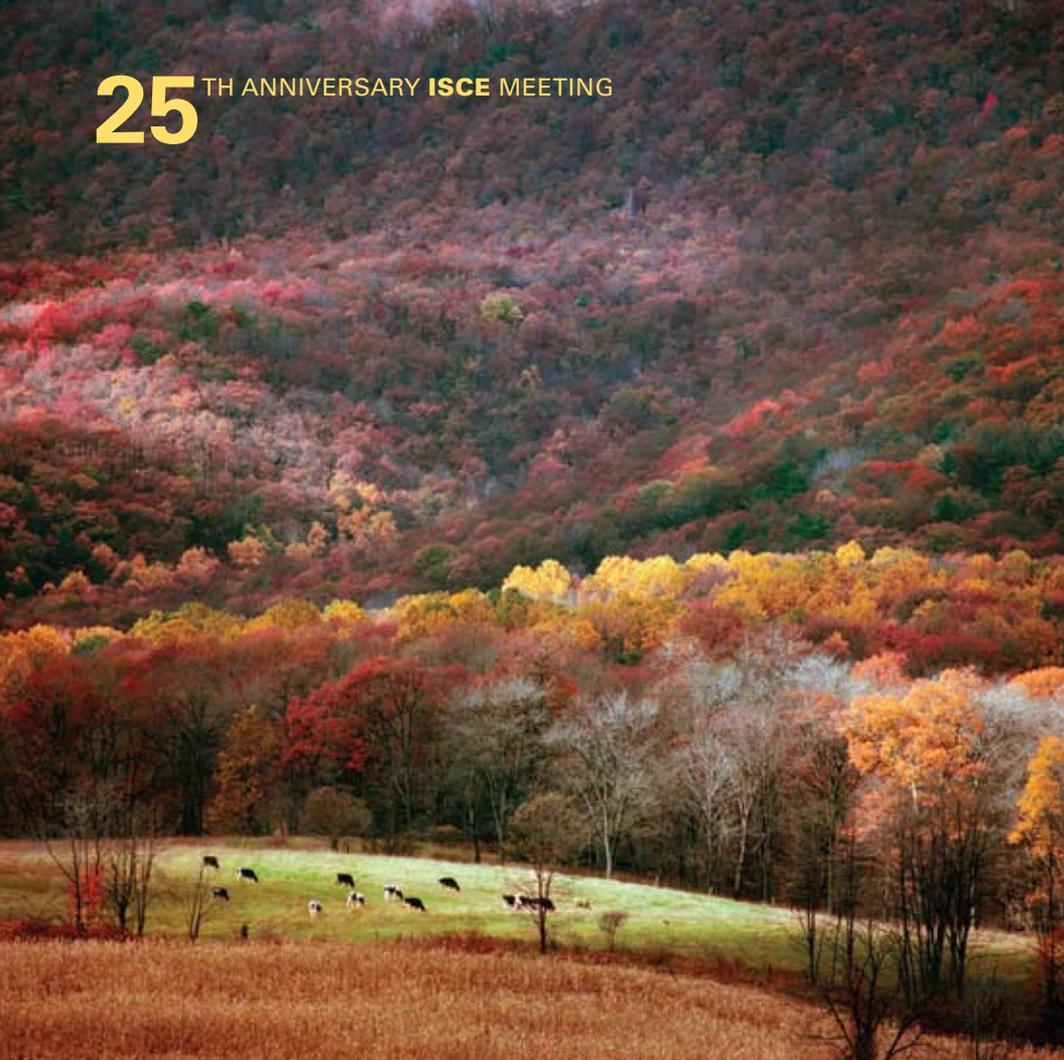


25TH ANNIVERSARY **ISCE** MEETING



STATE COLLEGE, PENNSYLVANIA, U.S.A.
AUGUST 17-22, 2008



INTERNATIONAL SOCIETY
OF CHEMICAL ECOLOGY



ANNIVERSARY MEETING



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Meeting Overview

25TH ANNIVERSARY ISCE MEETING



**SUNDAY, 17 AUGUST**

Executive committee meeting, Board Room 1, 15.00–17.00
 Registration, Nittany Lion Inn Rotunda, 15.00–20.00
 Welcome reception, Nittany Lion Inn, Alumni Lounge, 19.00–22.00, live music: jazz

Scientific Program, Monday–Thursday, 18–21 August (all in Life Sciences Building)

A registration desk will be outside of Berg Auditorium during the meeting

Plenary sessions, 100 Berg Auditorium
 Parallel afternoon sessions each day, Berg Auditorium and Room 11
 Poster viewing, 3rd and 4th Floor Bridges
 Oral presentations being judged for an award are highlighted in gray in the main program.

Symposium 1 (Monday): Chemical Ecology of Plant–Plant Interactions

Parallel Session 1 (Berg Auditorium): Plant–Insect Interactions (Belowground, Elicitors)

Parallel Session 2 (Room 11): Insect Pheromones (Olfaction, Evolution, Application)

Poster Session 1

Symposium 2 (Tuesday): Chemical Deception/Mimicry

Parallel Session 3 (Berg Auditorium): Plant–Insect Interactions

Parallel Session 4 (Room 11): Insect Defense and Prey Location

Poster Session 2

Symposium 3 (Wednesday): Phylogenetic Analyses of Plant Defense and Insect Host Range

Symposium 4 (Thursday): Chemical Ecology of Disease Transmission

Parallel Session 5 (Berg Auditorium): Plant–Insect Interactions

Parallel Session 6 (Room 11): Pheromone Identification, Biosynthesis

MONDAY, 18 AUGUST

Silverstein–Simeone Lecture, Leslie Vosshall, 100 Berg Auditorium,
 Life Sciences Building, 19.30–20.30
 Odd-numbered poster viewing, 20.30–22.00

TUESDAY, 19 AUGUST

Silver Medal Award Lecture, Gunnar Bergström (100 Berg Auditorium,
 Life Sciences Building, 19.30–20.30)
 Even-numbered poster viewing, 20.30–22.00

WEDNESDAY, 20 AUGUST

Morning session only (8.30–12.00) Free time starting at 12.00 into the evening)
 Outing to Black Moshannon (leave at 14.00 for hiking, swimming, boating,
 barbecue, live music: bluegrass)

THURSDAY, 21 AUGUST

Banquet, awards ceremony, speaker David Wood (President's Hall,
 The Penn Stater Conference Center Hotel, 19.00–24.00, live dance music: 80s rock)

FRIDAY, 22 AUGUST

Final business meeting (Nittany Lion Inn, Board Room 1), 10.00–11.00

Main Program



25TH ANNIVERSARY ISCE MEETING

SUNDAY, 17 AUGUST

15.00– 17.00	ISCE Executive Committee Meeting, Nittany Lion Inn, Board Room 1
15.00– 19.00	Free time for informal scientific discussions, walking around campus town
15.00– 20.00	Registration, Nittany Lion Inn, Rotunda Preregistered participants can pick up their printed program/ abstract books, name tags New registrants are welcome to register at this time The rest of the week, a registration desk will be outside the sessions in Berg Auditorium, Life Sciences Building.
19.00– 22.00	Welcome Reception, Nittany Lion Inn, Alumni Lounge

MONDAY, 18 AUGUST

8.30–8.35	Welcome, Jim Tumlinson	
8.35–8.50	Introduction, Dean Robert Steele, Penn State	
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☐ = oral presentations being judged for an award

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(100 Berg Auditorium, 14.00–17.20)

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 = oral presentations being judged for an award

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■ = oral presentations being judged for an award

WEDNESDAY, 20 AUGUST

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14.00–19.00	Outing to Black Moshannon State Park (hiking, boating, swimming, barbecue, live music: bluegrass)	
20.00–	Evening Free	

= oral presentations being judged for an award

THURSDAY, 21 AUGUST

SYMPOSIUM 4: Chemical Ecology of Disease Transmission

(100 Berg Auditorium, 8.30–11.40)

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■ = oral presentations being judged for an award

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FRIDAY, 22 AUGUST

10.00– 12.00	Final Business Meeting, Nittany Lion Inn, Board Room 1
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Chemical Ecology of Plant–Plant Interactions

Chair: Consuelo De Moraes,
Penn State University, University Park, PA
100 Berg Auditorium, Life Sciences Building

SYMPOSIUM | MONDAY, 18 AUGUST

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Constitutive and Induced Plant–Plant Signaling: Hypotheses and Practical Developments

Pickett JA

Department of Biological Chemistry, Rothamsted Research, Harpenden, Hertfordshire, AL5 2JQ, United Kingdom

Corresponding author: john.pickett@bbsrc.ac

By studying plant–insect interactions, particularly in multitrophic systems, it is possible to identify insect semiochemicals that also induce defence responses in plants (1). From such work, *cis*-jasmonone has been identified as having highly specific and persistent effects on up-regulating gene expression associated with plant defence. Originally, *cis*-jasmonone was thought to represent an inactive sink by which jasmonic acid was catabolised after its role as a plant hormone was over. However, the induction of defence, combined with a relatively restricted effect on other aspects of metabolism compared with the activity of jasmonic acid, suggests *cis*-jasmonone to be of significant agricultural value. The molecular genetic mechanisms involved are being investigated in *Arabidopsis thaliana* by microarray, the use of knockout lines, and by functional gene expression studies in *A. thaliana* and other organisms (2). In cereals, there are considerable varietal differences in the level of defence induced by *cis*-jasmonone. With some cultivars, long-term protection against aphids has been established in the field. Chemical studies and investigations with insects using electrophysiological and behavioural assays have shown one compound, the production of which is induced by *cis*-jasmonone, to be 6-methyl-5-hepten-2-one, which served both to repel herbivorous pests such as aphids but at the same time to increase foraging by their parasitoids. Differential induction between cultivars will provide useful means by which to elucidate the associated genetics. In both wheat and barley, there is also an induction with *cis*-jasmonone of antibiotic effects against aphids. In wheat, enhanced production of hydroxamic acids (benzoxazinoids) contributes to this observed resistance and is now being exploited in a breeding programme. With this system, the associated genes are known and quantitative RT-PCR is providing a means by which the induction of expression is investigated. HPLC and MSⁿ, after GC on trimethylsilylated derivatives, provide an analytical tool for the estimation of these and the unknown antibiotic agents in barley. Plants can be modified to report presence of plant defence activators such as *cis*-jasmonone, thereby acting as sentinel plants for pest and disease monitoring. Although the main work is directed to wheat, recently strong activation of defence has been found in both soya and cotton and will be developed particularly via collaboration in Brazil. In Africa, we have with collaborators, particularly the International Centre of Insect Physiology and Ecology (*icipe*), developed a push-pull system for controlling lepidopterous stemborers by using highly attractive trap crops and intercrops that repel the pests by producing stress-related defence semiochemicals (3). One type of intercrop comprises cattle forage legumes in the genus *Desmodium* and also dramatically controls the parasitic African witchweed *Striga hermonthica*. The combined effect on stemborers and witchweed has caused a rapid take-up by farmers in targeted regions and provides a clear demonstration of the value in exploiting this type of natural product defence chemistry, which has lessons for intensive agriculture.

1. Curr. Opin. Plant Biol. 10:387–392.

2. Proc. Natl. Acad. Sci. USA 103:10509–10513.

Volatile Communication among and between Sagebrush Individuals Affects Herbivory

Karban R

Department of Entomology, University of California, Davis, CA

Corresponding author: rkarban@ucdavis.edu

Communication between plants can affect their resistance to damage by herbivores. Following Farmer and Ryan's lab demonstration of volatile communication between sagebrush and potted tomato plants, we found that wild tobacco near clipped sagebrush experienced less damage by herbivores and produced as many or more fruit than tobacco near unclipped sagebrush in the field. This communication required air contact but was only found for plants in very close proximity, within 10–15 cm. Sagebrush individuals near clipped sagebrush neighbors also experienced less damage by herbivores at distances up to 60 cm. Volatiles released by clipped sagebrush caused multiple consequences, although they were not directly repellent to feeding herbivores. Volatiles attracted more predators and parasites although these did not necessarily reduce herbivory. Volatiles inhibited germination of potential competitors of sagebrush. Volatile communication was required for branches of the same plant to coordinate defenses as vascular communication was limited. Results from several systems indicate that plant–plant communication is not restricted to sagebrush although the generality of this phenomenon is still unclear.

Chemical Communication among Plants

De Moraes CM, Mescher MC, Runyon J, and Frost C

Center for Chemical Ecology, Department of Entomology, The Pennsylvania State University, University Park, PA

Corresponding author: czd10@psu.edu

Plants engage in continuous gas exchange with the surrounding atmosphere and, in so doing, emit airborne chemicals that can be perceived by other organisms. Because the makeup of the chemical blend emitted by a plant varies considerably across taxa and in response to environmental variation, plant volatiles can convey information about the identity and status of emitting plants to organisms that receive these airborne signals. Volatile-mediated interactions among plants and insects are widespread and have been extensively documented. In addition to serving as foraging cues for herbivorous insects and pollinators searching for plants on which to feed (1,2), plant volatiles function as an indirect mode of plant defense because of their role in attracting predatory and parasitic insects that attack feeding herbivores (3,4,5). Relative to plant–insect interactions, far fewer studies have documented plant-to-plant communication involving volatiles but, in recent years, a number of discoveries have contributed to an increased appreciation of the complexity and sophistication of volatile mediated plant–insect interactions and an enhanced understanding of the role of volatiles in plant-to-plant communication (6,7,8). This talk summarizes some of our recent findings on plant-to-plant communication via volatiles, including our studies examining the role of volatiles in host location by parasitic plants (9) and our recent work on the role of volatiles in within-plant signaling that primes defense responses in poplar saplings (10). The significance of these findings for broader conceptual issues in chemical ecology is discussed.

1. *Curr. Opin. Plant Biol.* 5:237–243.
2. *Trends Plant Sci.* (2002) 10:269–274.
3. *Science* (1990) 250:1251–1253.
5. *Plant Physiol.* (1999) 121:325–331.
7. *Oecologia* (2006) 148:280–292.
8. *Ecology* (2006) 87:922–930.
9. *Science* (2006) 311:812–815.
10. *Science* (2006) 313:1964–1967.

Chemical Interaction between Undamaged Plants in a Tritrophic Context

Glinwood R, Ninkovic V, Ahmed A, Qvarfordt E, and Kellner M

Department of Ecology, Swedish Agricultural University, Uppsala, Sweden

Corresponding author: robert.glinwood@ekol.slu.se

Plants damaged by herbivores or pathogens release volatiles active in defense signaling between or within plants, but undamaged plants also may engage in chemical interactions. We present results that contribute new perspectives on allelopathy and use of volatile cues by plants and insects in a tritrophic context. In a system consisting of different barley genotypes and some aggressive weeds we show that chemical interactions, mainly via volatiles, cause changes in plant status that modify biomass allocation (1), reduce acceptance by an aphid herbivore (2,3), and affect interaction with two aphid natural enemies: ladybirds and parasitic wasps (4). In barley, the volatile inducing and responding capacity is shown to be genotype-related. Increased understanding of chemical interaction between undamaged plants may give a new outlook on mixed genotype cropping and eventually provide solutions for sustainable management of insect pests (5).

1. *J. Exp. Bot.* (2003) 54:1931–1939.
2. *Plant Signal. Behav.* (2007) 2:205–210.
3. *Ecol. Entomol.* (2004) 29:188–195.
4. *Oikos* (2003) 100:65–70.
5. *Entomol. Exp. Appl.* (2002) 102:177–182.

Struggling for Light: Regulation of Plant–Plant Interactions

Pierik R

Plant Ecophysiology, Institute of Environmental Biology, Utrecht University, Sorbonnelaan 16, 3584 CA Utrecht, the Netherlands

Corresponding author: r.pierik@uu.nl

Plants growing in dense vegetations interact in various ways and compete with proximate neighbors for light. They can ensure growth and survival through an escape syndrome known as shade avoidance. Upon perception of neighbors, plants elongate their shoots and move their leaves upward. Until recently, neighbor detection was thought to occur exclusively through spectral changes in the light reflected from or transmitted through neighboring vegetation. Red light (R) is absorbed for photosynthesis, whereas far-red light (FR) is reflected, thus lowering the R:FR ratio, which can be sensed by the phytochrome photoreceptors. We showed recently that plant neighbor detection is far more complex and involves several other signals. Among these seemed to be a chemical signal: the volatile plant hormone ethylene. Ethylene was shown to accumulate within the atmosphere of very dense, greenhouse-grown tobacco stands. The levels found in these stands were sufficiently elevated to induce shade avoidance responses in isolated plants. Furthermore, transgenic ethylene insensitive genotypes showed delayed shade avoidance responses to neighbors and performed inferior to wild type in competition trials. The complexity of aboveground plant neighbor detection as well as the physiological mechanisms regulating the adaptive responses is discussed.

Allelopathy: Problems and Opportunities

Khan MA, Blackshaw RE, and Marwat KB

Agriculture and Agri-Food Canada, Lethbridge Research center, T1J 4B1, AB, Canada (AMK, REB); NWFP Agricultural University Peshawar, Pakistan (KBM)

Corresponding author: khanm@agr.gc.ca

Several invasive species were studied for their allelopathic properties in a series of experiments. The results indicated that *Prosopis juliflora*, *Acacia nilotica*, *Silybum marianum*, *Eucalyptus camaldulensis*, and *Parthenium hysterophorus* all have negative effects on the germination and growth of weeds and crops as well. All these species are invasive weeds in the region studied; thus, these weeds threaten biodiversity in several areas of Pakistan. In the present study, the allelopathic potential was examined in the laboratory and in the field. Laboratory-based experiments showed that with increasing concentration of these species, germination percentage, seedling length, and seedling weight of all the three species tested were significantly decreased. The tolerance order of the species against the extract concentration was *Triticum aestivum* > *Avena fatua* > *Lepidium* sp. Field experiments showed that there was no effect of any concentration either pre- or postemergence on weed density 25 days after sowing (DAS), fresh biomass, and dry biomass. However, different concentrations had a significant effect on weed density 50 DAS postemergence as well as pre-emergence. This might be due to delayed germination due to allelochemicals. Hence, the present study suggests that these species can be used as a bioherbicide, but they still need extensive study to fully explore their potential against different summer and winter weeds. As allelopathy leads to monoculture, therefore, the release of allelochemicals should be prevented to accumulate in the soil.

Talking Trees and Groaning Grasses: Past, Present, and Future**Schultz JC**

105f Life Sciences Center, University of Missouri, Columbia, MO

Corresponding author: schultzjc@missouri.edu

One of the first reports of airborne communication between plants occurred at the first ISCE meeting in Austin, TX. Reaction to that report was mixed, to say the least. I'll talk about the personal and professional impacts of the unexpected discovery of "plant BO" (as Peter Price dubbed it). Fast-forwarding through a long period of plant-communication research stasis, I'll characterize (critically) the current state of understanding of external communication by plants and suggest where research and particularly applications might go next, as we move from burping birches to flatulent flats of *Arabidopsis* (and back).

Plant–Insect Interactions (Belowground, Elicitors)

Moderators:

14.00–15.30 **Heidi Appel**, University of Missouri

15.50–17.20 **Andrew Stephenson**, Penn State

100 Berg Auditorium, Life Sciences Building



PARALLEL SESSION 1 | MONDAY, 18 AUGUST

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Above- and Belowground Direct and Indirect Defense Mechanisms of Transgenic Bt Maize and Implications for Biological Control

Zwahlen C, Léchet GS, and Turlings TCJ

Institute of Biology, University of Neuchâtel, CH-2009 Neuchâtel, Switzerland

Corresponding author: claudia.zwahlen@unine.ch

Herbivore-induced direct and indirect defense mechanisms are important for the plant's ability to combat attacking herbivores. Transgenic *Bacillus thuringiensis* (Bt) maize produces one or two Cry endotoxins against various pests, such as the European corn borer (Cry1Ab) and the corn rootworm (Cry3Bb1), which provide a highly effective direct defense mechanism against the target pests, but it is unclear whether the continuous production of these Cry proteins affects the plants' ability to allocate resources to fend off other, nontarget attackers. We present results from our studies that determine the capacity of Bt maize to defend themselves using direct defense compounds and inducible volatile emissions that attract natural enemies, such as entomopathogenic nematodes (EPN), against corn rootworm larvae. So far, we have found that when various Bt maize varieties and their near isogenic control were either artificially induced to produce volatiles in a similar manner as corn rootworm feeding or with corn rootworms themselves, they did produce (*E*)- β -caryophyllene, although in relatively low amounts. However, entomopathogenic nematodes did not seem to have a preference for induced versus noninduced maize roots. In some Bt varieties, corn rootworm- and artificially induced Bt maize produced significantly less Cry1Ab in their leaves and roots than untreated Bt maize. In a next step we will determine whether this has an effect on the plants' ability to fend off the aboveground herbivore *Spodoptera littoralis*. Our studies will contribute to a better understanding of the resource allocation of plants under herbivore attack. Moreover, they provide a first step toward comparing transgenic plants with biological control systems.

Enhancing the Attractiveness of Maize Roots for Entomopathogenic Nematodes

Hiltbold I and Turlings TCJ

Laboratory of fundamental and applied research in chemical ecology (FARCE), University of Neuchâtel, Neuchâtel, Switzerland

Corresponding author: ivan.hiltbold@unine.ch

When attacked by insects, plants emit volatiles that can serve as cues for predators and parasitoids in search of herbivorous prey. It has been proposed that the emissions of these volatile signals can be manipulated to improve crop protection. We demonstrated the full potential of this strategy by restoring a specific belowground signal emitted by insect-damaged maize roots. This signal, the sesquiterpene (*E*)- β -caryophyllene, is highly attractive to the entomopathogenic nematode *Heterorhabditis megidis* and is emitted by ancestral maize and European lines, but not by most American varieties. The loss of the signal was previously found to strongly reduce the effectiveness of the nematode as a control agent of the larvae of the western corn rootworm (WCR), a ferocious root pest. To restore nematode attraction, a nonproducing maize line was transformed, resulting in constitutive emissions of (*E*)- β -caryophyllene. In WCR-infested field plots in which we released nematodes, transformed lines received significantly less WCR damaged and had fewer adult beetles emerge than isogenic lines. These differences were not observed in plots without released nematodes. We have also been successful at selecting a strain of the highly infective nematode *H. bacteriophora* to better respond to (*E*)- β -caryophyllene. Field tests with this new strain show that by combining plants that emit the appropriate signal and nematodes that respond well to this signal, biological control can be just as effective as conventional WCR control strategies.

Involvement of ABA in Signaling Network Mediating Aboveground-Induced Resistance upon Infestation by Belowground Herbivores

Erb M, Karlen D, de Lange E, Planchamp C, D'Allessandro M, Flors V, Turlings TJC, and Ton J

Laboratory for Fundamental and Applied Research in Chemical Ecology (FARCE), Institute of Biology, University of Neuchâtel, CH-2000 Neuchâtel, Switzerland

Corresponding author: matthias.erb@unine.ch

Plants can develop enhanced resistance against pathogens and insects upon perception of biotic or abiotic stress, a phenomenon that can even link very distant multitrophic systems such as above- and belowground herbivores and their respective natural enemies. Induced resistance is often expressed systemically and involves complex regulation by the hormones salicylic acid (SA), jasmonic acid (JA), ethylene (ET), and abscisic acid (ABA). We examined the impact of belowground infestation by the root herbivore *Diabrotica virgifera virgifera* on aboveground resistance against the leaf herbivore *Spodoptera littoralis* and the pathogenic fungus *Setosphaeria turcica* in maize. To examine the role of plant hormones in this interaction, we first profiled the transcriptional and phenotypical specificity of induced resistance upon treatment with the salicylic acid analogue benzothiadiazole (BTH), JA, the ET precursor ACC, and ABA. Quantitative PCR analysis of 34 defense marker genes revealed distinct transcriptional patterns in the leaves upon root treatment with the various hormones, which shared similarities with the transcriptional responses to *S. turcica* and *S. littoralis*. Belowground attack by the root herbivore *D. virgifera* induced resistance to both the leaf herbivore and the fungus. *D. virgifera* infestation also led to up-regulation of several defense genes in the shoots of maize seedlings. Interestingly, the transcriptional patterns in leaves of *D. virgifera*-infested plants partially resembled an ABA-inducible transcriptional profile. In support of this, we found significantly enhanced levels of ABA, but not JA and SA in leaves of *D. virgifera*-infested plants. Hence, belowground attack by a root herbivore triggers systemic accumulation of ABA in the leaves, resulting in ABA-inducible gene expression and broad-spectrum-induced resistance.

Cardenolides, Induced Responses, and Interactions between Above- and Belowground Herbivores in the Milkweeds (*Asclepias* spp.)

Rasmann S, Agrawal AA, Cook SC, and Erwin AC

Department of Ecology and Evolutionary Biology, Cornell University, Ithaca, NY

Corresponding author: srasmann@gmail.com

Theory has long predicted allocation patterns for plant defense against herbivory, but only recently have both above- and belowground plant defenses been considered simultaneously. Milkweeds in the genus *Asclepias* are a classic chemically defended clade of plants with toxic cardenolides (cardiac glycosides) and pressurized latex used as antiherbivore weapons. Here, a comparative approach to investigate broad-scale patterns in allocation to root versus shoot defenses across species is combined with a species-specific experimental approach to identify the consequences of defense allocational shifts on a specialist herbivore. Results show a high level of evolutionarily lability in root cardenolides across 34 species of *Asclepias*, with four-fold variation across taxa. However, phylogenetic conservatism prevailed for inducibility of shoot cardenolides by an aboveground herbivore, with only four closely related tropical species showing significant induction, whereas the eight temperate species examined were not inducible. Allocation to root and shoot cardenolides was positively correlated across species, and this relationship was maintained after accounting for phylogenetic nonindependence. In contrast to long-standing theoretical predictions, no evidence was found for a trade-off between constitutive and induced cardenolides; indeed, the two were positively correlated across species. Finally, specialist root and shoot herbivores of common milkweed (*A. syriaca*) affected latex production, and these effects had consequences for caterpillar growth consistent with latex providing resistance. Although cardenolides were not affected by our treatments, *A. syriaca* allocated 40% more cardenolides to shoots over roots. In conclusion, constitutive and inducible defenses are not trading-off across plant species, and shoots of *Asclepias* are more inducible than roots. Phylogenetic conservatism cannot explain the observed patterns of cardenolide levels across species, but inducibility per se was strongly conserved in a tropical clade. Finally, given that above- and belowground herbivores can systemically alter the defensive phenotype of plants, we concur with recent calls for a whole plant perspective in testing models of plant defense allocation.

Intelligent Pest Monitoring: Creating a Database of Pest-Induced Plant Volatiles for Monitoring Greenhouse Crops by Electronic Chemo-Sensors

Miresmailli S and Isman MB

Center for Plant Research, Faculty of Land and Food Systems, The University of British Columbia, Vancouver, BC, Canada

Corresponding author: saber@interchange.ubc.ca

Developing new pest control methods has often been the center of attention for many researchers and far less has been done to develop more effective detection and identification techniques. It is well known that several plant species emit a wide array of volatiles when infected with pathogens or attacked by pests. Detecting these signals at an early stage could be a key factor for successful pest control. In this project, we suggest a novel approach to pest monitoring by shifting the attention from pests to plants. If interpreted correctly, plant-driven volatile chemical signals can provide more accurate information about the health of the plant. We focused on three greenhouse crops and their major pests. Significant qualitative and quantitative differences were found among some volatile chemicals emitted from clean plants versus infested plants. Our results indicate that pests can induce different plant responses on different host plants. We also demonstrate that plants are capable of formulating slightly different responses to different pests. Pest density and duration of damage have also been found to have direct effects on the emission of some volatile chemicals. Further experiment will be carried out in experimental and commercial greenhouses using a portable ultra-fast GC (zNose™) to create a volatile database. By using well-developed mechanical olfaction (known as the “electronic nose”) and other sensory technologies, we can follow some of these plant chemical cues to locate crop problems before they become visible to the naked eye of human scouts. It has been shown that plant driven volatiles might vary due to several biotic and abiotic factors. Therefore, the pest induced-plant volatile database will be designed in such a way that enables us to segregate pest-induced signals from nonpest-induced signals and also can provide a flexible and robust range of acceptable signals.

Tomato Plants Are Primed by *Helicoverpa zea* Oviposition for Impending Neonate Feeding

Kim J and Felton G

Center for Chemical Ecology, Department of Entomology, The Pennsylvania State University, University Park, PA

Corresponding author: gwf10@psu.edu

Plants initiate antiherbivore mechanisms when they are attacked by insects. For example, plants produce compounds that are toxic to insects and/or emit volatiles into the air that not only attract the natural enemies of the insect invaders but also warn neighboring plants of the possible subsequent danger. Most of the studies on the induction of plant defenses by insect herbivory have been carried out with simulated herbivory where host plants were mechanically wounded frequently combined with application of insect regurgitant or by “real” herbivory where insect herbivores were placed on and allowed to consume their host plants. However, considering that many of herbivores lay eggs on their host plants and that the larvae start feeding after hatching from the eggs, it would be plausible to speculate that plants may sense oviposition, initiate antiherbivory defenses and get ready for hatching and subsequent herbivory by neonates. Here, we demonstrate that tomato plants, *Lycopersicon esculentum* (Solanales: Solanaceae), up-regulate various antiherbivory genes only upon the oviposition by adults of tomato fruitworm, *Helicoverpa zea* (Lepidoptera: Noctuidae), and more interestingly, tomato plants that had been exposed to *H. zea* oviposition and were treated with simulated herbivory showed faster, stronger and longer levels of proteinase inhibitor gene 2 (*pin2*) induction than control plants. Moreover, the growth rate and survival of neonates of *H. zea* significantly decreased when they were placed on the tomato leaves where the eggs of *H. zea* had been laid. The survival of *H. zea* neonates was negatively correlated with the number of eggs that had been deposited on tomato plants. This study shows that tomato plants sense *H. zea* oviposition and prime themselves for the feeding by emerging neonates. As far as we know, this is the first report of priming of host plants by herbivore oviposition.

Diversity of Caeliferins in American Grasshoppers, What Possible Function?

Alborn HT, Brennan MM, and Teal PEA

Center for Medical, Agricultural, and Veterinary Entomology, Agricultural Research Service, U. S. Department of Agriculture, Gainesville, FL 32608

Corresponding author: hans.alborn@ars.usda.gov

Caeliferins is a new class of compounds recently identified from regurgitant of the American grasshopper (*Schistocerca americana*) (1). Two closely related caeliferins were shown to induce the release of volatiles in corn plants comparable with what earlier has been shown with volicitin and other fatty acid amides. However, *S. americana* regurgitant also contains other caeliferins with less activity on corn. Screening of regurgitant collected from several grasshopper species (2) collected in the wild in Florida revealed that regurgitant of all tested true grasshoppers contains caeliferins. However, the composition was more diverse than anticipated and even included new types of caeliferins not found in *S. americana* regurgitant. Despite this diversity between species, the patterns of caeliferins were remarkably constant within species. We present new types of caeliferins and discuss the possible functions of the complex but species-specific composition of caeliferins.

1. Proc. Natl. Acad. Sci. (2007) 104:12976–12981.

2. *Aptenopedes sphenarioides*, *Melanoplus bispinosus*, *M. propinquus*, *M. impudicus*, *Paroxya clavuliger*, *Romalea microptera*, *Schistocerca americana*, *S. obscura*, *S. damnifica*, *Dichromorpha viridis*, and *Locusta migratoria*.

Tomato Plant Gene Expression Altered by Caterpillar Labial Saliva Revealed by Microarray Analysis

Musser RO and Vogel H

Department of Biological Sciences, Western Illinois University, Macomb, IL 61455 (ROM);
Department of Entomology, Max Planck Institute for Chemical Ecology, Beutenberg Campus,
Jena, D-07745, Germany (HV)

Corresponding author: ro-musser@wiu.edu

We tested the effects *Helicoverpa zea* caterpillar labial saliva had on tomato plant gene expression. In the first experiment, leaves of tomato plants were wounded with scissors to simulate *H. zea* caterpillar herbivory and then applied with labial salivary gland extract, autoclaved labial salivary gland extract, GOX, water, or left nonwounded. Four hours after wounding, leaf tissue was harvested and then analyzed with tomato cDNA microarrays. In the second experiment, caterpillars with labial salivary glands (mock) and caterpillars without labial salivary glands (ablated) due to surgery fed on tomato plants for 24 h. Plant gene expression was analyzed with tomato cDNA microarrays. Approximately 395 annotated genes seemed to be significantly altered by wounding either by scissors to simulate caterpillar herbivory or by caterpillar herbivory ($P < 0.05$, one-way ANOVA). Of the 395 genes, approximately 273 were suppressed and 122 were stimulated in comparison to the nonwounded control. Sixty eight genes had trends that seemed to be stimulated by labial saliva particularly for herbivory by caterpillars with labial salivary glands. Verification of genes stimulated by labial saliva included acid phosphatase, acidic endochitinase, allene oxide cyclase, arginase, lipoxygenase, dehydrin TAS14, polyphenol oxidase F, and protease inhibitor 2. Many of the abiotic-related genes that seemed to be stimulated by labial saliva were also known to be abiotic acid-stimulated genes such as acidic endochitinase, acid phosphatase, and dehydrin.

Visualizing Caterpillar Oral Secretions on Leaves

Peiffer M and Felton G

Department of Entomology, The Pennsylvania State University, University Park, PA

Corresponding author: mlk101@psu.edu

For some caterpillar species, the components of oral secretions, or regurgitant, have been well characterized. Regurgitant is known to contain fatty acid conjugates such as volicitin, an elicitor of plant defense responses. It has been assumed that as caterpillars feed, they regurgitate onto the leaf, thereby allowing volicitin to come in contact with leaf tissue. However, regurgitation during feeding has not been adequately documented. In these experiments, we visualize regurgitant on the leaf by using fluorescent dye and confocal microscopy. Caterpillars were fed artificial diet to which the fluorescent dye Alexa 488 had been added. The caterpillars were then allowed to feed on leaves. Confocal microscopy confirmed that the caterpillar regurgitant was highly fluorescent, whereas salivary secretions did not fluoresce. Observation of the leaves by confocal microscopy allowed us to visualize regurgitant on the leaves. This study included *Helicoverpa zea*, *Heliothis virescens*, *Manduca sexta*, *Spodoptera frugiperda*, and *Spodoptera exigua* on a variety of host plants.

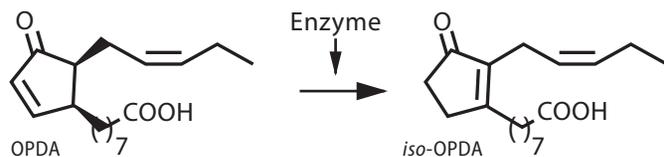
Enzyme-Assisted Isomerization of 12-Oxophytodienoic Acid in the Insect Gut and Its Significance for the Herbivore

Dąbrowska P, Freitak D, Vogel H, and Boland W

Department of Bioorganic Chemistry (PD, WB) and Department of Entomology (DF, HV), Max Planck Institute for Chemical Ecology, Jena, Germany

Corresponding author: pdabrowska@ice.mpg.de

Plant-derived compounds ingested by a feeding herbivore undergo different metabolic modifications in its gut. Most studies focus on the detoxification processes of poisonous plant secondary metabolites, which involve a variety of insect enzymes. Recently, more attention has been drawn to the potential role played by plant signaling molecules in the insect gut. Li et al. (1) proposed that plant hormones, in particular jasmonic and salicylic acids, can be used by feeding insects as early cues to activate their detoxification system against accumulating plant toxins. During our analysis of the fate of plant-derived oxylipins in the gut of a feeding Egyptian cotton leafworm (*Spodoptera littoralis*), we observed an enzyme-assisted isomerization of 12-oxophytodienoic acid (*cis*-OPDA), the biosynthetic precursor of jasmonic acid, to *iso*-OPDA. This rapid isomerization takes place in the foregut of many lepidopteran species (2).



Our current efforts are focused on elucidating the possible mechanism and significance of this isomerization process. First results indicate that the relevant enzyme belongs to the family of glutathione *S*-transferases, which are known to play a central role in detoxifying of insecticides in insects (3). Moreover, considerable structural similarities between OPDA and prostaglandins could suggest a potential interference of *cis*-OPDA with prostaglandin-linked effects in the insects. In plants *iso*-OPDA can serve a precursor for the famous fragrance *cis*-jasmane (4).

1. Nature (2002) 419:712–715.
2. Chembiochem (2007) 8:208–216.
3. Zool Stud (1996) 35(1):9–19.
4. Chembiochem (2007) 8:2281–2285.

Increased Universality of Lepidopteran Elicitor Compounds across Insects: Identification of Fatty Acid Amino Acid Conjugates

Yoshinaga N, Abe H, Fukui M, Nishida R, Alborn HT, Lait CG, Tumlinson JH, and Mori N

Applied Life Sciences, Graduate School of Agriculture, Kyoto University, Kyoto, 606-8502, Japan (NY, HA, RN, NM); Biological Sciences, Graduate School of Agriculture, Kyoto University, Kyoto, 606-8502, Japan (MF); Center of Medical, Agricultural, and Veterinary Entomology, Agricultural Research Service, U.S. Department of Agriculture, Gainesville, FL 32604 (HTA); Center for Chemical Ecology, Department of Entomology, Pennsylvania State University, University Park, PA 16802 (CGL, JHT)

Corresponding author: mokurin@kais.kyoto-u.ac.jp

Fatty acid amino acid conjugates (FACs) are known elicitors of induced release of volatile compounds in plants that, in turn, attract foraging parasitoids. Since the discovery of volicitin [*N*-(17-hydroxylinolenoyl)-L-glutamine] in the regurgitant of larval *Spodoptera exigua* (1), a series of related FACs have been identified in several other lepidopteran species. When screening 13 nonlepidopteran insects for the presence of FACs, we identified the same variety of FAC analogs previously found in lepidopteran caterpillars [*N*-linolenoyl- and *N*-linoleoyl-L-glutamic acids (main components), glutamine conjugates and hydroxylated derivatives of these corresponding FACs, including volicitin] in gut extracts of two closely related cricket species, *Teleogryllus taiwanemma* and *T. emma* (Orthoptera: Gryllidae), as well as in larval fruit fly, *Drosophila melanogaster* (Diptera: Drosophilidae). This suggests that FACs are more common than previously thought and may have physiological roles through a wide range of insects (2). In a previous study, we demonstrated that glutamine conjugates play an active role in nitrogen metabolism in *S. litura* larvae (manuscript in preparation). However, a physiological role or biosynthetic pathway has yet to be established for glutamic acid conjugates that are the major FAC components in crickets and fruit fly as well as in *Manduca sexta* (Lepidoptera). Here, we report an intriguing biosynthetic pathway of glutamic acid conjugates in *T. taiwanemma*, which might help us to understand why some insects have evolved such a variety of FACs, whereas others have not.

1. Science (1997) 276:945–949.
2. J. Chem. Ecol. (2007) 33:1376–1381.

Apocarotenoids—Signaling Compounds of Zygomycetes and Plants?**Schachtschabel D, Schlicht M, Baluska F, and Boland W**

Max-Planck-Institute for Chemical Ecology, Hans-Knöll-Strasse 8, D-07745 Jena, Germany (DS, WB); IZMB, University of Bonn, Kirschallee 1, 53115 Bonn, Germany (MS, FB)

Corresponding author: dschachtschabel@ice.mpg.de

Fungi of the division Zygomycetes are common heterotrophic microorganisms occurring on terrestrial habitats such as soil and decaying plant material. For sexual reproduction, the zygomycete fungi interact via an elaborate series of carotene-derived compounds, namely, trisporic acids and their biosynthetic precursors. The compounds are used for recognizing mating partners, for inducing sexual differentiation and for mediating the recognition between zygomycetes and some of their mycoparasites (1). However, details of their metabolism and the biological significance of the various intermediates remained unclear. Therefore, we generated a trisporoid library by a combination of synthesis and biotransformation cultures of the species *Blakeslea trispora* (2). These references enabled us to study the biosynthesis and the biological function of individual trisporoids in more detail (3). The results prompted us to postulate a new sequence of molecular interaction, which includes two different metabolic pathways: one pathway to initiate and stimulate the hormone production and the second pathway for the intrinsic production of the known trisporic acids. Because these fungi occupy the same habitats like plants and animals, we decided to test whether the apocarotenoids have an influence on plant or animal cells. Amazingly, the C18-ketone, an early trisporoid precursor, strongly inhibited root hair development of *Arabidopsis thaliana* in a nanomolar range. The inhibition is linked to disruption of the root-hair-associated NADPH-oxidase and caused a collapse of the actin cytoskeleton along with the actin-linked endosome trafficking system (4). The disruption of the actin cytoskeleton is also visible by treatment with the same compound on human cells.

1. Phytochemistry (2005) 66:1358–1365.

2. J. Org. Chem. (2007) 72:1366–1372.

3. Schachtschabel D, David A, Menzel K, Boland W (submitted) Who is talking—complex hormone signaling between (+)- and (–)-mating type of *Blakeslea trispora*. J. Biol. Chem.

4. Schlicht M, Samajová O, Schachtschabel D, Mancuso S, Menzel D, Boland W, Baluska F (in press) Dorenone blocks polarized tip-growth of root hairs by interfering with the PIN2-mediated auxin transport network in the root apex. Plant J.

Insect Pheromones (Olfaction, Evolution, Application)

Moderators:
14.00–15.30 **Wilhelm Boland**, Max Planck
15.50–17.20 **Jeffrey Aldrich**, USDA–ARS Beltsville
Room 11, Life Sciences Building



PARALLEL SESSION 2 | MONDAY, 18 AUGUST

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Wicher D, Schäfer R, Bauernfeind R, Stensmyr MC, Heller R, Heinemann SH, and Hansson BS

Max Planck Institute for Chemical Ecology, Hans-Knöll-St. 8, D-07745 Jena, Germany (DW, RS, RB, MCS, BSH); Center for Molecular Biomedicine, Institute of Molecular Cell Biology, Friedrich Schiller University Jena, Nonnenplan 2, D-07743 Jena, Germany (RH); Center for Molecular Biomedicine, Department of Biophysics, Friedrich Schiller University Jena, Hans-Knöll-St. 2, D-07745 Jena, Germany (SHH)

Corresponding author: hansson@ice.mpg.de

Odorant signals are detected by binding of odor molecules to odorant receptors. These belong to the G-protein-coupled receptor (GPCR) family. They in turn couple to G-proteins, most of which induce cAMP production. This second messenger activates ion channels to depolarise the olfactory receptor neuron, thus providing a signal for further neuronal processing. Recent findings, however, challenge this concept of olfactory signal transduction in insects, because their odorant receptors (ORs), which lack any sequence similarity to other GPCRs, are composed of conventional ORs (e.g., Or22a), dimerised with a ubiquitously expressed chaperone protein, such as Or83b in *Drosophila*. Or83b has a structure similar to GPCRs, but it has an inverted orientation in the plasma membrane. Still, G-proteins are expressed in insect olfactory receptor neurons, and olfactory perception is modified by mutations affecting the cAMP transduction pathway. In our experiments we could demonstrate that application of odorants to mammalian cells coexpressing Or22a and Or83b results in nonselective cation currents activated via both an ionotropic and a metabotropic pathway, and a subsequent increase in the intracellular Ca^{2+} concentration. Expression of Or83b alone leads to functional ion channels not directly responding to odorants, but directly activated by intracellular cAMP or cGMP. Insect ORs thus form ligand-gated channels as well as complexes of odorant sensing units and cyclic nucleotide-activated nonselective cation channels.

Nature (2008) 452:1007–1011.

Evidence for a Nano-Level “Olfactory Lens” Found on Individual Male Moth *Sensilla trichodea* by Using Atomic Force Microscopy

Baker TC, Maitani M, Park KC, Böröcsky K, Tumlinson J, and Allara D

Departments of Entomology (TB, KCP, KB, JT) and Chemistry (MM, DA) and the Huck Institutes of the Life Sciences, The Pennsylvania State University, University Park, PA 16802

Corresponding author: tcb10@psu.edu

Because male moths cannot sniff, they depend on the ability of their antennae and their tens of thousands of long, hairlike *sensilla trichodea* to adsorb airborne pheromone molecules contained in the odor strands comprising pheromone plumes. Karl-Ernst Kaissling’s group (1) first introduced the concept of an “olfactory lens” occurring on moth antennae when he and his colleagues found that the thousands of *sensilla trichodea* on moth antennae ended up adsorbing an inordinately high proportion of radiolabeled sex pheromone molecules relative to their surface area compared to the total antennal surface area. R. A. Steinbrecht (2) had shown how the sensilla have a fine surface structure comprised of ridges, flat inter-ridge areas, and regularly arranged pores on these flat areas. The pores lead to pore tubules through which the pheromone molecules, via one-dimensional surface diffusion, enter the sensillum lymph that is made up of binding proteins and pheromone-degrading enzymes. Kaissling’s group had shown, using dried antenna, that pheromone molecules land on the antennal surface and enter the pore tubules in less than 5 ms. We used chemical force and atomic force microscopy on individual *sensilla trichodea* of male *Helicoverpa zea* moth antennae and found that the ridges, flat areas, and pores described by Steinbrecht are chemically heterogeneous in a way that might possibly expedite the surface adsorption and one-dimensional diffusion of pheromone components into the pores. For *H. zea*, each sensillum is a sculptured, orderly array of terraces festooned with pores, with each terrace having at its edge a ridge. We found that the pores, the entry points into pore tubules on each sensillum, are more hydrophilic than are the flatter inter-ridge regions, and the bases below each ridge are also more hydrophilic than the inter-ridge regions. The chemical heterogeneity of the sensillar surface lipids may provide a way for the *H. zea* aldehyde pheromone component molecules, which have surfactant characteristics, to solubilize more readily in the pores than in the flat areas, and this may expedite transport down the pore tubules into the sensillum lumen.

This research was supported by a grant from the Office of Naval Research, and from the Keystone Alliance of the Commonwealth of Pennsylvania.

1. J. Insect Physiol. (1985) 31:71–81.
2. Int. J. Insect Morphol. Embryol. (1997) 26:229–245.

Trade-off between Sensitivity and Specificity in the Cabbage Looper Moth Response to Sex Pheromone

Allison JD, Hemmann DJ, and Haynes KF

Department of Entomology, University of Kentucky, Lexington, KY (JDA, KFH); John Ball Zoological Garden, 1300 W. Fulton, Grand Rapids, MI 49504 (DJH)

Corresponding author: jeremy.allison@uky.edu

The evolution of male moth responses to pheromone blends may be constrained by a trade-off between the response traits sensitivity and breadth of response. Population genetic simulations predict that if sensitivity and breadth of response are negatively correlated (i.e. a trade-off exists) that selection will favor males with narrow response phenotypes and high sensitivity. Although sensitivity-breadth of response trade-offs are generally assumed to exist and are implicit to the shape of male preference function, this study is the first to provide empirical support measuring behavior at the level of the individual. Previous studies with the cabbage looper, *Trichoplusia ni*, have documented the existence of a mutant pheromone strain. Although mutant females produce a pheromone blend significantly different from wild-type females, mutant males respond equally to the wild-type and mutant pheromone blends. This study used wind tunnel bioassays to document that relative to wild-type males, mutant males had broader response profiles but lower optimum pheromone sensitivity. Although wild-type male responses were highest to the wild-type pheromone blend, mutant males did not discriminate among pheromone blends. These results are consistent with a trade-off between breadth of response and sensitivity. Pure wild-type and mutant lines were crossed and hybrid males assayed. Both hybrid types (maternal wild-type and mutant hybrids) responded similarly. Hybrid males had response profiles similar to wild-type males and the reduced sensitivity observed in mutant males. These results suggest a possible hybrid disadvantage and a putative mechanism for reinforcement of male pheromone response traits.

Selection for Increased Proportion of “Rare” Responding Male *Ostrinia nubilalis* to the Sex Pheromone of *O. furnacalis*

Linn CE, Musto CJ, Dronney DC, and Roelofs WL

Department of Entomology, New York State Agricultural Experiment Station, Cornell University, Geneva, NY (CEL, CJM, WLR); Department of Biology, Hobart and William Smith Colleges, Geneva, NY (DCD)

Corresponding author: cel1@cornell.edu

In previous studies with *Ostrinia nubilalis*, the European corn borer (ECB) and *O. furnacalis*, the Asian corn borer (ACB) moths, we found that 3–5% of males tested in the flight tunnel are capable of flying upwind to the pheromone blends of both species. We report here on studies to determine whether this rare response phenotype could be increased via selection and to estimate the realized heritability of the response in our ECB Z colony. Because the rare response is sex-limited and occurs at low frequencies, we used a family selection protocol. We started with 21 families in which 20 males were flown to determine the family frequencies of unusual responders and the mean strength of response for each family. We scored ECB males by strength of response (no response, 0; fly upwind one fourth of the distance to the source, 1; 50%, 2; 75%, 3; and land on the source, 4). Male parents for subsequent generations were chosen based on the total male response score in a family. Realized heritability (h^2 ; calculated by dividing the mean response over the experiment by the cumulated selection intensity) after nine generations was estimated to be 0.7, and the proportion of rare responders rose from 3 to 30%. After generation 9, selection was relaxed by allowing 180 males and 180 females, chosen from the top-ranking families, to randomly mate. Over the next five generations of relaxed selection within the selection line, the proportion of responses to the ACB blend was maintained at 40 to 50%. The results clearly demonstrate a heritable genetic component to rare male response, and they lay the foundation for further genetic studies on the evolution of these moth pheromone systems.

Phyllophaga: Pheromones and Phylogenetics

Robbins PS

Department of Entomology, NYSAES, Cornell University, Geneva, NY

Corresponding author: psr1@cornell.edu

The *Phyllophaga* (s. lato) (Coleoptera: Scarabaeidae: Melolonthinae) are a very large New World genus, consisting of 860 species in 10 subgenera, whose distribution extends from Canada to South America and throughout the Caribbean. Historically, discerning relationships between and within groups has been difficult and somewhat contentious. The discovery of a large number of *Phyllophaga* species using various blends of the same compounds (methyl esters of L-valine, L-isoleucine, L-leucine, methyl 2-(methylthio) benzoate, and methyl 2-amino benzoate, methoxybenzene) as long-distance sex attractants has contributed substantially to group definition when paired with mitochondrial DNA. However, much remains to be done to understand mating behaviors in sympatric *Phyllophaga* species using the same long-distance pheromones during the same night.

How Important Is Sex for Females of a Haplodiploid Species under Local Mate Competition? An Olfactory Approach

Steiner S and Ruther J

Freie Universität Berlin, Institut für Biologie, Haderslebener Str. 9, 12163 Berlin

Corresponding author: svenst@zedat.fu-berlin.de

In haplodiploid species, even virgin females are able to reproduce by laying unfertilized eggs developing into male offspring. Thus, it has been proposed that females of haplodiploid species gain only little advantages from mating when population sex ratio is at equilibrium. However, in species where population sex ratio is female-biased and mating frequently occurs between siblings (local mate competition), females should invest comparatively more time and energy in mating activities. Here we studied the model organism *Nasonia vitripennis* Walker (Pteromalidae), a gregarious ectoparasitoid of several necrophorous Diptera and particularly suitable for the study of local mating competition. Males of this species use a substrate borne sex pheromone composed of (4*R*,5*R*)- and (4*R*,5*S*)-5-hydroxy-4-decanolide and 4-methquinazoline to attract virgin females. We tested in behavioural two-choice experiments whether females are also attracted toward the odour of host puparia. Furthermore, we investigated whether the pheromone or host odour is the stronger stimulus for females and studied possible interactions between both stimuli. We found that virgin females were attracted to the odour of a single host puparium. However, when given the choice between host odour and the pheromone released by a single male, virgin females preferred the pheromone irrespective of the number of host puparia offered. This preference was shown even by those females that had oviposition experience and thus, the chance to associate host odours with oviposition success. After mating, however, the female preference immediately switched to host odour. We conclude from the strong innate preference for the male sex pheromone that mating is crucial also for females of haplodiploid species due to indirect fitness gained by the reproductive success of daughters. Further results, investigating the correlation between male size and mating frequency with their pheromone status suggested that in *N. vitripennis* the pheromone functions as an indicator of male quality enabling female mate choice.

Pheromones Mediating Reproductive Conflicts in the Honey Bee, *Apis mellifera*

Malka O, Katzav-Gozansky T, and Hefetz A

Department of Zoology, George S. Wise Faculty of Life Sciences, Tel Aviv University, Ramat Aviv, Tel Aviv 69978, Israel

Corresponding author: hefetz@post.tau.ac.il

The honey bee, *Apis mellifera*, is renowned for the almost absolute reproductive monopolization by the queen. Workers, however, did not forgo reproduction completely, and under queenless conditions compete for the opportunity to produce males before colony collapse. There are two major pheromones that are associated with reproduction in honey bees, those of the mandibular (QMP) and Dufour's (QDP) glands. QMP is involved mostly in reproductive dominance, whereas QDP serves as a fertility signal. Accordingly, these pheromones are generally queen specific, but reproductive workers produce them as well, instead of their usual worker bouquet. Upon queenlessness some of the workers exhibit an early increase in both the QMP and QDP. The first presumably play a major role in establishing reproductive dominance by inhibiting ovarian development in worker nestmates, whereas the second first signal the worker's propensity to develop ovaries and subsequently delineate the degree of ovarian development. Reliability of the QDP fertility signal implies a close association with reproductive development. However, by treating callow workers with methoprene, we were able to inhibit ovarian development in such workers without affecting the pheromone systems. This uncoupling suggests that these processes may operate independently, but their initiation is triggered by a common event (e.g. the absence of the queen signal). We propose that these two processes evolved independently and that the pheromone systems were co-opted to emphasize the reproductive status of workers in the competition for reproduction. Fertility signals are favourably selected because they are beneficial both for the emitter (in recruiting helper workers for raising their brood) and the perceiver (in assisting them to make the appropriate reproductive decision to maximize fitness). Moreover, early pheromone producing workers are attacked by some of their nestmates, inflicting a cost on pheromone production and thus creating an honest signal. The evolution of QMP, in contrast, due to its coercive action on worker reproductive suppression, can be viewed as the result of queen worker sequential conflict.

Heading for Home: A Testable Hypothesis of How the Monarch Finds the Overwintering Sites in Mexico

McNeil JN and Frost BJ

Department of Biology, University of Western Ontario, London, ON, Canada (JNM); Department of Psychology, Queen's University Kingston, ON, Canada (BF)

Corresponding author: jmcneil2@uwo.ca

The seasonal migration of the monarch butterfly is well documented and although a great deal is known about this spectacular phenomenon, several important questions remain to be answered. Recently, Mouritsen and Frost showed that monarchs use sun compass orientation when migrating southward to Mexico in the fall. However, it is still unclear how they are able to locate the specific overwintering sites that are used year after year. One possibility is that chemical cues left at the site by previous generations play a role in this facet of migration. A testable hypothesis, based on different aspects of known monarch chemical ecology, the persistence of certain organophosphates in the waxes of conifer needles and adult flight behavior are presented.

Enhanced Pheromone-Based Trapping Method for Banana Weevil, *Cosmopolites sordidus* (Coleoptera: Curculionidae)

Reddy GVP, Cruz ZT, and Guerrero A

Western Pacific Tropical Research Center, College of Natural and Applied Sciences, University of Guam, Mangilao, Guam 96923 (GVPR, ZTC); Department of Biological Organic Chemistry, Institute of Chemical and Environmental Research (Consejo Superior de Investigaciones Cientificas), Jordi Girona 18-26, 08034-Barcelona, Spain (AG)

Corresponding author: reddy@uguam.uog.edu

The banana weevil, *Cosmopolites sordidus* (Germar) (Coleoptera: Curculionidae), is one of the main pests of banana plantations throughout the world (1). Among the existing control methods for this pest, chemical application is both undesirable and expensive, and biological control is so far limited. In addition, the pheromone-based trapping method using pitfall traps typically results in low capture rates (2). In this study, several important factors that affect pheromone-based catches, such as types of traps, trap dimensions, and color and position of the traps have been optimized. Ground traps baited with pheromone lures have been found to be superior to the ramp and pitfall traps, with the larger dimension traps (40 × 25 cm) being more efficient than those of smaller dimensions (30 × 15 cm). In a color-choice test, the banana weevil clearly preferred brown traps over yellow, red, gray, blue, black, white, and green, with the mahogany brown being more effective than other browns. In addition, pheromone-baited ground traps positioned in the shade of the canopy caught significantly more adults than traps placed in sunlight. Therefore, mahogany brown ground traps of 40 × 25 cm seem to be very efficient in catching *C. sordidus* adults, and they may be useful in mass trapping and eradication experiments for this pest.

1. Tropical Agriculture Series (1987) Scientific and Technical, UK (Bananas).
2. J Appl Entomol (2005) 129:265–271

Effects of Autodetection of Sex Pheromone on the Efficacy of Mating Disruption

Harari AR and Zahavi T

Department of Entomology, The Volcani Center, Israel

Corresponding author: ally@int.gov.il

Mating disruption, the use of species-specific sex pheromone for the control of insect pests, is used for the last 25 yr against a variety of species, in various levels of success. Mating disruption is achieved, in large, by permeating the area under focus with a synthetic pheromone, to reduce mate location, with the end results of suppressing the target population. The mechanisms proposed to be involved in achieving this goal are not completely understood, but traditionally use male attributes: adaptation of the antennal receptors, habituation of the central nervous system, masking the natural female's plum, and interrupting with the female's natural plum and imbalance in sensory input due to massive release of only partial pheromone blend. We suggest a mechanism based on female characteristics as an additional explanation for the observed reduction of pest populations, under the regime of mating disruption. After the repeated finding of autodetection of female sex pheromone in several species, the effect of self pheromone on the behavior of females was studied in some species, and evidence is accumulating on its end results on females' fitness. To verify the negative effect of self pheromone on females, experiments were conducted in vineyards treated with *Lobesia botrana* mating disruption pheromone and under laboratory conditions. The results were supported in testing fitness parameters of *Plodia interpunctella* and *Agrotis segetum* exposed to their own pheromone.

Endocrine Control of Sex Pheromone Response in a Long-Lived Moth, *Caloptilia fraxinella* (Lepidoptera: Gracillariidae)

Lemmen J and Evenden ML

Department of Biological Sciences, University of Alberta, Edmonton, AB, Canada

Corresponding author: jlemmen@ualberta.ca

Caloptilia fraxinella (Lepidoptera: Gracillariidae) is a long-lived moth that spends 9 months of its univoltine life cycle in a state of reproductive diapause. Throughout the adult life stage, males demonstrate plasticity in response to female sex pheromone and response is most acute when females are sexually receptive (1). Because juvenile hormone (JH) is known to regulate reproductive diapause in many lepidopteran species, we tested the hypothesis that exogenous application of a JH analog (methoprene) to male *C. fraxinella* in reproductive diapause would restore pheromone responsiveness. Separate groups of male *C. fraxinella* were treated with methoprene in early and mid-diapause, and their pheromone response was compared with solvent-treated males. Pheromone response was measured behaviorally using a wind tunnel bioassay and electrophysiologically using an electroantennogram assay. Methoprene application to males in mid-diapause restored male responsiveness to female sex pheromone in behavioral and electrophysiological assays. However, methoprene treatment of males in early diapause did not restore pheromone response in either assay. Our data indicate that juvenile hormone regulates the reproductive diapause syndrome in male *C. fraxinella*, but its effect varies throughout the extended diapause period, as has been shown previously for females (2). Furthermore, unlike what has been shown in other long-lived moths, juvenile hormone seems to act, at least in part, on the peripheral nervous system because antennae from methoprene-treated males in mid-diapause showed increased electrophysiological response to pheromone.

1. Annu Rev Entomol 47:93–122.

2. Physiol Entomol 32:275–282.

Novel Approaches Using Push-Pull Strategy for Stable Fly Control**Zhu JJ, Berkebile DR, and Zurek L**

USDA-ARS-AMRU, UNL, East Campus, Lincoln, Nebraska 68583 (JJZ, DRB);
Department of Entomology, Kansas State University, Manhattan, KS 66506 (LZ)

Corresponding author: jerry.zhu@ars.usda.gov

The stable fly, *Stomoxys calcitrans*, has been considered as the most important insect pest of cattle in the United States. The negative impacts of biting result in significant weight loss and milk production, which attribute to huge economic losses in cattle industry. The control of this pest heavily depends on the application of insecticides, but only provides with marginal effectiveness, and this practice is often not practical for the organic cattle farming. Push-pull strategy is a behavioral manipulation strategy in which behavior-modifying stimuli are integrated for reaching the sustainable pest management goal. The efficacy of using this strategy for the pest control is enhanced with a combination of attractants (“pull”) and repellent (“push”). Several successful cases have already been reported in agricultural and urban pest control. However, similar approach has barely been tested in stable fly control. In this presentation, we report our preliminary findings on efforts to identify effective stable fly repellents (botanical-based) and potential attractants (including oviposition attractants) that will be further developed into a integrated stable fly management tool. One or several chemical components have been identified from one herbal plant species, which shows with >98% repellency against starved stable flies (from feeding bioassays using K-D module). In addition, these compounds can effectively deter stable fly egg-laying behavior (>95%). At a dosage of 20 mg, it (vapor pressure) kills stable flies in a relative short period (with knockdown mean time between 6 and 8 min). The spatial repellency of these compounds against gravid stable flies also has been demonstrated. Stable flies use a variety of visual, olfactory, gustatory, and physical stimuli in host location and selection. Of these stimuli, volatile semiochemicals play a major role in mediating host location, including oviposition. Several manure and rumen associated odorants have been identified with strong sensory responses of stable flies. Further studies suggest that bacterially derived volatile compounds also play a role as oviposition stimulants for gravid stable flies. GC-MS analyses of odors collecting from *Citrobacter freundii* substrate (the most attractive medium for oviposition) show one or several volatile chemicals that may contribute to their ovipositional attractiveness. Potentials of using these identified repellent and attractant candidates is discussed for use in the future stable fly integrated management.

Genetics of Odor Perception in Insects

Speaker: Leslie B. Vosshall
Laboratory of Neurogenetics and Behavior,
The Rockefeller University, New York, NY
100 Berg Auditorium, Life Sciences Building

SILVERSTEIN-SIMEONE LECTURE



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Vosshall LB

Laboratory of Neurogenetics and Behavior, The Rockefeller University,
1230 York Avenue, Box 63, New York, NY 10065

Silverstein–Simeone Lecture: leslie@mail.rockefeller.edu

My laboratory has been studying the odorant receptors (ORs) governing recognition of food and host odors in *Drosophila* fruit flies and *Anopheles* mosquitoes. A major goal of this project is the development of novel chemical compounds that will inhibit these proteins and thus inhibit host-seeking behavior of vector insects. Insect ORs were first identified in *Drosophila* by a bioinformatic approach that sought multitransmembrane domain proteins selectively expressed in olfactory neurons (1,2,3). The *D. melanogaster* genome contains a family of 62 OR genes (4), each of which encodes a divergent seven transmembrane domain protein with no homology to chemosensory receptors in vertebrates or the nematode *C. elegans*. All members of the gene family except one are expressed in small subpopulations of olfactory neurons in the adult antenna and maxillary palp or in the olfactory organ of the larva. A single member of the family, Or83b, is broadly expressed in nearly all olfactory neurons, and forms a heteromultimeric complex with the other, selectively expressed Ors (5). We recently proposed that the insect ORs adopt an atypical membrane topology, with the N terminus facing the cell cytoplasm and the C terminus directed outside the cell (6), opposite to the topology of the G protein-coupled ORs mediating olfaction in vertebrates. We and others recently suggested that the insect ORs form ion channels that are directly gated by odors (7,8), although the extent and relevance of G protein-mediated second messengers in the signaling pathway of these receptors remains an ongoing and interesting controversy in the field. I discuss our efforts to identify small molecule inhibitors of insect ORs that have the potential to be safer and more effective insect repellents than DEET (9).

1. Neuron (1999) 22:327–338.
2. Genomics (1999) 60:31–39.
3. Cell (1999) 96:725–736.
4. Proc. Natl. Acad. Sci. USA (2003) 100(Suppl 2):14537–14542.
5. Neuron (2004) 43:703–714.
6. PLoS Biol (2006) 4:e20.
7. Nature (2008) 452:1002–1006.
8. Nature (2008) 452:1007–1011.
9. Science (2008) 319:1838–1842.

Poster Session 1

3rd and 4th Floor Bridges, Life Sciences Building

★ = poster presentations being judged for an award

Posters can be hung starting Monday afternoon, 18 August. Posters will remain in place until Thursday afternoon, 21 August, when they should be taken down by 12.00 (12:00 PM). Presenters assigned to odd-numbered poster sites will be expected to be present at their stations during the Monday evening (18 August) poster-viewing session. Viewing time both evenings is from 20.30 to 22.00 (8:30–10:00 PM).



POSTER SESSION 1 | MONDAY, 18 AUGUST

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Phospholipid Biosyntheses in *Spodoptera litura* Gut Tissues

Aboshi T, Yoshinaga N, Nishida R, and Mori N

Division of Applied Life Sciences, Graduate School of Agriculture, Kyoto University, Kyoto 606-8502, Japan

Corresponding author: frecher@kais.kyoto-u.ac.jp

In lepidopteran caterpillars, phospholipids and lysophosphatidylcholine (lysoPC) are known as biosurfactants present in the guts, and lysoPC is also thought to inhibit tannin-protein binding. Details on biosynthetic pathways of phospholipids in caterpillars, however, have not been studied, because phospholipids in their guts are thought to come from plant lipids. In this study, we investigated biosyntheses of phospholipids in *Spodoptera litura* larvae. Tracer experiments using [U-¹³C]linolenic acid clearly showed that some phospholipids were synthesized in midgut tissues of the larvae and then secreted into gut lumen to produce lysoPC. *S. litura* larvae were fed on artificial diets enriched with [U-¹³C]linolenic acid for 3 h, and then the gut contents were extracted by chloroform/methanol/water (2:1:0.8). The extracts were analyzed by liquid chromatography quadrupole ion trap time of flight mass spectrometry to investigate the phospholipid composition. Labeled linolenic acid was incorporated into lysoPC, lysophosphatidylethanolamine (lysoPE), and diacylPC, diacylPE, and diacylphosphatidylinositol (PI). To investigate details on the biosyntheses, supernatants of homogenates of gut tissues or contents were incubated with linoleoylCoA and lysoPC (18:3). Interestingly, PC (18:3-18:2) was biosynthesized in the supernatant of the tissues, but not in that of the gut contents. When each supernatant was incubated with PC (18:3-18:3), in contrast, a significant amount of lysoPC (18:3) was detected in the supernatant of the gut contents, but little in that of the tissues. These results suggested that [U-¹³C]linolenic acid was incorporated into phospholipids in gut tissues, and the biosynthesized phospholipids were released to the gut lumen, and then some PC was hydrolyzed in the gut lumen to produce lysoPC.

Relationship between Fruiting Phenology of Late Valencia Citrus (*Citrus sinensis*) and Abundance and Distribution of Mediterranean Fruit Fly, *Ceratitis capitata*

Appiah EF, Afreh-Nuamah K, and Obeng-Ofori D

Icipe, Nairobi, Kenya (EFA); Arppis Sub-Regional Centre for Western Africa, University of Ghana, Legon (KA-N); Department of Crop Science, College of Agriculture and Consumer Sciences, University of Ghana, Legon (DO-0)

Corresponding author: fappiah@icipe.org

The abundance and distribution of *Ceratitis capitata* in relation to the fruit phenology of Late Valencia citrus was investigated in citrus orchards at the University of Ghana's Agricultural Research Centre in the eastern region of Ghana. The study also investigated whether *C. capitata* used other crops within the study area and the effect of some climatic factors on the population dynamics of the pest. Results from the research showed an increase in the population of *C. capitata* from September through October 2006 and from February through March 2007, when Late Valencia citrus fruit were ripening or ripened. The fruit fly population was lowest from November 2006 through January 2007 when citrus fruit were in the immature green stage. *C. capitata* was not trapped in mango, pawpaw, and pepper fields and did not emerge from fruit of these crops. This suggests that the fruit fly population at the research centre is mainly dependent on citrus fruit. Abandoned citrus orchards, lack of fruit fly monitoring, incomplete harvesting, and poor orchard sanitation were identified as some of possible factors contributing to the increasing fruit fly population at the research centre. Multiple regression analysis on the effect of some climatic factors on the field population of *C. capitata* in the two citrus orchards showed a significant effect of rainfall and temperature ($P < 0.05$) on the pest population. The results of this study have important implications on the decision-making process for the safe, effective monitoring and management of *C. capitata* in Late Valencia citrus orchards at the research centre.



Volatiles of Citrus and Guava Plants: Something in the Air to the Psyllid *Diaphorina citri*

Noronha NG and Bento JMS

Universidade de São Paulo-USP, Escola Superior de Agricultura Luiz de Queiroz-Esalq, Dept Entomologia, Fitopatologia e Zoologia Agrícola, C.P. 09, Piracicaba-SP, 13418-900, Brazil,

Corresponding author: jmsbento@esalq.usp.br

Recent studies carried out in Vietnam showed that the intercropping of citrus and guava plants could reduce the infestation of the psyllid *Diaphorina citri*. This psyllid is the vector of “*huanglongbing*,” a very important bacterial disease in citrus. According to these studies volatiles from guava plants hypothetically could be repellent to *D. citri*. The aim of our work was therefore to study the olfactory response of *D. citri* to the volatiles of citrus and guava plants. We established experimental steps to test the repellent hypothesis asking the following questions: 1) Do guava volatiles alter the behavior in *D. citri*?; and 2) In which way do these volatiles affect *D. citri*? To answer 1, we offered volatiles of either citrus or guava plants and then offered a combination of both in Y-olfactometer bioassays. To answer 2, we used a 4-way-olfactometer setup in which we tested guava volatiles (3 fields of 4-way-olfactometer) against clean air (control). Twenty males and females were tested in each bioassay. The residence time in an odor field as well as the total number of choices for an odor field were recorded. Our results showed an alteration of the *D. citri* behavior after contact with volatiles of either guava or citrus. Compared with the control (clean air) individuals preferred odor fields containing citrus volatiles (total number of choices = 78.3%, $p < 0.01$ chi-square; residence time = 74.5%, $p < 0.01$ Friedman test). Interestingly, when offered guava alone and a mixture of guava and citrus volatiles in comparison with air in Y-olfactometer, we observed no response in the same parameters in both sexes. When testing *D. citri* to volatiles in the 4-way olfactometer setup, individuals significantly avoided guava leaf volatiles (total number of choices = 22.2%, $p < 0.01$ chi-square; residence time = 18.6%, $p < 0.01$ *t* test). Our results strongly suggest that guava leaves contain volatiles acting as effective repellants against *D. citri*.

Financial support: Fundação de Amparo à Pesquisa do Estado de São Paulo.

Identification of Male-Specific Volatiles Released by *Pissodes castaneus* (Coleoptera: Curculionidae): Evidence of a Sex Pheromone

Zaleski SRM, Frensch G, Lazzari SMN, Iede ET, Maia BHLNS, Tröger A, Francke W, and Marques FA

Federal University of Paraná, Curitiba, PR, Brazil (SRMZ, GF, SMNL, BHLNSM, FAM); EMBRAPA-FLORESTAS, Colombo, PR, Brazil (ETI); University of Hamburg, Hamburg, Germany (AT, WF)

Corresponding author: tic@ufpr.br (Marques)

In Brazil, pine plantations occupy an area of 1,840,050 ha, 57.6% of this area in the southern states of Paraná (32.9%), Rio Grande do Sul (7.4%), and Santa Catarina (17.3%). The pine species most frequently planted in the South are *Pinus taeda* and *P. elliottii*, which are attacked by the siricid woodwasp *Sirex noctilio*, aphids of the genus *Cinara*, and most recently by the banded pine weevil *Pissodes castaneus* (De Geer, 1775) (Coleoptera, Curculionidae), a forest pest introduced into Brazil in June 2001. Gas chromatographic (GC) analysis of airborne volatiles released by males or females of *P. castaneus* showed the presence of two male-specific compounds. Using GC-mass spectrometry (MS), these compounds were identified to be grandisol and grandisol. Treatment of the natural extract with LiAlH_4 produced grandisol, which was analyzed by enantioselective GC by using a β -cyclodextrin column and synthetic standards. As a result, (1*R*,2*S*)-grandisol was obtained in an enantiomeric excess >95%. This shows that in *P. castaneus* both grandisol and grandisol keep (1*R*,2*S*)-configuration in high enantiomeric purity. In laboratory bioassays using a Y-olfactometer, males attracted only females, indicating that these compounds are likely to be sex pheromones. The current status of the investigations is discussed.

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Black Fly *Simulium lineatum* (Diptera: Simuliidae) Possess Sex Pheromone

Baužienė V and Būda V

Laboratory of Chemical and Behavioural Ecology, Institute of Ecology, Vilnius University, Akademijos 2, LT-08412 Vilnius, Lithuania (VBa); Vilnius University, Faculty of Natural Sciences, Centre for Ecology and Environmental Studies, M.K. Čiurlionio 21/27, LT-03101, Vilnius, Lithuania (VBū)

Corresponding author: vinbuda@ekoi.lt

The aim of the present research was to establish whether black flies possess sex pheromones regulating their precopulatory behaviour. Bloodsucking stenogamous (ground coupling) species *Simulium lineatum* (Meigen, 1984) was chosen as a model. For comparative study, a phylogenetically closest bloodsucking eurygamous (aerial coupling) species *S. equinum* (L, 1758) has been chosen. Mating behaviour of *S. lineatum* was registered both under natural and laboratory conditions. In nature, *S. lineatum* males demonstrate courtship behaviour toward conspecific blackflies of any sex; however, quantitative characteristics of behavioural interactions differ depending on the sex in contact. Based on behavioural records both in nature and under laboratory conditions, and after a number of preliminary tests, an original bioassay for search of sex pheromones has been elaborated. A stimulus as a filter paper either with evaporated pure solvent or solvent containing female cuticular chemicals plus a female dummy attached thereon was provided to *S. lineatum* male for 1 min. Registered precopulatory behaviour elements were the following: approach, contact (a dummy touch) and attempt to copulate. The cuticle of *S. lineatum* females contains chemical compounds affecting pre-copulatory behaviour of conspecific males during the final phases of courtship only (touching and attempting to copulate). The effect is dose dependent. The cuticular chemicals are species specific, as those of phylogenetically closely related *S. equinum* females do not affect precopulatory behaviour of *S. lineatum* males. Chromatographic profiles of extracts of *S. lineatum* and *S. equinum* adults (both females and males) differ qualitatively and quantitatively: 27 compounds (10 are hydrocarbons with C_{17} , C_{26} , C_{37}) are present in *S. lineatum*, and 55 compounds (19 are hydrocarbons with $C_{11,19}$ and $C_{24,37}$) in *S. equinum* only. Bioassay demonstrated presence of sex pheromone in stenogamous *S. lineatum* females. This is the first sex pheromone record in the family Simuliidae.

Age, Gonotrophic State, and Cuticular Hydrocarbon Profiles of Mating House Flies, *Musca domestica* (Diptera: Muscidae), Collected on Dairies

Butler SM, Moon RD, Hinkle NC, Millar JG, McElfresh JS, and Mullens BA

Department of Entomology, University of Kentucky, Lexington, KY (SMB); Department of Entomology, University of California Riverside, CA (JGM, JSM, BAM); Department of Entomology, University of Minnesota, St. Paul, MN (RDM); Department of Entomology, University of Georgia, Athens, GA (NCH)

Corresponding author: sarah.butler@uky.edu

House flies, *Musca domestica* L. (Diptera: Muscidae), were collected in copula over two summers from six dairies located in three climatically distinct regions: southern California, Minnesota, and Georgia. The ages of 511 mating pairs were determined using pterin analysis. Mean age of mating males ranged from 54-100 degree-days (DD) (4-10 d), depending on the farm. Very young males (<10-20 DD) and old males (>200 DD) were rare in mating pairs. Mean female age at mating ranged from 20 to 46 DD (2.5-4 d). All mating females had eggs with partial yolk deposition (stages 2 and 3), 99.2% of which were nulliparous. Head width measurements of mating pairs suggest that assortative mating by size does not occur. The cuticular hydrocarbon profiles of females were determined, with emphasis on (Z)-9-tricosene (muscalure). Overall, only 55% of mating females had detectable amounts (>4 ng per fly) of (Z)-9-tricosene. Of the females that had detectable (Z)-9-tricosene, variation in amount per female was high and not related to the size or age of the mating female. The proportion of mating females with detectable levels of (Z)-9-tricosene was not independent of geographic region. Seventy-one, 63, and 27% of females from southern California, Minnesota, and Georgia had detectable amounts of (Z)-9-tricosene, respectively. A state-level distinctiveness of hydrocarbon profiles was observed in house fly populations, and it likely reflects genetic variation associated with environmental stresses in those geographic zones.

PBAN/Pyrokinin Peptides in Central Nervous System from Fire Ant *Solenopsis invicta*

Choi MY, Vander Meer RK, and Raina AK

Center of Medial, Agricultural and Veterinary Entomology, ARS–USDA, Gainesville, FL (MC, RKV); ARS–USDA, SRRRC, New Orleans, LA (AKR)

Corresponding author: mychoi@ars.usda.gov

Peptides from the pheromone biosynthesis activating neuropeptide (PBAN)/Pyrokinin family are expected to be found from all insect groups and some other arthropods. These neuropeptides are characterized by a conserved pentapeptide, FXPRLamide, at the C terminus. This amino acid sequence is required for physiological activity. The PBAN/Pyrokinin peptides have been found to 1) stimulate pheromone production (PBAN), 2) act as an embryonic diapause hormone (DH), 3) induce melanization and reddish coloration hormone (MRCH), 4) stimulate muscle contraction (myotropins or pyrokinins), 5) accelerate puparium formation, and 6) regulate pupal development and diapause. PBAN is synthesized in the subesophageal ganglion (SG) and released into the hemolymph where it stimulates sex pheromone production in many moths. However, virtually nothing is known about how pheromone production and release is regulated in social insects, which depend on sophisticated pheromonal communication to maintain colony cohesiveness and sociality. In the present study, we confirmed the presence of PBAN peptides and identified PBAN cDNA for the first time in an ant species, *Solenopsis invicta*. Brain-subesophageal extracts of male and female fire ant sexuals significantly stimulated sex pheromone production in a lepidopteran. Three groups of PBAN producing cell clusters were found in the SG that correspond to the mandibular, maxillary and labial neurons, as in Lepidoptera. PBAN-like immunoreactive neurons in the VNC were found in thoracic (two pairs) and abdominal ganglia (three pairs). Fire ant PBAN cDNA, encoding 176 amino acids, including *S. invicta* PBAN (Soi-PBAN) and three additional neuropeptides, was identified. The structure and preprohormone positions of the fire ant PBAN gene are more similar to lepidopteran PBANs than the PBAN/Pyrokinins found in other insect groups. Thus, the PBAN peptides in fire ants could function in pheromone production and/or regulation as they do in Lepidoptera.

A Fine-Tuned Strategy by *Trissolcus brochymenae* (Hymenoptera: Scelionidae) to Exploit Chemical Traces Left by *Murgantia histrionica* (Heteroptera: Pentatomidae)

Salerno G, Frati F, Conti E, De Pasquale C, Peri E, and Colazza S

Department of Scienze Agrarie e Ambientali, University of Perugia (GS, FF, EC); Department of C.I.S.A.C., University of Palermo (CDeP); Department of S.En.Fi.Mi.Zo., section of Acarology, Entomology and Zoology–University of Palermo (EP, SC)

Corresponding author: colazza@unipa.it

Indirect host-related cues are important signals used by egg parasitoid during host selection process. They guide parasitoid females in the areas where their hosts are potentially present, but they are not able to “promise” the presence of the suitable host stage. Among these cues, the presence of areas contaminated by traces left by walking pentatomid hosts trigger the arrestment response of parasitoids enhancing the searching in a restricted area and allowing the parasitoid to discriminate the host gender. However, the traces left on the substrate by mated females could give a more accurate information because they could “guarantee” the presence of suitable eggs in short time. Based on this premise, we hypothesized that changes in physiological status of adult host females, induced by copulation or oviposition, could be the key factors of a more fine-tuned strategy carried out by the parasitoids females to exploit the host chemical traces. To test this hypothesis *Murgantia histrionica*–*Trissolcus brochymenae* association was used. All experiments were conducted on an open arena, and recorded with a video tracking and motion analysis system. In a first experiment the arrestment responses of *T. brochymenae* females to host chemical traces left by virgin and mated males and females, mated females with interrupted copulation (after 30 min, 2 h, 4 h, and 8 h) and parous (one egg mass laid) females were evaluated. All *M. histrionica* females used for the bioassays were killed and dissected to evaluate the presence of male-derived secretions (pulpy substance) in the spermathecal duct and of the sperm in the spermathecal bulb. *T. brochymenae* showed the ability to discriminate the traces left by males and females of its host only when the host was mated. The arrestment responses of female wasps on traces left by *M. histrionica* females with interrupted copula were weaker than those obtained in the case of mated females. Furthermore, in all mated females the spermathecal duct was markedly swollen from being filled with “pulpy” substance and the sperm was found in the spermathecal bulb. On the contrary, in virgin females and females with interrupted copulation neither pulpy substance nor sperm was found. A second experiment was carried out to examine the searching behaviour of female wasps in arenas treated with tarsi and scutella dissected from mated adults. In these bioassays residues from tarsi of mated *M. histrionica* females elicited stronger responses than those from the corresponding body part of males, indicating that the source of kairomonal compounds could be at tarsi level. In a third experiment the *T. brochymenae* response to arenas treated with hexane extracts of the cuticles of virgin and mated host adults were evaluated. The hexane extracts of host cuticular lipids elicit a strong response of *T. brochymenae* females for extracts from mated females confirming that parasitoid arena residence times were significantly affected by the host physiological status. GC-MS analyses of hexane extracts of the cuticle of mated *M. histrionica* males and females showed quantitative differences in the composition of the cuticular hydrocarbons. We conclude that the exploitation of host chemical fingerprints by *T. brochymenae* is influenced by the host physiological status.

Plant Dependency on Rhizobia for Nitrogen Influences Herbivory and Accumulation of Phytohormones

Dean JM, Mescher MC, and De Moraes CM

Center for Chemical Ecology, Department of Entomology, The Pennsylvania State University, University Park, PA

Corresponding author: jmdean@psu.edu

Plants are central to most community interaction webs, and thus their ability to coordinate responses to many simultaneous interactions is an essential adaptation. Interaction with beneficial organisms, such as mycorrhizal fungi, can have a wide range of effects on plant–herbivore interactions (1,2,3). However, nitrogen-fixing bacteria (rhizobia), another class of ecologically important plant mutualists, have received little attention concerning their effects on plant–herbivore interactions. The association of legumes with rhizobia involves numerous physical and chemical changes in the host plant, such as initial induction of defense-like pathways, development of nodules on roots to house the bacteria, and the transport of different forms of nitrogen to the leaves (4). Many of these changes are lessened in a dose-dependent manner when plants receive a nitrogen fertilizer, as nitrate inhibits the plant's association with rhizobia (5). We explored the effects of rhizobia on herbivory and plant responses to herbivores by growing plants with a range of dependency on rhizobia versus N fertilizer for their nitrogen needs, while controlling for plant total nitrogen concentration. In soybean (*Glycine max*), this difference in nitrogen source has been shown to result in different ratios of organic ureide N to inorganic nitrate N. Soybean aphid (*Aphis glycines*) fitness was not affected by these differences, but soybean podworm (*Helicoverpa zea*) larvae grew better on plants completely dependent on rhizobia. In response to feeding by aphids and podworms, accumulations of the defense-related hormones salicylic acid and jasmonic acid, respectively, were more pronounced in plants completely dependent on rhizobia for nitrogen needs. Our results demonstrate that the rhizobia–legume mutualism can influence plant–herbivore interactions and contribute to a growing area of research exploring plant responses to multiple organisms.

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Walkingsticks as Models for Chemical Biodiversity and Biosynthesis (Order Phasmatodea)

Dossey AT and Edison AS

Department of Biochemistry and Molecular Biology, University of Florida, Gainesville, FL

Corresponding author: bugochem@gmail.com

Walkingsticks have evolved a variety of mechanisms to avoid predation—from morphology (camouflage) to behavior and mimicry to behavior to chemical defenses (1). Most phasmid species that use chemical weapons do so by spraying irritating substances from a pair of metathoracic glands just behind the head (1). A classic study by Meinwald and Eisner in 1962 made the first structural identification of a phasmid defensive compound (2). The species was *Anisomorpha buprestoides*, and the compound was a cyclopentanyl monoterpene dialdehyde they called anisomorpal (2). In earlier work by other groups, the chemistry of defensive sprays from eight species of phasmids had been analyzed (as cited in 3). Compounds found in those studies range from simple straight chain ketones to alkaloids (3). Our current work highlights the following recent discoveries in walkingstick chemical defense: 1) an overview of compounds found in walkingsticks and the species analyzed, 2) a developmental and geographic study of variability in *A. buprestoides* defensive chemistry (4,5), and 3) preliminary results demonstrating that *A. buprestoides* is a very promising model organism for chemical biosynthesis studies. Recently, we have analyzed the defensive sprays of 10 phasmid species. From this work, eight compounds, including spiroketals and dimethylpyrazines, have been identified for the first time from walkingsticks, and at least one novel compound has been discovered (3). We show these compounds, as well as other species analyzed that produce compounds already known to be produced by walkingsticks. In addition to small irritating molecules, evidence is presented that the defensive secretion of *A. buprestoides* also contains glucose (5) as well as a small number of proteins. Also, ¹³C labeling experiments, along with previous reports (6), demonstrate that *A. buprestoides* makes its own defensive monoterpene de novo (4). These results demonstrate that walkingsticks are a good model system for studying biosynthesis of a variety of small molecules.

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Unexplored Diversity of Bacteria in Arboreal Ants of Genus *Pseudomyrmex* and Their Putative Role in Nitrogen Fixation

Eilmus S and Heil M

Department of General Botany—Plant Ecology, FB BioGeo, Universität Duisburg-Essen, Universitätsstr. 5, D-45117 Essen, Germany (SE); Departamento de Ingeniería Genética, CINVESTAV—Irapuato, Km. 9.6 Libramiento Norte, Carretera Irapuato-León, 36821 Irapuato, Guanajuato, México (MH)

Corresponding author: sascha.eilmus@uni-due.de

Bacterial communities are highly diverse and have a great ecological importance. We studied the presence and potential ecological role of bacteria in Mexican acacia-ants of the genus *Pseudomyrmex*. Highly specialized species of this genus obligatorily live in hollow thorns of *Acacia* myrmecophytes and exclusively feed on plant-derived food sources, whereas other species have only loose associations with plants. The majority of bacteria cannot be cultivated, and knowledge on their ecological importance and even their mere existence is still in its infancy. We therefore chose a molecular approach. Unfortunately, even standard polymerase chain reaction (PCR) techniques followed by cloning and sequencing provide only a small glance into the bacterial community structure of an environmental sample. In contrast, the assignment tool TReFID (1) applies in silico analysis of terminal restriction fragments (tRF) obtained from digestions of fluorochrome-labeled PCR products with multiple restriction enzymes and allows the gross characterization of bacterial life in any complex bacterial community. Using TReFID, we could identify the bacterial taxa (phylum/division) Acidobacteria, Actinobacteria, Bacteroidetes, Firmicutes, Planctomycetes, Proteobacteria, and Spirochaetes and the genera *Burkholderia*, *Curtobacterium*, *Enterobacter*, *Erwinia*, *Pantoea*, *Serratia*, *Sphingomonas*, and *Wautersia* (*Cupravidus*) in arboreal *Pseudomyrmex* ants. The presence of most genera was confirmed via independent PCR and cultivation approaches. 1) The reduction of gaseous C₂H₂ to C₂H₄ by living workers and larvae of different *Pseudomyrmex* species confirmed the presence and functional activity of nitrogenase. 2) The *nifH* gene could be amplified from *Serratia* and *Pantoea* isolates, underlining their possible role as nitrogen-fixing symbionts. Nitrogen fixation in insects, which so far was believed to be restricted to termites, might therefore be much more common, and it might even explain the extraordinary abundance of tropical arboreal ants, which commonly feed on N-poor food sources such as exudates of hemipteran trophobionts, plant sap, or extrafloral nectar.

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Induction of Cell-Wall Invertase in *Arabidopsis thaliana* by Caterpillar Feeding—Implications for the Induced Sink Strength Model of Plant Defense?

Ferrieri A, Schultz JC, and Appel H

Division of Plant Sciences, University of Missouri, Columbia, MO 65211

Corresponding author: apfvfb@mizzou.edu

The ability of plants to respond to herbivores is determined by a highly integrative and interactive network of chemical signals and resources that flow between modules via the plant's vascular architecture. Although carbohydrates, translocated from source tissues to sinks, provide the necessary resources for growth and development in young leaves, they also have been shown to serve as a source of energy enabling the production of costly carbon-based defenses. For example, grazing by gypsy moth caterpillars on hybrid poplar saplings, as well as treatment using jasmonic acid, was shown to increase sink strength in developing leaves (1). This response manifested in elevated activities of cell wall bound invertase (cw-INV), an enzyme that facilitates phloem unloading at sink tissues by cleaving sucrose into hexose monomers, and increased tannin biosynthesis (2). This study evaluates the "induced sink strength" model in plant defense by *Arabidopsis thaliana* in response to caterpillar feeding by two species of Lepidoptera: *Pieris rapae* and *Spodoptera exigua*. cw-INV activities were measured for leaf cohorts of varying ages and compared with responsiveness of entire rosettes. Results for *P. rapae* show a substantial increase in the cw-INV activity in all leaf cohorts of attacked plants 48 h after treatment. This work not only provides a new perspective on the mechanism underlying resource allocation in response to environmental stimuli, but identifies a potentially unappreciated defense function—induced sink strength—and its ability to shape defense chemistry.

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A Field Trial Testing the Role of Various Diterpenoids in the Adult Host Preference and Larval Performance of Banded Sunflower Moth on Several Sunflower Lines

Foster SP, Ode PJ, and Charlet LD

Department of Entomology, North Dakota State University, P.O. Box 4356, Fargo, ND (SF, PJO); USDA-ARS, Northern Crop Science Laboratory, ND 58105

Corresponding author: stephen.foster@ndsu.edu

The banded sunflower moth (BSFM), *Cochylis hospes* Walsingham, (Lepidoptera: Cochylidae), is a major pest of cultivated sunflower in North America. Adult female moths oviposit on the outer whorl of involucre bracts of prebloom sunflower heads. After hatching, neonate larvae feed initially on bracts then, as the head begins to bloom, move inside the head to feed on pollen, florets, and ovaries. The latter causes direct loss of seed production. We have found that several diterpenoids, found on the bracts of sunflower heads, stimulate oviposition by female BSFM in laboratory bioassays. Moreover, cultivated sunflower contains relatively large amounts of two diterpenoid acids that, in laboratory trials, were found to be toxic to other lepidopteran species that feed on sunflower heads. We conducted a small field trial, using several lines of sunflower and a wild population of BSFM, to determine whether concentrations of these various diterpenoids in sunflower heads influenced adult female host selection and larval performance in the field.

Sex Pheromone of the Bud Borer *Epinotia aporema* (Walsingham) (Lepidoptera: Tortricidae): Chemical Identification and Male Behavioral Response

Altesor P, Horas VR, Rossini C, Zarbin PHG, and González A

Laboratorio de Ecología Química, Facultad de Química, Universidad de la República, Montevideo, Uruguay (PA, CR, AG); Laboratorio de Semioquímicos, Departamento de Química, Universidad Federal de Paraná, Curitiba, PR, Brazil (VRH, PHGZ)

Corresponding author: agonzal@fq.edu.uy

Epinotia aporema is a Neotropical pest of legumes of increasing importance in southern South America, owing to the significant growth of soybean production in this region. Monitoring of *E. aporema* is difficult given the cryptic behaviour of the larvae, and chemical control is hence done preventively. We studied the female sex pheromone of this species by combined chromatographic, spectroscopic, and electrophysiological methods, and we found that females produce two EAD-active compounds, (Z,Z)-7,9-dodecenol and (Z,Z)-7,9-dodecanyl acetate (15:1 ratio), which we identified based on the comparison of their retention indices, mass spectra, and EAD activity with those of synthetic standards. We also studied the behaviour of males in wind tunnel tests using different combinations of alcohol/acetate (15:1; 1:1, and 1:0; 1.6 µg). Although most males (60%) reached the pheromone source with the 15:1 mixture, only 10% did so for the other combinations. If taking into account other behaviours (wing fluttering, flying within the plume), 70% of the males responded to the 15:1 mixture, 60% to the alcohol alone, and 20% to the 1:1 mixture. The European congener, *E. tedella*, produces the same compounds (1), and most genera within the tribe Eucosmini (subfamily Olethreutinae) include species that are attracted to Δ7 and/or Δ9 dodecanyl/dodecadienyl alcohols and acetates.

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Attraction of Male Winterform Pear Psylla to Female-Produced Volatiles and to female Cuticular Extracts with Evidence of Male-Male Repellency

Guédot C, Horton DR, and Landolt PJ

U.S. Department of Agriculture, Agricultural Research Service, 5230 Konnowac Pass Road, Wapato, WA 98951

Corresponding author: christelle.guedot.ars.usda.gov

Pear psylla, *Cacopsylla pyricola* (Förster) (Hemiptera: Psyllidae), is a major pest of commercial pears in North America and Europe. Olfactometer trials have shown that males of both the summerform and winterform morphotype are attracted to female-infested host material (1,2,3). Additional work with the summerform morphotype has also shown that males are attracted to females even in the absence of the host plant, which is evidence that female *C. pyricola* produce a volatile sex attractant (3). We describe similar results with the winterform, confirming for this morphotype that the female psylla rather than the infested host material is the source of the attractants. Male winterforms displayed attraction to volatiles emitted by live females in the absence of the host plant, freshly killed females, and female cuticular extracts. The female cuticular extracts were as attractive as a comparable number of live females, suggesting that we were successful at extracting the important components of the attractant with this procedure. All previous olfactometer trials with *C. pyricola* used the insect as the attractant; the current study is the first to demonstrate that female-produced volatile chemicals, in the absence of the female insect, are attractive to male conspecifics. Winterform males also were assayed to odors produced by conspecific males. We found that male psylla avoided volatile odors from live males, freshly killed males, or cuticular extracts of males. To our knowledge, these results are the first indication that males of any Psyllidae avoid odors associated with conspecific males. Research is currently ongoing to isolate and identify the specific chemicals that are biologically active (attractant or repellent) to males.

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Studying the Role of Chitinase in Maize Insect Defense

Han Y, Chuang W-P, Herde M, Howe GA, and Luthe DS

Department of Crop and Soil Sciences, The Pennsylvania State University, University Park, PA (HY, DSL); Department of Biochemistry and Molecular Biology, Michigan State University, East Lansing, MI (MH, GAH)

Corresponding author: yxh158@psu.edu (Han)

A large percentage of crop loss yearly is due to insect damage, especially caterpillar damage. With the rapidly increasing world population, it is critical to minimize this yield loss. Therefore, the focus of this project is to study the function of defense-related chitinases in *Zea mays* (corn) inbred lines (insect-resistant line Mp708 and insect-susceptible line Tx601) and the defense mechanism against fall armyworm (*Spodoptera frugiperda*, FAW). Recent studies suggested there are four different chitinase genes present in maize plant that may target and destroy chitin associated with the caterpillar midgut. Using multidimensional protein identification technology of FAW frass, we determined that there were at least two chitinases (*Chitinase A* and *Chitinase I*) present in both insect-resistant and insect-susceptible maize lines. Quantitative real-time polymerase chain reaction analysis indicated that the levels of both chitinase transcripts were induced by at least 20-fold on FAW-fed and mechanically wounded plants. Similarly, chitinase enzymatic activity increased in FAW-infested and mechanically damaged plants as well. In the future, we plan to test the effectiveness of purified chitinase on caterpillar growth. Exploring plant defense proteins provides an opportunity to further understand the mechanism of plant insect resistance. The practical goal of this research is to find chitinases that could effectively inhibit caterpillar damage or improve plant resistance. Compared with chemical insecticides, this “natural” resistance provides more advantages both economically and environmentally.

Enzymatic Activity of Lipoxygenases, Isoflavone Contents, and Trichomes in Soybean Genotypes: Implication on Insect Plant Resistance

Graça, JP da, Salvador MC, Mello D, Mandarin JMG, and Hoffmann-Campo CB

Universidade Estadual do Norte do Paraná (JPG), UNESP/Jaboticabal (MCS) Universidade Federal do Paraná (DM), Embrapa Soja, Londrina, Paraná State, Brazil (JPG, MCS, DM, JMGM, CBHC)

Corresponding author: hoffmann@cnpso.embrapa.br

Soybean [*Glycine max* (L.) Merr.] is attacked by insects and other pests and constantly needs to activate its defenses against their injuries. Generally, plants use the synthesis of metabolites or morphological structures to deter or inhibit herbivorous activity. Plant lipoxygenases are enzymes involved in the biosynthesis of regulatory molecules, and in the response to insect and pathogen attacks. Trichomes are morphological structures generally related to physical plant defense. This work was carried out to evaluate lipoxygenases (LOX) enzymatic activity and density and length of trichomes in soybean genotypes ('BRS 155', 'BRS 213', 'BRS 257', 'BRS 134', 'BRS 133', 'BR 16', 'IAC 100', PI 227687, and 'Dowling'), relating these information with *Nezara viridula* (Hemiptera: Pentatomidae) developmental time and adult weigh. The genotypes were grown in greenhouse. Pods and seeds were morphologically and biochemically evaluated at soybean developmental stages R6, and R6 plus R8, respectively. Nymphs of stink-bugs were fed with R6-pods, from the second stage to adulthood. BRS 134, BR 16, Dowling, and BRS 155 showed longer trichomes, whereas IAC 100, BRS 213, and PI 227687 presented higher density of trichomes compared with the other genotypes. At R6, seeds of BRS 155 presented the highest LOX 1 activity, BRS 155, and PI 227687 presented the highest LOX 2 and LOX 3 activities, and Dowling presented higher LOX 2 activity. At R8, BR 16, IAC 100, BRS 133, and PI 227687 presented the highest activities of LOX 1, and BRS 133 presented the highest activity of LOX 2 and 3. Adults fed on BRS 155, whose seeds presented high LOXs (1, 2, and 3) activities were heavier (173.52) compared with PI 227687 (108.20 mg) and on BRS 257 (130.15 mg); developmental time varied from 21.46 to 22.57 days, but no statistical differences between treatments were detected. PI 227687 is a widely used source of insect resistance and in our trial showed higher LOX 2 and LOX 3 activities, higher trichome density and higher isoflavone contents. However, BRS-257, which presented low LOXs (1, 2, and 3) activity showed neither longer nor higher trichome density, intermediate isoflavone content compared with the other genotypes. Consequently, as indicated by this bioassay, chemical, biochemical, and mechanical factors can act synergistically on the defense of PI 227687 against insect pests. Regarding BRS 257, the plant defense likely comes from other types of LOXs. Consequently, the involvement of lipoxigenases in soybean plant defense against insects is not clearly elucidated.

Cucumber Volatile Blend Attractive to Female Melon Fly, *Bactrocera cucurbitae* (Coquillett)

Jang EB, Siderhurst MS, Carvalho LA, and Nagata JT

USDA-ARS-USPBARC, Hilo, HI (EBJ, LAC, JTN); Department of Chemistry, Eastern Mennonite University, Harrisonburg, VA (MSS)

Corresponding author: eric.jang@ars.usda.gov

The melon fly, *Bactrocera cucurbitae* (Coquillett), is a serious crop pest throughout the Pacific, subcontinental, and Southeast Asia, causing damage to a variety of solanaceous and cucurbit-related crops. Attractants for female melon flies are of particular interest because they could be used to reduce pest levels by killing both the female and her potential offspring. Previous work has shown that freshly sliced cucumbers are attractive to female melon fly, and the objective of this research was to identify a synthetic lure for *B. cucurbitae*. GC-EAD analysis of fresh and aged cucumber slurry volatiles identified 32 compounds that were detected by melon fly females. Active compounds were initially screened as single components in glass McPhail traps in outdoor rotating olfactometer experiments. Synthetic blends were composed based on initial screening results and EAD responses and tested in both an outdoor rotating olfactometer and field cage. Six- and 9-component blends were shown to have female-biased attraction in outdoor rotating olfactometer and field cage experiments. Initial field captures with the 9-component blend were 2 times greater than with solulys protein bait. Subsequently, a 7-component blend was formulated into plastic matrix plugs and caught more females than solulys in McPhail type traps. Besides being more attractive, this lure may have several other advantages over protein baits; it has the potential to be used with a dry trap, to be long-lasting, and it captures comparably low numbers of nontargets. Applications of this new female lure include trapping for detection (new infestations/biosecurity) and monitoring/delimitation (for treatment timing), as a control/eradication method with mass trapping or an attract-and-kill system, or as an attractant additive to existing protein insecticide bait sprays such as GF-120.

Seasonal Changes in the Cuticular Hydrocarbons of Bean Leaf Beetle, *Cerotoma trifurcata*: Multi-Methylbranched 1-Alkenes Present in Overwintering Adults

Jurenka R

Department of Entomology, Iowa State University, Ames, IA 50011-3222

Corresponding author: rjurenka@iastate.edu

Hydrocarbon profiles were determined for adult bean leaf beetles, *Cerotoma trifurcata* (Coleoptera, Chrysomelidae), at various times during the season. This beetle has two generations per year in Iowa with the adults of the last generation overwintering. Beetles were collected off soybean by using sweep nets during the months of May to September. The overwintering population was collected from the leaf litter of a nearby deciduous forest. The beetles were stored at -20°C within 24 h of collection and sexed based on the color of the frons being black for females and tan for males. Cuticular lipids were extracted with hexane and thin-layer chromatographic analysis indicated that >95% of the hexane extract contained hydrocarbon. Hydrocarbons were identified based on equivalent chain length indices and known mass spectrometer diagnostic ions. Double bond position in alkenes was determined by purification through argentation chromatography followed by derivatization with dimethyl disulfide and analysis by GC-MS. Hydrocarbon profiles obtained from adult beetles did not differ based on sex. However, profiles were different between beetles collected during the summer and overwintering beetles. Overwintering beetles had fewer alkanes and more monounsaturated alkenes. The alkenes were identified as all 1-alkenes based on dimethyl disulfide derivitization. Both alkenes and alkanes had the same mono-, di-, tri-, and tetra-methyl branching patterns.

Sexually Biased Synergism between Oligosaccharides and Phospholipids in Gustatory Perception of Nuptial Secretion in the German Cockroach, *Blattella germanica*

Wada-Katsumata A, Ozaki M, and Nishida R

Laboratory of Chemical Ecology, Graduate School of Agriculture, Kyoto University, Kyoto, Japan (AW-K, RN); Department of Biology, Graduate School of Science, Kobe University, Kobe, Japan (MO)

Corresponding author: awada@kais.kyoto-u.ac.jp

Females of the German cockroach, *Blattella germanica*, compulsively feed on pheromonal phagostimulants provided as a nuptial secretion from male's eighth tergal glands (TG-8) in its sequential courtship behavior. The "nuptial feeding" leads the female to keep herself in a precopulatory position. Using TG-8 extracts and its major ingredients, maltotriose (MT) and phosphatidylcholine (PC), we investigated the gustatory effect of the components on the perception of the secretions in both sexes by behavioral and electrophysiological tests. Both feeding and electrophysiological responses to TG-8 extracts were significantly higher in females than those in males. Four types of impulses, that were sugar-specific, salt-specific, and two types of nonspecific impulses, were recorded from a gustatory sensillum on the paraglossae when stimulated with TG-8 extracts in both sexes. The frequency of the sugar-specific and one type of nonspecific impulses were significantly higher in females than those in males. In contrast, there was no significant difference between the sexes in the responsiveness to MT. Phosphatidylcholine elicited no specific responses in both sexes. In the responses to a mixture of MT and PC, feeding response and the frequency of sugar-specific impulses increase in females but not in males. These results suggested that the synergistic effect of PC on MT in female's sugar receptor cells would lead her to compulsive nuptial feeding. A female-biased gustatory sensitivity to the nuptial secretion seems to ensure the successful precopulatory position before genital connection.

Development of the Pear Ester Kairomone as a Microencapsulated Spray Adjuvant for Insecticidal Control of Neonate *Cydia pomonella* (Lepidoptera: Tortricidae)

Light DM, Knight AL, Beck JJ, Haworth J, Downs J, and Lingren B

Western Regional Research Center, Plant Mycotoxin Research Unit, USDA-ARS, Albany, CA (DML, JJB); USDA-ARS-YARL, Wapoto, WA (ALK); Trécé, Inc., Adair, OK (JH, JD, BL)

Corresponding author: doug.light@ars.usda.gov

Food Quality Protection Act 1996 is banning the use of most organophosphate (OP) insecticides. Thus, there is a need for alternative, reduced-risk insecticides to be made more effective and affordable. The pear ester (PE), ethyl (2*E*,4*Z*)-2,4-decadienoate, kairomone was found to be highly attractive to neonate codling moth larvae (1). Moreover, it promotes "larval wandering" and retention and enhanced exposure time on sprayed surfaces. PE has been formulated within 2–8- μ m microcapsules (PE-MEC) that emit kairomone for weeks. Experiments using PE-MEC (CIDETRAK DA-MEC; Trécé, Inc.) have expanded over the past 4 yr to 65 worldwide field trials testing its efficacy as a bait-spray adjuvant with various insecticides, including OPs, pyrethroids, insect growth regulators, botanicals, and microbials. Low rates of PE-MEC adjuvant (<1.5 g PE [AI]/ha) have improved overall efficacy of 18 insecticides by 30 to 59% over paired trial sprays of insecticides alone under low to high codling moth pressure in apple, pear, and walnut orchards.

1. Naturwissenschaften 88:339–342.

Impact of Physiological Status on the Peripheral Olfactory System of the Cotton Leaf Worm *Spodoptera littoralis* (Lepidoptera: Noctuidae)

Martel V, Anderson P, Hansson B, and Schlyter F

Division of Chemical Ecology, Department of Plant Protection Biology, SLU, Alnarp, Sweden

Corresponding author: veronique.martel@mail.mcgill.ca

Physiological state of an insect (age, starvation, mating status, etc.) is known to have an impact on its behaviour. Virgin females generally call males, mated females look for oviposition sites, and hungry individuals look for food. However, such modulation in behaviour might have different physiological origins: from the peripheral sensory system to the central nervous system. In this study, we investigated the impact of different physiological factors (mating status, age, and access to food) on the antennal sensitivity of females of the cotton leaf worm, *Spodoptera littoralis*, by using electroantennography (EAG). The response to different plant odours (herbivore-damaged induced and noninduced cotton volatiles, and flower odours) and the main component of the sex pheromone was used to assess the factors modulating antennal response. Both mating status and age were found to affect the antennal sensitivity of females: virgin females were more sensitive and antennal sensitivity tends to increase in the first few days after emergence, and then decrease. Results are discussed in terms of their physiological and ecological meaning.



Changes in the Salivary Proteome and Gustatory Receptor Activities of Larval *Heliothis virescens* (Lepidoptera: Noctuidae) in Response to Tasting Plant Defenses

Szulewski KR, Marion-Poll F, and Felton GW

Department of Entomology, The Pennsylvania State University, University Park, PA (KRS, GWF); French National Institute for Agricultural Research, Versailles, France (FMP)

Corresponding author: krs287@psu.edu

Insect taste perception of their host plant mediates feeding behavior and physiology (1,2). It also may trigger changes in the insect's saliva related to plant defense activation or suppression. Glucose oxidase, a salivary enzyme in caterpillars, suppresses the wound-inducible defenses in plants (3), is present in higher quantities in caterpillars feeding on tobacco than on other host plants such as tomato or cotton (4), and saliva-deficient caterpillars perform poorly on plants (5). However, elicitors of plant defenses, such as volicitin, also have been found in caterpillar oral secretions (6). We present the gustatory responses of larval *Heliothis virescens* to a variety of known plant defenses, in addition to the corresponding changes in the insect's salivary proteome after exposure of gustatory receptors to selected plant defenses. These results will be used as a method to identify candidate insect counter-defenses and plant defense-elicitors by examining insect salivary proteins that are synthesized or degraded in response to the taste of plant defenses. Examining this dynamic interaction could open new avenues for the development of sustainable agricultural practices.

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Adults and Nymphs Do Not Smell the Same: The Different Defensive Compounds of the Giant Mesquite Bug (*Thasus neocalifornicus*: Coreidae)

Noge K, Purdic KL, and Becerra JX

Department of Entomology, University of Arizona, Tucson, AZ (KN, KLP, JXB); Ecology and Evolutionary Biology, Yale University, New Haven, CT (KLP)

Corresponding author: noge@email.arizona.edu

Aposematic animals commonly possess the chemical defensive systems. The nymphs of the giant mesquite bug (*Thasus neocalifornicus*: Coreidae) are brightly colored red with white spots, whereas the adults are large and cryptic, not aposematic. We investigated the chemical defense of the nymphs and also examined whether their chemical defense would change along with the change of the visual defensive strategies. The secretory compounds from the nymphs and the adults were isolated and identified by gas chromatography-mass spectrometry and NMR. The nymphs secrete (*E*)-2-hexenal and 4-oxo-(*E*)-2-hexenal, whereas the adults secrete hexyl acetate, hexanol, and hexanal. The results of the predator bioassays suggest that nymphs are chemically protected from insect predators, whereas adults are not. Of all the compounds, 4-oxo-(*E*)-2-hexenal was particularly deterrent and toxic to mantids. Some of the chemical components also function as alarm signals to conspecific mesquite bugs. The nymphs dispersed in the presence of either the nymph secretion, (*E*)-2-hexenal or 4-oxo-(*E*)-2-hexenal, but they did not respond to the adult secretion and its components. In contrast, the adult responded only to the adult secretions. Hexyl acetate and hexanal alone also elicited the adult dispersal behaviors, but hexanol did not. The differences of the chemical components and their functions during development may be due to the differences in predator guilds and visual defensive strategies between the nymph and the adults.

Influence of a Generalist Predator, *Orius insidiosus* (Heteroptera: Anthocoridae), on Induced Defenses in Tomato

Quaghebeur HM and Felton GW

Department of Entomology, The Pennsylvania State University, University Park, PA
Corresponding author: hmq1@psu.edu

Early detection of environmental cues indicating the closeness of herbivores, and the subsequent activation of specific plant defense mechanisms, that may be critical for plant ecological success. In tomato (*Solaneum lycopersicum*), leaf glandular trichomes are thought to play an important role in the early detection of herbivores: Peiffer and Felton demonstrated that the mechanical disruption of type VI glandular trichomes caused by caterpillars crawling was sufficient to induce the expression of the wound induced proteinase inhibitor II (Pin2) by activation of the jasmonate (JA) pathway, even before feeding damage occurs (Peiffer and Felton, unpublished data). Here, we investigate the potential role of the presence of a facultative predator, *Orius insidiosus*, in the early induction of tomato defense genes, in the absence of strict herbivores. We sampled the youngest leaf of 4-wk-old tomato plants caged with *O. insidiosus*, and quantified by real-time PCR the relative expression level of several defense genes associated with the JA and salicylic acid pathways. We examined the influence of parameters such as insect density and life stage on the relative level of induction of tomato defense genes. We discuss our results in the context of the ecological significance of this plant/natural enemy interaction.

Identification of Host-Plant Attractants for the Cranberry Weevil, *Anthonomus musculus* Say (Coleoptera: Curculionidae)

Szendrei Z, Malo E, and Rodriguez-Saona C

Rutgers, The State University of New Jersey, 125A Lake Oswego Road, Chatsworth NJ 08019 (ZS, CR-S); Departamento de Entomologia, El Colegio de la Frontera Sur, Tapachula, Chiapas, CP 30700, Mexico

Corresponding author: crodriguez@aesop.rutgers.edu

The cranberry weevil is an oligophagous flower bud feeder native to North America. It causes significant economic injury on cultivated highbush blueberry, *Vaccinium corymbosum* L. (Ericaceae), and on cranberry, *Vaccinium macrocarpon* Ait. (Ericaceae). This project was conducted in New Jersey, where the weevil causes major crop losses to blueberry growers. As part of an integrated response to this problem, we investigated the role of plant volatiles and pheromones for monitoring or trapping. We analyzed volatile compounds from blueberry leaves, buds, and flowers, which were subsequently tested in EAGs. Blueberry plant parts, compounds with high antennal responsiveness and the pepper weevil pheromone lure were tested in a Y-tube olfactometer for behavioral activity. A field study was set up at four commercial blueberry farms with cinnamyl alcohol and pepper weevil lure baited traps in 2008. More than 30 compounds were identified using GC-MS: *cis*-3-hexenyl butyrate and cinnamyl alcohol were emitted only from blueberry flowers with cinnamyl alcohol as the major blueberry flower volatile. Weevil antennae were highly responsive to hexyl acetate, *cis*-3-hexenyl acetate, *cis*-3-hexenyl butyrate, methyl salicylate, cinnamyl alcohol, linalool oxide, linalool, and α -humulene. Y-tube choice test did not support behavioral activity of methyl salicylate, hexyl acetate, or cinnamyl alcohol. Flowers were attractive to female weevils, and male weevils were attracted to the pepper weevil pheromone lure. The cinnamyl alcohol baited traps did not capture more weevils than control traps, but the pepper weevil lure attracted significantly more weevils than the control. This project has identified several volatile compounds from blueberries, but because they lack significant behavioral activity they alone are not likely to become important in IPM. We are further investigating the pepper weevil pheromone that could lead us to developing an attractant for this species.

Are Aphids Stealthy, or Are They Manipulative? Suppression of Caterpillar-Induced Volatile Emission in Broad Bean Plants by the Pea Aphid, *Acyrtosiphon pisum*

Schwartzberg EG and Tumlinson JH

Center for Chemical Ecology, The Pennsylvania State University, University Park, PA

Corresponding author: ezra@psu.edu

Plants are able to defend themselves against herbivory through several means, including the production of airborne volatile compounds that attract natural enemies. The pea aphid, *Acyrtosiphon pisum*, is able to feed on broad bean plants without inducing detectable changes in volatile emission. Additionally, pea aphids inhibit volatile emission normally increased in response to feeding by the beet armyworm caterpillar, *Spodoptera exigua*. Some of the caterpillar-induced volatiles that are inhibited by aphid feeding are known attractants to the aphid parasitoid *Aphidius ervi*. These compounds include the terpene (*E*)- β -ocimene and the green leafy compounds (*Z*)-3-hexen-1-ol and (*Z*)-3-hexenyl acetate. The ability of aphids to inhibit herbivore-induced volatile signals provides insight into a mechanism by which aphids can overcome induced plant defenses that attract parasitoids and predators by manipulating volatile production.

Alkylpyrazines: Alarm Pheromone Components of the Little Fire Ant, *Wasmannia auropunctata*

Showalter DN, Troyer EJ, and Siderhurst MS

Department of Chemistry, Eastern Mennonite University, Harrisonburg, VA

Corresponding author: dns265@emu.edu

Little fire ant (LFA), *Wasmannia auropunctata*, is an invasive pest occurring throughout the Western Hemisphere. Its range extends from northern Argentina through Central America and the Caribbean into the southern United States with infestations reported in West Africa and the Pacific. In addition to threatening native species, ants nesting in Hawaiian fruit trees pose health and commercial risks to fruit growers and workers. Howard et al. (1) identify 2,5-dimethyl-3-(3-methylbutyl)pyrazine as a component of the *W. auropunctata* mandibular alarm pheromone. However, a comparison of this compound with ant extracts suggests the identification is incorrect. Mass spectrometry and synthetic analysis indicate the presence of two pyrazines in ant extracts: a constitutional and/or regio-isomer of 2,5-dimethyl-3-(3-methylbutyl)pyrazine and a second disubstituted alkyl pyrazine. Ongoing synthetic work seeks to identify the observed ant pyrazines. Results of LFA responses to alkylpyrazines in bioassays are presented. Current *W. auropunctata* detection methods use peanut butter baits, which are known to contain alkylpyrazines. The possible relationship between peanut butter pyrazines and LFA attraction is discussed.

1. Insectes Soc. 29:369-374.



Teaching Chemical Ecology in High School: Rewards and Challenges

Smith S

The Roanoke Valley Governor's School for Science and Technology, Roanoke, VA

Corresponding author: ssmith@rvgs.k12.va.us

Chemical ecology is a relatively new and fast-growing field of research. Combining biology and chemistry in a unique way offers a window through which we can examine the many different ways in which animals and plants communicate with themselves and with each other. As a cutting-edge (and often fascinating) area of science—and one with manifold applications—it would seem to make for a wonderful way to introduce science to high school age students. A brief description of the elective research course (Chemical Ecology Research) is given, along with the lab activities and some of the research topics chosen by students. Both the successes and the challenges arising from this course are detailed, along with suggestions for prospective teachers on how to implement such a course at the high school level, and suggestions for local professors as to how they might be able to assist high school teachers who take on that challenge.

Feeding Stimulants from the Camphor Tree Leaves (*Cinnamomum camphora* Sieb) to the Green-Banded Swallowtail (*Graphium sarpedon* L.) Larva

Wakui R, Nishimura Y, Nishiyama Y, Ikeno Y, Tebayashi S, and Kim C-S

Faculty of Agriculture, Kochi University, 200B Monobe, Nankoku, Kochi, 783-8502, Japan

Corresponding author: tebayashi@kochi-u.ac.jp

Host selection of the Papilionidae has been well investigated; in particular, the oviposition stimulants for adult female butterflies have been identified from their host plants. However, there are only a few reports on feeding stimulants for butterfly larva from their host plants. We have been conducting a study on the host selection of the green-banded swallowtail (*Graphium sarpedon* L.) larvae from the camphor tree (*Cinnamomum camphora* Sieb) by investigating the feeding stimulants. The methanol extract of *C. camphora* leaves was first partitioned into hexane and water layers. The water layer and the hexane layer showed strong feeding stimulus activities by a choice test, which used *G. sarpedon* larva and thin foam polystyrene disks. Then, the water layer was fractionated into water fr., 20% MeOH-water fr., 40% MeOH-water fr. and MeOH fr. by an ODS column, but activity could not be detected in any of the four fractions. The combined fraction (water fr., 20% MeOH-water fr. and MeOH fr.) showed strong activity. Each fraction was then further purified with a reversed phase HPLC, and three compounds were isolated. Analysing by NMR, LC-MS, and UV, sucrose, chlorogenic acid, and quercetin 3-*O*- β -glucopyranoside were identified as feeding stimulants from the water fr., 20% MeOH-water fr. and MeOH fr., respectively. In contrast, the hexane layer was chromatographed on a silica gel column by eluting with following solvents: Hex, 50% Et₂O-Hex, Et₂O, and MeOH. Activity was recovered into only MeOH fr., and this fraction is being further purified with ODS column and reversed phase HPLC.

Biosynthetic Studies of the Male Marking Pheromone of Bumblebee Species *Bombus lucorum* and *B. lapidarius*

Valterová I, Žáček P, Kindl J, and Luxová A

Institute of Organic Chemistry and Biochemistry, Academy of Sciences of the Czech Republic, 166 10 Prague, Czech Republic (IV, PŽ, JK, AL); Department of Analytical Chemistry, Faculty of Science, Charles University, 128 40 Prague, Czech Republic (PŽ)

Corresponding author: irena@uochb.cas.cz

Marking pheromones of bumblebee males are produced by the cephalic part of the labial gland (1). *Bombus lucorum* and *B. lapidarius* were selected as model species. Both species produce aliphatic compounds in their pheromonal blends (ethyl tetradec-9-enoate, ethyl dodecanoate, and hexadecan-1-ol in *B. lucorum* [2]; hexadec-9-en-1-ol and hexadecan-1-ol in *B. lapidarius* [3]). The biosynthesis was studied using deuterium-labeled fatty acids as precursors. After 2–4 days of incubation of the labeled compounds, extracts of the dissected labial glands and fat bodies were analyzed by means of comprehensive two-dimensional gas chromatography. The following deuterated metabolites were found in the tissues: mono-, di-, and triacylglycerols (fat body), ethyl esters, alcohols, and hydrocarbons (labial gland). Desaturation of the carbon chain was found, too, as well as elongation of the carbon chain of the particular applied fatty acid. Chain shortening has not been observed in perdeuterated fatty acids, probably due to the isotopic effect. However, small amounts of labeled ethyl tetradecenoate were detected in the application of partially labeled hexadecanoid acid to *B. lucorum* males. Presence of the deuterated metabolites in different parts of the bumblebee body gives us an evidence of the presence of corresponding enzymes (ethyl ester synthase, desaturase, elongase, reductase, and decarboxylase).

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2. Eur J Entomol (2001) 98:111–115.
3. Nature (1969) 221:856–857.

Plant Volatiles Influence Electrophysiological and Behavioral Responses of *Lygus hesperus*

Williams III L, Blackmer JL, Rodriguez-Saona C, and Zhu S

USDA–ARS, EIWRU, Reno, NV (LW); USDA–ARS, ALARC, PMBCRU, Maricopa, AZ (JLB); Department of Entomology, Rutgers University, Chatsworth, NJ (CR-S); Department of Chemical, Biological, and Materials Engineering, University of Oklahoma, Norman, OK (SZ)

Corresponding author: livyw@unr.edu

The western tarnished plant bug, *Lygus hesperus*, inhabits agricultural regions in western North America, where it is an important pest on a wide range of crops. Host finding by *L. hesperus* is poorly understood, but it is at least partly mediated by plant volatiles. We examined the electrophysiological (EAG) and behavioral effects of plant volatiles on *L. hesperus*. These studies might lead to novel control strategies, as well as improve population monitoring of this pest. Results of EAG trials showed that *L. hesperus* male antennae are generally more responsive than female antennae. For both genders, the alcohols (*E*)-3-hexenol, (*Z*)-3-hexenol, and 1-hexenol elicited significantly higher EAG responses than the acetates, monoterpenes, and sesquiterpenes that were tested. Behavioral trials using a Y-tube olfactometer showed that *L. hesperus* females exhibit a significant attraction to the acetates (*E*)-2-hexenyl acetate and (*Z*)-3-hexenyl acetate, the monoterpenes (*R*)- α -pinene, (*R*)-(+)-limonene, and (*E*)- β -ocimene, and the sesquiterpene farnesene. Males, however, only exhibited a significant attraction to (*R*)- α -pinene. Our results show that male and female *L. hesperus* respond differentially to host plant volatiles under laboratory conditions; thus, plant volatiles might have potential for manipulating and monitoring *L. hesperus* populations in agroecosystems. Similarities and discrepancies between EAG and behavioral trials are discussed.



Indole: An Airborne Priming Signal of Indirect Defenses in Maize?

Zhang Y, Gordon-Weeks R, Pickett J, and Ton J

Biological Chemistry Department, Rothamsted Research, Harpenden, Hertfordshire, United Kingdom

Corresponding author: yuhua.zhang@bbsrc.ac.uk

Upon attack by herbivorous insects, plants activate various mechanisms to protect themselves. Apart from activation of direct defense mechanisms, plants can protect themselves indirectly by emitting volatile organic compounds (VOCs) that can attract natural enemies of the herbivore. Apart from a role in indirect defence, herbivore-induced VOCs have also been implicated as within-plant signals that mediate induced resistance in systemically connected plant parts and even neighbouring plants (1). In maize, this so-called VOC-induced resistance is based on priming, which mediates a faster and stronger activation of direct and indirect defenses upon insect attack (2,3). In this study, we investigated the role of indole as an airborne priming signal. We found that exposure of maize to indole directly enhanced the transcription of the sesquiterpene biosynthesis gene *TPS10*, whilst reducing the expression of other terpene synthase genes. GC analysis of air- and indole-exposed plants revealed that exposure of indole had no direct effect on VOC emission, but mediated a primed emission of the sesquiterpenes (*E*)- β -farnesene and (*E*)- α -bergamotene upon mechanical wounding and application of *S. littoralis* regurgitant. These data indicate that indole contributes to VOC-induced priming of indirect plant defenses in maize. Currently, we are also assessing the role of green leaf volatiles in this form of priming.

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2. Proc. Natl. Acad. Sci. USA (2004) 101:1781–1785.
3. Plant J. (2007) 49:16–26.

Chemical Deception/Mimicry

Chair: **Bill Hansson**,
Max Planck Institute for Chemical Ecology, Jena, Germany
100 Berg Auditorium, Life Sciences Building

SYMPOSIUM | TUESDAY, 19 AUGUST

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SUN MON TUE WED THU FRI

Pollinator Attraction and Deception in Wasp-Flowers

Ayasse M, Brodmann J, Twele R, and Francke W

Institute of Experimental Ecology, University of Ulm, Albert-Einstein-Allee 11, 89069 Ulm, Germany (MA, JB); Institute of Organic Chemistry, University of Hamburg, Hamburg, Deutschland (RT, WF)

Corresponding author: manfred.ayasse@uni-ulm.de

Wasp-flowers exhibit physiological and morphological adaptations for the attraction of pollinating social wasps. The mechanism for the attraction of wasps was something of a mystery. Using a combination of behavioral experiments, electrophysiological investigations, and chemical analyses, we investigated in a comparative approach the pollinator attracting floral volatiles in wasp-flowers of the orchid genus *Epipactis* and of the nonorchid genus *Scrophularia*. To find floral volatiles that are unique to wasp-pollinated species, we also investigated *Epipactis atrorubens*, a species that is pollinated by bees. We found that all wasp-pollinated species of *Epipactis* as well as of *Scrophularia* emit green leaf volatiles (GLVs), which are attractive to foragers of the social wasps *Vespa germanica* and *V. vulgaris*. GLVs, emitted by damaged plant tissues are known to guide parasitic wasps to their hosts (1). Several GLVs that induced response in the antennae of wasps also were emitted by cabbage leaves infested with caterpillars (*Pieris brassicae*), which are common prey items for social wasps. Our results showed that *Epipactis* flowers mimic GLVs to attract prey hunting wasps for pollination (2). This is the first example in which GLVs have been implicated in chemical mimicry for the attraction of pollinating insects. To test our prediction that the scent of *Epipactis* flowers does not attract other potential pollinators such as honey bees, we also performed electrophysiological investigations and behavioral experiments with workers of *Apis mellifera*. We found that antennae of *A. mellifera* workers respond to the same compounds as *V. germanica* and *V. vulgaris* in the electrophysiological investigations but were neither attracted by the synthetic mixture of *Epipactis* flowers nor by the mixture of the GLVs in the Y-tube experiment. In solvent extracts of flowers we identified several aldehydes and alcohols. Their bouquets were more similar to each other in the wasp-pollinated *Epipactis* species than in *E. atrorubens*, a species that is mainly pollinated by bees. We presently investigate whether these more specific compounds than the GLVs found in headspace samples are learned by visiting wasps and used as recognition signals while visiting other *Epipactis* plants.

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1. Science (1990) 250:1251–1253.
2. Curr. Biol. (2008) 18:1–5.

Floral Scent in Deceptive Apocynaceae-Asclepiadoideae

Dötterl S, Jürgens A, and Meve U

Department of Plant Systematics, University of Bayreuth, Germany (SD, UM); School of Biological and Conservation Sciences, University of KwaZulu-Natal, South Africa (AJ)

Corresponding author: stefan.doetterl@uni-bayreuth.de

Flowers are a symbol of beauty and their fragrances inspired poets like Shakespeare, Goethe, and others. However, floral volatiles are not produced by the plants to please the human nose; they are usually produced to attract animals for the goal of pollination. This becomes obvious in flowers pollinated by insects like carrion flies or dung beetles. These flowers emit to the human nose very unpleasant odours. Famous are the deceptive stapeliad (Apocynaceae-Asclepiadoideae) "carrion flowers" that emit odors reminiscent of rotting fish, carrion, or dung. These odors mimic the substrate to which especially some Coleoptera and Diptera (flesh flies, houseflies, and blowflies), i.e., the pollinators, are attracted to oviposit or feed. However, within Apocynaceae-Asclepiadoideae stapeliads are not the only group with food deceptive flowers. Well known and keenly collected by plant growers is also *Ceropegia*. All *Ceropegia* species have trap flowers (Kesselfallen-Blüten) and temporarily trap their pollinators (mainly small dipteran species). Pollinators are released after a period of about 24 h, during which time pollinaria have been picked up and/or deposited by them. It is assumed that *Ceropegia* flowers mimic brood sites or food supply for small (kleptoparasitic) flies. Interestingly, the scent emitted by *Ceropegia* is quite different to the scent emitted by stapeliads. In some species, it is sweetish/greenish, and it can easily be detected by the human nose, whereas other species are, at least to the human nose, nonscented. Nevertheless, in stapeliads as well as *Ceropegia* flowers scent is assumed to be responsible for attraction of flies. We discuss flower scent composition of different stapeliad as well as *Ceropegia* species determined by dynamic-headspace-GC/MS, and its importance for the attraction of different types of fly-pollinators.

Insult without Injury: The Evolution of Chemical Mimicry and Fly-Mediated Spore Dispersal in Dung Mosses (Splachnaceae)

Raguso RA, Marino PC, and Goffinet B

Department of Neurobiology and Behavior, Cornell University, Ithaca, NY (RAR); Memorial University, St. Johns, Newfoundland (PM), University of Connecticut, Storrs, CT (BG)

Corresponding author: rar229@cornell.edu

Brood site deception involving chemical mimicry of decaying organic matter is an effective pollination strategy among aristolochias, aroids, stapelioids, and other lineages of flowering plants. Salient features of this strategy include the universality of mimetic odorants as attractants, the presence of supplementary signal components (heat, texture) that manipulate vector behavior, and, often, the vector's detainment in a floral chamber or trap until both male and female functions are accomplished. We describe a simpler version of brood site deception and its role in the diversification of the so-called "dung mosses" (Splachnaceae), an unusual family of bryophytes. Unlike other mosses, which rely exclusively on wind and gravity for spore dispersal, many species of Splachnaceae are coprophilous—their gametophytes grow on decaying organic matter—and entomophilous—they co-opt insect vectors to deliver spores to appropriate substrates. Sporophytes of *Splachnum*, *Tetraplodon*, and many *Tayloria* species present masses of sticky spores protruding from a small sporangium mounted on a brightly colored and parasol-shaped or otherwise inflated sterile base, the apophysis, which emits pungent odors. Dung mosses partition diverse families of Diptera as spore dispersal agents through staggered phenology, microhabitat specialization and sporophyte odors that chemically mimic specific substrates for gametophytic growth. Across boreal North America, *Tetraplodon mnioides* grows on carnivore dung and bone and attracts calliphorid and muscid flies with the odors of rotting flesh, dimethyl disulfide and dimethyl trisulfide, whereas *Splachnum ampullaceum* and *S. sphaericum* grow on herbivore dung and attract diverse families of flies with sporophyte odors that include phenol, cresols, and cyclohexane carboxylic acids present in cervid urine and feces. These patterns are mirrored in austral South America, where *Tetraplodon fuegiensis* grows on fox dung and emits oligosulfides, whereas *Tayloria mirabilis* and *T. dubyi* grow on cow and goose dung, respectively, and emit sporophyte odors rich in phenol, cresols, and indole. Behavioral assays indicate that the relative importance of visual and olfactory display varies among fly visitors to these mosses, as it frequently does among insects that pollinate angiosperm flowers. However, the major difference between dung mosses and brood-deceptive flowers is that flies visit the mosses briefly and proceed to appropriate substrates without being trapped or detained. Entomophilous spore dispersal has evolved at least twice in the Splachnaceae; its prevalence at global antipodes underscores the universal effectiveness of chemical mimicry in the exploitation of saprophilous insects.

The Hitchhiker's Guide to Sexual Deception: *Colletes* Bees (Hym. Apoidea, Colletidae) and Their Associated "Chemical" Parasites

Vereecken NJ and Schiestl FP

Behavioural and Evolutionary Ecology, Free University of Brussels CP 160/12, Av. FD Roosevelt 50, B-1050 Brussels, Belgium (NJV); Institute of Systematic Botany, University of Zürich, Zollikerstrasse 107, CH-8008 Zürich, Switzerland (NJV, FPS)

Corresponding author: nicolas.vereecken@ulb.ac.be

Parasites are renowned for their various strategies of exploiting their hosts, sometimes in a highly specific way. Among parasites, several species have evolved complex adaptations that allow them to hijack chemical communication channels of their hosts. We introduce results from both recent and ongoing research on different species of *Colletes* bees whose males are sexually deceived by flowers of terrestrial orchids (1,2,3) or clusters of newly hatched beetle larvae (4). Specifically, we first show how an orchid mimic can benefit from its mimetic imperfection to its specific model, a female bee, by triggering supernormal stimulation in males of the targeted species (3). We then describe the natural history and the preliminary results on the chemical ecology of a new case of sexual deception found in the Old World, where clusters of parasitic oil beetle larvae attract male bees, their host, by imitating the female bee sex pheromone. Once on board the male bee, the beetle larvae gain access to their host nests in which they will ultimately forage on pollen and nectar to complete their life cycle (4).

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Arum Pollination—Function and Evolution

Urru I, Stökl J, Linz J, Stensmyr M, and Hansson BS

Department of Evolutionary Neuroethology, Max Planck Institute for Chemical Ecology, Jena, Germany

Corresponding author: iurru@ice.mpg.de

Pollination is usually regarded as a classical case of insect–plant mutualism. However, several pollinator/flower systems have evolved pollinator attraction by chemical mimicry without offering any kind of reward, i.e., pollination by deceit. The genus *Arum* represents a good example of flowers mimicking the scent of a valuable resource. Data available on the reproductive biology of the genus shows a high degree of variability (within and between species) regarding morphology, odour, and type and species of attracted insects. This is very likely a consequence of adaptations to different habitats and pollinators. We are investigating morphological characters, odour bouquets, and pollinating fauna of the *Arum* species and correlate these characters with a molecular phylogeny of the group. Furthermore, we are carrying out GC-EAD and GC-MS experiments to identify which compounds that are active in attracting insects to *Arum* floral-emitted blends. To clarify the phylogenetic relationships, a phylogenetic tree was constructed from sequences of three chloroplast markers (*matK*, *rbcl*, *trnL*) and one nuclear marker (ITS) from 24 species of *Arum*. Overall, the differences between species were very low, indicating a possible recent radiation of the genus. Among the *Arum* species, *A. dioscoridis* represents one of the best examples of geographical pollinator variation. In Turkey, this species traps coprophilous beetles (Staphilinidae: *Anotylus* and *Atheta*), whereas in Cyprus mainly coprophilous flies (Sphaeroceridae: *Coproica*) are caught. In Israel, the visitors are coprophilous flies (Sphaeroceridae, Sepsidae) and beetles (Staphilinidae, Scarabeidae). In Rhodos, as in Cyprus, mainly Sphaeroceridae flies are found. Floral volatiles of *A. dioscoridis* in four different locations (Cyprus, Turkey, Rhodos, and Israel) were collected via headspace, and a PCA analysis was performed. Three different constellations could be identified: the Cyprus and Rhodos population form a unique group, quite well separated from the Israel and Turkey populations. Considering the high pollinator variation that exists between the four populations, our findings suggest a possible ongoing process of adaptive speciation.

Chemical Mimicry in the Desert Tenebrionid Subtribe Stizopina (Coleoptera: Tenebrionidae)

Geiselhardt, S, Geiselhardt SF, and Peschke K

Institute of Biology, Free University of Berlin, Haderslebener Strasse 9, D-12163 Berlin, Germany (SG); Institute of Biology I; University of Freiburg, Hauptstrasse 1, D-79104 Freiburg, Germany (SG, SFG, KP)

Corresponding author: sven.geiselhardt@fu-berlin.de

Some members of the southern African darkling beetle subtribe Stizopina have evolved biparental brood care with division of labor, whereas other Stizopina are kleptoparasites (“cuckoos”) that exploit the subsocial species and lay their eggs inside the host burrows. The kleptoparasites use the volatiles emitted from the breeding burrows to locate their hosts. These volatiles originate from the pygidial defensive secretion of the host and consist of 1,4-benzoquinones, 1-alkenes, and monoterpene hydrocarbons (1). Chemical analysis of the pygidial defensive secretion of >50 species shows that the chemical composition is very similar within the subtribe. Moreover, behavioral studies reveal that the defensive secretions cofunction as aggregation pheromones and that the species are unable to discriminate between the secretions of different species. Thus, the kleptoparasites may mistake the host odor for that of conspecifics. Once the kleptoparasites have located a burrow, they have to sneak in, as the host species guard their breeding burrows and attack any intruders. When they were attacked by a host, they adopt a quiescent defensive position with retracted legs and antennae and sometimes emit defensive secretion. Once they are quiescent, the host pays no further attention to the intruders, and the kleptoparasites could start a new intrusion attempt. Bioassays have shown that both defensive secretion and cuticular hydrocarbons (CHCs) play a role in the intrusion and acceptance of the kleptoparasites. When defensive secretion of different Stizopina species was applied onto odorless dummies, these dummies were ignored by guarding males of the host species *Parastizopus armaticeps*, whereas dummies without defensive secretion were attacked or pulled into the larval food cage. Nevertheless, the defensive secretion is not sufficient to explain the discrimination between different Stizopina species. Only the kleptoparasite *Eremostibes opacus* is accepted by the host *P. armaticeps*; other species were attacked and thrown out of the burrow. In a bioassay, dummies treated with the CHC extract of *E. opacus* were almost ignored, whereas dummies with CHC extracts of other species were heavily attacked. The degree of aggression correlates with the similarity of the CHC profiles tested and that of *P. armaticeps* (2). Furthermore, a neighbor-joining analysis based on CHC similarities of >50 different Stizopina species shows that all host/parasite pairs cluster together, suggesting that the congruence in the CHC profiles are the key factor for the acceptance of the kleptoparasites.

1. J. Chem. Ecol. (2006) 32:767–778.

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Butterfly Olfactory Responses to Ant Volatiles: Potential Cues for Host Ant Recognition in the Obligately Ant-Associated Butterfly *Jalmenus evagoras* (Lepidoptera: Lycaenidae)

Fraser AM, Mutsaers AP, Bullard B, Beckett JS, and Hofferberth JE

Department of Biology, Kalamazoo College, Kalamazoo, MI (AMF, APM, BB); Department of Chemistry, Kenyon College, Gambier, OH (JSB, JEH)

Corresponding author: afraser@kzoo.edu

Nearly three-quarters of all butterfly species in the family Lycaenidae associate in some way with ants. Relationships range from facultative, mutualistic interactions with a phylogenetically diverse assemblage of ants, to obligately dependent mutualistic, or more rarely parasitic, associations with a single or several closely related species of ants. Obligately ant-associated lycaenids frequently use their ant partners as cues when selecting egg-laying sites. As a result, shifts in host ant associate may directly enhance speciation rates of these butterflies. The chemical ecology of lycaenid butterfly–ant interactions is poorly understood, however, and studies to date have focused on interactions between butterfly larvae and their attendant ants. To elucidate the proximate basis of host ant selection by adult butterflies, we used GC-EAD to evaluate the olfactory responses of the Australian butterfly *Jalmenus evagoras* to solvent extracts of both attendant and nonattendant ant species of *J. evagoras* caterpillars. Attendant ant species included *Iridomyrmex anceps* and *I. rufoniger* sp. 1. Nonattendant ant species included *I. purpureus* (attendant ant of *J. ictinus*), *I. rufoniger* sp. 2 and 3 (attendant ants of *J. daemeli*) and *Froggattella kirbii* (attendant ant of *J. pseudictinus*). Nonattendant ant species occur sympatrically with *J. evagoras* at selected localities but are indifferent or aggressive toward *J. evagoras* caterpillars. Using GC-MS, published spectra and authentic standards, we identified 6-methyl-5-hepten-2-one and iridodial as electrophysiologically active compounds present in extracts of all *Iridomyrmex* species. Multiple peaks corresponding to iridodial elicited EAD responses and iridodial peak profiles differed among ant species. Further work on identification of iridodial stereoisomers is needed. Extracts from *Froggattella kirbii* consistently elicited an EAD response to a single large peak we tentatively identify as dolichodial, an iridodial-related compound. Based on our chemical analyses, we hypothesize that *Jalmenus* butterflies differentiate among closely related ant partners of *Jalmenus* species based on relative proportions of iridodial and related compounds present in ant volatile blends. This hypothesis will be tested using laboratory-based behavioral bioassays.

Synthesis and GC-EAD Analysis of Semiochemicals Putatively Used by the Obligately Ant-Associated Butterfly *Jalmenus evagoras* in Host Ant Selection

Hofferberth JE, Beckett JS, Beckett JD, and Fraser AM

Department of Chemistry, Kenyon College, Gambier, OH (JEH, JSB, JDB); Department of Biology, Kalamazoo College, Kalamazoo, MI (AMF)

Corresponding author: hofferberthj@kenyon.edu

Butterflies of the genus *Jalmenus* exist in obligate mutualism with ants. This mutualism is made possible by accurate selection of an oviposition site by female butterflies. The presence of the attendant ant on an appropriate host plant (*Acacia*) has been shown to govern oviposition site selection. A well-characterized example of such a mutualism is the association of *Jalmenus evagoras* with the ant *Iridomyrmex anceps*. Although a substantial corpus of research has been reported on the behavioral aspects of oviposition site selection in this mutualism, little is known about the cues used by the female butterfly in this decision. We reason that a species-specific semiochemical (or semiochemical blend) produced by the ant is the cue used by the butterfly to identify an *Acacia* plant tended by *I. anceps*. To examine this hypothesis, we have collected the volatiles produced by *I. anceps* and three sympatric ant species known to tend other *Jalmenus* species. The components of these volatile blends were tentatively characterized by GC-MS to guide the preparation of authentic chemical standards. Here, we present the synthesis and electrophysiological analysis, via GC-EAD, of some of the natural products tentatively identified in these volatile blends. Specifically, we have prepared the monoterpenes iridodial, dihydronepetalactone, and iridomyrmecin in several stereoisomeric forms. In addition, we have devised a two-step enantiospecific synthesis of actinidine from citronellal. A small library of 3-alkyl-2,5-dimethylpyrazines also has been prepared using a Suzuki–Miyaura reaction as the key synthetic step. GC-EAD analysis revealed that the antennae of *J. evagoras* are capable of sensing all of the compounds we have prepared regardless of their presence in the volatile blends produced by the ants in this study.

Plant–Insect Interactions

Moderators:
14.00–15.30 **Jennifer Thaler**, Cornell University
15.50–17.20 **Judith Becerra**, University of Arizona
100 Berg Auditorium, Life Sciences Building



PARALLEL SESSION 3 | TUESDAY, 19 AUGUST

SUN MON

TUE

WED THU

FRI

Optimizing Chemical Deception: Geometric Multivariate Designs and Response Surfaces Support Noncompetitive Disruption of Mating by Pheromone Blends of the Leafminer *Phyllocnistis citrella* (Lepidoptera: Gracillariidae)

Lapointe SL, Stelinski LL, Evens TJ, Neidz RP, and Hall DG

USDA-ARS, U.S. Horticultural Research Laboratory, Ft. Pierce, FL (SLL, TJE, RPN, DGH);
Department of Entomology and Nematology, Citrus Research and Extension Center, University of Florida, Lake Alfred, FL (LLS)

Corresponding author: stephen.lapointe@ars.usda.gov

Geometric designs were used to examine the effect of proportionality and amount of pheromone blend components on attraction to lures and mating disruption for the leafminer *Phyllocnistis citrella*, an important pest of citrus throughout the world. Response surfaces constructed from field trials for attraction and mating disruption revealed divergence in optimal blends of the two major pheromone components, (Z,Z,E)-7,11,13-hexadecatrienal and (Z,Z)-7,11-hexadecadienal. Attraction of male moths in a citrus grove was greatest to traps baited with a 3:1 blend of triene:diene. Disruption, however, was driven primarily by the triene compound with little to no effect of the diene. Our results support a noncompetitive mechanism mediating disruption of sex pheromone communication in this moth species. The application of geometric statistical designs to optimize pheromone blends is, we believe, the first use of advanced statistical methods to address the question of optimization of pheromone blends as mixtures.

Components of Extrafloral Nectar: Their Function in Ant Attraction and Pathogen Defence

Gonzalez-Teuber M and Heil M

Department of General Botany-Plant Ecology, University of Duisburg-Essen, Germany (MG-T);
Departamento de Ingeniería Genética, CINVESTAV-Irapuato, México (MH)

Corresponding author: marcia.gonzalez@uni-due.de

Extrafloral nectar (EFN) is an aqueous plant secretion that mainly contains sugars and amino acids. Its main function is as plant indirect defence through the attraction of defending ants. Although sugars and amino acids seem to be the compounds mainly responsible for this attraction, a defensive function also seems important, because nectar composition makes it an appropriate medium for pathogen attack. The objectives of our study were 1) to determine chemical compounds of EFN related to ant attractiveness and antimicrobial defence and (2) to study whether these compounds differ among EFN of plant species with different types of mutualism with ants. In the *Acacia* species studied here, obligate myrmecophytes produce EFN constitutively to nourish their symbiotic ant inhabitants, whereas nonmyrmecophytes induce EFN secretion only in response to herbivore feeding to attract nonsymbiotic ants. EFN thus serves in the alimentation of obligate symbionts in the first case and in attraction of facultative mutualists in the second case. We focused on amino acid composition, protein content, and the activities of three pathogenesis-related (PR) enzymes (chitinase, β -1,3-glucanase and peroxidase) in EFN of two myrmecophytes (*Acacia cornigera* and *A. hindsii*) and two related nonmyrmecophytes (*A. farnesiana* and *Prosopis juliflora*). Myrmecophyte EFN contained significantly more proteins than EFN of nonmyrmecophytes. Also, amino acid concentration and composition differed among the species, allowing a grouping of myrmecophytes vs. nonmyrmecophytes. Behavioural assays demonstrated that amino acids indeed can affect the attraction of symbiotic or nonsymbiotic ants and may allow the ants to distinguish among different types of EFN. All three PR enzymes were found in all EFNs, with activities of chitinase and β -1,3-glucanase being significantly higher in myrmecophyte EFN. Assays indicated that chitinase indeed serves the protection of EFN from fungi and yeasts. Our study demonstrates that EFN components function both in ant attraction and the protection from microbial infection, and it seems to be the first report on PR enzymes in EFN. More studies are required to understand the yet unexplored chemical ecology of EFN.

Metabolomic Analysis of the Interaction between Plants and Herbivores

Jansen JJ, Allwood JW, Marsden-Edwards E, van der Putten WH, Goodacre R, and van Dam NM

Department of Multitrophic Interactions, Netherlands Institute of Ecology (NIOO-KNAW) (JJJ, WHvdP, NMvD); School of Chemistry and Manchester Interdisciplinary Biocentre, The University of Manchester (JWA, RG); Waters Corporation (EM-E)

Corresponding author: j.jansen2@nioo.knaw.nl

Insect herbivores by necessity have to deal with a large arsenal of plant defence metabolites. The levels of defence compounds may be increased by insect damage. These induced plant responses also may affect the metabolism and performance of successive insect herbivores. The chemical nature of induced responses is largely unknown and therefore unbiased metabolomic analyses are a valuable tool to gain more insight. We analyzed the interaction between feral cabbage (*Brassica oleracea*) and the small cabbage white (*Pieris rapae*) and how previous attacks to the plant affect the caterpillar metabolism. Because plants may be induced by shoot and root herbivory, we compared shoot and root induction by treating the plants on either plant part with jasmonic acid. Both the plants and caterpillars were chemically analysed using UPLCT/MS. The study revealed that the levels of three structurally related coumaroylquinic acids were elevated in plants treated on the shoot. The levels of these compounds in plants and caterpillars were highly correlated: these compounds were defined as the "metabolic interface." The role of these metabolites could only be discovered using simultaneous analysis of the plant and caterpillar metabolomes. We conclude that a metabolomics approach is useful in discovering unexpected bioactive compounds involved in ecological interactions between plants and their herbivores and higher trophic levels.

From Leaf to Bite: Spatial Distribution Mapping of Allelochemicals in Plants by Using MALDI Mass Spectrometric Imaging

Shroff R, Vergara V, Muck A, Hoelscher D, Gershenzon J, and Svatoš A

Mass Spectrometry Research Group (RS, AM, AS), Departments of Biochemistry (FV, JG) and Entomology (DH), Max Planck Institute for Chemical Ecology, Jena, Germany

Corresponding author: svatos@ice.mpg.de

The spatial distribution of plant defenses within a leaf may be critical in explaining patterns of herbivory. The larvae of generalist *Helicoverpa armigera* (the cotton bollworm) avoided the midvein and periphery of *Arabidopsis thaliana* rosette leaves and fed almost exclusively on the inner lamina. This feeding pattern was attributed to glucosinolates because it was not evident in a myrosinase mutant that lacks the ability to activate glucosinolate defenses by hydrolysis. Usually, leaves or their parts are extracted and extracts analyzed to determine distribution of plant allelochemicals. New method developed by us (1) is able to measure the spatial distribution of glucosinolates in *A. thaliana* leaves at a fine, caterpillar bite-like scale. Using matrix-assisted laser desorption ionization/time of flight (MALDI-TOF) mass imaging method (2), we constructed ion intensity maps from mass spectra at 200- μm resolution. The major glucosinolates were found to be more abundant in tissues of the midvein and the periphery of the leaf than the inner lamina, patterns that were validated by HPLC analyses of dissected leaves. In addition, there were differences in the proportions of the three major glucosinolates in different leaf regions. Hence, the distribution of glucosinolates within the leaf seems to control the feeding preference of *H. armigera* larvae. The preferential allocation of glucosinolates to the periphery may play a key role in the defense of leaves by creating a barrier to the feeding of chewing herbivores which frequently approach leaves from the edge. In addition, MALDI imaging was also applied to study the distribution of phenylpropanoids in *Monocoria elata* (Pontederiaceae), and special localization of pharmacologically active compounds such as hypericin in *Hypericum* species (Saint John's wort) was determined. In summary, MALDI-TOF/MS imaging is a powerful addition to the arsenal of label free imaging tools available to study the distribution of metabolites in plant tissues on a fine scale and evaluate the biological roles of the observed distribution.

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1. Proc. Natl. Acad. Sci. USA (2008) 105:6196–6201.
2. Anal. Chem. (1997) 69:4751–4760.

Host Location in the Black Bean Aphid, *Aphis fabae* (Homoptera: Aphididae): Host Recognition in the Absence of Host-Specific Volatile Compounds

Webster B, Bruce TJA, Birkett MA, Hardie J, and Pickett JA

Rothamsted Research, Harpenden, Hertfordshire, AL5 2JQ, United Kingdom (BW, TJAB, MAB); Imperial College London, division of Biology, Faculty of Natural Sciences, Silwood Park Campus, Ascot, Berkshire, SL5 7PY, United Kingdom (JH, JAP)

Corresponding author: ben.webster@bbsrc.ac.uk

Behavioural and electrophysiological responses of the black bean aphid, *Aphis fabae*, to volatile compounds of its summer host faba bean, *Vicia faba*, were studied, and semiochemicals used in host location were identified. In olfactometer bioassays, aphids were attracted to an air entrainment sample of adult *V. faba* plants. Coupled gas chromatography-electroantennography revealed the presence of 16 electrophysiologically active compounds in the air entrainment sample and 15 of these compounds were identified as (*Z*)-3-hexen-1-ol, 1-hexanol, (*E*)-2-hexenal, benzaldehyde, 6-methyl-5-hepten-2-one, octanal, (*Z*)-3-hexen-1-yl acetate, (–)-linalool, methyl salicylate, decanal, undecanal, (*E*)-caryophyllene, (*E*)-β-farnesene, (–)-germacrene D, and 4,8,12-trimethyl-1,3,7,11-tridecatetraene. When tested individually at the same concentrations as in the air entrainment sample, none of the compounds elicited a similar response to the air entrainment sample, but a synthetic blend composed all 15 compounds in the same quantities and ratio as in the air entrainment sample elicited a similar behavioural response to the air entrainment sample. This suggests that host location in *A. fabae* is mediated by blends rather than individual volatile compounds. Further olfactometer bioassays showed that subtraction of certain compounds from the blend does not result in loss of attraction, suggesting host location is mediated by a blend of a small number of those compounds identified in the air entrainment sample. None of the identified compounds are specific to *V. faba*. This raises the question of how host recognition can be achieved in the absence of host-specific volatile compounds. It has been suggested that when general rather than host-specific volatiles are used in host location, the ratios of the different compounds are used to provide host specificity (1). Attempts to investigate this hypothesis in respect to host-seeking *A. fabae* are discussed.

1. Trends Plant Sci (2005) 10:269–274.

Host Attractants for Ethiopian Fruit Fly, *Dacus ciliatus* Loew (Diptera: Tephritidae)

Soroker V, Alagarmalai J, Dragushich D, Anshelevich L, Nemny-Lavy E, Nestel D, and Zada A

Department of Entomology, ARO, The Volcani Center, Bet Dagan 50250, Israel

Corresponding author: sorokerv@agri.gov.il

The Ethiopian fruit fly, *Dacus ciliatus*, is an oligophagous, EPPO quarantine pest of cucurbit crops, especially of melons, cucumbers, and marrows. The present study aimed at identifying fruit attractants for *D. ciliatus*. The identification process was guided by two laboratory bioassays: behavioral and electrophysiological. We screened fruit volatile extracts from different host plants (e.g., zucchini, *Cucurbita pepo*; galia melon, *Cucumis melo* var. *reticulatus*) as well as nonhosts (e.g., strawberry, *Fragaria* sp.; banana, *Musa* sp.; and tomato, *Lycopersicon esculentum* L.). Both sexes were attracted to volatiles emitted by host-melon fruit but were not significantly attracted to volatiles of nonhosts strawberry and tomato. Attraction to zucchini volatiles was intermediate. Among melon volatiles, those of ripe melon were preferred. Antennae of both sexes responded in GC-EAD to about 10 components in melon volatiles and one different component in zucchini. Eight responsive compounds in melon and one compound in zucchini preparation were identified using GC-MS libraries, retention indices and authentic standards. The electrophysiological activity of identified compounds at 6 different doses was evaluated with electroantennography (EAG). EAG dose–response studies indicated differential sex specific antennae sensitivity of the identified compounds. Benzyl and hexenyl acetates elicited dose response only in males while other tested compounds elicited dose responses in both sexes. Maximum EAG response was usually attained between 100 ng and 10 μg. The attractiveness of synthetic octyl acetate, Z3-octenyl acetate, and Z3-decenyl acetate was confirmed by behavioral bioassay. Interactions between the bioactive compounds are currently evaluated.

Metabolic Flux Analysis Using ^{13}N and ^{11}C in *Nicotiana tabacum* Reveals New Insights into the Timeline by which Methyl Jasmonate Reconfigures Primary Metabolism

Hanik N, Gomez S, Schueller M, Orians CM, and Ferrieri RA

Fachbereich Chemie, Johannes Gutenberg Universität, 55099 Mainz, Germany (NH); Tufts University, Biology Department, Cambridge, MA (SG, CO); Brookhaven National Laboratory, Medical Department, Upton, NY (MS, RAF)

Corresponding author: rferrieri@bnl.gov

Higher plants have evolved with sophisticated defense mechanisms in response to herbivory. Specific signaling molecules, such as jasmonates, are key components of the defense response. Here, we examine how methyl jasmonate reconfigures primary metabolism. We use short-lived radioisotopes, including ^{13}N ($t_{1/2}$ 10 m) and ^{11}C ($t_{1/2}$ 20.4 m) to measure changes in the metabolic fluxes of amino acids (AAs) and soluble sugars. Nonbiological doses of $^{13}\text{NH}_3$ are administered to leaves, enabling rapid incorporation of radionitrogen into the plant's photorespiratory cycle. This isotope can be traced through the metabolic machinery using radio high-performance liquid chromatography on leaf extract to quantify levels of radiolabeled and unlabeled metabolites. Similarly, $^{11}\text{CO}_2$ is administered to leaves enabling rapid fixation with incorporation of radiocarbon into different insertion points of the same machinery. These tracers in combination give new insight into possible metabolic control points linking the timeline for defense induction with substrate supply for secondary metabolism. We applied a single spray treatment of 500 μM methyl jasmonate (MeJA) to aboveground foliar tissue in *Nicotiana tabacum* L. ('Samsun'). Flux analyses were carried out on leaf 3 and 4 at baseline and post-treatment, respectively. Control studies demonstrated that leaf excision at the petiole did not alter endogenous substrate concentrations, or their input fluxes. After MeJA treatment, there was an initial "flush" of metabolic activity at 4 h post-treatment reflected by increased fluxes of ^{13}N substrates, serine, glycine, and of ^{11}C substrates phenylalanine, tryptophan, orithinine and isoleucine. These AAs are involved in a host of defense-related metabolic processes, including phenolic biosynthesis, modification of cell wall architecture, indole acetic acid biosynthesis, and nicotine production within roots. By 15 h post-treatment the flush in metabolic activity involving phenylalanine and orithinine subsided, whereas fluxes of the other substrates continued to grow. Furthermore, ^{11}C fluxes of proline and arginine were now elevated. By unraveling the time dependence for reconfiguration of primary metabolism during plant interaction with herbivory, researchers will be in a better position to understand the complexity of the plant defense response.

This research was supported in part by the U.S. DOE, Office of Biological and Environmental Research; by Deutscher Akademischer Austauschdienst (DAAD), Bonn, for supporting N. Hannik; and in part by the National Research Initiative of the USDA Cooperative State Research, Education and Extension Service.

Is Chitin a Key Signal in Plant Responses to Insects?

Appel H, Wan J, Dayang H, Stacey G, Shyu C, and Schultz JC

Life Sciences Center (HA, JW, GS, JCS) and Bioinformatics Institute (HD, CS), The University of Missouri, Columbia, MO

Corresponding author: appelh@missouri.edu

Chitin is a potent elicitor of defense genes and chemistry in plants (1). Similarities in plant signaling in response to fungi and insects (2) may be explained by the presence of chitin in insect exoskeleton. We evaluated the importance of chitin to plant defenses against insects by determining caterpillar preference for, and induction of glucosinolates in, *Arabidopsis* mutants deficient in chitin-responsive genes. We also report a novel automated phenotyping system we developed to rapidly determine the amount of leaf area consumed by insects.

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Indole-3-Acetonitrile Production from Indole Glucosinolates Deters Oviposition by *Pieris rapae*

De Vos M, Kriksunov KL, and Jander G

Boyce Thompson Institute for Plant Research, Ithaca, New York 14853

Corresponding author: md285@cornell.edu

Like many crucifer-specialist herbivores, *Pieris rapae* uses the presence of glucosinolates as a signal for oviposition and larval feeding. *Arabidopsis thaliana* glucosinolate-related mutants provide a unique resource for studying the in vivo role of these compounds in affecting *P. rapae* oviposition. Low indole glucosinolate *cyp79B2 cyp79B3* mutants received fewer eggs than wild type, confirming prior research showing that indole glucosinolates are an important oviposition cue. Transgenic plants overexpressing epithiospecifier protein, which shifts glucosinolate breakdown toward nitrile formation, are less attractive to ovipositing *P. rapae* females. Exogenous application of indol-3-ylmethylglucosinolate breakdown products to *cyp79B2 cyp79B3* mutants showed that oviposition was increased by indole-3-carbinol and decreased by indole-3-acetonitrile (IAN). *P. rapae* larvae tolerate a cruciferous diet by using a gut enzyme to redirect glucosinolate breakdown toward less toxic nitriles, including IAN, rather than isothiocyanates. The presence of IAN in larval regurgitant contributes to reduced oviposition by adult females on larvae-infested plants. Therefore, production of nitriles via epithiospecifier protein in cruciferous plants, which makes the plants more sensitive to generalist herbivores, may be a counteradaptive mechanism for reducing oviposition by *P. rapae* and perhaps other crucifer-specialist insects.

Cardenolide Tolerance in Exposed Insects—New Approaches to an Old Challenge

Petschenka G and Dobler S

Biocenter Grindel, Department of Molecular Evolution, University of Hamburg, Martin-Luther-King Platz 3, 20146 Hamburg, Germany

Corresponding author: georg.petschenka@web.de

Insect tolerance for cardenolide containing plants has stimulated many studies because these compounds are highly toxic to animals. Cardenolides (cardiac glycosides) are highly specific inhibitors for the Na⁺K⁺-ATPase (sodium pump), which is a ubiquitous enzyme throughout the animal kingdom. Most of the research on insects and cardenolides has focussed on the monarch butterfly (*Danaus plexippus*) and some other model organisms. In several of these species, it could be shown that they possess Na⁺K⁺-ATPases, which are insensitive to cardenolides (target site insensitivity). Evidence for the enzymes' altered sensitivity is either provided by enzymological assays or by analyzing the gene sequence and screening for amino acid substitutions that have been shown to alter the binding capacity of ouabain (a commercially available cardenolide). In our recent approach, we set out to test whether this target site insensitivity is a common phenomenon in Lepidoptera feeding on cardenolide plants. Physiological investigations of their sodium pump enable us to directly measure ouabain sensitivity. We found that *Daphnis nerii* (Lepidoptera, Sphingidae) as well as the cardenolide sequestering *Empyreuma pugione* (Lepidoptera, Arctiidae) possess highly sensitive sodium pumps. Using a newly developed enzymological assay we can determine the cardenolide content in the hemolymph of sequestering species to get reliable estimates of the actual cardenolide exposition in the insect's tissue. Data obtained by this approach are compared with an established method of cardenolide quantification. Additionally, cardenolide profiles of larval hemolymph are determined by thin layer chromatography. To investigate the tissue distribution of the target site, the Na⁺K⁺-ATPase, we use immunohistochemical and molecular biological methods. The correlation of these data to the observed insensitivity of the enzyme lead us to an attractive hypothesis how these species tolerate the cardenolides that they ingest and sequester.

Detoxification of Isothiocyanates in the Generalist Lepidopteran Insect *Spodoptera littoralis*

Schramm K, Gershenson J, and Wittstock U

Department of Biochemistry, MPI for Chemical Ecology, Jena, Germany (KS, JG); Institut fuer Pharmazeutische Biologie, TU Braunschweig, Braunschweig, Germany (UW)

Corresponding author: kschramm@ice.mpg.de

The defensive properties of the glucosinolate-myrosinase system in plants of the order Brassicales have been attributed to the formation of toxic isothiocyanates generated upon tissue damage. Lepidopteran herbivores specialised on brassicaceous plants have been shown to possess biochemical adaptations preventing the formation of isothiocyanates. Yet, no such adaptational mechanisms are known for generalist lepidopterans that also occasionally feed on plants of the Brassicales. Analysis of the faeces of the generalist larvae of *Spodoptera littoralis*, which had fed on different Brassicaceae, revealed that this species forms glutathione conjugates of glucosinolate-derived isothiocyanates whenever isothiocyanates are formed in the food plant. The caterpillars are able to form glutathione conjugates from a large variety of isothiocyanate structures. The conjugation was shown to be catalysed by glutathione *S*-transferases in the larval gut. The identification of the specific enzymes responsible for isothiocyanate detoxification by two-dimensional protein separation is in progress, as is a molecular approach for identifying the corresponding genes. Future investigations will explore the costs of this detoxification ability and determine whether glutathione *S*-transferase activity is sufficient to cope with the range and quantity of isothiocyanates present in potential hosts.

Interspecific and Intraspecific Diversity of Latex Ingredients in Moraceae Plants: Chemicals and Proteins with Defensive Roles against Herbivorous Insects

Konno K, Wasano N, Hirayama C, Tateishi K, Nakamura M, and Ono H

National Institute of Agrobiological Sciences, Tsukuba, Ibaraki, Japan (KK, NW, CH, KT, MN); National Food Research Institute, Tsukuba, Ibaraki, Japan (HO)

Corresponding author: konno@affrc.go.jp

Plant latex, a white sap exuded from damaged leaf veins, is widely found in plant species (ca. 12,000-35,000). The hypothesis that the role of plant latex is defense against insect herbivory is prevalent, but the ecological roles of latex and their material bases remain unstudied in most latex-exuding plants. Moraceae includes >1,000 species of latex-exuding plants such as mulberry trees, and fig trees. Although the appearances of latexes from Moraceae species are similar as milky saps and the latexes often show toxicity and play defensive roles, we found that the ingredients of latexes are diverse both among and within species. For example, latex of mulberry trees (*Morus* spp.) contains sugar-mimic alkaloids such as 1,4-dideoxy-1,4-imino-D-arabinitol (D-AB1), 1-deoxy nojirimycin (DNJ) in high concentrations (1.5-2.5% to wet latex, 8-18% to dry latex) (1), which inhibit the sugar-metabolizing enzymes of insects such as sucrose in the midgut tissue and trehalase in various tissues (but not those of mulberry specialist *Bombyx mori*) (2). Also, mulberry latex contains a unique defense protein with extensin and chitin-binding moieties, which shows toxicity to insect in very low concentrations (0.01% to wet diet). Mulberry latex further contains strong Gal/GalNAc binding lectin activity and chitinase activity, which may have defensive roles. *Ficus* is a very large group of Moraceae (>800 species). *Ficus* species such as the common fig, *Ficus carica*, and a wild fig, *Ficus virgata*, one of ca. 10 wild figs in Okinawa, Japan, exude latex rich in cysteine protease called ficin. Our study showed ficin is toxic to lepidopteran larvae and is substantial in defense of *F. virgata* (3). Among other wild fig species in Okinawa, *F. ampelas* exude latex rich in cysteine protease and latex protease is responsible for defense. However, much weaker protease activity was detected from latex of *F. macrocarpa*, and no detectable protease activity was observed in *F. septica*, *F. variegata*, and *F. erecta*. In spite of absence of protease activity, removal of latex decreased the defense activity of *F. septica*, *F. variegata* and *F. erecta* (most apparently in *F. variegata*), suggesting that latex still plays defensive roles in these species. However, latex of these three species differs considerably. *F. variegata* exudes large amount of white sticky latex, which is tasteless and less toxic to insect when added to artificial diets. In contrast, *F. septica* exude small amount of pale yellow sticky latex, which is strongly bitter and highly toxic to insect when added to artificial diet in 2% concentration. The major toxic component of *F. septica* latex is soluble and stable in chloroform suggesting that the toxic component is likely to be a low molecular weight compound rather than a defense protein. Our results show the diversities of defensive ingredients (proteins or chemicals) in latex even in the same family, genus and species, which may have arisen from longstanding hostile relationships between plants and herbivores.

1. Proc. Natl. Acad. Sci. USA 103:1337-1341.

2. Insect Biochem. Mol. Biol. 37:1341-1351.

3. Plant J. 37:370-378.

Insect Defense and Prey Location

Moderators:
15.00–15.30 **Allard A. Cossé**,
National Center for Agricultural Utilization Research
15.50–17.20 **Martha Weiss**, Georgetown University
Room 11, Life Sciences Building



PARALLEL SESSION 4 | TUESDAY, 19 AUGUST

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Semiochemical Investigations of Lacewings (Neuroptera: Chrysopidae)

Aldrich JR, Chauhan K, Torres J, Zhang Q-H, and Winterton SL

USDA-ARS Invasive Insect Biocontrol & Behavior Laboratory, 10300 Baltimore Ave., Bldg. 007, rm301, BARC-West, Beltsville, MD 20705 (JRA, KC); DEPA-Entomologia, Universidade Federal Rural de Pernambuco, Av. Dom Manoel de Medeiros, s/n-Dois Irmãos 52171-900 Recife, PE, Brasil (JT); Sterling International, Inc., 3808 N. Sullivan Rd., Bldg. 16BV, Spokane, WA 99216-16130 (Q-HZ); Department of Primary Industries & Fisheries, Entomology Collection, 80 Meiers Rd., Indooroopilly, Queensland 4068, Australia (SLW)

Corresponding author: jeffrey.aldrich@ars.usda.gov

Adult chrysopids possess paired prothoracic glands (PG) thought to produce defensive secretions (allomones). We analyzed PG extracts of the following lacewing species from North and South America, Australia, and China: *Ceraeochrysa cubana* (Brazil); *Chrysopa* (= *Co.*) *oculata*, *Co. nigricornis*, *Co. incompleta*, *Co. quadripunctata* (USA) and *Co. septempunctata* (China); *Chrysoperla* (= *Cl.*) *rufilabris* (USA) and *Cl. sp.* (Brazil); and *Plesiochrysa ramburi* and *Mallada* spp. (Australia). PG secretions are characteristic for species within a genus, except for *Chrysopa* spp., and (Z)-4-tridecene is ubiquitous; earlier reports in the literature that *Co. oculata* and *Co. nigricornis* produce 1-tridecene were shown to be in error. *Chrysopa* PG secretions are marked by the presence or absence of *N*-(3-methylbutyl)acetamide plus skatole (3-methylindole). Skatole also is identified for the first time from the genus *Ceraeochrysa*. The PG secretion in *Plesiochrysa ramburi* is characterized by the presence of 4-undecene instead of (Z)-4-tridecene and *N*-(3-methylbutyl)propanamide instead of the acetamide, resembling the PG secretions of *Chrysopa nigricornis* and *C. incompleta*.

Production of Alarm Pheromone by Developing Aphids Varies in Response to Their Social Environment

Verheggen FJ, Mescher MC, Haubruge E, and De Moraes CM

Department of Functional and Evolutionary Entomology, Gembloux Agricultural University, Gembloux, Belgium (FJV, EH); Department of Entomology, The Pennsylvania State University, University Park, PA (MCM, CMDM)

Corresponding author: verheggen.f@fsagx.ac.be

Aphid alarm pheromone—the volatile sesquiterpene (*E*)- β -farnesene (E β f) in most species—is released in response to predation and other stresses and typically causes nearby aphids that receive the signal to cease feeding, drop from their host plant, and disperse. However, E β f also serves as a foraging cue for a syrphid predator of aphids, suggesting that the adaptive benefits of signaling via E β f must be weighed against the ecological cost of increasing apparency to predators. Because alarm signaling confers apparent fitness benefits on recipients, but the production and release of E β f likely entails costs for the emitting aphid, it could be adaptive for aphids to regulate E β f production in response to variation in the social environment. To explore this possibility, we compared the production of E β f by *Acyrtosiphon pisum* (Harris) individuals reared from first instars to the adult stage in isolation to that of individuals reared among conspecifics or among individuals of a different aphid species, *Myzus persicae* (Sulzer). Levels of E β f produced in each treatment were assayed by gas chromatograph-flame ionization detector quantification of E β f in volatiles collected from crushed aphids. Production of E β f by *A. pisum* reared in isolation was significantly lower than that of aphids reared in a colony of conspecifics, in an *M. persicae* colony, or in a colony made up of conspecifics of another aphid clone. *A. pisum* individuals also produced less E β f when reared among *M. persicae* than among conspecifics, although this difference was not statistically significant. In a second experiment, we reared *A. pisum* individuals in isolation and exposed them to the odor of conspecifics. Under these conditions, E β f production was similar to that of aphids reared among conspecifics, suggesting that aphids use volatile cues to assess their social environment and regulate their production of alarm pheromone accordingly. Finally, to explore the potential ecological effects of reduced E β f production, we examined the attraction of a predatory hoverfly to groups of aphids reared in isolation or in a colony and found that groups comprising individuals reared in isolation were significantly less attractive to the predator.

Chemical Characterization of the Footprints Left by Adult *Nezara viridula* That Induce Arrestment in the Egg Parasitoid *Trissolcus basalis*

Lo Giudice D, Mcelfresh JS, Millar JG, Peri E, and Colazza S

Department of S.En.Fi.Mi.Zo.-Section of Acarology, Entomology and Zoology-Università di Palermo, Italy (DLG, EP, SC); Department of Entomology, University of California, Riverside, CA 92521 (DLG, JSM, JGM)

Corresponding author: entela13@hotmail.it

Females of the egg parasitoid *Trissolcus basalis* that have touched an area contaminated by adult footprints of their host *Nezara viridula* initiate searching behavior in and around the contaminated patches. Female wasps discriminate between footprints left by adult female or male *N. viridula*. The chemical cues in the host's footprints are hexane soluble, suggesting that cuticular lipids might be involved. Chemical analysis of extracts of *N. viridula* cuticular lipids revealed linear alkanes with quantitative and qualitative differences between the sexes. The linear alkane n-nonadecane (nC19) was recovered only from extracts of males and from solid-phase microextraction of residues left by *N. viridula* males walking on glass plates. This alkane, when added to crude extracts of *N. viridula* females, reduced host-searching responses by *T. basalis* females, similar to what occurs when female wasps contact hexane extracts of male hosts. However, a reconstructed blend of the straight-chain hydrocarbons extracted from female *N. viridula* induced only weak arrestment responses by *T. basalis* females. Thus, other components of the wax layer of *N. viridula*, such as branched hydrocarbons or more polar components of the wax layer, must be important in elicitation of full response. To better characterize the cues that induce arrestment of female wasps, crude extracts were fractionated by several methods. Solvent extracts of dissected body parts of *N. viridula* were fractionated on a silica gel solid phase extraction cartridge, and the biological activity of the resulting fractions was tested in open arena bioassays coupled with a video tracking and motion analysis system, by using female *T. basalis* as test animals. A second fractionation was carried out by normal phase HPLC on silica, eluting with a hexane-isopropanol gradient. In both fractionations, several fractions elicited partial responses from female wasps, with one of the more polar fractions eliciting responses similar to those induced by the crude extract of *N. viridula* females. Thus, the most active compounds cannot be cuticular hydrocarbons.

A Free Meal: No Costs for Acquiring Defensive Plant Alkaloids in an Arctiid Moth (*Utetheisa ornatrix*)

Cogni R, Trigo JR, and Futuyama DJ

Department of Ecology and Evolution, Stony Brook University, Stony Brook, NY (RC, DJF); Departamento de Zoologia, Instituto de Biologia, Universidade Estadual de Campinas, Campinas, Brazil (JRT)

Corresponding author: rcogni@life.bio.sunysb.edu

Many specialist herbivores can overcome plant chemical resistance traits. For example, several aposematic butterfly and diurnal moth species sequester unpalatable or toxic substances from their host plants and use them as defensive substances against their own natural enemies. *Utetheisa ornatrix* (Lepidoptera: Arctiidae) uses the plant alkaloids (PAs) sequestered from *Crotalaria* (Fabaceae: Papilionoideae) host plants as protection against different predators. *U. ornatrix* males also modify PAs acquired from the host plant and use them as a courtship pheromone. Arctiid moths adapted to sequester PAs possess a monooxygenase enzyme in the hemolymph that oxidize pro-toxic free base PAs into nontoxic *N*-oxides. Even though the physiology and the benefits of sequestration have been well established in several systems, very few studies have addressed the costs associated with sequestration. By feeding larvae with an artificial diet with different concentrations of pure PAs added, we tested whether PAs have any negative effect on *U. ornatrix* performance. We extracted a mixture of intergerrimine, retrorsine, and seneconine from *Senecio* species and added the PAs to an artificial diet. These PAs are the same type found on *Crotalaria pallida*, the main host plant of *U. ornatrix*. Five treatments were used: 0% dry weight of PAs added, 0.0048, 0.024, 0.12, and 2.4. Larvae were fed on each PA concentration from hatching to pupation. The total amount of PAs sequestered was measured on recently emerged adults by colorimetric method. *U. ornatrix* larval survival and diet consumption were not affected by the PA concentration. Three weeks after hatching, *U. ornatrix* larvae eating the diet with the highest PA concentration were smaller than the larvae eating diets with less PAs. Larvae eating the highest PA concentration also took longer to pupate. In contrast, pupal and adult weights were not affected by PA concentration. The amount of PAs sequestered greatly increased with increasing PA concentration on the diet. As a control, we used larvae of the generalist *Heliothis virescens*, an insect that is not adapted to use PA. Survival of *H. virescens* was strongly reduced at the two highest PA concentrations, and this moth did not sequester PAs. Our results show that PAs are toxic to a nonadapted herbivore, and that even high PA concentration on the diet does not represent a cost for the specialist *U. ornatrix*. At the highest PA concentration, larval growth was reduced, but due to an increased developmental time, these larvae resulted in pupae and adults with the same weight as the larvae in the other treatments. We show that sequestration of a secondary metabolism substance by a specialist herbivore used as antipredator defense and sexual pheromone does not have a cost.

Response of *Trichogramma* Egg Parasitoids toward the Synthetic Sex Pheromone (Z)-7-Tetradecenal, of the Olive Moth, *Prays oleae* (Bern)

Mylonas PG, Martinou A, Kontodimas DC, and Konstantopoulou MA

Laboratory of Biological Control, Department of Entomology, Benaki Phytopathological Institute, Kifissia, Attikis, Greece (MPG, MA, KDC); Chemical Ecology and Natural Products Laboratory, Institute of Biology, NCSR "Demokritos," Greece (KMA)

Corresponding author: p.milonas@bpi.gr

The response of four *Trichogramma* egg parasitoid strains (two strains of *T. oleae* Voegelé & Pointel, *T. cacoeciae* Marshall, and *T. bourarachae* Pintureau & Babault) to the synthetic sex pheromone of their host *Prays oleae* (Bern) (Lepidoptera, Yponomeutidae) was studied in olfactometer bioassays. All the species tested originated from parasitized olive moth eggs in Tunisian olive groves. A Y-tube glass olfactometer was used (3 cm ID, 10-cm stem, 8-cm arms). The synthetic sex pheromone of the olive moth, Z7-14:Ald, was obtained from Sociedad Española de Desarrollos Químicos (SEDQ, Barcelona, Spain), and a series of solutions in hexane with different doses of the pheromone (0.01, 0.1, 1, 10, and 100 µg/10 µl) was prepared and stored at -20°C until bioassay. More than 40 parasitoids of each strain and pheromone dose were individually tested for a 5-min period. When exposed to the synthetic sex pheromone of the olive moth, parasitoid females of *T. cacoeciae* spent significantly longer time walking in the pheromone treated arm at all but one concentration (1 µg/10 µl). Positive response toward the pheromone was observed for one strain of *T. oleae* (I047) and for *T. bourarachae*, but their response varied with pheromone concentration. *T. oleae* females (strain I047) spent longer time in the pheromone treated arm at concentrations 10 and 100 µg/10 µl, whereas those of *T. bourarachae* at 1 and 100 µg/10 µl. *T. oleae* females (strain I024) did not respond to the pheromone at the concentrations examined. There was no negative response observed toward the pheromone by the parasitoids in any case. Female parasitoids either spent longer time in the pheromone treated arm, or the difference in time spent between pheromone and control was not significantly different. Adults of the olive moth (either males or females) as well as fully grown larvae were extracted using three solvents of different polarity, and these crude extracts were then used in olfactometer bioassays with *T. cacoeciae*. This species was chosen as it was highly responsive during the bioassays with the synthetic sex pheromone Z7-14:Ald. Females of *T. cacoeciae* were found to spend significantly longer time in the arm treated with the methanol crude extract of male olive moths. In all other crude extracts, parasitoids showed neither positive or negative response. The results of this research are discussed in view that they may be considered as alternatives in the biological control of *P. oleae*.

How a Generalist Predator of Bark Beetles Can Deal with a Variety of Prey Signals: Olfactory Experience Modifies Responses to a Prey Pheromone, Ipsenol, in a Clerid Predator

Costa A and Reeve JD

Laboratory of Insect Ecology, Department of Zoology, Center for Ecology, Southern Illinois University, Carbondale, IL

Corresponding author: arnaudcosta@yahoo.fr

Learning has been widely studied in parasitic wasps that can associate host pheromones and herbivore-induced volatiles with the presence of a host (1). Surprisingly, this research remains largely unexplored in a system involving a predator that attacks bark beetles in pine forests. This system presents interesting features that could promote learning of cues associated with prey items. Among bark beetle predators that occur throughout North America, *Thanasimus dubius* is a generalist that can attack several *Ips* species and at least one *Dendroctonus* sp. This predator could be a good candidate to learn cues from its environment because of its broad diet, its unpredictably distributed resources in both space and time (2), and its long-lived adult stage. Previous field experiments using mark recapture suggested that these predators can display a substantial flexibility in their response to three prey pheromones (ipenol, ipsdienol, and frontalin). To confirm these field observations and to investigate at which extent this behavior can occur, we measured responses of *T. dubius* in a wind tunnel that permits to test a wider range of kairomones on a larger number of individuals. Anemotactic behaviors were observed using a wind tunnel using frontalin, ipenol, ipsdienol, and other prey pheromones/semiochemicals. Results showed that these predators can display a large flexibility in their response, confirming a potential for switching in the field (3). Whether this predator could learn prey pheromones was then examined in a second wind tunnel experiment. We tested two major prey pheromones, frontalin and ipenol, that were presented alone or associated with a reward. The percentage of *T. dubius* flying upwind was used to measure its preference toward the source. Naïve predators that were trained to ipenol showed a dramatic increase of attractiveness after having experienced two conditioning associated with a reward. The preference rose from 16% of upwind flights for naïve individuals to 63% for those conditioned. Interestingly, simple exposure produced a moderate increase that was not significantly different from the control group. Response toward frontalin was slightly stronger for trained predators, but it was not significantly higher compared with a control. We discuss possible implications of such learning mechanism in this system. Our study constitutes to our knowledge the first evidence that a bark-beetle predator can modify its response toward prey pheromones after an olfactory experience.

1. Science (1990) 250:1251–1253.
2. Environ Entomol (1991) 20:401–409.
3. Reeve et al. (2008) Ecol Entomol (submitted)

Spiders' Cuticular Hydrocarbons Mediate Prey Recognition by Mud-Dauber Wasps

Uma D, Phelan L, and Weiss M

Department of Biology, Georgetown University, Washington DC (DU, MW); Department of Entomology, The Ohio State University, Wooster, OH (LP)

Corresponding author: dbu@georgetown.edu

Although spiders are predators, they are themselves heavily preyed upon by mud-dauber wasps. Spiders' web architecture has been considered to be a defensive behavior against wasp predation, with three-dimensional (3D) webs thought to provide more effective physical barriers than their two-dimensional (2D) counterparts. When we offered *Sceliphron caementarium* (Sphecidae) wasps a choice of *Larinioides sclopetarius* (Araneidae, 2D) and *Parasteatoda tepidariorum* (Theridiidae, 3D) spiders on their respective webs, wasps took significantly more 2D than 3D spiders, a result that initially seemed to support the importance of web architecture. However, when offered a choice of freshly killed 2D and 3D spiders without their webs, wasps rarely took 3D spiders. Furthermore, wasps spent more time on filter papers containing silk and chemical residues from 2D than 3D spiders, suggesting that prey recognition involves chemotactile cues. Analysis of cuticular extracts of three 2D and three 3D species by gas chromatography/mass spectrometry revealed distinct differences in hydrocarbon profiles between 2D and 3D spiders. We found that wasps rejected hexane-washed 2D spiders, suggesting that a cuticular chemical cue is necessary for prey recognition. Furthermore, when we offered individual wasps a choice of paper balls treated with cuticular extracts of 2D spiders, 3D spiders, or hexane controls, the wasps spent significantly more time antennating 2D extract-coated balls. These results provide the first evidence that cuticular cues are involved in recognition and differentiation of prey species by sphecid wasps.

Fire Ant Cuticular Hydrocarbons: A Research Tool and Nestmate Recognition Cues?

Vander Meer RK, Choi MY, and Anderson JA

USDA-ARS-CMAVE, Gainesville, FL (RKVM, MYC); Center for Wetland and Water Resources, The University of Mississippi, Abbeville, MS (JAA)

Corresponding author: bob.vandermeer@ars.usda.gov

Social insects use complex pheromone systems to maintain sociality, and, in most ant species, colony integrity. Colony integrity is expressed as nestmate recognition, where colony members can discriminate between nestmates and conspecific non-nestmates by matching an experience based neural template of their colony odor with the cues detected on the cuticle of an intruder. If the cues and template do not match, e.g., a non-nestmate intruder, then aggression occurs. There is a large body of correlative data linking nestmate recognition and cuticular hydrocarbons, but little evidence of direct cuticular hydrocarbon involvement. Multivariate analyses of hydrocarbon patterns can differentiate one colony from another; however, it is not clear whether the ants also can differentiate colonies based on cuticular hydrocarbons. We present a review of the use of fire ant cuticular hydrocarbons as models for heritable nestmate recognition cues and as tools for monitoring movement of cuticular hydrocarbons in a colony. We then describe a new method for putting physiologically relevant amounts of treatment hydrocarbons on the cuticle of workers, which we used to evaluate the role of hydrocarbons in fire ant nestmate recognition.

Changes in Cuticular Hydrocarbon Composition in Relation to Changes in Ambient Temperature in the Red Imported Fire Ant

Anderson J and Marquess J

The University of Mississippi Center for Water and Wetland Resources, Abbeville, MS
Corresponding author: jandersn@olemiss.edu

Because of scaling, insect surface area is much larger in proportion to volume than in larger animals, increasing the hazard of water loss. Cuticular hydrocarbons (HCs) function primarily to prevent water loss through the cuticle, but they may have other adaptive characteristics, such as recognition cues in many hymenopterans and mating pheromones in beetles. They have long been implicated as cues in the complex nest-mate recognition systems of ants. Gibbs et al. (1) suggested that temperature may be a factor determining the composition of hydrocarbons in some insects, because the melting point of the mixture coincides with loss of function in preventing water loss. In ants, where the hydrocarbons have been found to function as a colony “gestalt organ,” necessarily passed around and blended together for nestmate recognition to function, we predicted that the compositional response to ambient temperature might be faster and more homeostatic than in other insects. We tested this by comparing the HC compositions of recently collected red imported fire ant colonies subjected to shifts in three temperature regimes, and we present our results.

1. Am. Zool. (1998) 38:471–482.
2. Water Relations of Terrestrial Arthropods (1994) Academic Press, San Diego, CA.
3. Ann. Entomol. Soc. Am. (1995) 88:485–495.

Function and Complexity of Species-Specific Aggregation Pheromones in Two Species of Related Fish, the Goldfish and Common Carp

Levesque H and Sorensen PW

Department of Fisheries, Wildlife and Conservation Biology, University of Minnesota, 1980 Folwell Avenue, Saint Paul, MN 55108
Corresponding author: leves006@umn.edu

The common carp (*Cyprinus carpio*) is a cyprinid fish that thrives in turbid water and relies heavily on odorous cues to find food and mates. Previous studies in our laboratory discovered that both immature carp and goldfish (*Carassius auratus*, a close relative that hybridizes with carp) are attracted to conspecific body washings in a species-specific manner (1). Likely, the body odors of these species serve as aggregational pheromones. We explored the function and origin, potency, and chemical complexity of this pheromone system. Initial experiments tested the effect of maturational status on pheromone production and perception in the goldfish using two-choice behavioural mazes. We discovered that juvenile, immature, and mature goldfish are all attracted to the odor of conspecifics, irrespective of fish maturational state; likely, this pheromone functions independently of the endocrine system (2) and mediates social attraction amongst all life stages of goldfish, with the possible exception of sexually receptive mature females, which are only attracted to mature males (3). Tests using anosmic goldfish demonstrated that responses to the attraction pheromone are mediated by the sense of smell, whereas dilution tests found goldfish body odor to be potent even when diluted >10 times, seemingly confirming a role as a long-distance attractant. Chemical characterization using C18 resin found that both the carp and goldfish cues have nonpolar and polar components that synergize each other's actions. Bile acids, strong olfactory stimulants long suspected of have pheromonal function in fishes, were identified in both goldfish and carp holding water and tested on juveniles but found not to be species specific or attractive. HPLC fractionation of the carp body odor now suggests that the carp aggregation pheromone has at least two novel, nonpolar components. Ongoing work is focused on identifying the carp pheromone as species is a highly damaging invasive species across North America and might be controlled with species-specific pheromonal attractants. This work was funded by the Minnesota Environmental and Natural Resources Trust Fund.

1. Behaviour (in press).
2. Lim et al. (this conference).
3. Horm. Brain Behav. (2002) 2:375–435.

Sensitivity to an Attractive Male Sex Pheromone in the Common Carp (*Cyprinus carpio*) Is Tightly Correlated with Endocrine State and Sexual Receptivity

Lim H, Levesque H, and Sorensen PW

Department of Fisheries, Wildlife & Conservation Biology, University of Minnesota, St. Paul, MN
Corresponding author: limxx148@umn.edu

Gender recognition in externally fertilizing fishes such as the common carp, *Cyprinus carpio*, which thrives in turbid water, needs to be precisely regulated, especially for female carp that may only spawn once a year and must locate males quickly because egg viability is short-lived. Previous studies of this species have shown that species-recognition is mediated by a complex species-specific aroma released by individuals at all stages of maturity (1,2) and that males discern females by using a variety of hormonally-derived stimuli (3, ongoing). We tested whether female carp specifically recognize mature males by using a male-derived pheromone composed of nonpolar sex steroids, sensitivity to which might be gated by female receptivity and regulated by hormonal condition. Experiments using a two-choice maze found that mature but nonreceptive females are attracted to the odors of conspecifics regardless of their reproductive state, likely reflecting their perception of a species-specific aggregation pheromone. However, sexually receptive females (a condition induced by prostaglandin F₂ α hormone injection) are only attracted to mature male odor, suggesting that compound(s) released only by mature males become uniquely attractive to receptive females. Experiments using different concentrations of male odor indicated that the male pheromone is likely to be used for short-range orientation. Furthermore, although the male pheromone seems to be species-specific, females are modestly responsive to the odors of male heterospecifics, suggesting it mediates gender recognition in spawning groups in which additional sensory cues are active. Nanomolar concentration of androstenedione, a male hormonal pheromone in the goldfish, also seems to play a role in the carp pheromone because it attracts receptive female carp, although not as actively as whole male odor; other components must exist. Ongoing studies seek to identify these components and to test them in the field. The pheromone has promise for controlling the common carp, an extremely damaging invasive fish in North American waters. Thus work was funded by the Invasive Animals Cooperative Research Centre (Australia) and the Minnesota Environmental and Natural Resources Trust Fund.

1. Behaviour (in press).
2. This conference.
3. Can. J. Physiol. Pharmacol. 81:329–341.

The Urine of Mozambique Tilapia Males Signals Social Dominance

Barata EN, Santos A, Hubbard PC, and Canário AVM

Centro de Ciências do Mar, Universidade do Algarve, Faro, Portugal (ENB, PCH, AVMC);
Departamento de Biologia, Universidade de Évora, Portugal (ENB, AS)
Corresponding author: ebarata@ualg.pt

Many species of freshwater fish with relatively simple mating strategies release hormonally derived sex pheromones in their urine. However, species with more complex mating and social systems may use specialized urinary chemical signals during reproductive behaviour and/or other social interactions. We addressed this question in the Mozambique tilapia (*Oreochromis mossambicus* Peters 1852), a lek-breeding species wherein males establish dominance hierarchies and visiting females mate preferentially with dominant males. We measured urination frequency of males in social isolation and whilst interacting with each-other, or with females ready to spawn or post-spawn females (1,2). In groups of fish, we monitored the volume of urine stored in subordinate and dominant males to determine whether urine volume and olfactory potency (assessed by the electro-olfactogram [EOG] recorded in both sexes) are related to the male's social rank (1,2). We also assessed the effect of male urine on females' spawning decision and on aggression of males fighting their own image in a mirror. Dominant males stored significantly more urine than subordinates and released it in short duration pulses (1 s on average), the frequency of which increased whilst being aggressive toward opponent males but not when submissive (1). Urination frequency increased also in the presence of females ready to spawn but not of post-spawn females (2). Females preferred to spawn next to males that were associated with male urine rather than next to males that conveyed only visual information. Also, males were less aggressive to their own image in a mirror when in the presence of urine from dominant males than when in the presence of urine from subordinate males or in water-only control. The olfactory potency of urine was positively correlated with the social rank of the male donor (1,2). The results suggest that dominant male tilapia store and use their urine for chemical communication both in male–male and male–female interactions. One urinary odorant, which has been tentatively identified as a 610-Da sulfated aminosterol (2), may act as a pheromonal signal of dominance influencing female spawning and inhibiting aggression in competitor males.

We acknowledge funding by Fundação para a Ciência e Tecnologia-FCT (POCTI/BSE/38815/2001 and POCTI/BIA-BDE/55463/2004), Portugal.

1. BMC Biol. (2007) 5 (doi:10.1186/1741-7007-5-54).
2. J. Chem. Ecol. (2008) 34:438–449.



Molecular Specificity: Recognition vs Deception

Speaker: Gunnar Bergström,
Ethological Chemistry, Göteborg University, Göteborg, Sweden
100 Berg Auditorium, Life Sciences Building

Bergström G

Ethological Chemistry, Göteborg University, Göteborg, Sweden

Silver Medal Award Lecture: oddsoxinventions@comhem.se

My talk is based primarily on our own results from studies of behavior-releasing olfactory signals in insects and flowering plants (about 300 species studied altogether). It consists of five parts.

1. Background and Techniques. I emphasize the great (in my experience) importance of adequate techniques and methods for the outcome of our analytical work. I then make a resumé of the development of chemical ecology in Sweden, point out some principles for our endeavours, and summarize our main research objects, with an emphasis on bees, ants, forest insects, and analytical techniques.
2. Recognition among Insects. The objects chosen to exemplify chemical species specificity are bumble bees, antlions, bark beetles, and pine sawflies. Different ways of achieving specificity in these, and other, organisms are contrasted.
3. Recognition between Insects and Flowering Plants. The role of volatile compounds in the strong coevolutionary relationships between the two groups is exemplified by *Rosa rugosa* and *Ranunculus acris*. Specific pollen odors are mentioned.
4. Deception and Mimicry. Chemical mimetism between host and parasitic bees (cleptoparasitism) is taken as an example from insects. In flowering plants, we have studied chemical signals involved with deception vis-à-vis pollinators. Examples: *Ophrys* and *Cypripedium*.
5. Theory—Future. I argue for the development of a general theory for the structure, function, and evolution of behavior-guiding olfactory signals, which can comprise the essence of our current knowledge and which also can have a predictive value. I also mention some personal thoughts about possible future research in chemical ecology.

Much of the material presented in this talk can be found in the references below.

Pure Appl. Chem. (2007) 79(12):2305–2323 (Chemical ecology = chemistry + ecology!
Bergström G; contribution to IUPAC project Chemistry for Biology)
Chem. Commun. (2008) (in press) Chemical communication by behaviour-guiding olfactory signals. Bergström G (invited feature article)

Poster Session 2

3rd and 4th Floor Bridges, Life Sciences Building

★ = poster presentations being judged for an award

Posters can be hung starting Monday afternoon, 18 August. Posters will remain in place until Thursday afternoon, 21 August, when they should be taken down by 12.00 (12:00 PM). Presenters assigned to even-numbered sites will be expected to be present at their stations during the Tuesday evening (19 August) viewing session. Viewing time both evenings is from 20.30 to 22.00 (8:30–10:00 PM).



POSTER SESSION 2 | TUESDAY, 19 AUGUST

SUN

MON

TUE

WED

THU

FRI

Ahn S-J, Badenes-Perez FR, and Heckel DG

Department of Entomology, Max Planck Institute for Chemical Ecology, Jena, 07745, Germany
Corresponding author: sahn@ice.mpg.de

Capsaicin (8-methyl-*N*-vanillyl-6-nonenamide), an alkaloid found only in *Capsicum* spp. (Solanaceae), is responsible for the pungency of hot pepper fruit. The oriental tobacco budworm, *Helicoverpa assulta* (Lepidoptera: Noctuidae), feeds on a few solanaceous plants, including *Capsicum* spp. Larvae consume the tissue inside of the fruit, where capsaicin concentrations are highest. The purpose of this study was to determine both the toxicity of capsaicin on the development of *H. assulta* and other noctuids as well as how insects detoxify this compound. Feeding experiments with capsaicin-spiked artificial diet showed that capsaicin was toxic to all insect herbivores tested except *H. assulta*. The larval growth of *H. armigera*, *H. zea*, *H. virescens*, *H. subflexa*, and *S. frugiperda* was delayed when these insects were fed capsaicin. Larval survival was decreased by capsaicin in *H. zea* and *H. subflexa*. In *H. assulta*, larval growth and survival rates were even enhanced by capsaicin, suggesting that capsaicin plays a role in this species' adaptation to its host plant. Furthermore, when capsaicin was injected into the hemocoel of fifth-instar larvae, pupal weights decreased in *H. armigera* and *H. zea*, but not in *H. assulta*. Capsaicin seems to act as a feeding deterrent to *H. zea*, but not to *H. assulta* and *H. armigera*. The relative consumption rate was decreased by capsaicin in *H. zea*, but not in *H. assulta* and *H. armigera*. This suggests that capsaicin may deter *H. zea*, but not *H. assulta* and *H. armigera*. The amount of capsaicin in feces was measured by HPLC. Only small amounts of the capsaicin ingested were excreted in the feces of *H. assulta*, suggesting that capsaicin is sequestered, modified, or degraded into nontoxic compounds before being excreted in feces. To evaluate whether cytochrome-P450 monooxygenases was involved in the capsaicin detoxification in *H. assulta*, piperonyl butoxide (PBO; a potent inhibitor of the enzyme) was topically administered on third-instar larvae and then larval growth was measured with capsaicin-supplemented diet for 5 days. When the two chemicals (PBO and capsaicin) were combined, they reduced larval growth significantly more than in the control, where PBO was treated but capsaicin was not incorporated in the diet. This indirectly shows that PBO blocks the enzyme, which is responsible for capsaicin detoxification, suggesting, that the tolerance of *H. assulta* to capsaicin relies on the activity of the cytochrome-P450 monooxygenases. Such detoxification mechanisms are now being further investigated using biochemical and genetic approaches.





Methyl Jasmonate-Induced Responses Reduce Natural Herbivory on *Brugmansia suaveolens* (Solanaceae): Is the Tropane Alkaloid Scopolamine Responsible?

Arab A, Alves MN, Sartoratto A, Ogasawara DC, Massane G, and Trigo JR

Laboratório de Ecologia Química, Departamento de Zoologia, Instituto de Biologia, Unicamp, Caixa Postal 6109, 13083-970, Campinas, São Paulo, Brazil (AA, GM, JRT); Centro Pluridisciplinar de Pesquisas Químicas, Biológicas e Agrícolas, Unicamp, Campinas, São Paulo, Brazil (MNA, AS, DCO)

Corresponding author: alberto@unicamp.br

Brugmansia suaveolens (Solanaceae) contains tropane alkaloids, with scopolamine and atropine as the main components, which have been suggested to act as a chemical defense. To assess the effect of increased scopolamine in leaves of *B. suaveolens* on herbivore attack, we conducted a field experiment where we induced scopolamine synthesis by adding methyl jasmonate (MJ) to potted plants. After 10 days, MJ-induced plants suffered significantly less herbivory and showed increased scopolamine content than field control plants. However, herbivory in the field was not correlated with scopolamine content. To determine whether scopolamine affects the performance of insect herbivores, we conducted a laboratory experiment using 4th instars of the specialist *Placidina euryanassa* (Lepidoptera: Ithomiinae) and the model generalist *Spodoptera frugiperda* (Lepidoptera: Noctuidae). Larvae were fed with leaf discs of *B. suaveolens* (*P. euryanassa*) or *Zea mays* (*S. frugiperda*) impregnated with scopolamine at the concentrations found in leaves after mechanical and MJ induction. After 24 h, the scopolamine at the concentration found in MJ-induced plants failed to affect the performance of the herbivores. These results suggested that scopolamine is not responsible for the reduction in herbivory observed in the field. Moreover, total tropane alkaloids only affected the performance of *P. euryanassa*. It is possible that a single compound failed to affect insect herbivores and a synergistic interaction among different tropane alkaloids would be necessary in order to play a major role in herbivore defense in this plant species.

Financial support was provided by Fundação de Amparo à Pesquisa do Estado de São Paulo (Fapesp), processes nos. 07/0304-1, 07-07607-7, 08-01924-3.

Host Recognition in *Contarinia nasturtii* and *Dasineura brassicae*: Two Sympatric Gall Midge Species in Crucifers

Boddum T, Birgersson G, Hansson BS, and Hillbur Y

IC-E3. Department of Plant Protection Biology, Swedish University of Agricultural Sciences, SLU, PO Box 102, 230 53 Alnarp, Sweden (TB, BSH,YH); Department of Evolutionary Neuroethology, Max Planck Institute for Chemical Ecology, MPI, Hans-Knöll-Straße 8, D-07745 Jena, Germany (BSH)

Corresponding author: tina.boddum@ltj.slu.se

The family Cecidomyiidae (Diptera), gall midges, contains >5,000 species. They form galls on almost all plant parts and are widely distributed among host plants, occurring on gymnosperms, angiosperms, monocotyledons, and dicotyledons. They are of evolutionary interest because of their rapid rate of speciation compared with related families of Diptera. At the generic level, gall midges are often polyphagous, especially in the large genera. However, at species level many gall midges are monophagous or oligophagous. We study possible evolutionary mechanisms behind the great diversity of the gall midges. Gall midge behavior is guided by olfactory cues; they use pheromones when locating a suitable mate and host plant volatiles for host plant recognition. Thus, host plant volatiles might be important when gall midges shift between hosts and subsequently in the formation of new gall midge species. GC-EAD is used to test the antennal response of two distantly related gall midge species, the swede midge, *Contarinia nasturtii*, and the brassica pod midge, *Dasineura brassicae*, when stimulated with plant volatiles. Both species oviposit on cruciferous plants and the larvae develop on the plant. A comparison of the key volatiles indicates whether host recognition in distantly related gall midges—that coexist on the same plant—is mediated by similar odors or by different compounds.



Detection and Identification of Estrogenic Compounds in a *Polygonum* Species

Brennan JC, Denison MS, Holstege DM, and Millam JR

Department of Animal Science, University of California Davis, Davis, California (JCB, JRM); Department of Environmental Toxicology, University of California Davis, Davis California (MSD); Agriculture and Natural Resources Laboratory, University of California Davis, Davis, California (DMH).

Corresponding author: jcbrennan@ucdavis.edu

Seed extracts of selected weeds (including *Polygonum aviculare*) induced estrogenic activity in vitro and may contain novel estrogens (1). We have used analytical chemistry techniques with guidance from an in vitro estrogen bioassay (ovarian carcinoma cell line using a luciferase reporter gene inserted downstream of the estrogen receptor response element) to isolate estrogenic compounds in a related *Polygonum*, *P. convolvulus*. A crude separation of *P. convolvulus* seed extract yielded multiple estrogen-positive fractions when cross-referenced against the bioassay, which led us to hypothesize there are multiple estrogenic compounds in *P. convolvulus* seed. At the ranges tested, *P. convolvulus* seed extract induces greater response than *P. aviculare* seed extract in the estrogen bioassay. Estradiol equivalency of *P. convolvulus* seed extract is 2.21 pmol 17 β -estradiol (E2) eq/g seed. Effective concentration at half-maximal response (EC₅₀) is 0.65 mg seed. Estradiol equivalencies of positive fractions are 0.085, 0.13, and 0.21 pmol E2/g seed. Through high-performance liquid chromatography coupled with diode array detection, we have tentatively identified resveratrol, polydatin, and emodin in estrogen-positive fractions. To our knowledge, no previous studies have confirmed estrogenic properties in seeds of weed *P. convolvulus*; therefore *P. convolvulus* seed would be a novel source of phytoestrogens.

1. Rochester JR (2004) M.S. thesis, University of California-Davis.

Reaction of Leafminer *Liriomyza bryoniae* to Plant Volatiles

Būda V and Radžiūtė S

Laboratory of Chemical and Behavioural Ecology, Institute of Ecology, Vilnius University, Akademijos 2, LT-08412 Vilnius, Lithuania

Corresponding author: vinbuda@eko.lt

Polyphagous leafminer *Liriomyza bryoniae* (Diptera, Agromyzidae) is economically important pest of tomatoes. In Lithuania, this species was included into the list of quarantine species and remained there until 2003. Since then, it has become widespread throughout Lithuania. EAG reactions of adults of *L. bryoniae* were recorded after stimulation by host plant odours. The source of the odours was 6 mg of fresh juice of any of the following host plants: bittersweet (*Solanum dulcamara* L.), tomato (*Lycopersicon esculentum* Mill.), deadnettle (*Lamium album* L.), downy ground-cherry (*Physalis pubescens* L.), white goosefoot (*Chenopodium album* L.), as well as that of nonhost plant wrinkled rose (*Rosa rugosa* Thunb.). There was noted some sexual dimorphism in reactions. In females the highest EAG responses were evoked by deadnettle, downy ground-cherry, and bittersweet volatiles, whereas only downy ground-cherry and bittersweet evoked the highest responses in males. Behavioural tests in Y-olfactometer were carried out using pairs of four host plants: bittersweet, tomato, deadnettle, and downy ground-cherry. The source of the odours was airstream passing over leaves of undamaged plant on a branch top from undamaged plant. The number of the leaves varied from 12 to 16, depending on their size. Reaction both of females and males depended on their rearing at larval stage. Females reared on a bittersweet, significantly more prefer odour of bittersweet compare either to tomato or deadnettle, whereas no difference was recorded in choice between bittersweet and downy ground-cherry. Males reared on bittersweet, were significantly more attracted to odour of bittersweet compare with that of tomato, deadnettle, and downy ground-cherry. In males, there were recorded no preferences when they were allowed to choose between downy ground-cherry and tomato or between tomato and deadnettle. Females and males reared on a field bean demonstrated no preference to odour either of bittersweet or tomato. Behavioural test under field conditions revealed that methyl salicylate, herbivore-induced plant volatile, was attractive for *L. bryoniae*. The test was carried out in industrial tomato greenhouses late in summer. All tested dosages (from 0.25 to 1 ml/dispenser were attractive; Kruskal–Wallis ANOVA test). This is the first attractant of kairomone origin for the species. Plant preference for adult feeding and egg-laying was analysed.

Preliminary Results on the Trail Pheromone of the Termite *Microcerotermes exiguus* by Using Direct Contact Solid Phase Microextraction

Lubes G and Cabrera A

Dpto. de Química, Universidad Simón Bolívar, Valle de Sartenejas, Caracas 1080A, Venezuela

Corresponding author: acabrera@usb.ve

Microcerotermes exiguus termites are commonly found in Central and South America, and they usually build nests in trees or on the ground surface. With the aim of identifying the chemical compounds responsible for trail-following behavior in *M. exiguus*, first, 50 workers were extracted with *n*-hexane for 3 h, and then this extract was fractionated through column chromatography. The more polar fraction was biologically active and not significantly different from the fraction obtained with the crude hexane extract. So far, we have preliminary results after comparing samples from 40 worker dorsal and ventral surfaces, by using direct contact solid phase microextraction (SPME) with a 100- μ m PDMS-coated fiber. GC-MS analysis showed several exclusive peaks from the ventral samples, which indicates the presence of three aldehydes. Further studies being carried out to establish the action of these compounds, and their confirmed identity.

Effect of Caterpillar Saliva on Maize Defense Gene Expression

Chuang W-P, Herde M, Howe GA, Boston RS, and Luthe DS

Department of Crop and Soil Sciences, The Pennsylvania State University, University Park, PA (WPC, DSL); Department of Biochemistry and Molecular Biology, Michigan State University, East Lansing, MI (MH, GAH); Department of Plant Biology, North Carolina State University, Raleigh, NC (RSB)

Corresponding author: wxc212@psu.edu (Chuang)

The United States is a major worldwide producer of maize. Maize can be damaged heavily by insects and one of the major pests is *Spodoptera frugiperda* (fall armyworm, FAW). Maize exhibits general plant defenses to insect damage; however, we are specifically interested in whether FAW saliva induces plant defense genes. We used FAW-resistant and FAW-susceptible maize genotypes to distinguish general from insect-specific plant defenses. To prevent salivation, the spinneret of the caterpillars was cauterized or ablated, preventing salivary secretions. Insect-resistant and -susceptible maize lines were infested with ablated and unablated larvae. The expression of three groups of genes was measured by quantitative real-time polymerase chain reaction (QRT-PCR). In group 1, we studied genes in the JA-biosynthesis pathway. In group 2, we analyzed plant-induced defense genes, such as maize insect resistance 1-cysteine proteinase (Mir1-CP) (1). In group 3, we measured the expression of genes encoding plant proteins found in FAW frass (2) after ingestion of maize whorl tissue. The caterpillar frass was collected and analyzed by multidimensional protein identification technology (MudPIT). The QRT-PCR results showed that JA-biosynthesis pathway genes transcripts (allene oxide synthase, lipoxygenase 1, 3, and 6) in maize were induced >20 times after caterpillar feeding than uninfested plants. The plant defense genes (maize proteinase inhibitor, chitinase A, and chitinase I) and genes presented in frass (ribosome-inactivating protein 2 [RIP2], leucine aminopeptidase, and β -D-glucosidase precursor) had the same trend as JA-biosynthesis pathway genes. Interestingly, nonablated could induce maize gene expression up to 5 times more than ablated caterpillar attacked. Immunoblot analysis showed that RIP2 protein in maize leaves was significantly induced by FAW saliva in nonablated compared with ablated caterpillar. Hence, caterpillar saliva seems to be critical for eliciting the maximum levels of insect defense genes in maize.

1. Proc. Natl. Acad. Sci. USA (2002) 20:13319–13323.
2. Plant Physiol. (2007) 143:1954–1967.



Plant Protection: Using Chemical Mechanism of Plant Defense and Chemical Interactions between Plants and Other Organisms

Cui H, Wei J, and Lei J

College of Biological Science, China Agricultural University, Beijing 100193, China.

Corresponding author: cuihuiting@cau.edu.cn

All organisms use chemical signals to transmit information; “chemical languages” are the oldest forms of communication. There are chemical interactions, which act as plant chemical defenses on pests in cropping ecosystems. Plants may turn on the defense mechanism to resist or inhibit pests. Furthermore, the chemical mechanism of plant defense may be induced under pest stress. Recent studies have showed that there are manipulating interactions among crops, weeds, and pests in cropping systems. Chemical recognition and information transfer may occur among inter- and intraplant species. Clarification of chemical interactions between plant and other organisms not only broadens the insight of species interactions but also develops new strategy for pest management. We review prospects for application in plant protection by using chemical interactions between plants and other organisms.

Interaction of Chemical, Photic, and Aeolic Stimuli in the Behavior of the Tropical Root Weevil, *Diaprepes abbreviatus*, on a Servosphere

Otálora-Luna F, Lapointe SL, and Dickens JC

Invasive Insect Biocontrol and Behavior Laboratory, Plant Sciences Institute, USDA-ARS, Beltsville, MD (FO-L, JCD); U.S. Horticultural Research Laboratory, USDA-ARS, Fort Pierce, FL 34945 (SLL)

Corresponding author: fernando.otalora-luna@ars.usda.gov

Although the use of different sensory modalities to search and identify appropriate resources is common among insects, most laboratory studies of orientation behavior consider only one sensory modality. Here, we investigated behavior of the tropical root weevil *Diaprepes abbreviatus* (Coleoptera: Curculionidae) to chemical, photic, and aeolic (wind) signals as well as combinations of these signals. Behavioral experiments were performed in a dual choice arena formed by a crossroad of two corridors adapted to an open-loop tracking device (servosphere), where a walking weevil was able to visualize but unable to reach any of the four arms. Emissive colors of narrow wavelength with a maximal peak at 351 nm (UV), 472 nm (blue), 570 nm (green), 585 nm (yellow), or 660 nm (red) were presented to the insect in paired combinations, perpendicular to each other. In still air, both sexes chose green light over UV or blue, and yellow over blue; there was no preference between yellow and green. In the presence of an aeolic stimulus, weevils walked more upwind (37.9% of males and females) compared with still air (3.1%). When an attractive photic stimulus (green) was presented perpendicular to the air current and an unattractive photic stimulus (blue) was presented in the same corridor with the current, upwind displacement decreased to 22.8%, indicating dominance of the attractive photic stimulus. An attractive chemical stimulus (volatiles from the headspace of males feeding on young citrus leaves) injected into an airstream increased upwind walking to 67.0% compared with the aeolic stimulus alone. When green was presented perpendicular to the air current and blue was presented in the same corridor with the chemical and aeolic attractant combination, upwind displacement decreased to 11.1%, again indicating dominance of the attractive photic stimulus. Our observations correspond to behavioral manifestations of the processing and integration of multiple inputs to the central nervous system of the insect. These results facilitate the development of detection tools and control strategies for *D. abbreviatus* and provide a framework for investigations already in progress of multimodal stimuli in other insects such as the Colorado potato beetle.

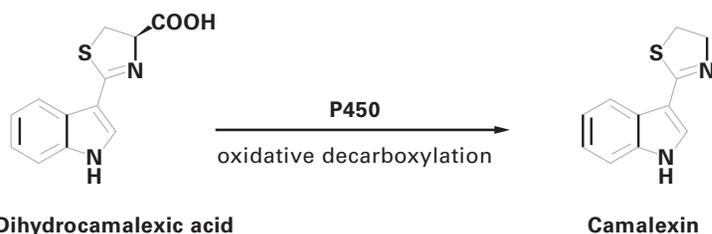
Dihydrocamalexin Acid as an Intermediate in the Final Step of Camalexin Biosynthesis

Doubský J, Bednarek P, Glawischnig E, and Svatoš A

Max Planck Institute for Chemical Ecology, Research Group Mass Spectrometry, Beutenberg Campus, D-07745 Jena, Germany (JD, AS); Max Planck Institute for Plant Breeding Research, Department of Plant–Microbe Interactions, D-50829 Cologne, Germany (PB); Lehrstuhl für Genetik, Technische Universität München, D-85350 Freising, Germany (EG)

Corresponding author: jdoubsky@ice.mpg.de

Camalexin is the principal phytoalexin of *Arabidopsis thaliana*, induced by a great variety of plant pathogens, which has been shown to inhibit growth of particular pathogens, as well as a human tumor cell line (1). Recently, a putative camalexin precursor, dihydrocamalexin acid, has been isolated and identified (2). Additionally, it has been shown that cytochrome CYP71B15 is able to convert dihydrocamalexin acid into camalexin in yeast CYP71B15-expressing cells and also in *A. thaliana* plants (3). We report synthesis of both enantiomers of dihydrocamalexin acid, determination of the absolute configuration of the natural acid, and some details of the final decarboxylative step of camalexin formation. The target acids were synthesized in several steps starting from indole-3-carboxylic acid and methyl esters of L- or D-cysteine via corresponding amides. The resulting amides upon treatment with the Burgess reagent underwent intramolecular cyclization, and a subsequent hydrolysis of the esters with Me₃SnOH afforded pure enantiomers of dihydrocamalexin acid. Isomeric purity of both synthetic and natural samples was determined using ¹H NMR and/or HPLC measurements. The natural dihydrocamalexin acid was found to be (*R*)-isomer predominantly, corresponding to natural L-cysteine, containing 15% of (*S*)-isomer, presumably due to high sensitivity of thiazoline-ring to epimerization. The epimerization process might explain the fact that both isomers are converted into camalexin, although the efficiency for the (*R*)-isomer is significantly higher (3). Biotransformation of a mixture of deuterated and unlabeled dihydrocamalexin acids was performed to elucidate the mechanism of the decarboxylation step. The obtained value of kinetic isotopic effect indicates that the hydride abstraction is not the rate-determining step of this process as proposed before (3).



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Comprehensive Study of *Centella asiatica* Leaves (Family: Apiaceae) Extract against Insects as a Potential Natural Antifeedant

Abdullah F, Fauzee F, Syakirin F, and Kanyan Enchang F

Institute of Biological Science, Faculty of Science, University of Malaya
50603 Kuala Lumpur, Malaysia

Corresponding author: fatma_fauzee@perdana.um.edu.my

The behavioral responses of the three different insect *Epilachna indica* (Coleoptera: Coccinellidae), *Crematogaster acrocoelia* (Hymenoptera: Myrmicinae), and *Camponotus* species ant (Hymenoptera: Formicidae) were tested using *Centella asiatica* crude extract. *C. asiatica* is a well-known medicinal plant valued in ayurvedic treatment and Chinese medicine. This study is carried out to investigate the antifeedant properties instead of their medicinal value. A T-shaped olfactometer method was used to test the behavior of the three insects as mentioned above toward *C. asiatica* leaves extract. The leaves were extracted by n-hexane, dichloromethane (DCM), and methanol (MeOH) to achieve comparison and the best extraction with the strong antifeedant activities. The percentages of *C. acrocoelia* ants that avoided going toward the dichloromethane extract and methanol extract were 84% (n = 21) and 72% (n = 18), respectively. This shows positive antifeedant behavior toward both treatments. In *Camponotus* species, 68% (n = 17) and 72% (n = 18) showed antifeedant behavior toward DCM extract and methanol extract. However, hexane extract evoked only 28% (n = 7), and 24% (n = 6) antifeedant behavior from *E. indica* and both ant species, respectively. *E. indica* exhibited 80% (n = 20) response of avoidance toward DCM and MeOH extract equally. The antifeedant properties were equally exhibited by isolated extract eluted with mixtures of DCM and MeOH at 95:5, 98:2, and 90:10 (χ^2 ; $p < 0.05$). Two spots achieved from isolation of DCM extract in mixtures of 98:2 DCM:MeOH by using thin layer chromatography analysis shows the strong antifeedant properties. Further studies are required to identify the active compounds that give *C. asiatica* its antifeedant properties.

Alternative Splicing of a Flight Muscle Gene Reflects the Flight Phenotypic Response to Body Weight and Nutrition

Fescemyer HW and Marden JH

Center for Chemical Ecology, Department of Entomology, The Pennsylvania State University, University Park, PA

Corresponding author: hif1@psu.edu

Alternative splicing is a prominent feature of gene expression that creates distinct transcripts from single genes, thereby amplifying functional diversity. The force and power of insect and vertebrate muscles is affected by alternative splicing of the muscle contractile gene *troponin-t* (*tnt*). Powerful muscles improve flight ability and are necessary to counteract body weight, but excessive force and power consume energy at an unnecessarily high rate. Alternative splicing of *tnt* may be a mechanism by which some animals, such as moths, modulate flight muscle power in relation to organism-level or environmental variables influencing the development of flight phenotypic variation among individuals. Therefore, we tested the hypothesis that alternative splicing of *tnt* responds in a quantitative fashion to adult body weight and larval nutrition. Restricting larval diet strongly affected adult body weight, relative abundance of *tnt* isoforms in flight muscle, and peak metabolic rate (PMR) during flight of *Spodoptera frugiperda* moths. Adult body weight (i.e., mass) was strongly correlated with the composition of *tnt* isoforms and PMR. By uncoupling the influence of diet restriction and body weight, we showed that moths use alternative splicing to adjust their flight muscles to changes in weight that occur during adult life. Moths probably possess mechanisms to sense their weight and nutritional state. Alternative splicing of *tnt* appears to allow muscle performance to be adjusted according to energy availability and need to counteract gravity in order to fly. Differences in *tnt* isoform composition and PMR also were observed in *Helicoverpa zea* moths from larvae that ate field corn, sweet corn, or field corn expressing Bt-toxin. Field and sweet corn differ in carbohydrate abundance and, thereby provide another level of variation in nutrition. The Bt-toxin in corn acts on digestive cells in larvae, thereby decreasing feeding efficiency and nutrient acquisition. Body weight and corn variety together explained 95% of the variation in *tnt* isoform composition. Moths from larvae that ate Bt-corn were smallest and had *tnt* isoform composition and PMRs associated with reduced flight muscle performance. Moths from larvae that ate conventional field and sweet corn had PMRs significantly higher than the Bt-corn treatment, and sweet corn moths had a *tnt* isoform composition that suggested they had the most powerful flight muscles. These results indicate that muscles respond via alternative splicing at the pre-mRNA level to whole-organism variables such as weight and nutrition. At a given body weight, insects in a poorer nutritional condition have a molecular composition indicative of weaker muscles. Similar adjustments may occur in vertebrate muscles, but these types of questions have not yet been addressed in other animals. Alternative splicing responds in a precise way to organism-level traits, is an epigenetic mechanism carrying effects of past nutritional history, and it is a central component of phenotypic plasticity and life history variation.

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Short-Term Resource Allocation in *Nicotiana tabacum* in Response to Herbivory

Gómez S, Ferrieri RA, Schueller M, and Orians CM

Biology Department, Tufts University, Medford, MA (SG, CMO); Medical Department, Brookhaven National Laboratory, Upton, NY (MS, RAF)

Corresponding author: sara.gomez@tufts.edu

As sessile organisms, plants have evolved a wide array of defense mechanisms to reduce the impact or withstand enemy attacks. Upon damage, herbivores are confronted by the production of chemical compounds or physical structures by their host. This may reduce herbivore preference or performance through reduced tissue digestibility, increased tissue toxicity, or both. However, plants can use an alternative, not mutually exclusive, strategy in response to damage. Through so-called tolerance mechanisms, plants can undergo changes in their primary metabolism allowing them to fine-tune allocation of new or existing resources and direct them to plant organs where they are less accessible for attackers. One of the main aims of this project is to investigate short-term resource allocation in tobacco and tomato in response to generalist and specialist herbivores. We make use of short-lived radiotracers (^{14}C and ^{15}N) to study the distribution *in planta* of newly incorporated $^{14}\text{CO}_2$ and $^{15}\text{NH}_3$, avoiding the use of intrusive techniques. Preliminary results applying *Manduca sexta* regurgitant on tobacco plants indicate differential whole-plant allocation of carbon and nitrogen. In addition, the comparison of these results on resource allocation with a companion methyl jasmonate study, suggests that different elicitors yield very different responses in terms of short-term resource dynamics. The study of short-term dynamics of whole-plant resource allocation provides novel insight that will allow for a better understanding of defense and tolerance mechanisms.

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Response of the Invasive Emerald Ash Borer, *Agrilus planipennis* (Coleoptera: Buprestidae), to Foliage Volatiles of Its North American Hosts

Grant GG, de Groot P, Poland TM, Buchan L, Abou-Zaid MM, and Pitt D

Canadian Forest Service, Sault Ste. Marie, ON, Canada P6A 2E5 (GGG, PdeG, LB, MMA-Z, DP); USDA Forest Service, East Lansing MI (TMP)

Corresponding author: ggrant@nrcan.gc.ca

Adult borers feed on ash (*Fraxinus* spp.) foliage. In situ sampling of foliage volatiles followed by gas chromatography-electroantennographic detection (EAD) bioassays, revealed that up to eight green leaf volatiles (GLVs) and several sesquiterpenes were consistent EAD-active components, with the unsaturated GLV alcohols and aldehydes producing the largest responses. Comparisons were made with the native bronze birch borer, *Agrilus anxius*, and foliage volatiles from birch (*Betula* spp.). Because GLVs serve as host attractants for other coleopteran folivores, we conducted field tests to evaluate the attractiveness of GLV lure blends and dosages for the emerald ash borer.

Interactions among *Lygus lineolaris* and Its Host Plants: A Study of Volatile Induction and Implications for Biological Control

Halloran ST and Tumlinson JH

Center for Chemical Ecology, Department of Entomology, The Pennsylvania State University, University Park, PA

Corresponding author: sth150@psu.edu

The tarnished plant bug, *Lygus lineolaris*, is an important generalist hemipteran pest in a number of fruit and agricultural systems. Generalist and hemipteran insects are both understudied in terms of their chemical ecology, and a greater understanding of *L. lineolaris* would benefit biological control efforts and contribute to basic knowledge of plant–insect interactions. Preliminary work described here demonstrates that *L. lineolaris* induces host plants to release specific blends of compounds that are unique relative to undamaged and mechanically damaged plants. This was observed in both weedy hosts (*Melilotus officinalis* and *Erigeron annuus*) as well as cultivated hosts (*Medicago sativa*) and suggests that compounds in the salivary secretions of this piercing–sucking insect elicit the observed volatile response. Previous work in the area of *L. lineolaris* biological control has demonstrated that the success of parasitoid natural enemies varies depending on the weed and crop community. This is potentially due to variation in the ability of natural enemies to locate hosts through volatile cues elicited from their food plants. If certain plants more easily attract natural enemies when elicited by *L. lineolaris* feeding, then edge habitats can be manipulated to enhance the success of biological controls. Future work will examine the attractiveness of *Lygus*-induced volatile profiles to native and introduced *L. lineolaris* parasitoids with this goal in mind.





Reinvestigation of Female Sex Pheromone of the Legume Pod Borer, *Maruca vitrata* (Lepidoptera: Crambidae)

Hassan MN, Hall DR, and Downham MCA

Natural Resources Institute, the University of Greenwich, United Kingdom

Corresponding author: nayem@russellipm.net

The legume podborer, *Maruca vitrata* (Lepidoptera: Pyralidae), is a serious pest of legumes, particularly, cowpea, pigeon pea, black gram, green gram, beans, and soybean throughout Asia and Africa. Losses have been reported at 20–80% in different parts of Africa (Singh and Allen 1980) (1), with yield loss of US\$30 million worldwide. Control of *M. vitrata* mainly relies on use of chemical insecticides some of them are tolerance effective but simultaneously killed natural predators and parasitoids. Recently, *M. vitrata* has acquired reduced susceptibility to insecticides that have been previously effective (2). Adult male *M. vitrata* were previously shown to be attracted to traps baited with live virgin females, which suggested the females produce a sex pheromone. Adati and Tatsuki (3) identified (*E,E*)-10,12,16:Ald is the major component of the female sex pheromone of legume pod bore moth. Later, Downham et al. (4) reported a 3-component blend of (*E,E*)-10,12-hexadecadienal with (*E,E*)-10,2-16 hexadecadienol, (*E*)-10-hexadecadienal in a ratio of 100:5:5. They developed a lure that attracts male moths in Benin (West Africa) but failed to produce a blend of synthetic compounds that attracts male moths in laboratory, or a lure that attracts male moth in Asia. We found that volatiles produced by virgin female *M. vitrata* contained an additional component that elicited EAG responses from males in analyses by linked GC-EAG, E10-16:OH, which was identified by comparison of GC retention times and mass spectra with those of synthetic standards. Ovipositor tip extract of virgin female was also showed a new EAG-active compound that was also identified as unsaturated hydrocarbon triene ZZZ3,6,9-23:H by comparison of GC retention times and mass spectra with those of synthetic standards. To date two new pheromone components has been detected and identified. Using these, a synthetic lure has been developed that is as attractive to male *M. vitrata* moths as the natural pheromone in a wind tunnel for the first time. Field trials were carried out using various traps baited with all possible combination of newly identified unsaturated alcohol E10-16:OH with previously identified compounds by using different combinations of dispensing systems. Catches of male *M. vitrata* were significantly higher than those of unbaited traps. Significantly higher male were caught on traps baited with EE1012:16-Ald and E10:16-10 in ration 90:10 than baited (*E,E*)-10,12-hexadecadienal with (*E,E*)-10,2-16 hexadecadienol and (*E*)-10-hexadecadienal in a ratio of 100:5:5. The newly identified unsaturated hydrocarbon in combinations with other pheromone components is being tested in field now. We found that the new candidate pheromone compound would able to increase trap catch in the field worldwide. The role of the two newly identified pheromone compounds and possible reasons for the increase of trap catch to males are discussed.

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Identification of Novel Volicitin-Related Compounds from *Manduca sexta* and Their Elicitor Activity

Ishikawa C, Yoshinaga N, Aboshi T, Lait CG, Ono H, Nishida R, Tumlinson JH, and Mori N

Division of Applied Life Sciences, Graduate School of Agriculture, Kyoto University, Kyoto 606-8502, Japan; Center of Medical, Agricultural, and Veterinary Entomology, Agricultural Research Service, U.S. Department of Agriculture, Gainesville, FL 32608; Center for Chemical Ecology, Department of Entomology, Pennsylvania State University, University Park, PA 16802

Corresponding author: c0727@kais.kyoto-u.ac.jp (Ishikawa)

Plants damaged by caterpillar feeding release a blend of volatiles that guide parasitoids, natural enemies of caterpillars, to locate their host. Since the discovery of volicitin [*N*-(17-hydroxylinolenoyl)-L-glutamine] in the oral secretions of larval *Spodoptera exigua* (Noctuidae), a series of related fatty acid amides (FACs) have been identified in numerous lepidopteran species as well as in crickets, larval fruit flies, and in katydids. In this study, we report previously unknown volicitin-related compounds from the oral secretions of *Manduca sexta* (Sphingidae) and compare their elicitor activity on corn (*Zea mays*) plants. Previously, *N*-linolenoyl-L-glutamine as well as *N*-linolenoyl-L-glutamic acid were found in the oral secretions of *M. sexta* and identified as elicitor of volatiles in corn plants. By liquid chromatography quadrupole ion trap time of flight mass spectrometry analyses, we found additional hydroxylated FACs in the secretions. These novel compounds, that were isolated and identified by the help of ¹H NMR and gas chromatography-mass spectrometry after methanolysis, were shown to be composed of 18-hydroxylated linolenic acid. A bioassay of elicitor activity on *Z. mays* showed that *N*-(18-hydroxylinolenoyl)-L-glutamine was approximately 40% as active as volicitin and *N*-(18-hydroxylinolenoyl)-L-glutamic acid was approximately 20% as active as volicitin. These results show not only that *M. sexta* regurgitant contains volicitin-like hydroxylated FACs but also that the metabolic mechanisms resulting in the hydroxylation seems to be fairly specific. Furthermore, just a one carbon shift of the hydroxylation had an remarkable effect on the perception of these compounds by the plants.





Wing Glands Secretion of the Bee Moth *Aphomia sociella*

Jiros P, Kalinova B, Kindl J, Zacek P, Cvacka J, Vasickova S, and Valterová I

UOCHB AV CR vvi, Flemingovo nam. 2. Praha 6, 166 10, Czech Republic

Corresponding author: jiros@uochb.cas.cz

Aphomia sociella (Lepidoptera, Pyralidae, Galleriinae) is a wild parasite of social bees, wasps, and bumblebees. The larvae attack the nests, eat and destroy not only the brood comb but also the offspring of the host. As in other members of subfamily Galleriinae, mating behavior in *A. sociella* is mediated by sex pheromone produced in male-specific wing glands. The pheromone is released by males during calling behavior and it induces attraction of female moths. Previously, the composition of wing-gland secretion has been identified as a mixture of mellein and (2Z,6Z)-2,6-nonadien-4-olide in the approximate ratio 95:1 (1). Although wing-gland extracts was attractive to females by inducing walking, no convincing evidence was provided with respect to synthetic compounds. In a search for pheromone-based control and/or a monitoring strategy of *A. sociella*, we extracted wing glands by hexane and analyzed the extract composition using gas chromatographic-electroantennographic detection (GC-EAD), GC-GC-mass spectrometry (MS), and GC-infrared spectrometry. The analyses revealed seven antennally active compounds: 1-hexanol, 2-phenylethanol, (2Z,6Z)-2,6-nonadien-4-olide, unknown lactone, mellein, phytone, and an inseparable mixture of C18 fatty acids (with zero, one, two, and three double bonds). Mellein and nonadienolide were the most abundant compounds present in approximately 4:1 ratio. The absolute configuration of (2Z,6Z)-2,6-nonadien-4-olide was determined as (*R*) by means of enantioselective GC separation. Interestingly, the antennal sensitivity to (*R*)-enantiomer was higher than to the (*S*)-enantiomer. Our results show that male wing-gland pheromone has a complex structure. Solid phase microextraction (SPME) technique was used to determine which of the identified compounds are likely used for communication between sexes. SPME signals analyzed using GC-EAD and GC-GC-MS confirmed all compounds as being airborne and thus potential pheromone components. Our results provide a reliable background for a prospective development of pheromone-based monitoring or control method for *A. sociella*.

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Fitness of the Viral Resistance (VR) Transgene (t) during Introgression

Kariyat RR, Guirand AS, Verbano JD, Wall KL, Sasuclark MM, and Stephenson AG

Department of Biology, The Pennsylvania State University, University Park, PA

Corresponding author: ruk157@psu.edu

In the mid-1990s, the USDA deregulated the growth of transgenic squash containing a DNA construct that was engineered to express the viral coat protein (CP) genes of WMV-2, ZYMV, and CMV. When these genes are expressed in squash cells they undergo posttranscriptional silencing (RNAi) that protects the plants from the intact viruses. In this cultivar, the virus resistant (VR) transgene is hemizygous, and, importantly, the *NPTII* gene conferring resistance to neomycin (a selective marker to identify *Agrobacterium* that have picked up the transgene during transformation) has not been deactivated and is still tightly linked (flanked on each side) by the CP genes of the three viruses. Consequently, we have been able to introgress the transgene (CP genes and *NPTII*) into wild gourds (*C. pepo* ssp. *texana*) because the presence of the transgene in hybrid progeny can be detected using a DAS-ELISA kit for detecting NPTII protein. To examine the fitness of the transgene during the initial stages of introgression into the wild gourd, we grew 9 outbred (X), 9 inbred (S) wild gourds, 3 F1 (transgenic), 3 F1, 3 BC1 (t), 3 BC1, 3BC2 (t), and 3 BC2 from each of 5 families in each of two fields in 2006 and 2007 (180 plants/field; 45 X, 45 S, 45 transgenic introgressives, and 45 non-t introgressives). In both years, the first plants to show symptoms of viral disease (WMV-2 and ZYMV in 2006; ZYMV only in 2007) occurred in mid- to late July and spread through the nontransgenic plants. We found that the transgenic plants produced significantly more male flowers in both years and more mature fruit in 2006 than the either the nontransgenic introgressives or the wild gourds. In each year, we also harvested two mature fruit from each of the nontransgenic plants (S, X, Introgressives), germinated a random sample of 480 seeds per field, and scored for the presence of NPTII. If fertilization is random we expected that 12.5% of the seeds would be NPTII positive (because 25% of the plants in each field were hemizygous for the transgene). In both years significantly more of the seeds were NPTII positive than expected by chance alone (26.7, 26.4%) and the difference cannot be entirely explained by the increase in male flower production alone, suggesting that the viral free transgenic plants may attract more pollinators, produce more pollen per male flower, or produce competitively superior pollen.



Herbivore-Induced Emissions from *Pinus tobulaeformis* Cones and Host Selection by *Dioryctria pryeri*

Li XG and Yang LJ

College of Forestry, Northwest A & F University, Yangling, Shaanxi 712100, P. R. China

Corresponding author: xingangle@nwsuaf.edu.cn

Pine coneworm, *Dioryctria pryeri* Ragonot (Lepidoptera: Pyralidae), is an important insect pest of coniferous cones and shoots in China. In Chinese pine (*Pinus tobulaeformis* Carr.) orchards of northern China, the coneworm, in association with *Gravitar-mata margarotana* (Heinemann) (Lepidoptera: Tortricidae), seriously damages cones of Chinese pine and causes more than 60% of the seed losses. Experiments showed that larvae and female moths selected cones previously infested by *G. maigarotana* for feeding and oviposition, respectively. This phenomenon is known as induced susceptibility (1) or herbivore-induced negative defense (2). We used headspace collections and gas chromatography-mass spectrometry to study the composition and quantity of terpene volatiles from cones infested by *G. maigarotana* larvae throughout the larval feeding and moth oviposition period of *D. pryeri*. Cones emitted specific blends of 1*R*(+)- α -pinene, β -myrcene, and D-(+)-limonene. Electrophysiological and behavioral bioassays indicated that (+)- α -pinene, β -myrcene, and D-(+)-limonene acted as adult host attractants. Our experimental results further show that polyphenol oxidase and protein inhibitor activities, and resin acid content, increased, whereas water content decreased after the cone was damaged by *G. maigarotana* larvae. These results demonstrate that the enzyme activities of resistance in the infested cone increased and the nutritive quality of the cone decreased. Therefore, our question is how do pine coneworm larvae overcome this induced resistance? Maybe this is a key example of herbivore-induced negative defenses.

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Olfaction: A Neglected Sense in the Praying Mantis

Mallory HS and Weiss MR

Department of Biology, Georgetown University, Washington, DC

Corresponding author: hsm3@georgetown.edu

As sit-and-wait predators, praying mantids spend most of their time immobile, yet move to a new hunting site in response to hunger when local prey abundances decrease. The cues that mantids use to select a foraging site are poorly understood; both visual and olfactory information may be important. Vision in mantids has been well studied, perhaps due to the prominence of their large, compound eyes. Olfaction, in contrast, has received relatively little attention, despite that mantids have long, filamentous antennae that they orient toward prey. Indeed, it would be surprising if mantids did not use olfaction when foraging, because they are sister taxa to the cockroaches, which rely heavily on chemical cues. In addition, many arthropod predators, including wasps and crab spiders, exploit volatile cues to locate prey habitats. Although male mantids are known to depend on pheromones for mate location, to our knowledge no one has explored the use of olfaction by mantids in location of prey or prey habitat. Using electroantennograms (EAGs), we show that a mantis antenna can detect headspace volatiles collected from a variety of flowers and grasses. We use EAGs coupled with gas chromatography-mass spectrophotometry on two mantis species to develop a clearer picture of the range of volatiles (including herbivore-induced plant volatiles and floral volatiles) that a mantis antenna can detect. We also carried out behavioral experiments to determine the extent to which volatiles are used by mantids to locate foraging sites. The combination of physiological data with behavioral experiments may yield insight into the foraging behavior of these important sit-and-wait predators.





Virus-Induced Changes in Host Chemistry: Do Plant Viruses Manipulate Insect Vectors through a Shared Host?

Mauck KM, Mescher M, and De Moraes C

Center for Chemical Ecology, Department of Entomology, The Pennsylvania State University, University Park, PA

Corresponding author: kem315@psu.edu

Improved understanding of the complex interactions among plants, pathogens, and insect vectors should inform the development of effective and sustainable disease management strategies, which often focus on manipulating vector populations to limit opportunities for pathogen transmission. The impact of infection on plant quality and attractiveness to insect vectors is a key issue, and the dependence of pathogens and herbivores on a common host plant creates the potential for both mutualistic and antagonistic interactions. We explored these interactions by using the ubiquitous, nonpersistently transmitted pathogen, *Cucumber mosaic virus* (CMV) and a cultivated host, *Cucurbita pepo* cv. 'Dixie', documenting the effects of CMV on host plant quality and production of volatile chemical cues in both field and greenhouse environments. We further examined interactions between CMV-infected host plants and two common vectors of CMV (the aphids *Myzus persicae* and *Aphis gossypii*) through experiments that assessed aphid population growth, plant preferences, and behavioral responses to plant odors and surfaces. Analyses of plant volatile indicate that infected plants release more total volatiles per unit of plant tissue than healthy plants. Additionally, the composition of infected plant blends is similar to that of healthy plants in the absence of heat stress. These results suggest that, infected plants likely have similar odor-based apparency to insect vectors relative to healthy plants under normal field conditions. Growth experiments with aphid vectors found that aphid populations are lower on infected leaf tissue, and field observations revealed that aphids prefer to arrest and establish on healthy rather than infected *C. pepo*. Overall, our findings suggest that CMV alters host chemistry in ways that affect both the attraction and arrestment of vectors. This manipulation of the host seems to be beneficial for the virus, favoring transmission by vectors that locate infected plants and acquire CMV, but rapidly disperse to higher quality (uninfected) hosts.

Chemical Characterization of 4,8-Tetradecadien-1-yl Acetates and Corresponding Alcohols and Their Use in Chemical Communication by Moths of the Genus *Phyllonorycter*

Mozūraitis R, Liblikas I, Noreika R, and Borg-Karlson A-K

Ecological Chemistry Group, Division of Organic Chemistry, Department of Chemistry, School of Chemical Science and Engineering, Royal Institute of Technology, SE-10044 Stockholm, Sweden (RM, IL, A-KB-K); Laboratory of Chemical and Behavioural Ecology, Institute of Ecology, Vilnius University, Akademijos 2, LT-2600 Vilnius, Lithuania (RM); Department of Botany, Faculty of Natural Sciences, Vilnius Pedagogical University, LT-08106 Vilnius, Lithuania (RN)

Corresponding author: raimis@kth.se

Altogether, 688 *Phyllonorycter* species are known throughout the world (1); so, high chemodiversity of sex pheromone components is expected. A few years ago, molecular phylogeny of 77 European *Phyllonorycter* species was published (2), which provided a good opportunity to compare the chemical pattern of compounds used for search of a mate with the phylogeny of the genus. However, data concerning sex attractants of *Phyllonorycter* are too scattered to be mapped onto the moth phylogeny, and more efforts searching for new attractants including structurally new compounds are needed. GC-MS analysis of two compounds released during calling periods of virgin *Phyllonorycter cerasicolella* females indicated presence of 4,8-tetradecadien-1-yl acetate and alcohol previously unknown as sex pheromones in Lepidoptera. Synthetic products were characterized by gas chromatography, carbon and hydrogen nuclear magnetic resonance as well as mass spectroscopy methods. All four isomers gave distinctive mass spectra where m/z 81 fragments clearly dominated. Principal component analysis revealed that all isomers of 4,8-tetradecadien-1-ols and their acetates could be identified basing on ratios of diagnostic mass ions. Elution order of 4,8-tetradecadien-1-ols followed by retention index presented in parentheses was determined as (Z,Z)- (2082.1), (Z,E)- (2082.8), (E,E)- (2083.1), and (E,Z)- (2083.2) from unpolar SPB-1 column and as (E,E)- (2210.2), (Z,E)- (2222.1), (E,Z)- (2223.4), and (Z,Z)- (2224.7) from polar DB-WAX column. The isomers of 4,8-tetradecadien-1-yl acetates eluted in the order of (Z,Z)- (2176.1), (Z,E)- (2178.4), (E,Z)- (2185.9), and (E,E)- (2186.4) from SPB-1 and (Z,E)- (2124.3), (E,E)- (2157.7), (Z,Z)- (2128.9), and (E,Z)- (2135.9) from DB-WAX columns. Field screening tests for attractiveness of 4,8-tetradecadien-1-yl acetates showed that (4Z,8E)-tetradeca-4,8-dien-1-yl acetate significantly attracted *Phyllonorycter coryli* and *Chrysoesthia drurella* males. (4E,8E)-Tetradeca-4,8-dien-1-yl acetate was the most efficient attractant for *Ph. esperella* and *Ph. saportella* males. (4E,8Z)-Tetradeca-4,8-dien-1-yl acetate was attractive to *Ph. cerasicolella* males, and it has to be considered as a sex pheromone component for leaf-mining moth of this species.

1. Global Taxonomic Database of Gracillariidae, <http://gc.bebif.be>.

2. Evolution 57:1804–1821.



Fall Armyworm, *Spodoptera frugiperda* (J.E. Smith) (Lep., Noctuidae), Oviposition Induces Volatile Emission in Maize That Attracts the Egg Parasitoid *Telenomus remus* Nixon (Hym., Scelionidae)

Peñaflor MFGV and Bento JMS

Insect Behavior Laboratory, Departamento de Entomologia, Fitopatologia e Zoologia Agrícola, Escola Superior de Agricultura "Luiz de Queiroz", Universidade de São Paulo, ESALQ/USP, Piracicaba, São Paulo, Brazil

Corresponding author: penaflor@esalq.usp.br

Attraction of natural enemies to plant volatiles induced by herbivory has been intensely studied over the past years. By contrast, little is known about the parasitoid's response to plant volatiles released after the herbivore's oviposition (1). This research aimed to study the olfactory response of the specialist egg-parasitoid *Telenomus remus* Nixon to volatiles from plants induced by *Spodoptera frugiperda* oviposition at intervals of 10–14, 18–22 and 34–38 h. The effect of maize volatiles and egg kairomones on parasitoid searching was investigated in a Y-tube olfactometer. Naïve 3-day-old females were tested singly, and each replicate lasted a maximum period of 10 min. The airflow was adjusted to 200 ml/min. Bioassays consisted of 20 replicates, and olfactory stimuli derived from the following treatments were tested: 1) undamaged plant, 2) egg-induced plants, and 3) egg masses at 10–14, 18–22, and 34–38 h. Odors emitted by host egg masses at different ages (according to the intervals of induction) also were tested to verify whether the stimuli derived from the egg-induced plants were due to residues of kairomones from eggs, scales, or both. To obtain induced maize, plants were placed in cages with four 4-day-old females to oviposit on them for a period of 2 h after scotophase. Only plants with two to three egg masses were considered, and they were removed minutes before starting the bioassay. Choices between test and control arm were analyzed using chi-square test. The results showed that the odors derived from the induced plants attracted the parasitoid at the intervals 18–22 and 34–38 h, at 5 and 1% of significance, respectively. In contrast, the egg masses only attracted the parasitoid at 18–22 h, which indicate that the parasitoid's response to the induced plant at 34–38 h is definitely not due to kairomonal effect of residues from host eggs and scales, but rather to the release of egg induced volatiles. However, we cannot affirm that induced plants at 18–22 h release attractive volatiles to *T. remus*. Further studies will focus on the chemical analysis of egg-induced volatiles released by maize.

1. Entomol Exp Appl (2002) 104:181–192.

Aphid-Induced Aphid Susceptibility in *Nicotiana benthamiana*

Ramsey JS and Jander GJ

Boyce Thompson Institute for Plant Research, Tower Road, Ithaca, NY (JR, GJ); Department of Plant Biology, Cornell University, Ithaca, NY (JR, GJ)

Corresponding author: jsr47@cornell.edu

Although aphid feeding can result in the activation of plant defense pathways, enhanced performance of aphids (*Myzus persicae* and *Sipha flava*) and caterpillars (*Spodoptera exigua*) has been reported on plants previously infested with aphids. This aphid-induced plant susceptibility may be due to nutritional changes resulting from aphid manipulation of plant source–sink relationships. We compared the fecundity of single *M. persicae* adults caged on naïve and previously infested *Nicotiana benthamiana* leaves, and we found that adult aphids of tobacco-adapted (red) and nonadapted (green) lineages experience greater survivorship and fecundity on previously infested leaves. The induced susceptibility is a local effect; aphid fecundity is significantly greater on previously infested leaves compared with systemic leaves of the same plant. The relative significance of induction of plant secondary metabolites and changes in leaf nutritional value triggered by aphid feeding is considered.

Inhibiting *Ips typographus* Attack on Norway Spruce Stand Edges by Nonhost Volatiles

Schiebe C, Blaženc M, Jakuš R, Unelius CR, and Schlyter F

Swedish University of Agricultural Sciences, Chemical Ecology, Alnarp, Sweden (CS, FS); Institute of Forest Ecology of the Slovak Academy Sciences, Zvolen, Slovakia (MB, RJ); University of Kalmar, School of Pure and Applied Natural Sciences, Biology, Kalmar, Sweden (CRU)

Corresponding author: christian.schiebe@hik.se

Until now, no methods were developed to actively protect Norway spruce (*Picea abies*) forests against mass attacks of the aggressive bark beetle *Ips typographus*. The well-known negative response of *I. typographus* to nonhost volatiles (NHV) from angiosperm trees may be a signal serving in habitat selection, and these volatiles could be used to deter flying beetles from detecting vulnerable host trees. Experiments to protect stand edges with NHV were performed in 2006 and 2007 in the Tatra Mountains in Slovakia and in 2007 in southeastern Sweden. The results in Slovakia support previous results from the Czech Republic in 2001 (1). A statistically significant reduction of attacked trees could be reported in groups of trees in stand edges that were treated with IT-Rep dispensers (Fytofarm, Slovakia) containing verbenone and NHV. The Swedish data indicate an active inhibiting range exceeding the previous assumed range, thus supporting the hypothesis of NHV serving as a signal in habitat selection for *I. typographus* in flight. No attacks in experimental areas were recorded, only in adjacent areas.

1. J Pest Sci (2003) 76:89–99.

Impacts of Pathogen-Induced Plant Volatiles on the Behavior of Insect Vectors

Shapiro LR, Stephenson AG, De Moraes CM, and Mescher MC

Center for Chemical Ecology, Department of Entomology, The Pennsylvania State University, University Park, PA (LRS, CDM, MCM); Center for Chemical Ecology, Department of Biology, The Pennsylvania State University, University Park, PA (AGS)

Corresponding author: lrs200@psu.edu

Plant volatiles cues are known to mediate interactions among plants, insect herbivores, and herbivore natural enemies, but only recently have investigators begun to examine how pathogen-induced volatiles influence the behavior of insect disease vectors and thus mediate disease transmission. We examined volatile induction in the wild gourd *Cucurbita pepo* ssp. *texana*, native progenitor of the cultivated squashes, in response to infections by *Erwinia tracheiphila*, the causal agent of bacterial wilt disease, and zucchini yellow mosaic virus (ZYMV), and we documented distinct volatile signatures for each pathogen. Plants infected by both pathogens emitted a volatile profile similar to that of plants infected by ZYMV alone, a potentially significant finding as previous studies have shown that viral infection reduces subsequent rates of infection by *E. tracheiphila*. We are currently exploring the effects of pathogen-induced volatile profiles on the behavior of the cucumber beetle, *Acalymma vittatum*, one of only two confirmed vectors of *E. tracheiphila*, and on several generalist aphid species that vector ZYMV in a nonpersistent manner. We are also exploring the effects of the NPTIII transgene, which confers viral resistance and thus may increase susceptibility to *E. tracheiphila*, on plant–pathogen–vector interactions. There is significant gene flow between wild and cultivated cucurbit taxa and introgression of the NPTIII transgene from cultivated squashes into wild populations has potentially significant implications for disease dynamics in natural systems.



Phenology and Population Radiation of the Nettle Caterpillar, *Darna pallivitta* (Moore), in Hawai'i**Siderhurst MS, Jang EB, Conant P, and Siderhurst LA**

Department of Chemistry, Eastern Mennonite University, Harrisonburg, VA (MSS); USDA–APHIS–PBARC, Hilo, HI (EBJ); Hawaii Department of Agriculture, Hilo, HI (PC), Department of Biology, James Madison University, Harrisonburg, VA (LAS)

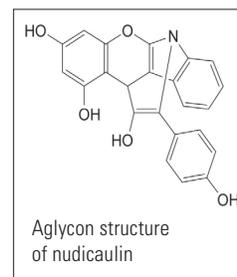
Corresponding author: ms826@emu.edu

The nettle caterpillar, *Darna pallivitta* (Moore) (Lepidoptera: Limacodidae), is an invasive pest with established populations on three Hawai'iian islands. Indigenous to Southeast Asia, *D. pallivitta* caterpillars cause defoliation of ornamental nursery stock and poses a human health hazard due to their urticating hairs that can cause painful stings. Identification of the pheromone component n-butyl (*E*)-7,9-decadienoate from *D. pallivitta* has made it possible to investigate the phenology or population dynamics by using this attractive lure. Male captures in Jackson traps baited with the synthetic lure showed a vegetation preference for tall-grass fields and forest/grass interfaces over forest. Microlocation preferences were also found for trap height, with >65% of males being caught in traps suspended at 1 m, compared with the traps at 3 and 5 m. Captures of male moths in traps baited with live females, and direct observations of female calling behavior, showed peak activities 6–7 h after the onset of scotophase. This is a much later communication period than for *D. bradleyi* and *D. trima* and may provide a mechanism by which *D. pallivitta* maintains reproductive isolation in areas where all three species are present. Mountain and coastal transects established in eastern Hawai'i measured aspects of population fluctuations and radiation into new areas with relation to elevation and microclimate. Comparing the 80 and 90% population boundaries of the moth populations along these transects showed significant differences in population expansion. The coastal transect population radiated at least twice a fast as the mountain population. Both the behavioral and ecological data collected can be used to optimize deployment of detection/control strategies and to predict population-expansion/risk-assessment for establishing quarantine protocols for the nettle caterpillar.

Effect of UV-B Radiation on the Phytochemical Pattern in Petals of *Papaver nudicaule* Flowers**Tatsis EC and Schneider B**

Max Planck Institute for Chemical Ecology, Jena, Germany

Corresponding author: etatsis@ice.mpg.de



Iceland poppy (*Papaver nudicaule*) is a plant that is native to Northern Arctic landscapes and high altitudes (alpine landscapes). Garden varieties of *P. nudicaule* show red, orange, dark and pale yellow and white flowers. Red-coloured petals are due to the presence of glycosides of pelargonidin (1), and pale yellow petals of the wild form are attributed mainly to the occurrence of nudicaulins (2). The nudicaulins (see structure) contain an indolic substructure, and the nonindolic part of the molecule seems to be derived the phenylpropanoid-polyketide route. The

similarity of the nonindolic nudicaulin substructure to flavonoids that are present also in yellow flower petals (2) evokes the hypothesis that both nudicaulins and flavonols are present in *P. nudicaule* petals may be formed from a common C₆-C₃-C₆ precursor, as naringenin chalcone. Generally, alkaloids are generally accepted as protective substances discouraging animal or insect attacks or playing diverse roles in ecological interaction between different organisms. Nudicaulin accumulation in petals seems to enhance the attractiveness of flowers to pollinators and therefore can be considered as a fitness trait. Epidermal located phenolic compounds, such as flavonoids, are well known for protecting plants against harmful UV-B radiation (3). Hence, the occurrence of nudicaulins, which are structurally related to flavonoids, in petals of the Icelandic poppy raises the question of whether nudicaulins are involved in protection against harmful solar radiation. The investigation of the potential influence of UV radiation on the chemical composition of flowers of different coloured petal varieties will be important to determine the ecological role of nudicaulins as UV screening compounds. In the present work, the influence of UV-B radiation on chemical composition of different coloured varieties of *P. nudicaule* is studied in nature and under controlled growth chamber conditions. The qualitative and quantitative differences of flower pigments are examined of the different coloured flower garden varieties, which were grown with or without supplemental UV-B radiation. The metabolites are identified using HPLC-DAD, LC-MS, and NMR data and quantitatively analyzed by the use of HPLC-DAD.

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1. Phytochemistry (1981) 20:1461–1462.
2. Phytochemistry (2006) 67:191–201.
3. Planta (2004) 218:721–728.

Inducing Tomato Defense by *Helicoverpa zea*: Role of Glandular Trichomes

Tian D and Felton G

Center for Chemical Ecology, Department of Entomology, Penn State University,
University Park, PA 16801

Corresponding author: dut3@psu.edu

Trichomes, both glandular and nonglandular, are hair-like structures that extend from the epidermis of aerial tissues; they can serve several functions, including protection against damage from herbivores. Although most plants produce trichomes constitutively, some species respond to damage by increasing trichome density in new leaves. We tested the effect of saliva on the induction of trichomes by surgically manipulating caterpillars to affect their ability to secrete saliva. We also tested the effects of trichome density on the growth and survival of early instars of *H. zea*. Quantitative real-time PCR was used to test a series of defensive proteins, e.g., proteinase inhibitors (pin1), arginase, allene oxide cyclase, and lipoxygenase (Lox), in the trichome.

Retention of Olfactory Memory through Metamorphosis: Can a Moth Remember What It Smelled as a Caterpillar?

Blackiston DJ, Casey ES, and Weiss MR

Department of Biology, Georgetown University, Washington, DC (DJB, ESC, MRW); The
Forsyth Institute, Boston, MA (DJB)

Corresponding author: weissm@georgetown.edu

Insects that undergo complete metamorphosis experience enormous changes in both morphology and lifestyle. The current study examines whether larval experience can persist through pupation into adulthood in Lepidoptera, and assesses two possible mechanisms that could underlie such behavior: exposure of emerging adults to chemicals from the larval environment, or associative learning transferred to adulthood via maintenance of intact synaptic connections. Fifth-instar *Manduca sexta* caterpillars received an electrical shock associatively paired with the odor of ethyl acetate to create a conditioned odor aversion, and were assayed for learning in a Y-choice apparatus as larvae and again as adult moths. We show that larvae learned to avoid the training odor, and that this aversion was still present in the adults. The adult aversion did not result from carryover of chemicals from the larval environment, as neither application of odorants to naïve pupae nor washing the pupae of trained caterpillars resulted in a change in behavior. In addition, we report that larvae trained as third instars still showed odor aversion after two molts, as fifth instars, but did not avoid the odor as adults, consistent with the idea that postmetamorphic recall involves regions of the brain that are not produced until later in larval development. The present study, the first to demonstrate conclusively that associative memory survives metamorphosis in Lepidoptera, provokes intriguing new questions about the organization and persistence of the central nervous system during metamorphosis. Our results have both ecological and evolutionary implications, because retention of memory through metamorphosis could influence host choice by polyphagous insects, shape habitat selection, and lead to eventual sympatric speciation.



Function and Evolutionary Diversity of Fatty Acid Amino Acid Conjugates in Lepidopteran Caterpillars

Yoshinaga N, Nishida R, Alborn HT, Tumlinson JH, and Mori N

Applied Life Sciences, Graduate School of Agriculture, Kyoto University, Kyoto, 606-8502, Japan (NY, RN, NM); Center of Medical, Agricultural, and Veterinary Entomology, Agricultural Research Service, U.S. Department of Agriculture, Gainesville, FL 32604 (HTA); Center for Chemical Ecology, Department of Entomology, The Pennsylvania State University, University Park, PA 16802 (JHT)

Corresponding author: nuy3@psu.edu

Fatty acid amino acid conjugates (FACs) in regurgitant of larval *Spodoptera exigua* (1) were initially identified as plant volatile elicitors and research has been focused on this apparent ecological disadvantage rather than on possible benefit for the caterpillar itself. Recently, we demonstrated that *N*-linolenoyl- and *N*-linoleoyl-L-glutamine function as important intermediates that enhance the efficiency of nitrogen assimilation in *S. litura* larvae, one of the most notorious pests in Japan. This result might be best understood in the context of the *lepidopteran* larval stages that are thoroughly specialized morphology and ecology to maximized feeding, metabolism, and growth. Efficient use of nitrogen resources might be a vital issue for certain phytophagous insects living on nitrogen-poor leaves. In this investigation, we screened FACs through 26 lepidopteran species and found 17 of these species to have FACs in various patterns in their gut contents. Glutamine conjugates such as *N*-linolenoyl- and *N*-linoleoyl-L-glutamine seemed to be common to all these species, suggesting that these molecules are the primary structure of FACs. However, there were several additional evolutionary patterns based on acquirement of 1) glutamate conjugates, 2) hydroxylated fatty acid conjugates, and 3) both. Interestingly, hydroxylated FACs, including volicitin, were found only in Macrolepidoptera species (which are well developed and relatively big caterpillars), and most of which are vastly polyphagous. These results may give us a hint to understand the evolutionary interplay between a plants ability to detect and respond to different fatty acid amides and the insect's dependence on these same compounds for maximized nitrogen assimilation.

1. Science 276:945–949.

Fungal Infection and Methyl Jasmonate Application Induce Chemical Changes in the Phloem of Norway Spruce, *Picea abies*

Zhao T, Borg-Karlson AK, Långström B, Solheim H, and Christiansen E

Ecological Chemistry Group, Department of Chemistry, Royal Institute of Technology, SE-100 44 Stockholm, Sweden (TZ, AKBK); Department of Ecology, Swedish University of Agricultural Sciences, SE-750 07 Uppsala, Sweden (BL); Norwegian Forest Research and landsacpe Institute, N-1432 Ås, Norway (HS, EC)

Corresponding author: taozhao@kth.se

Induced reactions in the phloem is a basic mechanism of conifer resistance to bark beetle and their associated fungi (1,2). Previous research has proved that certain doses of *Ceratocystis polonica* infection or methyl jasmonate (MeJA) application could induce acquired resistance and decrease subsequent fungal or bark beetle colonization (3,4,5). To study the induced chemical changes after fungal infection and MeJA application in the phloem of mature Norway spruce, three groups, each of 24 *P. abies* trees of similar size, were chosen in Tönnersjöheden, southern Sweden, in May 2006. The three groups were then inoculated with *C. polonica*, sprayed with MeJA, or used as untreated control, respectively. Phloem samples were taken twice from each tree: on the same day as treatment and 1 mo later. The terpene composition of all the samples was analyzed by GC-MS, and the enantiomeric compositions of α -pinene, β -pinene, and limonene were analyzed by 2D-GC (6). The result indicated that both MeJA application and *C. polonica* infection had certain effects on the terpene composition. *C. polonica* infection significantly increased the biosynthesis of 3-carene, sabinene, and terpinolene. Both mean absolute amounts and relative amounts of these monoterpenes increased in samples from fungus inoculated trees, similar to what is observed in Scots pine after *Leptographium wingfieldii* inoculation (7). MeJA application increased the absolute amount of α -pinene, β -pinene, limonene, and some other major terpenes, but it did not change the relative amount of these terpenes. However, neither MeJA application nor fungal infection changed the enantiomeric compositions of α -pinene, β -pinene, and limonene in the phloem of Norway spruce.

1. For. Ecol. Manag. (1987) 22:89–106.
2. Agric. For. Entomol. (2001) 3:211–216.
3. Plant Physiol. (1999) 121:565–569.
4. Oecologia (2006) 148:426–436.
5. Tree Physiol. (2006) 26:977–988.
6. Acta Chem. Scand. (1993) 47:138–144.
7. J. Chem. Ecol. (2006) 32:1779–1795.



Phylogenetic Analyses of Plant Defense and Insect Host Range

Chair: Anurag Agrawal,
Department of Ecology and Evolutionary Biology,
Cornell University, Ithaca, NY
100 Berg Auditorium, Life Sciences Building

SYMPOSIUM | WEDNESDAY, 20 AUGUST

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Secondary Chemistry Links the Macroevolution of Defense and Pollination Systems in *Dalechampia* Vines (Euphorbiaceae)

Armbruster WS

School of Biological Sciences, University of Portsmouth, Portsmouth, UK; and Institute of Arctic Biology, University of Alaska, Fairbanks, AK

Corresponding author: scott.armbruster@port.ac.uk

Although a number of recent studies have shown that herbivores have important effects on plant reproduction in ecological time, it is less clear whether herbivores have major effects on the long-term evolution of plant–pollinator interactions or whether adaptations for pollination can influence plant–herbivore interactions. Studies of the floral chemistry, pollination, and herbivory of *Dalechampia* vines (Euphorbiaceae) suggest that adaptations for pollination can influence relationships with herbivores, as well as vice versa, at both micro- and macroevolutionary scales. Evolution of the color of the involucre bracts involved in advertising flowers to pollinators may have been influenced by the evolution of protective anthocyanins: macroevolutionary analyses show correlated evolution of blossom pigments and pleiotropically related vegetative pigments (as seen also in maples). The pollinator reward (mixtures of oxygenated terpenoid resins collected by bees for nest construction) used by most extant *Dalechampia* species seems to be a key innovation that originated from pre-existing resins involved in defending staminate flowers when in bud. Morphological adaptations that improved the attractiveness of the resin rendered it useless for floral defense. Subsequently, however, this same resin was deployed in defense of developing seeds, with at least two independent origins of this novel defense system. One clade has also deployed the same resin in a third function: defense of leaves from specialized lepidopteran larvae and leaf-cutting ants. Examination of the whole genus also reveals a pattern of escalating defenses of flowers and developing seeds: 1) closed, enveloping involucre bracts, followed by the addition of 2) enveloping pistillate sepals, 3) sticky terpenoid resins secreted by enveloping sepals, 4) detachable irritating hairs on the enveloping sepals, and 5) nocturnal closure of involucre bracts during anthesis. Despite these multiple layers of defense, flowers and developing seeds of most species are still fed on successfully by specialized *Dynamine* (neotropics) and *Neptidopsis* (paleotropics) larvae (both Lepidoptera: Nymphalidae) and curculionid beetles. *Dynamine* larvae seem to redeploy the resins for their own defense.

The Evolutionary Play and the Ecological Theater: *Blepharida* Beetles on *Bursera*

Becerra JX

Department of Biosphere 2, University of Arizona, Tucson, AZ 85721

Corresponding author: becerra@ag.arizona.edu

One of the central paradigms in the field of plant–herbivore interactions is that the diversity and complexity of defenses in plants have escalated over evolutionary time and that the abilities for herbivores to counteradapt also have become more effective. Nevertheless, there is almost no data yet confirming this arms race paradigm. I used the coevolved interaction between the tropical genus of plants *Bursera* and their chrysomelid herbivores to test whether there has been a historical increase in the diversity and complexity of chemical compounds produced. Results show that the average diversity and complexity of chemical defenses in these plants have increased slightly through time, but they also show that there has been a decrease of new compounds produced per species. Also, many lineages have not increased their chemical diversity or complexity. Overall, the degree of diversity/complexity seems to be related to the ecological interactions between these plants and insects. In communities where these beetles are highly specialized and the probability of high damage to plant increases, species tend to be more chemically diverse and complex.

Phylogenetic Patterns of Insect-Herbivore/Plant Interaction and Their Inferred Chemical Basis: A Synopsis of Recent Evidence

Mitter C and Winkler I

Department of Entomology, University of Maryland, College Park, MD

Corresponding author: cmitter@umd.edu

The dramatic expansion of research on insect/plant interactions prompted by Ehrlich and Raven's (1) essay on coevolution focused at first mainly on the proximate mechanisms of those interactions, especially the role of plant secondary chemistry, and their ecological consequences. Subsequently, in parallel with the resurgence of phylogenetics beginning in the 1970s and 1980s, there arose increasing interest in the long-term evolutionary process envisioned by Ehrlich and Raven. Since the early 1990s, spurred in part by the increasing accessibility of molecular systematic data and methods, there has been rapid proliferation of phylogenetic studies of interacting insect and plant lineages. Given the great diversity of phytophagous insect life histories and chemical, full characterization of the evolution of insect host plant use, and the role of chemistry therein, will require both in-depth analysis of selected systems and estimation of the relative frequencies of alternative evolutionary patterns across a broad sampling of lineages. Our emphasis here is on the second, "meta-phylogenetic" approach. We attempt to 1) enumerate and classify the profusion of broad range of evolutionary questions and hypotheses that has emerged from this literature, 2) catalog (or at least extensively sample) the recent accumulation of phylogenetic studies of insect-herbivore/host plant associations, and 3) determine the extent to which quantifiable answers/generalizations about those questions are emerging from that evidence.

1. *Evolution* (1964) 18:586–608.

Evolutionary Origins and Genetic Basis of a Plant–Insect Coevolutionary Key Innovation

Vogel H, Fischer HM, Heckel DG, and Wheat CW

Department of Entomology, Max-Planck Institute of Chemical Ecology, Beutenberg Campus, Jena, Germany (HV, HMF, DGH); Department of Biological Sciences, The Pennsylvania State University, University Park, PA (CWW)

Corresponding author: hvogel@ice.mpg.de

The interactions between plants and their specialist insect herbivores provide an excellent opportunity to study the evolution of species interaction on a molecular level. The unresolved central tenet of Ehrlich and Raven's theory is that evolution of plant chemical defenses is followed closely by biochemical adaptation in insect herbivores, and that newly evolved detoxification mechanisms result in adaptive radiation of herbivore lineages. Using one of their original butterfly-host plant systems, the Pieridae, we identify a pierid glucosinolate detoxification mechanism, nitrile-specifier protein (NSP), as a key innovation. NSP redirects the hydrolysis of plant chemical defense glucosinolates to nitriles rather than toxic isothiocyanates in caterpillar guts, enabling them to feed on plants in the order Brassicales. Butterfly NSP activity matches the distribution of glucosinolates in host plants across Pieridae. Moreover, by using five different temporal estimates, NSP seems to have evolved shortly after the evolution of the host plant group (Brassicales) (10 Myr). An adaptive radiation of these glucosinolate-feeding Pierinae followed, resulting in significantly elevated species numbers compared with related clades. We found NSP to be a member of an insect specific gene family we characterized and named NSP-related gene family. Members of this family can be found across Insecta. Generation of cDNA libraries and genomic libraries allowed us a robust phylogenetic analysis. NSP-like gene family evolution is a dynamic, ongoing birth death process and we provide direct evidence that gene duplication is one of the driving forces for speciation and adaptation. We have generated a broad and detailed sketch of the evolutionary origins and ongoing selection pressures of an adaptive trait, which facilitated an ecologically important host shift and the diversification of the Pieridae butterfly family.

Parasites of a Mutualism: Strategies and Phylogenetic Histories in the *Acacia*–*Pseudomyrmex* System

Heil M

Depto. de Ingeniería Genética, CINVESTAV-Irapuato, Km. 9.6 Libramiento Norte, CP 36821, Irapuato, Guanajuato, México

Corresponding author: mheil @ira.cinvestav.mx

Many defensive plant strategies depend on plant–carnivore mutualisms. Mutualisms are, however, believed to be in continuous danger of destabilization by exploiters, species that use the host-derived rewards without rendering a service. Models combining the Prisoner's dilemma approach with natural features of mutualisms predicted that, for mutualism to evolve, increased investment in a partner must yield increased returns (1), whereas others predicted high exploitation rates for high-reward mutualisms (2). I argue that the phylogenetic history of an exploiter determines which mechanism can stabilize a mutualism against it and use the term “cheater” exclusively for exploiters that evolved from former mutualists that ceased the service, whereas “parasites” are exploiters that invaded the system without having an evolutionary history as a mutualist. If this distinction is made, it becomes clear that, in an obvious contradiction to theoretical expectations, cheaters are uncommon (3). *Acacia* myrmecophytes and their *Pseudomyrmex* ant inhabitants are an ideal system to study questions on the stability of such defensive mutualisms and the strategies that are used by exploiters of mutualisms, because the Mesoamerican species of these two genera form different associations: facultative and obligate ant–plant mutualisms and facultative and obligate exploitation of host plants by nondefending ants. A molecular phylogeny of *Pseudomyrmex* revealed no evidence for the existence of cheaters, and the system, thus, obviously comprises mechanisms for the stabilization of mutualisms. *Acacia* myrmecophytes secrete sucrose-free extrafloral nectar (EFN) (4). Mutualist workers discriminated against sucrose, whereas parasites and generalists preferred sucrose. These preferences are caused by the ants' digestive physiology: mutualist workers lacked the sucrose-cleaving enzyme invertase, whereas workers of parasites and generalists possessed enzymatic activity. Sucrose induced invertase in larvae of all species and in workers of parasites and generalists, but not mutualists. Invertase in mutualists is lost during ontogeny, and mutualists fully depend on the host-derived diet, whereas parasites also use external food sources and thus are less dependent on host quality. On the plant side, host species differed in the amount of EFN, food bodies, and hollow thorns that they produce for defending ant mutualists. Hosts producing more EFN were more aggressively defended by their inhabitants, and a mutualist, but not a parasite, could induce EFN flow. A closed loop of positive feedback mechanisms among fitness-relevant traits of both partners can stabilize mutualisms against less well adapted exploiters, which can, however, have higher success on low-quality hosts. This model is confirmed by the observation that high-reward host species were less commonly exploited.

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Phylogenetic Escalation and Decline of Plant Defense Strategies

Agrawal AA and Fishbein M

Department of Ecology and Evolutionary Biology, Cornell Center for a Sustainable Future, and Department of Entomology, Cornell University, Ithaca, NY (AAA); Department of Biology, Portland State University, Portland, OR (MF)

Corresponding author: agrawal@cornell.edu

Being the basal resource in most food webs, plants have evolved myriad strategies to battle consumption by herbivores. Over the past 50 years, plant defense theories have been formulated to explain the remarkable variation in abundance, distribution, and diversity of secondary chemistry and other defensive traits. For example, in their classic propositions of enemy driven evolutionary dynamics, both Ehrlich and Raven and Vermeij have hypothesized that defensive traits escalate through the diversification process. Despite macroevolutionary patterns being an explicit part of defense theories, phylogenetic analyses have not been previously attempted to disentangle specific predictions concerning 1) investment in resistance traits, 2) recovery after damage, and 3) plant growth rate. We construct a molecular phylogeny of 38 species of milkweed and test four major predictions of defense theory by using maximum likelihood methods. Our main finding is a pattern of phyletic decline in the three most potent resistance traits (cardenolides, latex, and trichomes), and an escalation of regrowth ability. Our neontological approach complements more common paleontological approaches to discover directional trends in the evolution of life, and points to the importance of natural enemies in the macroevolution of species. The finding of macroevolutionary escalating regrowth ability and declining resistance provides a new window into the ongoing coevolutionary dynamics between plants and herbivores, and suggests a revision of classic plant defense theory.

The Macroevolution of Leaf Defensive Traits in Oaks

Pearse IS

Department of Entomology, University of California, One Shields Ave., Davis, CA 95616

Corresponding author: ispearse@ucdavis.edu

The macroevolution of antiherbivore defenses has been a long-standing topic in chemical ecology. Macroevolutionary studies have predominantly focused on the conservatism of defensive traits, as evolutionarily conserved defenses could partially explain the patterns of host use by specialist herbivores. Conversely microevolutionary studies focus on the variation of traits within species—often to explain local adaptation to differing herbivore regimes. This study attempts to assess the degree of evolutionary conservatism or lability of leaf defensive traits in oaks (*Quercus*) by using an explicitly phylogenetic framework while assessing the effectiveness of these defenses against herbivores. Nine leaf defensive traits as well as leaf damage were measured across 57 species of mature, non-native oaks in a common garden. Each of these aspects of plant–herbivore interactions were mapped onto a phylogeny of oaks. Phylogenetic distance to the local native oak (*Quercus lobata*) predicted the presence and abundance of specialist gallers, but it did not predict chewing, mining, and phloem feeding (i.e., more generalist) leaf damage. A principal components axis (PCA-1) of all nine defensive traits predicted the presence and damage caused by these more generalized feeders. A phylogenetic independent contrast of PCA-1 revealed a high degree of convergence on total leaf defense; however, analysis of each of the individual defensive traits suggested trends ranging from weak conservatism to lability. This study shows that within *Quercus*, many leaf defenses that are effective against generalist herbivory have evolved in convergence. That defensive traits are both gained and lost throughout evolutionary history suggests both positive and negative selection pressures acting on antiherbivore defenses in oaks.

Chemical Ecology of Disease Transmission

Chairs:
Sanford Eigenbrode, University of Idaho, Moscow, ID;
and **Mark Mescher**, Penn State University, University Park, PA
100 Berg Auditorium, Life Sciences Building

SYMPOSIUM | THURSDAY, 21 AUGUST

4

SUN MON TUE WED THU

Disease Progression and the Chemical Ecology of *Potato leaf roll virus* in Potato

Eigenbrode SD, Werner BJ, Bosque-Pérez NA, and Ding H

Plant, Soil and Entomological Sciences, University of Idaho, Moscow, ID.

Corresponding author: sanforde@uidaho.edu

The green peach aphid, *Myzus persicae*, is the principal vector of *Potato leaf roll virus* (PLRV). These aphids preferentially settle on potato plants (*Solanum tuberosum*) infected with PLRV compared with noninfected control plants (1,3). Both greater rates of immigration toward, and lower rates of emigration away from, leaflets of PLRV-infected plants occur (2). Differential emigration away from PLRV-infected leaflets occurs even in the dark in a bioassay in which aphid contact with the leaflets is prevented, thus implicating virus infection-induced changes in release of volatile organic compounds (VOC) from the plants as the stimulus to which the aphids are responding (2,3). Combinations of these VOC, rather than individual components of the blend, are required to elicit an arrestant response by the aphids similar to that observed in infected plant headspace (4). In this study, we examined the changes in *M. persicae* responses to PLRV-infected potato plants during the course of infection from 2, 4, 6 and 8 wk after inoculation. Noninfected plants, which were sham-inoculated with nonviruliferous aphids were used as controls. Plants of all infection stages were available simultaneously during the experiment. Tests were conducted in darkness with thirty aphids being placed on a screened floor suspended over the surface of treatment leaflets. Observations were recorded every 10 min for 1 h documenting the number of aphids that emigrated from the treatment leaf. Aphids were considered to have emigrated if they were no longer over the leaflet surface and were subsequently removed from the test arena. Emigration rates were estimated by fitting cumulative number of aphids emigrating to an exponential decay function. Immediately after the bioassay, headspace volatiles were collected from entire plants (2, 4, and 8 wk after infection only) and plant tissues were collected for ELISA to confirm infection status. Leaflets from the two locations in the plant were tested: upper (4th node from terminus) and lower (4th node from the soil line). In bioassays using upper leaflets, aphid emigration was reduced on infected plants as compared with controls at 4 wk after infection, but not at 2, 6 or 8 wk after infection. In bioassays using lower leaflets emigration rates did not differ between PLRV-infected plants and sham-inoculated controls at any stage of infection. Headspace VOC changed during development and infection based on total amount of VOC trapped per gram of plant tissue and on the relative composition. VOC were indistinguishable between treatments 2 weeks after infection. Thereafter, total trapped VOC per gram increased more rapidly from infected plants than from sham-inoculated plants. The relative proportion of green leaf volatiles, monoterpenes and sesquiterpenes in the blend shifted during disease progression. The blend detected at 4 weeks had the greatest relative proportion of monoterpenes. The requirement for a VOC blend that includes monoterpenes to reduce *M. persicae* emigration (4) may explain this pattern in aphid response to plants during the progression of the potato leaf roll disease. Varying vector responsiveness to VOC from plants at different stages of infection could affect patterns of virus spread.

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2. Proc. R. Soc. Ser. B (1992) 269:455–460.

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Plant Viruses Benefit Their Herbivore Vectors

Belliure B, Albiach-Martí MR, Rubio L, Janssen A, and Sabelis MW

Valencian Institute for Agricultural Research (IVIA), Center for Plant Protection and Biotechnology, Moncada (Valencia), Spain (BB, LR); Fundación Agroalimed-IVIA, Moncada (Valencia), Spain (MRAM); Institute for Biodiversity and Ecosystem Dynamics (IBED), University of Amsterdam, Amsterdam, The Netherlands (AJ, MWS)

Corresponding author: belliure@ivia.es

Vector-borne plant viruses depend on their herbivore vectors for dispersal. Virus spread requires vector attraction to infected source plants, acquisition, dispersal of vectors to healthy plants, and inoculation. Acquisition and inoculation involve vector feeding, which triggers induction of anti-herbivore plant defences, whereas virus infection induces anti-pathogen defences in plants. Anti-herbivore defences decrease host quality of herbivore-infested plants and render them unattractive to conspecific herbivores. In spite of this, infected plants are often attractive to their herbivore vectors. To test whether this attraction is beneficial to vectors, we studied the effect of a plant virus on its vector through changes in the quality of infected plants (direct anti-herbivore plant defence), changes in performance of infected vectors, and changes in efficiency of natural enemies of the vectors. We used the system *Tomato spotted wilt virus* (TSWV)–*Frankliniella occidentalis*–pepper plants. Our results indicate that thrips induce a response in pepper plants that is negative for thrips, and that TSWV alleviates this anti-thrips response in infected plants, rendering them as good hosts as healthy plants. These results might be explained by a negative cross-talk between anti-herbivore and anti-pathogen plant defences. The effect of virus infection of plants also was beneficial for a nonvector herbivore arthropod, the spider mite *Tetranychus urticae*. To explore whether beneficial effects for vectors are related to virus virulence, we compared performance of vectors on plants infected with a mild and a resistance-breaking TSWV isolate. In another system, we also compared performance of the vector *Aphis gossypii* on citrus plants infected with isolates of *Citrus tristeza virus* (CTV) differing in virulence and geographical origin. We did not find differences in benefits from different TSWV-isolates for *F. occidentalis*. However, *A. gossypii* performed better on citrus plants infected with some CTV isolates than with others. This difference was neither related to isolate virulence nor to its geographical origin. Nevertheless, *A. gossypii* had one of the highest developmental rates on citrus infected with one of the most spread CTV-isolates worldwide. This might indicate adaptation of the vector population tested to this specific virus isolate. We hypothesize that mechanisms to alleviate anti-herbivore plant defences may have evolved in vector-borne plant viruses to promote their own spread. These mechanisms would involve metabolic changes that promote performance of vectors on infected plants and enhance attraction to them, thus increasing the probability of virus dispersal. Further research is needed to understand the effect of virus on attraction of natural enemies toward infected plants, the effect of infection of vectors on their attraction to infected and healthy plants, coevolution of plant–virus–vector interactions, evolution of virulence in viruses in relation to vector selection, and interactions of the plant–virus–vector system within the food web. All these processes affect vector dispersal and virus epidemiology. To unravel them would contribute to the design of adequate pest and viral control strategies.

Pathogen-Induced Plant Volatiles and Disease Ecology

Mescher MC, De Moraes CM, Stephenson AG, Mauck KM, and Shapiro LR

Center for Chemical Ecology, Departments of Entomology (MCM, CMD, KMM, LRS) and Biology (AGS), The Pennsylvania State University, University Park, PA

Corresponding author: mcmescher@psu.edu

Many of the most important plant diseases impacting crop production in the United States and worldwide are vectored, often obligately, by insects. Yet, the chemical ecology of pathogen–plant–vector interactions is not well explored. Although plant volatiles are likely a major factor influencing the behavior, distribution, and movement of insect vectors (1) relative to infected plants, the implications for disease ecology of pathogen-induced changes in plant volatile emissions and other plant characteristics are not well understood. We are currently examining the chemical ecology of disease transmission in the wild gourd *Cucurbita pepo* ssp. *texana* and in cultivated *C. pepo* cv. ‘Dixie’. In the wild gourd, we have documented distinct volatile signatures associated with infection by *Erwinia tracheiphila*, the causal agent of bacterial wilt disease, and zucchini yellow mosaic virus (ZYMV). Notably, the volatile profiles of plants suffering mixed infections were similar to those induced by ZYMV infection alone, suggesting that ZYMV infection suppresses the volatile response otherwise induced by *E. tracheiphila*. This has potential ecological significance as we have previously observed that virus-infected plants rarely or never contract wilt. We are exploring the influence of pathogen-induced volatiles on the behavior of the cucumber beetle *Acalymma vittatum*, one of the two confirmed vectors of *E. tracheiphila*, and on several generalist aphid species that vector ZYMV. In cultivated *C. pepo* cv. Dixie, we have found that infection by the viral pathogen *Cucumber mosaic virus* decreases plant quality for the aphids *Myzus persicae* and *Aphis gossypii*, resulting in reduced aphid population growth and increased dispersal. Analysis of volatiles indicates that infected plants release elevated amounts of a volatile blend similar to that of healthy plants, which may increase the apparency of infected plants without providing cues regarding infection status. The contrast of our findings with those of previous studies that found aphid attraction to characteristic volatile signatures associated with viruses that increase plant aphid performance (1,2) suggest that the attractiveness of pathogen-induced volatiles can vary with details of the natural history of pathogen–plant–vector interactions and argues for more fully documenting these interactions in additional systems.

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Interrelationships among Inbreeding, Herbivory, and Pathogen Transmission in *Cucurbita pepo* ssp. *texana*: Bacterial Wilt Disease as a Sexually Transmitted Disease

Sasuclark M, Seidl-Adams I, Winsor J, and Stephenson A

Corresponding author: mas816@psu.edu

Many plant pathogens are vectored by insect herbivores. In the genus *Cucurbita*, the bacterium *Erwinia tracheiphila* causes wilt disease, a fatal disease that arrests water transport; is economically devastating to cultivars; and also attacks wild species. *Erwinia tracheiphila* is vectored by specialist beetles *Acalymma vittata* and *Diabrotica undecimpunctata howardi* (Coleoptera). Transmission occurs when fecal pellets containing *Erwinia* fall onto the sites of feeding damage. Our studies of *Cucurbita texana*, a wild gourd, show no difference between inbred and outbred lines in susceptibility to the pathogen when it is injected into stem and foliar tissue. However, in the field, inbred lines were significantly less likely to develop wilt disease (in three of the 4 yr studied), although they suffered greater herbivore damage over the growing season ($p \geq 0.04$). This paradox is explained by transmission of the pathogen through floral tissue: 1) outbred plants produced greater numbers of flowers, in which the beetles congregate in a lek-like behavior; 2) they produced significantly higher levels of attracting volatiles (e.g., 1,2,4-trimethoxybenzene); 3) and they attracted greater numbers of beetles to their flowers. Greenhouse studies showed that 23% of plants inoculated with *Erwinia* suspension through staminate flowers and 31% of plants inoculated through pistillate flowers developed wilt disease, and fluorescence studies of GFP-transformed *Erwinia* show that the bacterium entered flowers through intracellular spaces and nectarthodes in the nectaries and traversed the peduncle to the stem within 48 h. Our data demonstrate another example of the risk of reproduction and suggest that the trade-off between reproduction and pathogenicity warrants greater attention.

Metabolomics and Proteomics of Systemic Induced Resistance in Pine

Bonello P

Department of Plant Pathology, The Ohio State University, Columbus, OH
Corresponding author: bonello.2@osu.edu

Systemic induced resistance (SIR) against pathogens is a poorly understood phenomenon in trees, compared with its related subsets, systemic acquired resistance (SAR) and induced systemic resistance (ISR), in herbaceous model plants. Nevertheless, recent work has highlighted the potential ecological significance of SIR in pine, particularly as it pertains to host-mediated systemic interactions between fungal pathogens and phytophagous insects, such as bark beetles and defoliators (1). Furthermore, the large size and longevity of these organisms make them ideal to study the role of relative spatial and temporal separation of inducers and challengers in phenotypic expression. Evidence shows that induced resistance is not always the endpoint phenotype; indeed, we have demonstrated that whether SIR or systemic induced susceptibility (SIS) is expressed is dependent on which organs are induced and which ones are challenged (2,3). In this talk, I present evidence pointing to integration of resistance mechanisms in pine across temporal and spatial scales involving several defense compartments in these complex organisms, including phenolic and terpenoid metabolism (3–6), classically defined defense proteins (7), and other novel proteins of potential significance in these interactions (8). An improved understanding of bottom-up forces driving fundamental ecological interactions centered around these important components of boreal forests should lead to development of more rational forest management strategies in the future.

1. *Physiol. Mol. Plant Pathol.* (2006) 68:95–104.
2. *Tree Physiol.* (2007) 27:511–517.
3. *Tree Physiol.* (2008) in press.
4. *Physiol. Mol. Plant Pathol.* (2003) 63:249–261.
5. *New Phytol.* (2008) 177:767–778.
6. *Planta* (2005) 221:75–84.
7. *J. Chem. Ecol.* in review.
8. *Physiol. Mol. Plant Pathol.* (2006) 68:149–157.

Metabolomics of Plant–Pathogen Interaction

Kushalappa AC and Ajjamada C

Plant Science Department, McGill University, Ste. Anne de Bellevue, QC, Canada H9X3V9
Corresponding author: ajjamada.kushalappa@mcgill.ca

Metabolomics is the identification and quantification of all endogenous and exogenous metabolites in a biological situation or system. Plants produce ca. 200,000 metabolites. Metabolomics analysis can give comprehensive information on the strategies that plants use to defend against the attacking pathogen. However, quantification of all metabolites produced by a plant is still very challenging. There is no single solvent that can extract all the metabolites and no analytical platform that can detect all the metabolites in a sample. In spite of these limitations significant amount of knowledge has been generated using metabolomics. Recent advances made in the analytical platforms, along with bioinformatics tools, have enabled detection and identification of thousands of metabolites. Nanoelectron spray ionization, high resolution and high mass accuracy determination using liquid chromatography and mass spectrometry now can detect >1,000 compounds in a sample. These have been used to study plant–pathogen interaction. The resistance in plants to defend against biotic pathogen stress can be grouped into structural and biochemical, and within each into constitutive and induced. Metabolomics analysis can reveal not only biochemical but also structural defense. The resistance-related metabolites (higher in abundance in the resistant genotype than in the susceptible) have antimicrobial, pathotoxin degradation, signaling, and cell wall enforcement properties. The metabolic pathways of production of these metabolites can be searched using databases. The relation of resistance related metabolites to their precursors can lead to better understanding of the plant defense mechanisms. The resistant plant genotypes have a better repertoire of metabolites and metabolic pathway activities to defend against the pathogen than that by the susceptible. The problems and progress made in the application of metabolomics technology to investigate the wheat (1,2) and barley interactions with *Fusarium graminearum*, causal agent of fusarium head blight, are discussed.

1. *Physiol. Mol. Plant Pathol.* 66:119–133.
2. *Can. J. Plant Pathol.* 30:24–36.

Identification of Bacteria and Bacteria-Associated Chemical Cues That Mediate Oviposition Site Preferences by *Aedes aegypti*

Ponnusamy L, Xu N, Nojima S, Wesson DM, Schal C, and Apperson CS

Department of Entomology and W. M. Keck Center for Behavioral Biology, North Carolina State University, Raleigh, NC (CS, LP, NX, SN, CSA); Department of Tropical Medicine, Tulane University, New Orleans, LA (DMW)

Corresponding author: coby_schal@ncsu.edu

The mosquito *Aedes aegypti* is the principal vector of important global diseases, including dengue and yellow fever viruses. Dengue fever is a major public health problem in tropical countries worldwide, and the World Health Organization estimates that 51 million infections occur annually and 2.5–3 billion people are at risk in the 100 countries where dengue fever occurs. The dramatic rise in the number of cases of dengue hemorrhagic fever in Asia and its recent introduction into Central and South America have stimulated interest in biorational approaches, including identification and implementation in mosquito-control programs of behavior-modifying compounds, such as host attractants, repellents, and oviposition site attractants and stimulants. *Ae. aegypti* females are inexorably linked to water-filled human-made containers for egg laying and production of progeny. Oviposition is stimulated by cues from water containers, but the nature and origin of these cues have not been elucidated. Microbial metabolites have been implicated as mosquito attractants, but these odors do not induce egg laying. We show that mosquito females direct most of their eggs to bamboo and white-oak leaf infusions, and only a small fraction of the eggs are laid in plain water containers. In binary choice assays, we demonstrated that microorganisms in leaf infusions produced oviposition-stimulating kairomones, and using a combination of bacterial culturing approaches, bioassay-guided fractionation of bacterial extracts, and chemical analyses, we now demonstrate (1) that specific bacteria-associated carboxylic acids and methyl esters serve as potent oviposition stimulants for gravid *Ae. aegypti*. Elucidation of these compounds will not only serve to better understand the chemical basis of egg-laying behavior of *Ae. aegypti* but also these kairomones will likely enhance the efficacy of surveillance and control programs for this disease vector of substantial global public health importance.

This work was supported in part by a National Institutes of Health–National Institute of Allergy and Infectious Diseases grant U01-AI-58303-01, the Blanton J. Whitmire Endowment, and the W. M. Keck Center for Behavioral Biology at North Carolina State University.

1. Proc. Natl. Acad. Sci. USA (in press).

Plant–Insect Interactions

Moderators:

14.00–15.30 **Ted Turlings**, University of Neuchâtel

15.50–17.20 **Rob Raguso**, Cornell University

100 Berg Auditorium, Life Sciences Building



PARALLEL SESSION 5 | THURSDAY, 21 AUGUST

SUN MON TUE WED THU

***De Novo* Biosynthesis Versus Sequestration: A Network of Transport Systems Supports Both Modes of Defense in Iridoid-Producing Leaf Beetle Larvae**

Boland W, Kunert M, Discher S, Tolzin-Banasch K, Burse A, and Pasteels J

Max Planck Institute for Chemical Ecology, Hans-Knöll-Str. 8, 07745 Jena, Germany (WB, MK, SD, KT-B, AB); Etho-Ecologie Evolutive, CP 160/12 Université Libre de Bruxelles, B-1050 Bruxelles, Belgium (JP)

Corresponding author: boland@ice.mpg.de

In the larval chrysomelids the *de novo* synthesis of monoterpenoids (iridoids) is believed to represent the ancestral state in the evolution of chemical defenses (1). Here, we demonstrate that the iridoid-producing larvae of *Plagiodera versicolora* and *Phratora laticollis* have the potential to sequester precursors from food (2). In the natural condition, iridoids may even have a dual origin, namely, plant-derived and *de novo*-produced (3). The ability to sequester plant-derived precursors was proved by 1) ¹³C-labeling of the terpenoids in the food plant, 2) by larval feeding on leaves impregnated with analogs and labeled putative precursors for iridoid biosynthesis; and 3) by injection of the precursors into the hemolymph followed by mass spectroscopic analysis of their distribution in the hemolymph, defensive secretion, and faeces. The experimental findings support a network of transport systems that allows a broader range of glucosides to enter and to leave the hemocoel, whereas only the appropriate precursor, 8-hydroxy-geraniol-8-*O*-β-D-glucoside, is channeled to the reservoir and processed to iridoids (4). The dual system of *de novo* biosynthesis and sequestration of phytogetic precursors may have favoured the larvae to shift from one host plant to another without losing their defense.

1. Insect Biochem. Mol. Biol. (2008) 38:76–88.
2. Isotopes Environ. Health Stud. (2004) 40:175–180
3. Proc. Natl. Acad. Sci. USA (2001) 98:3909–3914.
4. Insect Biochem. Mol. Biol. (2008) in press.

Heavy Metal Stress Primes for Herbivore-Induced Volatile Emission

Rostás M, Kaiser K, and Borkowski L

Department of Botany II, University of Würzburg, Germany

Corresponding author: rostas@botanik.uni-wuerzburg.de

Plant volatile emission in response to herbivory has multiple functions. Induced volatiles can serve as signals for parasitoids, predators, or herbivores. In addition, they may be perceived by neighbouring plants or act as signals in remote plant parts of the same individual. Volatiles involved in such plant–plant communication may directly induce responses or prime the affected plant (part) to respond stronger or earlier upon later herbivory. Abiotic factors may change the composition of herbivore-induced volatiles and can potentially alter interactions between emitting and perceiving individuals. High levels of heavy metals in some natural habitats or in anthropogenically degraded soils exert stress on most plants. We investigated the effects of two levels of copper (10 and 80 μM) and cadmium (5 and 50 μM) on the performance of hydroponically grown maize, the accumulation of reactive oxygen species (ROS), the kinetics of jasmonic acid (JA), and the time course of herbivore-induced volatiles. In maize plants, Cu was completely retained in the roots, whereas Cd accumulated also in the shoots. Plant growth and photosynthesis were reduced at higher metal concentrations (80 μM Cu, 50 μM Cd). Measurements of JA kinetics revealed that this endogenous signal did not increase above control levels in only Cu- or Cd-treated plants. However, herbivory resulted in significant JA accumulation after 6 h of feeding in control plants without heavy metal supplementation and already after 3 h of feeding in both Cu treatments. Interestingly, a similar priming effect was observed in Cd-treated maize. DAB-staining of roots and leaves showed that ROS were visible only in the roots of plants treated with 80 μM Cu. Plant volatile emission was exclusively found in herbivore-damaged maize, whereas heavy metals per se did not induce volatiles. However, priming was observed in herbivore-damaged plants that were pre-treated with 80 μM Cu as the amounts of released compounds were significantly higher than in herbivore-damaged control plants without additional Cu. We conclude that Cu and Cd stress may have adverse effects on many plant functions but their impact on volatile release is neutral or even positive.

Identification of Branch Point Enzymes within the Iridoid Biosynthesis in *Chrysomelina* Larvae *Phaedon cochleariae*

Frick S, Burse A, Schmidt A, and Boland W

Department of Bioorganic Chemistry, Max-Planck-Institute for Chemical Ecology, Hans-Knöll-Strasse 8, 07745 Jena, Germany

Corresponding author: sfrick@ice.mpg.de

Larvae of the leaf beetle *Phaedon cochleariae* produce iridoids (cyclopentanoic monoterpenes) as chemical weapons against predators. They are capable of synthesizing deterrent compounds *de novo* or by sequestering the iridoid precursor 8-hydroxygeraniol-8-*O*- β -D-glucoside from their host plant. The precursor is assembled from isopentenyl diphosphate (IDP) and dimethylallyl diphosphate (DMADP), derived from the mevalonate pathway where 3-hydroxy-3-methylglutaryl-CoA reductase (HMGR) is one of the key enzymes. Real-time data indicate a high impact of HMGR activity in larval fat body for *de novo* production (1). Enzyme assays with recombinant protein revealed attenuated activity by addition of the aglucon 8-hydroxygeraniol, whereas no effect has been observed for the glucoside or geraniol. Subsequent homology modeling and docking experiments of the catalytic domain demonstrate binding of 8-hydroxygeraniol to the active site that indicate a competitive inhibition mechanism. Furthermore, not only enhanced HMGR activity but also elevated geranyl diphosphate synthase (GDPS) activity is detectable in larval fat body (2). Whereas HMGR constitutes a key enzyme of the early steps, short-chain *E*-isoprenyl diphosphate synthases act as later regulatory branch point enzymes within terpenoid biosynthesis (3). GDPS catalyzes the single condensation of IDP and DMADP, resulting in geranyl diphosphate (GDP). In iridoid-producing *Chrysomelina* larvae, 8-hydroxygeraniol-8-*O*- β -D-glucoside is derived from GDP, which implies participation of GDPS in *de novo* synthesis (4). GDPS represents the branch point between the synthesis of defensive compound and the remaining terpenoid pathway. Identification and characterization of a GDPS will point out the enzymatic processes and regulatory mechanisms of iridoid production with regard to homeostasis of *de novo* synthesis and sequestration processes. We want to establish the feedback control system of the iridoid biosynthesis with a view on the regulatory impact of several enzymes.

1. Insect Biochem. Mol. Biol. (2007) 37:255–265.
2. Mol. Biol. (2008) 38:76–88.
3. Eur. J. Biochem. (2002) 269:3339–3354.
4. Tetrahedron (1994) 50:6859–6874.

Mechanistic Aspects of a Multiproduct Sesquiterpene Synthase of *Medicago truncatula*

Garms S, Arimura GI, and Boland W

Max Planck Institute for Chemical Ecology, Department of Bioorganic Chemistry, Hans-Knoell-Str. 8, D-07745 Jena, Germany

Corresponding author: sgarms@ice.mpg.de

Sesquiterpenoids comprise a huge variety of cyclic and acyclic compounds generated from three "isoprene-units" and have the molecular formula $C_{15}H_{24}$. The structural diversity of the sesquiterpenoids known today are derived from 300 hydrocarbon skeletons, formed by so called sesquiterpene cyclases, utilizing farnesyl diphosphate (FDP) as the substrate. Beside single product enzymes producing only one major product, also multiproduct enzymes are known, generating up to 52 sesquiterpenes (1). To understand to which extent these multiproduct enzymes exert control over the different reaction pathways, we investigated the multiproduct enzyme MtTPS5 from *Medicago truncatula* (2) by analyzing the stereochemical course of the reaction pathways leading to the enzyme products. The incubation of the recombinant MtTPS5 with FDP provided 18 sesquiterpene hydrocarbons and 10 alcohols with different carbon skeletons such as Cadinane, Germacrane, Cubebane, or Muurolane. The stereochemical analysis of 17 of the enzyme products by chiral gas chromatography showed that all products were enantiomerically pure. Additionally we could observe that products derived from a common precursor shared the same configuration of their stereocenters. This results demonstrated that each single cyclization step is controlled by the enzyme guiding the reaction in a particular direction. Further experiments with specifically labeled FDP (deuterium) and mass spectrometric analysis of the released products gave information on the stereochemical course of previously proposed hydride shifts in the biosynthesis of the intermediate Germacradienyl cations.

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Molecular Cloning and Functional Expression of an Extracellular Salicyl Alcohol Oxidase of Defensive Secretions of *Chrysomela populi* and *Chrysomela tremulae*

Michalski C, Mohagheghi H, and Ober D

Christian-Albrechts-University of Kiel, Biochemical Ecology and Molecular Evolution, Olshausenstrasse 40, 24098 Kiel, Germany (CM, DO); Technical University of Braunschweig, Pharmaceutical Biologie, Mendelssohnstrasse 1, 38106 Braunschweig, Germany (HM)

Corresponding author: cmichalski@bot.uni-kiel.de

Larvae of *Chrysomela populi* and *C. tremulae* use a specific defense strategy to displace their predators. Upon attack, they release defensive chemical from specialized glands that are located on the meso- and metathorax and on the first seven abdominal segments. Each gland consists of several gland cells that are attached to a glandular reservoir whose content is everted in case of irritation. To produce the defensive secretions, larvae need to take up salicin as an herbal precursor from leaves of their host plant (poplar trees). This plant-derived precursor is transported via the hemolymph into the glandular reservoir where two extracellular enzymes convert it into salicylaldehyde: a β -glucosidase transforms salicin into salicyl alcohol that is immediately oxidized into salicylaldehyde by a salicyl alcohol oxidase (SAO). We were able to identify the cDNA encoding SAO from the two related species, *C. tremulae* and *C. populi*. The sequences were successfully cloned and expressed in *Escherichia coli*. Enzyme activity and quantification of the produced salicylaldehyde was measured by gas chromatography. Sequence comparison revealed SAO to belong to the family of glucose-methanol-choline oxidoreductase-like sequences with mostly unknown function. Enzymes of this family share similar overall structure with an essentially identical FAD binding site but possess different catalytic activities. The data suggest that SAO, essential for activation of the plant-derived precursor salicin, was recruited from an oxidase involved in the autogenous biosynthesis of iridoid monoterpenes that is found in related chrysomelid leaf beetle species.

Investigation of a Specific Detoxifying Enzyme from the African Grasshopper *Zonocerus variegatus* (Orthoptera) Adapted to Pyrrolizidine Alkaloids

Wang L and Ober D

Botanisches Institut, Abt. Biochemische Ökologie und Molekulare Evolution, Universität Kiel, Olshausenstrasse 40, 24098 Kiel Germany

Corresponding author: lwang@bot.uni-kiel.de

The grasshopper *Zonocerus variegatus* (Orthoptera) from West Africa is a polyphagous feeder with a predilection for poisonous food plants. This species can sequester and store various secondary plant substances such as cardiac glycosides and pyrrolizidine alkaloids (PAs) that function as predator deterrents. PAs are hepatotoxic to mammals and have been responsible for animal and human poisonings in many parts of the world. Sequestration of PAs is widespread within the arctiids (tiger moths). However, we surprisingly found that the unrelated orthopteran *Z. variegatus* also has the ability to sequester PAs. We study, how the grasshopper is able to feed on chemically defended plants without negative effects and in addition is able to accumulate plant-derived toxins for their own defense. Previous experiments showed that senecione-*N*-oxygenase (SNO) is the key enzyme for PA detoxification and accumulation in arctiids. SNO is a flavin-dependent monooxygenase that uses NADPH as cofactor. This enzyme catalyzes the *N*-oxidation of toxic PAs to nontoxic PA *N*-oxides and enables the insects to accumulate the PA-*N*-oxide as a metabolically safe form. To understand the evolutionary origin of SNO in more detail, FMO-like sequences of *Z. variegatus* were identified and investigated in respect to their biochemical activity. For this purpose, we established an *Escherichia coli* expression system. The results show that the gene product of one of the FMO-like sequences of *Z. variegatus* catalyzes the *N*-oxygenation of PAs. For the other FMO-like sequences, we were unable to predict a function. Analyzing the substrate specificity of the PA *N*-oxygenase, the enzymes showed broad substrate specificity for various PA structures, including other alkaloid structures such as atropine, in contrast to the specificity of the SNO of the specialist *T. jacobaeae*. Additional investigations of other PA-adapted insects are necessary to get a better glimpse in this field of mechanistic adaptation processes of insects to plant toxins.

Effect of *Fusarium graminearum* Infestation on Carbon Delivery to the Roots and *de novo* Biosynthesis of Defense Root Exudates in Barley

Henkes GJ, Lanoue A, Thorpe MR, Burlat V, Schurr U, and Röse USR

ICG-3, Institute Phytosphere, Research Center Jülich, D-52425 Jülich, Germany, (GJH, MRT, US, USRR); Université François de Tours, EA 2106, Biomolécules and Plant Biotechnology (A.L., V.B.), 31 Avenue Monge, F-37200 Tours, France

Corresponding author: u.roese@fz-juelich.de

We investigated the role of carbon allocation and chemical signals in the mediation of belowground plant defense. We studied how infection of barley roots with the pathogenic fungus *Fusarium graminearum* affects allocation of recently fixed carbon from the shoot to infested and uninfested roots by using the radioisotope ¹⁴C in a sterile split-root system. We hypothesized that detrimental effects of *F. graminearum* on carbon allocation will be attenuated by colonization of roots with the biocontrol bacterium *Pseudomonas fluorescens*. Plants reduced carbon allocation toward infected roots for the benefit of the noninfected roots. When the sink strength of one root half was reduced by cooling alone, there was no enhanced partitioning to the untreated roots (1). Local or systemic preinoculation with the *P. fluorescens* strain CHA0 annihilated the effect of *F. graminearum* on carbon allocation and were of similar intensity, whereas the mutant CHA19 defective in the production of antibiotics did not repress the pathogen effect. Our results show that roots divert carbon from infected towards uninfected roots, suggesting rapid first line defence against pathogens (2). We further evaluated the potential of barley to secrete defense root exudates when challenged by *F. graminearum*. Liquid chromatography with photodiode array detection (LC-DAD) was used to profile small molecular weight exudates that are induced in response to root attack. Among the induced exudates, compounds were identified and used as marker to investigate the kinetics of the interaction of pathogenic fungus with the plant roots. The plant or fungal origin of the compounds was determined by detection of exudates under noninduced conditions, i.e., culture of plant or fungus alone. Thus, *t*-cinnamic, *p*-coumaric, ferulic, syringic, and vanillic acids were assigned to the plant metabolism and were induced within 2 d after *Fusarium* inoculation. Biological tests confirmed the capacity of induced root exudates to specifically inhibit the germination of *F. graminearum*. Simultaneously to its root exudation, *t*-cinnamic acid was accumulated in the intracellular compartment. In vivo labeling experiment demonstrated that the secreted *t*-cinnamic acid was *de novo* biosynthesized within 2 d during fungal infection. Microscopic analysis revealed that after *Fusarium* attack nonlignin cell wall phenolics were induced not only in necrotic zones but in all root tissues. Results suggest that the plant recognized the pathogen attack and responds by reallocation of carbon, *de novo* biosynthesis and secretion of compounds with repellent functions.

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Volatile Chemical Cues Involved in Tomato–Whitefly Interactions

Bleeker P, Diergaarde P, Ament K, Haring M, de Both M, and Schuurink R

Keygene NV, Agrobusiness Park 90. P.O. Box 216, 6700 AE Wageningen, NL (PB, PD, MdB); University of Amsterdam, Swammerdam Institute for Life Sciences, Dept. Plant Physiology, Kruislaan 318, 1098 SM Amsterdam, NL (PB, PD, KA, MH, RS)

Corresponding author: r.s.schuurink@uva.nl

Plant semiochemicals play an important role for insects in locating a host plant. We investigated which volatile cues might be involved in repellence of the pest *Bemisia tabaci* (sweet potato whitefly), a well-known vector of many devastating viruses. Free-choice bioassays with 16 wild and 5 cultivated tomatoes revealed a clear preference for the cultivated plants and a differential preference for wild tomatoes. Host choice was not different for whitefly biotype B and Q. We also analyzed the complete headspace-metabolome of all tomatoes by GC-MS. This data set was combined with the preference behaviour of the whiteflies and subjected to multiple-linear regression and MANOVA analyses. This resulted in the identification of 8 terpenoids that putatively influenced the choice behaviour of *B. tabaci*. Indeed, cultivated tomato plants could be made significantly less attractive by some of these terpenoids, which are normally produced by the glandular trichomes. To identify the terpene synthase that produce these terpenoids, we sequenced the trichome-transcriptome of one cultivated and two wild tomatoes. Using the massive parallel sequencing technology of 454 Life Sciences (GS-flex), we could annotate 20 contigs as terpene synthases. The cDNAs of the sesquiterpene synthases were expressed in *E. coli* to determine the activities of the recombinant proteins. We also cloned the promoters of three terpene synthases and produced transgenic tomato plants with these promoters driving the GUS-reporter gene. The transgenic plants show that these promoters are exclusively expressed in the glandular trichomes. In summary, we now have the tools to engineer the production of volatile terpenoids in tomato trichomes. This will enable us to investigate whether the *in planta* produced terpenes do indeed influence the choice behaviour of *B. tabaci*.

Volatile Signatures from Potato Mediate Oviposition in Females of the Guatemalan Moth *Tecia solanivora* (Lepidoptera: Gelechiidae)

Karlsson MF, Birgersson G, Bosa CF, Cotes AM, Bengtsson M, and Witzgall P

Chemical Ecology Group, Department of Plant Protection Biology, SLU Alnarp, Sweden (MFK, GB, MB, PW); Biological Control Laboratory, Colombian Corporation of Agricultural Research, Las Palmas, Colombia (CFB, AMC)

Corresponding author: peter.witzgall@phero.net

The Guatemalan potato moth *Tecia solanivora* has become the most devastating pest of potato in Central America and adjacent South American countries. Guatemalan potato moth has only recently emerged as a pest of potato, probably due to host plant shift from a native solanaceous plant to potato (1,2). In potato fields, gravid *T. solanivora* females lay eggs close to plants in soil crevices, the larvae feed inside the tubers. In storage facilities, females oviposit directly on tubers. Potato foliage, in three phenological stages from sprouting to flowering, releases a wide range of aliphatic compounds, benzenoids, phenylpropanoids, and monoterpene hydrocarbons. Most of these compounds were also released from tubers. In contrast, a few compounds were characteristic for tuber headspace. Most interestingly, behaviourally active compounds were present in headspace of flowering potato plants and in tuber headspace. These compounds may thus elicit attraction of females to both flowering plants and tubers. Flowering coincides with the onset of tuber formation in most potato cultivars and females of Guatemalan potato moth are attracted to flowering potato plants in the field as well as to potato tubers in storage. The headspace of green plants is dominated by ca. 40 sesquiterpene compounds that are almost entirely absent from tuber headspace. This is remarkable, in view of *T. solanivora* oviposition behaviour in response to potted potato plants in a greenhouse. Significantly more eggs were deposited at flowering plants, compared to plants in the preflowering stage. Females were attracted to flowering plants by upwind flight, but the eggs were laid in the soil surrounding the plants, not on the stems or foliage. Further bioassays established a repellent effect of green leaf potato volatiles on ovipositing female moths. Some of the active compounds are biosynthetically related to toxic compounds that decrease larval survival. The occurrence of these toxic compounds in different plant organs correlates to the emission of repellent volatiles that signal the presence of deleterious nonvolatile compounds. Female attraction and oviposition behaviour in Guatemalan potato moth is encoded by a combination of attractant and repellent volatiles.

This work is supported by SIDA (www.sida.se) and IC-E3 (www.ice3.se).

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2. J. Econ. Entomol. (2006) 99:1245–1250.

Plasticity in the Self-Incompatibility System of *Solanum carolinense* and Its Impact on Volatile Production

Stephenson AG, Delphia CM, Mena-Ali JI, Mescher MC, and DeMoraes CM

Center for Chemical Ecology and Department of Biology, The Pennsylvania State University, University Park, PA (AGS); Center for Chemical Ecology and Department of Entomology, The Pennsylvania State University, University Park, PA (CMD, MCM, CMM); Department of Biology, Amherst College, Amherst MA (JIM)

Corresponding author: as4@psu.edu

Horsenettle weed, like other solanaceous plants, exhibits RNase-mediated gametophytic self-incompatibility (SI). SI is uncommon in weedy species that are short lived and undergo repeated cycles of colonization and extinction. We examined the effects of floral age, prior fruit production, and genotype on the strength of SI and found that the SI response in horsenettle is a plastic trait: self-fertility varies with flower age and conditions of low fruit production. Consequently, horsenettle can self-fertilize when outcross pollen is limited, but avoid the adverse effects of inbreeding when outcross pollen is available. Moreover, genotypes bearing the S9 allele have higher self-fertility than genotypes bearing other S-alleles. To examine the consequences of inbreeding on plant–insect interactions, we grew ramets of inbred and outbred plants from 8 maternal families under field conditions; collected the VOCs that were produced by these ramets; and analyzed the volatiles using GC-MS. We found that inbred plants released significantly lower quantities of volatiles compared with outbred plants and that there was a significant maternal family effect on the total amount and blend of volatiles released. In addition, we found a breeding-by-family interaction for the total amount and blend of volatiles, indicating genetic variation among families for inbreeding depression. Analyses also revealed broad-sense heritability of volatiles among genets. In the lab, we found that caterpillars preferred leaf disks from inbred plants and that caterpillars had higher relative growth rates (RGR) and total leaf consumption (TC) on the inbred plants. We also found a breeding-by-family interaction for insect RGR and TC, indicating variation among families for inbreeding depression. Our findings show that inbreeding can alter the volatile cues available to foraging insects in wild plants and they demonstrate genetic variation for volatiles in a noncultivated species under field conditions. Thus inbreeding can potentially alter volatile mediated plant–herbivore interactions.

Plasticity in Volatile-Based Host Recognition of a Specialist Moth, Grape Berry Moth (*Paralobesia viteana*)

Cha DH, Linn CE, Roelofs WL, and Loeb GM

Department of Entomology, New York State Agricultural Experimental Station, Cornell University, Geneva, NY

Corresponding author: dhc28@cornell.edu

The role of host volatiles in host recognition has been suggested to be more pronounced in specialist than generalist herbivores (1). However, whether generalist and specialist herbivores use different modes of volatile-mediated host recognition has received little attention. As a first step, we tested whether a specialist moth, grape berry moth (GBM, *Paralobesia viteana*), uses nonubiquitous species-specific plant volatiles or can use ubiquitous plant volatiles to locate its preferred host tissue, grape shoots (2), and we examined which volatile compounds are essential to elicit upwind flight in the flight tunnel. From grape shoots, using SPME and GC-EAD, we found 11 ubiquitous plant volatiles {(Z)-3-hexen-1-yl acetate, [Z]; (E)-linalool oxide, [ELO]; (Z)-linalool oxide, [ZLO]; nonanal, [N]; linalool, [L]; (E)-4,8-dimethyl-1,3,7-nonatriene, [T]; methyl salicylate, [M]; decanal, [D]; β -caryophyllene, [C]; germacrene-D, [G]; α -farnesene, [F]} that elicited consistent EAD activity. In the flight tunnel, the complete 11-component blend elicited 68.6% upwind flight, which was not statistically different from live shoots (73.6%). However, not all 11 volatile compounds were necessary for eliciting the upwind flight response. In subtraction tests, we identified two different 7-component blends [blend 1: ELO, ZLO, N, T, D, C & G; blend 2: Z, L, T, M, C, G & F] that elicited the same level of upwind flight compared with the complete blend (66.7 and 51.6%, respectively). Interestingly, for some compounds, context seemed to be an important factor in determining whether it was essential to elicit strong upwind flight. For example, we could remove Z, L, M, and F from the 11-component blend and still achieve equivalent levels of attraction. Removing any of the remaining 7 compounds singly from Blend 1 significantly reduced upwind flight levels, suggesting that some or all of these seven compounds in blend 1 are essential to maintain attractiveness. However, with blend 2, we found that Z, T, and M were essential components. The finding that some compounds, such as Z and M, acted as essential components in one blend but not in another suggests that some plasticity exists in the volatile-based host recognition system of this specialist moth. Our results further suggest that the context based on the presence of different volatile compounds may play a role in providing specificity in host recognition to the specialist herbivores that use ubiquitous volatiles as host finding cues. The role of background volatiles in the host recognition will be further tested in comparison with generalist herbivores.

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2. J. Chem. Ecol. (2008) 34:622–627.

Relationship of Antennal ORN Response Profiles with Host- and Nonhost Plant Volatiles in the Codling Moth, *Cydia pomonella* (Lepidoptera: Tortricidae)**Park KC, Mitchell VJ, White N, El-Sayed AM, and Suckling DM**

HortResearch, Lincoln, New Zealand

Corresponding author: kpark@hortresearch.co.nz

Olfaction is a major sensory modality in insects, and many have species-specific sets of olfactory receptor neurons (ORNs) for locating their proper hosts. The response profiles of these ORNs are directly related to their host specificity and location process. In single cell recording studies using *Cydia* species as model species, several different types of ORN populations were identified from both male and female codling moths. The major ORN populations could be classified into three different groups. Each of the first group of ORNs was highly sensitive to ethyl-2,4-decadienoate (pear ester) and α -farnesene, respectively, that are major behaviourally active volatile compounds in apple or pear trees, the host plants of the codling moth. Each of the second group of ORNs was highly sensitive to citral, geraniol, linalool, and α -terpineol, respectively, that are not present in apple or pear trees but are the major volatiles in other nonhost fruit trees or vines, such as kiwifruit, plum, apricot, nectarine, peach, lemon, orange, and grape. The third group of ORNs was sensitive to benzaldehyde, ethyl butanoate and green-leaf volatiles, such as (*Z*)-3-hexenol and (*E*)-2-hexenal that are common in many plants. We hypothesize that the first group of ORNs as agonist ORNs detecting host-specific volatile compounds and the second group of ORNs as antagonist ORNs detecting nonhost-specific volatile compounds. Combinational input from these two different groups of ORNs may play a key role in discriminating host plants from non-host plants. We also hypothesize that the third group of ORNs, responding to common volatiles, may not be used to distinguish between host plants and nonhost plants, but to recognise a target as a plant. Therefore, input from these ORNs may also be required to be recognised as a suitable host-plant. Based on this finding, it is suggested that the primary recognition as a proper host plant could be made using a combination of information from these three groups of ORNs. The results also indicate that the range of host plants may be predictable by using information on ORN response profiles of a given species and on volatile chemical profiles of the candidate plants.

Pheromone Identification, Biosynthesis

Moderator:

14.00–15.30 **Ring Cardé**, University of California-Riverside
15.50–17.20 **Jocelyn Millar**, University of California-Riverside

Room 11, Life Sciences Building



PARALLEL SESSION 6 | THURSDAY, 21 AUGUST

SUN MON TUE WED THU

Proactive Chemical Ecology: Identification of a Novel Dienynal Structure as the Sex Pheromone of an Invasive Pest Species, the Avocado Seed Moth, *Stenoma catenifer*

Millar JG, Hoddle M, McElfresh JS, Zou Y, and Hoddle C

Department of Entomology, University of California, Riverside, CA 92521

Corresponding author: jocelyn.millar@ucr.edu

The avocado seed moth, *Stenoma catenifer*, is one of the major insect pests infesting commercial avocado orchards from Mexico to South America. *Stenoma catenifer* can cause so much damage that it has limited and even prevented commercial avocado cultivation. This insect has not yet been found in avocado production areas of the United States in southern California, Florida, or Hawaii, but U.S. Department of Agriculture risk assessments have identified *S. catenifer* as one of the most serious potential threats to the U.S. avocado industry (1). As part of a proactive effort to provide growers, exporters, and regulatory agencies with a sensitive and reliable method for detection of *S. catenifer* before it arrives in the United States, we initiated a project to identify and synthesize the pheromone of this insect. We report here the identification, synthesis, and preliminary testing of this novel pheromone structure, and several other potential components identified from pheromone gland extracts (2). Practical problems related to carrying out a project of this type, where the target insect is not yet present in the country sponsoring the work, are discussed.

We thank the California Avocado Commission for financial support of this work.

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2. Tetrahedron Lett. (2008), doi: 10.1016/j.tetlet.2008.06.019.

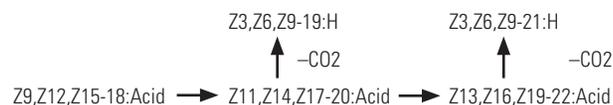
Identification of C₂₁ Type II Sex Pheromone Components and Novel C₂₀ and C₂₂ Trienyl Biosynthetic Precursors from a Wasp Moth, *Syntomoides imaon* (Arctiidae: Syntominiæ)

Ando T, Matsuoka K, Yamamoto M, Muramatsu M, and Naka H

Graduate School of Bio-Applications and Systems Engineering (BASE), Tokyo University of Agriculture and Technology, Koganei, Tokyo 184-8588, Japan (TA, KM, MY); The Ayamiha-biru Museum, Yonaguni-cho, Yaeyama-gun, Okinawa 907-1801, Japan (MM); JT Biohistory Research Hall, Takatsuki, Osaka 569-1125, Japan (HN)

Corresponding author: antetsu@cc.tuat.ac.jp

Gas chromatography-mass spectrometry (GC-MS) and GC-electroantennographic detection analyses of the pheromone extract from a wasp moth, *Syntomoides imaon* (Arctiidae: Syntominiæ), showed that the virgin females produced (Z,Z,Z)-3,6,9-henicosatriene (Z₃,Z₆,Z₉-21:H) and (Z,Z,Z)-1,3,6,9-henicosatetraene with a trace amount of their C₂₀ analogues. The chemical structures were elucidated by comparing with authentic standards (1); furthermore, the positions of the double bonds were confirmed by chemical reactions, i.e., dimethyl disulfide derivatization of monoens produced by a diimide reduction. In a field in the Yonaguni-jima Islands, males of the diurnal species were captured by traps baited with a 1:2 mixture of the synthetic C₂₁ polyenes. In addition to the pheromone components, lipids extracted from abdominal integuments, which were associated with oenocytes and peripheral fat bodies, were examined. After fractionation of fatty acid methyl esters (FAMES) derived from the lipids by high-performance liquid chromatography equipped with an ODS column, GC-MS analysis identified methyl esters of (Z,Z,Z)-11,14,17-icosatrienoic acid (Z₁₁,Z₁₄,Z₁₇-20:Acid) and (Z,Z,Z)-13,16,19-docosatrienoic acid (Z₁₃,Z₁₆,Z₁₉-22:Acid). These novel C₂₀ and C₂₂ acids are longer chain analogues of linolenic acid, (Z,Z,Z)-9,12,15-octadecatrienoic acid (Z₉,Z₁₂,Z₁₅-18:Acid). The C₂₂ acid is presumed to be a direct biosynthetic precursor of the *S. imaon* pheromone because the C₂₁ trienyl component can be formed by decarboxylation of the C₂₂ acid. Furthermore, the C₂₀ acid, but not the C₂₂ acid, was found in FAMES of *Ascotis selenaria cretacea* (Geometridae), which secreted C₁₉ pheromone components (Z,Z,Z)-3,6,9-nonadecatriene (Z₃,Z₆,Z₉-19:H) and the monoepoxy derivative (2). These results indicate that difference of their chain elongation systems might play an important role in producing species-specific type II lepidopteran sex pheromones (3) composed of polyunsaturated hydrocarbons and/or epoxy derivatives, as shown in the proposed biosynthetic pathways for polyenyl pheromone components starting from linolenic acid.



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Response of Females Toward a Combination of Male Sex Pheromone and Host Volatiles in *Hedypathes betulinus* (Coleoptera: Cerambycidae)

Fonseca MG and Zarbin PHG

Laboratory of Semiochemicals, Department of Chemistry, PO 19081, Centro Politecnico, Curitiba-PR-Brazil

Corresponding author: pzarbin@quimica.ufpr.br

Hedypathes betulinus (Klug, 1825) is the most serious pest of green mate, *Ilex paraguariensis*, of the southern region of Brazil. Damage inflicted to the branches and trunks by wood-feeding larvae causes mortality of plants and reduction in production. Three male-specific compounds were identified as (*E*)-6,10-dimethyl-5,9-undecadien-2-acetate, (*E*)-6,10-dimethyl-5,9-undecadien-2-one, and (*E*)-6,10-dimethyl-5,9-undecadien-2-ol (1). The objectives were to test the attractiveness of the male sex pheromone of *H. betulinus* in the laboratory and to identify the site of pheromone-producing glands. The behavioural response of females to synthetic racemates, host volatiles alone, and synthetic racemates plus host volatiles in two dosages was evaluated using Y olfactometer. The results showed that the host plant, major component alone and ternary mixture were not significantly attractive to female, but the combination of major component plus host volatiles attracted significantly more females. The ternary mixture at higher dosage was attractive by itself, but when combined with host volatiles attracted ten times more than ternary mixture alone, suggesting that host volatiles enhance female attraction to male sex pheromone. To identify the site of pheromone-producing glands, volatiles were collected from different male body regions: head, prothorax, elytra, meso + metathorax, and abdomen. Pheromone was detected in high abundance on the prothorax, suggesting that this is the region where pheromone glands are found. To confirm this observation, studies with scanning electron microscopy and histological sectioning are now in progress.

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Behavioral Evidence for a Contact Cue Used by Male Emerald Ash Borers, *Agrilus planipennis* Fairmaire (Coleoptera: Buprestidae), to Determine Conspecific Gender

Lelito JP, Böröczky K, Jones TH, Fraser I, Mastro VC, Tumlinson JH, and Baker TC

Center for Chemical Ecology, Department of Entomology, The Pennsylvania State University, University Park, PA (JPL, JHT, KB, TCB); USDA-APHIS-PPQ, Brighton, MI (IF, VCM); VMI Chemistry Department, Lexington, VA (THJ)

Corresponding author: jpl207@psu.edu

Emerald ash borers (EAB), *Agrilus planipennis*, were sampled for cuticular hydrocarbon profile using modified solid-phase microextraction (SPME) fibers to determine whether there are differences in these compounds between genders. We then assessed feral male EAB for behavioral changes based on the application of a female specific compound to dead, solvent-washed beetles. Males spent significantly more time attempting copulation with dead, pinned female beetles coated with a three-beetle-equivalent dose of 3-methyl-tricosane than with solvent-washed beetles or with those coated in 3-methyl-tricosane at lower concentrations. Males spent the most time investigating dead, unwashed female pinned beetles. In the laboratory, sexually mature males were presented with one of several mixtures applied in hexane to filter paper disks or to the elytra of dead female beetles first washed in solvent. The presence of a beetle as a substrate for chemical application made a significant difference in male investigation time in some cases, compared with applying a droplet of the same solution to the filter paper. Male EAB also spent more time in investigation of beetles and solution applications that contained 3-methyl-tricosane than solutions containing male body wash or a straight-chain hydrocarbon not found on the cuticle of EAB.

What a Difference a Coat Makes: Cuticular Hydrocarbons as Contact Sex Pheromone Components of the Woodwasp *Sirex noctilio*

Böröczky K, Crook DJ, Jones TH, Zylstra KE, Mastro VC, and Tumlinson JH

Center for Chemical Ecology, Department of Entomology, The Pennsylvania State University, University Park, PA (KB, JHT); USDA, APHIS, PPQ, Otis ANGB, MA (DJC, VCM); Virginia Military Institute Chemistry Department, Lexington, VA (THJ); USDA, APHIS, PPQ, North Syracuse, NY (KEZ)

Corresponding author: kub13@psu.edu

In North America, *S. noctilio* is a major pest of several pine species. Previous studies reported that mating occurs on the trunk higher up in the canopy and that antennal contact plays a key role in the courtship behavior (1). To test this in a bioassay, freeze-killed females were dipped in hexane. Most of the males attempted copulation after tapping female models with reapplied body-wash, whereas washed females did not elicit such response. Analysis of the hexane extract revealed saturated and unsaturated hydrocarbons as the major components of the female cuticle. Behavior-guided fractionation of the female body-wash lead to the identification of several contact sex pheromone components of which the most active compounds are (Z)-monoalkenes in the range of C27–C29.

1. Trans. R. Soc. N. Z., Zool. 7:195–204.

Behavioral and Electrophysiological Responses of Emerald Ash Borer, *Agrilus planipennis* (Coleoptera: Buprestidae) to Female-Produced Macrocyclic Lactone and to Ash Bark Volatiles

Cossé AA, Bartelt RJ, Zilkowski BW, Fraser I, Crook DJ, Khrimian A, and Mastro VC

Crop Bioprotection Unit, USDA–ARS–NCAUR, Peoria, IL (AAC, RJB, BWZ); USDA–APHIS–PPQ, Brighton, MI (IF); USDA–APHIS–PPQ, Otis, MA (DJC, VCM); USDA–ARS–IIBBL, Beltsville, MD (AK)

Corresponding author: allard.cosse@ars.usda.gov

Emerald ash borer (EAB), *Agrilus planipennis* Fairmaire (Coleoptera: Buprestidae), is an invasive beetle species from Asia that has caused extensive mortality of ash trees (*Fraxinus* spp.) since arriving in the United States in 2002. Especially hard hit are green ash (*F. pennsylvanica*), black ash (*F. nigra*), and white ash (*F. americana*). Since its detection in the Detroit area, the beetles have rapidly spread throughout Michigan and into portions of Illinois, Indiana, Ohio, Pennsylvania, Maryland, West Virginia, and Ontario, Canada. EAB detection methods, especially in low infestation areas, relied heavily on visual surveys and the use of girdled trap trees to detect the presence of the beetles. Both methods are labor-intensive and relatively expensive and improved methods of beetle detection are urgently needed. Semiochemical-baited traps for monitoring EAB are currently under development (1,2,3). Pheromones have been used to detect pest insects, but none have so far been identified in the Buprestidae. We have identified a predominantly female-produced macrocyclic lactone, (3Z)-dodecen-12-olide, that could serve as a pheromone in EAB (4). The behavioral effects of this lactone is discussed. Girdled green ash trees have an elevated release of volatile bark sesquiterpenes compared with ungirdled trees, and GC-EAD studies showed six antennally active sesquiterpenes for both male and female EAB. Five of these six compounds were identified as β -cubebene, β -copaene, 7-epi-sesquithujene, trans- β -caryophyllene, and β -humulene (2). At NCAUR, GC-EAD studies with white ash bark volatiles showed two active sesquiterpenes, 7-epi-sesquithujene and previously unidentified (–)-eremophilene. The key isolation procedure for the two compounds was silver nitrate/silica HPLC. Identification was by GC-MS, NMR, polarimetry, and microchemical reactions. The essential oil of the Manuka tree (*Leptospermum scoparium*) contains many different volatile compounds including five of identified sesquiterpenes with the exception of 7-epi-sesquithujene (5). Similarly, the oil from the Brazilian walnut tree, *Phoebe porosa*, contains at least five of the identified compounds (6). Sticky traps baited with either Manuka or Phoebe oil have been shown to attract male and female EAB in the field (2). Isolation and purification methods are being developed to obtain field bait quantities of (–)-eremophilene from Buddah wood (*Eremophila mitchelli*) oil and the behavior effects of this compound and others will be discussed in light of an effective semiochemical-based monitoring system for EAB.

1. J. For. (2006) 104:118–124.
2. Environ. Entomol. (2008) 37:356–365.
3. J. Entomol. Sci. (2005) 40:93–95.
4. J. Chem. Ecol. (2007) 33:1299–1302.
5. Phytochemistry (2004) 65:1255–1264.
6. Flavour Fragrance J. (1994) 9: 179–186.

Sex Pheromone of the Plant Bug *Phytocoris calli* Knight (Heteroptera: Miridae)

Zhang Q-H and Aldrich JR

Sterling International, Inc., Bldg 16BV, 3808 N. Sullivan Rd., Spokane, WA 99216 (Q-HZ); USDA–ARS Invasive Insect Biocontrol and Behavior Laboratory, BARC-West, B-007, 10300 Baltimore Ave., Beltsville, MD 20705 (JRA)

Corresponding author: qing-he@rescue.com

Female *Phytocoris calli* Knight produce a sex pheromone from metathoracic scent glands. The pheromone consists of hexyl acetate (HA; present in both sexes), with the female-specific compounds (*E*)-2-hexenyl acetate (E2HA), octyl acetate (OA), and (*E*)-2-octenyl acetate (E2OA). HA and E2OA are key components of the pheromone, because deletion of either ester from the blend resulted in a total shutoff of conspecific male attraction. However, the binary blend of HA and E2OA was only slightly attractive to males, and it was significantly less active than the four-component blend. The two ternary blends, HA/OA/E2OA and HA/E2HA/E2OA, were each as attractive as the full four-component blend, but removal of both E2HA and OA from the full blend dramatically reduces the attraction of *P. calli* males. Evidence from previous research on the pheromones of *Phytocoris* species suggests that the apparent chemical redundancy in the pheromone of *P. calli* may actually be involved in maintaining reproductive isolation from other sympatric species. The patterns observed for pheromones of the five *Phytocoris* species whose pheromones have been directly (*P. californicus*, *P. relativus*, *P. difficilis*, and *P. calli*) or indirectly (*P. brevisculus*) studied are discussed vis-à-vis the pheromone intractable species of *Lygus* and *Lygocoris* plant bugs.

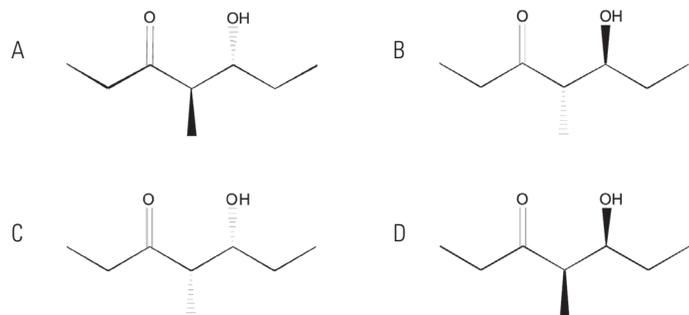
Use of Plant Tissues Toward a New Synthesis of All Stereoisomers of 5-Hydroxy-4-methyl-3-heptanone—Potential Sex Pheromones of *Sitona discoideus*

Bohman B and Unelius CR

School of Pure and Applied Natural Sciences, University of Kalmar, Kalmar, Sweden

Corresponding author: bjorn.bohman@hik.se

The *syn* isomers (C and D) were synthesized by a selective Diels Alder reaction from 3-pentanone and propanal (1) and the *anti* isomers (A and B) from a Baker's yeast reduction of 4-methyl-3,5-heptadione (2), which was prepared from 3,5-heptanedione and iodomethane (3). Commercial lipases were used to obtain the pure enantiomers of hydroxy ketones and keto acetates from the pair of diastereomers, but the keto acetates could not be transformed back to the corresponding alcohols using lipases. Chemical hydrolysis failed in alkaline as well as acidic conditions due to the acidic α hydrogen and elimination of the formed alcohol occurred instantly. We found that the hydrolysis of keto acetates was easily carried out using whole plant tissues from various roots, e.g., carrot and beetroot. The hydrolysis of the corresponding keto benzoates, which are diastereomerically separable by column chromatography, also was investigated. This reaction, which also failed using lipases and chemical hydrolysis, succeeded using potato and sweet potato tissues and is potentially interesting for hydrolysis in general deprotection sequences of alcohols. Finally, we attempted to replace the low yielding Baker's yeast in the diketone reduction in the preparation of the *anti* isomers. Manioc, sunroot, and parsnip tissues carried out this reaction in various yields. All reactions were enantioselective. Our preliminary results show that the reduction of diketone gave the *S,S*-hydroxy ketone at >90% *ee* without any optimisation.



Four stereoisomers of 5-hydroxy-4-methyl-3-heptanone

1. JACS (1991) 113:1047.
2. Tetrahedron. Lett. (1986) 27:565.
3. Eur. J. Org. Chem. (2006) 2309.

Terminal Steps in Ipsdienol Biosynthesis in *Ips* spp.: An Oxidoreductase Determines the Final Stereospecificity of the Pheromone Blend

Blomquist GJ, Figueroa Teran R, Gilg A, Sandstrom P, Vanderwel D, and Tittiger C

Department of Biochemistry and Molecular Biology, University of Nevada, Reno, NV (GJB, TRF, AG, PS, CT); Department of Chemistry, University of Winnipeg, Winnipeg, Canada R3B2E9 (DV)

Corresponding author: garyb@cabnr.unr.edu

Ips spp. produce most of their pheromone components *de novo* in midgut tissue through a process that involves a geranyl diphosphate synthase (1) that also functions as a myrcene synthase. A cytochrome P450 (2) hydroxylates myrcene to ipsdienol, producing an 80:20 mixture of the (-)/(+) enantiomers in both *Ips pini* [which uses a pheromone blend of 95:5 (-)/(+)] and *Ips confusus* [which uses a pheromone blend of 10:90 (-)/(+)]. The final step in pheromone production that determines ipsdienol stereospecificity seems to involve an oxidoreductase. Microarray and RT-PCR data showed that three oxidoreductases were present in midgut tissue, and one is up-regulated in midgut tissue and demonstrates a much higher basal level in males compared with females, similar to other pheromone producing genes. This expressed oxidoreductase readily converts ipsdienol to ipsdienone in the presence of NADP, and reduces ipsdienone to ipsdienol in the presence of NADPH. The stereochemistry of the ipsdienol substrates and products from ipsdienone is under investigation.

This work was supported by National Science Foundation grant 0719279 and the Nevada Agricultural Experiment Station.

1. Proc. Natl. Acad. Sci. USA (2005) 102:9760–9765.
2. Insect Biochem. Mol. Biol. (2006) 36:835–845.

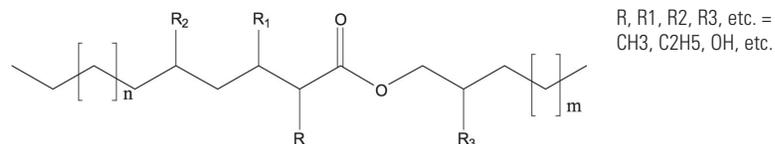
1D and 2D NMR Techniques to Elucidate Structures of Complex Mixtures of Long Chain Esters of Uropygial Gland Secretions of Birds

Haribal MM, Oswald RE, Dhondt AA, and Rodriguez E

Cornell Lab of Ornithology Cornell (MMH, AAD); Department of Molecular Medicine (RO); Plant Biology, Cornell University, Ithaca, NY (ER)

Corresponding author: mmh3@cornell.edu

Birds possess a holocrine gland on the rump, known as uropygial gland. The gland produces semi fluid secretion, which is used to apply to the feathers to maintain them in good conditions. The secretion contains mixtures of both polar and major nonpolar waxy components. Waxy components are mostly made up of esters of long-chain acids and alcohols. These long-chain components are usually substituted by methyl, ethyl, and sometimes hydroxyl groups at various positions of the carbon chains of both alcohol and acid components of the esters.



GC-MS is one of the most commonly used techniques to separate and identify the mixture. Due to complexity of the mixtures, individual components cannot be easily separated from each other by GC-MS. MS-MS technique does give some limited insight to some details of the structures of these components. We used NMR techniques (1D and 2D) to reveal the structural complexity of the mixture. ^1H NMR helps in locating types of substitutions close (α to γ substitutions) to carbonyl and alcohol groups of esters. ^{13}C NMR is much more useful in deducing branching as ^{13}C shifts are based on electronegativity of the substitutions, hybridization and γ -gauche effects. The substitutions can have affect on the chemical shifts of carbons that are as far removed as the δ position. Thus, highly branched compounds can be recognized by the carbon shifts of the substituted carbons. Furthermore, the 2D techniques such as DQCOSY, HSQC can help in elucidation of the structures. Using ^1H and ^{13}C predictions (Chemdraw software) and comparing with a known long chain ester hexadecyl hexadecanoate, we analyzed the components of the uropygial gland secretion of house sparrows. We also show that the phylogenetically different species show different NMR patterns, thus proving that the components differ widely in different species, although they have very similar physical properties. Thus, NMR techniques could be used as an analytical tool for studying chemophylogeny of uropygial gland secretions of birds.

Mating Behavior and Pheromone Studies of *Lasiodactus pictus* (Coleoptera: Nitidulidae)

Abdullah F

Institute of Biological Sciences, Faculty of Science, University of Malaya, 50603 Kuala Lumpur
Corresponding author: fauziah@um.edu.my

Adult *Lasiodactus pictus* ($N = 364$) were obtained from damaged mango fruit ($N = 104$) collected from six *Mangifera indica* farms in Perlis, Malaysia. Mating was observed between intact beetles in cut fruit and in petri dishes. Males were attracted to females in a T-shaped olfactometer. Mating between deantennated and intact beetles showed that antennae and vision are important for mate recognition. Three minutes 31 s is needed before mounting occurs between intact pairs, which lasts for 3 min 21 s, with peak mating time at 11:00 AM. The significance of this finding toward management of mango pest is discussed.

Chemical Ecology Memories

Speaker: David L. Wood,
Department of Environmental Science, Policy and Management,
University of California, Berkeley, CA
President's Hall, The Penn Stater Conference Center Hotel



BANQUET SPEECH | THURSDAY, 21 AUGUST

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CHEMICAL ECOLOGY MEMORIES

Wood DL

Department of Environmental Science, Policy and Management, University of California, Berkeley, CA 94720

Banquet and Awards Ceremony Speaker: bigwood@nature.berkeley.edu

Chemical communication systems play a major role in the survival of all organisms, from bacteria to primates. The identification of the silkworm moth sex attractant by Butenandt and colleagues in 1959 is often cited as the founding study in chemical ecology. In the same year, Karlson and Lüscher proposed a new term, “pheromones,” for a class of biologically active substances that affect individuals of the same species. Wilson and Bossert in 1963 described these compounds as “releasers” of a behavioral response by the receiving individual or as “primers” that alter development of the receiver. Brown, Eisner, and Whittaker in 1970 proposed new terms for interspecific chemical messages where the message benefits the sender (allomone) or the receiver (kairomone). Whittaker and Feeny in 1971 classified the chemical interactions that affect the growth, health, behavior, or population biology of individuals of other species as “allelochemical” interactions. In 1971, Law and Regnier defined all chemicals acting as messages between organisms as “semiochemicals.” Within a decade the “language” of chemical ecology had been created to characterize chemical interactions among and between species. These interactions are among the most important components of ecological investigations.

In the period leading up to the first issue of the *Journal of Chemical Ecology* in 1975, biologically active compounds were identified from many organisms in diverse taxa: water mold, slime mold, honey bee, bark and ambrosia beetles, carpet beetle, boll weevil, ground beetle, cabbage looper, redbanded leaf roller, Indianmeal moth, pink bollworm, European corn borer, monarch, leaf-cutting ant, house fly, millipede, whip-scorpion, minnow, boar, black-tailed deer, pronghorn antelope, rhesus monkey, and many more. Also during this period many new biological phenomena were discovered, e.g., multicomponent chemical stimuli, synergism among components, interruption/disruption, structural and enantiomeric specificity, interspecific interactions, releaser and primer effects by the same compound, and others. At the same time, an amazing diversity of new biologically active chemicals was discovered. These studies were summarized in more than 10 books published during this period.

The International Society of Chemical Ecology (ISCE) was founded in 1983 and the first annual meeting was held in Austin, TX. Lincoln Brower was the first president. The Silver Medal for career achievement in chemical ecology was first awarded in 1984 in Berkeley, CA, to Robert M. (Milt) Silverstein, and in 1995 in Los Andes, Chile Wilhelm Boland received the first Silverstein/Simeone Lecture Award for significant mid-career scientific achievement. I chose to review the work of the winners of these awards as an indicator of the trends in research of the *ISCE*. I assumed that this would be representative of the membership. Awardees were classified by scientific discipline, organism(s) studied, ecosystem function (producers, consumers, and de-

composers), feeding habits of consumers, and ecosystem elements. An analysis of the contributions of the 37 award winners reveals that chemists/biochemists/molecular biologists were dominant (27/37) and insects were, by far, the most studied organisms (34/37), especially, beetles, moths, and ants. All organisms studied are consumers and most are herbivores. However, the *ISCE* awards probably under represent investigations with a greater focus on ecology. Many frontiers remain to be explored with other taxa in order to build a foundation for the study of interacting species at the guild and community levels of biological organization. The few investigations of tritrophic interactions among herbivorous insects, parasitoids and predators, and plants are important steps towards this goal. There is a significant trend in research on the physiology and molecular biology of the transduction of chemical signals among organisms. Meanwhile, we applaud our colleagues who have discovered many new compounds (alkaloids, amino acids, fatty acid derivatives, proteins, shikimic acid derivatives, terpenes, and others) that serve and may serve to benefit human survival.

This theme was developed with the collaboration of Jim Tumlinson, Julie Tillman, and Steve Seybold.

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Cover photo by
BILL COLEMAN

“Pastorale” is one of the most beautiful images ever made by local photographer Bill Coleman. Best known for his photos of a remote and barely known Amish village, his work shows a sensitivity to, and a genuine enthusiasm for, the gestures and character of the Amish people. Through these images he reveals to us an America that once was - and possibly still is.

For more information about Bill Coleman's 36 year photographic odyssey, visit www.amishphoto.com



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