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## **Chemical Ecology Symposium, Hawaii**

In early March, 2006, the Pacific Branch of the Entomological Society of America held its annual meeting on the island of Maui. During this meeting, a half-day symposium was held entitled "Symposium: Chemical Ecology – Discoveries and Applications in the Pacific States". Organizers were: Leonardo De A. Camelo, Christelle Guedot, Peter J. Landolt, Richard S. Zack.

Below are the abstracts from this symposium.

### **MODELS AND METHODS IN CHEMICAL ECOLOGY**

John A. Byers

USDA-ARS, US Arid-Land Agricultural Research Center  
21881 North Cardon Lane, Maricopa, AZ

**Abstract** – "Chemical ecology concerns the origin, function, and significance of natural chemicals that mediate interactions within and between organisms" (adapted from the Journal of Chemical Ecology). Chemical ecology is an interdisciplinary area primarily based on ecology, which includes interfaces to physiology, evolution, genetics, and behavior. In addition, the natural chemicals are studied by chemical means (analytical chemistry for identification and synthetic chemistry for synthesis). Therefore, the models and methods in chemical ecology are as varied as the subject areas above and cannot be covered in any detail in a short presentation. In this presentation I will cover only a few selected models and methods. The study of the chemical ecology of a species usually begins with observations of the behavioral/physiological phenomenon and then identification of the semiochemicals involved. Little progress is possible without knowing which semiochemicals occur in a particular case, and without having quantities for experiments. Thus, many methods involve the identification process (chemical analysis and bioassay). The GC-EAD (gas chromatography-electroantennographic detection) or behavioral bioassays are routinely used for identifying the compounds of interest that are then elucidated by GC-MS and other chemical methods. Software that aids building heated transfer lines for GC-EAD and in developing GC-oven temperature programs are presented. For determining the best blend of components, the subtractive-combination bioassay is the most efficient fractionation and assay method. The synthetic compounds, if they are suspected to be an attractive pheromone, are then released from traps placed in the field to verify the semiochemical identifications. The rotor trap evens stochastic variation that is normally encountered in the field. The EAR (effective attraction radius) can be used to determine an index of attraction for a particular blend and trap that can be used in optimizing mass trapping. The "diffusion-dilution" method of chemical release from dispensers also aids in optimizing the release rates of semiochemicals. Spatial analysis programs such as MAD can aid in discovery of semiochemicals (for example in bark beetles). Computer-implemented models of

behavior aid our understanding of chemical ecology systems and various semiochemical control methods.

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## **HOW DO THE PATTERNS OF TEMPORAL AND SPATIAL CONTACT WITH AN ODORANT MODULATE BEHAVIORAL RESPONSE?**

Ring T. Cardé

University of California, Department of Entomology, Riverside, CA

As a plume of odor is transported downwind, it is shredded by turbulent forces into a highly discontinuous signal, comprised of filaments of odor of varying concentration interspersed with packets of clean air. Such fluctuations in signal intensity seem requisite for successful upwind orientation in several moths. Males of *Cadra cautella*, however, can fly upwind in a homogenous cloud of pheromone. How generally applicable is a model of filament-induced response? The female mosquito of *Aedes aegypti* also orients upwind after brief contact with a single filament of CO<sub>2</sub>, but its orientation to human skin odor is improved when this odorant encountered as a relatively homogeneous plume. Brief contact with a single filament of CO<sub>2</sub> instantly lowers the threshold for orientation to skin odor, and such sensitization suggests that CO<sub>2</sub> may be the initial odor used by mosquitoes in host orientation. Finally, conditioning of the proboscis extension response of the honeybee seems contingent on 100s of ms contact with the conditioning odorant. Complex and variable mixtures of odors appear to require a comparatively long exposure time before behavioral response, whereas relatively simple and invariant odorants may induce response following a brief encounter.

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## **A VOLATILE SEX PHEROMONE IN *PRIONUS CALIFORNICUS* (MOTS) (COLEOPTERA: CERAMBYCIDAE) BEETLES**

Daniel E. Cervantes<sup>1</sup>, Emerson S. Lacey<sup>2</sup>, Lawrence M. Hanks<sup>2</sup>, Jocelyn G. Millar<sup>3</sup> and, James D. Barbour<sup>1</sup>

<sup>1</sup>University of Idaho, Department of Plant, Soil, and Entomological Sciences, Parma Research and Extension Center, Parma, ID 83660-6699.

<sup>2</sup>University of Illinois at Urbana-Champaign, Department of Entomology, Urbana, IL 61801

<sup>3</sup>University of California-Riverside, Department of Entomology, Riverside, CA 92521

The California prionus (*Prionus californicus* Mots), is widely distributed in western North America from Baja Calif. to British Columbia and Alberta and known to attack many plants including oak, pine, poplar, apple, apricot, grape and it is a serious pest of hop in Idaho. It has been thought for many years that female *P. californicus* use a sex pheromone to attract males for mating. Some traits of *P. californicus* presumably are associated with pheromone production by adult females: 1) males are smaller than females and have larger, more strongly serrate antennae; 2) males are active fliers and frequently are captured in light traps whereas females are more sedentary, apparently

rarely fly, and are rarely captured in traps; 3) adults of both sexes apparently do not feed, having atrophied digestive tracts, and consequently are short-lived (2-3 wk under laboratory conditions). Laboratory and field experiments were conducted during July 2004 and 2005 to determine if male beetles respond to olfactory signals from female beetles or female beetle body parts. Males responded strongly to odors from live females, dead females, and ovipositors excised from females in laboratory olfactometer bioassays. Males were also strongly attracted to cages baited with female beetles experiments conducted in hop fields. We conclude that female-produced sex pheromones allow actively dispersing male *P. californicus* to locate their sedentary mates. Once the sexes are brought into proximity, however, mate recognition apparently is mediated by contact pheromones on the cuticle of the female (Barbour et al. 2006b). This is the first conclusive demonstration of a volatile sex pheromone in a cerambycid species of the subfamily Prioninae, or any of the other more primitive subfamilies of the Cerambycidae.

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## **CHEMICAL ECOLOGY OF PEAR PSYLLA, *CACOPSYLLA PYRICOLA* (HOMOPTERA: PSYLLIDAE)**

Christelle Guédot, David R. Horton, and Peter J. Landolt

USDA-ARS Yakima Agricultural Research Laboratory, Konnowac Pass Road, Wapato,  
WA

*Cacopsylla pyricola* has been a pest of pear trees in the western U.S. for several decades. Insecticides are the main means of control; however, pear psyllids are known to rapidly develop resistance. *Cacopsylla pyricola* is multivoltine and seasonally dimorphic, with a dark overwintering adult and a small, light-colored summerform. *Cacopsylla pyricola* is found in aggregations among trees and in different areas within trees. It has been suggested that summerform males move to plants where high female densities are present. This study addresses the chemical ecology of pear psylla *C. pyricola* in relation to mate location.

To determine if *C. pyricola* females of either morphotype attract males by means of volatile chemicals, a Y-tube olfactometer assay was used. Virgin summerform pear psylla males were attracted by virgin summerform females on pear seedlings (64%) compare to uninfested pear seedlings (36%). Post-diapause winterform males showed similar attraction towards female infested pear shoots (68% vs. 32%). These results strongly support the hypothesis that *C. pyricola* females of either morphotype attract males with volatile chemicals.

Several factors, such as insect age, ovarian maturation (diapause status), presence of host plant, mating status, and circadian rhythm could affect pheromone production and perception. Virgin summerform males showed a preference for 8-10 day old virgin females (76%) versus 2-4 day old virgin females (24%). To address the diapause status, winterform psylla undergoing diapause were treated with fenoxycarb to terminate diapause and tested in the olfactometer. Treated males showed a significant attraction

towards treated females (58%) versus untreated females (42%). Untreated males did not show a preference for either treated (47%) or untreated (53%) females. The presence of the host plant is currently under investigation. Collection of the volatile chemical attractants emitted by pear psylla females will begin as soon as the best conditions for pheromone production and perception have been identified.

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### **7,9-DECADIENOATES: SEX PHEROMONE COMPONENTS OF THE NETTLE MOTH, *DARNA PALLIVITTA* (MOORE)**

Eric B. Jang<sup>1</sup>, Matthew S. Siderhurst<sup>1</sup>, Arnold H. Hara<sup>2</sup>, Patrick Conant<sup>3</sup>, Janice T. Nagata<sup>1</sup>, Esther L. Schneider<sup>1</sup>, & Lori A. Carvalho<sup>1</sup>

<sup>1</sup>US Pacific Basin Agric. Res. Center, USDA-ARS, P.O. Box 4459, Hilo, HI

<sup>2</sup>Dept. Plant Environ. Protect. Sci., CTAHR, University of Hawaii at Manoa, 461 West Lanikaula Street, Hilo, HI

<sup>3</sup> Hawaii Dept. Agric., 16 East Lanikaula St., Hilo, HI

The nettle caterpillar, *Darna pallivitta* (Moore), is an invasive pest on the island of Hawaii, causing defoliation of ornamental nursery stock and posing a human health hazard due to their painful sting. Wind tunnel and field tests with 2 day old moths revealed male behavioral responses to caged females consistent with a female released sex pheromone. Coupled gas chromatography-electroantennogram detection (GC-EAD) analysis of abdominal tip extracts revealed two male electroantennographically active compounds produced by female *D. pallivitta*. Mass spectral analysis and subsequent synthesis identified the active compounds as (*E*)-7,9-decadienoates, both structurally similar to sex pheromone components previous reported from related *Darna* sp. Additionally, a third decadienoate was identified from female abdominal extracts and a strong EAD response was elicited by the synthetic compound. The most abundant decadienoate was the only component detected by solid phase microextraction (SPME) collections from single calling female moths, however the absence of the minor components may be a result of their lower abundance. Field trials showed significant attraction to all lures containing the major pheromone component while the minor components did not increase trap captures at the levels and ratios tested. Synthetic pheromone lures outperformed virgin moths as attractant baits and could be developed for monitoring *D. pallivitta* populations of the island of Hawaii and detection on the other Hawaiian islands.

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### **MANIPULATING PLANT-ARTHROPOD CONVERSATIONS TO IMPROVE CONSERVATION BIOLOGICAL CONTROL**

David G. James, Sandra C. Castle del Conte, Tanya S. Price, Tessa Grasswitz, Victor Reyna, Lawrence C. Wright, Joe Perez, Oscar Garcia and Deborah Brooks.

Department of Entomology, Washington State University, Irrigated Agriculture Research and Extension Center, 24106 North Bunn Road, Prosser, Washington 99350, USA.

Plants talk. Plants listen. Elegant scientific studies in Europe, Japan and the US have demonstrated that plants, when attacked by insects and mites, respond by emitting distress signals. These signals take the form of a complex bouquet of volatile chemicals which is the 'language' plants use to warn neighboring plants that they may too be attacked and should begin defending themselves.

Arthropods listen too. Predatory and parasitic insects and mites are also fluent in plant language and plants 'know' this. Predators and parasitoids understand what the plants are saying and importantly, they know it means there will be food and hosts available. Thus, the chemical dialogue plants produce when attacked benefits the plants as well as the 'bodyguards' they recruit for protection.

Scientists have been eavesdropping on plant-plant and plant-insect conversations in the laboratory for about two decades now. While we are by no means fluent in plant language yet, we do know some of the key 'words' (volatile chemicals) that are used in these conversations. There is no universal plant language, different plant species appear to have their own unique languages, thus grapevine 'conversations' are different from potato 'conversations'. However, there are some words that appear to be fairly common in plant distress conversations. For example, different kinds of plants attacked by spider mites usually emit blends containing methyl salicylate (MeSA). Plants attacked by caterpillars usually emit blends containing methyl jasmonate (MeJA).

This natural defense system perfected over millennia is no doubt a very important part of the armory used by plants for protection. But can we perhaps improve upon it in pest management? Is it possible that we could improve the conversation that plants have with each other and with beneficial insects by eliciting distress calls earlier and making them louder?

It was with these questions in mind that we commenced some simple field experiments in 2002 to see whether synthetic forms of some of the 'words' used by plants to signal their distress when attacked, could be used as attractants for beneficial insects. The results from simple baited sticky card trapping experiments indicated that synthetic MeSA was indeed a potent attractant for a number of predatory insects including green lacewings (Chrysopidae), mite-eating ladybeetles (*Stethorus punctum picipes*), minute pirate bugs, (*Orius tristicolor*), bigeyed bugs (*Geocoris pallens*), predatory mirids (*Deraeocoris brevis*) and hover flies (Syrphidae). In 2003 we expanded our field studies to test the effect of controlled-release dispensers of MeSA in recruiting and retaining predators in vineyards and hop yards. All of the above insects were attracted and retained in greater numbers in baited than in unbaited crops. In addition we attracted a number of other beneficial insects including brown lacewings (*Hemerobius* spp.), parasitic wasps (Braconidae), and predatory flies (Empididae, Sarcophagidae). At some MeSA-baited sites overall beneficial insect populations were nearly four times greater than at

comparable unbaited sites. In some MeSA-baited hop yards large populations of predatory insects were associated with dramatic declines in spider mites and aphids.

The wide array of beneficial insects attracted to MeSA-baited hops and grapes suggested that more than direct attraction was occurring. Many of these insects were not attracted to MeSA in simple trapping experiments. Had the MeSA we dispensed in the crops communicated to nearby plants that pest attack was imminent and therefore chemical dialogue with bodyguards should begin? Based on what we know about MeSA as a widely occurring plant distress signal, together with published laboratory observations, this is a reasonable assumption. In 2004 we took a closer look at the potential signaling effect of MeSA, MeJA and hexenyl acetate (HA) (another plant distress signal) on grapevines. Blocks in which these chemicals were deployed via controlled-release dispensers recruited significantly greater numbers of two species of parasitic wasps (*Metaphycus* sp., *Anagrus* sp.), compared to control blocks. These wasps do not respond directly to MeSA, MeJA or HA-baited traps. We speculate that recruitment of these wasps occurred via the emission of natural parasitoid-attracting volatiles from grapevines, mediated by exposure to synthetic MeSA, MeJA and HA. Further evidence for a signaling function of synthetic MeSA was gained in a 2005 experiment when hop plants treated with a spray oil/MeSA mixture recruited five times as many minute pirate bugs (*O. tristicolor*) as untreated plants.

Field experiments during 2002-2005 in Washington State have provided strong evidence that we can indeed improve the conversations plants have with each other and with beneficial insects by using synthetic versions of naturally occurring plant distress signals like MeSA. Experiments conducted in commercial hops and grapes have consistently shown elevated populations of beneficial insects and improved biological control in MeSA-baited crops. In hops, a number of MeSA-baited yards have not required any miticide use compared to 1-3 applications in unbaited yards.

The use of synthetic plant distress signals either as direct or indirect enhancers of natural enemy population levels in crops (i.e. Herbivore-Induced Plant Protection Odors: HIPPO), is an exciting prospect. However, many questions surrounding the use of these materials in integrated pest management remain to be answered. For example, what are the ecological consequences of providing synthetic distress signals to predators and parasitoids in the absence (or relative absence) of their prey? Will this 'misinformation' result ultimately in non-response by natural enemies to plant distress signals? Fortunately, most if not all of the predatory insects attracted to synthetic MeSA are generalist-feeding species and the misinformation issue may not be as important with these species as it might be to specialist parasitoid species like *Anagrus* spp. which only parasitize leafhoppers. Defining and understanding the mechanism(s) of attraction and recruitment of predatory and parasitic insects by synthetic distress signals, will be of paramount importance in the effective use of these materials in crop pest management. Optimal deployment (release rates, dispenser density) of HIPPOs for natural enemy recruitment and retention, will require a good understanding of the precise mechanisms mediating attraction of predators and parasitoids. Comprehensive studies are planned and will be reported in due course.

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**RESPONSE OF MYZUS PERSICAE TO POTATO VOLATILES:  
DECIPHERING THE EFFECTS OF POTATO LEAF ROLL VIRUS INFECTION**

Sanford D. Eigenbrode<sup>1</sup>, Esther Ngumbi<sup>1</sup>, Hongjian Ding<sup>1</sup>, Thomas M. Mowry<sup>2</sup>, Analiz Rodriguez<sup>1</sup>, and Brent Werner<sup>1</sup>

<sup>1</sup>University of Idaho, Division of Entomology, Ag. Sci. 242, Moscow ID

<sup>2</sup>University of Idaho, Division of Entomology, Parma Research and Extension Center,  
Parma ID

Potato plants infected by *Potato leaf roll virus* (PLRV) become attractive or arrestant for alate and apterous green peach aphids (*Myzus persicae* Sulzer). Our prior work has shown that this effect is at least partly mediated by changes in the volatile organic compounds released by infected plants, but the specific components responsible for the aphid responses have not been identified. Infection of potato by *Potato virus Y* (PVY) or *Potato virus X* (PVX) causes some elevated release of green leaf volatiles and other small molecular weight volatile compounds, but not the terpenoids elevated in headspace of PLRV-infected plants. Potato plants treated with methyl jasmonate (MeJA) produce elevated terpenoids, but do not produce elevated small molecular weight compounds. In our bioassays, VOC from PVY-infected, PVX-infected and MeJA-treated plants are not arrestant for *M. persicae*, despite the fact that each causes elevation of some of the components whose release increases in PLRV infection. Individual components found to be elevated in PLRV-infected plants show a range of physiological activity based on electroantennography (EAG) with *M. persicae*. None of these individual components, however, has significant and substantial behavioral activity at a range of physiologically realistic concentrations. For example, the most active component by EAG, 2 hexene-1-ol, and one elevated 7-fold in headspace of infected plants, is only mildly arrestant for *M. persicae*, and only at some concentrations. Direct observations of the aphids reveals some differences in behavior in response to individual components of the potato blend, but none of these effects is strong. Nonetheless, aphids are arrested by the intact headspace from PLRV-infected plants and by a synthetic blend of the most prominently elevated components of the blend. Evidently the blend is important for generating the full behavioral response by *M. persicae* to headspace of PLRV-infected plants. Other experiments corroborate this conclusion. As the PLRV infection process proceeds in the plant, aphid responses and volatiles in headspace are dynamic. The maximum response by aphids is not correlated with the concentration of specific components but may be related to the ratios of specific components. Potato plants transformed with individual open reading frames of PLRV virus cause changes in released volatiles, but the PLRV genes tested so far predominantly affect terpenoids and the volatiles from these plants are not arrestant for the aphids. All this evidence together indicates the aphid response depends on some aspects of the blend of elevated volatile compounds released by the plants. The compounds in the blend originate from different metabolic pathways indicating that the PLRV infection has multiple effects on the plant due to different types of physiological injury, elicitor mediated induction, or both.

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## **DEVELOPMENT AND ADOPTION OF PHEROMONE TECHNOLOGY FOR MANAGEMENT OF KEY PESTS IN WASHINGTON APPLE ORCHARDS**

Jay F. Brunner<sup>1</sup>, John E. Dunley<sup>1</sup>, Elizabeth Beers<sup>1</sup>, Vince P. Jones<sup>1</sup> and Mike Doerr<sup>1</sup>

Washington State University, Tree Fruit Research and Extension Center, 1100 N. Western Ave., Wenatchee, WA 98801

Pheromones were shown effective against the key pest, *Cydia pomonella* (L.), the codling moth, in the early 1990s. The initial studies showed that pheromones alone could provide control equal to conventional insecticide-based programs in some orchard but not in others. Failure of pheromones was related to codling moth density. In the mid-1990s pheromones plus tebufenozide (IGR), a highly selective insecticide, were shown to be effective as conventional insecticides in apple orchards. In 1995 the codling moth areawide codling moth management project (CAMP) was initiated through USDA funding. CAMP demonstrated that when used over large areas pheromones could result in significant reductions in crop loss from codling moth, reduced insecticide use (50%), and even allow for a reduced use of pheromones thus reducing the cost of a pheromone-based pest management program. Adoption of a highly selective control technology for codling moth led to increased problems with leafrollers but not other pests. From 1990 through 2000 there was a steady increase in adoption of pheromones as part of the apple pest management program in WA. From 2001-2003 there was a slight decline in the use of pheromones but in 2005 ca. 120,000 acres were treated with pheromone for codling moth control in WA, or about 65% of the total acres. Insecticides supplement pheromone treatments and recent efforts have been directed at demonstrating how to replace organophosphate insecticides with newly registered alternatives. Research is currently focusing on new pheromone delivery technologies that could automate applications, reduce costs or increase efficacy.

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## **CHEMISTRY AND APPLICATIONS OF NEW MEALYBUG PHEROMONES**

Jocelyn G. Millar<sup>1</sup>, J. Stephen McElfresh<sup>1</sup>, Kent M. Daane<sup>2</sup>, Walter J. Bentley<sup>3</sup>, and Jardel Moreira<sup>1</sup>

<sup>1</sup> Department of Entomology, University of California, Riverside CA 92521

<sup>2</sup> Division of Insect Biology, Center for Biological Control, University of California, Berkeley, CA 94720 CA

<sup>3</sup> University of California Integrated Pest Management Program, Kearney Agricultural Center, Parlier, CA 93648

Various species of mealybugs are major pests in a wide variety of cropping systems worldwide. Heavy infestations can result in direct damage and death of crop plants, particularly with those mealybug species that vector plant diseases. Equally or more important in many crops is the production of honeydew by mealybugs, and the subsequent development of sooty mold. Effective sampling and control of mealybugs

can be problematic because the insects are often concealed in cracks and crevices, where they are hard to spot, and where adequate pesticide coverage is difficult to achieve.

For the past several years, we have been working on the identification and development of applications of pheromones of several mealybug species, including the vine, obscure, longtailed, and grape mealybugs, and very recently, a new mealybug pest in California, *Ferrisia gilli*. Pheromones for the first three species have now been identified, and work on the pheromones of the other two species is in progress. The unique chemistry of these pheromones, and the pheromones of related mealybug species, will be discussed, along with important implications. Ongoing work on applications for these pheromones in various cropping systems also will be described.

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**CUCUMBER VOLATILE BLEND ATTRACTIVE TO FEMALE MELON FLY,  
*BACTROCERA CUCURBITAE* COQUILLET**

Matthew S. Siderhurst<sup>1</sup>, Eric B. Jang<sup>1</sup>, Janice T. Nagata<sup>1</sup>, & Lori A. Carvalho<sup>1</sup>

<sup>1</sup>US Pacific Basin Agric. Res. Center, USDA-ARS, P.O. Box 4459, Hilo, HI

The melon fly, *Bactrocera cucurbitae* Coquillett, is a serious crop pest throughout sub-continental and southeast Asia and the Pacific, causing damage to cucurbits and related crops and some tree fruits. Female attractants for melon fly have been a promising, if illusive, goal for control programs since a female lure removes both females and 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*. Coupled gas chromatography-electroantennogram detection (GC-EAD) analysis of fresh and aged cucumber slurry volatiles identified 32 compounds which 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 nine-component blends were shown to have female biased attraction in outdoor rotating olfactometer, field cage and field experiments.

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**C. W. WOODWORTH AWARD**

At the same Maui meeting, the Society announced the recipient of the C. W. Woodworth Award, which annually recognizes a person in the Pacific Branch of the Entomological Society of America who has made outstanding contributions to entomology during the past decade. The 2005 Woodworth Award went to Dr. Jocelyn Millar of the Department of Entomology, University of California, Riverside, CA.

The following is the abstract for Dr. Millar's presentation at the Opening Session of the meeting.

**DECIPHERING INSECT COMMUNICATION SYSTEMS:  
A LONG AND WINDING ROAD**

Jocelyn G. Millar

Department of Entomology, University of California, Riverside CA 92521

Humans normally think of communication in terms of sound or vision, such as speech and writing. However, for many organisms, exchange of chemical signals constitutes their primary method of communication, and chemical signaling was probably the first type of communication to evolve. For these microbes, plants, and animals, individual chemicals might be viewed as our equivalent of letters or words, that can be combined in various ways to create many different messages. Thus, in the same way that we use an alphabet of 26 letters to make thousands of words, a large number of different chemical signals can be generated by blending a small number of chemicals together in different combinations.

Chemical signals and cues mediate many aspects of insect behavior and life history, including reproduction, feeding and oviposition, and defense. Our research has been focused primarily on the identification, synthesis, and development of practical applications of insect pheromones and related chemicals that mediate insect behaviors. To give an idea of how ubiquitous this type of communication is within the Insecta, in my group alone we have worked on chemical signaling in insects as diverse as moths, flies, mosquitoes, beetles, true bugs, scales, mealybugs, ants, termites, bees, and parasitic wasps. Along the way, our research has gone in some unexpected directions when it has become clear that the organisms under study were using more than one medium for communication, for example, by combining chemical signals with visual or acoustic signals. Other projects have stopped and restarted several times, as new ideas suggested possibilities for further research. Illustrative examples have been taken from three projects.

As a first example, we began identifying pheromones for a series of true bugs in the late 1990's, and that work is still continuing. The pheromones of some species (e.g., mirid bugs in the genus *Phytocoris*) were straightforward, both in their chemistry and how they worked. However, the pheromones of phytophagous stink bugs were more complicated, and at first, it appeared that they did not work very well at all (although they did attract bug parasites and predators!). However, more careful observation revealed that these bugs were actually using two overlapping sets of signals. That is, pheromones were used to get males and females onto the same plant, but once on the plant, the bugs used substrate-borne vibrational signals to locate each other, and for courtship and recognition once they were in contact. In fact, each species and sex produced its own unique repertoire of 3-5 different types of vibrational signals, for use in different contexts. Overall, use of these vibrational signals appears to be an efficient method of mate location, while simultaneously limiting eavesdropping by predators and parasitoids.

The second example had its genesis in a collaborative project on invasive cerambycid beetles that started in 1989 with Drs. Tim Paine, Larry Hanks, and myself. We wanted to learn about the biology and ecology of *Phoracantha punctata*, and later *Phoracantha recurva*, two serious pests of Eucalyptus that had been introduced into California from Australia. We have studied many aspects of the life history and biological control of these beetles, but one of the offshoots from this project was that we became increasingly interested in cerambycid beetles overall, and particularly, their use of chemical signals. This has now blossomed into a longterm, multiparticipant project, from which we hope to develop an overall outline of pheromone occurrence and use in this large insect family, including how the pheromones are used, the types of chemicals that are involved, and how the chemicals are mixed and matched in subsets by different species to generate unique chemical signals.

The third project, on the sex pheromone of navel orangeworm, a major pest of nut crops in California, also began in 1989 but for years we made little progress. In the last couple of years, using a surrogate species as a model we were finally able to prove that there were missing components to the pheromone blend. Now, looking back over data from many years, it is clear that those trace components were there all along, but were missed because they were not where or what we expected them to be. This project served as a salutary lesson in the value of conducting research with an open mind, rather than expecting results to fall into known or predicted patterns.