several proteins associated with olfaction.

**Nonchiral and chiral GC×GC/TOFMS analysis of antennally active compounds from gut and frass extracts of bark beetles *Ips shangrila* and *Ips nitidus***

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The present study shows the applicability of two-dimensional GC with TOF-mass spectrometric detection (GC×GC/TOFMS) for enantioselective insect pheromone analysis. The insect species of our interests were two major bark beetle representatives of the genus *Ips* in coniferous forests of Tibetan Plateau (NW China) - *Ips nitidus* and newly discovered *Ips shangrila*. Both bark beetle species are serious pests of thick leaf spruce (*Picea crassifolia*) in NW China mountain areas.

Samples were obtained by SPME collection and extraction of frass (sawdust and excreta produced by bark beetles boring in bark that presumably contain pheromone) and guts (expected source of bark beetle pheromone) of *I. nitidus* and *I. shangrila*. The experiments we performed aimed to i) determine the main composition of bark beetle synusia of *Picea crassifolia*, and ii) using GC×GC/TOFMS to analyze the bark beetle gut extracts and effluvia in order to get a information about the composition and chirality of putative aggregation pheromone.

The pilot non-chiral GC×GC/TOFMS and GC-EAD analyses of extracts and collected SPME-samples gave initial information about presence of antennally active compounds in studied *Ips* species. Ipsenol, ipsdienol and *cis/trans*-verbenols were found as antennally active compounds in analyzed samples obtained from both species. Subsequent chiral GC×GC/TOFMS analyses provided us by detail information about enantiomeric composition of all studied samples from both *Ips* species. We found significant differences in enantiomeric composition of antennally active compounds produced by both species. We hope that obtained data will serve in design of pheromone based monitoring system usable in IPM of both species.

## Phytochemical Investigation of an Endemic Polynesian *Alstonia* Species

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The genus *Alstonia* (Apocynaceae) is a pantropical plant, well-known to be a rich source of original secondary metabolites including bioactive compounds. We conducted the first phytochemical investigation of *Alstonia marquisensis*- an endemic species growing in the Marquesas archipelago. Since only a very limited amount of plant material (leaf and bark) was available, HPLC-SPE-NMR together with HPLC-MS was used for the analysis of the main components: bark and leaf material was investigated for the presence of alkaloids. The identified structures belong to the akuammicine type such as alstovine [1] and vincanidine [2].

References: 1. Legseir, B., *Phytochemistry*, Vol.25, No.7, pp. 1735-1738, 1986. 2. Yagudaev, M.R., *Khimiya Prirodnykh Soedinenii*, No. 2, pp. 210-212, 1983.

## Phytochemical Investigation of *Rauvolfia sachetiae* Fosberg (Apocynaceae)

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*Rauvolfia sachetiae* Fosberg (Apocynaceae) is an endemic plant species growing in the Marquesas archipelago (Nuku Hiva and Hiva Oa), which has been used in traditional folk-medicine. The excessive removal of the bark for the preparation of macerates is one of the threats to the species. Other negative influences are the predation of seeds by rats, the overgrazing of feral cattle and the increased occurrence of invasive plants. Together with a drier climate a natural regeneration of *Rauvolfia* is not likely. Today the species is very rare on Nuku Hiva and perhaps extinct on Hiva Oa. It is now protected by law in French Polynesia and its IUCN status is CR (critical rare).

It was not clear which pharmacological principles are responsible for the use in the past. A phytochemical investigation was necessary to get information about major active compounds- factors that might support the urgent need of a future conservation program. For this purpose, we focussed on extractable bioactive ingredients of the natural bark and the macerate used by the Nuku-Hiva natives. Chemical profiles were created with modern analytical methods: HPLC-MS and HPLC-SPE-NMR. As one result, sandwicine-type compounds could be identified as main components of the alkaloidal fraction of the extracts. The first occurrence of sandwicine had been reported from Hawaiian *Rauvolfia* species (*R. sandwicensis* and *R. mauiensis*). This finding establishes a close phytochemical relationship between Hawaiian and Marquesan *Rauvolfia* species.

## Detection dog recognize pheromone from spruce bark beetle and follows it to source: a new tool from chemical ecology to forest protection

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Chemical ecology has already provided tools for monitoring, mass-trapping, and relocation of forest insects, especially in conifer bark beetles.

Here we demonstrate the use of detector dog to be effective for finding and locating spruces that has been recently infested by the European spruce bark beetle (*Ips typographus* L.), one of the most aggressive forest pests.


Attacked trees must be promptly removed (weeks) but are difficult to spot. Humans, who rely on visual cues for target identification of recent tree kills (still green), need to approach within <1 m, dogs use primarily olfactory cues and can therefore locate remote targets that are not visually obvious. The ability to search for target odour and then go to its source makes dogs ideal for rapid target recognition in field settings.

A dog was trained on synthetic pheromone on an educational platform (video) and later on trees before beetle swarming. Dog movement and detection distance data were collected during experiments with GPS. After beetle flight started, the dog showed rapid and accurate orientation to single or groups of trees attacked, often over >50 m distance (video).

We observed detection distances ranging from 0.5 m to 150 m. Attacks of different ages (day –weeks) and standing or wind-felled trees were all detected. Scents from synthetic pheromone blends or natural pheromone seemed equally detectable for the dog.

Further applications may include detection of low-level attacks of alien or recently introduced pest insects.





Poster Session  
S2. Mechanisms of intraspecific  
communication in animals





## **Pheromones and signature mixtures: defining species-wide signals and variable cues for individuality in both invertebrates and vertebrates**

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Despite being found across the whole animal kingdom, the definition of ‘pheromone’ remains controversial for some researchers (1, 2). The problem seems to be confusion between pheromones and signature mixtures (variable subsets of molecules of an animal’s chemical profile which are learnt by other animals, allowing them to distinguish individuals or social insect colonies). What are the distinguishing characteristics of pheromones and signature mixtures (≡ mosaic signals (3))? It is not the innateness of responses to pheromones, though this is common to most if not all pheromones (4). Paradoxically, nor is it the specificity of pheromone receptor proteins – male moths may not be typical of all pheromone processing. Instead, the distinguishing characteristics of signature mixtures are the combination of a requirement for learning and the variability of the cues learnt. In contrast to a species-wide pheromone, there is no single signature mixture to find, as signature mixtures are a ‘receiver-side’ phenomenon and it is the differences in signature mixtures which allow animals to distinguish each other.

All signature mixtures, and almost all pheromones, whatever the size of molecules, are detected by olfaction (as defined by receptor families and glomerular processing). There is convergence on a glomerular organization of olfaction. The processing of all signature mixtures, and most pheromones, is combinatorial across a number of glomeruli, even for some sex pheromones which appear to have ‘labeled lines’. A small minority of pheromones act directly on target tissues (allohormone pheromones) or are detected by non-glomerular chemoreceptors such as taste.

### References

1. Wyatt, TD (2009) Fifty years of pheromones. *Nature* 457: 262-263
2. Wyatt TD (2003) Pheromones and animal behaviour. Cambridge University Press
3. Johnston RE (2003) Chemical communication in rodents: from pheromones to individual recognition. *J Mammal* 84:1141-1162
4. Wyatt TD (in press) Pheromones and signature mixtures: defining species-wide signals and variable cues for individuality in both invertebrates and vertebrates. *J Comp Physiol A Sens Neural Behav Physiol*

## Monitoring the Neotropical brown stink bug *Euschistus heros* (F.) (Hemiptera: Pentatomidae) with pheromone-baited traps in soybean fields

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The effectiveness of the synthetic sex pheromone of the Neotropical brown stink bug, *Euschistus heros*, was evaluated both in laboratory and field assays. Lures loaded with 1 mg of methyl 2,6,10-trimethyltridecanoate continuously attracted female bugs for more than thirty days to pheromone-baited traps in field trials. The pheromone-baited traps were effective in field tests even at low bug population densities, as compared with the usual monitoring technique, shake cloth sampling. Traps around borders or in the centre of soybean fields caught similar numbers of bugs. Trap captures showed a positive relation with field populations, as monitored with the shake cloth technique, during the reproductive phase of the soybean crop, i.e., from the R1 to R5 developmental stage (pod formation to pod fill). The physiological state of the trapped migrating insects was determined. The first insects arriving in the field had fewer eggs in the reproductive tract compared to later arrivals. Some cross-attraction was also observed, with *Piezodorus guildinii* and *Edessa meditabunda* also being caught in pheromone-baited traps, suggesting that these insects respond to the sex pheromone or to the defensive compounds released by *E. heros* captured in traps. The results showed that traps baited with 1 mg of the sex pheromone efficiently caught bugs, that the lures lasted for more than one month under field conditions and that placement of traps around the borders of the crop area was as effective as placement inside the crop area. Border-placed traps were effective at a density of one trap every 200 meters.

**An individual and sex odour signature in kittiwakes****LECLAIRE S<sup>a</sup>, MERKLING T<sup>a</sup>, DANCHIN E<sup>a</sup>**<sup>a</sup> *Laboratoire Evolution & Diversité Biologique, Toulouse, FRANCE**sarahlecl@hotmail.com*

Black-legged kittiwakes *Rissa tridactyla* preferentially mate with genetically dissimilar individuals but the cues used to assess genetic characteristics remains unknown. In other vertebrates, olfactory cues have been identified as implicated in the advertisement of genetic compatibility. We thus hypothesized that kittiwake body odours may carry individual and sexual signatures thus reliably signalling individuals' genetic makeup. Here, we test whether odours in preen secretion and feathers of kittiwakes may provide an individual signature, by the use of gas chromatography-flame ionization detector and mass spectrometry. We first found that male and female odours differ quantitatively, suggesting that scent may be one of the multiple cues used by birds to discriminate between sexes. We further detected an individual signature in the volatile and non-volatile fractions of preen secretion and feathers. These results suggest that kittiwake body odours may broadcast the genetic makeup of individuals and might be used to assess the genetic compatibility between potential mates in mate choice.

## Effect of 20-hydroxyecdysone on contact recognition pheromones and sexual behaviour in spider, *Tegenaria atrica*

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For spiders, there are two prerequisites for the exchange of gametes: inhibition of cannibalism and attract of the partner. Both events must occur simultaneously. *Tegenaria atrica* initially defined as essentially solitary can modify their agonistic behavior in order to interact with their conspecifics during reproductive phases. Unmated female spiders attract a sexual partner with web. The attracted males come into contact with pheromone on the web, which are embedded in a specific lipid matrix. Our ethological tests showed relationship between female receptivity and levels of lipids on web and cuticle. In some arthropods ecdysteroids stimulate sex pheromone activity, but in spiders, the endocrine regulation of reproduction is not known and the role of ecdysteroids is a much neglected field of research. In *Tegenaria atrica*, our results showed that the highest levels of total circulating 20-hydroxyecdysone (20E) were detected in unmated females during the transition between the pre-vitellogenic and the early vitellogenic phases of oocyte development. In unmated females, the ovaries did not initiate vitellogenesis, the frequency of sexual receptivity was low and cannibalism of male was observable. Our results showed that injection of 20E in unmated female induces a vitellogenesis process and variation of female cannibalism parallels sexual receptivity, and could be related to changes in web and cuticular compounds. We showed that 20E altered the production of contact sex polar compound levels (methylesters and fatty acids). Our results delineate the compounds involved in female sexual attractivity and receptivity in *Tegenaria atrica*.

## Identification of the two sex pheromone components of sugarcane borer, *Diatraea flavipennella* (Lepidoptera: Crambidae)

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GC-EAD and two-dimensional gas chromatography–time-of-flight mass spectrometric (GC×GC-TOFMS) analysis of pheromone gland extracts of sugarcane borer, *Diatraea flavipennella*, revealed two antennally active compounds identified as (Z)-9-hexadecenal (Z9-16:Ald) and (Z)-11-hexadecenal (Z11-16:Ald). (Z)-11-hexadecenal comprises 75% of the secretion whereas (Z)-9-hexadecenal was found to be the second most abundant component (25%). GC-EAD experiments with synthetic compounds confirmed antennal activity of the identified hexadecenal isomers. (Z)-11-hexadecenal elicited the highest EAD response and therefore was tentatively considered as major pheromone component. Trace amounts of tetradecanal (14:Ald), hexadecanal (16:Ald), (Z)-9-hexadecen-1-ol (Z9-16:OH) and (Z)-11-hexadecen-1-ol (Z11-16:OH) were also found in the extract, however these compounds did not show any antennal activity. Laboratory-based bioassays showed that the binary blend composed of (Z11-16:Ald), and (Z9-16:Ald) in a ratio of 75:25 elicited in *D. flavipennella* virgin males a response similar to that observed to virgin females and to hexane extracts of pheromone glands, therefore this mixture constitutes the sex pheromone of this species.

**First identification of termite trail pheromones by using GC-EAD**

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Gas chromatography coupled with electroantennographic detection (GC-EAD) gave successful results by identifying insect pheromones. This technique has been used for the first time to identify the trail pheromone of two termite species, *Psammotermes hybostoma* (Rhinotermitidae, Psammotermitinae) and *Prorhinotermes simplex* (Rhinotermitidae, Prorhinotermitinae). (3Z,6Z,8E)-Dodeca-3,6,8-trien-1-ol (hereinafter referred to as dodecatrienol) is the only component of the trail pheromone of *P. hybostoma*, whereas it is a quantitatively minor component but a qualitatively important component of the trail pheromone of *P. simplex*. Neocembrene is the major component of the two-component trail pheromone of *P. simplex*. Trail-following bioassays and GC-MS analysis confirmed the nature of the trail pheromone of both species, except that dodecatrienol could not be detected in sternal gland extracts of *P. simplex* by GC-MS analysis. These original results underline once again the special phylogenetic status of *Prorhinotermes* among Rhinotermitidae since all other Rhinotermitidae species studied, including *Psammotermes*, have only dodecatrienol as trail pheromone.

**Female pheromonal chorusing and aggregation in an arctiid moth,  
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A unique intrasexual communal sexual display and attraction mediated by female sex advertisement pheromone is found in *Utetheisa ornatrix* (Lepidoptera: Arctiidae). The female adjust the temporal aspects of their calling behavior in response to the long-distance pheromone released from neighboring conspecific females during a nightly activity period. The perception of the pheromone is directly responsible for the induction and adjustment of calling behavior of the receiver females. Intriguingly, some females even initiate calling during photophase in response to the pheromone and its individual components. They also orient toward the higher concentrations of the pheromone with a sensitivity and reaction intensity equivalent to that exhibited by males in the laboratory. However, contrary to the predictions that rhythmic abdominal pumping serves to increase the peak pheromone release rate and heighten response in perceiving females, *U. ornatrix* females do not respond more strongly to the pulsed pheromone stimulus than to the constant release of pheromone at the same average rate. This behavior differing from the usual form of sexual communication in moths may be influenced by mating system of this species. At mating, the male *U. ornatrix* transfer a large spermatophore that may enhance female reproductive success. This may extend post-mating male refractory period, which bias the operational sex ratio. The biased OSR would generate intrasexual competition among females in which they respond by elevating signaling effort. Females can also benefit from aggregation, which creates a concentrated pheromone plume that attracts many males and higher opportunities for remating and multiple spermatophores.



**Nasvi-EH1 – an epoxide hydrolase involved in the biosynthesis of the male sex attractant in the jewel wasp *Nasonia vitripennis*****RUTHER J<sup>a</sup>, KOCH M<sup>b</sup>, GARBE LA<sup>c</sup>, ABDEL-LATIEF M<sup>d</sup>**<sup>a</sup> *University of Regensburg, Regensburg, GERMANY*<sup>b</sup> *University of Bonn, Bonn, GERMANY*<sup>c</sup> *Technical University of Berlin, Berlin, GERMANY*<sup>d</sup> *Free University of Berlin, Berlin, GERMANY**joachim.ruther@biologie.uni-regensburg.de*

Epoxide hydrolases (EH) are enzymes occurring in virtually any living organism. They catalyze the hydrolysis of epoxide containing lipids and are involved in crucial mechanisms like the detoxification of xenobiotics or the regulation of inflammation and blood pressure. We describe an EH gene involved in the biosynthesis of a sex attractant in *Nasonia vitripennis*. Males of this parasitic wasp have been shown to release a mixture of (4*R*,5*R*)- and (4*R*,5*S*)-5-hydroxy-4-decanolides (HDL) to attract virgin females [1]. By using stable isotope labeling techniques, we have demonstrated that vernolic acid (= *erythro*-12,13-epoxy-octadec-9*Z*-enoic acid) is incorporated by *N. vitripennis* males into the HDL molecule. This suggests the involvement of an EH hydrolyzing the fatty acid epoxide into the respective diol, which might be further processed by chain shortening and lactonization to HDL. We have cloned a putative *N. vitripennis* EH gene (*Nasvi-EH1*) and localized its transcripts in the male rectal papillae by *in situ* RT-PCR. Involvement of *Nasvi-EH1* in HDL biosynthesis was established by RNAi-mediated gene silencing. Injection of *Nasvi-EH1* dsRNA into the male abdomen inhibited pheromone biosynthesis and suppressed the targeted gene transcripts in the rectal vesicle [2].

*References cited:*

1. Ruther, J. et al. 2007. J. Exp. Biol. 210: 2163-2169.
2. Abdel-latif, M et al. 2008. Proc. Natl. Acad. Sci. USA 105: 8914-8919.

## **A signal emitted by females promotes activation and orientation in *Rhodnius prolixus* males**

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Chagas disease is caused by *Trypanosoma cruzi* and affects humans. *Rhodnius prolixus* (Hemiptera: Reduviidae) is its main vector in Northern South America and Central America. A pheromone emitted during copula was reported for this species. Adult triatomines possess metasternal glands, whose volatile products were identified and reported to be released by females at night. Thus, our study investigates whether a relationship between locomotory activity and female emitted volatile signals exists. We used a shelter based bioassay to characterize the activation pattern and locomotory activity of groups of males or females in the presence of potential chemical signals emitted by opposite sex conspecifics. Further, we used a dual-choice walking compensator bioassay to investigate whether female or male *R. prolixus* orientates in response to adult emitted odors. Finally, we characterized the source of compounds that promoted orientation responses in this context. Males left their shelters significantly more frequently when groups of females were present outside the arena. Besides, copulation attempts between males increased significantly under the same condition. Additionally, males confronted with a clean air current versus an air current carrying female odors oriented significantly in the latter direction. Females did not show orientation when confronted with the different stimuli tested. Finally, females with occluded metasternal glands did not promote significantly oriented trajectories by males. Our results suggest that a chemical signal is emitted by female metasternal glands and, that this signal promotes the activation of sheltered *R. prolixus* males and their subsequent orientation when the volatiles are carried by air currents.

**Elongases involved in male pheromone biosynthesis in *Drosophila*****BONTONOU G<sup>a</sup>, DENIS B<sup>a</sup>, WICKER-THOMAS C<sup>a</sup>**<sup>a</sup> CNRS - LEGS - UPR9034, Gif sur Yvette, FRANCE*wicker@legs.cnrs-gif.fr*

*Drosophila* pheromones are long-chain hydrocarbons secreted by the epidermis. They act by contact or at short distances. *Drosophila melanogaster* male produces two main pheromones, 7-tricosene (7-T) and 7-pentacosene (7-P). There is a geographical polymorphism with male hydrocarbons: African males display a majority of 7-P, whereas males from other countries (cosmopolitan males) display a majority of 7-T. This difference is hypothesized to be due to a difference in the regulation of an elongase involved in 7-P biosynthesis.

There are twenty elongases in the *Drosophila* genome. The function of these elongases on male pheromone biosynthesis will be analysed by their inactivation through RNAi. We present some data concerning pheromone analysis of RNAi males. In parallel, real-time PCR has been performed on males from two populations, Tai and Canton-S, characterized by high levels of 7-P and 7-T, respectively. These experiments may help to characterize the elongase(s) involved in 7-T and 7-P biosynthesis.

**Potential peripheral modulation of olfactory processing by ecdysteroids in males of the Egyptian cotton leafworm, *Spodoptera littoralis***

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The physiological and behavioural plasticity presents a critical importance to permit adaptation of animals to the modification of environmental parameters and internal factors as e.g. mating status or age. In vertebrates, the olfactory sensitivity for odorant molecules is clearly modulated by as steroids which act on peripheral or central olfactory processing. In insects, the physiological state is a major intrinsic factor modulating adult behaviour in response of different stimuli such as pheromones, food or host plant. However, although a peripheral modulation by the physiological state was previously suggested, a lack of information concerning the influence of hormones on the peripheral olfactory processing. The aim of our work is to demonstrate that the peripheral olfactory processing as for e.g. sex pheromone could be regulated by ecdysteroids. After a bioinformatic analysis of a *S. littoralis* male antennal expressed sequence tag (EST) library, we first realized a molecular characterization of *S. littoralis* EcR, USP and E75 cDNAs by RT-PCR strategy. The expression patterns of these genes were then determined in different tissues by RT-PCR and *in situ* hybridization in olfactory *sensillae* of adult male antennae. Finally, injections of the well-known most active form of ecdysteroid, 20-hydroxyecdysone or 20E, were realized in order to confirm inducibility of the ecdysteroid signalling pathway in antennae and its impact on several peripheral actors of the olfactory processing. The results of our study suggested a modulation of the peripheral olfactory processing by ecdysteroids in a potential modification of the physiological state in the moth *S. littoralis*.

## **Binding specificity of recombinant Odorant-Binding Protein isoforms is governed by phosphorylation**

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Native porcine OBP bears eleven sites of phosphorylation, not always occupied in the molecular population, suggesting that different isoforms could co-exist in animal tissues. As phosphorylation is dynamic and result in temporary conformational changes that regulate the function of target proteins, we have investigated the possibility that OBP isoforms could display different binding affinities to biologically relevant ligands. The availability of recombinant proteins is of particular interest to study protein/ligand structure-function relationships, but prokaryotic systems are unable to perform post-translational modifications. To investigate the role of phosphorylation in the binding capacities of OBP isoforms, we have produced recombinant porcine OBP in two eukaryotic systems, the yeast *Pichia pastoris* and the CHO cell line. Isoforms were separated by anion exchange HPLC, and their phosphorylation sites were mapped and compared to those of the native protein. Binding experiments with ligands of biological relevance in the porcine species were conducted by fluorescence spectroscopy on two isoforms of recombinant OBP expressed in the yeast. The two isoforms, differing only by their phosphorylation pattern, displayed different binding properties, suggesting that binding specificity is governed by phosphorylation.

## Sex differences in the volatile composition of the Moroccan locust *Dociostaurus maroccanus* (Orthoptera: Acrididae): Identification and electrophysiological responses

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The Moroccan locust, *Dociostaurus maroccanus* (Orthoptera: Acrididae) is an important pest of crops and pastures with a high economic impact in many countries of the Mediterranean Basin, including the South of France and the Middle East. The lack of biorational methods and the certainty that chemical signals play an important role in the species-specific communication of Acrididae both in nymphs and adults in solitary and gregarious phase, has led us to investigate for the first time the possible presence of pheromones and their role in the chemical communication of *D. maroccanus*.

To determine possible sources of pheromones, volatiles of males and females were collected and extracts of egg-froth and eggs were prepared and analyzed by GC-MS (EI and CI) and GC-EAD. Our results indicate that qualitative differences exist between male and female volatile compounds from which five (n-tetradecanol, hexadecanal, 1-hexadecanol, 2-octadecanone and one unknown) elicited significant responses in female antennae and one of them (structurally unknown so far) also in male antennae. Both sexes displayed dose-dependent EAG responses to the known pheromone compounds phenylacetone nitrile, acetophenone and veratrole of other acridid species, such as *Schistocerca gregaria* and *Locusta migratoria*.

## Utilization of plants as a source of medicines for behavioral nutrition and physical activity in the Chittagong Hill Tracts of Bangladesh

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Indigenous peoples are often considered potential allies in the conservation of biological diversity. The present paper deals with the field observations recorded on the traditional indigenous therapeutic applications of the plants used by the inhabitants of the Chittagong Hill Tracts in Bangladesh. These inhabitants have been dependent on the ambient plant resources for food, fuel, fiber, timber, household articles, and medicines to a great extent for ages. Information was collected by interviewing native people, mainly elderly: engaged in farming & stock-raising activities, and housewives. The plants collected, indicated by the locals, have been identified according to "Flora of Bangladesh". The exsiccata vouchers are preserved in the Bangladesh National Herbarium. The collected information indicates that the following plants are used to treat behavioral nutrition and physical activity: *Olea europaea*, *Nigella sativa*, *Camellia sinensis*, *Piper nigrum*, *Ocimum gratissimum*, *Saccharum officinarum*, *Cocos nucifera*, *Plantago ovata*, *Brassica napus*, *Datura metel*, *Azadirachta indica*, *Bacopa monnieri*, *Abrus precatorius*, *Citrus acida*, *Cicer arietinum*, *Ipomoea aquatica*, *Aegle marmelos*, *Daucus carota*, *Curcuma longa*, *Musa sapientum*, *Carica papaya*, *Withania somnifera*, and *Euphorbia hirta*. The inhabitants of the Chittagong Hill Tracts in Bangladesh mostly does not have access to primary medical facilities; the above-plants can form the basis of treatment for behavioral nutrition and physical activity without resorting to costly urban visits or allopathic medical practitioners. Information on indigenous use of plants has led to discovery of many medicines in use today. Scientific studies conducted on the plants may lead to discovery of more effective drugs than in use at present.

**Olfactory responses of male and female Mozambique tilapia (*Oreochromis mossambicus*, Peters 1852) to a putative urinary pheromone that signals male dominance**

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The Mozambique tilapia is a lek-breeding cichlid wherein males establish dominance hierarchies and visiting females mate preferentially with dominant territorial males. Dominant males increase urination frequency during courtship and aggression (1). A sulphated amino-sterol urinary odorant for females has been suggested to act as pheromonal signal of dominance, thereby influencing female spawning (1). Here, we address whether both sexes detect the same odorants in the urine of males of different social rank by recording of the electro-olfactogram (EOG) in response to HPLC fractions of male urine.

Urine volume collected from dominant males was larger than that collected from subordinate males and positively correlated with relative bladder tissue weight. The olfactory system of both sexes was more sensitive to dominant male urine and corresponding C18 solid phase extracts than to those of subordinates. HPLC of these extracts revealed a well retained peak on a Evaporative Light Scattering detector with significantly larger relative area in dominant than in subordinate males. The HPLC fractions containing this peak, assumed to be the amino-sterol odorant, evoked concentration-dependent EOG amplitudes in both sexes. In contrast, the EOG amplitudes elicited in both sexes by another HPLC fraction containing non-retained peaks were not significantly different among males of different social rank. The results suggest that an increase of the amino-sterol urinary odorant signals dominance to both sexes in a blend comprising another not yet identified urinary odorant(s).

*References cited*

1. Barata, E.N. et al. 2008. *Journal of Chemical Ecology* 34:438–449.

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## Maturation studies in nymphs grouped with adults of the desert locust *Schistocerca gregaria* (Orthoptera: Acrididae): Analysis of morphological and chemical markers

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Previous studies had demonstrated delayed maturation in immature adults of the gregarious desert locust *Schistocerca gregaria* (Orthoptera: Acrididae) grouped with conspecific nymphs in the presence of visual, tactile and chemical stimuli<sup>1</sup>, while maturation was accelerated in the young adults grouped with mature adult males<sup>2</sup>. These studies also demonstrated that volatiles, some of them described as the major pheromonal compounds<sup>3</sup>, were involved in the different maturation effects. Despite this knowledge, the effect of grouping nymphs with immature adults on the maturation of nymphs in the presence of the full spectrum of these cues is unknown. We investigated this grouping effect and assessed maturation in nymphs regarding: (a) body weight, (b) body length, (c) formation of wings, (d) developmental time, (e) titer of the pheromone phenylacetoneitrile in adult males, and (f) number of egg pods laid by adult females. Nymphs grouped with adults displayed accelerated maturation compared to those grouped without adults with respect to all the parameters monitored. These results confirm a previous finding of synchronous maturation in the gregarious locust, that is, a delayed maturation in immature adults, with accelerated maturation in nymphs in a mixed population of nymphs and immature adults. These results also have important implications for understanding gregarization and swarm formation in the desert locust.

### *References cited*

1. Assad, Y.O.H. et al. 1997. *Journal of Chemical Ecology* 23:1373-1388.
2. Mahamat, H. et al. 1993. *Chemoecology* 4:159-164
3. Torto, B. et al. 1994. *Journal of Chemical Ecology* 20:1749-1762

**Age-dependent olfactory responses in *Apis mellifera carnica* Pollm workers treated with synthetic queen mandibular pheromone****BLAZYTE-CERESKIENE L<sup>a</sup>, VAITKEVICIENE G<sup>a</sup>, APSEGAITE V<sup>a</sup>, BUDA V<sup>a</sup>**

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In order to reveal the role of the queen mandibular pheromone (QMP) (Bee Boost, PheroTech Inc., Canada) in the ontogeny of the queen pheromone perception by a honeybee workers, the olfactory receptor (EAG) and conditioned proboscis extension responses were recorded in QMP-treated (QMP<sup>+</sup>) and untreated (QMP<sup>-</sup>) *Apis mellifera carnica* workers of different age (0 – 10-day old). Ethanol queen extract (10<sup>-3</sup> Qeq) was used for testing.

Significant increase in olfactory learning during the first 10 days of adult life in QMP<sup>+</sup> workers but no in those QMP<sup>-</sup> was revealed. Difference in the levels of conditioned response of QMP<sup>+</sup> and QMP<sup>-</sup> workers was statistically significant in both 8- and 10-day old adults. EAG responses increased both in QMP<sup>+</sup> and QMP<sup>-</sup> workers during the 10 days period, and no significant differences were recorded.

These results suggest that increase in olfactory learning performance was caused by the modulator effect of queen mandibular pheromone on CNS of the worker's olfactory system. The effect of synthetic QMP on the learning performance of worker bees is not identical to that produced by an alive mated queen: the highest learning performance in queenright colonies was observed already in 3-day old workers (1), whereas in QMP<sup>+</sup> colonies – in 8-day old workers only.

*References cited*

1. SKIRKEVICIUS A., BLAZYTE-CERESKIENE L. 2009. *Biologija* 55: 125-132.

## Sex specific differences in perception of the pheromone in *Anastrepha fraterculus*

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Males of the South American fruit fly, *Anastrepha fraterculus*, typically form leks to attract females by releasing a multicomponent volatile pheromone. Previous pheromone analyses identified two monoterpenes limonene and  $\beta$ -ocimene, three isomers of the sesquiterpene  $\alpha$ -farnesene, the sesquiterpenes  $\beta$ -bisabolene and  $\alpha$ -bergamotene, benzoic acid, nonanal, nine-carbon alcohols, the lactones anastrephin, epianastrephin and suspensolide, and four alkyl pyrazines<sup>1,2</sup>.

The present study reports the antennal activities of pheromone components, the sex specific differences in pheromone perception, and age and circadian - dependent pheromone production. Laboratory-reared flies, supplied by FAO/IAEA, Seilsberdoff, Austria, were used for collection of volatiles released by calling males. The compounds were trapped on SuperQ, hexane eluted, analyzed by gas chromatography with electronantennographic detection (GC-EAD on WAX and DB-1 columns), and two dimensional gas chromatography with TOF mass spectrometric detection (GC $\times$ GC/TOF MS). Antennal activities were found for 6 components, namely (Z)-3-nonenol, (Z,Z)-3,6-nonadien-1-ol, geranyl acetone, (E,E)- $\alpha$ -farnesene, suspensolide and epianastrephin. Sex specific differences in pheromone perception were observed, females were more sensitive to nine-carbon alcohols than males, sensitivity to other EAD active compounds was equal for both sexes. Age and circadian - dependent pheromone production – is now being investigated.

1. Cáceres, C. et al. 2009. Biological Journal of the Linnean Society 97:152-165.
2. Lima, I. S. et al. 2001. Journal of the Brazilian Chemica Society 12:196-201.

## **A sex pheromone triggers courtship behaviour in the egg parasitoid *Trissolcus brochymenae***

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The male of the egg parasitoid *Trissolcus brochymenae* exploits a short range pheromone for recognition of the other gender. To evaluate the role of this pheromone, the behavior of virgin males was studied in closed arena when exposed to: virgin females (alive, dead “washed” with solvents, “unwashed”); dissected and re-assembled virgin females (females dissected into head, mesosoma, and gaster, then reassembled using only one “unwashed” part); “washed” females treated with acetone extracts (of virgin females or legs). The chemicals have short time activity as females elicited a stronger male antennation and mounted only when they were 1-2 days old. Males antennated and mounted virgin “washed” females with lower frequency and duration compared to dead “unwashed” females. Stimuli from females with only the mesosoma “unwashed”, including legs and wings, induced more intense male response in terms of antennation, mounting and penis extrusion, compared to stimuli from females assembled with “unwashed” head or gastro, indicating that this region is the site of production and/or release of the sex pheromone. The males antennated intensely and mounted the dead females treated with extracts of virgin females. The chemical analysis of acetone extracts of virgin males and females showed qualitative differences. The polar fractions of the extracts triggered male antennation, but only those with intermediate polarity were able to elicit male mount. The present study shows that the first signal that triggers the courtship behaviour in *T. brochymenae* males is a female-derived sex pheromone acting at- a short distance.

## Biological role of a compound produced by *Acanthoscelides obtectus* males

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The dry bean weevil, *Acanthoscelides obtectus* (Say), is one of the most important pests of beans (*Phaseolus vulgaris* L.). Economic damage caused by larvae developing inside beans is extremely important in countries where seeds of Leguminosae represent a fundamental food resource.

The control of this stored product pest is based on chemical, physical and cultural methods. Novel control methods based on semiochemicals may provide an interesting alternative. The sex pheromone, that is produced by the males, was identified in 1970 as methyl (E)-2,4,5-tetradecatrienoate. However, this compound is not currently used for pest management, mainly owing to the difficulty of its synthesis and instability.

Here we show that males produce another compound that was identified as octadecanal. Octadecanal elicited significant attraction of female weevils in two different bioassays although attractiveness was less than that of the natural male extract. Fractionation of the male extract suggested that octadecanal is a synergist for methyl (E)-2,4,5-tetradecatrienoate as attraction to the extract was reduced when octadecanal was removed.

**Disturbance of the chemical communication by non-lethal dose of spinosad in *Drosophila melanogaster***

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In a previous study we have evidenced the effects of spinosad on the *Blatella germanica* behaviour (Habbachi et al. 2009 a & b). In the present work, we have extended this study to the standard biological model, *Drosophila melanogaster*. We observed that the application of a sub-lethal dose of spinosad on 3rd instar larvae disturb the sexual behaviour of the resulting adults.

Habbachi, W., Bensafi, H., Adjami, Y., Ouakid, M.L. & Everaerts, C. 2009a. Spinosad effects on chemically mediated behaviours in *Blatella germanica*. ISCE 25th annual meeting (Neuchâtel, CH, 23-28/8)

Habbachi, W., Bensafi H., Adjami Y., Ouakid M.L., Farine, J.P. & Everaerts, C. 2009b. Spinosad affects chemical communication in the German cockroach, *Blattella germanica* (L). *J. Chem. Ecol.* 35: 1423-1426.

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**Female sex pheromone of *Matsumuraeses phaseoli* (Lepidoptera: Tortricidae) and comparison with that of *M. falcana***JUNG JK<sup>a</sup>, SEO BY<sup>a</sup>, CHO JR<sup>b</sup>, PARK CG<sup>b</sup>, YUM KH<sup>b</sup><sup>a</sup> National Institute of Crop Science, Suwon, KOREA<sup>b</sup> National Academy of Agricultural Science, Suwon, KOREA*jungjk@korea.kr*

*Matsumuraeses phaseoli* and *M. falcana* are not discriminated with external morphology each other. A few differences have been observed in genitalia morphology and in the mt-COI DNA sequences between the two species. Sex pheromone composition of *M. falcana* has been reported previously (1). In this study, identification for the sex pheromone of *M. phaseoli* was conducted to develop a monitoring tool. Adult mating was observed during the scotophase in a laboratory condition. GC and GC-MS analyses revealed that the extract of female abdominal tips contained three candidate compounds, (*E*)-8-dodecenyl acetate (E8-12Ac), (*E,E*)-8,10-dodecadienyl acetate (E8E10-12Ac) and (*E,Z*)-7,9-dodecadienyl acetate (E7Z9-12Ac) at a ratio of 1:7:1. In GC-EAD analyses, male antennae responded to E8-12Ac and E8E10-12Ac, while those antennae didn't respond to E7Z9-12Ac. EAG tests also provided similar results to those of GC-EAD. Final composition was determined with field-trapping tests. Male genitalia morphology was used for species-identification, because *M. falcana* males were caught in the traps together. The three individual candidates didn't show any attractiveness to males of *M. phaseoli*, while a binary mixture of E8-12Ac and E8E10-12Ac showed a significantly higher attractiveness. When E7Z9-12Ac was added to the mixture, the highest attractiveness was resulted. Finally, field-tests with several blends of three components determined that the 1:7:2 composition of E8-12Ac, E8E10-12Ac and E7Z9-12Ac was the sex pheromone of *M. phaseoli*. However, there remained a problem that the sex pheromone lure for *M. phaseoli* still attracted *M. falcana* together.

Cho, J.-R. et al. 2007. J. Asia-Pacific Entomol. 10: 345-349.

## Comparison study of volatiles produced by calling males of *Ceratitis capitata* from different populations

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*Ceratitis capitata* is one of the most worldspread members from the fruit fly family (Tephritidae). There have been numerous SIT (Sterile Insect Techniques) programs running all around the world to control natural populations of this pest. In these programs, males originating from mass produced laboratory colony are sterilized by irradiation and released into the field. It is supposed that sterile males which mate with wild females generate no progeny. The efficiency of these programs is based on the assumption that laboratory males reared on artificial diet are able to compete with wild males. In fruit flies, mating behavior is quite complex and involves chemical, visual and acoustic communication<sup>1</sup>. Chemical communication is based on sex pheromone released by males to attract females<sup>2</sup>. In order to provide adequate studies concerning compatibility or incompatibility of laboratory and wild populations of *C. capitata*, we analyzed and compared the chemical composition of volatiles released by calling males of one laboratory and two wild populations of *C. capitata* using GC×GC/TOFMS technique and Principal Component Analyses (PCA). Our results indicate that there is a strong correlation between laboratory and wild populations of *C. capitata* studied, suggesting that males from these populations use the same chemical language to attract females. These results are of great significance for the successful implementation of the SIT program.

1. Vera, M. T. et al. 2003. Annals of the Entomological Society of America 96:567-570.

2. Goncalves, G. B. et al. 2006. Florida Entomologist 89:375-379.



## De novo biosynthesis of components of the male sexual marking pheromone of bumblebees by means of *in vitro* incubation with [1,2-<sup>14</sup>C]acetate

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Components of the sexual marking pheromone of the bumblebee males (*Bombus lucorum* and *Bombus terrestris*) are produced and stored in the cephalic part of the labial gland (1). Both species differ in the composition of the labial gland secretions. *B. lucorum* males produce aliphatic compounds with long carbon chain (ethyl (Z)-tetradec-9-enoate, 53%) (2). Labial gland of *B. terrestris* males contains mixture of terpenic and aliphatic compounds (3) where 2,3-dihydrofarnesol and ethyl dodecanoate are the most abundant. It was proposed (4) that fatty acids are precursors of aliphatic components of the labial gland. Their source can be triacylglycerols from the fat body or they can be synthesized *de novo* in labial gland.

Labial glands and fat bodies were incubated *in vitro* with radioactive [1,2-<sup>14</sup>C]acetate. Incubation conditions were optimized by changing pH, non-labelled acetate addition, incubation time and temperature. After incubation, the gland extracts were analysed by means of thin layer chromatography with non-destructive detection of <sup>14</sup>C-distribution by position sensitive detector. Analysis showed incorporation of the [1,2-<sup>14</sup>C]acetate into various kind of compounds such as fatty acids, triacylglycerols, esters and hydrocarbons. Our results indicate that the labial gland and fat body are able to produce fatty acids *de novo* in both species.

Financial support by the Czech Science Foundation (#203/09/1446).

### References cited

1. Kullenberg, G. et al. 1973. Zoon Suppl. 1:25.
2. Urbanová, K. et al. 2001. European Journal of Entomology 98:111-115.
3. Kullenberg, G. et al. 1970. Acta Chemica Scandinavica 24:1481.
4. Luxová, A. et al. 2003. Chemoecology 13:81-87.

## **Mating behavior of *Aphomia sociella* – evidence for close range female sex pheromone that triggers ultrasound production in males**

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Bumble bee wax moth, *Aphomia sociella* L., is an important parasite of bumblebees. The larvae destroy the host nest by consuming the brood combs, the food stores and the offspring. Unlike most Lepidoptera, reproductive behavior of *A. sociella* and other members of the subfamily Galleriinae (family Pyralidae) is initiated by males and includes quite variable combinations of pheromone and ultrasonic signals. Seven pheromone component candidates e.g. 1-hexanol, 2-phenylethanol, [(R),(Z)]-nona-2,6-dien-4-olide, [(S),(Z)]-nona-6-en-4-olide, mellein and phytone were identified in male wing pheromone glands and in emanations released by calling males<sup>1</sup>. Behavioral experiments revealed that in *A. sociella* male sex pheromone mediates long range attraction, while ultrasound is involved in close range courtship and male competitions.

Observation of mating behavior indicates that male ultrasound signaling is triggered only when other conspecifics are nearby. We will report evidence of the existence of female sex pheromone. The pheromone is produced by so far unidentified gland(s) on female body and triggers ultrasound production when perceived by a male. The chemical identity of the female pheromone will be presented using data obtained from GCxGC-MS, GC-EAD, EAG and behavioral analysis.

Financial support by the Ministry of Education of the Czech Republic (#2B06007) is gratefully acknowledged.

### References

1. Journal of Natural Products 72:8-13.

### **Long range attraction and short range preferences in *Drosophila melanogaster* males**

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In its natural environment, the fruit fly *Drosophila melanogaster* aggregates on decaying fruits. The flies are attracted by chemical compounds, products of the fermentation process, which indicate the presence of yeasts, their main nutritional resources. These fruits are thus used as a food source for both males and females. They also serve as an oviposition substrate for mated females and copulations occur in the same place. In order to find a mate, a male should therefore locate fruits on which other flies, and especially females, are present. However, it remains unclear whether flies are able to distinguish between fruits already colonized by conspecifics from other fruits at long ranges or shorter distances.

In a series of experiments, combining a Y-tube olfactometer with a wind tunnel, we investigated the different cues involved in long and short range attraction of *Drosophila* males.

Long range attraction is mainly mediated by food odours rather than by the presence of other flies. However, at shorter distances, males can perceive the presence of conspecifics and exhibit a higher preference towards such food sources. In this case, chemical compounds produced by flies as well as the presence of the flies themselves play a role in the attraction of other males. Both food and fly odours, acting successively during the searching period, are therefore important for the habitat choice of *Drosophila melanogaster* males.

## Coding and interaction of sex pheromone and plant volatile signals in the antennal lobe of the codling moth *Cydia pomonella*

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The codling moth *Cydia pomonella*, a worldwide pest of pome fruit, has long been a model for the development of behaviour-modifying chemicals for sustainable insect control. Studies of the behavioural and chemical ecology of this species emphasize the close evolutionary relationship in the detection and communication with sex pheromones and host plant chemicals. To improve our understanding of how information from social and environmental cues is processed, we performed an anatomical and functional study of the antennal lobe (AL), the primary olfactory centre, of *C. pomonella*. A three-dimensional reconstruction of the glomerular structure of the AL led to the identification of  $50 \pm 2$  and  $49 \pm 2$  glomeruli in males and females, respectively. By means of intracellular recordings and staining technique, we physiologically characterized ca. 50 interneurons in each sex, revealing complex patterns of activation and a wide variation in response dynamics to the tested compounds (13 plants volatiles, 4 pheromone components). Stimulation with single chemicals and their 2-component blends produced both synergistic and inhibitory interactions in PNs innervating ordinary glomeruli and the macroglomerular complex. Our results demonstrate that social and plant odours in the codling moth are seemingly processed in across-glomerular coding patterns, suggesting a more complex level of interaction between the two olfactory subsystems than previously observed in other species. These findings have consequences for our understanding of the reproductive behaviour of codling moth and the design of sustainable control methods.

## **Amniotic fluid is important for the maintenance of maternal responsiveness and the establishment of maternal selectivity in sheep.**

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Amniotic fluid (AF) is important for the establishment of maternal behaviour in inexperienced ewes, but its role in experienced mothers remains to be studied. Here, the maintenance of postpartum maternal responsiveness and the establishment of exclusive bonding was investigated in multiparous ewes when AF was removed from the neonate or/and physical contact with the young was precluded for the first 4 h postpartum. Maintenance of maternal responsiveness and establishment of exclusive bonding were measured by the proportion of mothers accepting their own lamb and alien lambs that had been either washed or not washed, and by comparing an acceptance score for each type of lamb. Washing the neonate reduced its acceptance score, but the proportion of mothers rejecting their own lamb was reduced only when washing the neonate and prevention of physical contact for 4 h were combined (7/15 vs 0/10 in controls,  $P = 0.02$ ). In addition, washing the neonate increased the acceptance score of the washed alien lamb, but not of the unwashed alien. However, washing and privation of physical contact did not increase significantly the proportion of mothers accepting an alien lamb at 4h postpartum. We conclude that AF is important in experienced ewes for the establishment of maternal responsiveness, as already found in primiparous mothers. In addition, our results indicate that AF carries also some chemosensory information facilitating exclusive bonding.

## **Towards to sex pheromone identification of *Diatraea indigenella* Dyar & Heinrich 1927 (Lepidoptera: Crambidae)**

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Behavioral aspects such as calling start time as well as length and calling bouts of *Diatraea indigenella* Dyar & Heinrich 1927 (Lepidoptera: Crambidae) were evaluated during seven scotophases. Calling behavior was observed since the emergence of the females with a decrease in calling bouts and length after the sixth scotophase. The highest percentage of calling females was verified passed six hours after the lights off. The main sex pheromone component was identified from extracts of female glands, using GC-EAD and GC-MS. The major component, identified as (Z,E)-9,11-hexadecadienal, and two other unidentified minor components elicited antennal responses. This major component concentration varied from 2.53 to 13.7 ng gland<sup>1</sup>, and the highest concentration was detected at the sixth hour of the scotophase. Although we could estimate the retention times of the other two active components present in extracts due to the EAD response, their chemical structures could not be confirmed because of their low concentration in the extracts. Olfactometer bioassays showed that gland extracts and the synthetic major component were able to attract 86% and 68% of males, respectively, when tested individually against hexane. Significant attraction (77%) was also observed when the gland extract was tested against the synthetic major component. Our results corroborate the literature with respect to the importance of minor components in male attractiveness. The identification of the major sex pheromone component carried out in the present study represents an important step towards the complete elucidation related to the composition of the sex pheromone of *D. indigenella*.

**Characterization of sexual behavior, volatile collections, and attempts to identify semiochemicals in *Callisphyris apicicornis* (Coleoptera: Cerambycidae)**

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We have characterized *Callisphyris apicicornis* sexual behavior (calling, searching, courtship, and mating), finding evidence for semiochemicals as cues eliciting responses in males. Statistical analysis and Stereotypy Index have shown that all behavioral phases are stereotyped behaviors. Likewise, binary logistic regression has helped to identify significant environmental and endogenous variables occurring during those behaviors. This knowledge has oriented us to perform volatile sample collections from virgin females at different ages and times of the day, and also from males. Aeration apparatus consists on a cylindrical aeration chamber where the absorbent was activated charcoal. The air was purified and the flux was 1 l/min. The charcoal was eluted with hexane. The solution was reduced to 1-2 uL with gentle N<sub>2</sub> stream, and submitted to GC-MS (Shimadzu GC 17A-GCMS QP5050A). Chromatograms of a blank aeration vs. *C. apicicornis* aerations have shown no reproducible differences of either males or females at different ages and times of the day. Laboratory conditions might not be appropriate to stimulate calling and release of pheromone. Bioassays with these extracts are needed in order to confirm presence or absence of pheromone.

We acknowledge the support to the grant FONDECYT 11070072 from the Chilean Commission for Research in Science and Technology.

## Enhancing Bull Libido By Female Urinary Chemical Cues with Special Reference to Estrus

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In non human mammals, males are attracted to females in heat through exchange of chemical signals, which sources are urine, faeces, vaginal secretions and exocrine glandular products. Bulls, through the investigation of female's urine and anogenital region, are able to detect heating cow and determine the stage of estrus cycle. In order to identify chemical compounds implicated in such a process, urines from 30 cows were collected at specific stages of the estrus cycle and their chemical composition has been determined by GC/MS analysis. No systematic estrus specific compounds could be characterized but six molecules were characterized as pre-estrus and/or estrus specific in some animals. Commercially available identified compounds, 2H-benzopyrazone, 1,2-dichloroethylene and squalene were tested for their biological activity and ability to elicit sexual behaviour in the male. These molecules were sprayed in the nasal cavity of bull at physiological concentration. Two of these compounds in mix, squalene and 1,2-dichloromethane, significantly lowered mating and ejaculating times, and interestingly, improved sperm quality. Effects are observed in both young and experimented bulls with less efficiency in young ones, suggesting that learning could be important. Experiments are on-going to optimize the observed biological and behavioral effects testing various concentration and mixes of identified molecules. Identification of such chemical cues will permit to develop new biotechnological tools for estrus detection but also in process used in sperm collection (registered patent).

Patent n° DI-RV-10-0001 (C. Le Danvic, P. Nagan-Le Meillour, P. Humblot, P. Chemineau, O. Gérard, C. Briant)



**MALE *Metisa plana* (Lepidoptera:Psychidae) response towards female pheromone lure in wind tunnel bioassays****ABDULLAH F<sup>a</sup>, FAUZEE FF<sup>a</sup>, IBNU S<sup>a</sup>, MAT ISA S<sup>a</sup>, MOHD SABRI MS<sup>a</sup>**<sup>a</sup> UNIVERSITY MALAYA, KUALA LUMPUR, MALAYSIA*fauziah@um.edu.my*

The bagworm *Metisa plana* Walker is one of the major pests of economic importance causing 43% decline of yield in oil palm plantation *Elaeis guineensis* Jacq.in Malaysia. Observation in the field in oil palm plantation and a number of behavioral bioassays using T-shaped olfactometer at close range in petri dishes showed the existence of a female pheromone. This study was conducted to investigate male moth *M. plana* behavior towards female bagworm extract in the wind tunnel. A known concentration of the extract was tested in the wind tunnel with the presence of wind and without wind. The effect of distance was also determined by testing male response towards extract placed 50 cm and 100 cm from the lure in the wind tunnel. A number of concentrations was tested to determine the optimal dosage in the wind tunnel. Results of the bioassays showed that a minimum of 0.0005 female equivalent(FE) was needed to elicit male response. The lure placed at 100 cm significantly elicited a positive response from male. Males did not show aggressive behavior with the presence of other males near the lure. Different concentrations of the extract should be tested in the field in order to determine the best formulations for pheromone lure in the presence of wind in the field.

## Biosynthesis and absolute configuration of the sex pheromone of *Hedypathes betulinus* (Coleoptera: Cerambycidae)

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*Hedypathes betulinus* (Coleoptera: Cerambycidae) (Klug, 1825) is the most serious pest of green maté (*Ilex paraguariensis*) in the southern region of Brazil. The male-produced sex pheromone of the species was previously described as a mixture of the compounds (*E*)-6,10-dimethyl-5,9-undecadien-2-yl acetate (major), (*E*)-6,10-dimethyl-5,9-undecadien-2-one (geranylacetone), and (*E*)-6,10-dimethyl-5,9-undecadien-2-ol (minor). We are reporting now the absolute configuration of the chiral molecules as well as initial studies on the biosynthesis of the sex pheromone components. An enantioselective synthesis was carried out by kinetic resolution promoted by CAL-B. Three organic solvents, TBME, hexane and THF were employed in the assays, in five different reaction times. GC analysis in a modified  $\beta$ -CD column was employed to compare the retention times of the natural products with the standards obtained from CAL-B resolution. The alcohol (minor component of the pheromone) is produced in both enantiomeric forms in a ratio of 82.3% (*R*)-(-)-(*E*)-6,10-dimethyl-5,9-undecadien-2-ol and 17.6% (*S*)- (*R*)-(-)-(*E*)-6,10-dimethyl-5,9-undecadien-2-ol. The major component acetate is produced as a single isomer, (*R*)-(-)-(*E*)-6,10-dimethyl-5,9-undecadien-2-acetate. The pheromone components are produced in higher amount in the prothorax of males, suggesting that pheromone production source is located in this region of the insect. The presence of small pores distributed throughout the prothorax was detected by SEM, suggesting that these pores are associated with pheromone release. Labeled-geranylacetone was applied topically in the prothorax of males and females. We detected that males readily incorporated the labeled compound, confirming that the glands responsible for pheromone production are located in prothorax and that the main pheromone component is biosynthesized from geranylacetone.

## Mating behavior of giant sugarcane borer, *Telchin licus licus* (Drury) (Lepidoptera: Castniidae)

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The giant sugarcane borer is considered an important pest in sugarcane. It was firstly registered in 2007 in the state of São Paulo, which comprises 70% of Brazilian sugar cane crops. So far, there are no efficient methods for its management and control of this pest. A better understanding of the poorly known reproductive behavior of the giant sugar cane borer is needed in order to develop a more efficient management strategy mediated by semiochemicals. Thus, this research aimed to characterize the reproductive behavior of *T. licus*. These studies will give support to the next steps of isolation, identification and synthesis of a possible sex pheromone in *T. licus*. We observed in greenhouse that males and females remain still until 11:00A.M. when they become active until 3:00P.M. The occurrence of insect mating was noted only during sunlight, however on cloudy days the insects remained motionless. During the warmest period of the day, the male perceive the presence of a female flying next to him (around 10 cm, or less), and pursues her in serpentine movements at heights that varied from 50 to 370 cm. This flight lasts, on average, two minutes. Once the female lands, the male also lands, walks towards her moving wings up and down until they are parallel to each other. Subsequently, the male curves its abdomen in direction to the final portion of the female's abdomen initiating mating, which lasts 2h on average.

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## Pheromone titre influences mating behavior in both sexes of the desert locust (*Schistocerca gregaria*; Orthoptera)

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Recent findings demonstrate involvement of pheromones in mediating gregarious desert locust *Schistocerca gregaria* (Orthoptera: Acrididae) behavior. The gregarious adult male pheromone phenylacetone nitrile (PAN) has been observed to act as an adult aggregant for both sexes<sup>1,3</sup> and a male homosexual avoidance pheromone<sup>2,3</sup>. Wings and legs have been shown to be the site of production of PAN<sup>3</sup>. However, the role of PAN in mate selection by females remains unknown. The current study examined this by using bioassays and chemical analysis of PAN titres from volatiles and body tissues of adult males and females of different ages. The results indicated that 14 day-old females preferred mating with 14- and 21-day old males, which correlated with elevated levels of PAN titres in the volatiles and body tissues of these males. PAN was detected in the body parts and tissues of females, with the highest levels detected in 14-day old females in contrary to results from female volatiles from previous studies<sup>1</sup>. The bioassays also showed that mating occurred more during the day than at night. This concurred with the levels of PAN emitted by males during the day. These results suggest that mating behavior in the gregarious desert locust is influenced by the levels of PAN in both sexes of adults, and that PAN titers could be used to identify genes regulating reproduction behavior in *S. gregaria*.

1 Journal of Chemical Ecology (1994) 22: 2273–2281

2 Journal of Insect Physiology (2003) 49:1125–1133

3. Journal of Insect Physiology (2008) 54:162-168





Poster Session  
S3. Chemical communication within, among  
and around plants



## S3. Chemical communication within, among and around plants

Poster P045

### Effect of Bt genetic modification on indirect defense in cotton via a tritrophic interaction

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Despite the benefits of the Bt technology, genetic modification potentially can generate unpredictable unintended effects in non-target insects such as natural enemies (parasitoids or predators) of the pests, altering the natural biological control capacity existing in the agroecosystem. We present a tritrophic analysis of the potential non-intended pleiotropic effects of *cry1Ac* gene derived from *Bacillus thuringiensis* (Bt) insertion in cotton (DeltaPine 404 Bt Bollgard® variety) on the emission of herbivore induced volatile compounds and on the attraction of the egg parasitoid *Trichogramma pretiosum* (Hymenoptera: Trichogrammatidae). Both the herbivore damaged Bt variety and its nonBt isolate (DeltaPine DP4049 variety) produced volatiles in higher quantity when compared to undamaged plants and significantly attracted the egg parasitoids (*T. pretiosum*) when compared to undamaged plants. However, *Trichogramma pretiosum* did not differentiate between the transgenic and nontransgenic varieties, suggesting that the ratios between the compounds released by herbivory damaged -Bt cotton and herbivory damaged-nonBt cotton did not change significantly.



### Comparison allelopathic effects of some Brassica species in two growth stages on germination and growth of sunflower

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Brassica species contain allelochemical compounds as glucosinolate that under special condition is released to environment and affects seed germination and plant growth. Glucosinolates are degraded by the enzyme myrosinase, which results in the release of various hydrolysis products such as isothiocyanates, nitriles and others. This specific plant defense determines various interactions with other organisms. This study investigated the allelopathic potential of three species of Brassica including *Brassica napus*, *B. rapa* and *B. juncea* on the sunflower seed germination and seedling growth. Extracts were made from plants in full flowering stage and straw. Aqueous extracts of three species from two stages of sampling were separately made with 0 (distilled water), 10, 20, 30 and 40% concentrations. This experiment was conducted in 2×3×5 factorial arrangement based on completely randomized design with five replications. There was a highly significant difference among different concentrations of extracts and also between two stages of extraction. All aqueous extracts significantly affected sunflower germination, germination rate, seedling root and hypocotyl length, fresh and dry matter weight when compared with distilled water control. The greatest concentration showed more inhibitory effect. Root length was more sensitive to extracts than hypocotyl length. Water uptake was also reduced by increasing the concentration of aqueous extracts. The results demonstrated that sunflower seeds are sensitive to allelopathic compounds released by mentioned plants especially *B. juncea*. Thus future plans are to conduct field experiments to assess their potential to reduce sunflower growth and their persistence in soil.

**Keywords:** Allelopathy, Brassica, Germination, Sunflower

## Manipulating from the inside: Impact of leaf-miner insects on the sugar metabolism of their host-plant

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Endophytophagous insects, such as stem-boring, gall-forming and leaf-mining insects, live within plant tissues and feed internally. This life-style presumably provides adaptive advantages for the insect over other external-feeding modes by allowing access to most nutritional tissues while avoiding main plant defensive compounds. This selective feeding behavior can be reinforced by manipulating the plant physiology, as suggested by the autumnal formation of 'green islands' around mining caterpillars in yellow leaves. We study this so-called 'nutrition hypothesis' on the biological system *Malus domestica* (Rosaceae) / *Phyllonorycter blancardella* (Lepidoptera: Gracillariidae). Thanks to colorimetric assays and capillary electrophoresis, we quantified starch, total soluble carbohydrates and main individual carbohydrates on green and yellow mined leaves. In this system, we found that the leaf-miner larva has the ability to manipulate its host plant in order to generate a microenvironment with all the nutrient supply needed for its growth and its survival. Our results suggest that insects impact source-sink relationships through manipulation of cytokinin levels leading to nutrient translocation. We seek to identify underlying physiological and molecular mechanisms and to estimate the impact of the endophagous life-style on major insect life history traits.

**The Feeding response of *Epilachna indica* (Coleoptera: Epilachninae) towards *Solanum nigrum* (Family Solanaceae) leave extract**

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Feeding bioassays of the 12 spotted ladybird beetle *Epilachna indica* (Coccinellidae: Epilachninae) showed that the ladybird did not show a feeding preference when given choice between leaf discs of egg plant *Solanum melongena* and *Solanum nigrum* (Family: Solanaceae). *E. indica* feeding response towards *S. nigrum* extracts in agar and towards Thin layer chromatography spots were investigated. The number of bites made by *E indica* on the agar containing *S. nigrum* extract showed that the ladybirds consumed the agar containing *S. nigrum* extract and fractions. A further study was conducted to identify the feeding stimulant found in *S. nigrum*. This shows the potential of using the wild solanaceous plant *S. nigrum* as a trap plant against pest attack of the egg plant *S. melongena*.

## S3. Chemical communication within, among and around plants

Poster P049

### Oviposition pheromone of gall midge, *Mycodiplosis coniophaga* (Winnertz)

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The female oviposition pheromone of gall midge, *Mycodiplosis coniophaga* (Winnertz) was identified to be heptyl butyrate from the fruit of the Chinese crabapple, *Malus hupehensis* (Pamp.) Rehder (Rosaceae), covered with aecia of cedar apple rust, *Gymnosporangium* sp. (Uredinales: Pucciniaceae). Preliminary field assays demonstrated that a single compound, heptyl butyrate, itself was significantly attractive than the heptyl isobuturate, an analog of the pheromone. However, attraction could be significantly inhibited by addition of *trans-beta*-ocimene, which was another component identified from the same volatile source. In a period of 2-wk in 2009, more than 10,000 *M. coniophaga* females were captured in 10 Jackson traps (Trécé Inc., Salinas, CA) baited with synthetic heptyl butyrate in hedgerow, which made up of many kinds of trees, shrubs and vines, but mainly by *M. hupehensis* in Beltsville, Maryland.

**Repellent effect of *Andrographis paniculata* (family: Acanthaceae)  
towards the rice weevil *Sitophilus oryzae* (Coleoptera:  
Tenebrionidae)**

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A study on the repellence effect of *Andrographis paniculata* (Family: Acanthaceae) towards the rice weevil *Sitophilus oryzae* (Coleoptera: Tenebrionidae) was conducted from July 2008 to March 2009. Behavioral bioassay of weevils showed increased repellent behavior with exposure to increased concentration of extract. In no choice bioassay 50% of weevils were repelled from extract of *A. paniculata* in water at 100,000 ppm. 85% of weevils were repelled towards *A. paniculata* methanol extract. Fractions E7, E8 and E9 after column chromatography have the most repellent effect. After undergoing Thin layer chromatography, fraction E7 and E8 has spot with R<sub>f</sub> value of 0.90 while the R<sub>f</sub> value for spot from fraction E9 was 0.94. The spots were scratched out, dissolved in methanol and again tested with the weevils. Analysis using Gas Chromatography-Mass Spectrometry revealed four compounds found in the purified extract which caused repellence response from *Sitophilus oryzae* were methyl ester-(Z)-9-octadecenoic, 4-hydroxyl-3-methyl-2-butanone, 2,3-dihydro-3,5-dihydroxy-6-methyl-4H-pyran-4-one and 2,4-bis (1,1-dimethylethyl)-phenol.

keyword: *Andrographis paniculata*, *Sitophilus oryzae*, repellent behavior

## The chemical defenses in living fossil conifer cones

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Conifer cones are important parts for the self-propagation of coniferous species. It has been suggested the existence of bioactive components against several ecological influences. *Taxodium distichum* and *Metasequoia glyptostroboides* are well known as living fossil conifers in Taxodiaceae species. We have reported that cones of *T. distichum* contain characteristic bioactive abietane-type diterpenes (1). In this study, we selected the cones of three Taxodiaceae species, *T. distichum*, *M. glyptostroboides* and *Cryptomeria japonica*, and one Cupressaceae species, *Chamaecyparis obtusa*, and investigated the chemical compositions of each *n*-hexane extract. In addition, antifungal activities of each extract against two wood decay fungi, *Trametes versicolor* (white-rot) and *Fomitopsis palustris* (brown-rot), were evaluated. The yield of *n*-hexane extract from *T. distichum* cones was much higher than the extracts obtained from other cones. We also revealed that more than 70% of diterpenes were contained in this extract. The main components of *M. glyptostroboides* were fatty acids, and *C. japonica* and *C. obtusa* were sesqui- and diterpenes. *C. obtusa* also contained some monoterpenes. The results of antifungal tests against *T. versicolor* were as follows: the growth inhibition of *T. distichum*, *C. japonica*, *M. glyptostroboides*, and *C. obtusa* were  $33.7 \pm 2.64\%$ ,  $24.7 \pm 0.64\%$ ,  $18.3 \pm 1.64\%$ , and  $14.0 \pm 0.60\%$ , respectively. Consequently, it was suggested that the diterpenes of *T. distichum* cones significantly concerned with their chemical defenses.

### References cited

1. Kusumoto, N. et al. 2009. Journal of Chemical Ecology 35 :635-642.

## Longifolene as a precursor of plant self-defensive compounds

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Longifolene is known as a main component in heart wood of pine tree (*Pinus thunbergii* etc.) and has none or week bioactivities for plant pests, ex. termite and wood decay fungi. It is also known that the longifolene has unique tricyclic structure and easily autoxidized in natural condition. However, the bioactivities of the autoxidation products have not been studied. In order to consider the ecological meaning of longifolene, we analyzed the structures of autoxidation product and examined these bioactivities for termite (*Reticulitermes speratus*), white rot fungi (*Trametes versicolor*) and blown rot fungi (*Fomitopsis palustris*). Longicamphenilone, longicamphenilol and *sec*-norlongilactone were identified as the autoxidation products from the longifolene sample oxidized by air at the room temperature. Bioassays against termite and wood decay fungi were performed according to the previous reports<sup>1</sup>. Longifolene had no bioactivities against the termite and the fungi. The autoxidation products, longicamphenilone, longicamphenilol and *sec*-norlongilactone, had termicidal and/or anti-feedant activities in the termite tests. In case of fungi tests, longicamphenilol showed potent anti-fungal activities on mycerial growth of *T. versicolor* and *F. palustris*. Thus, the autoxidation products of longifolene were recognized as plant self-defensive compounds. It was suggested that the longifolene was stored as a precursor of plant self-defensive compounds in sound heart wood and changed into the bioactive compounds when it was exposed to air by some reason of damage.

### Acknowledgments

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### Reference

Sekine N. et al., J. Agric. Food Chem. 57:5707-5712

### **Volatiles from apple at early phenological stages and their effect on the apple blossom weevil**

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Apple volatiles emitted at early phenological stages are little investigated, although they may influence behaviour of early season pests, including the apple blossom weevil *Anthonomus pomorum* (Coleoptera: Curculionidae). This species colonizes apple trees at their early phenological stages and oviposits into developing flower buds, often leading to economic damage.

Using *in situ* radial diffusive sampling and thermal desorption followed by gas chromatography-mass spectrometry, we identified and quantified headspace volatiles from apple twigs with flower buds at three early phenological tree stages. The volatile blend consisted of 14 compounds, for the first, and increased to 16 compounds for the third phenological stage sampled.

A recombined synthetic blend served as the odour source in a still-air dual-choice olfactometer bioassay, in which we tested individual male and female weevils. Results from this behavioural test document a non-sex-specific attraction of this herbivore to volatiles of its host plant, suggesting that apple volatiles emitted early season serve as olfactory cues for host location of *A. pomorum* in the field.



#### **Behavioral and chemical factors involved in the choice of *Solanum lycopersicon* genotypes by *Tuta absoluta* (Meyrick) (Lepidoptera: Gelechiidae) females**

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Higher plants release a wide variety of volatile compounds which are perceived by specialized neurons chemo-receptor in the insect antenna. It is believed that chemical stimuli produced by tomato plants should regulate their interspecific relationship with pest insects, including the tomato pinworm, *Tuta absoluta* (Meyrick) (Lepidoptera: Gelechiidae). Therefore, the objective of this study was to carry out behavior studies in a wind tunnel and oviposition experiments. First, five genotypes of *Solanum lycopersicon* were chosen: BGH-674, BGH-1497, BGH-1708, BGH-489 and the Santa Clara variety. In the wind tunnel, the response of mated *T. absoluta* females to these genotypes was studied resulting in a preference of the Santa Clara variety ( $p=0.004$ ). We were also able to show that they reach the susceptible plants faster than the resistant ones. After that, mated females were submitted to three different kinds of choice-experiments: no-choice, free-choice contrasting all genotypes and a free-choice contrasting only two genotypes (one resistant and one susceptible). In the no-choice experiments, females didn't prefer to lay eggs in any of the genotypes ( $p=0.06$ ). In the first free-choice experiment, the number of eggs was not influenced by genotype ( $p=0.924$ ). In the second free-choice experiment, only for the Santa Clara/BGH-674 contrast, the number of eggs laid on Santa Clara variety was higher than in BGH-674 ( $p=0.025$ ). As a result, it was possible to establish a female preference for this variety. Moreover, subsamples BGH-1497, BGH-674 and BGH-1708 were considered resistant to antixenosis and could be used in the host plant resistance.

## S3. Chemical communication within, among and around plants

Poster P055

### **Responses of Insects to Understory Air's Volatile Organic Compounds Distributed in a Tropical Forest of Northern Thailand**

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The northern part of Thailand is rich in biodiversity and tropical forests with unique ecosystems. Presently, very little information is available on the interactions between volatile organic compounds (VOCs) and insects present in the forests. We, therefore, carried on-site experiments to collect samples of VOCs and insects in the understory air level and to analyze the chemical composition of VOCs in relationship with the distribution of insect types. Simple air traps, each filled with Super Q adsorbent, were placed together with insect traps at selected fifteen sites in Doi-Phu- Kha National Park for simultaneous studies of the VOC components and insect population. The samples were collected both in the warm season (March 07) and cool season (November 07). The results showed that six VOCs, including  $\alpha$ -thujene, camphene, *p*-cymene, limonene, (1,8)-cineol and camphor were the major components in both seasons, with variation of 4 insect types, namely Hymenoptera, Diptera, Coleoptera and Homoptera. The correlation between the VOC components and the existence of the flying insects were then analyzed. Based on the data obtained from the two seasons of the year 2007, it was found that Hymenoptera and Diptera were attracted by most of the components, whereas Coleoptera were repelled by the same compounds. For the group of Homoptera, the relationship between the VOCs and the biological effect was not conclusive. Discussion with respect to the interaction of chemically mediated communication between plants and insects in the Doi Phu Kha tropical forest will be given in the presentation.

## Waterborne cues by grazed algae induce chemical anti-herbivory responses in con- and heterospecific neighbors

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Plants are known to become more resistant in responses to cues released from other plants damaged by herbivores. Here, we examined whether macroalgae triggered such anti-herbivory responses via waterborne cues from either conspecific or heterospecific neighbors attacked by herbivores, in comparison with direct grazing. The algal trait modifications were detected by feeding preference tests by grazer between control and treated alga in types of fresh and reconstituted foods. Experiments were conducted in Portugal and Germany with non-native *Sargassum muticum* and two local natives, using local grazers. Directly grazed local algae, but not *S. muticum*, were less consumed than ungrazed conspecifics. Exposure to waterborne cues of grazed conspecifics decreased the palatability in *Fucus spiralis* (Portugal) and *F. vesiculosus* (Germany), except for other seaweeds. The palatability of *S. muticum* was not affected when exposed to waterborne cues from grazed heterospecific algae. In contrast, exposure to waterborne cues from grazed *S. muticum* lowered the palatability of *Cystoseira humilis* (Portugal) and *Halidrys siliquosa* (Germany) but not that of *Fucus* spp. Moreover, the cues emitted from grazed *F. spiralis* increased the resistant traits of *C. humilis*. Our results indicate that waterborne cues by con- and heterospecific algae can induce chemical anti-herbivory responses but these responses are species-specific. The non-indigenous *S. muticum* appeared to be insensitive to direct grazing and waterborne cues, but was used for eavesdropping by local seaweed species that are relatively closed in phylogenetic relatedness.

**Antennal sensory structures in *Scaphoideus titanus* Ball  
(Hemiptera: Cicadomorpha)**

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A scanning and transmission electron-microscope study has been carried out on the antennae of the leafhopper *Scaphoideus titanus* Ball (Hemiptera: Cicadomorpha). This cicadellid species is the vector of a phytoplasma disease, the Flavescence dorée (FD), belonging to the vine yellows group. The antennae of *S. titanus* bear few sensilla, mostly located on the flagellum. The scape is enlarged and is completely devoid of sensilla. The pedicel presents some scattered hairs and, like the scape, is covered by cuticular scales. The flagellum consists in a unique elongated segment in which numerous subunits can be recognized, being separated by a sort of cuticular crown. The proximal five subunits bear most of the sensilla. We discovered the presence of single and double walled coeloconic sensilla, campaniform sensilla, basiconic sensilla and trichoid sensilla. A scolopidium is also located within the proximal region of the flagellum. Ultrastructural investigations suggest that the antennal sensilla could be involved in the perception of air-borne vibrations, temperature and humidity variations and CO<sub>2</sub> concentration. The most relevant feature is the extreme reduction of the olfactory sensilla, both in terms of number of sensory structures and sensory neurons per sensillum. This could be related to the perception of specific stimuli from the host plant. The strong reduction in antennal olfactory sensilla to which this specie has undergone is discussed as possible consequence of the specificity towards the host plant.

## How cheating affects relationships

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Mutualisms are characterized by benefiting both participants. In a pollination mutualism, animals ensure plant reproduction by transferring pollen. The plant rewards the pollinator with nectar. While the pollinator transports pollen in a self-serving behavior, the plant has to invest into nectar production. A key question is what stabilizes plant-pollinator mutualisms.

We assessed whether hawkmoths discriminate between cheating and honest petunias and if their behavior limits reproductive success of cheaters.

We bred *Petunia* lines that contain low nectar volumes but resemble the rewarding species in a backcross breeding design. The goal was to obtain low nectar lines which can be used in behavioral assays and for fitness analysis. We have isolated a promising line with nectar volumes of 10.4  $\mu$ l, displaying all other floral characteristics of *P. axillaris*.

In behavioral assays, the foraging behavior of hawkmoths was tested on "cheaters". The only significant difference was a reduction of drinking duration on low nectar lines. We determined how this behavior affects seed set. Low nectar lines sired more seeds when hand-pollinated than *P. axillaris*. However this effect is neutralized in a moth pollinated set up. We propose that the reduction in drinking time might act as a partner control mechanism to promote nectar production in *Petunia axillaris*.

## The inhibition effects of norlignan (C<sub>17</sub> phenolic compound) on plant growth

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The norlignan is C<sub>17</sub> phenolic compound which is distributed mainly in the wood plant in Taxodiaceae. The structure of this type compound is less a carbon than lignan (C<sub>18</sub> phenolic compound). A norlignan which was firstly isolated from sugi (*Cryptomeria japonica*) was named to "sugiresinol"<sup>1)</sup>. The other main norlignans that were contained in sugi, were hydroxysugiresinol, agatharesinol, sequirin-C. Besides *Metasequoia* and *Taxodium* in Taxodiaceae, a norlignan, hinokiresinol, was reported at hinoki (*Chamaecyparis obtusa*) in Cupressaceae. Thus, a lot of norlignans were reported in conifer as well as sugi.

We investigated the inhibition effects of sugi heartwood extracts on plant growth, and found out that there was a heavy activity of methanol extracts except n-hexane, ethyl acetate extracts. So, we analyzed the methanol extracts, and isolated and identified agatharesinol, sequirin-C, hydroxymetasequirin-A. Those norlignans and sugi main norlignans (sugiresinol, hydroxysugiresinol) were tested to the inhibition of the growth of seedlings using Lettuce (*Lactuca sativa* L.), Shirotsunekusa (*Trifolium repens* L.), Italian raigururu (*Lolium multiflorum* Lam.).

The result showed the different activity of five norlignans tested clearly. It was confirmed that structure of norlignan had effects to the germination and growth. We will discuss the inhibition effects on plant growth for the isolated norlignans, and relation to the structure.

### References

*cited*

1. Yuji Kai (1965) : On the phenolic constituents from *Cryptomeria japonica* D. Don.. – The structure of sugiresinol – J. Jap. Wood Research Soc.,11(2), 23-26.

## Pear ester-based female-targeted lures - responses of non-codling moth Lepidoptera

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Pear ester (ethyl-2,4-decadienoate) has been described as a powerful attractant for female codling moth (*Cydia pomonella*, Lepidoptera, Tortricidae) in North America. In Hungary, parallel to other reports, we failed to show significant attraction. However, in the presence of acetic acid, catches of codling moths increased dramatically in our tests, confirming earlier results from non-European sites.

During our field tests we found that catches of other, non-codling moth tortricids (i.e. *Hedya nubiferana*, *Cydia pyrivora*) were also increased when acetic acid was added.

It was more surprising that significant catches of the apple clearwing *Synanthedon myopeformis* (Sesiidae) were also recorded. This sesiid counts among the important pests of apple in Europe. Pear ester + acetic acid lures were active no matter whether the two compounds were dispensed from separate dispensers or from a single dispenser, and the majority of the clearwings caught were females.

To our knowledge this is the first report on the activity of pear ester-based lures on non-tortricid Lepidoptera, and suggests that the compound may be exploited as a host location stimulus by a wider array of insects than thought before. This is supported by preliminary results showing similar trends of captures of a day flying butterfly, *Coenonympha arcania* (Satyridae), in traps baited with pear ester + acetic acid lures, suggesting that more surprises can be expected from the study of pear ester-based lures in the future.

## Phytotoxicity of 2-Amino-5-Hydroxyhexanoic Acid

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A non-protein amino acid, 2-amino-5-hydroxyhexanoic acid (5-HNL), extracted from *Crotalaria juncea* (sunn hemp, var. Tropic Sun) seeds inhibits the growth of many agronomical important weeds at low dosage levels. This compound is unique among allelochemicals in that when topically applied to weeds that reproduce by root buds, the treated plants symptomatically lose chlorophyll (bleach) and plantlets from the root buds are also devoid of chlorophyll, indicating translocation of the compound (source to sink). When compared to the organic herbicide, Amitrol (3-amino-1,2,4-triazole), a carotenoid synthesis inhibitor, in a *Lemna obscura* bioassay, 50uM of the 5-HNL inhibited growth of *Lemna* to the same extent as 100uM of Amitrol. *Lemna minor* was more sensitive to the 5-HNL in bioassay with a 90% inhibition of growth at 12 uM. The *in vitro* deamination of poly-L-lysine by nitrite at low pH, produces 5-HNL and the 6-hydroxy (6-HNL) isomer along with the isomer chlorosubstitution products (5-CNL & 6-CNL). We are currently assessing if the *in vitro* produced 5-HNL has the same level of phytotoxicity as the *in vivo* produced 5-HNL and if any of the other products of poly-L-lysine deamination are phytotoxic.



### **Inhibitory activity and chemical investigation on aqueous extracts of several common crop field weeds in Bogra district of Bangladesh and comparison with the reference values**

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An experiment was conducted to investigate the biological effect of several common crop field weeds in Bogra district of Bangladesh. The effect of naturally occurring growth substances in aqueous extracts of several common weeds e.g. *Chenopodium album*, *Striga densiflora*, *Leucas aspera*, *Cyperus rotundus*, *Eleusine indica*, and *Digitaria ischaemum* were studied on germination and growth of wheat and jute seeds. The weeds viz. *Leersia hexandra*, *Fimbristylis miliacea*, *Marsilea quadrifolia*, *Vicia hirsuta*, *Solanum nigrum*, and *Gnaphalium affine* were also separately studied for rice and mustard seeds. In the first experiment, boiled and unboiled extracts of the entire weeds significantly reduced and delayed the rate of germination of wheat and jute seeds in compared with control. The highest germination (96% and 85.3%) and maximum growth (root and shoot length) of wheat and jute seeds were observed in control. Similarly, the second experiment showed that boiled and unboiled extracts of the weeds tested here also significantly reduced and delayed the rate of germination of rice and mustered seeds. The unboiled extract of *Solanum nigrum* had negative effect on mustered seeds germination by 50.6%. The thin layer chromatography examination of crude extracts of *Digitaria ischaemum*, *Chenopodium album*, and *Solanum nigrum* were showed the existence of different compounds at different solvent systems. After purification of crude extract of *Solanum nigrum* by column chromatography, three distinct compounds were isolated. It is suggested that associated weeds should be removed from the crop field during the germination of jute, wheat, rice, and mustered seeds to check the negative effect.

## S3. Chemical communication within, among and around plants

Poster P063

### **Purification of insect-resistant proteins from the wax gourd, *Benincasa hispida***

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Latex and exudates contain various proteins that are thought to take important roles in physiological mechanisms in plant. Proteins which have toxicity or growth-inhibitory activity have been isolated from latex of papaya and mulberry, and they have been suggested to act as defensive substances. Therefore, it appears that wide a variety of plants exude latex or exudates that contain insect-resistant proteins.

In order to find novel insect-resistant proteins from plants, we observed the sieve exudates of cucurbitaceous plants and examined defensive activities using sieve exudates derived from six Cucurbitaceas species. As a result, sieve exudates of wax gourd *Benincasa hispida* showed growth-inhibitory activity. Currently, we are trying to isolate insect-resistant factors from the sieve exudates of the wax gourd.

## ***Pieris napi* olfactory receptor neurons tuned to pheromones and floral plant odorants**

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*Pieris napi* use volatile signals both for intraspecific communication and to localize host plants for nutrition and oviposition. The male produces and emits citral (1:1 geraniol and nerol) from glucose as an aphrodisiac enhancing the female's willingness to mate (1). The male also produces methyl salicylate from phenylalanine, transfers it during mating to the female and she emits the transferred compound as an anti-aphrodisiac when subsequently courted (2). Interestingly, these pheromone compounds are also constituents of host plants and might serve as plant attractants or repellents.

We have collected nectar and flower volatiles from host-plants and identified the constituents and their relative amounts by GC-MS. The test protocol to characterise functionally *P. napi* olfactory receptor neurons included blends of volatiles produced by hosts, non-hosts, and chemical standards including the insect produced volatiles. The volatiles were separated in GC and tested on single receptor neurons recorded extracellularly on the antenna (GC-SCR). So far, all identified olfactory receptor neuron types were tuned to odorants present in the floral volatiles of host-plants. One neuron recorded from a female responded to methyl salicylate. The question is whether the female can smell her own anti-aphrodisiac signal or if this odorant is a cue for locating host-plants.

### *References cited*

1. Andersson et al. 2007 J Exp Biol 210:964-970
2. Andersson et al. 2000 Proc Biol Sci 267:1271-1275

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### **Attraction Effect and Electrophysiological Response of Spearmint Oil against Lantern fly, *Lycorma delicatula* (Hemiptera: Fulgoridae)**

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*Lycorma delicatula* is a subtropical pest introduced in South Korea from China and Southeast. This pest receives little attention from the people until 2006, but surged throughout the grapevine yard of Mid-West regions of South Korea. The adults infested many hosts including an *Ailanthus altissimum*, and *Vitis vinifera*. To know their attractiveness, this study was performed to investigate the attraction effect of six plant essential oils and to confirm their electrophysiological response from 1st to 4th instar of *L. delicatula*. Among the tested oils, spearmint oil (94.1%) significantly attracted 4th instar of *L. delicatula* at a dose of 1.25 $\mu$ l/cm<sup>2</sup> by using an olfactometer. However, 1st instar was not significantly attracted at the same dose. In dose response to spearmint oil, a dose of 2.5 $\mu$ l/cm<sup>2</sup> was very effective for 1st and 4th instar of *L. delicatula*. GC-MS analysis revealed that the active components responsible for the effective attraction effect of spearmint oil were carvone (70.6%) and limonene (54.8%). Of the two active components, carvone was more significant than limonene. Analysis by GC-EAD showed, major components of spearmint oil that elicited response in *L. delicatula* antennae, indicating the potential role of the essential oil as attractant that determine the choice of the attraction material. In the field test, spearmint oil exhibited attraction effect up to 5 days. The effect differently showed in test places and treatment dose.

### **Chemical communication between *Cephalotaxus harringtonia*, its endophytes and fungal pathogens: the role of fungal secondary metabolites**

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Most plants in natural and anthropogenic ecosystems are colonized by unapparent and symptomless microscopic fungi called endophytes. They invade the living tissues of the host plant for all or part of their life cycles and do not cause any symptom of disease. In the symbiotic association, the endophytic fungi are thought to confer the plants an ability to resist to various environmental stresses while they benefit in turn from the plant protection. It is likely that fungal secondary metabolites are involved in these processes. Concerning host defense against pathogens, two potential mechanisms have been proposed: (1) induction or increase of the expression of intrinsic host defense mechanisms and (2) supply of additional mechanism of defense, extrinsic to those of the host (chemical antibiosis, competition and parasitism). In this context, we focus on the chemical ecology of the conifer *Cephalotaxus harringtonia* in association with its endophytic fungi, and against fungal phytopathogens. To date, 650 isolates from *Cephalotaxus harringtonia* trees, introduced in the living collections of the Muséum National d'Histoire Naturelle (France) have been identified using ITS and 28S rDNA sequencing and classified in 94 operational taxonomic units (OTUs). One of the endophytic isolate, *Paraconiothyrium variable*, showed a striking antagonism against common plant pathogens and we undertook the chemical characterization of the fungal metabolites implied on the mutualistic relationship between the host plant and the endophyte.

## Bumblebees (*Bombus terrestris*) discriminate the floral scents of two wild snapdragon subspecies (*Antirrhinum majus*)

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Two wild subspecies of snapdragon, *Antirrhinum majus*, subspecies *pseudomajus* and *striatum*, differ in floral color and can be visually discriminated by insect visitors. The extent to which olfactory cues derived from floral scents contribute to discrimination between snapdragon subspecies remains unknown. We tested whether the snapdragon subspecies differ in floral scent, and whether olfactory differences help bumblebees *Bombus terrestris* to discriminate between them. The progeny of both subspecies collected from a total of 7 wild populations was grown under controlled conditions. We quantified the volatile organic compounds (VOCs) emitted by the flowers using gas chromatography-mass spectrometry-flame ionization detection (GC-MS-FID) analyses and measured the volume of flower nectar. In bumblebees, we studied antennal detection of VOCs by means of electroantennograms (EAG), and performed behavioral experiments in a Y-maze to determine the innate response to the main floral VOCs. We found that *A.m. pseudomajus* produced more nectar and presented a floral scent that contained three volatile benzenoids absent in the floral scent of *A.m. striatum*. These benzenoids elicited a significantly higher EAG response compared with other VOCs. In the Y-maze, bumblebees were significantly less attracted by one of the benzenoids, acetophenone, suggesting an aversive effect of this chemical. Differences in flower scent between the snapdragon subspecies, and olfactory bumblebee preferences, are discussed in the light of biochemical constraints on VOCs synthesis in the plant. Our findings indicate that bumblebees discriminate between *Antirrhinum* subspecies using olfactory cues, thus underlining the important role of flower scent in the evolutionary ecology of *Antirrhinum majus*.

## S3. Chemical communication within, among and around plants

Poster P068

### **Can interplant communication prime jack pine against future attack? Effects of defoliation by jack pine budworm on healthy neighbouring conspecifics.**

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It is well documented that in response to herbivory, plants produce volatile chemical signals which are used for plant defence. However, it is not well understood in conifers, nor is it clear whether these signals can also be interpreted by conspecific plants to prime healthy neighbours for defence. Jack pine (JP) seedlings are used as a model to test the still poorly understood mechanisms of plant-plant communication; and the jack pine budworm (JPB) as the model herbivore to elicit this plant communication via defoliation. In the greenhouse, effects of low, medium and high levels of defoliation by late instar JPB larvae as well as medium levels of mechanical wounding are observed by volatile collection daily. Volatiles are collected on wounded and unwounded branches (representing the response *de novo* and systemically) of the defoliated seedling, as well as two branches on a neighbouring undefoliated plant. These are compared by percentage defoliation over time for quantity and quality of monoterpenes. All plants are examined for resistance to a challenge; this being a simulation attack via *Grosmania clavigera*, a well known fungal symbiont of mountain pine beetle (MPB). A general defence response in conifers is sought, after exposure to induced plant volatiles. This project has significant implications for conifer forest management, to identify which forest stands are most at risk to future MPB attack, and explore a potential solution: priming trees prior to attack.

#### The signaling effect of volatile monoterpenes emitted from elicited *Cupressus lusitanica* cultured cells

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Terpenes emitted after insect attack, mechanical wounding, fungal invasion or artificial elicitation are thought to have biological and ecological functions such as plant defense and plant communication. The volatiles emitted after herbivore attack transfer the chemical information from damaged plants to undamaged plants or distant plants of the same plants. Most of these studies examine the effect of total volatiles blend, few of them showed the effect of individual compounds.

Volatile compounds including some monoterpenes are produced when *Cupressus lucitanica* (Mexican cypress) cultured cell incubated with fungal elicitor (partially purified yeast extracts). Then, in this study, it was performed that the qualitative analysis of gas phase of incubated cells which were exposed to individual artificial monoterpene vapor. As a result, the cascade of stimulation and production by gas phase monoterpenes was observed; i. e. artificial terpinene feeding initializes the production of *p*-cymene, then, feeding of *p*-cymene results consequently in terpinolene production. Because terpinolene is a putative precursor of  $\beta$ -thujaplicin which has strong antimicrobial activity, this gas phase monoterpene production sequence indicates the plant communications for defense preparation against fungal invasion into woody plants. And it was also shown that even cultured cell have the ability to communicate with volatile chemical compounds and can be utilized as a model of inter-plant signaling experiments.



## S3. Chemical communication within, among and around plants

Poster P070

### **Allelopathy and Biodiversity: impact of Aleppo pine colonization on plant diversity of Mediterranean abandoned agricultural lands**

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The Mediterranean region is one of the main areas of biodiversity in the World. However an important natural reforestation directly threatens the mosaics of open middles. *Pinus halepensis* (Mill.), a pioneer and expansionist species, colonizes abandoned agricultural lands that present a high species richness. The potential impact of *P. halepensis* on plant diversity through allelopathy, and the role of microorganisms in these interactions are studied through *in vitro* bioassays. Germination and growth of twelve target species were tested according to the concentration of aqueous extracts obtained from branchlets of young pines (about 5 years), with or without the presence of soil microorganisms. In absence of microbial community and with a high concentration of extract, ten species are inhibited, a species exhibit contrasting effects and a species is stimulated. Under conditions representing at best the natural ecosystem, the different target species have more contrasting reactions against to the allelochemicals of pine. Six species are inhibited, three are insensitive, and five are stimulated. *P. halepensis* has the potential to significantly alter the composition and the structure of plant communities. However, microorganisms play an important role in the plant-plant interactions by altering the expression of allelochemicals released in the ecosystems.

Key words: Allelopathy, biodiversity, bioassay, soil microorganisms, *Pinus halepensis*

**Tomato plant damage by economically important leafminer *Liriomyza bryoniae* induces changes in tomato volatile emissions**

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Comparative analysis on the composition of headspace volatiles in *Liriomyza bryoniae* (Kaltenbach, 1858) (Diptera, Agromyzidae) infested and uninfested tomato plants was carried out. Headspace SPME sampling was applied with the aim to reveal tomato plant response to *L. bryoniae* adult feeding and larvae mining activity. Herbivorous pest infestation changed GC profile of volatile compounds quantitatively. The significant differences in the emission of volatiles by healthy plants, those damaged by adult leafminers and damaged by second instar larvae were registered. After herbivory by *L. bryoniae* adults emission of three compounds ( $\beta$ -phellandrene, 3-hexenyl acetate and unidentified sesquiterpene) increased significantly while emission of two terpene compounds (limonene and  $\alpha$ -copaene) decreased. After plant damage by *L. bryoniae* second instar larvae the emission of  $\beta$ -phellandrene increased and the emission of three compounds (limonene,  $\alpha$ -copaene and  $\alpha$ -humulene) decreased.

The total amount of volatiles emitted from second instar larvae damaged tomatoes was significantly lower compare to that released by undamaged tomato plants or those damaged by adult flies.

**Reaction to linalool in two species of potato cyst nematodes  
*Globodera rostochiensis* and *Globodera pallida***

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Potato cyst nematodes *Globodera rostochiensis* (Wollenweber, 1923) Behrens, 1975 and *G. pallida* (Stone, 1973) Behrens, 1975 are the major pests causing losses of potato harvest worldwide. Plant-nematode interactions by chemical signals have not been widely investigated. Potatoes release into environment various chemicals including linalool. The latter is known as toxic for some nematodes. To reveal the effect of linalool on two species of potato cyst nematodes at the stage J2 (juveniles move to host roots) was our aim.

**Toxicity test.** No significant nematocidal activity of linalool was recorded compared to control (water) when J2 were put even into saturated linalool (Sigma-Aldrich, 97% purity) solution and were observed for 10 days. Nematodes were defined as dead if their body was straight and they did not move after mechanical prodding.

**Behavioural test.** As linalool turned to be nontoxic, repellency effect was expected. Bioassay was carried out in Petri dishes and linalool concentrations in the range from  $10^0$  to  $10^{-6}$  ng/mL were tested. Both potato root diffusate (PRD) and water were used as control. Attractivity of linalool to J2 of both species was revealed close to that of PRD. *G. rostochiensis* was more sensitive as was attracted to  $10^{-2}$  ng/mL in 0.5h, while *G. pallida* was attracted to  $10^{-1}$  ng/mL in 1h (attractivity reached that of PRD). GC analysis revealed low concentration of linalool in PRD. Other compounds attractive for the nematodes in PRD are present. Linalool we attribute to kairomones for both nematode species tested.

**Behavioural Identification of Electrophysiologically Active Compounds From Damaged Cotton Affecting Oviposition Decision in Female *Spodoptera littoralis***

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Nocturnal insects rely on olfaction for selection of a suitable site for oviposition. Female moths of *Spodoptera littoralis* have shown oviposition avoidance from caterpillar damaged cotton plant – *Gossypium hirsutum* – patches in dual choice bioassay. Night headspace volatile analyses showed that larval damaged cotton plants tend to emit a complex mixture of induced compounds belonging to different classes such as: green leaf volatiles (GLVs), terpenoids, as well as cis-jasmone and indole. Gas chromatographic coupled with electroantennographic detection (GC-EAD) studies on headspace collections from damaged cotton plants revealed antennal response to at least eighteen compounds in gravid *S. littoralis* females. Electroantennographic (EAG) studies on a synthetic mixture of these antennal active compounds showed similar response as natural headspace. Further studies are in process to identify the behaviourally active components, affecting the oviposition decision in *S. littoralis* females, under the application of different synthetic mixtures of the peripherally detected compounds from caterpillar damaged cotton plants.

## **Pheromone-based mating and aggregation in the sorghum chafer, *Pachnoda interrupta***

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Adults of the sorghum chafer, *Pachnoda interrupta* Olivier (Coleoptera: Scarabaeidae: Cetoniinae), form aggregations during the mating period in July and in October when they feed before going into aestivation. In July aggregations are mainly seen on *Acacia* spp. trees, where the beetles mate and feed on pollen, and in October they feed in large numbers on ripening sorghum, causing severe crop losses for Ethiopian farmers. During the mating season, field trapping experiments with live beetles as bait demonstrated attraction of males to unmated females, but not to mated females or males, indicating the presence of a female-emitted sex pheromone. Unmated females combined with a food source, banana, attracted high numbers of both male and female beetles. Other combinations of beetles with banana were not more attractive than banana alone. The mechanism behind the aggregation behavior in July thus seems to be attraction to a combination of pheromone and host volatiles. Female and male beetles were extracted with hexane during the mating period, and the extracts were compared using GC-MS. In a field trapping experiment, 19 compounds found only in female beetles were tested, both singly and in a mixture. Traps baited with one of the female-associated compounds, phenylacetaldehyde, caught significantly more beetles than any other treatment; 1191 beetles/trap during three days compared to the second most attractive compound that caught 64 beetles/trap.

### Plasticity of olfactory host plant preferences in the mustard leaf beetle *Phaedon cochleariae*

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The geno- and phenotypic plasticity of Brassicacean plants with respect to glucosinolates is well-known. However, little knowledge is available on the plasticity of the chemosensory response of herbivorous insects using glucosinolates and their volatile breakdown products (e.g. isothiocyanates, ITC) to locate their hosts. We studied whether olfactory preferences of the adult mustard leaf beetle *Phaedon cochleariae* for host plants with differing ITC emission are dependent on the beetle's experience with these plant species during larval, pupal, and adult development. We offered the following host plants to *P. cochleariae*: watercress (*Nasturtium officinale*) emitting phenethyl isothiocyanate (PE-ITC) and a Chinese cabbage variety (*Brassica rapa* var. *pekinensis*) releasing no ITC. Dual choice tests showed that beetles kept on watercress for several generations were attracted by watercress odor, whereas beetles reared on Chinese cabbage showed no preference for a plant odor. A switch between these plants during larval, pupal, or adult stage revealed that adults needed experience with watercress during both pupal and adult stage to show preference for watercress odor. The adult's olfactory preference was independent of the plant species experienced during larval development. We tested whether the adult's olfactory preference is dependent on experience with PE-ITC by offering PE-ITC as odor and ITC-free Chinese cabbage for food to *P. cochleariae* during pupal and adult stage. The PE-ITC-experienced adults were attracted by PE-ITC *per se*. Our study shows that the olfactory response of an herbivorous insect specialized to Brassicacean plants is phenotypically plastic and dependent on pupal and adult experience with ITC.

**Olfactory responses and sensilla morphology of *Hyalesthes obsoletus* signoret (Hemiptera: Fulgoromorpha: Cixiidae)**

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*Hyalesthes obsoletus* is a palearctic planthopper, natural vector of a yellow disease (Bois noir) to grapevine.

Grapevine represents only an accidental plant host for this planthopper while its whole life cycle occurs mainly on bindweed and hedge bindweed (Germany and France), nettle (Germany and Italy) and chaste tree (Israel).

Behavioural, morphological and electrophysiological studies were carried out in order to deepen the knowledge on the role of volatile organic compounds in *H. obsoletus* host plant recognition.

Bioassays were performed by using a Y-olfactometer, testing the following plant species: nettle, bindweed, hedge bindweed, chaste tree and grapevine. Results showed a significant attraction of females to nettle and of males to chaste tree; males showed also a significant "non-attractiveness" for hedge bindweed.

Antennal ultrastructural studies showed the presence of at least two typologies of olfactory sensilla, at the level of the pedicel: "plaque organs", and trichoid sensilla.

Volatile organic compounds (VOCs) from nettle and chaste tree were collected, and the extracts analysed by coupling gas-cromatography to both electroantennography (GC-EAD) and mass-spectrometry (GC-MS).

The VOCs were identified, and some of them were shown to elicit significant electrophysiological responses on male and female antennae.

The identification of biologically active compounds and the study of behavioural mechanisms involved in host recognition are of fundamental importance to develop a new possible strategy of monitoring and control of *H. obsoletus*.

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## Role of plant volatiles in host selection: attraction and oviposition behavior of *Tuta absoluta* females (Lepidoptera: Gelechiidae) in response to tomato leaves

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*Tuta absoluta* (Lepidoptera: Gelechiidae) is a major pest of the tomato (*Lycopersicon esculentum*) throughout South America and recently in Europe. In the present study, by combining wind tunnel experiments, oviposition bioassays as well as tomato volatile analyses, we aimed to characterize the behavioral mechanisms as well as the cues involved in host selection of *T. absoluta* females. Wind tunnel experiments showed that tomato leaves elicit upwind oriented flight followed by landing of mated females. These results demonstrate the essential role of plant volatiles in distance attraction for *T. absoluta*. In addition, volatiles are also important cues for female egg-laying as shown by the oviposition experiments. However, females lay more eggs while in contact with tomato leaf suggesting the role of tactile and/or non-volatile cues in addition to plant volatiles. Moreover, we have shown that females both with and without contact with tomato leaf are able to discriminate among three different cultivars of tomato and oviposit preferentially in two of them. Finally, headspace collections of tomato leaves of the three cultivars contained mainly  $\beta$ -phellandrene, followed by limonene, 2-carene and  $\beta$ -caryophyllene, which together accounted for more than 70 % of the total release rate. The three cultivars released the same compounds, but showed a significant difference in the volatile blend proportion. Our results provide a first description of the cues involved in attraction and oviposition of *T. absoluta* to its favorite host *L. esculentum* and suggest promising potential to control this important pest.



### **Comparative locomotory response of the red palm weevil, *Rhynchophorus ferrugineus* (Coleoptera, Curculionidae) to biogenic odours presented alone or combined**

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The red palm weevil (RPW), *Rhynchophorus ferrugineus*, a major pest of palm trees in Monsoon Asia and the Middle-East has been recently introduced in the Western Mediterranean Basin.

The combination of male aggregation pheromone and natural host plant odours (from pieces of fermenting date palm tissue) has been shown to be highly attractive to RPW in the field. Traps containing this bait are currently the most efficient means to lure adults. Addition of ethyl acetate, a ubiquitous fermentation product, increases the captures. Field tests have shown that trap captures are female-biased. Palm trees near such traps often suffer a higher infestation than other plants suggesting that some weevils are attracted by the odours released by the traps without reaching the odour sources. A thorough understanding of the type of locomotory responses involved in orientation of the insect towards odour sources, either from conspecifics or host plant, is indispensable to optimize the control of this pest. With a walking simulation device the locomotory behaviour of RPW was analysed in response to stimulation with aggregation pheromone, a natural host plant odour (a fermenting piece of date tissue) and ethyl acetate, each evaluated at two doses, and the mixtures of pheromone with either plant odour.

With pure odours clear differences of locomotor effects were established depending on the odour and the sex of the individuals. Mixtures between pheromone and plant odours also led to changes in the responses as compared to the pure odours, which partly agreed with field trapping data.

## Manipulation of the phenolic chemistry of apple leaves by leaf-mining caterpillars

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The apple leaf-miner *Phyllonorycter blancardella*, is able to manipulate its host as suggested by the autumnal formation of 'green-islands' in yellow leaves. Recent studies showed that physiological manipulations for nutritional purposes provide the larva with an optimal environment in an otherwise degenerating context<sup>1,2</sup>. However the ability of leaf-miner insects to alter host plant defenses remained unknown. The aim of this study was to characterize the impact of leaf-miners on plant defense compounds in infested leaves. We realized a quantification of phenylpropanoids (hydroxycinnamates, flavonols and dihydrochalcones) in field-collected samples at an appropriate spatial scale using liquid chromatography with photodiode array detection (LC-DAD). In green leaves, we observed no induction of phenylpropanoids in the mined tissues suggesting that the insect was either not recognized as a parasite (plant tolerance) or was able to defeat plant defense mechanisms. In yellow leaves, a low concentration of phenolic compounds was observed in mined zones compared to the surrounding tissues where a large accumulation was observed. Combined with high nutritional contents<sup>1</sup>, the ability to alter plant defense expression in the mined area most likely provides key fitness benefits and potentially explain the evolution and the adaptative significance of the endophagous-feeding life history mode.

Key words: endophagous insects, green-island, plant defense, *Phyllonorycter blancardella*, *Malus domestica*.

1. Giron, D. et al. 2007. *Biology Letters* 3: 340-343.

2. Kaiser, W. et al. 2010. *Proceedings of The Royal Society B. in press*

#### **Oviposition preference of *Ceratitis capitata* (Diptera: Tephritidae) to *Coffea arabica* and *Coffea canephora* cultivars.**

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Finding a suitable place for oviposition is a challenging task for a female insect herbivore. The decision of the site has positive or negative consequences on their offspring. The Mediterranean fruit fly (medfly), *Ceratitis capitata* (Wiedemann), is a high polyphagous tephritid that oviposits and develops in more than 300 species of fruits and vegetables. We have the evidence that medfly oviposits and develops in coffee fruits of *Coffea arabica* but was never found in fruits of *Coffea canephora*. The aim of this study was to evaluate the chemical cues for oviposition of *C. capitata* with combinations of *Coffea arabica* and *Coffea canephora* cultivars. The headspace volatiles were collected in all combinations of cultivars and analyzed by GC-MS. To assess the oviposition preference we used three cultivars of *C. arabica* (Mundo Novo, Bourbon Vermelho and Catuaí Amarelo in combination with two cultivars of *C. canephora*, (Conilon and Robusta). At least 10 couples of *C. capitata* were used in cages for different combinations of coffee fruits. When females reached a peak of oviposition (the third day after emergence), rosettes containing five mature fruits of each cultivar were introduced in cages. After 44 hours, the rosettes were removed from the cages and the eggs counted. The headspace volatiles analysed by GC-MS show a strong divergence in the chemical profile of coffee species. *C. arabica* cultivars were strongly preferred to oviposition when compared to the *C. canephora* cultivars. Acknowledgments to CNPq, INCT-Semioquímicos na Agricultura and FAPEMIG.

## **Plant volatile compounds: Cues or noise for mating in *Spodoptera littoralis*?**

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Pheromone communication in moths serves for mate recognition and finding. The pheromone is detected by olfactory receptor neurons (ORNs) expressing narrowly tuned olfactory receptors. In natural conditions, pheromone is perceived in a complex background of plant volatile compounds (PVCs). PVCs synergise or decrease attraction to pheromone, according to species, but the mechanisms are unknown. To understand how PVCs can be cues or noise for mate finding, we analyzed their effects on response to pheromone to in *Spodoptera littoralis*.

First, behavioural experiments with a locomotion compensator showed that the addition of linalool during pheromone stimulation reversibly disrupted the orientation of males, mimicking the effects of stopping the pheromone stimulus.

Second, we analyzed the firing responses of ORNs to the main pheromone component, Z9E11-14:Ac, in the presence of linalool and other monoterpenes, under different temporal patterns. Linalool reversibly reduced the response to Z9E11-14:Ac and produced an off effect. Other compounds reduced the response with a longer time course or had no effect. Pulses of linalool over a prolonged stimulation with Z9E11-14:Ac interrupted firing activity. In the reverse experiment, pulses of pheromone were better separated over a background of linalool, compared to odourless air. We concluded that PVCs reduce the sensitivity, but may improve the temporal resolution of pheromone pulses.

These complex effects suggest that terpenes might affect differently pheromone perception according to the patterns of PVCs and pheromone plumes encountered by male moths. Experiments aiming to understand how they adapt their orientation behaviour according to changing chemical cues are in progress.

## Monoterpene biosynthesis induction in *Origanum x majoricum* by soil bacteria

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Italian oregano (*Origanum x majoricum*) was subjected to root system inoculation with three species of plant growth-promoting rhizobacteria (PGPRs) (*Pseudomonas fluorescens*, *Bacillus subtilis*, *Azospirillum brasilense*), and essential oil (EO) content and plant growth were measured. Composition of monoterpenes, a major EO component, was analyzed qualitative and quantitatively by gas chromatography. Plants inoculated with *P. fluorescens* and *A. brasilense* showed a 2.5-fold increase in EO yield relative to controls, without change of oil composition. The major EO compounds showed increased biosynthesis. Plant growth parameters (shoot and root fresh and dry weights, numbers of leaves and nodes) were evaluated. Shoot fresh weight was significantly increased by all three PGPR species, but only *P. fluorescens* and *A. brasilense* increased root dry weight. These two species have clear commercial potential for economic cultivation of *O. x majoricum*. Knowledge of the factors affecting yield and accumulation of monoterpenes is essential for improving production of these economically important plant compounds.

### Seasonal monoterpene emissions of *Quercus coccifera* after sewage sludge compost spreading in a mediterranean scrubland.

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French production of urban sewage sludges increases each year, overloading existing recovering ways. In such context, the spreading of this waste in natural environment would be an alternative to burying or incineration. This study aims at assessing the short term effect of sewage sludge compost spreading, in a scrubland, on monoterpene emissions of *Quercus coccifera*. These compounds are involved in atmospheric photochemical reactions, favouring tropospheric ozone accumulation and therefore air pollution episodes.

The study was carried out next to Aix-en-Provence (Southern France). The compost was spread in July 2007, under 50 or 100 tonnes per hectare. The emitted compounds were collected using the dynamic headspace method. Seven samplings were performed between summer 2007 and summer 2009.

Results showed a strong seasonality of monoterpene emissions with normalized rates globally 3 times stronger in November 2007 and late June 2008 than for the other sampling dates. Early summer emissions varied from a year to another, and seemed to be related to past day temperature. The two major compounds,  $\alpha$ -pinene and limonene, were emitted in different proportions, according to the season.  $\alpha$ -pinene was mostly emitted in autumn and winter while limonene was rather emitted in spring and summer. Compost increased normalized emission rate in summer. Plant growth was favoured on amended plots but leaf nutrient content remained unaffected, two years after spreading, and thus does not explain emission variations. On the short-term, the physical properties of the compost probably had dominant effects on monoterpene normalized emission rates.

### **Disruption of aphid colonization behaviour and reproduction on plant treated by mineral oil.**

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Although mineral oil spray is one of the most effective way to control the transmission of non persistent aphid borne viruses in the fields, its mode of action is poorly understood. In the present study, the effects of mineral oil treatment on potato plants on the host plant selection behaviour, growth and reproduction of the potato aphid alates *Macrosiphum euphorbiae* were investigated. The effects were assessed 30 min, one and seven days after treatment. First, aphid orientation behaviour was studied by using a Y olfactometer to investigate potential attractant or repellent effects of the treated plants. Secondly, electrical penetration graph (EPG) technique was used to investigate potential deterrent or phagostimulant effects of the treated plants on aphid feeding behaviour. Olfactory experiments showed that the oil had a repulsive effect only 30 min after spraying. EPG experiments showed a slight modification of the aphid feeding behaviour mainly 7 days after treatment. The number of both salivation and sap ingestion events during the phloem phases were increased 7 days after treatment. In addition, whatever the time after treatment, xylem ingestion time was increased. Clip cage experiments were set up to assess potential effects of the oil treatment on aphid survival and demographic parameters. Nymphal mortality was increased on treated plant while fecundity of surviving insects was enhanced. Implications of semiochemicals in the antagonistic effects of oil treatment on aphids are discussed.

## S3. Chemical communication within, among and around plants

Poster P181

### **Behavioral and electrophysiological responses of the Egyptian cotton leaf worm, *Spodoptera littoralis* to plant and floral volatiles**

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Most insect species, moths in particular, rely on their chemosensory abilities to locate and navigate towards biologically relevant resources such as food, mates and oviposition sites. Our goal is to understand the attraction of the polyphagous Egyptian cotton leaf worm, *Spodoptera littoralis* (Lepidoptera: Noctuidae) towards the larval host-plant, cotton (*Gossypium hirsutum*) and flowers of Lilac (*Syringa vulgaris*) in a flight tunnel. Our findings show that both males and females exhibit odor-mediated anemotactic flights to flowers and larval host-plants. Male attraction to flowers attained 46%, while 38% of males were attracted to the larval food plant cotton. Up to 74% of the females were attracted to flowers for feeding, and 42% were attracted to the cotton plant for oviposition. Gas chromatography (GC) coupled with electroantennographic detection (EAD) was used to identify physiologically active odorants from the flowers of lilac and cotton foliage. We found nine antennal active compounds in the lilac flower headspace and ten compounds from cotton foliage. Identification of plant semiochemicals mediating host recognition and foraging behavior will help us to get better understanding of the sensory physiology and behavioral modulation in *S. littoralis*.



## S3. Chemical communication within, among and around plants

Poster P184

### **Does fluctuating asymmetry in male sex pheromones affect female choice?**

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There are a number of documented cases where the degree of asymmetry in bilateral male traits significantly affected female mate choice in a wide variety of organisms including insects. However, while it has been postulated that the degree of fluctuating asymmetry (FA) in pheromone content of male hairpencils may influence mating success these has been hard evidence to support this claim.

We tested this hypothesis using the armyworm, *Pseudaletia unipuncta* by providing a receptive virgin female with two virgin males and then analysing the content of the left and right hairpencils of both the successful and unsuccessful male. When insects were reared under ideal conditions on artificial there was no difference in the level of pheromone FA in successful and unsuccessful males, nor did females mate with the more symmetrical male of the two. However, when reared in poor quality diet the successful males had lower FA than unsuccessful males, and females mated significantly more with the male in the pair that had more symmetrical pheromone content.

This is the first example showing that FA in male pheromone content affects mating success. It also underlines the importance of considering the ecological context when studying the importance of FA in mate choice.



Poster Session  
S4. The diverse roles of non volatile  
compounds



**Role of chemical cues in the host-plant ranking by gravid *Polygonia c-album* females**MOZURAITIS R<sup>a</sup>, MURTAZINA R<sup>b</sup>, SOREN N<sup>c</sup>, BORG-KARLSON AK<sup>b</sup><sup>a</sup> 1. Royal Institute of Technology; 2. Institute of Ecology of Nature Research Centre, Lithuania, Stockholm, SWEDEN<sup>b</sup> Royal Institute of Technology, Stockholm, SWEDEN<sup>c</sup> Stockholm University, Stockholm, SWEDEN

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Despite the ability of oligo- and polyphagous insects to utilize a wide range of plants, a certain preference hierarchy within this range is typically observed (1). The aim of this study is to determine the role of chemical cues in oviposition and host-plant ranking by polyphagous comma butterfly, *Polygonia c-album* to be able to address questions regarding specialization, host plant shifts and ecologically driven speciation.

In a multiple choice test, groups of egg-laying *P. c-album* females ranked plants bearing surrogate leaves treated with crude methanol extracts obtained from leaves of seven host-plant species in descending order: *Humulus lupulus*, *Urtica dioica*, *Ulmus glabra*, *Ribes nigrum*, *Salix caprea*, *Corylus avellana* and *Betula pubescens*. The ranking order of surrogate leaves treated with host-plant extracts corresponded well to that reported on natural foliage, except *R. nigrum* (2). Thus, host plant choice in this species seems to be highly dependent on chemical cues. Moreover, after two subsequent fractionations using reversed phase chromatographic medium the non-volatile chemical cues residing in the most polar water-soluble fractions evidently provided sufficient information for egg-laying females to discriminate and rank between the samples of more and less preferred plants, since the ranking in these assays was similar to that for natural foliage or whole-methanol extracts, while the physical traits of the surrogate leaves remained uniform.

*References cited*

1. Schoonhoven, L.M. et al. 2005. Insect-Plant Biology, 2nd edn. Oxford University Press, Oxford.
2. Nylin, S. 1989. Ecological Entomology 14:209-218.

**Biosynthetical pathway of 8-hydroxyquinoline-2-carboxylic acid, a compound from the gut of *Spodoptera* larvae**PESEK J<sup>a</sup>, SVOBODA J<sup>a</sup>, BOLAND W<sup>a</sup><sup>a</sup> Max-Planck-Institute for Chemical Ecology, Jena, GERMANY

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In the regurgitant (foregut content) of *Spodoptera* larvae we observed high concentrations (0,5-5 mM) of 8-hydroxyquinoline-2-carboxylic acid (8-HQA). In a survey of different lepidopteran species, this compound was only detected in species belonging to the family of Noctuidae. Quinolinic derivatives in general are well-known from various environments and very distinct organisms (insects, plants, bacteria).<sup>1-3</sup> Therefore the origin of 8-HQA was investigated by chemical studies with labelled precursors. Here, it could be shown that 8-HQA is formed by the larvae themselves and not by their commensal gut bacteria. Antibiotics supplemented to the larval diet had no effect onto the biosynthesis of this compound. In addition, neither selected gut bacteria nor the whole gut microbiota were able to synthesise 8-HQA.

8-HQA was shown to derive from tryptophan metabolism. The amount of 8-HQA in the regurgitant was strongly dependent from the tryptophan content of the diet. Here it could be shown that 8-HQA is formed via the branch of kynurenine and 3-hydroxykynurenine. Tryptophan, kynurenine and 3-hydroxykynurenine were shown to be precursors, whereas xanthurenic acid, quinaldic acid or kynurenic acid were not. Thus 8-HQA is formed in a similar way as xanthurenic acid, a compound that is presumably used by mosquito larvae to detoxify the toxic precursor 3-hydroxykynurenine.<sup>4</sup> The investigation of different life stages of *Spodoptera* larvae revealed that 8-HQA is only formed during the larval stage, whereas its precursor 3-hydroxykynurenine accumulated during the pupal stage.

1. Schildknecht *et al.* (1969) *Z.Naturforsch.* 24b
2. Mossialos *et al.* (2000) *Appl. Environ. Microbiol.* 66,487-492
3. Lee *et al.* (2009) *J.Korean Soc. Appl. Biol. Chem.* 52,331-335
4. Han *et al.* (2007) *J. Insect Physiol.* 53,254-263

### **Caterpillar saliva: does it induce herbivore defenses in maize?**

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Plants are frequently attacked by insect herbivores and have evolved a range of defense mechanisms to counter their attack. Although chewing insects cause mechanical damage to plants, this does not account for the entire effect of herbivory. Caterpillar oral secretions, including saliva and regurgitant, trigger herbivore defense responses in plants. We examined the effect of fall armyworm (*Spodoptera frugiperda*) oral secretions on the maize (*Zea mays*) defense response. Analyses indicated that very little regurgitant was deposited on the maize leaf during caterpillar feeding. Whereas, leaf tissue immunoblots indicated that glucose oxidase, an abundant saliva protein, was deposited when caterpillars fed on the leaf. The effect of caterpillar saliva on maize defense gene expression was determined by allowing ablated (no saliva) and non-ablated (saliva present) caterpillars to feed in the maize whorl. The expression of a group of maize defense genes including those from the jasmonate biosynthesis pathway and those involved in direct defenses were monitored using qRT-PCR. The results showed that feeding by unablated caterpillars significantly increased the expression of these genes. Differential accumulation of maize proteins in response to ablated and unablated caterpillar feeding was analyzed by 2D Fluorescence Difference Gel Electrophoresis (DIGE). Out of 87 protein spots, 81 were identified with 49 proteins being up-regulated and 32 down-regulated when unablated and ablated samples were compared. These data show the profile of proteins that respond to fall armyworm larvae saliva. The results of this study show that caterpillar saliva is an important elicitor herbivore defenses in maize.

**Intraspecific alkaloid variation in ladybird eggs and its effects on con- and heterospecific intraguild predators**KAJITA Y<sup>a</sup>, OBRYCKI JJ<sup>a</sup>, SLOGGETT JJ<sup>a</sup>, HAYNES KF<sup>a</sup><sup>a</sup> University of Kentucky, Lexington, KY, USA

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Egg predation and cannibalism are common phenomena in predatory ladybirds despite the presence of defensive alkaloids. Consumption of heterospecific eggs negatively affects survivorship and development; however, intraspecific variation in quantities of alkaloids and post-ingestion responses to con- and heterospecific alkaloids, are not well understood. We examined variation in the quantity of alkaloids in eggs of *Harmonia axyridis* (Pallas), *Coccinella septempunctata* L., and *Hippodamia convergens* (Guérin) using gas chromatography-mass spectrometry, and show a link between heterospecific alkaloids and their toxicity and/or costs by feeding high and low alkaloid eggs to first instar *H. axyridis* and *C. septempunctata*. The repeatability of alkaloids measurements in eggs in an egg cluster was high, however, the amount of alkaloids varied significantly between egg clutches within and among females. This variation affected egg consumption by *C. septempunctata* when fed on *H. axyridis* eggs. *Harmonia axyridis* accumulated and synthesized their own alkaloid *de novo* by cannibalism, but *C. septempunctata* lost some portion of consumed conspecific alkaloids. Both species lost (e.g. defecated or metabolized) most of consumed heterospecific alkaloids, but *C. septempunctata* died within three days. Most of *H. axyridis* survived to the second instar, but *C. septempunctata* alkaloids led to a significant reduction in weight gain compared to aphid control. In addition, high alkaloid ingestion of *C. septempunctata* extended development of *H. axyridis* compared to aphid control, or conspecific eggs. *Harmonia axyridis* had greater abilities to process ingested con- and heterospecific alkaloids compared with *C. septempunctata*, which may, in part, explain their interspecific interactions in nature.

### Chemical ecology and behavioral complexity in Ant-Termite interactions

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A great number of species coexist, sometimes with reciprocal benefits (ex. mutualism) and sometimes for the benefit of only one (ex. predation). Ants are generally described as the main predators of termites. They do not usually live together but can in some cases. This preliminary study considered two ant species - *Monomorium carbonarium*, described as a very aggressive predator, and *Hypoponera eduardi*, thought to be a commensal parasite - and two termite species (*Reticulitermes flavipes* and *R. grassei*), living in sympatry on the Ile d'Oléron (France). It has been suggested that they may be able to live in close proximity owing to a change in their chemical signatures (i.e. cuticular hydrocarbons (CHCs) known to be involved in the recognition process).

To test this hypothesis, CHC profiles of ants and termites were analyzed by GC and GC-MS. Individuals were taken from different colonies of ants and fed with *R. santonensis* or *R. grassei* or worms. Initial results showed that food seemed to affect the colonial signatures: colonies fed with different diets had different profiles and different colonies fed with the same species of termite had similar profiles. In parallel, dyadic encounters were studied to see whether ants behaved in a different way towards termites depending on whether they had previously been in contact. Both species of ants developed a different predatory strategy. However, no significant difference in ant behavior was noted whether they were living close to termites or not. This project will be continued using different behavioral analysis protocols.



### **Dietetic constraint of host-plant specialisation in bee evolution (Hymenoptera, Apoidea)**

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Represented by more than 16 000 described species, bees are the major pollinators of angiosperms in most ecosystems. Since early Cretaceous, they share a long and intimate evolutionary history with flowering plants.

Bees forage on pollen and nectar as the exclusive food source. Interactions between flowering plants and wild bees are highly diverse. Different foraging strategies have been described. Some taxa are specialized in their floral choices (oligolectic species or pollen specialists) while other bee species are more opportunistic and forage on a large plant spectrum (polylectic species or pollen generalists). Oligolecty is frequent in solitary bees (around 50 % of described species).

Some clades of wild bees like the Melittidae include only oligolectic bees. Ancestral host-plant and specialist behavior seem both highly inherited. However some rare “host-plant shifts” occurred during evolution inside clades. The origin and the mechanism of these host-plant shifts remain misunderstood. They can be based on morphological or phylogenetical similarity with ancestral host-plant but host switches to unrelated plant families are also common. The constraints of these switches are not well known. Similar morphology of alternative host-plants could make the shift easier but the need of particular chemical (sterol, protein, ...) in pollen could reduce the range of suitable hosts.

We present our first results in the analyses of chemical composition of pollen host-plants.

### **Chemically mediated sexual isolation between Zimbabwean endemic and cosmopolitan populations of *Drosophila melanogaster*.**

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The partial reproductive isolation of conspecific populations is an initial step towards speciation. We have analysed the reciprocal mating propensities of 6 Zimbabwean endemic and 2 cosmopolitan strains of *Drosophila melanogaster* and evidenced a strong asymmetrical sexual isolation between the first and the last ones: while cosmopolitan males mate with either female types, the endemic females almost never copulate with cosmopolitan males.

We have characterized this sexual isolation and correlated it with the differences in the cuticular hydrocarbons profiles of the Zimbabwean and cosmopolitan strains.

We evidenced that the  $\omega$ 7- and  $\omega$ 5-alkenes (and of the corresponding *desat-1* and *desat-2* genes) are involved in this process while visual and acoustic cues only play an accessory role in it.

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**Biosynthetical pathway of 8-hydroxyquinoline-2-carboxylic acid, a compound from the gut of *Spodoptera* larvae**PESEK J<sup>a</sup>, SVOBODA J<sup>a</sup>, BOLAND W<sup>a</sup><sup>a</sup> Max-Planck-Institute for Chemical Ecology, Jena, GERMANY

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In the regurgitant (foregut content) of *Spodoptera* larvae we observed high concentrations (0,5-5 mM) of 8-hydroxyquinoline-2-carboxylic acid (8-HQA). In a survey of different lepidopteran species, this compound was only detected in species belonging to the family of Noctuidae. Quinolinic derivatives in general are well-known from various environments and very distinct organisms (insects, plants, bacteria).<sup>1-3</sup> Therefore the origin of 8-HQA was investigated by chemical studies with labelled precursors. Here, it could be shown that 8-HQA is formed by the larvae themselves and not by their commensal gut bacteria. Antibiotics supplemented to the larval diet had no effect onto the biosynthesis of this compound. In addition, neither selected gut bacteria nor the whole gut microbiota were able to synthesise 8-HQA.

8-HQA was shown to derive from tryptophan metabolism. The amount of 8-HQA in the regurgitant was strongly dependent from the tryptophan content of the diet. Here it could be shown that 8-HQA is formed via the branch of kynurenine and 3-hydroxykynurenine. Tryptophan, kynurenine and 3-hydroxykynurenine were shown to be precursors, whereas xanthurenic acid, quinaldic acid or kynurenic acid were not. Thus 8-HQA is formed in a similar way as xanthurenic acid, a compound that is presumably used by mosquito larvae to detoxify the toxic precursor 3-hydroxykynurenine.<sup>4</sup> The investigation of different life stages of *Spodoptera* larvae revealed that 8-HQA is only formed during the larval stage, whereas its precursor 3-hydroxykynurenine accumulated during the pupal stage.

1. H.Schildknecht *et al.*, Z.Naturforsch.B:Chem.Sci.,1969,24b,28-47
2. D.Mossialos *et al.*, Appl.Env.Microbiol., 2000,66,487-492
3. C.H.Lee *et al.*, J.KoreanSoc.Appl.Biol.Chem.,2009,52,331-335
4. Q.Han *et al.*, J.InsectPhysiol., 2007,53,254-263

**Caterpillar mediated glucosinolate and gene responses in ecotype crosses of *Arabidopsis***ROHR F<sup>a</sup>, ULRICHS C<sup>b</sup>, SCHREINER M<sup>a</sup>, MEWIS I<sup>a</sup><sup>a</sup> *Leibniz-Institute of Vegetable and Ornamental Crops Großbeeren/Erfurt e.V., Quality Department, Großbeeren, GERMANY*<sup>b</sup> *Humboldt Universität zu Berlin, Faculty for Agriculture and Horticulture, Berlin, GERMANY*

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Plants have developed effective strategies in order to overcome their enemies. Glucosinolates are sulphur-rich secondary metabolites mostly found in families in the order Brassicales. They are an important model system for studying insect-plant interactions. Glucosinolates are the non-volatile part of a two component defence system that is activated after tissue damage either by chewing insects or mechanical wounding when cleavage of the glucose moiety of the glucosinolate-core structure occurs by endogenous myrosinases. An important determining factor of the defence activity of glucosinolates against lepidopteran herbivores, bacteria, and aphids is the side chain structure. Especially the side chain of aliphatic methionine-derived glucosinolates is highly variable in cruciferous plants that is attributed to only a small set of polymorphic loci (*GS-AOP*, *GS-ELONG*) generating a modular alteration of the aliphatic glucosinolate profile.

In order to investigate the impact of side-chain modifications (*AOP* genes) of glucosinolates on insect resistance in the same genetic background, we have studied the plant response of 30 lines of *Arabidopsis thaliana* L. crosses with a different aliphatic glucosinolate profile. Crosses between the methylsulfinyl glucosinolates producing C<sub>3</sub> ecotype Gie-0 and the ecotypes Mr-0 (allyl glucosinolate containing) and Sap-0 (3-hydroxypropyl glucosinolate containing), respectively, were used to obtain homozygote lines for *AOP0*, *AOP2*, and *AOP3* in the F<sub>3</sub> generation. Feeding by two different lepidopteran larvae, the generalist *Spodoptera exigua* (Hübner) and the specialist *Pieris brassicae* L. resulted in typically glucosinolate induction and transcript levels of related genes and genes of signalling pathways. However, ecotype lines were differently responsive to herbivore attack.

## Cuticular hydrocarbons analyses in the Asian hornet *Vespa velutina*

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The Asian hornet *Vespa velutina nigrithorax* (Vespidae; vespinae) was introduced in South-West of France in 2004. Since this period, this invasive species is found in several locations in the country. *V. velutina* is an important problem in France, because it is a honeybee predator. The hornet has emptied several hives in South-West of the country.

Some studies are performed to analyse its biology and to try to eliminate this species from our country. More particularly, we wanted to analyse if workers from different colonies could predate the same bee hives. This result is important for bee keepers to know if they have to search and eliminate one or more hornet colonies when their bees are predated. For this purpose, we analysed cuticular hydrocarbons (CHCs) from workers, males and young queens produced from 8 colonies, by GC-FID and GC-MS. This technique was used to analyse workers caught in front of hives to determine if they are from one or more colonies. Our preliminary results showed that some workers could originate from different colonies. These results are important to the studies of this new hornet species present in France, and probably in Europe in the future. They also present species specific chemical signatures different from the European *Vespa crabro*, and queen and males have quantitative different signatures.

### **Induced Response of Soybeans to *Phakopsora pachyrhizi* inoculation and alternative products used in organic production system**

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Alternative products for soybean Asian rust (*Phakopsora pachyrhizi*) management in organic production system were tested. Among them, calda viçosa (CV, mixture of Cu, Zn and Mg sulfates), Fish Fertil® (FF, hydrolyzed organic based on crustacean shell and marine fish, rich in chitosan), and sodium silicate (SS) were selected for defense induction studies. Soybean sown in greenhouse, at V3 development stage, was sprayed with alternative products and inoculated or not-inoculated with spores of *P. pachyrhizi*. Control plants were neither sprayed with products nor fungus inoculated. At 0 (before treatment) and 72, 96 and 120 hours after treatments, leaves were collected, extracted in MeOH, filtered, and HPLC injected for identification and quantification of compounds. In general, FF + fungus plant treated increased isoflavone concentrations and responded faster than the other treatments. Malonyl genistin, malonyl daidzin and malonyl glycitin concentrations were approximately, four and 90 times (72h) and two (96h) times greater, respectively, compared to previous analysis, in fungus + FF treated plants. In this treatment, genistein and daidzein concentration also increased at 72h after treatment. Acethyl daidzin concentration increased in most of treatments, but was bigger in FF + fungus, 72h after treatment. Aglycones (genistein, daidzein and glycitein) concentration was lower compared to glycosides. However, FF has been exhaustively tested for management of phytopathogens in flowers and fruits, its role in soybean disease management needs additional experiments. Thus, for further elucidation *P. pachyrhizi* management by using FF, tests with pure chitosan is the next step of our studies.

### Using chemical tools to discriminate *Tapinoma* species.

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Communication among social insects is fundamental between individuals, castes and societies. This is achieved through chemical recognition and ensures that altruistic acts are directed towards relatives. The nestmate and species discrimination in ants is based on antennal detection of non-volatile chemicals found on the cuticle. These cuticular compounds are species specific and can be used as biosystematic tools. Actually, discriminating some of the *Tapinoma* species is a difficult undertaking with only variations in the clypeal cleft shape of queen or workers. Currently male genitalia analysis provides the only secure method for differentiation. In order to simplify the differentiation of five species of *Tapinoma* (*T. erraticum*, *T. israelis*, *T. madeirense*, *T. nigerrimum*, and *T. simrothi*) we used gas chromatography coupled with GC-mass spectrometry to identify, compare and quantify cuticular hydrocarbons (CHCs). We found that each species possesses unique pattern of CHCs. Across the five species, 154 CHCs were identified. We found n-alkanes, monomethylalkanes, dimethylalkanes and trimethylalkanes occurring between C23 and C33. Unlike the n-alkanes and monomethylalkanes, there was a large diversity of specific dimethylalkanes that makes them likely candidates for nestmate and species discrimination signals.

**Keywords:** *Tapinoma*, Nestmate recognition, cuticular hydrocarbons, gas chromatography, GC-mass spectrometry, biosystematic.

### Identification of an ant queen pheromone regulating worker sterility

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Social insect societies range from paragons of cooperation to uneasy alliances in which conflict is rife. The interplay between altruism and self-interest is especially apparent in the evolution of worker sterility. In many species, queens are thought to produce primer pheromones that modulate worker sterility; these may either be honest signals of quality to which it benefits workers to respond, or manipulative agents of control. Although queen pheromones underpin both the proximate and ultimate causes of reproductive division of labour, progress has been hampered by the fact that they have only been conclusively identified in the honeybee. By synthesising candidate pheromones, we experimentally demonstrate that the queen surface hydrocarbon 3-MeC<sub>31</sub> reduces worker ovarian development and aggression in the ant *Lasius niger*. Production of 3-MeC<sub>31</sub> is lower in immune-challenged queens, showing that it is condition-dependent and potentially costly. The chemical is also correlated with queen fertility and ovarian activation, and is potentially used by workers to identify and eliminate unproductive queens in multi-queen colonies. The pheromone appears to be an honest signal of the queen's value as a reproductive, suggesting that workers altruistically remain sterile as long as their queen is healthy and fertile.



### Male pheromones in *Drosophila*

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*Drosophila melanogaster* produces sexually dimorphic pheromones, with C23 and C25 monoenes produced in males and C27 and C29 dienes produced in females. In non-African (cosmopolitan) flies, 7-tricosene (7-T) and 7,11-heptacosadiene (7,11-HD) are the main hydrocarbons in males and females, respectively, whereas some African populations have high amounts of 7-pentacosene (7-P) and 5,9-heptacosadiene (5,9-HD) in males and females, respectively. A strong reproductive isolation has been demonstrated between these two types of populations.

Interestingly, some African populations show high levels of 7-P in males while 7,11-HD is the major hydrocarbon in females. We do not yet know whether there is a reproductive isolation between these populations and cosmopolitan ones. Here we study a population from Cotonou, which displays this peculiar pheromonal type. The courtship behavior of these flies is studied and discrimination of Cotonou or cosmopolitan flies against mates from another pheromonal type is tested.

Results show that there is a reproductive isolation between the two pheromone races. Moreover, the Cotonou flies are more resistant to heat and desiccation, suggesting that this population could be more adapted to the hot and dry conditions occurring during the winter season.

## Chemical diversity in the cuticular lipids of adult papilionid butterflies

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Adult butterflies use visual cues for mating. Moreover, in several butterfly species, male adults emit characteristic volatile compounds, which serve as sex pheromones. In contrast, the effects of cuticular lipids have been little investigated in the mating system of butterfly. Several papilionid butterflies sympatrically distribute and the certain species are similar to each other in appearance. For example, *Papilio xuthus* and *P. machaon* are commonly adorn with a black-yellow striped coloration. While *Papilio polytes* and *P. protenor* have black wings, *Atrophaneura alcinous* and *Pachliopta aristolochiae* possess red-spotted black wings. As the sympatric existence of related species with a similar color pattern would disrupt mating selection based on visual cues, these adults are expected to use cuticular lipids as the additional cues. In the present study, these six papilionid butterflies were examined for chemical composition of cuticular lipid.

Cuticular lipids were extracted with dichloromethane from each 3-d-old adult. Total 20 samples (10 males and 10 females) were prepared for each species and were subjected to GC-MS analyses. The six species showed different compositions of cuticular lipids, from which several hydrocarbons (C<sub>23</sub> to C<sub>31</sub>) and hexadecanoic acid were identified as the major components. In principal component analyses for the cuticular composition, *P. xuthus* and *P. machaon* showed sexual similarity, whereas the other four displayed sexual dimorphism. These results suggest that the papilionid butterflies possess species-specific cuticular lipids and that the four species with black wings possibly use the chemical signature for recognition of different sex.

### Is the clue to low attractivity of the female winter psylla hidden in her cuticle?

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The pear psylla, *Cacopsylla bidens*, is seasonally dimorphic, producing a dark overwintering adults (winterform- WF) and the light-colored summerforms (SF). Summerform males are attracted to the odors of summerform female (1). The winterform morph is produced by nymphs that were exposed to short photoperiods and cold temperatures. Winterform exhibits slow ovarian development and reduced rates of mating (2.). The aim of this study was to evaluate the effect of psylla sex and morphotype on the composition of chemical cues and to elucidate the role of these chemicals in sexual attraction. Hexane body washes were prepared from males and females summer and winterform psylla and analyzed by GC-MS. The analyses revealed a series of long straight chain and branch hydrocarbons along with long chain aldehydes. Most of the compounds were in common to both sexes and morphs. However, discriminant analysis revealed morph and sex effects on chemical composition. It clearly discriminated between the Male-SF and Female-SF extracts. Moreover, summerforms appear distinct from winterforms yet, overlap between Male-WF and Female-WF extracts was noted. Further analysis confirms high similarity in profile of male and female winterforms. These data are consistent with the observations that winterform females are less attractive to males than summerform females. The significance of specific compounds in this process will be discussed.

#### *Acknowledgments*

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#### *References cited*

1. Soroker, V. et al. 2004 J. Insect Behavior, 17: 613-626.
2. Soroker, V. et al. 2003. IOBC wprs Bulletin 26: 83-89.

### **Plant epicuticular waxes mediate the interaction between oviposition induced synomone and egg parasitoid**

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Oviposition-induced plant synomones are used by egg parasitoids during host selection. Here we show that, in the tri-trophic system *Brassica oleracea* - *Murgantia histrionica* - *Trissolcus brochymenae*, the latter responds, in a closed arena and in a static olfactometer, to induced chemicals that are perceived from a very short range. An additive or synergistic effect due to egg deposition, feeding punctures and chemical footprints of *M. histrionica* was observed. When all such three phases were present, the parasitoid reacted to the induced synomone locally on the treated leaf area, at a close distance from the treated area, and systemically on the leaf above the treated one. When plants with host footprints combined with feeding punctures or with oviposition were tested, responses were obtained both locally and at close distance, whereas in the remaining assays only local responses were observed. Induction time was lower than 24h whereas signal duration was apparently related to the period of suitability of the host eggs. The role of epicuticular waxes in synomone perception by the parasitoid was investigated. Mechanically and chemically removing the wax layer resulted in the removing of oviposition-induced synomones, as indicated by the lack of behavioral response by the parasitoid. Conversely, chloroform extracts of the wax surface, applied on filter paper, induced arrestment behavior in the parasitoid. Oviposition-induced contact synomones have an important role in the host location process by the egg parasitoids in combination with contact kairomones, and therefore they show a relevant ecological role as indirect plant defenses.


### Visitors and social parasites of wasp colonies use different chemical integration strategies

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Social parasites enter well-guarded insect colonies. They evade detection by hosts and succeed into integrating fully into the host social hierarchy. Social parasites usually attain social integration into host colonies via chemical similarity, a process which requires time. Instead, chemical concealing strategies of cleptoparasites are rarely studied. Colonies of the social wasp *Polistes biglumis* are targeted by both *Mutilla europaea* (Hymenoptera: Mutillidae), possibly a cleptoparasite, and by *P. atrimandibularis* (Hymenoptera: Vespidae), an obligate social parasite. Chemical analyses of epicuticular hydrocarbons show that the chemical profile of *M. europaea* is species-specific, qualitatively and quantitatively poor, has species-specific proportions of alkenes and branched alkanes and some degree of similarity to that of the target species *P. biglumis*. In contrast, the chemical profile of the social parasite *P. atrimandibularis* adjusts to perfectly matching that of its *P. biglumis* host. The chemical strategies of concealment in social wasp colonies parallel how exploiters interact with the target species; the visitor *M. europaea* has a neutral and weak recognition signal which may limit detection whereas the social parasite *P. atrimandibularis* attains a strong signal which allows detection and stable integration into the host social structure.



Poster Session  
S5. Evolutionary aspects of chemical  
communication



## Cineole synthases in *Nicotiana*. sec. *Alatae*- a synapomorphic marker

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The genus *Nicotiana* belongs to the family of the Solanaceae. The genus is divided into the subgenera *Petunoides*, *Rustica* and *Tabacum*, furthermore into fifteen sections, and 75 species are known (1).

The scent volatiles from the sections *Alatae*, *Rusticae* and *Suaveolentes* were analysed (1). Species of *N.* section *Alatae* present a monoterpene dominated bouquet (simultaneous emission of 1.8-cineole, limonene,  $\beta$ -myrcene,  $\alpha$ -pinene,  $\beta$ -pinene,  $\alpha$ -terpineole, sabinene; the „cineole cassette“), while plants of *N.* section *Suaveolentes* predominantly emit benzenoid compounds. Only the eponymous species *N. suaveolens* is an exception and emits the „cineole cassette“ (2). A cineole synthase (CIN) was isolated from *N. suaveolens*, it is a multi product enzyme releasing the “cineole cassette” monoterpenes (3).

To investigate whether the CIN is a synapomorphic marker of *N.* section *Alatae* we started the isolation of CINs from various species of *N.* section *Alatae*. A homology based RT-PCR strategy was used to find CINs in *N. alata* and *N. langsdorffii*. cDNAs of both species contained 1632 bp and encoded a 550 amino acid protein. Both enzymes were overexpressed and produced the „cineole cassette“ monoterpenes. To investigate whether a CIN occurs in the common progenitor, various species from *N.* section *Alatae* and *Suaveolentes* will be screened successively and respective enzymes will be biochemically characterized.

1. Raguso, R. et al. 2006. *Phytochemistry* 67:1931-1942
2. Chase et al. 2003. *Annals of Botany* 92:107-127
3. Roeder, S. et al. 2007. *Plant molecular biology* 65: 107- 124



**Pheromones variation in the context of incipient speciation****BUATOIS B<sup>a</sup>, GANEM G<sup>b</sup>, DAVIDIAN CE<sup>b</sup>**

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Two subspecies of house mice (*Mus musculus domesticus* & *M. m. musculus*) share a hybrid zone in Europe. Divergences of mate preference and of urinary signals were pointed out between the two subspecies in the context of maladaptive hybridization. A recent study revealed quantitative differences between males of the two subspecies involving several organic molecules two of which are: 2,3-dehydro-*exo*-brevicomine (DHB) and 2-*sec*-butyl-4,5-dihydrothiazole (SBT), two male pheromones related to dominance and present in larger proportions in *M.m.d.* than in *M.m.m.*

The present study aimed to i) confirm chemical divergence, assess relative concentrations and within subspecies variation; ii) test whether pheromone differences relate to male dominance and female preference; iii) test potential for conflict between information on quality (dominance) versus compatibility (subspecies).

We used the SPME procedure to sample volatiles in 10µl urine at 22°C during 10 min. Calibration curves were obtained after injections of known concentrations of SBT and DHB directly in the GC/MS, using the quantitative procedure implemented in the machine programme (standards provided by Prof. Mori, Univ. of Tokyo). Behavioural responses were assessed during dyadic encounters and two-ways choice tests.

Our results i) confirm differences in DHB/SBT; ii) female *M.m.m.* prefer *M.m.d.* rather than *M.m.m.* dominant males and DHB/SBT are higher in dominant *M.m.d.*, however consistent correlation between levels of pheromones and social rank was not found; iii) divergence between subspecies seems to predate their contact and potential for a conflict between quality/compatibility information could exist. We discuss the implications of these results for the speciation process.

## Chemical phenotypic divergence: Diet-related modification of cuticular hydrocarbon profiles in a leaf beetle

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Host shifts by herbivorous insects can promote speciation. Genetic divergence of herbivores on the ancestral and the novel host plant may result in reproductive isolation which is reinforcing divergence and driving formation of new host races. Here, we investigated the impact of a host species on phenotypic divergence of herbivores and tested the hypothesis that plant-induced phenotypic divergence leads to assortative mating. First, we studied whether a plant-induced change of the beetles' cuticular hydrocarbon (CHC) profile is a phenotypic change rather than a genotypic one due to selection of certain genotypes on the different plant species. Therefore, we addressed the question for how long the leaf beetle *Phaedon cochleariae* needed to feed on a novel host plant to significantly change its CHC profile. Adults which have spent their juvenile stage on Chinese cabbage and fed on watercress for only 14 days displayed a significant (phenotypic) shift of their CHC profile. Second, we investigated whether a host shift affects mate recognition which is known to be mediated by CHC <sup>[1]</sup>. Bioassays showed that males significantly preferred females or glass dummies treated with CHC extract from females reared on the same host plant as males. Hence, a plant-induced phenotypic change of mating signals of an herbivorous insect leads to changes of mate preferences. We suggest that in addition to genotypic divergence also plant-induced phenotypic divergence of insects may play an important role in speciation processes.

<sup>[1]</sup> Geiselhardt S, Otte T, Hilker M (2009) *J Chem Ecol* 35:1162-71

**An uncommon volatile oil-marker in oil-producing plants?**SCHAEFFLER I<sup>a</sup>, DOETTERL S<sup>a</sup><sup>a</sup> University, Bayreuth, GERMANY

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Floral oil is an alternative reward to pollen and nectar, which is only used from specialized bees to provide their offspring and to line their cells. Besides several interactions worldwide only one occurs in the Holarctic, which consists of the bee genus *Macropis* (Melittidae) and species in the plant genus *Lysimachia* (Myrsinaceae) (1).

Until now it is not clear how the bees find their oil hosts, but we could show that pentane solvent extracts of *L. punctata* flowers are highly attractive to *Macropis* bees (2). Further we analyzed solvent extracts of several Holarctic and non-Holarctic oil plants by GC-MS (Gas chromatography - Mass spectrometry) and tested several on the antennae of *M. fulvipes* by using GC-EAD (GC-Electro-Antennographic Detection). EAD-active compounds were identified and tested for their attractiveness to *M. fulvipes* bees.

In the GC-MS analyses we found in most of the oil-producing plants glycerols esterified with one, two or three acetic acid molecules (Monoacetin, Diacetin, Triacetin). These compounds could be by-products in the biosynthesis of the fatty floral oil (3), and were not described as natural compounds before. Di- and Triacetin elicited a high response on *M. fulvipes* antennae and in preliminary bioassays, few bees were attracted by Diacetin. This compound may be a global mediator in the oil-flower oil-bee pollination system.

1. Vogel S. 1986 Tropische und subtropische Pflanzenwelt 54: 1-168.
2. Dötterl S., Schäffler I. 2007 Journal of Chemical Ecology 33: 441-445.
3. Dumri K. 2008 Ph.D. Thesis, Leibniz Institute of Plant Biochemistry and Martin-Luther-Universität Halle-Wittenberg, Halle.

**Chemistry and anatomy of the frontal gland in termite imagoes**

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The frontal gland of termites represents a prominent defensive organ with no equivalent among other insects. It is a synapomorphy of advanced isopteran families Serritermitidae, Rhinotermitidae, and Termitidae. While the frontal gland of termite soldiers was studied in detail, its presence in imagoes was quite ignored. Bearing in mind the occurrence of this gland in imagoes, we ask the following questions: What is the anatomy and ultrastructure of the frontal gland in termite imagoes and the chemical composition of its secretion? How are these related to the situation in soldiers? Consequently, what is the purpose and evolutionary significance of this gland in isopteran imagoes – the future kings and queens? We describe here the results of our investigations on the anatomy of the frontal gland and chemical composition of its secretion in imagoes of the rhinotermitids *Prorhinotermes simplex*, *P. canalifrons*, *Coptotermes testaceus*, *Glossotermes oculatus*, *Psammotermes hybostoma*, *Dolichorhinotermes longinasus*, and *Heterotermes tenuis*. We compare these observations with the situation in soldiers of the studied genera and discuss the relevant evolutionary scenarios.

**Effect of coniferous bark components on aquatic organisms**SAIJO H<sup>a</sup>, TSURUTA K<sup>a</sup>, KUSUMOTO N<sup>b</sup>, ASHITANI T<sup>a</sup>, TAKAHASHI K<sup>a</sup><sup>a</sup> Yamagata University, Tsuruoka, JAPAN<sup>b</sup> Iwate University, Tsuruoka, JAPAN

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In Japanese forest, a conifer tree of Sugi (Japanese cedar: *Cryptomeria japonica* D.Don) have been planted and accumulated in large amounts to make housing materials. The Sugi tree contains several bioactive components in their bark, and heart wood <sup>1)</sup>. The components of Sugi have influences on organisms in Japanese forest. However, the activities of these components have not been studied against aquatic organisms. In this study, the bioactivities of the components contained in Sugi bark were examined against aquatic planktons, red tide plankton *Heterosigma akashiwo* as a phytoplankton and brine shrimp *Artemia salina* larva as a zooplankton. The inner bark and outer bark of Sugi were successively extracted with *n*-hexane, ethyl acetate, and methanol.

In the assay of *H. akashiwo*, growth inhibition activities were shown in low polar components (i. e. *n*-hexane and ethyl acetate extracts from inner bark, and *n*-hexane extract from outer bark). Cubebol, phyllocladanol, 6,7-dehydroferruginol, ferruginol, and sugiol were detected as main components in the active extracts by GC-MS analysis. The each main component was isolated by SiO<sub>2</sub> column chromatography, and the activities against *H. akashiwo* were examined. Strong growth inhibitions against *H.akashiwo* were observed in cubebol and ferruginol. Growth inhibition activities against brine shrimp larva were also observed in low polar components, *n*-hexane and ethyl acetate extracts from inner bark. Therefore, in Japanese forest, it was suggested that the Sugi trees were related to the water ecosystems through the low polar components contained in their bark.

## Reference

1) Kofujita, H. et al. 2006. *Holzforschung*, 60 :20-23.

## The evolution of venom alkaloids in *Megalomyrmex ants*, from predators to social parasites

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A number of Myrmicine ant species in the tribe Solenopsidini are known to synthesize volatile alkaloids excreted from their sting. In *Monomorium*, *Solenopsis*, and *Megalomyrmex*, venom includes a diversity of monocyclic and bicyclic alkaloids, but also species-specific heterocyclic compounds that can be used as taxonomic characters. Some of these alkaloids have been shown to function as repellents against other ant species but for most, the function is unknown. The genus *Megalomyrmex* comprises 31 described species.

Eight *Megalomyrmex* species, classified in the *silvestrii* species group, are social parasites of fungus-growing ants while the other *Megalomyrmex* species are free-living predators. These fungus-growing ant associates infiltrate then consume the fungus garden and brood of the host colony. In this study we have identified 21 alkaloids found in 14 species spanning the *Megalomyrmex* phylogeny. Using a phylogeny constructed from five genes (protein-coding EF-1 alpha F1, EF-1 alpha F2, wingless, CAD, and mtCOI) alkaloids are examined in a phylogenetic context. Among the species studied, pyrrolines are found only in predatory free-living species, piperidines are found only in social parasites, and pyrrolizidines and pyrrolidines are found in both types of ants. The evolutionary implications of these results are discussed.

**Novel sex pheromones produced by emerald moths**YAMAKAWA R<sup>a</sup>, NGUYEN DD<sup>b</sup>, KINJO M<sup>c</sup>, ANDO T<sup>b</sup><sup>a</sup> Tokyo University of Agriculture and Technology, Tokyo, JAPAN<sup>b</sup> Tokyo University of Agriculture and Technology Graduate School of Bio-Applications and Systems, Tokyo, JAPAN<sup>c</sup> University of the Ryukyus, Okinawa, JAPAN

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Lepidopteran Type II sex pheromones, which are biosynthesized from dietary linolenic and linolic acids, are mainly composed of 6,9-dienes, 3,6,9-trienes, and their epoxides and identified from the species in highly evolved families, such as Geometridae. This group is classified into several subfamilies, such as Larentiinae, Ennominae, and Geometrinae, and sex pheromones have been exclusively studied in species in the first two subfamilies. Geometrinae includes many species called emerald moths with green wings; however, the pheromones from these species have not been identified, indicating a high probability of finding new pheromone compounds in the emerald moths. In order to understand Type II pheromones in depth, we collected male and females in several emerald moths using a light trap and analyzed their pheromone extracts using GC-EAD and GC-MS. Consequently, in addition to known unsaturated hydrocarbons, we could find novel Type II pheromones, (*Z,Z,Z*)-6,9,12-octadecatriene from *Hemithea tritonaria* and (*Z,Z,Z,Z*)-3,6,9,12-icosatetraene from *Thalassodes immissaria intaminata*. The triene was synthesized by a double Wittig reaction between hexanal and an ylide derived from (*Z*)-1,6-diiodo-3-hexene. The tetraene was synthesized by a coupling between (*Z*)-3-undecenal and an ylide derived from (*Z,Z*)-1-iodo-3,6-nonadiene. Furthermore, pheromone gland extracts of both species interestingly contained (*E,E*)- $\alpha$ -farnesene as an EAG-active component. A field evaluation of the synthetic compounds showed that the Type II components were essential for male attraction and (*E,E*)- $\alpha$ -farnesene acted as a synergist. This is the first mating communication system of lepidopteran species mediated by farnesene, a representative sesquiterpene.

## Macroevolutionary Chemical Escalation In An Ancient Plant-Herbivore Arms Race

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A central paradigm in the field of plant-herbivore interactions is that the diversity and complexity of secondary compounds in plants have intensified over evolutionary time, resulting in the great variety of secondary products that currently exists. Progressive steps of herbivore attack and plant defense are thought to be largely responsible for the incremental elaboration, proliferation, and intricacy of plant secondary compounds. Despite the prominent conceptual role of the herbivore-plant arms race paradigm, very few studies have tested for chemical escalation in plants. Another important question is whether this ostensible chemical escalation may have resulted in an overall increase in plant chemical diversity over time. We tested whether the defensive chemistry of a group of plants, the tropical genus *Bursera*, has intensified over evolutionary time and determined whether chemical escalation in each species has resulted in an increase of the total number of chemical secondary structures in the genus over time. The results confirm that as new species diverged over time they tended to be armed not only with more compounds/species, but also with compounds that could potentially be more difficult for herbivores to adapt to since they belong to an increasing variety of chemical pathways. Also, as predicted by theory, overall chemical diversity in the group increased over time. If this is representative of other plant groups, it could explain the high number of plant chemical secondary structures that currently exists.







Poster Session  
S6. Chemical ecology of multitrophic  
interactions



**Pheromone production and perception in *Anastrepha fraterculus***VANÍČKOVÁ L<sup>a</sup><sup>a</sup> *Institute of Organic Chemistry and Biochemistry AC CR, v.v.i., Prague, CZECH REPUBLIC**vanickova@uochb.cas.cz*

Males of the South American fruit fly, *Anastrepha fraterculus*, typically form leks to attract females by releasing a multi-component volatile pheromone. Experiments showed that the volatiles were produced by the salivary and rectal glands and are released from the mouth and anus. Previous pheromone analysis identified two monoterpenes limonene and  $\beta$ -ocymene, three isomers of the sesquiterpene  $\alpha$ -farnesene, the sesquiterpenes  $\beta$ -bisabolene and  $\alpha$ -bergamotene, benzoic acid, nonanal, C9-alcohols, the lactones anastrephin, epianastrephin and suspensolide, and four alkyl pyrazines (Cáceres *et. al.*, 2009; Lima *et. al.*, 2001).

The present study report the antennal activities of pheromone components, the sex specific differences in pheromone perception, and age and circadian - dependent pheromone production. The compounds were trapped on SuperQ, hexane eluted, measured by gas chromatography – electronantennography (GC-EAD on WAX and DB-1 columns), and two dimensional gas chromatography with TOF-mass spectrometric detection (GC $\times$ GC-TOFMS). Antennal activities were found for 6 components, namely for (Z)-3-nonenol, (Z,Z)-3,6-nonadien-1-ol, geranyl acetone, (E,E)- $\alpha$ -farnesene, suspensolide and epianastrephin. Sex specific differences in pheromone perception were observed: females were more sensitive to C9- alcohols than males, sensitivity to other EAD active compounds were equal for both sexes. Age and circadian - dependent pheromone production is now under investigation.

*References*

- Cáceres, C. *et. al.* 2009. *Biological Journal of the Linnean Society* 97 :152-165.  
Lima, I. S. *et. al.* 2001. *Journal of Brazilian Chemical Society* 15:196-201.

## **Pyrrrolizidine alkaloids (PA) - Deterrent effects on honey bees (*Apis mellifera*)?**

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PA constitute a group of secondary plant defense compounds of a variety of plants generally belonging to one of four plant families within the angiosperms; the Asteraceae, the Boraginaceae, the Apocynaceae and the genus *Crotalaria* within the Fabaceae. So far, more than 400 different structures are known<sup>1</sup>. The 1,2-unsaturated PA are known to be toxic and were responsible for a number of intoxications of humans and livestock in the past. Recent studies have demonstrated the occurrence of PA in honey and pollen loads of bees intended for human consumption. Genotoxicity of chronic exposure of low levels of PA in the food chain are subject of current scientific and legislative discussions<sup>2</sup>.

Little is known about the influence of PA on the fitness and the collecting behavior of honeybees which are the primary consumers and vehicles for the transfer of these toxic plant compounds into food products.

Against this background the collecting and feeding behavior of trained honeybees in choice-test outdoor tests with plant-PA in their naturally occurring forms (tertiary PA and the PA-*N*-oxides) were studied. The applied concentrations ranged from 0.2 to 0.0002% PA in sugar solutions.

The results for the different PA-forms are discussed in terms of concentration dependent deterrence effects and possible implications for beekeepers on management practice like hive location, harvest conditions and general impacts on the overall health effects on beehive.

[1] Hartmann, T., et al. 1995. Pelletier, S.W. Pergamon Press, Oxford Vol.9:155–233.

[2] Kempf, M., et al. 2010. Mol. Nutr. Food Res. 54: 1–11.

## Geographical variation of odour profiles between two *Arum* species deceiving their insect pollinators.

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We investigated geographical variations of a plant/pollinator interaction among populations of two *Arum* species which show different degrees of specificity for their fly pollinators.

In two consecutive years, we collected insect pollinators and compared inflorescences odour profiles collected by Solid Phase Micro Extraction (SPME) in these *Arum* populations. We confirmed that from a pollination point of view, *Arum italicum* is an opportunist species, as it is mainly pollinated by insects of the families Psychodidae, Chironomidae and Sciaridae, whereas *Arum maculatum* is a specialist species, as it is 90% pollinated by Psychodidae. In all populations, *A. italicum* was less attractive to pollinators than *A. maculatum*. Floral odour profiles of *A. italicum* were not geographically structured among populations, suggesting a high gene flow or adaptation to a fluctuant guild of pollinators. On the contrary, odour profiles of *A. maculatum* varied between the two populations studied suggesting a lower gene flow or adaptation to different local pollinator preferences.

In conclusion, the interaction between these two *Arum* species and their pollinators forms a geographic mosaic of coevolution, where a floral syndrome, the floral odour composition, and pollinator numbers and diversity vary geographically between populations, leading to different outcomes of the interaction among sites.

## Flavin-dependent monooxygenases as a detoxification mechanism in insects: New insights from the Arctiids (Lepidoptera)

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Insects experience a wide array of chemical pressures from plant allelochemicals and pesticides and have developed several effective counterstrategies to cope with such toxins. Flavin-dependent monooxygenases (FMOs) seem not to play a central role in xenobiotic detoxification in insects, in contrast to mammals. However, the previously identified senecionine *N*-oxygenase of the arctiid moth *Tyria jacobaeae* (Lepidoptera) indicates that FMOs have been recruited during the adaptation of this insect to plants that accumulate toxic pyrrolizidine alkaloids. Identification of related FMO-like sequences of various arctiids and other Lepidoptera show that FMOs in Lepidoptera form a gene family with three members (FMO1 to FMO3). Within the FMO1 gene cluster, a gene duplication early in the arctiid lineage provided the basis for the evolution of the highly specific biochemical, physiological, and behavioral adaptations of these butterflies to pyrrolizidine-alkaloid-producing plants. The genes encoding pyrrolizidine-alkaloid-*N*-oxygenizing enzymes (PNOs) are transcribed in the fat body and the head of the larvae. An *N*-terminal signal peptide mediates the transport of the soluble proteins into the hemolymph where PNOs efficiently convert pro-toxic pyrrolizidine alkaloids into their non-toxic *N*-oxide derivatives. Heterologous expression of a PNO of the generalist arctiid *Grammia geneura* produced an *N*-oxygenizing enzyme that shows noticeably expanded substrate specificity compared with the related enzyme of the specialist *Tyria jacobaeae*. The data about the evolution of FMOs within lepidopteran insects and the functional characterization of a further member of this enzyme family shed light on this almost uncharacterized detoxification system in insects.

### **Effect of drought on plant defenses mediating interactions between the Mountain Pine Beetle and mature pine trees**

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The Mountain Pine Beetle (*Dendroctonus ponderosae*) has destroyed 16.3 million ha of lodgepole pine (*Pinus contorta*; historical host) forest in British Columbia, Canada and has moved eastwardly into the hybrid zone of lodgepole x jack pine (*Pinus banksiana*; potential host) in Central Alberta. Some studies suggest that the beetle has the potential to survive and establish in jack pine and may spread all across the boreal forest to Eastern Canada. This study is part of an interdisciplinary project ([www.thetriaproject.ca](http://www.thetriaproject.ca)) that focuses on the genomics and chemical ecology of the three interacting organisms: the mountain pine beetle, its host pine trees and its fungal associates (e.g. *Grosmannia clavigera*).

In the summer of 2009, we investigated the chemical ecology of mature lodgepole x jack pine hybrids in response to different environmental conditions (water vs. no water) and biological treatments stimulating tree defences and emulating beetle attack. We tested whether monoterpene release from pine trees varies with water regime and biological treatments: control, mechanical wounding, inoculation with *G. clavigera* and beetle mash. The chemical profile of volatiles emitted from the boles of hybrid pines resembles a mixture of chemical profiles of the pure species. Monoterpene emission was higher in the fungal inoculated trees compared to the controls. Fungal growth resulted in reduced phloem moisture. Host chemical response to treatments will be linked to beetle fitness. This field season a similar experiment is conducted on pure lodgepole pine and jack pine at two field sites.



**Impact of insect generalist predators on tomato induced defenses**QUAGHEBEUR H<sup>a</sup>, FELTON G<sup>a</sup><sup>a</sup> Penn State University, University Park, USA*h.quaghebur@gmail.com*

Plant anti-herbivory defenses may be activated by various environmental clues associated to the presence of herbivores. Feeding, as well as less traumatic behaviors, such as oviposition or walking, have been documented to be detected as clues for upcoming herbivore damage in various plant species. They may lead to the activation of defense pathways and subsequently to transcriptome changes. In this study, we explored the possible impact of the presence of generalist predators on the induction of defense genes in tomato (*Solanum lycopersicum*). We originally focused on 2 Hemiptera predaceous species: the spined soldier bug *Podisus maculiventris* (Pentatomidae) and the minute pirate bug *Orius insidiosus* (Anthocoridae). The predator was caged on the youngest leaf of 4-week old plants. Leaf RNA was extracted and changes in gene expression were quantified by RQ-RT-PCR. We originally used the proteinase inhibitor II (*pin2*) as our marker for the induction of the jasmonic acid (JA) pathway. We documented the impact of the predators' gender, reproductive status, life stage, density or time of presence on the relative expression of *pin2* in the leaves. For each species, we also investigated the response of other genes associated to the JA or ethylene pathways to nymphs and adults. Finally, the impact of other predatory species from different orders on the induction of the JA pathway was tested. We will discuss the potential ecological relevance of our findings.

**Past or present: parasitoid attractants from *Brassica rapa* under caterpillar attacks**KUGIMIYA S<sup>a</sup>, SHIMODA T<sup>b</sup>, TAKABAYASHI J<sup>c</sup><sup>a</sup> National Institute for Agro-Environmental Sciences, Tsukuba, JAPAN<sup>b</sup> National Agricultural Research Center, Tsukuba, JAPAN<sup>c</sup> Center for Ecological Research, Kyoto University, Otsu, JAPAN*kugimiya@affrc.go.jp*

In order to find hosts, females of the solitary endoparasitoid *Cotesia vestalis* are known to use a blend of volatile organic compounds (VOCs) released from cruciferous plants infested with larvae of their host, the diamondback moth (*Plutella xylostella*). We investigated the flight response of female parasitoids to a cruciferous plant, *Brassica rapa*, using two-choice tests under laboratory conditions. The parasitoids significantly preferred plants that had been infested for 6 hr or 24 hr by the host larvae to intact plants. Parasitoids also significantly preferred formerly infested plants, 1 and 2 days after herbivory, to intact plants even though hosts were absent. Yet, parasitoids significantly preferred plants that had been infested for 24 hr to formerly infested plants, implying that parasitoids can distinguish between the VOC profiles of currently and formerly infested plants. Gas chromatography-mass spectrometry analysis of the headspace VOCs revealed that levels of benzyl cyanide and dimethyl trisulfide significantly decreased after removal of the host larvae, whereas terpenoids and their related compounds continued to be released at high levels. We tested the flight response of parasitoids to synthetic compounds presented with intact plants, and found that benzyl cyanide and dimethyl trisulfide attracted them in a dose-dependent manner, whereas the other compounds were not attractive. These results suggest that nitrile and sulfide compounds temporarily released from plants under current attack by host larvae are more effective attractants for the parasitoids than other VOCs that are continuously released from infested plants.

## **Non-pollinators use the volatile chemicals emitted by Mediterranean orchids**

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Orchids and their pollinators offer great examples of the role of chemical ecology in plant-insect interactions. Depending on the species considered, pollinators are attracted more or less specifically by volatile compounds emitted by orchids. We asked here whether non-pollinators also use these emitted scents to exploit orchids. We describe a field olfactometer device that allows us to test behavioural choices performed by insects (collected locally and very recently) when confronted with volatiles emitted by orchids under natural conditions, and thus to avoid experiments performed with stressed animals and/or transplanted plants. Non-pollinators tested here were 1) small grasshoppers that eat the labellum of *Ophrys araneola*, 2) crab spiders that capture insect visitors of *Orchis simia*, and 3) *Myrmica* sp. ants that exploit nectar produced by *Epipactis helleborine*. In all these cases, non-pollinators were significantly attracted by the volatiles emitted by the orchids concerned. These results demonstrate that non-pollinators, like pollinators, may also use chemical signals to exploit food sources associated with orchids.

## Attraction of flower visitors to plants that express indirect defence can minimize ecological costs of ant-pollinator conflicts

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Thousands of plant species throughout tropical and temperate zones secrete extrafloral nectar (EFN) to attract ants, whose presence leads to an indirect defence against herbivores. Because ants tend to defend reliable food sources against all types of putative competitors, it has been hypothesized that the presence of extrafloral nectaries close to flowers may lead to competition between ants and pollinators, or even to direct ant-pollinator conflicts. Such antagonistic interactions would reduce the access of pollinators to flowers and, thereby, may cause significant 'ecological costs' of indirect, ant-mediated defences.

We used Lima bean (*Phaseolus lunatus* L.) to study the effect of EFN induction within the inflorescences on the interaction between ants and flower visitors. EFN secretion was induced by applying an aqueous solution of jasmonic acid to the shoots. The results confirmed earlier observations that jasmonic acid (JA) treatment increased EFN secretion and that this leads to increased numbers of ants visiting the inflorescences. Increased ant presence resulted in a reduction of pollinator visits which supports the hypothesis of an ant-pollinator conflict. Future studies on ant-pollinator conflicts should consider the potential positive effect on flower visitors of a resistance induction within the inflorescences and its consequence for pollination efficiency.

## **Adaptation of battery-operated aquarium air pumps for on-site collection of air samples in a tropical forest for studying the responses of insects to the understory air's volatile organic compounds**

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Plants emit volatile organic compounds (VOCs) that play important roles in their interactions with other organisms in the ecosystem and have a major impact on atmospheric chemistry. Presently, very little is known about the effect of VOCs on insects present in the tropical forests of Northern Thailand, the area that is rich in biodiversity and ecosystems. We, therefore, carried out field-experiments on collecting samples of VOCs and insects in the understory air level and analyzed for the chemical composition of VOCs in relationship with the distribution of insect types in the area. In doing this, a battery-operated aquarium air pump was adapted to be a simple air-trap portable to be installed in the forest. This battery-operated device was connected with a plastic syringe tube packed with Super Q (Divinylbenzene/ Ethylvinylbenzene) polymer as VOC sorbent. The air-trap was tested for its efficiency and capacity of VOC collecting, and recovery of the volatile elution using head-space GC-MS. With 30 sets of the adapted air-traps installed together with insect traps at different 15 sites in Doi Phu Kha tropical forest of Northern Thailand, it was found that six VOCs, including  $\alpha$ -thujene, camphene, *p*-cymene, limonene, (1,8)-cineol and camphor were the major components, with the distribution of 4 insect types, namely Hymenoptera, Diptera, Coleoptera and Homoptera. The correlation between the individual VOCs and the existence of these insects was then analyzed, and the results will be presented.

**Kairomonal source from the safflower aphid *Uroleucon (Dactynotus) carthami* (L) host-plant complex to the generalist predator, *Coccinella septempunctata* (L).**

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Herbivore-attacked plants release chemicals that serve as olfactory cues for parasites and predators. The relation between volatile emissions from the safflower aphid, *Uroleucon (Dactynotus) carthami*(L) (Homoptera: Aphididae) its host plant , Safflower, *Carthamus tinctorius L* and the foraging behavior of its prey, *Coccinella septempunctata* (L) (Coleoptera : Coccinellidae) were studied in the laboratory. The predator's response towards the chemical cues emitted by the host plant, aphid alone and/or combinations were assessed. The leaf dip extracts of 1) the uninfested leaf of the host plant *C. tinctorius.*, 2) leaves of the safflower plants consisting 1000–1500 safflower aphids of all stages along with the frass, exuvia and other related materials, 3) leaves of the plants previously infested with the aphids and then cleaned with a wet paint brush to remove all the traces of aphids and rinsed in distilled water and 4) cuticular extracts of the aphids of all stages were utilized. We found that the predator, *C. septempunctata* was strongly attracted to the volatiles from cuticular extracts of the whole aphids. The safflower plants infested with aphids also evoked a quick directional response in the predators and caused arrestment in the close vicinity of the odour source while the leaf extracts of *C. tinctorius* plant and the solvent treatment used as controls failed to evoke any positive responses in *C. septempunctata*. These results indicate that foraging ladybird beetles, utilize the odour stimuli from the aphids themselves and the aphid-infested plants in order to locate their prey.

**Identification of chemical cues from the larvae of Scarabaeidae that mediate the host-seeking behaviour by the larva of the robber fly *Mallophora ruficauda* (Diptera: Asilidae)**

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For parasitoids host finding is a central problem that has been solved through a variety of behavioural mechanisms. Among species in which females do not contact directly the host, as in many dipteran parasitoids, larvae have to locate the host in the habitat through chemical cues. *Mallophora ruficauda* is a pestiferous species common to the open grasslands of the Pampas region of South America. Adults are predators of bees and larvae are solitary parasitoids of soil-dwelling scarab beetle larvae (Coleoptera: Scarabaeidae). Host searching in *M. ruficauda* is carried out by both larvae and adults. Females lay eggs in clusters on tall grasses. Upon eclosion, larvae fall to the ground, moult to the second instar and actively search for their hosts using chemical cues that originate in the posterior half of the host's body. There are 9 species of potential hosts in the distribution area of this parasitoid. A high preference has been observed for *Cyclocephala signaticollis* (87% of field parasitism), although the fly parasites other species. The aims of this work were to describe the chemical profile of all potential host species using body homogenates and GC analysis, and to determine which homogenates are attractive for the larvae through behavioral assays in a stationary olfactometer. Analytical procedures revealed the presence of large saturated hydrocarbons in the homogenates as potential components of the attractive cue. Also, the larvae of *M. ruficauda* showed positive orientation to 4 homogenates, and these results were concordant with the presence of these compounds in the chromatograms.

**Foraging behaviors and prey findings of *Harmonia axyridis* (Pallas) (Coleoptera: Coccinellidae) to in laboratory conditions**KANG TJ<sup>a</sup>, CHO MR<sup>a</sup>, CHOI DR<sup>a</sup><sup>a</sup> National Institute of Horticultural & Herbal Science, RDA, Suwon, REP. OF KOREA

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Foraging behaviors and prey findings of 4<sup>th</sup> and female adult *Harmonia axyridis* (Pallas) to *Aphis gossypii* Glover in laboratory conditions were examined. All the *H. axyridis* were isolated individually for 18~24 h before using for test. Each treatment was set up in the arena and then investigated the foraging behavior of *H. axyridis* within arenas for 12 hours by Insect activity meter. Treatment significantly affected the turning numbers, average velocities and turning angles of 4<sup>th</sup> instars of *H. axyridis* ( $p < 0.05$ ). Both 4<sup>th</sup> instar and female adult of *H. axyridis* exhibited two foraging patterns (extensive and intensive search) during searching behaviors. The response of female adult *H. axyridis* to the odor of different conditions of cucumber leaves were investigated in a Y-tube olfactometer. Dual-choice tests revealed that *H. axyridis* preferred aphid-infested cucumber leaves to other conditions ( $p < 0.05$ ). In small experimental cages in which different densities of *H. axyridis* and *A. gossypii* were released, correlations between the densities of *A. gossypii* and *H. axyridis* within cucumber plants were high. From the previous results, it was supposed that *H. axyridis* probably use physical contacts and herbivore-induced plant volatiles during foraging behaviors. In the future, more studies should be conducted under laboratory and field condition for using biological control agents.

Keywords: *Harmonia axyridis*; *Aphis gossypii*; foraging behavior, herbivore-induced plant volatiles



## **Involvement of microorganisms hosted in two-spotted spider mites (TSSMs) in the production of TSSM-induced volatiles in Lima bean plants**

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In response to herbivory, plants emit a specific blend of volatiles that attracts carnivorous natural enemies of the herbivores. These volatiles are called herbivore-induced plant volatiles (HIPVs). When infested by TSSMs, the accumulation of salicylic acid (SA) and the expression of SA-inducible genes were observed in Lima bean plants. Thus, we hypothesized that microorganisms hosted in TSSM were involved in the induction of specific blends of HIPVs. To address this hypothesis, we prepared axenic TSSMs.

Microorganisms hosted by TSSM were divided into two categories: exobacteria, i.e. gut bacteria, and endobacteria i.e. symbionts. We prepared mites without exobacteria (-Exo mites), mites without both bacterial kinds (-All mites), and control mites (non-axenic mites). In a Y-tube olfactometer, Lima bean plants infested by -Exo mites were more attractive than those infested by control mites, while plants infested by -All mites were less attractive than those infested by control mites. The amounts of jasmonic acid (JA) in plants infested by the three different TSSMs did not differ significantly, while those of SA were higher in -Exo mites infested plants than the other two infested plants. Based on these results, we discuss the possible involvement of the bacterial community in the production of a specific blend of TSSMs-induced lima bean leaf volatiles.

## The predatory mite *Amblyseius swirskii* avoids ovipositing near its dangerous thrips prey *Frankliniella occidentalis*

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Oviposition behaviour may be affected by presence of predators or killer prey. Here, we study the oviposition behaviour of the predatory mite *Amblyseius swirskii* in reaction to the presence of its counterattacking prey, the western flower thrips *Frankliniella occidentalis*. We offered the mites a choice between two oviposition sites, one with and one without food. In absence of the prey, adult female *A. swirskii* preferred ovipositing on a site with pollen, which is food for the predatory mite. This might facilitate the foraging for food by the immature offspring that will emerge from the eggs. When one of the sites contained thrips larvae, which are also food for the adult predators, female predators preferred ovipositing on the site without thrips. The number of thrips larvae consumed on the number of eggs produced was no significant suggesting that thrips density was not limiting egg production. In contrast, there was a significant effect of the number of thrips consumed on the fraction of eggs oviposited on the disc with thrips larvae. Because there was no effect of consumption of thrips larvae on total oviposition, this suggests that the predators that oviposited more on the disc with thrips larvae killed more thrips larvae, but this did not result in higher oviposition. Future studies should verify if the parental care displayed by predator in response to prey counterattack and, killing eggs of predators by thrips larvae because predators that move to another site to oviposit do not necessarily return.

**Host-chemical orientation in a dipteran soil dwelling larva****CRESPO JE<sup>a</sup>, LAZZARI C<sup>b</sup>, CASTELO MK<sup>a</sup>**

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The robber fly *Mallophora ruficauda* is one of the main apicultural pest in the Pampas region of Argentina. As adults, flies prey on honey bees and other insects, while as larvae they are solitary ectoparasitoids of the larvae of scarab-beetles. Females of *M. ruficauda* lay their eggs on tall grasses, i.e., away from the hosts. Larvae are dispersed by the wind, and once on the ground, they dig and search for a host. The preferred hosts of the second-instar larvae of *M. ruficauda* are third instar larvae of *Cyclocephala signaticollis*, and in the proximity, the parasitoid exhibit active searching behaviour. Although host-location seems to be mediated by chemical cues, neither the orientation mechanism, nor the sensory organs involved in host-location have been revealed. We carried out behavioural experiments in the laboratory to determine which is the main sensory input and how the parasitoid larvae make use of the chemical information to find their host. Our results show that larvae of *M. ruficauda* detect the host-associated chemicals by means of chemosensilia located in their maxillary palps. Only one functional maxillary palp was sufficient to successfully locate a host. The fact that bilateral integration is not necessary for orientation, suggests that the larvae of *M. ruficauda* orientate to their hosts by chemoklinotaxis. The capability to detect and orientate to odours based on successive sampling seems to be an efficient orienting strategy for a soil-dwelling larvae as *M. ruficauda*.

**Behavioral manipulation of stink bug egg parasitoids  
(Hymenoptera: Scelionidae) by field applications of  
semiochemicals.**

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The manipulation of the abundance and distribution of natural enemies by semiochemicals can improve biological control strategies (1). Here are presented two strategies for scelionid wasp's management in soybean crops. One is the use of (*E*)-2-hexenal, a green leaf volatile present in defensive secretions of stink bugs and used as a kairomon by scelionid wasps (2). The application of different doses (4 mg and 10 mg/plot) of (*E*)-2-hexenal on soybean fields with 0,25 ha showed that the abundance of parasitoids did not differed from control plots. However, in the (*E*)-2-hexenal treated plots the recruitment of parasitoids started in early phenological stage of the crop. A second strategy was the use of *cis*-jasmone, a phytohormone that induces indirect defenses in soybean (3). Field experiments in small plots (2 m<sup>2</sup>) was conducted spraying 6 ml of *cis*-jasmone solution (0,25 g *cis*-jasmone + 0,1 g de Tween20, in 1 L of water). The abundance and parasitism indexes in sentinel eggs of parasitoids in these plots was higher than in control plots during the tree initial weeks of the experiments. These preliminary results showed the potential of semiochemicals to help in conservative biological control. However, the application technique of these compounds need to be precisely established prior their incorporation in IPM.

*Acknowledgments.* FAPDF, CNPq and Embrapa.

*References cited*

1. Khan, Z. R. et al. 2006. *Biological Control* 45:210-224.2.
2. Laumann, R. A. 2009. *Journal of Chemical Ecology* 35:8-19.
3. Moraes, M. C. B. et al. 2009. *Entomologia Experimentalis et Applicata* 131:178-188.

## Optimising the use of allelochemicals for thrips pest management

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Thrips (Thysanoptera) are important pests of a range of plants in agriculture, horticulture and forestry. They are vectors of several important plant tospoviruses. Semiochemical-based strategies show great potential for reducing the current reliance on insecticides for controlling thrips. Only a limited number of pheromones have been identified for a few thrips species. However, a range of allelochemicals attractive and/or repellent to several thrips species have been identified, including pyridine compounds which form the basis for the commercially available thrips lure LUREM-TR. To date, this lure and its active ingredients have shown activity for nine different thrips species: *Frankliniella occidentalis* (Pergande), *Frankliniella schultzei* (Trybom), *Hydatothrips adolffriderici* Karny, *Megalurothrips sjostedti* (Trybom), *Thrips coloratus* Schmutz, *Thrips imaginis* Bagnall, *Thrips major* Uzel, *Thrips obscuratus* (Crawford) and *Thrips tabaci* Lindeman in a range of countries around the world. This lure is most commonly used in conjunction with coloured traps for improved thrips monitoring, but there is much potential for its use in other forms of thrips pest management including mass trapping, lure and kill, and lure and infect. A greater understanding of the intrinsic (e.g. hungry vs. starved, migrating vs. residing individuals) and extrinsic (e.g. humidity, temperature) factors influencing the response of thrips to these allelochemicals will be important for optimising their use in thrips pest management. This poster summarises some of the current knowledge in this area as well as key areas for future research.

## Inhibitory effects of synthetic insecticides on the foraging behavior of *Cotesia vestalis*, a larval parasitoid of the diamondback moth, *Plutella xylostella*

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Laboratory and field studies suggest that parasitoid wasps can find host-infested plants efficiently by using herbivore-induced plant volatiles (HIPVs) as a foraging cue. In some agro-ecosystems, however, their foraging behavior might be inhibited when broad-spectrum insecticides are sprayed on host-infested plants. In this study, we investigated the inhibitory effects of eight insecticides (diazinon, malathion, permethrin, etofenprox, methomyl, alanycarb, clothianidin, and emamectin benzoate) on flight response of female parasitoids (*Cotesia vestalis*) to cruciferous plants (*Brassica rapa*) infested with host larvae, the diamond back moth (*Plutella xylostella*), using two-choice tests in a laboratory. The female wasps showed a significant preference for host-infested plants that had been sprayed with distilled water to uninfested plants that had been sprayed with distilled water ("control plants"), supporting our previous study suggesting that *C. vestalis* can use HIPVs to find host-infested plants. The same tendency was obtained when infested plants treated with permethrin (1), emamectin benzoate or clothianidin vs. control plants were offered. The wasps did not discriminate between host-infested plants treated with alanycarb or diazinon and control plants. Interestingly, significantly more wasps selected control plants than host-infested plants treated with etofenprox, methomyl or malathion. These results suggest that there are different degrees of inhibitory effect of insecticides on the flight response of *C. vestalis* females. We will show how long insecticides affect their flight response under a laboratory condition.

*References* Kawazu, K. et al. 2010. Journal of Applied Entomology 134:313-322.

## Phenanthroindolizidine alkaloids: Defense chemicals found in the latex of *Ficus septica*

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Latex of a wild fig, *Ficus septica*, native to subtropical forests of Ishigaki Islands, Okinawa, Japan, shows a strong toxicity against larvae of Lepidopteran insects. In order to identify the chemicals responsible for the toxicity, we performed purification, chemical analyses and bioassays. The major components responsible for the toxicity were several phenanthroindolizidine alkaloids (PIAs), with the most abundant one being antofine. Antofine was highly concentrated in latex. The concentration of antofine in *F. septica* latex was high (0.67%), and was 50 times higher than that observed in *F. septica* leaves. Antofine showed very strong growth inhibition in very low concentrations. Thirty ppm of antofine (in fresh artificial diet) showed significant growth inhibition to the larvae of the Eri silkmoth, *Samia ricini*, and only 3 ppm of antofine showed significant growth inhibition to the larvae of the cabbage moth, *Mamestra brassicae*, and the silkmoth, *Bombyx mori*. Among the 8 *Ficus* species native to Ishigaki Island, only 2 species had PIA in latex, while some of the remaining species had cystein protease in latex as defense substance. The present results on diverse nature of defense substances in latex of *Ficus* species will help us understand the evolution of plant-herbivore interaction concerning *Ficus* species that are very important keystone species in the tropical ecosystem.

## Parasitoids discriminate healthy from parasitized aphid hosts using aphid semiochemicals

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The ability of an aphid parasitoid female to quickly find a healthy non-parasitized host provides a selective advantage as females avoid wasting foraging time and reduce the number of eggs laid in lower quality hosts. To avoid superparasitism, aphid parasitic wasps use markers present on the host either externally and/or internally. However, no information was available on the possible volatile odorant cues involved in this discrimination process. In the present study, we observed in a Y-olfactometer that *Aphidius ervi* and *Aphidius rhopalosiphi* (Hymenoptera, Braconidae), two generalist aphid parasitoids, were able to discriminate non-parasitized and parasitized *Sitobion avenae* (Hemiptera, Aphididae). In order to identify the chemical cues that guide their foraging behavior, we collected the odors released by healthy *S. avenae*, as well as by *S. avenae* parasitized for 2 and 6 days, with the use of an electronic nose. *Sitobion avenae* alarm pheromone, (*E*)- $\beta$ -farnesene (*EBF*), was the only chemical identified, and was found in lower quantities in parasitized aphids. Both parasitoid species provided pronounced electrical depolarizations to *EBF* in electroantennography (EAG), and both were significantly attracted to the latter compound in the Y-olfactometer. Parasitoid attraction were previously known to be guided by a wide variety of odorant cues released by plants and hosts, and our results support the hypothesis that the aphid alarm pheromone acts as a kairomone for *A. ervi* and *A. rhopalosiphi* leading them to discriminate healthy from already parasitized hosts.



## Food specialization of two oligolectic bumblebees on the toxic *Aconitum* genus (Ranunculaceae)

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Chemical protection is a widespread phenomenon in plants that develop under pathogen and herbivore pressures through plant evolution. The molecules involved are secondary metabolites that are found in key organs like roots, seeds and flowers. These compounds are highly diversified among plant groups. The *Aconitum* genus (Ranunculaceae) includes more than 250 toxic species also known as monkshoods with regard to their floral morphology. The toxicity derives from polycyclic diterpene alkaloids that are known to be neurotoxic. The most abundant compound is aconitine but a lot of other aconitine-type alkaloids are also present in plant tissues. Despite their high toxicity, the monkshoods are exclusively pollinated by bumblebees.

In this study, we focus on two oligolectic species highly specialized on *Aconitum* sp.: *Bombus gerstaeckeri* and *Bombus consobrinus*. These long-tongued subalpine bees are able to use nectar and pollen of *Aconitum* species as sole source of food for their colonies. We are developing a HPLC method to extract, identify and quantify the aconitine-type alkaloids from the pollen and nectar of two monkshoods species visited by *B. consobrinus* and *B. gerstaeckeri*, respectively *A. septentrionale* and *A. lycoctonum*. We assume that the toxin could provide protection against micro-organism and/or predator attacks to the bumblebees. From the plant point of view, this intimate association could specialize pollinators to improve pollination efficiency.

## The effect of variation in resistant traits of wild bean seeds on the performance and population dynamics of bruchids and their parasitoids.

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In order to predict new pest species and develop pest control techniques aimed at the disruption of host finding/acceptance, it is essential to understand the factors that determine how insects are attracted to and accept their host plants. Variation in resistance traits of plants can select for specialization in phytophagous insects. This could result in local adaptation where insects from different populations may adapt to the plants that are available in that locality. The common bean *Phaseolus vulgaris* originates from Central America, where the greatest diversity of wild plants and their associated insects is found. *P. vulgaris* seeds contain resistant compounds that result in decreased performance of the bruchid beetles feeding on them. However, the impact of these resistant traits on the third trophic level remains poorly understood. We conducted an extensive sampling of bean populations in Mexico known to vary in their resistant traits against bruchid beetles. Performance tests and population dynamics studies were conducted in order to determine the extent to which the resistance levels of the plant affect both bruchid beetles and their parasitoids. Bruchid and parasitoid performance was lower on higher resistant seeds; slower development time, lower survival rate and male biased sex ratio in parasitoids. The resistance pattern of the plants and the associated consequences affecting the insects suggest a great genetic variability in the wild seeds. These results will be further explored with local adaptation experiments.

## Possible manipulation of maize plant physiology by the Western corn rootworm

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Upon herbivory, plants mobilize systemic defenses and may reallocate resources to undamaged parts. The defense responses and resource reallocation can lead to plant-mediated interactions between herbivores that share a common host. Such effects can be positive or negative and are usually studied in the context of one herbivore species affecting the performance of another species. Here we report on a positive plant-mediated intraspecific interaction among temporally separated western corn rootworms (WCR), *Diabrotica virgifera virgifera*. WCR larvae were placed on roots from either healthy plants or from plants that had already been infested for four days with WCR larvae. We measured larval weight gain after six hours and found it to be considerably higher for larvae fed on previously infested plants compared to larvae fed on healthy plants. As possible mechanisms underlying this improved performance we propose that WCR larvae may manipulate the resource allocation of their host and thereby enhance the nutritional quality of the roots. Choice tests suggest that WCR feeding also increases the attractiveness of maize roots. It remains to be tested if at close range they prefer eating roots on which they perform best. Elucidating the physiological processes that allow WCR to perform so well on maize will not only contribute to our fundamental understanding of insect-plant interactions, but should also help in the development of novel varieties that can resist the pest.

## **Infochemical volatiles stabilizes multitrophic interaction**

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A phenomenon in which plants may respond to herbivore feeding activities by producing infochemical volatiles that in turn attract carnivorous enemies of the herbivores has been reported. These infochemical volatiles are not the mere result of mechanical damage, but are produced by the plant as a specific response to herbivore damage. We modify Lotka-Votrerra model by introducing infochemical mediated interaction and investigate the role of infochemical for multitrophic interactions theoretically and show that it stabilizes the interactions. We believe that infochemical plays important role for sustaining symbiotic relations in the eco-system.

## Chemical ecology of mosquitoes: a review of multitrophic interactions in Culicidae

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Early mentions about chemical ecology are not new with early observations of attractants in mammals from ancient Greeks or later by Butler (1609) in insects but serious investigations have only started recently with mentions of chemical signals in vertebrates by Darwin (1871) and insects by Fabre (1870). A major step has been passed in the fifties with Karlson, Luscher (1959) and Butenandt *et al.* (1959) introducing a new vocabulary and defining the major concepts of chemical communication. Since, progresses were made in the isolation, chemical identification, synthesis, confirmation of activity on animals in parallel with the development of analytical technologies (GC, HPLC, MS, SPME, NMR...). In the world of mosquitoes, it is not until 1922 with Rudolfs's work on the chemotropism of mosquitoes that the role of chemicals in their biology was explored. In between, studies by Sandhlo and Price (1962), Thorsteinson and Brust (1962), Hancock and Forster (1982), Healy and Jepson (1988) and Clements's textbook on mosquitoes (1999) synthesized several aspects of olfaction, sensory reception and behaviour. Since, odor-mediated host interactions have been investigated but it is recently that behavioural aspects like mosquito-plant interactions have been studied. This paper gives an overview of the past, present and future developments in the chemical ecology at different levels: insect-host preferences (attraction to vertebrates), insect-plant relationships (attractants and repellents), insect-insect (swarms...), insect-environment using new tools like the molecular biology of mosquito olfaction, electrophysiology and covering various aspects of emission, perception, odor coding...The results have implications in integrated vector management strategies.

## Intra- and interspecific interactions modulate the emission of alarm pheromone in pea aphids

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During parthenogenetic reproduction, the pea aphid, *Acyrtosiphon pisum*, can produce both winged and unwinged morphs. This phenotypic plasticity allows aphids to quickly adapt to changes in environmental conditions. When attacked by natural enemies, pea aphids produce more winged offspring, which is indirectly mediated by the aphid alarm pheromone, the sesquiterpene (*E*)- $\beta$ -farnesene (E $\beta$ F). Under natural conditions, alarmed aphids produced a higher proportion of winged offspring than undisturbed aphids. However, survival rates in the field were low compared to lab results, especially if aphids were alarmed by E $\beta$ F. Since E $\beta$ F emission incurs a large cost, it is likely that aphids reduce the emission of alarm pheromone under certain conditions to avoid being detected by natural enemies. By manipulating the colony size, we found that the emission of alarm pheromone was regulated by intraspecific interactions; aphids in groups released less E $\beta$ F than isolated individuals. The amounts of E $\beta$ F emitted in each colony were different, but sufficient to induce wing formation in groups of these respective sizes. The emission of alarm pheromone was also influenced by the type of predator. Pea aphids attacked by a larva of *Chrysoperla carnea* released higher amounts of E $\beta$ F and for longer duration than aphids that were attacked by a larva of *Coccinella septempunctata*. Furthermore, a higher proportion of cornicle droplets did not contain E $\beta$ F when aphids were attacked by *C. septempunctata* than by *C. carnea*. Therefore, the emission of E $\beta$ F was not only affected by innate traits of the pea aphids but also by inter- and intraspecific interactions.

## Reduced foliage herbivory in Bt cotton benefits phloem-feeding insects

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Genetically modified cotton plants that express Lepidoptera-active Cry toxins from *Bacillus thuringiensis* (*Bt*) are grown on 15 millions hectares worldwide. Numerous studies have established that these plants pose a negligible risk to non-target arthropods due to the narrow spectrum of activity of the expressed Cry toxins. However, potential indirect effects of *Bt* cotton have received little attention. We have thus studied the natural inducible defence mechanisms of cotton, specifically the induction of plant terpenoids, and whether they are affected by the introduced insecticidal trait. We hypothesize that the reduced damage caused by caterpillars in *Bt* cotton would lead to a lower concentration of cotton terpenoids. This could leave the plant vulnerable to attack by other herbivores such as aphids, which do not induce a defence response by the plant. We tested this hypothesis by monitoring the population dynamics of cotton aphids (*Aphis gossypii* Glover) on Lepidoptera-damaged and undamaged *Bt*- and non-*Bt* cotton plants in the greenhouse and in a field experiment. As hypothesized, aphids performed better on *Bt* cotton that were less damaged by caterpillars compared to the non-transgenic control plants. In a next step of the project cotton terpenoids will be analysed by HPLC to test whether cotton terpenoids are responsible for the observed differences.

**Molecular dialogue between cytokinin-producing *Wolbachia*, leafminer insects and associated host plants**KAISER W<sup>a</sup>, HUGUET E<sup>a</sup>, CASAS J<sup>a</sup>, GIRON D<sup>a</sup><sup>a</sup> *Institut de recherche sur la biologie de l'insecte IRBI, UMR CNRS, Tours, FRANCE**kaiser@univ-tours.fr*

The life-cycles of many organisms are constrained by the seasonality of resources. This is particularly true for leaf-mining herbivorous insects who use deciduous leaves to fuel growth and reproduction even beyond leaf fall. Our results suggest that an intimate association with bacterial endosymbionts might be their way of coping with nutritional constraints to ensure successful development in an otherwise senescent environment. We show that the phytophagous leaf-mining moth *Phyllonorycter blancardella* (Lepidoptera) relies on bacterial endosymbionts, most likely *Wolbachia*, to manipulate the physiology of its host plant resulting in the "green-island" phenotype - photosynthetically active green patches in otherwise senescent leaves - and to increase its fitness. Curing leaf-miners of their symbiotic partner resulted in the absence of green-island formation on leaves, increased compensatory larval feeding, and higher insect mortality. Our results suggest that bacteria impact green-island induction through manipulation of cytokinin levels. All individuals analyzed so far are closely associated with *Wolbachia* and a key enzyme of the cytokinin biosynthetic pathway (ipt: isopentenyl transferase) has been isolated, cloned and sequenced from the *Wolbachia* genome (tRNA-ipt Wo). This key enzyme is clearly expressed in *Wolbachia*-infected insects. This is the first time that insect bacterial endosymbionts are associated with plant physiology and we currently explore the presence of cytokinin-producing *Wolbachia* in different plant-leafminer systems and associated plant physiological alterations.



**Strategies of a generalist and a specialist egg parasitoid:  
Herbivore-induced plant volatiles in host location.**PEÑAFLORES MF<sup>a</sup>, WERNEBURG AG<sup>a</sup>, BENTO JMS<sup>a</sup><sup>a</sup> University of São Paulo, Piracicaba, BRAZIL*fernanda.penaflor@gmail.com*

Host location behavior of larval parasitoids has been intensively studied since herbivore-induced plant volatiles (HIPVs) have been shown to be important chemical cues. Recently, studies revealed that insect oviposition also elicits plant volatiles which are attractive to egg parasitoids, although this defense mechanism seems not to be widespread. Thus, egg parasitoids must be guided by other cues to locate host, such as host odors, that seem to play a role especially at short-range distance. The current study aimed to assess the olfactory response of a generalist egg parasitoid, *Trichogramma pretiosum* (Tricogrammatidae), and a specialist, *Telenomus remus* (Scelionidae), towards HIPVs released by maize attacked by their host *Spodoptera frugiperda* (Noctuidae) in Y-tube olfactometer. As fall armyworm is a multivoltine species we expected that HIPVs can be informative to egg parasitoids to locate host community. We also assessed if *S. frugiperda* females prefer to oviposit on attacked by conspecifics or undamaged plants. The results showed that *S. frugiperda* oviposits on conspecific-attacked plants; generalist egg parasitoid is innately attracted by volatiles released by freshly-damaged plants (mainly green leaf volatiles) and it is able to learn more complex blends latter released by plants. In contrast, specialist egg parasitoids were only attracted to volatiles emitted from freshly- and old-damaged plants after experience. These results revealed that egg parasitoids employ HIPVs to locate its host, however, depending on the host range, egg parasitoids differently exploit HIPVs.

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## Volatiles from infected citrus plant with 'Huanglongbing' affect response of psyllid *Diaphorina citri*

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'Huanglongbing' is currently considered the most serious disease of citrus, having as causal agent the bacteria *Candidatus Liberibacter* spp., exclusively transmitted by *Diaphorina citri* Kuwayama (Hemiptera: Psyllidae). With the advance of disease in the main regions of citrus in world, the interest of better understand the insect-plant-bacteria interactions is mandatory and it was raised the hypothesis that insect *D. citri* is able to recognize volatile compounds from infected citrus by pathogen. For this, the olfactory responses of *D. citri* were measured in Y-tube olfactometer, allowing the free choice of psyllids to odor fields. The presence of *Ca. L. asiaticus* in treated *Citrus sinensis* (L.) Osbeck variety Pêra, grafted on *Citrus limonia* (L.) Osbeck was confirmed by molecular biology. Only when plants became symptomatic the experiments were started and *D. citri* males and females were individually exposed to: (i) Healthy plants, (ii) plants infected by *Ca. L. asiaticus*, and (iii) clean air. The proportion of choices of *D. citri* to volatiles from health plants versus infected plants was 79 and 71% of males and females responses, respectively to infected plants (chi-square test,  $P \leq 0.05$ ). The results provide evidence that *D. citri* is not only able to distinguish, but also is attracted to volatiles induced by infection of bacteria *Ca. L. asiaticus* in citrus. Furthermore, this pathogen appears to have evolved in a way to promote changes in citrus plants, making them more attractive to sucking insect *D. citri* as a survival strategy to disseminate.

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Poster Session  
Outside session subjects



**HPLC-MS Analysis of Phytoalexins from *Salvadora persica***ELTAYEB E<sup>a</sup>, KHAN T<sup>a</sup><sup>a</sup> SULTAN QABOOS UNIVERSITY, MUSCAT, OMAN

Many plants defend themselves by accumulating newly synthesized antifungal compounds called phytoalexins. As part of a larger project, screening Omani plants for phytoalexin induction, we have looked at phytoalexin induction in *Salvadora persica*. Parts of this plant have been used as therapeutic agents against leprosy, eye and skin diseases. *In vitro* antimicrobial activity of extracts from the roots and leaf was studied against some bacteria. *S. persica* is used as chewing stick in the Middle East and Africa as an oral hygiene device for cleaning teeth and gum. The use of these sticks as a tooth brush is known to be effective in lowering candidiasis. No studies have been reported on phytoalexins production in Salvadoraceae.

Chemical induction of phytoalexins by the leaves of *S. persica* was achieved using CuCl<sub>2</sub> solution, and UV induction with wavelength 254 nm. Extracted phytoalexins were detected using (TLC)-*Cladosporium* bioassay followed by large scale preparative TLC. Eluted antifungal compounds were purified by TLC and identified by HPLC-MS.

*S. persica* leaves were induced with 10mM of CuCl<sub>2</sub> solution and exposure to U.V. for 20 minutes as abiotic elicitor. *Botrytis cinerea* was also used as biotic agent. Results showed the presence of a number of phytoalexins; Psorospermin, Gratiogenin and 1-Caffeoyl-β-D-glucoside and Diferulic acid produced by the leaves of *S. persica*. These were analyzed and identified by High Performance Liquid Chromatography-Mass Spectrometry (HPLC-MS). Other phytoanticipin antifungal compounds have also been detected and identified e.g., lupeolic acid.

Some aspects of the biological activities of these phytoalexins have also been studied.

**Short and long term effects of nasal obstruction: physiological and structural adaptation of diaphragm and orofacial muscles in rats****TRABALON M<sup>a</sup>, PADZYS GS<sup>a</sup>, MARTRETTE JM<sup>a</sup>, TANKOSIC C<sup>a</sup>, THORNTON SN<sup>b</sup>**<sup>a</sup> UMR 7178 CNRS, IPHC-DEPE, Vandoeuvre-Les-Nancy<sup>b</sup> INSERM U961, Vandoeuvre-Les-Nancy, FRANCE*trabalon@scbiol.uhp-nancy.fr*

We studied ultra-structural adaptation of diaphragm and orofacial muscles as well as hormonal responses to early chronic oral breathing (lasting for only 4 days) following reversible bilateral nasal obstruction performed on day 8 post-natal male rats. Muscle myosin heavy chain (MHC) composition and hormone levels were analyzed during two periods; 1 and 3 days after obstruction (days 9 and 11 post-natal), and following 3 months recovery with nasal breathing (90 days, adult). Diaphragm muscle showed significant increases in adult isoforms (MHC 1, 2a). We observed increases in MHC neonatal and adult type 1 isoforms in muscles involved with oral breathing, *Masseter Superficialis* and *Anterior Digastric*. No changes were observed in the *Levator Nasolabialis* muscle involved with nasal breathing. Reversible nasal obstruction was associated with reduced growth of the olfactory bulbs lasting into adulthood, and an initial decrease in lung growth followed by recovery at 90 days. Adrenal hypertrophy was observed after 1 day of nasal obstruction and lasted into adulthood. The “stress” hormone response was variable, increased (over 1000%) during the obstruction but normal by adulthood. An increase in plasma testosterone was observed throughout as was a decrease in thyroid hormone levels. Very short term nasal obstruction, *i.e* oral breathing, leads to long term hormonal changes and respiratory muscle adaptation.

## Metabolism of terpinolene which is produced by fungal attack in *Cupressus lusitanica* culture cells

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Secondary metabolism, especially related to monoterpenes, has been researched in *Cupressus lusitanica* (Mexican cypress) culture cells. The cells were derived from cambium of *C. lusitanica* tree and maintained in modified Gamborg B5 medium for more than ten years in our laboratory. The cells incubated in the medium don't produce any monoterpenes. However, when cells are incubated with fungal elicitor (partially purified yeast extracts), some volatile and non-volatile monoterpenes including  $\beta$ -thujaplicin (hinoktiol) are produced.  $\beta$ -Thujaplicin is one of the extracts of Cupressaceae heartwood and is a tropolone with a conjugated seven-membered ring. This compound has strong and broad antimicrobial spectrum.

Among some monoterpene synthases, expression of terpinolene synthase was dominant. Then, we revealed that terpinolene receives two steps oxidation with the microsomal fraction of elicited *C. lusitanica* cells and proved 1,6-epoxy-4(8)-*p*-menthen-2-ol was produced. The reaction caused by chytrome P450 monooxygenases and had strict stereoselectivity and stereo-regulation. The role and effect of the enzyme reaction product against fungi are not known yet, but we expect that this product is one of the intermediate of the biosynthesis of  $\beta$ -thujaplicin.



## Quantification of flavonol glycosides and studies on degradation of phenolics in fermented *Betula pendula* leaves extracts

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Quercetin glycosides are widespread in nature and occur in appreciable amounts in some medicinal plants, such as *Betula pendula* leaves (1). These natural compounds show a variety of biological activities such as – among others – the antioxidant activity deserves special interest (2). Moreover, they are also used as quality parameters of plant preparations.

Here, we report an easy to use validated RP–HPLC method allowing a reliable quantitation of quercetin aglycone and glycosides in birch leaf extracts. This method was applied to study the stability of flavonols in different batches of fermented aqueous extracts prepared from fresh leaves. Interestingly, a loss of nearly 100 % of the flavonol fraction was observed over one year, indicating that flavonol glycosides are quite unstable in aqueous solution compared to ethanolic preparations (3). HPLC–DAD and LC–MS runs from these samples revealed the presence of eight metabolites of phenolics, out of which quercetin and 3,4'-dihydroxypropiophenone were the most important one. Quercetin, accumulating in the first days of fermentation due to deglycosylation, was readily decomposed afterwards. These data broaden the knowledge about possible metabolites of phenolics from birch leaves.

### References cited

1. Bedgood, D. R. et al. 2005. Analyst 130 (6), 809-823.
2. Havsteen, B. 1983. Biochemical Pharmacology 32 (7), 1141-1148.
3. Bilia, A. R. et al. 2002. Journal of Pharmaceutical and Biomedical Analysis 30 (3), 613-624.

## Plasticity of antennal and behavioural response of *Caloptilia fraxinella* (Lepidoptera: Gracillariidae) to host volatiles

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*Caloptilia fraxinella* (Lepidoptera: Gracillariidae) is a long-lived moth that undergoes a period of reproductive inactivity from eclosion in July until reproductive activity in April. *C. fraxinella* can be collected at three different physiological stages during their adult life: summer, when moths eclose and the majority of the population is reproductively inactive; fall, when moths search for overwintering sites and the entire population is reproductively inactive; and spring, when moths emerge from overwintering and are reproductively active and fly to host ash trees (*Fraxinus* spp.). Male *C. fraxinella* display plasticity in antennal and behavioural response to sex pheromone during different physiological stages, with male response being most acute when moths are reproductively active in spring. Here we test if male and female response to host volatiles is plastic. We measured male and female antennal response to ash tree volatiles in the spring, summer and fall, and female behavioural response in the spring. Both males and females display plasticity in antennal response to host volatiles. Females have lower response to methyl salicylate and linalool in the fall than in spring and summer. Males have higher response to linalool in the summer than in spring and fall, and lower response to both (*E*)-2-hexenal and methyl salicylate in the fall than at other times of year. In the wind tunnel, more females flew upwind to ash seedlings compared to plastic control seedlings when tested in the spring. Further experiments testing the plasticity of behavioural response with physiological state need to be conducted.

## The Impact of Pyrrolizidine Alkaloids on Honey Bees

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Pyrrolizidine alkaloids (PAs) are a structural diverse group of toxic secondary plant metabolites which occur in an estimate of 3% of the flowering plants. Recently, studies connected to potential consumer health risks have demonstrated the occurrence of PAs in honey [1]. The natural PA sources are most likely pollen and nectar of PA plants. Therefore, the question arises how honey bees cope with detrimental PAs and to what extent they are adapted to the occurrence of PA in their natural food sources. For tertiary 1,2-unsaturated PAs increased mortality was observed for concentrations above 0.2%. Corresponding PA-N-oxides were found to be non-toxic even at levels of 2.0% PA in the diet. Instead, PA-N-oxides, which represent the dominant form of PAs show feeding deterrent effects at levels above 0.2% [2]. The results indicated food exchange (trophallaxis) among worker bees. Using this model system we could confirm the route from the floral PA via the stored nectar into the final product (honey) [2]. The development of bee larvae depends to a great extent on protein rich processed pollen supplied by nursing bees. Pollen is known to contain quite high amounts of PAs [1]. Feeding experiments with bee larvae showed that larvae are quite sensitive to tertiary 1,2-unsaturated PAs (factor of 10 compared to adult worker bees). Tracer experiments with <sup>14</sup>C-labeled senecionine showed that only minute amounts of PAs were absorbed into the haemolymph of nursing bees.

### References

- [1] Kempf, et al., 2010, MolNutrFoodRes, 54, 158-168.
- [2] Reinhard, et al., 2009, JChemEcol 35, 1086-1095.

**Sex Pheromone Biosynthesis in the Fall Webworm Moth,  
*Hyphantria cunea* (Arctiidae) - Part 1: Conversion of Linolenyl  
Alcohol into an Aldehyde component and Substrate Specificity of  
the Oxidation**

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The fall webworm is a harmful polyphagous defoliator. The female moths produce the following four pheromone components in a ratio of about 5:4:10:2; (Z,Z)-9,12-octadecadienal (**I**), (Z,Z,Z)-9,12,15-octadecatrienal (**II**), *cis*-9,10-epoxy-(Z,Z)-3,6-henicosadiene (**III**), and *cis*-9,10-epoxy-(Z,Z)-1,3,6-henicosatriene (**IV**). All of the components are expected to be biosynthesized from linoleic or linolenic acids in the diets, but different pathways are proposed for the aldehydes and epoxides noted above. It is interesting to identify the enzymes involved in biosynthesis as well as the mechanisms of the regulation of the mixing ratio. Before starting these studies, we confirmed the biosynthetic pathways and the substrate specificity of the enzymes.

As the first step of the study on the biosynthesis of the aldehyde **II**, the pheromone glands of virgin females were treated with <sup>13</sup>C<sub>18</sub>-linolenic acid or <sup>13</sup>C<sub>18</sub>-linolenyl alcohol. Products in the glands were extracted with hexane at 4 or 20 hr after the treatment, and then analyzed by GC-MS. While **II** including 18 <sup>13</sup>C showed almost the same Rt as unlabeled endogenous **II**, the labeled aldehyde with M<sup>+</sup> at *m/z* 280 was found in the pheromone glands treated with alcohol but not with acid. This result showed that **II** was biosynthesized *via* the corresponding alcohol by oxidation. Furthermore, long-chain analogs (10,13-nonadecadien-1-ol and 10,13,16-nonadecatrien-1-ol) were converted into the corresponding aldehydes in the pheromone glands, suggesting the low substrate specificity of the alcohol oxidase in the pheromone gland.

## Sex Pheromone Biosynthesis in the Fall Webworm Moth, *Hyphantria cunea* (Arctiidae) Part 2: Conversion of C<sub>21</sub> Triene into the Epoxydiene Component and Substrate Specificity of the Oxidation

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The female moths secrete *cis*-9,10-epoxy-(*Z,Z*)-3,6-henicosadiene (Z<sub>3</sub>Z<sub>6</sub>epo9-21:H) as a major pheromone component. This epoxydiene is expected to be produced by epoxidation of the corresponding C<sub>21</sub> triene (Z<sub>3</sub>Z<sub>6</sub>Z<sub>9</sub>-21:H), which is biosynthesized from dietary linolenic acid in oenocytes and transported to a pheromone gland *via* the hemolymph with the help of lipophorin. To confirm the final biosynthetic step in the pheromone gland, [19,20,21,21,21]-D<sub>5</sub>-Z<sub>3</sub>Z<sub>6</sub>Z<sub>9</sub>-21:H was synthesized by chain elongation of linolenic acid and LiAlD<sub>4</sub> reduction as key reactions. GC-MS analysis of the extract of pheromone glands topically applied with D<sub>5</sub>-Z<sub>3</sub>Z<sub>6</sub>Z<sub>9</sub>-21:H revealed the formation of D<sub>5</sub>-Z<sub>3</sub>Z<sub>6</sub>epo9-21:H, indicating specific epoxidation of the double bond at the 9-position in the female glands. Based on this result, the substrate specificity of epoxidase was examined by treatments with mixtures of D<sub>5</sub>-Z<sub>3</sub>Z<sub>6</sub>epo9-21:H and some other hydrocarbons, such as C<sub>19</sub>-C<sub>23</sub> trienes, C<sub>21</sub> diene (Z<sub>6</sub>Z<sub>9</sub>-21:H), and C<sub>21</sub> monoene (Z<sub>9</sub>-21:H). The 9,10-epoxy derivative of each unsaturated hydrocarbon was detected in the GC-MS analysis, while the yields of the epoxides from the C<sub>22</sub> and C<sub>23</sub> trienes and the C<sub>21</sub> monoene were poorer than those from the other trienes and diene. This experiment indicates that the substrate specificity of the epoxidase in the pheromone gland is low and the species-specific pheromone of *H. cunea* females is mainly owed to the critical formation of polyenyl hydrocarbons restricted to the precursors of epoxy components. By injection of the mixed DMSO solutions, we are examining the selectivity of the step for the transportation of the precursors incorporated into the pheromone gland from the hemolymph.

**Absolute stereochemistry of iridoid monoterpene widely distributed among Oribotritriidae mites (Acari: Oribatida)**SHIMIZU N<sup>a</sup>, YAKUMARU R<sup>a</sup>, SAKATA T<sup>b</sup>, KUWAHARA Y<sup>a</sup><sup>a</sup> *Kyoto Gakuen University, kameoka, Kyoto, JAPAN*<sup>b</sup> *Stanford University School of Medicine, Stanford, USA**shimizu@kyotogakuen.ac.jp*

The opisthonotal gland secretions of Astigmatid mites contain monoterpenes, sesquiterpenes, aromatics, hydrocarbons, aliphatic compounds, and other components. Through investigation of natural product chemistry, components of the excretory glands of oribatid mites collected from the field have been examined using mass spectrometry coupled with gas chromatography (GC/MS). Although major components are identified as "Astigmata compounds" together with aromatics not yet found in Astigmata, the secretions of two species (*Scheloribates azumaensis* and unidentified *Scheloribates* sp.) belonging to Brachypylina (one of the four glandulate cohorts of Oribatida) were found to contain pumiliotoxins, coccinellid alkaloids, and related alkaloids, which strongly suggests that oribatid mites belonging to Brachypylina are one important origin of pumiliotoxins and other alkaloids in poison frogs.

This study analyzed the secretion of an unidentified *Oribotritia* sp. belonging to Mixonomata; one detected major compound was apparently an iridoid monoterpene dial. Its planar structure and relative stereochemistry were confirmed by comparison of the GC/MS spectrum and retention time of natural compound with those of synthetic racemates. To determine the absolute stereochemistry, we investigated an enantioselective synthesis to obtain an optically active target diol using the Mukaiyama–Michael reaction as a key step. Consequently, the absolute configuration was determined as (5*S*,8*S*)-chrysomelidial through GC analysis with a chiral column. Chrysomelidial, which is widely distributed among Oribotritriidae species as a common defensive component, is known to serve as a defensive compound in leaf beetle larvae.

## Chemical warfare from invasive corals *Tubastraea* spp against potential competitors

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Around the world marine exotic species are modifying native communities and can cause irreversible negative ecological and economic impacts. Competition for space affects species distribution and community organization on tropical rocky shores and the presence of secondary metabolites in tissues of exotic species may aid them in establishing and expand their range. The aim of this study was to describe the range of chemical substances produced by the exotic sun corals *Tubastraea coccinea* and *Tubastraea tagusensis* and to experimentally test whether these varied in the field when these corals were placed in direct contact with two common native potential competitors (the coral *Mussismilia hispida* and the sponge *Desmapsamma anchorata*). After field experiments the colonies of *M. hispida* showed necrosis of areas of tissue where in contact with *Tubastraea* spp. Through GC/MS we detected 70% of sterols and fatty acids in the tissues of both species. In the competitive interaction experiment the treatment employing *T. coccinea* and *M. hispida* induced variation in the concentration of the sterol Ergosta-5,24-dien-3 $\beta$ -ol between control and treatment. For *T. tagusensis*, the concentration of the alkaloid 5-Bromoindole-3-carbaldehyde showed a decrease between control and treatment employing *D. anchorata*. This study demonstrated that the exotic corals produce allelopathic substances against a native coral which probably add to the effectiveness of this exotic genus in invading native rocky shore communities in the tropical southwest Atlantic. However, any action of chemical compounds produced in the presence of *D. anchorata* was not effective as the sponge overgrew the sun corals.

## Sex pheromone communication of the members of the subfamily Procridinae (Lepidoptera: Zygaenidae): new sex attractants

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The members of the subfamily Procridinae (Lepidoptera: Zygaenidae) developed sex pheromone systems – position of the pheromone gland, female calling posture and chemistry of the sex pheromones - that are significantly different from other lepidopterans and even from sex pheromone systems of other zygaenids. After identification of the main sex pheromone components in *Harrisina metallica* (= *H. brillians*) (1) and *Theresimima ampellophaga* (2), two new compounds, 2-butyl (7Z)-dodecenoate and 2-butyl (9Z)-tetradecenoate, were identified in a third member of the subfamily Procridinae – *Illiberis rotundata*, and both (*R*)- and (*S*)-enantiomers of each compound were synthesized (3) and screened in the field in Bulgaria, Hungary, Ukraine (Crimea), Armenia and Turkey. As a result new sex attractants were found for the following Procridinae species: *Rhagades pruni*, *Zygaenoprocris taftana*, *Adscita geryon*, *A. albanica*, *A. manni*, *A. obscura*, *Jordanita notata*, *J. anatolica* and *J. horni*.

References cited:

1. Myerson J. Et al. 1982. Tetrahedron Lett **23**: 2757–2760
2. Subchev, M et al. 1998. J Chem Ecol **24**: 1141–1151 and J Chem Ecol **25**: 1203 (erratum 1999)
3. Subchev M et al. 2009. Chemoecology **19**:47-54

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**Chlorophyll degradation in the gut of herbivorous insects****BADGAA A<sup>a</sup>, BOLAND W<sup>a</sup>**<sup>a</sup> *Max Planck Institute for Chemical Ecology, Jena, GERMANY**abadgaa@ice.mpg.de*

Loss of green color is the most obvious sign of nature. A pathway of chlorophyll breakdown, comprising several enzymatic reactions, has been elucidated in recent years [1]. Natural breakdown of chlorophylls occurs during specific plant developmental stages, such as leaf senescence and fruit ripening. A putative ecological role of chlorophyll degradation products has been discovered only recently by their deterrent function in fecal shields of larval tortoise beetles. Identified were pheophorbide, pyropheophorbide and other early degradation products [2]. This finding suggested a wider occurrence of pigments in feeding insects and still unknown functions in ecological interactions. To investigate the process of chlorophyll degradation in feeding insects we analysed five Lepidopteran larvae, *Spodoptera littoralis*, *S. eridania*, *Helicoverpa armigera*, *Heliothis virescens* and *Manduca sexta*, for the spectrum of chlorophyll catabolites in their gut and feces. Four early-step products in the feces, two products in the regurgitate, and chlorophylls *a* and *b* were identified and quantified in larvae of the five species after rearing on fresh leaves of *Phaseolus lunatus* and/or *Nicotiana attenuata*. The degradation products were not detected in fresh leaves of the food plants except of pheophytin *a/b*. The catabolites were determined as pheophorbide *a/b* and pyropheophorbide *a/b* by using LC-MS, LC-MS/MS, and UV absorption. The spectrum of metabolites can be attributed to the combined action of gut enzymes and the strongly alkaline milieu in the digestive tract.

1. Kräutler, B., Hörtensteiner, S., *Advanc. Photosynthesis. Respiration*, 2006. **25**: p. 237-260.

2. VencI, F.V., et al., *J. Chem Ecol.*, 2009. **35**(3): p. 281-288.

### **Effect of population density on mate finding behaviour of the forest tent caterpillar, *Malacosoma disstria* (Lepidoptera: Lasiocampidae)**

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This project examined the effect of population density on chemically-mediated mate finding behaviour in forest tent caterpillar moths, *Malacosoma disstria* (Lepidoptera: Lasiocampidae). In particular, I tried to find mechanisms to explain reduced trap capture observed at high population densities in the field. Two hypotheses were tested: 1) at increased female density male moth capture in pheromone-baited traps is reduced due to competition for pheromone sources and 2) moths from high density populations will be in poor condition and less likely to respond to pheromone-baited traps. Field cage studies over two field seasons demonstrated support for hypothesis 1 as female density affected male moth trap capture at high (35 and 50 per cage) but not low (18 per cage) female densities. There was no effect of male population density on the proportion of released males that oriented to female-baited traps. Moth condition was successfully manipulated by varying the levels of food provided to larvae. Moth condition (size) did not influence mate finding behaviour in field cages but did impact close range mating behaviour and the distance flown in flight mill studies. In the field, it is likely that poor moth condition and high female densities during population outbreaks reduce the efficacy of pheromone-baited trap capture.

## Nutrient enrichment: sublethal effects on marine benthic communities

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The effects of anthropogenic activities on marine coasts have become a worldwide concern. One of the major stresses comes from the discharge of excessive nutrients from sewage into coastal waters. At the moment, there is no report in the Brazilian literature on the use of lipids from benthic organisms as markers for contamination of marine environments by organic pollutants. A manipulative *in situ* bioassay was conducted to investigate the impact of nutrient enrichment on the metabolisms of *Palythoa caribaeorum* (Zoanthidea), *Mussismilia hispida* (Scleractinia) and *Desmapsamma anchorata* (Porifera) at Ilha Grande Bay, RJ state. A randomized design comprised the fixation of fertilizer around the colonies along the coast. After 1 month, the lipids of these organisms were extracted with organic solvents, separately. The triacylglycerols were converted to fatty acid methyl esters by saponification and esterification. The FAMES were analyzed by HRGC/MS, and showed predominance of palmitic, stearic, arachidic, arachidonic and EPA acids in *P. caribaeorum*; those fatty acids and behenic acid in *M. hispida*, and 5,9-hexacosadienoic acid in *D. anchorata*. Monohydroxylated sterols were identified in all organisms. The preliminary data on impact of the nutrient enrichment seems to show difference in the capacity of synthesis of these metabolites in *P. caribaeorum*, *M. hispida* and *D. anchorata*. The fatty acid and sterols revealed a good indicator of cnidaria and porifera's response to stress. However, future studies are important to confirm these results. These studies may help the development of strategies for appropriate management for the water quality in this environment.

**Repellency of Lavender oil against Lantern fly, *Lycorma delicatula*  
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On the growing concerns worldwide of climate change, new infestation of the lantern fly, *Lycorma delicatula* have recently been reported in South Korea. Among the major pests, this pest is the most important ones of invasive species. This study was performed to investigate the olfactory response of 62 kinds of plant essential oils against *Lycorma delicatula*. Among tested oils, lavender oil was showed high repellency against 1st and 4th instar of *L. delicatula*. Lavender oil significantly repelled 1st instar (76.5%) and 4th instar (80.0%) of *L. delicatula* at a dose of 1.25ul/cm<sup>2</sup> by using an olfactometer. By analyzing of GC-MS, the components are revealed as linalyl acetate (49.4%), linalool (42.2%), terpinen-4-ol (5.0%), and caryophylleneoxide (3.4%), and active components responsible for the active repellency of lavender oil were revealed as linalool at a dose of 1.25ul/cm<sup>2</sup> against 1st and 4th instar of *L. delicatula* as 66.7, and 76.5%, respectively. Using a GC-EAD, antennae of *L. delicatula* were responded in linalool. Lavender oil showing higher repellency was tested to an *Ailanthus altissimum* in the field. Resulting that plot tested lavender oil was repelled the *L. delicatula* effectively.

## Repellency and Electrophysiological Response of Clove Bud Oil against *Culex pipiens pallens* (Diptera: Culicidae)

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*N,N*-Diethyl-*m*-methylbenzamide (DEET)-based repellents may provoke dermal toxicity in infants and children. For use against mosquitoes, various plant-derived essential oils are considered useful alternatives to such synthetic chemicals. The present study was undertaken to scientifically evaluate the repellency of 33 essential oils and evaluated the electrophysiological response against female *Culex pipiens pallens* adults. By using the ASTM E951-94 apparatus with human volunteers, we showed that the 33 essential oils exhibited varying degrees of mosquito repellency. Four oils, namely, clove bud, clove leaf, juniperberry and majoram, showed good repellency at a concentration of 0.005 mg/cm<sup>2</sup>. The clove bud and clove leaf oils had higher repellency than citronella known as a commercial repellent ingredient. Analysis of the clove bud and clove leaf oils by GC and GC-MS revealed their major components. Repellency analysis of these individual components indicated that isoeugenol, a major component of clove bud oil, had the highest repellency, while eugenol, a major component of both clove oils, was also more repellent than citronella. Clove bud oil, which was the most repellent of all tested oils, exhibited the prolonged repellency when mixed with vanillin for long-term use. Clove bud oil clearly had higher repellency than commercial repellents. These compounds were showed higher repellency in eugenol and isoeugenol by using the ASTM and simultaneously responded by GC-EAD.

## Effects of a stress-related plant compound on thrips behaviour

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*Frankliniella occidentalis* (Pergande) (Thysanoptera: Thripidae), the western flower thrips (WFT), a highly polyphagous cosmopolitan, is one of the most destructive agricultural and horticultural insect pests world-wide.

Methyl salicylate, a volatile metabolite of salicylic acid, is a common compound in essential oils, in particular in wintergreen (*Gaultheria procumbens* L.) oil. This phenylpropanoid has various biological functions in insect-plant interactions. Previous studies have identified methyl salicylate as olfactory repellent against *F. occidentalis* (1).

In this study we investigated behavioural responses of adult western flower thrips females to direct contact with methyl salicylate applied on bean leaves in leaf disc bioassays and behavioural observations. When they had the choice thrips females spent significantly less time on leaf discs treated with 1 % methyl salicylate within a 5-hour period. Beyond that time period, methyl salicylate applied on bean significantly prevented thrips females from oviposition and reduced the percentage of damaged area caused by their feeding activity for 24 hours. Series of behavioural observations of thrips females confirmed that methyl salicylate modifies their feeding and egg-laying behaviours on a treated plant.

Behaviour-modifying secondary compounds may be used in various strategies to contribute to the enhancement of current biological control measures and/or form novel components of Integrated Pest Management (IPM) strategies.

(1) Chermenskaya, T.D. et al. 2001. Behavioural responses of western flower thrips, *Frankliniella occidentalis* (Pergande), to volatiles from three aromatic plants. *Insect Science and its Application* 21: 67-72.

## Attractants toward the olive weevil, *Dyscerus perforatus*, from the olive

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The olive weevil (*Dyscerus perforatus*, Coleoptera, Curculionidae) is a native species in Japan and inhabits Honshu, Shikoku, and Kyusyu. Originally, this weevil seemed to colonize on *Ligustrum japonicum* and *L. obtusifolium*, both of which belong to the oleacea family like the olive. However, when olive trees were introduced to Japan and planted on large scale, this weevil immediately attacked the olive and soon preferred the plant to the former hosts. The population density is extraordinary high in the case of the olive tree unlike in the former hosts where the weevils live in low density and the assault become seriously damaged for the host plant. Therefore, this weevil is now the most serious pest of the olive tree in Japan. During the course of our study on the relationship between the olive tree and the olive weevil, we have been interested in the possible chemical constituents of this plant, that are responsible for host selection and attraction of the olive weevil. Using insect behavior regulators seems to be one of promising way to protect the olive against the weevils. In this study, we found that the olive trees release some volatiles as attractants to the weevils. We collected them by the headspace method and found that they showed attractant activity to the weevils. Therefore, we are trying to identify the attractive components. Here, the characterization and the activity of such attractants will be discussed.

**Semiochemicals in the *Varroa destructor* A. & T. – *Apis mellifera* L. relationship: host selection and cell invasion behaviour.****DEL PICCOLO F<sup>a</sup>, NAZZI F<sup>b</sup>, ANNOSCIA D<sup>b</sup>, DELLA VEDOVA G<sup>b</sup>**<sup>a</sup> *Università degli Studi di Udine (University of Udine), UDINE, ITALY*<sup>b</sup> *Dipartimento di Biologia e Protezione delle Piante - Università di Udine, UDINE, ITALY**fabio.delpiccolo@uniud.it*

*Varroa destructor* Anderson & Trueman is an ectoparasitic mite of *Apis* spp. and is responsible for huge losses in many bee-pollinated crops. The varroa mite is blind but well equipped with an efficient sensory system, suggesting that several chemical signals are involved in the high level of synchronization between the life cycles of the parasite and its host.

Cell invasion is a crucial step in the life cycle of the mite, as it represents the beginning of the reproductive phase. The parasite reaches the brood cell carried out by a bee, thus the choice of the host can heavily affect the success of invading mites.

The varroa mite prefers nurse bees over pollen foragers, and the reasons of this preference have been investigated. Adult bees have an age-dependent cuticular hydrocarbons profile: (Z)-8-heptadecene, an alkene present on pollen bees cuticle, has a repellent effect towards the mite.

The parasite invades mostly worker and drone cells, while queen cells are rarely infested. The effects of larval food in the cell invasion behaviour have been investigated: 2-hydroxyhexanoic acid, a component of brood larval food, positively influences the invasion process while octanoic acid, the major volatile component of royal jelly, is repellent to the mite and interferes with the process of cell invasion.



**Responses of *Rhynchophorus ferrugineus* adults to selected synthetic Palm esters: electroantennographic studies and trap catches in an urban environment**

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The Red Palm Weevil (RPW), *Rhynchophorus ferrugineus* Olivier, accidentally introduced in the European countries facing the Mediterranean basin, is becoming the most serious pest for ornamental palms in the urban environment. In our work we tested electroantennogram (EAG) and behavioural responses of RPW adults to five selected synthetic palm esters: ethyl propionate, ethyl butyrate, ethyl isobutyrate, ethyl lactate and ethyl acetate. EAG bioassays showed higher sensitivity of female antennae compared to male antennae for test compounds. Differences were also recorded in the EAG responses using different esters and doses. Ethyl propionate was the synthetic ester that elicited the strongest relative EAG response from both sexes. For all the synthetic esters, the weakest EAG response was recorded at the dose of 0.1 µg. RPW behavioural responses in the urban environment were evaluated over six biweekly observations on pheromone baited traps. Results showed that pheromone baited traps supplemented with either 10% (v/v) solution of ethyl propionate and 10% (v/v) solution of ethyl acetate caught more adults than pheromone traps baited with only ethyl propionate, while there were not different catches between pheromone baited traps supplemented with ethyl propionate or ethyl acetate. This study supports the application of the mass trapping implemented with palm esters as a potential tool for the management of RPW populations to protect ornamental palms in urban environments.

**The possible reasons of low trapping efficiency for the second generation of the Asian corn borer, *Ostrinia furnacalis*, by sex pheromone trap in corn field**

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The adult occurrence monitoring of Asian corn borer (ACB), *Ostrinia furnacalis* (Guenée) (Lepidoptera: Crambidae) by using sex pheromone trap has been conducted for 5 years in the corn field in Suwon, Korea. The pheromone lure consisted of (*E*)-12-tetradecenyl acetate and (*Z*)-12-tetradecenyl acetate at a ratio of 1:2. The rubber septa as pheromone releaser and wing-type sticky trap at a height of about 1~1.5 meters were used in order to attract and catch adults. From the monitoring results, the occurrence time of the first generation of ACB could be discriminated clearly as usually mid-June. However, although high increase of population density in the ACB second generation was usually expected considering nutritious corn stage at this time, the insect number caught in the sex pheromone trap from the second generation was much less than that of the first generation, and adult occurrence pattern of the second generation was not always clear. Change of trap height and position didn't affect trapping efficiency. As the possible reasons of relatively low trapping efficiency for the later generations of ACB by sex pheromone trap, it was presumed that synchronized emergence of over-wintering larvae dependent on ambient temperature, high mortality of the first generation of ACB by basically physiological disorder, insecticides and natural enemies, and early initiation of overwintering mode cause the decrease of adult population density in the ACB later generations.

## Chemical defense of terpenoids in boreal conifer against herbivorous vole

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Woody plants in the boreal-forest ecosystem should be seriously pressured by the predation of generalist mammalian herbivores over winter, because the overground part of the most herbaceous plants, as summer foods of these animals, dieback over winter. Hence parts of the woody plants have been suggested to gain strong chemical defense against the mammalian herbivory via evolutionary processes in plant-animal interactions. In the indoor two-choice feeding bioassay of coniferous tolerance to the herbivory, we have evaluated the dietary behaviors of the boreal small herbivore, gray red-backed vole (*Myodes rufocanus bedfordiae*) to the artificial woody pelleted foods, which were untreated or contained several extractives or compounds from the bark of the boreal conifer (*Larix gmelinii* var. *japonica*). The dietary amount of foods, which were added with the extractives, their fractions, and some compounds within their natural concentration levels of the bark, were significantly lower than those of untreated control foods. On the other hand, the dietary amount of food treated under the natural concentration levels didn't differ from those of the control foods. We show that several terpenoids<sup>1, 2</sup> of *L. gmelinii* var. *japonica* and their concentrations are some causes of its tolerance to vole herbivory.

### Acknowledgements

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### References

1. Seki, K. et al. 2009. International Society of Chemical Ecology Annual Meeting 2009, Nuechâtel, Switzerland.
2. Seki, K., Orihashi, K. 2010. British Ecological Society Annual Symposium 2010, Brighton, UK.

## Differential responses to two human kairomonal cues in *Aedes aegypti* and *Culex quinquefasciatus* mosquitoes

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The southern house mosquito, *Culex quinquefasciatus*, and the yellow fever mosquito, *Aedes aegypti*, are vectors of numerous human diseases that are among the main causes of human mortality and morbidity worldwide. Carbon dioxide (CO<sub>2</sub>), a main component of the host-released odor plume, is a major cue for activation and host attraction in these species. In addition these mosquitoes are behaviorally attracted to 1-octen-3-ol, a component of human sweat. Single sensillum recordings (SSR) were performed on the maxillary palp basiconic sensilla from both *Aedes* and *Culex* to define the sensitivity of olfactory receptor neurons (ORNs) to CO<sub>2</sub> and enantiomers of 1-octen-3-ol. Olfactory receptor neurons housed basiconic sensilla are differentially tuned CO<sub>2</sub> exceeding 600 ppm and 1-octen-3-ol isomers. To understand the kairomonal cues released from the human body, we collected whole-body volatiles from different volunteers. Pooled extracts were used in no-choice behavioral assays to test the attraction of intact mosquitoes, compared with mosquitoes either ablated antennae, maxillary palp or tarsi. Intact *Aedes* and *Culex* were 70% and 50% attracted to human extract respectively. *Aedes* and *Culex* showed 15/5% attraction with ablated maxillary palp and 5/0% with ablated antennae, respectively. In further studies, we will determine the compounds that in human extract are attractive/repellent to the mosquitoes by using gas chromatography coupled SSR. Behavioral and electrophysiological studies show that *Aedes* and *Culex* exhibit differential responses to human extract.

## Does prescribed burning affect secondary metabolism for two Mediterranean pines?

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Forest fires play a major role in Mediterranean plant dynamic. Their frequency is executed on a long time range but tend to accelerate because of increased anthropogenic impacts, enhancing ecosystem vulnerability. To preserve forests with economical and/or cultural value, forest management organisms have developed various techniques to prevent fires. Prescribed burning is used more and more but these effects on tree physiology are poorly understood. The aim of the "GIS fire" project was to describe the effect of prescribed burning on primary and secondary metabolism for two Mediterranean pines (*Pinus halepensis* and *P. laricio*). Here, we only present results obtained for secondary metabolism which is known to be induced mainly under stress conditions to increase defense processes. Would prescribed burning change content and/or emissions of these compounds in plots burning several times? Pine patches were divided in plots regarding to the number of prescribed burning (0, 1 or 2). Secondary metabolites (terpenes and phenols) were analyzed in needles.

For *P. halepensis*, double burning plots showed an upper foliar phenol and terpene contents than control plots. These contents also had a tendency to decrease with the burning age: The more recent was the burning; the highest was foliar metabolites content. *P. halepensis* appeared to be responsive to this forest management technique, by increasing defense processes in a "more-stressful" environment. Conversely, no significant impact of prescribed burning was observed for *P. laricio*. This species seemed less sensitive to prescribed burning or perhaps her resilience was faster (less than one year).

## Determination of the Absolute Configuration of a Pyrethroid-related Mealybug Sex Pheromone

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The determination of the absolute configuration of the Citrophilous mealybug sex pheromone proved to a real challenge as it contains two ester groups. Air entrainments from thousands of mealybugs gave a MS-spectrum but did not give enough material for an NMR sample.

But by hydrolysis and transesterification reactions in the microscale followed by synthesis, we could finally deduce the structure of the three stereocenters in this evasive diester pheromone related to a pyrethroid.

An overview of the structures of all 12 mealybug pheromones that have been discovered until now will be presented and their interesting resemblances discussed.

**Absolute configuration of 7-*epi*-sesquithujene, a plant volatile antennally active to emerald ash borer, *Agrilus planipennis*****KHRIMIAN A<sup>a</sup>, COSSE A<sup>b</sup>, CROOK D<sup>c</sup>**<sup>a</sup> USDA-ARS, Beltsville, Maryland, USA<sup>b</sup> USDA, Agricultural Research Service, Peoria, Illinois, USA<sup>c</sup> USDA, Animal and Plant Health Inspection Service, Otis ANGB, Massachusetts, USA*ashot.khrimian@ars.usda.gov*

Emerald ash borer, *Agrilus planipennis* Fairmaire (Coleoptera: Buprestidae), is an invasive beetle that has been causing extensive mortality of ash trees since arriving in the U.S. in 2002. 7-*epi*-Sesquithujene is among few compounds eliciting strong EAD responses on the emerald ash borer antenna. It is believed that a significant field attractiveness of the essential oil from the Brazilian walnut tree, *Phoebe porosa*, to emerald ash borer is due to 7-*epi*-sesquithujene, which is present in the oil among other EAD -active sesquiterpenes. In the course of making a synthetic 7-*epi*-sesquithujene for field studies, we found that the absolute configuration of this compound has not been yet determined. We purified 7-*epi*-sesquithujene from the phoebe oil via successive fractionation, conventional and argentation (HPLC) chromatographies. Based on available synthetic data, specific rotation, microscale hydrogenations, and chiral GC analyses, we established that 7-*epi*-sesquithujene, present in the phoebe oil and emitted by the stressed ash bark, has the 4*S*,6*S*,7*R* configuration.

## Identification of sex pheromone components of the pear barkminer moth (Gracillariidae)

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By GC-EAD analysis, three EAG-active components (I–III) were found in a pheromone gland extract of the female moths of the pear barkminer, *Spulerina astaurota*. This insect is a harmful pest of pear trees, *Pyrus pyrifolia* (Rosaceae) in Japan, and it also inhabits Korea and India. The larvae mine and feed inside the bark of branches, causing injuries, which might contribute to infection by other pest insects and diseases. On column chromatography with Florisil, the minor components I and II were mainly eluted with 10% ether in hexane and the major component III with 30% ether, indicating differences in the terminal functional groups of Type I lepidopteran pheromones. While the content of I was too small to determine the structure, GC-MS analysis indicated that II and III were C<sub>14</sub> dienyl acetate and alcohol, respectively. Their spectra included diagnostic ions for 9,11-dienyl compounds at *m/z* 82 and 95, and the double bond position was confirmed by an experiment with 4-methyl-1,2,4-triazoline-3,5-dione. On a GC system with a polar capillary column, the natural pheromone components showed the same retention times as those of synthetic standards with a (Z9,Z11)-configuration among the four geometrical isomers of 9,11-dienes. Therefore, we synthesized the (Z9,Z11)-isomers by using a Sonogashira coupling and hydroboration protonolysis as the key reactions. In this year, we will evaluate the synthetic lures and examine application of the sex pheromone for the IPM programs.



## Predator-released hydrocarbons repel oviposition by a mosquito

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Prey species commonly use predator-released kairomones (PRKs) to detect risk of predation, yet the chemical identity of PRKs remains elusive. Chemical identification of PRKs will facilitate the study of predator-prey interactions and the risk of predation, and when the prey are pests, will potentially provide environmentally friendly means of pest control. In temporary pools of the Mediterranean and Middle East, larvae of the mosquito *Culiseta longiareolata* Macquart are highly vulnerable to the common predatory backswimmer, *Notonecta maculata* Fabricius. We demonstrate that *N. maculata* releases two hydrocarbons, *n*-heneicosane and *n*-tricosane, which repel ovipositing females of *C. longiareolata*. In behavioral tests with environmentally relevant chemical concentrations in outdoor mesocosm experiments, the repellent effects of the two compounds were additive at the tested concentrations.

## Effects of pathogen/NtCDPK2-mediated signalling on later herbivore-induced defences in two *Nicotiana* species

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Plants have to optimize costs and benefits of their specific defence responses to various enemies and these responses may interact on different levels. Here we investigate whether and how a previous bacterial infection plus the additional activation of NtCDPK2, a protein kinase involved in the induction of plant defence reactions to bacterial pathogens, also cause alteration in a later plant defence response to herbivore attack. In two *Nicotiana* species we found opposing effects: In *N. benthamiana* native to Australia, upon bacterial infection and NtCDPK2 expression several plant defence compounds (e.g. plant protease inhibitors) showed a stronger induction in response to secondary herbivore attack by the tobacco hornworm *Manduca sexta*. In *N. attenuata*, a with *M. sexta* co-evolved species native to America, bacterial infection and NtCDPK2 expression prior to *M. sexta* herbivory caused a lower induction of protease inhibitor activity but no other changes in induced defences measured. Accordingly, performance of *M. sexta* was not affected. However, when the generalist herbivore *Spodoptera exigua* was tested instead on similarly pre-treated *N. attenuata* plants a higher mortality and mass gain of the herbivore was observed, which was then accompanied by altered levels in plant defence compounds. Our results demonstrate that defence reactions caused by a previous stress experience modify the plant's response toward the defence of a later different attacking herbivore in a plant and herbivore species-specific manner.

## Ecochemolibrary of chemical mediators: principle and first steps

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Ecochemolibrary's project is born from the prospects for evolution of the "GDR d'écologie chimique", managed by Martine Hossaert-McKey, and from an opening will from the CEFE laboratory to ecological chemistry (Pr. Claude Grison).

Ecochemolibrary's is aiming at uniting and organizing the biological, chemical and physicochemical knowledge, in the field of chemical mediators.

Our main goals are to extract, classify and analyze scientific data available from researchers to integrate them in a chemical information system, specialized in chemical ecology.

To achieve this, a secured database, accessible on the Internet after registration, is under development. It will allow any registered researcher to examine ecological information (studied species, mediation's type, specificity...), chemical information (chemical analysis, molecules' structure and nomenclature...) and bibliography stored in the knowledge database or increase data by adding its own results.

This tool's development should allow in the medium term to implement a chemotaxonomy of fungal species, plants and animals, and promote an interdisciplinary approach of chemical ecology involving ecology, molecular biology, biochemistry, analytical and organic chemistry.

Once established, the ecochemolibrary has the vocation to be integrated to the CNRS' National Chemolibrary managed by Marcel Hibert (UPS 3095 CNRS).

## Manure/Bacterial-associated volatile compounds as fly oviposition attractants

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The present study reports new discoveries on the isolation and identification of oviposition attractant compounds for the screwworm, *Cochliomyia hominivorax* and the stable fly, *Stomoxys calcitrans* (L.), which are serious livestock pests that feed/infect on cattle and cause significant economic loss in the cattle industry. Volatiles from their natural oviposition media (manures, vegetation and SPENT larvae diet) were collected and analyzed with gas chromatography, and then their chemical structures were tentatively identified by gas chromatography combined with mass spectrometry. Electroantennogram was also tested with those potential attractant compounds on gravid female antennae. Behavioral activity of these compounds was demonstrated in lab bioassays. Bacterial species were identified from the selected oviposition media, and their volatile emission was further analyzed from the bovine blood and other culturing media. Lures containing blends of attractant compounds with the newly-designed ovi-traps were further tested in the field. In addition, the exploration of using these potential filth fly attractants together with already-identified fly repellent compounds used in the Push-Pull strategy will be discussed.





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