



2nd International Conference on Pollinator Biology, Health and Policy  
August 14 - 17 2013

Pennsylvania State University

**Center for Pollinator Research**

The conference organizers would like to thank

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for the graphics used on the front cover  
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# WEDNESDAY

2:00 PM	<b>Registration check-in begins</b> (outside Boardroom, lower level of Nittany Lion Inn)
4:30 PM	<b>Welcome Reception</b> (Boardroom)
<b>Keynote Session</b> (Ballroom, main floor of NLI, open to the public)	
6:00 PM	<b>Introduction</b> Christina Grozinger, Penn State University
6:15 PM	<b>Understanding the impact of sublethal exposure to pesticides on bee colony health</b> David Goulson, University of Stirling
7:00 PM	<b>Endocrine disruption of the social brain</b> Heather Patisaul, North Carolina State University

# THURSDAY

7:00 AM	<b>Registration check in</b> Poster set up begins
7:30 PM	<b>Continental Breakfast</b> (outside Ballroom)
<b>Morning Session: Behavioral Ecology</b> Session Organizers: Christina Grozinger, Theresa Pitts-Singer (Ballroom)	
8:45 AM	<b>The role of uncertainty in foraging by pollinators</b> Dan Papaj, University of Arizona
9:20 AM	<b>Fragments, fragrance and fitness: translating pollinator sensory biology into gene flow</b> Rob Raguso, Cornell University
9:40 AM	<b>Individual bumble bees are locavores in a Rocky Mountain meadow</b> Jane Ogilvie, University of Toronto and Rocky Mountain Biological Laboratory
9:55 AM	<b>Bombus impatiens foragers exhibit predictable daily patterns in pollen foraging preferences</b> Anthony Vaudo, Penn State University
10:10 AM	<b>Break</b>
10:40 AM	<b>Colony-level burden of environmental pollutants for honey bees</b> Chris Mullin, Penn State University
11:00 AM	<b>Sipping from a poisoned chalice: plant drugs, toxins, and pesticides in nectar and their influence on bee behaviour</b> Jeri Wright, Newcastle University
11:20 AM	<b>Impact of combined pesticide exposure on individual and colony-level traits in bees</b> Nigel Raine, Royal Holloway, University of London
11:40 AM	<b>Effects of two commonly used fungicides on the nesting and foraging behavior of two managed solitary bees, <i>Osmia lignaria</i> and <i>Megachile rotundata</i> (Megachilidae)</b> Derek Artz, USDA-ARS Bee Biology & Systematics Laboratory
11:55	<b>Lunch</b>

## Afternoon Session: Ecosystem Services

Session Organizer: Neal Williams  
(Ballroom)

1:45 PM	<b>The integrated crop pollination project: supporting production of U.S. specialty crops</b> Rufus Isaacs, Michigan State University
2:10 PM	<b>Biodiversity and crop pollination services</b> Alexandra Klein, Leuphana University in Lüneburg
2:30 PM	<b>Sustainable pollination services for UK crops</b> Michael Garrat, University of Reading
2:50 PM	<b>Raising wild pollinators to maximize crop yields: how much pollinator-friendly habitat does one need?</b> Eric Lonsdorf, Chicago Botanic Garden
3:10 PM	<b>Break</b>
3:30 PM	<b>The conservation and use of pollinator diversity in addressing global food security and nutrition</b> Barbara Gemmill-Herren, Food and Agriculture Organization of the United Nations
3:50 PM	<b>Local management and landscape structure drive pollinator community and crop pollination in East Africa</b> Mark Otieno, Penn State University
4:05 PM	<b>Natural areas buffer impact of pesticides on wild pollinators of a perennial crop</b> Mia Park, Cornell University
4:20 PM	<b>Retention and reproduction of alfalfa leafcutting bees, <i>Megachile rotundata</i> (Megachilidae), in alfalfa fields</b> Theresa Pitts-Singer, USDA ARS Bee Biology & Systematics Laboratory
4:35 PM	<b>USDA Farm Service Agency efforts to expand foraging opportunities for honey bees on land enrolled in the Conservation Reserve Program</b> Skip Hyberg, Farm Service Agency
4:50 PM	<b>Session Ends</b>
6:00 – 8:00 PM	<b>Reception at the Arboretum at Penn State</b>

# FRIDAY

7:30 AM	<b>Continental Breakfast</b> (outside Ballroom)
<b>Morning Session: Ecology &amp; Conservation</b> Session Organizers: Neal Williams (Ballroom)	
8:45 AM	<b>Native bee responses to anthropogenic land use change</b> Neal Williams, University of California, Davis
9:10 AM	<b>Bumble bee conservation: applying molecular techniques to preserve biodiversity</b> James Strange, USDA-ARS Bee Biology & Systematics Laboratory
9:30 AM	<b>Urban land use limits regional bumble bee gene flow</b> Shalene Jha, University of Texas, Austin
9:50 AM	<b>Effects of an invasive plant on native bumble bee populations</b> Jessamyn Manson, University of Alberta
10:10 AM	<b>Break</b>
10:35 AM	<b>Citizen scientists document geographic patterns in pollinator communities</b> Alison Parker, University of Toronto
10:55 AM	<b>Stingless bee species' traits alter responses to deforestation</b> Elinor Lichtenberg, Washington State University
11:15 AM	<b>Pollinator conservation grows up: Lessons learned from the field</b> Mace Vaughan, Xerces Society / USDA NRCS
11:35 AM	<b>Lunch</b>
<b>Afternoon Session: Policy &amp; Public Outreach</b> Session Organizers: Ed Rajotte, Chris Mullin (Ballroom)	
1:30 PM	<b>Introduction</b> Chris Mullin, Penn State University

1:35 PM	<b>Progress and future prospects for assessing the risks posed to pollinators by pesticides &amp; science needs</b> Helen Thompson, FERA
2:05 PM	<b>How to make decisions when you don't know the facts: the interface of science and pesticide policy</b> Jim Frazier, Penn State University
2:20 PM	<b>Observations of a commercial beekeeper</b> David Mendes, National Honey Bee Advisory Board
2:35 PM	<b>Pesticides and pollinators: A 2013 view toward the future</b> Laurie Adams, Pollinator Partnership
2:50 PM	<b>Break</b>
3:10 PM	<b>Overview of USDA programs that inform pesticide policy</b> Mary Purcell-Miramontes, USDA-NIFA
3:25 PM	<b>Reevaluation of neonicotinoid pesticides in California</b> Richard Bireley, CA Department of Pesticide Regulation
3:40 PM	<b>The U.S. Environmental Protection Agency and pollinator health</b> Don Brady, Environmental Fate & Effects Division, US EPA
3:55 PM	<b>Evolving policies related to pesticides and pollinator conservation</b> Gabriele Ludwig, Almond Board of California
4:10 PM	<b>Pollinators and pesticides: complementary components of sustainable agriculture</b> Paul Hoekstra, CropLife Canada
4:25 PM	<b>Development of an improved testing and pollinator risk assessment process in North America: a progress report from the perspective of the crop protection industry</b> David Fischer, CropLife America
4:40 PM	<b>Panel discussion</b>
5:15 – 8:00	<b>Poster Session</b> (Ballroom)

# SATURDAY

7:30 PM	<p><b>Continental Breakfast</b> Posters must be removed by 10:00 a.m. (outside Ballroom)</p>
<p><b>Morning Session: Physiology &amp; Development</b> Session Organizers: Susan Fahrbach, Heather Hines (Ballroom)</p>	
8:35 AM	<p><b>Endocrine regulation of brain structure and function in the honey bee</b> Susan Fahrbach, Wake Forest University</p>
9:10 AM	<p><b>The genetic architecture of complex traits and selective breeding for honey bee health</b> Olav Rueppell, University of North Carolina at Greensboro</p>
9:30 AM	<p><b>The role of pesticides in queen health</b> Jeff Pettis, USDA-ARS Bee Research Laboratory</p>
9:50 AM	<p><b>The Effects of miticides on the mating health of honey bee (<i>Apis mellifera</i> L.) queens</b> Julianna Rangel, Department of Entomology, Texas A&amp;M University</p>
10:10 AM	<p><b>Break</b></p>
10:35 AM	<p><b>Solitary bees as a model system for predicting the impacts of climate warming on pollination services</b> Jordi Bosch, CREAF, Autonomous University of Barcelona</p>
10:55 AM	<p><b>How toxins impact flight performance</b> Jim Marden, Penn State University</p>
11:15 AM	<p><b>The impact of neonicotinoid insecticides on pollinators in tree fruit IPM programs</b> David Biddinger, Penn State University</p>
11:35 AM	<p><b>Transcriptional markers of sub-optimal nutrition in developing <i>apis mellifera</i> nurse workers</b> Vanessa Corby-Harris, USDA-ARS Carl Hayden Bee Research Center</p>

11:55 AM	<b>Lunch</b> (Alumni Lounge and Lobby)
<b>Afternoon Session: Host-Parasite Interactions</b> Session Organizers: Diana Cox-Foster, Elina Lastro Niño (Assembly Room)	
1:45 PM	<b>The gut microbial communities in honey bees and bumble bees</b> Nancy Moran, University of Texas, Austin
2:20 PM	<b>Pathogen-host-interactions in American foulbrood disease</b> Elke Genersch, Institute for Bee Research, Germany
2:40 PM	<b>Risks associated with invasive parasites of bumblebees and molecular haplotyping of <i>Apicystis bombi</i> from Europe and Argentina</b> Ivan Meeus, Ghent University
3:00 PM	<b>Break</b>
3:30 PM	<b>From resins to propolis: biological origins and role in honey bee social immunity and health</b> Marla Spivak, University of Minnesota
3:50 PM	<b>Transcriptomic analyses of <i>Varroa</i> x virus x bee interactions in a resistant population</b> Jay Evans, USDA-ARS Bee Research Laboratory
4:10 PM	<b>Multiple genetic lineages involved in a recent host-switch to European honeybees by the parasitic mite, <i>Varroa jacobsoni</i></b> John Roberts, CSIRO Ecosystem Science
4:30 PM	<b>Closing Remarks</b> Christina Grozinger



# WEDNESDAY SPEAKERS

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# Understanding the impact of sublethal exposure to pesticides on bee colony health

**Prof Dave Goulson,**  
Sussex University, UK

Both wild and managed pollinators in farmland are routinely exposed to complex mixtures of agrochemicals at low concentrations. Of particular concern is exposure to neonicotinoids, a widely-used class of systemic insecticides that are toxic to bees at very low concentrations and are found in the nectar and pollen of treated crops such as canola. It has recently become apparent that exposure of bees to these compounds has subtle but important sublethal effects on individual behaviour, effects that are not revealed by the safety tests that are used by regulators to evaluate the impacts of agrochemicals on bees. These behavioural effects include impaired navigation, reduced capacity to gather pollen, and also reduced egg laying. In combination, these effects appear to be sufficient to result in major reductions in colony-level performance and reproduction in bumblebees at field-realistic levels of exposure. Accumulation of neonicotinoids in soils, and evidence for their uptake by non-target wildflowers growing near crops, suggest that they may also be having broader impacts on farmland biodiversity that have not yet been adequately investigated.

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# Endocrine Disruption of the Social Brain

**Heather B. Patisaul**

Associate Professor, Department of Biology, NC State University, Raleigh, NC 27695

Endocrine disrupting chemicals (EDCs) are pervasive in the wild and built environment. Chemicals with endocrine disrupting properties include plasticizers, fire retardants, and pesticides, but also naturally occurring compounds such as the soy phytoestrogens. Emerging evidence in a wide range of vertebrates suggests that EDCs have subtle but significant effects on brain development and, ultimately, behavior. Intriguingly, they often have non-monotonic dose response curves suggesting that effects observed at high doses may not be predictive of health effects at lower, more environmentally relevant, doses. Concerns about environmental influences on the brain and behavior have also been raised because the incidence of some neurobehavioral disorders is rapidly and inexplicably increasing in the US and other industrialized nations. These include autism spectrum disorders (ASDs), attention deficit hyperactivity disorder (ADHD), anxiety, and depression. Because hormones play such a critical role in brain development, it is hypothesized that the brain is particularly vulnerable to EDC exposure, especially during gestation and early life while circuits critical for social and other complex behaviors are still forming. This talk will provide an overview of EDC effects on vertebrate brain development focusing on impacts on the social brain and the neural circuitry underlying them.

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# THURSDAY SPEAKERS

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# The Role of Uncertainty in Foraging by Pollinators

**Dan Papaj**

Pollinators, like any organism, must contend with uncertainty in their environment. For example, pollinators such as bees may experience uncertainty about visual or olfactory cues produced by flowers. I will discuss two kinds of uncertainty in floral signals – first, uncertainty related to the detectability of floral signals and second, uncertainty related to the information about the resource conveyed by those signals. In the first part, data from experiments on nectar-foraging in bumble bees will be presented to show that, under realistic experimental conditions, bees perceive floral signals as difficult to detect, and use multiple mechanisms to improve detection accuracy. In the second part, uncertainty about two kinds of information about the floral resource will be discussed, first, so-called ‘personal’ information gathered by the bumble bee itself and, second, social information gathered in encounters with other bees. Bees respond to experimental manipulations of the reliability of personal versus social information in a highly functionally dynamic fashion, indicative of an organism adapted to use the kind of boom-bust resource that flowers in nature represent. I will attempt to place the flexibility of bee behavior into the context of challenges faced by pollinators in an environment increasingly impacted by human activity.

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# **Fragments, fragrance and fitness: translating pollinator sensory biology into gene flow**

**Robert A. Raguso**

Department of Neurobiology and Behavior, Cornell University, Ithaca NY, USA

A current challenge facing pollination biologists is to integrate discoveries across different levels of analysis to reach a predictive understanding of how pollinator foraging behavior translates into ecological goods and services. Over the past three decades, a wealth of studies has accumulated on the sensory biology, nutritional physiology and learning abilities of model pollinators from honey bees to honey creepers, often under controlled (or contrived) environmental conditions. With my students and collaborators, I have worked extensively on the foraging behavior of nectar-feeding hawkmoths (Lepidoptera: Sphingidae), with a focus on their responses to floral signals and cues and their modification by environmental context. Due to their strong flight capability and high energetic demands, sensitivity to light pollution and pesticide use, and potential as long distance pollen vectors, hawkmoths are useful models for studying the effects of climate and human disturbance on pollinator services. My talk will draw together results from several ongoing studies concerning the responses of hawkmoths to floral scent as a distance attractant, their use of carbon dioxide and relative humidity at different spatial scales to choose nectar sources, patches and habitats, and their impacts as drivers of gene flow between increasingly fragmented plant populations.

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# Individual bumble bees are locavores in a Rocky Mountain meadow

**Jane E. Ogilvie**<sup>1,2\*</sup>, **Takashi T. Makino**<sup>1,3</sup>, and **James D. Thomson**<sup>1,2</sup>

1. Department of Ecology and Evolutionary Biology, University of Toronto, 25 Harbord Street, Toronto, Ontario, M5S 3G5, Canada
2. Rocky Mountain Biological Laboratory, Post Office Box 519, Crested Butte, Colorado, 81224-0519, USA
3. Current address: Department of Biology, Faculty of Science, Yamagata University, 1-4-12 Kojirakawa, Yamagata, 990-8560, Japan

Pollinators must make decisions about where to forage in landscapes where floral resources are patchy. These foraging decisions can influence plant pollination and reproductive success. Despite this importance, we still have a poor understanding of how pollinators use patches that vary in floral abundance and isolation, at broad spatial scales in natural habitats. To address this, we marked and resighted known individual bumble bees (*Bombus* spp.) visiting 12 *Delphinium barbeyi* flower patches that varied in size (4 to 400 m<sup>2</sup>) and isolation (5 to 50 m), in a subalpine meadow in the Colorado Rocky Mountains. At the patch-level, bumble bee visits per flower increased with flower patch size, and for small flower patches, bee visits per flower declined with increasing patch isolation. At the individual bee-level, half of the individuals we saw many times foraged solely in one of 12 patches in the meadow, with a median patch-use of one and a range from one to four patches. Overall, we show that when floral resources are abundant, many individual bumble bees commonly forage very locally in a meadow, and that they concentrate their foraging in large and close patches, which may have implications for the pollination of *D. barbeyi*.

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# ***Bombus impatiens* Foragers Exhibit Predictable Daily Patterns in Pollen Foraging Preferences**

**Anthony D. Vaudo, MSc**

Department of Entomology, The Pennsylvania State University

**Christina Grozinger, PhD**

Department of Entomology, The Pennsylvania State University

**David Mortensen, PhD**

Department of Plant Science, The Pennsylvania State University

**Harland Patch, PhD**

Department of Entomology, The Pennsylvania State University

**John Tooker, PhD**

Department of Entomology, The Pennsylvania State University

Understanding foraging preferences of bee species is critically important for designing agroecosystems and habitat restoration projects that attract and conserve these vital community members. Foraging preferences are crucial for success of the species because the survival of subsequent generations depends on proper foraging decisions. Unfortunately, fully characterizing bee host plant preferences and patterns of visitation to host plants has been challenging because many factors influence foraging decision-making. Moreover, determining preferences of generalist bee species is difficult because of their catholic tastes and the available resources are sensitive to spatiotemporal variability. To better understand patterns of floral visitation, we have used novel metrics to measure preferences of *Bombus impatiens* to seven perennial plant species native to central Pennsylvania. These metrics include time-of-day based observations of pollen foraging at scales of the community and individual bees, while simultaneously accounting for the number of available foragers in the environment and the differences in floral display of each plant species. Our results thus far indicate that *B. impatiens* make predictable daily patterns in their pollen foraging choices. Accounting for circadian cycles of host plant visitation should be considered in future analyses to encourage precise interpretations of host plant preference and optimal bee conservation decisions.

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# Colony-level Burden of Environmental Pollutants for Honey Bees

**Chris Mullin, Jing Chen, Wanyi Zhu, Maryann Frazier and James Frazier**

Department of Entomology, Center for Pollinator Research, The Pennsylvania State University, University Park, PA 16802

Assessment of the total toxic chemical load or burden for an organism requires monitoring all the relevant exposures, including environmental pesticides, miticide and antibiotic treatments, and dietary toxicants. Colony burden of contaminants must include the adjuvant and co-formulants in modern pesticides that are largely assumed to be biologically inert. Numerous pesticides and more recently co-formulants have been found in beehive samples. Formulations usually contain inerts at higher amounts than active ingredients, and tend to be more toxic to honey bees than the active ingredient alone. Impacts of 'inerts' in pollen, nectar or wax alone or in combination with coincident pesticide residues on honey bee survival and behavior are largely unknown. We have shown that honey bees are unusually sensitive to organosilicone spray adjuvants and the solvent N-methyl-2-pyrrolidone, common co-formulants used in agrochemicals and spray adjuvants. Effects include learning impairment for adult bees using the proboscis extension reflex assay, and chronic toxicity in larval feeding bioassays. Most formulations we tested were more toxic to bees than their respective active ingredients. We anticipate that if 'inerts' are influencing pesticide levels and general hive stress, formulation recommendations can be optimized for use in bee foraging areas.

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# **Sipping from a poisoned chalice: plant drugs, toxins, and pesticides in nectar and their influence on bee behaviour**

**Geraldine A. Wright**

Institute of Neuroscience, Newcastle University, Newcastle upon Tyne, NE1 7RU

Insect pollinators like bees have a long-standing mutualism with plants in which they visit flowers to obtain fuel for flight (nectar) and protein and fatty acids (pollen) for larvae and reproduction. This mutualism is complicated by the fact that nectar and pollen are often laced with toxic compounds that plants produce as a means of defence against herbivores. Toxins could provide plants with a means of protecting investments in floral resources or may simply occur as a result of their induction in other tissues throughout the plant, but potentially have serious consequences for pollinators. Here, I will describe the mechanisms that bees have for detecting and learning to avoid naturally occurring plant toxins. I will also describe a few examples where plants have used drugs to manipulate the behaviour of pollinators. In modern agriculture, we also inadvertently expose bees to pesticides in nectar and pollen; I will also discuss the consequences of pesticide-laced floral resources for the behaviour and nutritional ecology of bees.

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# Impact of combined pesticide exposure on individual- and colony-level traits in bees

**Nigel E Raine and Richard J Gill**

School of Biological Sciences, Royal Holloway University of London, Egham, Surrey, TW20 0EX, UK

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Bees play a critical role in the pollination of both food crops and wild flowers. Therefore, understanding the causes of widespread declines in bee populations has important consequences for our ecology, economy and food security. Our reliance on agricultural chemicals, such as pesticides, to boost both crop quality and yield is one factor which could have a significant detrimental impact on both wild and managed bees. Indeed, a number of recent studies have shown pesticide exposure is associated with changes in bee behaviour and reductions in colony queen production. However, the key link between changes in individual behaviour and the consequent impact at the colony level has not been shown. Social bee colonies depend on the collective performance of many individual workers. Thus, although field-level pesticide concentrations can have subtle or sublethal effects at the individual level, it is not known whether bee societies can buffer such effects or whether it results in a severe cumulative effect at the colony level. Furthermore, widespread agricultural intensification means that bees are exposed to numerous pesticides when foraging, yet the possible combinatorial effects of pesticide exposure have rarely been investigated. Here we show that chronic exposure of bumblebees (*Bombus terrestris*) to two pesticides, a neonicotinoid (imidacloprid) and a pyrethroid (lambda-cyhalothrin), at concentrations that approximate field-level exposure impairs natural foraging behaviour and increases worker mortality leading to significant reductions in brood development and colony success. Using Radio Frequency Identification (RFID) tagging technology we examined detailed foraging patterns of over 1000 individuals from 40 colonies under natural conditions in real time. We found that worker foraging performance, particularly pollen collecting efficiency, was significantly reduced with observed knock-on effects for forager recruitment, worker losses and overall worker productivity. Moreover, we provide evidence that combinatorial exposure to pesticides increases the propensity of colonies to fail.

This work was supported by the Insect Pollinator Initiative (funded under the auspices of the Living with Environmental Change programme, Biotechnology and Biological Sciences Research Council (BBSRC), Wellcome Trust, Scottish Government, Department for Environment, Food and Rural Affairs (DEFRA) and Natural Environment Research Council (NERC): grant BB/I000178/1).

Gill, R. J., Ramos-Rodriguez, O. & Raine, N. E. 2012. Combined pesticide exposure severely affects individual- and colony-level traits in bees. *Nature* 491, 105-108.

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# Effects of Two Commonly Used Fungicides on the Nesting and Foraging Behavior of Two Managed Solitary Bees, *Osmia lignaria* and *Megachile rotundata* (Megachilidae)

**Derek R. Artz and Theresa L. Pitts-Singer**

USDA-Agricultural Research Service, Pollinating Insect Research Unit, Logan, UT 84322

There is a growing body of empirical evidence showing that both wild and managed bees are negatively impacted by various pesticides that are applied in agroecosystems around the world. Exposure to pesticides can affect foraging and nesting behavior in both social and solitary bees. *Osmia lignaria* (blue orchard bee) and *Megachile rotundata* (alfalfa leafcutting bee) are two solitary managed bees that are used in various cropping systems in the U.S. In this study, we determine how two commonly used fungicides, Rovral (iprodione) and Pristine (pyraclostrobin plus boscalid), influence the foraging and nesting behavior in *O. lignaria* (Rovral and Pristine) and *M. rotundata* (Pristine only). In separate trials in California (for *O. lignaria*) and Utah (for *M. rotundata*), we set up 20' x 20' cages and released 20-25 females plus 30-50 males in each cage to document any changes in nesting and/or foraging behavior after fungicide sprays. We recorded mean time spent inside the nest, mean time spent pollen foraging outside the nest, mean time spent gathering mud (*O. lignaria*) or leaf pieces (*M. rotundata*), and the mean number of attempts to enter a nest cavity. Considerable variation in behavioral responses after fungicide sprays was found.

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# The Integrated Crop Pollination Project: supporting pollination in U.S. specialty crops

**Rufus Isaacs<sup>1</sup>, Jason Gibbs<sup>1</sup>, Neal Williams<sup>2</sup>, Theresa Pitts-Singer<sup>3</sup>,  
Mace Vaughan<sup>4</sup>, Kelly Garbach<sup>5</sup>, Eric Lonsdorf<sup>6</sup>, and Taylor Ricketts<sup>7</sup>**

1. Michigan State University, East Lansing, MI;
2. University of California, Davis, CA,
3. USDA-ARS, Logan, UT
4. Xerces Society, Portland, OR;
5. Loyola University, Chicago, IL;
6. Chicago Botanic Gardens, Chicago, IL;
7. University of Vermont, Burlington, VT.

Sustainable and economical pollination of specialty crops is increasingly at risk from the counteracting forces of declining bee populations and increased production of these fruit, nut, and vegetable crops. To proactively address this issue, a team of university researchers and extension staff, commercial pollination service providers, government scientists, and a non-profit conservation organization are working to develop, test, and implement Integrated Crop Pollination strategies at farms across the United States and in Canada. Building on recent and ongoing research to enhance farmland for wild pollinators and to incorporate the use of non-Apis managed pollinators, the Project ICP team is using a common experimental design at multiple farms. These locations are being used for a multi-year comparison of the economics of pollination strategies employing honey bees, alternative pollinators, or pollinator habitat. These analyses will be coupled with landscape modeling analyses, education-outreach programs, and social science investigations of farmer decision-making to determine the situations where different tactics will provide reliable and economical pollination of the target crops. This presentation will introduce the project, highlight initial results, and describe the future direction of our project. We encourage those interested in this topic to visit our website at [www.icpbees.org](http://www.icpbees.org) for more information.

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## Biodiversity and crop pollination services

### Alexandra-Maria Klein

Leuphana University of Lüneburg, Germany

Global declines in honeybees have led to concerns about negative impacts on food production due to low levels of pollination. This is exemplified in California where the demand for honeybees *Apis mellifera* to pollinate almond *Prunus dulcis* is increasing, but problems with honeybee health suggest it may not be sustainable to rely solely on the pollination service of a single species. I will discuss the potential of landscape and organic management to restore wild pollinators in intensive almond production landscapes of California, USA.

We found species richness and flower-visitation frequency of wild pollinators, but not of honeybees, being related to fruit set. I will show various methods and results exploring the mechanisms of this unexpected finding. This will include spatial complementarity in different environmental conditions and species interactions.

Although my talk will mainly present results of a case study system, I will discuss and highlight some results for wider implications across countries and agricultural systems.

I will conclude that honeybees, although by far the most efficient pollinator taxa in many agricultural systems cannot fully substitute the functional performance of diverse pollinator communities. Restoring semi-natural perennial habitat strips implemented in intensive agricultural landscapes may have potential to promote sustainable agro-pollination services.

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# Sustainable Pollination Services for UK Crops

**Dr Michael P. D. Garratt<sup>1</sup>, Dr Jacobus C. Biesmeijer<sup>2</sup>  
and Prof Simon G. Potts<sup>1</sup>**

1. Centre for Agri-Environmental Research, School of Agriculture, Policy and Development, University of Reading, UK.
2. Naturalis Biodiversity Centre, 2300 RA, Leiden, Netherlands

Crop pollination is a vital ecosystem service and essential in maintaining agricultural and horticultural productivity, worth \$910 m/year to UK agriculture. Pollination services are provided by wild pollinators including bees, hoverflies and butterflies and managed bee species such as the honey bee. Evidence shows that many wild pollinators are in decline and the number of UK managed honey bee colonies has fallen by 54% in two decades with resulting implication for food production. This talk presents research from the 'Sustainable Pollination Services for UK Crops' project, part of the UK Insect Pollinators Initiative. We report findings from fieldwork and experimental manipulations focussing on some key insect pollinated crops, showing that pollination service requirements vary between crops and in some cases pollination deficits exist, limiting crop yield and importantly crop quality. The impacts of landscape composition and pollinator communities on pollination service has been investigated, as well as testing on-farm mitigation strategies to boost pollination and crop production. These findings have important implications for agriculture and food security in the UK and globally by providing the evidence required to manage pollination services sustainably into the future.

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# Raising wild pollinators to maximize crop yields: how much pollinator-friendly habitat does one need?

**Eric Lonsdorf<sup>1</sup> and Taylor Ricketts<sup>2</sup>**

1. Franklin and Marshall College, Lancaster, PA;
2. University of Vermont, Burlington, VT

The supply of managed honey bees for crop pollination service is both increasingly uncertain and being out-paced by a growing demand. It is increasingly recognized that farmers need should respond by integrating native pollinator-friendly habitat into standard farming practices. Furthermore, recent findings indicate that both farm and landscape-level factors affect the supply of pollinators. This begs the question, how much pollinator-friendly is needed in the landscape to maximize yields? Here, we apply a quantitative model of pollination service to answer this question as a function of a crop's dependence on native pollination. Not surprisingly, we show that as the dependence on pollination increases, the optimal amount of pollinator-friendly habitat in the landscape increases. Moreover, as the quality of on-farm habitat for pollinators increases, the optimal amount of crop in the landscape increases. We uncover a pollination service version of the "tragedy of the commons" in which an individual may increase their profits by converting pollinator-providing habitat to crop while the entire landscape's value declines.

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# The Conservation and Use of Pollinator Diversity in Addressing Global Food Security and Nutrition

**Barbara Gemmill-Herren**

Food and Agriculture Organization of the United Nations

The contribution of pollination services to human livelihoods will be highlighted, with a particular stress on the high importance of these services in developing economies and in the tropics. The unique characteristics of pollination as an ecosystem function will be considered, with respect to how these should be reflected in the management and conservation of pollinators. Amongst these are the patterns of bee diversity, which diverge from general biodiversity patterns; the severity of pollen limitation in biodiversity hotspots; the contribution of pollinators to ecosystem architecture/design; the lack of conventional density dependence responses to disturbance; and the proclivity of bees for agricultural environments. Experiences in developing and applying a protocol to assess pollination deficits in crops will be presented, including its use in a pilot study for the newly formulated Intergovernmental Platform on Biodiversity and Ecosystem Services. A simple methodology to assess the context of pesticide exposure for bees, applied in Kenya, Brazil and the Netherlands will be discussed. Efforts to develop a suite of best management practices to sustain pollinators in smallholder agricultural systems will be described, and mention will be made of building on traditional knowledge of managing and encouraging pollinators.

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# Local management and landscape structure drive pollinator community and crop pollination in East Africa

**Mark Otieno**<sup>1</sup>, **Ben A. Woodcock**<sup>2</sup>, **Andrew Wilby**<sup>3</sup>,  
**Ioannis N. Vogiatzakis**<sup>4</sup>, **Alice L. Mauchline**<sup>5</sup>,  
**Mary W. Gikungu**<sup>6</sup> and **Simon G. Potts**<sup>5</sup>

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Environmental change has led to severe shortfalls in the availability of pollinators in many regions, threatening environmental stability and future food security. Identification of factors that structure bee communities in agricultural fields is critical to our understanding of how to manage pollination of crops and wild plants; similarly the structure of landscapes surrounding agricultural fields affect pollinator communities and pollination services. We investigated the functional responses of bee communities on pigeon pea crops to local and landscape drivers in six paired farms along a gradient of landscape structure in Kenya. At a local management scale, the number of insecticide applications had significant negative effects on the abundance of carpenter bees and bees nesting in soil. At the landscape scale, the complexity of habitat patches, together with patch size and edge density had a significant positive impact on bee abundance. An increase in bee abundance translated through to a corresponding increase in fruit set in pigeon pea. Based on these findings, bee community functional diversity and abundance, as well as pollination services, can be safeguarded, and potentially enhanced, through conservation management maintaining complex landscape structures surrounding pigeon pea crop fields, in conjunction with a reduction in insecticide inputs.

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# Natural areas buffer impact of pesticides on wild pollinators of a perennial crop

**Mia G. Park<sup>1</sup>, E.J. Blitzer<sup>1</sup>, Jason Gibbs<sup>2</sup>, John Losey<sup>1</sup>, Bryan Danforth<sup>1</sup>**

1. Department of Entomology, Cornell University, Ithaca, NY 14853
2. Department of Entomology, Michigan State University, East Lansing, NY 48824

Wild bees not only provide free pollination services for crops, they can improve efficiency of managed bees and increase the spatio-temporal stability of such services. Given predicted global shortages in pollination services, identifying factors that support thriving wild bee communities is central to ensuring sustainable and affordable food production. Natural areas surrounding farms generally benefit wild bees, but the effect of on-farm management and its interaction with the landscape are not well understood. Here, we assess the impact of pest management and landscape composition (1km) on wild bees visiting apple. We surveyed wild bee abundance and species richness in 18 conventional apple orchards, across a gradient of landscape complexity and pest management intensity in Western New York 2011 and 2012. Wild bee abundance and richness were measurably impacted by pesticides applied during or near the bloom, but only in agriculturally simplified landscapes. We establish a clear impact of pesticides on wild bees at the community level, as well as the ability of surrounding natural areas to support pollinator communities resilient to this on-farm disturbance. Maintaining wild crop pollination services, therefore, requires minimizing wild bee exposure to pesticides and promoting landscape complexity within agro-ecosystems.

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# Retention and Reproduction of Alfalfa Leafcutting Bees, *Megachile rotundata* (Megachilidae), in Alfalfa Fields

**Theresa L. Pitts-Singer**

USDA-Agricultural Research Service, Pollinating Insect Research Unit, Logan, UT 84322

*Megachile rotundata* has been used as a commercial pollinator for alfalfa seed production for more than 40 years. However, the factors that influence on-farm reproductive success are poorly understood. We know how to manage the bees and effect pollination of the crop, but we do not understand what exactly causes variation in bee reproductive success. Contributing factors for reproductive success (sustaining or increasing healthy bee population) involve management decisions, field and habitat characteristics, weather, and bee behavior. Multiyear studies of *M. rotundata* in the field have shown that the number of surviving bees is never as high as the intended number of those released in the field (45-79% of released bees survive) and that the number of established females is as low as 25% of the intended release number. Reproductive success is not necessarily limited by floral resources or availability of nesting cavities, and environmental conditions can influence progeny fate. If management can be altered to provide more attractive or optimal bee nesting conditions, then reproductive success of *M. rotundata* may be more reliable and predictable.

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# **USDA Farm Service Agency efforts to expand foraging opportunities for honey bees on land enrolled in the Conservation Reserve Program**

**Skip Hyberg**

Farm Service Agency

Abstract not submitted.

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# FRIDAY SPEAKERS

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# Native bee responses to anthropogenic land use change

## **Neal Williams,**

University of California-Davis, CA, USA

## **Eric Lonsdorf,**

Chicago Botanical Garden, IL, USA

## **Jessica Forrest,**

University of Ottawa, Ontario, Canada

Bee responses to anthropogenic land use change are determined by the interaction of specific life-history/ ecological traits (i.e., “response traits”) and underlying environmental attributes associated with the change, such as differing resource availability among habitats. As a result, land use change is expected to affect not only bee abundance and diversity, but also the composition of communities with potential implications for pollination function. The importance of trait-based responses also suggests that knowledge of bee traits might allow us to predict community responses to global change and inform conservation action. I report on a study examining bee species and guild responses to agricultural land transformation in Northern CA. I then use a spatially-explicit model to dissect the importance of different bee traits and forage versus nesting resources in determining bee community responses. Agriculture in general filtered bee communities based on specific traits, but its effect on abundance and species richness depended on farm management practices. The model revealed the differential sensitivity among nesting guilds, as well as differences in the spatial scale over which forage versus nesting resources operate. The model also shows promise for determining alternative restoration and mitigation actions to bolster bee populations and pollination function.

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# Bumble bee conservation: applying molecular techniques to preserve biodiversity

**James P. Strange**<sup>1</sup> and **Jonathan B. Koch**<sup>1,2</sup>

1. United States Department of Agriculture-Agricultural Research Service, Pollinating Insects Research Unit, Utah State University, Logan, UT 84322
2. Department of Biology, Utah State University, Logan, UT 84321

Worldwide many bumble bee species are declining in range and abundance due to various causes including pathogens, climate change, urbanization and agricultural intensification. In fact, the full extent of bumble bee declines is not well understood due the number of species at risk, the rapidity of the declines and the various factors contributing to this biodiversity crisis. Because of the complicated nature of the problem, many techniques are needed to evaluate these declines. Using population genetic techniques to study ecological phenomena is an approach that has grown in popularity over the past two decades and now the ability to apply molecular tools to species conservation are being realized. Here we present the results of recent efforts to apply molecular approaches to aid in non-destructive sampling and identification of bumble bees in surveys of bumble bee populations, and discuss how these techniques can reveal evolutionary significant units (ESUs) important to species conservation.

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# Urban land use limits regional bumble bee gene flow

**S. Jha<sup>1</sup> and C. Kremen<sup>2</sup>**

1. Integrative Biology, 401 Biological Laboratories, University of Texas, Austin, TX 78712, U.S.A.
2. Environmental Science, Policy & Management, 130 Mulford Hall, University of California, Berkeley, CA 94720, U.S.A.

Potential declines in native pollinator communities and increased reliance on pollinator-dependent crops have raised concerns about pollinator conservation and dispersal. Bumble bees are one of the most effective native pollinators and are often the first to be extirpated in human-altered habitats, yet little is known about what landscapes promote or limit their gene flow. In this study, we examine regional genetic differentiation and fine-scale relatedness patterns of the yellow-faced bumble bee, *Bombus vosnesenskii*, across natural and human-altered landscapes to investigate how current and historic habitat composition impact gene flow. We show that *B. vosnesenskii* exhibits low but significant levels of genetic differentiation across the study system ( $F_{ST}=0.019$ ,  $D_{est}=0.049$ ). Most importantly, we reveal significant relationships between pairwise  $F_{ST}$  and resistance models created from contemporary land use maps. Specifically, *B. vosnesenskii* gene flow is most limited by commercial, industrial, and transportation-related impervious cover. Finally, our fine-scale analysis reveals that pair-wise relatedness declines significantly with increasing distance between individuals at the 1- 9km spatial scale, most likely due to local queen dispersal. Overall, our results indicate that *B. vosnesenskii* exhibits considerable local dispersal and that regional gene flow is significantly limited by impervious cover associated with urbanization.

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# Effects of an invasive plant on native bumble bee populations

**Jessamyn S. Manson**<sup>1,2,3</sup>, **James P. Strange**<sup>4</sup> and **Rebecca E. Irwin**<sup>1,2</sup>

1. Dartmouth College, Department of Biology, Hanover NH 03755
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4. USDA-ARS Pollinating Insects Research Unit, Utah State University, Logan, UT 84333

Invasive plants can alter native plant communities directly and indirectly via changes in pollination services. However, little is known about the repercussions of plant invasions for native pollinator populations. An increase in the abundance of *Linaria vulgaris* in the western United States has been concurrent with changes in the foraging behavior of bumble bees, with workers demonstrating a preference for this invasive plant over native plants. To assess the effects of *L. vulgaris* on native pollinators, we compared the colony density and genetic diversity of bumble bees at sites with and without *L. vulgaris* in southwestern Colorado. We sampled *Bombus bifarius* and *B. appositus* workers at paired sites in 2010-2011 and genotyped approximately 2100 workers to evaluate kinship and colony density. Sites with *L. vulgaris* had higher bee abundance than sites without *L. vulgaris* and workers from more colonies foraged in sites with vs. without *L. vulgaris*. However, abundance of the two bee species varied substantially between field seasons and between sites with and without *L. vulgaris*. Results suggest that pollinators can benefit from foraging on *L. vulgaris*, and the invasive plant may play an important role in maintaining the size, number and genetic diversity of bumble bee colonies.

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# Citizen scientists document geographic patterns in pollinator communities

**Alison J. Parker and James D. Thomson**

Department of Ecology and Evolutionary Biology, University of Toronto

The community of pollinators that visit a plant species can show extensive variation across the plant's geographic range; this variation will influence patterns of pollen transfer in plant populations. However, geographic patterns in pollinator communities are difficult to document and often will change from year to year. To document the community of pollinators visiting the spring ephemeral *Claytonia virginica*, we assembled a team of citizen scientists to monitor pollinator visitation to *C. virginica* plants throughout the species' range. Citizen scientists documented some interesting differences in pollinator communities; specifically, these data support our observation that northern *C. virginica* populations are visited more often by the pollen specialist bee *Andrena erigeniae*. In addition, we collected data in three populations on the rate of pollen depletion, which is one important measurement of patterns of pollen transfer in *C. virginica* populations. We found that the rate of pollen depletion varies geographically, and that the rate of pollen depletion is consistent with differences in *A. erigeniae*'s visitation rate. Differences in pollinator communities throughout *C. virginica*'s range may affect the male fitness of *C. virginica* plants or the reproductive success of *C. virginica* populations, or both; these differences may affect selection on floral traits and potentially result in pollinator-mediated local adaptation.

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# Stingless bee species' traits alter responses to deforestation

Elinor M. Lichtenberg<sup>1</sup>, Berry J. Brosi<sup>2</sup>

1. Department of Entomology, Washington State University, Pullman, WA
2. Department of Environmental Studies, Emory University, Atlanta, GA

Evidence is rapidly accumulating that land use change alters pollinator abundance and richness, with significant effects on pollination. However, we still do not have a comprehensive view of how landscape modifications act within bee communities. This is particularly true for the tropics, where pollen limitation is most severe and land use change is the largest driver of biodiversity loss. The >500 species of stingless bees (Meliponini) contribute to pollination of 60 tropical crops, and are vital pollinators of natural areas throughout the tropics. While all eusocial, stingless bee species show high variation in key life history and behavioral traits. We used data on Costa Rican stingless bees to investigate whether such traits filter effects of forest loss. Trees provide important nesting and floral resources for this taxon. Our results show that effects on species presence of both forest cover and the competitive environment alter with species' traits. This suggests that some groups of pollinators are more susceptible to land use change than others. Given recent demonstrations that traits affect how bees interact with plants and function as pollinators, it is important to better understand relationship between land use change, life histories, behaviors and pollination.

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# Pollinator Conservation Grows Up: Lessons Learned from the Field

**Mace Vaughan, Eric Mader, Jessa Guisse, Jennifer Hopwood,  
Brianna Borders, and Nancy Adamson,**

The Xerces Society for Invertebrate Conservation

In 2008, in partnership with the USDA Natural Resource Conservation Service, and key researchers, the Xerces Society launched a series of pilot projects to restore pollinator habitat on farms from New England to Florida to California. This presentation follows those case studies—from initial landowner meetings—to mass seeding native wildflowers—to the ongoing management of maturing habitat.

Lessons learned include some of the human barriers to conservation adoption, the economic costs and benefits of habitat restoration, and novel approaches to establishing wildflower-rich landscapes. Through these lessons, the Xerces Society and agency partners have been able to refine the process of pollinator habitat conservation. The results are increasingly successful project implementation, greater motivation by farmers to take action, and a growing understanding that pollinator conservation can enhance other ecosystem services, such as supporting other beneficial insects for pest control (“conservation biological control”).

New technology, including a Pollinator Habitat Assessment Tool for in-field decision-making by conservation planners, and region-specific habitat restoration guidelines will also be introduced as part of the presentation.

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# Colony-level Burden of Environmental Pollutants for Honey Bees

**Chris Mullin, Jing Chen, Wanyi Zhu, Maryann Frazier and James Frazier**

Department of Entomology, Center for Pollinator Research, The Pennsylvania State University, University Park, PA 16802

Assessment of the total toxic chemical load or burden for an organism requires monitoring all the relevant exposures, including environmental pesticides, miticide and antibiotic treatments, and dietary toxicants. Colony burden of contaminants must include the adjuvant and co-formulants in modern pesticides that are largely assumed to be biologically inert. Numerous pesticides and more recently co-formulants have been found in beehive samples. Formulations usually contain inerts at higher amounts than active ingredients, and tend to be more toxic to honey bees than the active ingredient alone. Impacts of 'inerts' in pollen, nectar or wax alone or in combination with coincident pesticide residues on honey bee survival and behavior are largely unknown. We have shown that honey bees are unusually sensitive to organosilicone spray adjuvants and the solvent N-methyl-2-pyrrolidone, common co-formulants used in agrochemicals and spray adjuvants. Effects include learning impairment for adult bees using the proboscis extension reflex assay, and chronic toxicity in larval feeding bioassays. Most formulations we tested were more toxic to bees than their respective active ingredients. We anticipate that if 'inerts' are influencing pesticide levels and general hive stress, formulation recommendations can be optimized for use in bee foraging areas.

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# Progress and future prospects for assessing the risks posed to pollinators by pesticides – science needs

**Helen Thompson,**

Food and Environment Research Agency, Sand Hutton, York YO41 1LZ UK

There is a wealth of laboratory generated toxicity data (both acute and sublethal) of pesticides to honeybees; data for other bee species are far more limited. Acute toxicity comparisons suggests there are no major differences in species on a per weight basis. However, although testing can be readily undertaken to generate these data, there are inherent weakness in our ability to extrapolate from effects seen in the laboratory to those which occur in the field, particularly for social species which are dependent on communication but also may exhibit greater reliance in responding to stressors. A major gap in relates to our understanding of the realistic exposure of pollinators to pesticides, at both the individual and for social and eusocial bees at the colony level. Current risk assessment for single chemicals relies on major extrapolations from a very limited dataset, e.g. residues in pollen and nectar for systemic pesticides from application rates and predicted intake over the course of a day. More realistic scenarios include applications of multiple active ingredients, and both multiple pesticides and mixtures with in-hive medicines within colonies; some approaches to assessing risks of these will be discussed.

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# How to make decisions when you don't know the facts: the interface of science and pesticide policy

**James L Frazier,**

Department of Entomology, The Pennsylvania State University, University Park, PA 16802

Public policy making occurs at the interfaces of government, economics, and public welfare with all the attendant difficulties one can imagine. Participants count on the latest scientific understanding to inform the process, but the necessary details are often lacking. The downside of a bad decision from this process guarantees a lose-lose-lose scenario for everyone. Current pesticide policy attempts to balance the impacts of pesticides on people, the food system and its economics, and the environment, but seems to be failing us with regard to the new generation of reduced - risk pesticides. Is the current system of pesticide risk assessment, registration, monitoring, and enforcement working adequately? Many would say not, and the ensuing controversies that fill the current news headlines offer us the opportunity to seek a change direction. Can we improve current pesticide policy by working cooperatively rather than continuing to debate from individual group viewpoints? Some perspectives on how to change direction and adjust to a dynamic world of agricultural food production while minimizing risks for producers, consumers, and the environment will be presented.

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# Observations of a commercial beekeeper

## David Mendes

National Honey Bee Advisory Board

I was very fortunate to attend this conference 3 years ago and pleased to offer an update on commercial beekeeping since then. Although 2012 winter bee losses were reduced, excessive mortality this past winter (2013) proves that managed honey bees are still in trouble. While researchers and policy makers continue to debate what must be done to reverse this trend, very little real progress is reaching beekeepers. Many will tell you that in addition to higher losses, surviving hives are often slow to grow and make honey, and queen losses are at an all time high. Hives kept away from agricultural areas tend to have less health problems but it is harder to find "safe" places for bees as high commodity prices push out pollinator friendly and conservation habitats. Although beekeepers are reluctant to ask for government help, without a concerted effort to address loss of good forage areas and better pesticide management it will be increasingly difficult to maintain an adequate supply of managed hives to supply the crop requirements in the US.

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# Pesticides and Pollinators; A 2013 View Toward the Future

**Laurie Davies Adams**

Executive Director, Pollinator Partnership

Since 2010 changes and challenges have ensued in pesticide related policy. The SETAC Pellston met, issued its executive summary, and will release its completed book in 2013. That international gathering suggested protocols and additional tests for pesticide registration. The US EPA convened a Scientific Advisory Panel to examine the SETAC recommendations and advise them on which of the proposals should be adopted as part of the US regulatory process, including assessment of sub-lethal effects, effects on non-adult bees, and non-Apis species. The pollinator-pesticide discussion has focused on neonicotinoids, buttressed by continued unsustainable honey bee mortality ascribed to neonicotinoids by two 2012 studies in Science (Henry et al. and Whitehall et al.) and a 2012 letter to Nature (Gill et al.). Though these conclusions have been called into question, the European Union has determined through its precautionary principal to ban certain neonicotinoids for two years beginning November 2013. Finding irrefutable science to support intelligent, enforceable and beneficial policies will take commitment, resolve and funding. It will also require specific questions so study designs replicate real conditions, provide replicable conclusions, and signal a direction for policy supporting pollinators and engendering confidence within the regulatory, beekeeping, producer, and environmental communities.

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# Overview of USDA programs that Inform Pesticide Policy

**Mary Purcell-Miramontes**

USDA-NIFA, Washington DC

Given the importance of pollinators to agriculture, the USDA is actively involved in programs which address issues affecting health of pollinators and associated specialty crops. Activities range from surveys and monitoring programs, regulatory programs to minimize introduction of invasive pests and diseases, intramural and extramural support for research and extension, to educational programs that teach practices to conserve and manage pollinators. In 2007, several USDA agencies and EPA formed the Colony Collapse Disorder Steering Committee in response to widespread reports of a phenomenon believed to be causing sudden losses of honey bees. Increased investments were made to determine the importance of pests, diseases, nutritional stresses, pesticides and other factors suspected to be contributing to the disorder. However, honey bee losses still continue to be unacceptably high; beekeepers and crop growers are still concerned about how these losses will impact their livelihood and costs of producing specialty crops. Potential pesticide impacts on honey bees are one of the suspected factors contributing to bee declines and the subject of ongoing debate. This presentation gives an overview of key USDA-supported projects that should inform pesticide policy. Highlights of more targeted efforts will also be presented.

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# Reevaluation of Neonicotinoid Pesticides in California

**Richard Bireley, Denise Alder, John Troiano**

California Department of Pesticide Regulation

In 2009, the California Department of Pesticide Regulation (DPR) initiated a data call-in based on the hazards the neonicotinoid pesticides clothianidin, dinotefuran, imidacloprid, and thiamethoxam pose to honey bees. These four insecticides are systemic, meaning they translocate through plant tissues and can appear in pollen and nectar. The neonicotinoid reevaluation was initiated as a result of data submitted by Bayer CropScience showing residues above the LC50 in leaves of treated plants as well as a small number of honey bees and bumblebees. DPR has been focused on pesticides and particularly insecticides used on almonds as this crop was identified as a honey bee bottleneck to the extent that all commercial almonds are grown in California and more than one half of the United States supply of honey bees is required for pollination. Imidacloprid was registered for use on almonds. The reevaluation initially required registrants to submit leaf, pollen, and nectar residue data following the application to crops in California at the maximum allowable label rate and preferably directly to the soil. Information on the soil type was also required. In addition, larval toxicity data was required to assess the hazards these compounds may pose to larval honey bees. DPR has received larval toxicity data and limited residue data for three of the four compounds. Residue data requirements have been modified to reflect the addition of new crops to specific neonicotinoid labels, neonicotinoid pesticide use, and to ensure the uncertainties have been addressed.

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# The U.S. Environmental Protection Agency and pollinator health

**Don Brady**

Environmental Fate & Effects Division, US EPA

Abstract not submitted.

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## California Almonds: Growers Perspectives

**Gabriele Ludwig and Robert Curtis**

Almond Board of California

California almond orchards are by far the largest user of honey bee pollination services in the USA. Almonds are vulnerable to numerous plant pathogens during bloom, especially when it rains. Thus, the dilemma in terms of pesticides and honey bees in almonds is how to manage the need for fungicides at the same time there is a need for honey bee pollination. The presentation discusses trends in fungicide use in almonds along with research and outreach efforts on best management practices. Almond growers through the Almond Board of California have been funding research on honey bee health for about 20 years ranging from improving honey bee feed, better Varroa mite control, improving honey bee stocks, to assessing the impact of various fungicides on honey bees. The presentation discusses research needs and experience from the almond industry's perspective. A more recent issue has been the reduced quality of habitat for honey bees during the summer months and how that impacts colony health and almond pollination.

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# Pollinators and Pesticides: Complementary Components of Sustainable Agriculture

**CropLife Canada**

**Paul Hoekstra**

Syngenta

Bees and crop protection products are integral and complementary components of a sustainable, healthy, and productive agricultural system. As a result, the agrochemical industry has invested heavily in pollinator research and is playing an integral role in improving bee health across the world.

Pollinator health is a complex issue that requires a multi-faceted approach in order to realize long-term improvements. To that end, the agrochemical industry has invested in programs to promote product stewardship; biodiversity initiatives designed to re-establish bee foraging habitat; and research programs to study bee biology and develop treatments to protect bees from parasites and disease.

Neonicotinoids are one of the fastest growing classes of insecticides used in modern crop protection. Selectively targeting the insect nicotinic acetylcholine receptor (*nAChR*), neonicotinoids have high target specificity and low mammalian toxicity with no cross-resistance to conventional insecticide classes. Furthermore, neonicotinoids are systemic compounds that translocate throughout the treated plant when applied to the seed, greatly reducing overall chemical usage and minimizing acute exposures to sensitive non-target organisms.

Pesticides are one of the most stringently regulated products in North America and only those products that meet strict health and safety standards are registered for sale and use. Part of this assessment includes a rigorous evaluation of the potential impact on wildlife and other non-target organisms, such as bees. The agrochemical industry is strongly supportive of a rigorous, evidence-based regulatory system; decisions based in science are the most protective of human health and the environment.

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# **Development of an improved testing and pollinator risk assessment process in North America: a progress report from the perspective of the crop protection industry**

**CropLife America**

**David Fischer**

Bayer CropScience

A great deal of progress has been made toward improving pesticide-pollinator risk assessment since the 1st International Conference on Pollinator Biology, Health and Policy was held in 2010. A significant milestone was the convening of the SETAC Pellston Workshop on Pollinator Risk Assessment in January 2011 which reviewed existing methodology, identified research gaps and proposed a framework for international use. This was followed up by symposia at national meetings of several scientific societies and culminated with the EPA Science Advisory Panel review in September 2012 of the White Paper on Pollinator Risk Assessment that was issued jointly by US EPA, Canada PMRA and California DPR. Key elements of the new risk assessment process will be reviewed and some initial experiences from its implementation will be presented. While much progress has been made, numerous opportunities exist for research in order to reduce uncertainty and increase stakeholder confidence in pesticide-pollinator risk assessments. Key sources of uncertainty and areas of potential research will be presented.

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# SATURDAY SPEAKERS

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# Endocrine Regulation of Brain Structure and Function in the Honey Bee

**Susan E. Fahrbach,**

Department of Biology, Wake Forest University, Winston-Salem, NC 27109 USA

A focus of current research is to identify mechanisms that permit Kenyon cells in the adult honey bee brain to grow larger, more branched dendritic arborizations during experience-independent and foraging experience-dependent phases of neuronal growth. Previous studies have revealed that pharmacological activation of muscarinic cholinergic receptors mimics the effects of foraging experience on the mushroom bodies, suggesting that release of acetylcholine by afferents to the mushroom bodies is one factor that drives growth. We are now testing a model that highlights another factor, signaling via brain nuclear receptors. Past studies have identified the members of the nuclear receptor superfamily encoded in the honey bee genome and demonstrated that nuclear receptors are abundantly expressed in the mushroom bodies of the adult bee brain. Present studies are designed to ascertain the specific isoforms of two key nuclear receptors (EcR and USP) expressed by the Kenyon cells *in vivo* and *in vitro*. A proposed causal relationship between activation of a gene cascade triggered by ecdysteroids and process outgrowth has been tested by using RNA interference in primary cultures of Kenyon cells. These studies are embedded in a larger research context emphasizing the role of the so-called insect "molting hormones" in regulating adult physiology.

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# The Genetic Architecture of Complex Traits and Selective Breeding for Honey Bee Health

**Olav Rueppell**

Department of Biology, University of North Carolina at Greensboro, USA

Selective breeding for desirable traits has been successfully practiced for centuries in domesticated animals, including the Western honey bee, *Apis mellifera* (L). However, honey bee breeding and selection are complicated by honey bee biology and therefore comparatively underdeveloped. The current pollinator crisis has increased the need for genetically improving honey bees, and the growing genomic resources have increased the tools for artificial selection. Quantitative trait loci that influence complex traits can be identified to understand the genetic architecture of relevant traits and develop direct marker assisted selection. I will discuss the implications of the genetic architecture of the pollen hoarding syndrome for the prospect of genetic improvement of honey bees. Specifically, I will discuss the phenomena of pleiotropy, epistasis, and genetic heterogeneity. Finally, I will attempt to outline several alternative strategies that may lead to successful honey bee breeding programs.

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# The role of pesticides in queen health

**Jeff Pettis**

USDA-ARS Bee Research Laboratory

In honeybees, queen health is vital to the survival of the colony. In many parts of the world queens are failing or being superseded after as little as six months instead of the 1-2 year life span that is often reported. To explore possible causes of queen failure we dissected queens from failing colonies, including drone laying queens and measured mating success and sperm viability. Additionally, we treated healthy queens with various low doses of pesticides, based on the amounts found in adult bees and wax, and then measured sperm viability. We found that many drone laying queens still had sperm present but at a mortality rate above normal. Similarly, we found that queens from failing colonies had high sperm mortality compared to queens in healthy colonies. Lastly, when we artificially treated queens with sub-lethal dosages of pesticides we could kill up to 50% of sperm in only 7 days. Experiments are underway to look at the relationship between failing colonies, poor brood patterns and sperm viability. We have demonstrated that queens from failing colonies can have high levels of dead sperm. Experiments are underway to look at both pesticide exposures to drones and or mated queens to determine the source of the dying sperm relative to pesticide exposure. We hope to be able to report on the role that several pesticides may play in sperm viability and queen survivorship. We have data to support the conclusion that queens in failing colonies have high levels of dead sperm; the possible reasons, including pesticide exposure, for the dying sperm will be discussed

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# The Effects of Miticides on the Mating Health of Honey Bee (*Apis mellifera* L.) Queens

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The honey bee, *Apis mellifera* L., plays a pivotal role in the U. S. economy, contributing an estimated \$17 billion annually, primarily through crop pollination. Despite their importance, the number of managed honey bee colonies available for pollination services has dropped dramatically during the last decade, largely due to problems associated with *Varroa destructor* mites. For 20 years *Varroa* mites have been controlled primarily with two in-hive miticides: the pyrethroid tau-fluvalinate (Apistan) and the organophosphate coumaphos (Checkmite+). Studies have revealed that exposure of honey bees to sublethal levels of these miticides can lead to colony-wide health problems. In this study, we looked at the effects of fluvalinate and coumaphos on the reproductive health of honey bee queens. We did so by raising queens in either miticide-free beeswax or beeswax containing known concentrations of both coumaphos and fluvalinate. Upon their emergence and successful mating, we took several standard measures of queen reproductive health. We found that queens reared in miticide-laden beeswax were not significantly smaller in size but had significantly lower sperm counts, lower sperm viability, and higher mating frequency, compared to queens reared in miticide-free beeswax. Our results indicate that exposure to miticides during development severely compromises queen reproductive health.

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# Solitary bees as a model system for predicting the impacts of climate warming on pollination services

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Climate change is considered to be one of the major threats to biodiversity, and global warming in particular is predicted to have a strong impact on survival and fitness of insect populations. Climate warming is also likely to influence insect and plant phenology, potentially disrupting plant-insect interactions and threatening ecosystem services such as pollination. Surprisingly, our knowledge of how insect pollinator populations respond to increased temperature is very scarce, and is mostly limited to butterflies and honey bees. Several solitary bee species in the subgenus *Osmia* (*Osmia*) have been developed as pollinators of orchard crops. For this reason, a fair amount of information is available on the physiological mechanisms (respiration rates, weight loss, fat body depletion, response to various temperature regimes) underlying their respective life cycles. We can use this information to identify phases of the life cycle most likely to be affected by climate warming, including summer (prepupal) diapause, winter (adult) diapause, timing of emergence in the spring, and post-emergence performance (nesting and reproductive success). We can also use the available information on the timing of bloom of the crops pollinated by these bee species to explore potential plant-pollinator phenological mismatches.

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# Developmental, physiological and genomic variation affecting the flight and dispersal of lepidopteran pollinators

**James H. Marden,**

Department of Biology, Penn State University

I will discuss recent work from my lab and others in which Lepidoptera have been examined from developmental, physiological and genomic perspectives in relation to flight and dispersal. In a checkerspot butterfly, genetic variation in two metabolic genes affects flight metabolic rate, dispersal, and the dynamics of natural populations. Alleles at one of these loci affects tracheal development, oxygen supply to the flight muscles and the degree to which mitochondria are damaged by short bouts of intense flight. Using moths, we have found that aspects of plant chemistry, ranging from subtle changes (plant inbreeding) to profound changes (BT toxin) affect adult flight performance and the molecular composition of the flight muscles. This body of work may be useful for pollination biologists pondering the dispersal of pollen, the role of genetic variation in pollinator population biology, and the ways that plant chemistry can affect pollinators.

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# The Impact of Neonicotinoid Insecticides on Pollinators in Tree Fruit IPM Programs

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The conservation of biological control agents and pollinators is a key component of Integrated Pest Management in fruit orchards. As replacements for organophosphate and carbamate insecticides, neonicotinoid insecticides are considered much safer to consumers and the environment and better for fruit IPM than the products they replaced. However, because of their systemic movement into plant tissues, this class of insecticides is of special concern for impacts on pollinators. Through topical bioassays of formulated product of two neonicotinoid and three other insecticides, we compared their toxicity to adult honey bees and *Osmia cornifrons*. Responses to pesticides were not consistent between species. For example, *Osmia* was 26-fold less susceptible to imidacloprid than the honey bee, but 12-fold more susceptible to acetimidiprid. Unlike a previous report of up to 1,800-fold level of synergism of neonicotinoids with certain fungicides, our bioassays found only a 2-5-fold level of synergism with commercial formulations. Concerns over the movement of neonicotinoid insecticides into pollen and nectar from pre-bloom sprays in apple and their persistence over multiple seasons within the tree were addressed by collecting nectar, pollen, and whole flower samples after application for analysis over two seasons. These results will be presented and the integration of neonicotinoids into fruit IPM will be discussed.

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# Transcriptional markers of sub-optimal nutrition in developing *Apis mellifera* nurse workers

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Pollen is an essential component of the honey bee (*Apis mellifera*) diet, providing protein, lipids, and essential micronutrients. The dramatic shifts in physiology, anatomy, and behavior that accompany normal worker development are plastic and recent work demonstrates that development, particularly the transition from nurse to foraging roles, is greatly impacted by diet. However, the subtle role of diet, particularly as newly eclosed bees become nurse workers, is poorly understood. To further understand the effects of poor diet and the role of diet in mediating this key developmental transition, we used RNA sequencing to assay gene expression changes in the abdomens of 3 day and 8 day old worker bees fed either honey and stored pollen (rich diet) or honey alone (poor diet). Substantial changes in gene expression occurred during early adult development and due to starvation. Diet-induced changes in gene transcription occurring in early age were largely a subset of those occurring at later age. The aging process itself differed due to diet and the data suggest that poor diet causes normal age-related development to go awry. The transcriptional markers found in the present study provide a starting point for understanding the subtleties of starvation in *A. mellifera*.

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# The gut microbial communities in honey bees and bumble bees

**Nancy A. Moran**

A central role of microbial partners, particularly gut bacteria, in animal ecology and evolution is increasingly evident. Honey bees and bumble bees have characteristic bacterial species in their guts most of which are absent from most other bees. In honey bees, gut communities are dominated by 8 or 9 bacterial species, each consisting of numerous strains that differ in gene repertoires. Experiments show that these bacteria are transmitted through social interactions, and that each worker is fully colonized before leaving the hive for the first time. Three of these species are Gram negative bacteria that have recently been described as *Gilliamella apicola*, *Snodgrassella alvi*, and *Frischella perrera*; these dominate in the ileum region of the hindgut. Related bacteria occur in bumble bees, in which they have been shown to confer protection against trypanosome parasites. Genomic analyses and experimental assays indicate that *Gilliamella* is able to digest pectin, suggesting a possible role in pollen wall digestion. The history of antibiotic use in beekeeping in the United States has led to an accumulation of tetracycline resistance genes in all gut bacteria in American honey bees. The full extent of favorable or harmful effects of these bacteria is not yet known.

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# Pathogen-host-interactions in American Foulbrood disease

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Honey bees are attacked by numerous pathogens, some of them just causing covert infections others causing overt disease symptoms and even death of individuals and entire colonies. Among the latter group is the bacterium *Paenibacillus larvae*, the etiological agent of the epizootic American Foulbrood of honey bees (AFB). As the name suggests, AFB is a bacterial disease affecting only the larval stages of honey bees. *P. larvae* is an obligate killer because death of larvae and conversion of larval biomass into bacterial biomass are prerequisites for disease transmission within and between colonies. Hence, *P. larvae* must have evolved effective means to attack larvae, to circumvent the larval immune response and to finally kill and decompose larvae. We recently identified and characterized some of these virulence factors of *P. larvae*. We will present a model for molecular pathogenesis of *P. larvae* infections built upon these novel findings in order to further the understanding of the molecular basis of pathogen-host-interactions in American Foulbrood disease.

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# Host-Parasite Interactions: Risks associated with invasive parasites of bumblebees and molecular haplotyping of *Apicystis bombi* from Europe and Argentina.

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Within the *Apidae*, a family of bees with an important ecological value in maintaining plant diversity and providing pollination services, numerous species are declining. Bumblebees are no exception to this phenomenon; their distributions declined since the 1950, and numerous mechanisms could be behind this decline. For the recent declines of bumblebees in South and North America, it is proposed that the spread of parasites from commercial bumblebee colonies into wild populations could further deteriorate these already threatened populations. Within this context, we looked at the different viruses and protozoa bumblebees carry, and give an overview of the complex host-parasite networks these species live in. We evaluate the risks associated with spillover of pathogens from commercial bumblebees to wild populations and suggest mitigation actions. Second, we look for evidence of whether spillovers indeed occurred with the introduction of European bumblebees into South America by using molecular haplotyping of *Apicystis bombi*, a neogregarine parasite of bumblebees reported to occur in Europe and the Americas.

# From Resins to Propolis: Biological origins and role in honey bee social immunity and health

**Marla Spivak<sup>1</sup>, Renata Borba<sup>1</sup>, Michael Wilson<sup>2</sup>, Adrian Hegeman<sup>2</sup> and Jerry Cohen<sup>2</sup>**

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We are studying how honey bees, *Apis mellifera*, select and exploit the pharmacological properties of resin to benefit the health of the colony. Honey bees collect resin and deposit it in the nest where it is called propolis. A propolis envelope lining the inner walls of the nest acts as an antimicrobial layer surrounding the colony, benefiting bee immune defenses and colony-level social immunity. We are currently exploring if plant-derived chemical components in resin provide an important colony defense against both general microorganisms and specific pathogens of honey bees. We also are asking if bees increase resin collection and/or switch botanical sources of resin to those with greater biological activity after challenge with a bacterial pathogen. Ultimately, our findings will shed light on the behavioral mechanisms underlying the collection of resin by a relatively rare subset of bees, and establish the link between the origin of the biologically active substances and their ultimate impacts on honey bee health. More broadly, we hope to open new research avenues into the role of resins as pharmacological agents in the ecology and evolution of plant-animal interactions.

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# Transcriptomic analyses of *Varroa* x virus x bee interactions in a resistant population

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*Varroa* mites can have direct impacts on honey bee physiology but there is a consensus that their most important role for bee health is in transmitting viruses within and between honey bee colonies. Here we discuss an effort to decouple the effects of mite parasitism and viral presence on honey bee immunity. The study population was comprised of bee lineages with strong tolerance of high mite numbers, and an apparent resistance to symptomatic Deformed wing virus, compared to co-occurring colonies without these traits. Using qPCR we showed an expected higher viral titer in susceptible colonies. Using controlled injections of DWV, we found substantial variation in viral growth, coupled with strong variation in the expression levels of several bee antimicrobial peptides. We also noted differential expression for the gene encoding the bee ortholog for Eater, a protein hypothesized to be involved in cellular immunity. Using RNAseq analyses of 16 pooled samples we found that bees from putatively resistant lines showed expression profiles across a diverse set of transcripts that more closely resembled control samples that had not been exposed to viruses. The results are compared with two recent studies that also measured transcriptional changes in resistant bees exposed to mite parasitism.

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## Multiple genetic lineages involved in a recent host-switch to European honeybees by the parasitic mite, *Varroa jacobsoni*

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The parasitic mites, *Varroa destructor* and *V. jacobsoni* are native to Asia and parasitise the Asian honeybee *Apis cerana*. Until recently, only *V. destructor* had successfully switched hosts to become one of the most important global pests of the European honeybee *Apis mellifera*. In 2008, *V. jacobsoni* in Papua New Guinea (PNG) was found for the first time parasitising the local *A. mellifera* and causing serious damage to colonies. To better understand the evolution of this host-switch we used 12 microsatellite markers to genetically characterise *V. jacobsoni* on *A. mellifera* in PNG, and compared this genetic signature to mites on *A. cerana* both in PNG and the surrounding Asia-Pacific region. Our results identified two distinct genetic lineages reproducing on *A. mellifera* that appear to be independent host-switch events involving small numbers of mites from the local *A. cerana*. Significant genetic variation between mites on the different hosts was also detected, thereby indicating host-switched *V. jacobsoni* populations no longer reproduce on *A. cerana*. These findings are important for understanding the potential impact of *V. jacobsoni* on *A. mellifera* apiculture worldwide.

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# POSTERS

Note:

**Posters are listed alphabetically**

by presenter's last name.

## **Cellular responses in the fat body of *Scaptotrigona postica* (Latreille, 1807) exposed to low doses of fipronil and boric acid.**

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Studies of sublethal effects of pesticide residues in stingless bees are scarce and morphological analysis of organs would add information to toxicological analysis in order to clarify the continuous exposure of *Scaptotrigona postica* to insecticides. The aim of this study was to evaluate the morphology and histochemistry of fat body of *S. postica* exposed to fipronil and boric acid, to detect cellular responses. New-emerged bees were submitted to toxicological bioassays and morphological analysis by optical microscopy and Transmission Electron Microscopy as well as histochemical methods for proteins and glycoconjugates detection. Immunohistochemical detection of DNA fragmentation and HSP70 were performed to detect cell death and stress response, respectively. Statistical analysis for the bioassays conducted with ingestion of contaminated diet with boric acid at 0.75% (w/w) or with fipronil at 0.1 g/kg of food showed that the survival of bees that ingested the contaminated diets were significantly different to survival tax presented by the control group ( $P < 0.0001$ ). The data from this study reinforce the importance of research on sublethal effects of low doses of pesticides on bees in an attempt to assess possible dose realistic and evaluate the risk assessment of stingless bees foraging in the vicinity of cultivated fields.

## Genomic and physiological analysis of diapause in bumblebee queens

**Etya Amsalem**<sup>1</sup>, **Jonathan Cnaani**<sup>2</sup>, **Abraham Hefetz**<sup>3</sup>,  
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2. Beezbee Ltd., Ein Yahav, Israel
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Bumblebees provide critical commercial and wild pollination services throughout the world. However, populations of many wild bumblebee species are declining, with similar tendencies being reported in commercial mass rearing operations, evidenced by reduced survival, fertility and health.

The majority of queen-loss occurs during diapause, a period of 6-9 months of dormancy associated with physiological changes in hormones, fats, metabolism and immunity. Managed bumblebees are treated with CO<sub>2</sub> to bypass diapause though there is only ~50% success during the process and evidence for negative effects on queen survival and performance. The underlying molecular mechanisms associated with diapause or the impact of CO<sub>2</sub> on queens has not been characterized.

Comparison of physiological parameters and gene expression in diapaused *B. terrestris* queens during their life cycle and under CO<sub>2</sub> treatment revealed that genes involved in nutrient storage, core metabolic and insulin pathways seem to play a major role in the process. This is similar to non-social insects, despite differences in social organization and patterns of diapause (complete vs. reproductive diapause). Notwithstanding, other genes linked to reproduction and nutrition in bees, such as *vitellogenin*, do not show a typical pattern of expression. CO<sub>2</sub> treatment affected the expression levels of several genes and was associated with lower lipid mass. Understanding the molecular processes underlying diapause and CO<sub>2</sub> narcosis provides us with important information about commercial and wild bumblebee management.

# An Assessment of Bumblebee Health in Maine Blueberry Fields

**K.E. Bickerman and F. Drummond**

University of Maine, Orono

Bumblebees are extremely important pollinators in wild blueberry fields in Maine due to their ability to forage during cooler temperatures than honeybees and their use of sonication to more effectively extract pollen from the poricidal anthers of blueberry flowers. Declines in bumblebee populations have been documented for the past 60 years in Europe, and similar species range reductions and population downturns have also been observed in North America. Possible causes include habitat fragmentation, introduced parasites and pathogens from the commercial bee business, and the use of broad-spectrum pesticides on crops and managed areas. We assessed parasite and pathogen loads of bumblebee workers at a variety of wild blueberry fields with varying crop management strategies, from organic to conventional, to gather current baseline data of the health of bumblebees that forage in the extra floral resources around blueberry fields. *Nosema bombi* and *Crithidia bombi* were both present at very low levels, but approximately a quarter of workers presented with conopid fly pupae in the abdomen. This parasitoid could be a significant source of early and mid-season worker loss, potentially affecting colony growth and overall health. Successful visual identification of *C. bombi* from frozen specimens was also confirmed using DNA sequencing.

## Effects of the Herbicide Dicamba on Pollinators

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Weed management tactics play an important role in shaping plant communities in agroecosystems, but the role of herbicides in influencing plant-insect interactions remains unclear. Currently, 23 weed species are resistant to the herbicide glyphosate. In response to the evolution of glyphosate-resistant weeds, agricultural industry will soon release soybean and cotton varieties resistant to dicamba, a drift-prone herbicide that could have substantial non-target effects on plants and insects. To understand the risk posed by dicamba drift to pollinators, we conducted field experiments with two perennial plant species, one agricultural, alfalfa (*Medicago sativa*), and one native, common boneset (*Eupatorium perfoliatum*), and exposed each species to simulated dicamba drift events (doses < 0.1 of the label rate). We are also pursuing laboratory behavioral assays with *Apis mellifera* to determine if sub-lethal exposure to dicamba influences *A. mellifera* learning. From our results thus far, we found in an alfalfa field experiment that flowering decreased in plots dosed with relatively high drift doses of dicamba, and, as a result, fewer *A. mellifera* visited these plots. In plots exposed to lower doses of dicamba, we did not detect differences in the number of flowers per stem, or visitation by bees. Further work is underway.

## Identification of three Halloween genes from the varroa mite and their expression during brood cell invasion

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Biosynthesis of 20-hydroxyecdysone (20E) in insects involves the action of five cytochrome P450s collectively known as Halloween genes. Transcripts from 3 Halloween genes [spook (*Vdspo*), disembodied (*Vddib*) and shade (*Vdshd*)] from the varroa mite were identified, sequenced and mapped to their genomic sequences. Predicted proteins from the coding regions of *Vdspo*, *Vddib* and *Vdshd* shared 33.3, 32.1 and 29.6% identity with their orthologs from *Drosophila melanogaster*, respectively. The *Vdspo* transcript was observed in gut preparations, while *Vddib* was present in the ovary/lyrate organ and *Vdshd* was present in ovary/lyrate organ, Malpighian tubules and gut samples. Transcript levels between phoretic mites and early reproductive mites, from pre-capping brood cells, were not significantly different for *Vdspo*. However, *Vddib* and *Vdshd* were significantly up-regulated by 87 and 105%, respectively. A cell invasion assay showed transcript levels from *Vdspo*, *Vddib* and *Vdshd* were not significantly different between mites that entered a brood cell within 4 hr compared to mites that remained on adult bees. These results suggest that the expression of *Vddib* and *Vdshd* does not direct the varroa mite cell invasion behavior, but is associated with the physiological shift from phoretic to reproductive state.

## Causal Factors in Bumble Bee Decline: Testing the Role of *Nosema Bombi*

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**Jeffrey D. Lozier**

Department of Biological Sciences, University of Alabama

**Robbin Thorp**

Department of Entomology, University of California

**Sydney A. Cameron**

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Emerging infectious diseases, often introduced from foreign sources, pose major threats to wildlife conservation, thus to ecosystem services important to the welfare of the planet. We have shown recently that multiple species of N Am bumble bees, a group of important wild pollinators, have undergone drastic declines in range and abundance over recent decades. This decline appears to correlate with the fungal pathogen *Nosema bombi* since *N. bombi* prevalence is higher in populations of declining species. Here, we tested the hypothesis that *N. bombi* found in N Am bumble bees was introduced from Europe in early 1990's through commercial activities. We used a novel approach to extract DNA from historical bumble bee specimens (museum specimens) nondestructively and tested for the presence of *Nosema*. We showed that *Nosema* prevalence increased after 1992 in declining bumble bee species, supporting the notion that they experienced an invasion event. In addition, using sequence data from SSU rRNA and anonymous genomic loci, we conducted phylogenetic and population genomic analyses of *Nosema* derived from N Am, Europe and Asia. Our results indicated that *Nosema* in N Am has low phylogenetic diversity compared to those in Asia. Further, *Nosema* found in N Am bees were closely related to those obtained in Europe, suggesting that the latter was the source of invasion. Our findings have important implications for the movement of commercial insect species and the conservation of bumble bees here in N Am.

## Rapid ecological replacement of a native bumble bee by invasive species

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Despite rising global concerns over the potential impacts of non-native bumble bee (*Bombus*) introductions on native species, large-scale and long-term assessments of the consequences are lacking. *Bombus ruderatus* and *Bombus terrestris* were sequentially introduced into Chile and later entered Argentina's Patagonian region. A recent large-scale survey in Patagonia reveals that, in 5 years post-arrival, the highly invasive *B. terrestris* has become the most abundant and widespread *Bombus* species, and its southward spread is concurrent with the geographic retraction of the only native species, *Bombus dahlbomii*. Furthermore, a 20-year survey of pollinators of the endemic herb *Alstroemeria aurea* in northern Patagonia indicates that *B. ruderatus* and *B. terrestris* have replaced *B. dahlbomii*, formerly the most abundant pollinator. Although the decline's actual mechanisms remain unknown, the potential roles of exploitation competition, and pathogen co-introduction cannot be ruled out. Given that invasive bumble bees can rapidly extirpate native congeners, further introductions should be discouraged.

## How much sampling is needed to assess pollinator habitat restorations?

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Plantings of pollinator habitat have been widely implemented in agricultural landscapes throughout the United States following the prioritization of pollinator conservation in the 2008 Farm Bill. Detailed, time-intensive assessments of the effectiveness of these plantings are underway and will provide a general understanding of how these plantings influence bee communities. However, because most plantings will not be the subject of scientific study, landowners, land managers and staff of the Natural Resources Conservation Service need streamlined methods to assess whether their restorations support increased pollinator abundance and species richness. To develop and evaluate different approaches to pollinator habitat evaluation, we compared streamlined, observation-based protocols designed for landowners and conservation practitioners with more detailed, specimen-based collections obtained by trained scientists. We then used sub-sampling analysis to explore the effect of time of day, time of year, and sampling intensity on the accuracy of the streamlined protocols. Results of this study will provide land managers and land owners with a rigorously-tested protocol for assessing and managing pollinator habitat restorations.

## The immune response of honey bee queens (*Apis mellifera*) following *Nosema ceranae* inoculation

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The microsporidian parasite, *Nosema ceranae*, was found to infect both European and Asian honey bee species. All honey bee castes, queen, worker and drone can be infected with *N. ceranae*. In this study, the immune responses in honey bee queens of different ages (1, 6 and 12 days post adult emergence) after inoculation with *N. ceranae* was investigated. The results showed that the expression of the antimicrobial peptide genes apidaecin, eater, and vitellogenin in the gut and the remaining abdomen were different among queens of different ages when inoculated with *N. ceranae*. All three ages of queens inoculated by *N. ceranae* showed up-regulation of apidaecin in gut tissue 6 days after inoculation, but only in queens aged 1 day post adult emergence were the differences significant. However, transcript levels of eater were increased in all three ages of queens when sampled on day 12, and significant differences were obtained in queens inoculated at 6 and 12 days post emergence. The variation of vitellogenin expression was observed between queens of different ages. Finally, immune gene responses in the remaining abdomen of the queens were different amongst the three ages. We clearly show that immune responses to *N. ceranae* changes as queen age and this knowledge may provide clues for understanding the ability of queens to resist infection by this gut parasite.

Keyword: *Nosema ceranae*; microsporidian parasite; honey bee queen; *Apis mellifera*; immune expression

## **The application of spatial modeling tools to assess the effect of landscape pattern and arrangement on native bee abundance in Maine's wild blueberries**

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U.S. Geological Survey, Maine Cooperative Fish and Wildlife Research Unit

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Non-native honeybees historically have been used to pollinate many crops throughout the United States, however, recent population declines have brought to light the need for a more sustainable pollination plan. Native bees are an available resource that can play an important role in crop pollination. We are investigating the landscape factors that influence native bee richness and abundance, with a focus on the native bees that pollinate Maine's wild blueberries. By coupling the InVEST Crop Pollination modeling tool, which predicts pollinator abundance based on available floral resources and nesting habitat, with generated neutral landscape models, we seek to understand relationships between native bee abundance and landscape pattern and arrangement. Field collected data on bee abundance is being used to validate the model's assumptions, and a sensitivity analysis will be conducted to determine how uncertainty in parameter choice influences model output. Our research is one component of an interdisciplinary multi-state, multi-institution project that is exploring the ecology, sociology and economics of native bee conservation in the Northeast.

# Identification of Organosilicone and Alkylphenol Polyethoxylates Surfactants in Beehive Environments by Liquid Chromatography-Mass Spectrometry

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Surfactants are increasingly applied in agro-ecosystems, where bees are exposed when they gather nectar and pollen from flowers. Over the last six years, one third of honey bee colonies in the US were lost each winter under the combination of many stressors. Our previous work has demonstrated that agricultural spray additives containing organosilicone or alkylphenol polyethoxylate surfactants cause learning impairment in honey bees, and they (or their degradation products) may be important chemical stress factors associated with the ongoing reduction in pollinator health. Here, an analysis method for those surfactants was developed using a QuEChERS extraction approach and liquid chromatography coupled to mass spectrometry (LC-MS). The occurrence of organosilicone and alkylphenol polyethoxylate surfactants in the beehive environment will be discussed.

## Native Bees on a Superfund Site

**Anita M. Collins**

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Lehigh Gap Nature Center, Slatington, PA is a unique remediation property within a Superfund Site. From 1898 to 1980 a waste plume from the NJ Zinc Co. plant, Palmerton, PA, deposited heavy metals for 20 miles downwind. The north side of the Kittatinny Ridge, the southern-most of the PA Appalachian Mts., has been virtually denuded in most of this area. Following seeding with native warm season grasses in 2003, the 750 acres of mountainside within the Nature Center has gone from bare rocks to green. In collaboration with USGS, a survey of native bees on the LGNC began in 2006. Transects of pan traps were sited across shrub habitat, around a small pond and along a former railroad bed. As of the writing of this abstract, 37 species of native bee have been identified, including species of *Andrena*, *Anthidium*, *Augochlora*, *Augochlorella*, *Bombus*, *Caliopsis*, *Certina*, *Colletes*, *Halictus*, many *Lasioglossum*, *Megachile*, *Nomada*, *Osmia*, *Xylocopa*, and *Lithrugus crysurus*, an exotic. Additional pan trapping is planned for summer of 2013 to include more habitat areas, the EPA ongoing test plots where major heavy metal depositions were made, and within a 10 acre patch of a controlled burn to be done in April 2013.

# Reproductive biology and pollination of Cambuci (*Campomanesia phaea* - Myrtaceae), a commercial fruit of the Atlantic Rain Forest

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*Campomanesia phaea* is a native plant of the Atlantic Rainforest, which produces fruit with economic interest. We studied the floral biology, the pollinators and the reproductive system in natural habitat and cultivated area in Mogi das Cruzes, São Paulo State, Brazil (23°45'S/46°09'W). The flowers last three days and the stigma remains all the time receptive. The pollen, the only floral resource, is available on the first day of anthesis. *C. phaea* just formed fruits after cross-pollinations, thus is a self-incompatible species. Fruit set of hand-cross pollination (38%) was higher than open-pollination (28%) in the two localities. Then, probably, there's pollination deficit. Among the floral visitors, bees were dominant (41 spp.). Five flies species, 2 wasps species and one butterfly species were also observed in the flowers. The large size bees (*Bombus brasiliensis*, *Bombus morio*, *Centris* sp., *Epicharis* sp., *Ptiloglossa latecalcarata* e *Ptiloglossa* sp.) were considered effective pollinators, because they always touch the stigma during visits. Other bee species sporadically contacted the stigma and then were considered occasional pollinators. The others insects visitor don't touch the stigma and were considered opportunistic. These results must support studies with pollinators management to increase the fruit productivity in cambuci crops.

## **Viruses in Honey Bees: Impact of the antiviral drug ribavirin on viral infections in individual bees and in the colony**

**Michael Freiberg and Diana L. Cox-Foster**

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Small picorna-like viruses represent a significant threat to honey bee health and are associated with a variety of pathologies such as dead brood, deformed wings, and general colony mortality. It has also been suggested that Colony Collapse disorder is caused by a virus. These viruses also infect other hymenopteran pollinators and can be detected in commercial bumble bee colonies. Despite the threat posed by these RNA viruses, there are no chemotherapeutic agents currently in use to treat these viruses and so treatment remains limited to management techniques, which are largely ineffective. We explored the broad-spectrum antiviral drug ribavirin for its potential as an antiviral treatment in honeybees. We present evidence that ribavirin may be active against Deformed wing virus (DWV), Black queen cell virus (BQCV), and Sacbrood virus (SBV); however, the impact does not remove the viruses from the colony. Decreased levels of these viruses were detected in individual bees after feeding a colony with the ribavirin and after the colony was no longer actively foraging during the winter. In the spring, the viruses reappeared in the colonies once active foraging began, suggesting reinfection from neighboring colonies or other pollinators. The impact of the viral mutagen on the viral sequences will be discussed.

## **Prevalence of RNA viruses in the commercial greenhouse bumble bee (*Bombus impatiens*), and the pathology, health impacts and transmission of Israeli acute paralysis virus (IAPV) in bumble bees and honey bees**

**Rajwinder Singh, Edward Rajotte, Diana L. Cox-Foster**

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Widespread declines in bee populations have become a major cause of concern. RNA viruses, including recently described Israeli acute paralysis virus (IAPV), are linked to honey bee (*Apis mellifera*) losses worldwide; however, very little is known about the viral diseases in other managed bees. We investigated the ecology and epidemiology of RNA viruses in the common eastern bumble bee, *Bombus impatiens*, the major pollinator of commercial greenhouse crops in the US, with the main focus on IAPV. Greenhouse experiments involving the commercial bumble and honey bee colonies were conducted to study the health impacts, pathology and inter-taxa transmission dynamics of IAPV. RNA viruses were prevalent in the newly purchased, commercial colonies of the common eastern bumble bee, with DWV being the most common virus. IAPV can potentially spread in bumble bees through various horizontal and vertical transmission routes. IAPV moved from infected honey bees to IAPV-free bumble bees and from infected bumble bees to honey bees within a week to 10 days, when bees were allowed to forage together on common flowering plants. There was no species barrier and directionality involved in the movement of RNA viruses, between honey bees and bumble bees. Weeks after feeding IAPV to bumble bee queens, the virus was detected in different tissues besides the mid-gut, indicating virus dissemination throughout the queen bee's body. The colony survival and the foraging activity of IAPV-infected bumble bees were lower than the control colonies. IAPV also resulted in heavy adult mortality and poor colony build up in infected honey bees. Overall, IAPV negatively impacted the colony health of both bumble bees and honey bees under greenhouse conditions. The role of the IAPV and other RNA viruses needs to be studied in the recent declines in field populations of these pollinators.

## **Anatomical investigation of regular dorsal dimples on *Varroa destructor*, a devastating parasitic mite of honey bees**

**Daniel J. Wiens and Arthur R. Davis**

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*Varroa destructor* parasitizes immature and adult stages of the European honey bee (*Apis mellifera* L.), posing a serious threat worldwide. Regular dorsal dimples occur up to twice per mite, and are developmental faults in about 10% of adult females. These dimples exist as indentations in predictable locations directly above a set of obliquely-oriented muscles which run dorso-ventrally on each bilateral side within the mite's abdomen. Because other work indicated that dimples may decrease the reproductive success of female mites, this study concentrated on preparing resin sections to track the abdominal musculature situated below these dimples. An increased width (i.e., cross-sectional area) compared to regions where dimples were absent, could indicate sustained muscle contraction that becomes fixed beneath a hardened, permanent dimple during the adult mite's sclerotization process. Non-dimpled mites were also examined. The attachment points and paths of dorso-ventral muscles varied from literature reports. Six such muscles exist in each side of a mite, but only the outer few muscles showed a significant increase in area, reflecting the muscles' relative position beneath the deepest portion of a dimple. Further study is required to determine whether dimples constrain progeny development within the female's abdomen.

## Comparison of techniques to rank pollination effectiveness of insects visiting inflorescences of purple coneflower (*Echinacea angustifolia*; Asteraceae)

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As the largest dicot family, the Asteraceae (Compositae) represents 1/10th of all flowering plants. Inflorescences (heads) of purple coneflower [*Echinacea angustifolia* (DC) Cronq.] were visited by insects from four orders (Coleoptera, Diptera, Hymenoptera, Lepidoptera), in accordance with a generalist pollination syndrome. Pollinator effectiveness was evaluated by permitting solitary visits to receptive disc florets of virgin (previously-bagged) heads. After insect departure, four parameters were quantified: total stigmatic pollen load and proportion of pollen grains germinated; numbers of pollen tubes at style bases; and percentages of total florets that had retracted (i.e., shriveled, senescent) styles. Quantifying pollen and its germination proved ineffective to discriminate insect visitors from control (bagged) heads. However, the proportion of a head's florets that had styles retracted, and/or the numbers of pollen tubes at the style base, provided this descending ranking (on a per visit basis) of pollinator effectiveness: Apidae (*Bombus* spp., *Apis mellifera* L.); Pieridae (*Phoebis sennae* L.); Meloidae (*Epicauta ferruginea* Say); Bombyliidae (*Systoechus vulgaris* Loew); Megachilidae (*Megachile pugnata* Say). Analysis of the proportion of retracted styles per head was completed more rapidly, and in the field, without a microscope. Retracted-style analysis following single insect visits to previously-unvisited inflorescences may allow prompt evaluation of insects as pollinators of many asteracean taxa.

## **Varroa Population Dynamics in Commercial Honey Bee Colonies and the Implications for Control**

**Gloria DeGrandi-Hoffman<sup>1</sup>, Fabiana Segura<sup>2</sup>, Robert Curry<sup>3</sup>**

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Varroa is a major cause of colony losses throughout the world. Though Varroa has devastating effects on colonies, it can take a year or more for populations to reach exponential growth and significantly reduce colony survival. Theoretically then, well timed control measures that prevent mite populations from achieving exponential growth should prevent colony losses. To test this, we treated hives during broodless periods at three commercial bee yards and achieved better than 90% control. Additional treatments were applied throughout the year to further reduce mite populations. Predictions generated from our Varroa-honey bee colony population dynamics model that was initialized using data from the study and included our treatment schedule indicated that mite populations should not have reached exponential growth during the experimental period. However, within two months after the initial treatment, Varroa populations were 2-4 times higher than predictions based solely on the mite's intrinsic rate of increase. The results suggest that Varroa may be immigrating into colonies at sufficiently high frequency that populations can reach levels that threaten colony survival within a season.

## Examination of the accuracy of different methods for *Varroa destructor* population estimation in honeybee hives

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The examination of the accuracy of different methods for *Varroa destructor* population estimation in honeybee hives was conducted in the frame of the international research project "STRANOVA".

The following methods were tested: evaluation of live samples (*Vivo* evaluation) of adults (method 1), larvae and pupae (method 2) of bees for the presence of mites; evaluation with the killing of: adult bees in hot 1% water solution of soda (method 3) or brood taken from cells (method 4) for the presence of mites; evaluation of natural mite mortality using sticky sheets in hives) (method 5). All the tested methods have their advantages and disadvantages. The methods using *vivo* evaluation are more sparing for bees but less precise. The methods using killing of bees or brood are the most precise but less sparing.

Method 3 was 1.1 - 1.3 times more accurate than method 1 for evaluation of adult bees, method 4 - for immature bees (1.2 - 1.5 times more accurate than method 2). The indirect method 5 was the most sparing but less precise than the previous ones.

Natural mortality expressed by one mite drop per 24-hours corresponds to the presence of about 160 mites on bees per colony.

## Pesticide-Pollinator Relationships

**N.D. Dobrynin**

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The risk of pollinator poisoning by pesticides has become an important factor both for pollinator survival in general and sustainable yields of entomophilic crops in particular. The necessity to solve this problem forces to seek special approaches based on the study of ecological factors determining the possibility of pollinator intoxication by pesticides, the toxicity and rate of pesticide hazard to pollinators to develop measures for pollinator protection under pesticide application.

The results of more than 15-years studies on the topic for different kinds of pollinators (honey bee, solitary bees, bumble bees) are presented.

The role of abiotic and biotic ecological factors in pesticide-pollinator relationships is analyzed. The influence of pesticide properties, methods and target objects of application on possibility of pollinator intoxication is reviewed. The data on toxicity of insecticides for different kinds of pollinators are provided. The length of the period of residual hazard of different groups of insecticides to different kinds of pollinators is given.

## Factors Impacting Overwintering Success of Honey Bees

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Populations of honey bees experience dramatic losses over the winter, with beekeepers in the US losing an average of 30% of their colonies in surveys conducted from 2006 to 2011. Honey bees overwinter in a thermoregulating cluster, and overwintering honey bee workers are in a distinct physiological state compared to summer bees, including altered hormone levels, reduced metabolism, high nutrient stores, and dramatically increased lifespan. Previous studies have suggested that levels of *Varroa* mites and viruses are strongly correlated with colony losses, but the effects of these factors on overwintering bees at the molecular and physiological level have not been well studied. Here, we examine the impact of genetic stock (Northern vs Southern reared) on the physiology, parasite and pathogen load, and survival of overwintering honey bees, and characterize the interactions between these factors. This study will not only shed light on the mechanisms regulating overwintering and underlying overwintering success in honey bees, but will also produce valuable information that can be used to develop better management practices for the beekeepers.

## **Diversity of pollen diet and viral infection profiles affect survival and physiology in caged honey bees**

**Adam G. Dolezal, Jimena Carrillo-Tripp, W. Allen Miller, Bryony C. Bonning, Amy L. Toth**

Iowa State University

Honey bees are exposed to a variety of environmental stressors that impact their health. While important individually, these factors likely interact to produce the greatest effects. Of particular interest is the interaction between nutritional stress and viral infection. When honey bees are starved of pollen, or receive pollen from a single plant source, their lifespan and immune functions decrease. Changes in landscape usage have decreased the variety of pollen forage available to honey bees, making this a particularly pertinent issue. Viral pathogens, especially Israeli acute paralysis virus (IAPV), have also been implicated in honey bee losses, including symptomatic Colony Collapse Disorder, though neither viruses nor nutrition fully explain this problem. Thus, the interaction between nutritional deprivation and viral infection is an important question in honey bee health. To better understand how viral infection and nutritional stress interact, we created cages of bees made up of individuals from colonies with different viral infection profiles, and fed the cages diets of no pollen, a polyfloral pollen mixture, or pollen from single sources. We then measured mortality, lipid levels, and viral titers of the caged bees. Our results suggest that lifespan and physiology are affected by both pollen diversity and viruses.

# Influence of climatic factors on *Nosema* infections in honey bee colonies in Germany

**Anto Raja Dominic<sup>1</sup>, Sebastian Gisder<sup>2</sup>, Elke Genersch<sup>2</sup>, Andreas Linde<sup>1</sup>**

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Colony Collapse Disorder (CCD) is a relatively recent syndrome in the European honey bee (*Apis mellifera*) that has resulted in significant loss of honey bee colonies world-wide. Microsporidial infections (*Nosema apis* and *Nosema ceranae*) have been identified as one of the potential contributing elements leading to CCD.

Identification of factors that influence the incidence of *Nosema* spp. is necessary to forecast the infection risk in the coming season and take adequate preventive measures. Studies have shown that *Nosema* spores are influenced by temperature and the differential distribution of the two species in Europe indicate an influence of climate.

The data used comes from a study of about 1200 colonies from 120 apiaries in north-east Germany monitored twice annually since 2005. The incidence and infection levels of both *Nosema* spp. were analysed against daily weather variables (temperature, precipitation, relative humidity, cloudiness and sunshine duration). Aggregates of weather variables for different time window sizes (10-180 days) were extracted for the months prior to the collection of the bee samples. The relationship between the aggregated weather variables and the pathogens were analysed using data mining and statistical tests. The results of analysis will be presented.

## Phylogeography of *Bombus ephippiatus*, a Mesoamerican bumble bee species complex

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Little is known about the biology of bumble bees (*Bombus Latreille*) in southern portions of the Americas, especially in Mesoamerica, a region of geological and ecological complexity from Mexico through Central America. One ubiquitous Mesoamerican species, *Bombus ephippiatus*, is enigmatic. Like many other *Bombus*, this species is homogeneous in body structure yet exhibits striking intraspecific color pattern polymorphism across its range, leading to uncertainty about its genealogical boundaries. It has been grouped taxonomically with *B. wilmattae*, a species narrowly restricted to southern Mexico and northern Guatemala. Furthermore, the relationships between these two taxa and a third species, *B. impatiens*, found only in America north of Mexico, have been controversial. Our phylogenetic analysis of DNA sequences from mitochondrial COI and nuclear PEPCK and CAD resolves the phylogeny of these three taxa as (*B. impatiens*, (*B. ephippiatus*, *B. wilmattae*)). Additional data from eight nuclear microsatellite markers reveal complex patterns of genetic divergence and isolation among populations of *B. ephippiatus* across its extensive geographic range, providing evidence for multiple independent evolutionary lineages. These lineages correspond not only to geographic and habitat variation across their range, but also to distinct color pattern groups present in the species.

## **Text messaging pollinator observations in peri-urban food producing gardens: measurable pathways, participatory development and ecosystems (Bolivia and Peru)**

**Medora Ebersole**

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This paper examines the potential of public domain messaging software as a networking tool for participatory citizen-environmental science projects (in this case aggregating pollinator observation data) for marginalized residents of peri-urban locales. Peri-urban food producing gardens have become a focus of food security projects and policies while they also present vital habitat to pollinators on their increasingly threatened migratory flyways. A text messaging approach toward data-gathering related to this environmental-functioning of peri-urban gardens is the focus of a collaborative environmental sustainability project. It is designed to include participants across a wide range of groups knowledgeable about nature, with the goal of bringing their intrinsic environmental knowledge into a fuller dialog with social, environmental and biodiversity sciences. Their shared messaging and emergent observations are displayed alongside natural history collections. This study is based on five field visits to Cochabamba during 2008-12 and collaboration with personnel of the Museo Alcide d'Orbigny in Cochabamba, Bolivia.

## **Evaluation of native bee visitation and floral resources of early-season canola (*Brassica napus*) cover crop mixtures**

**Katherine Ellis, Mary Barbercheck**

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This research aims to evaluate the potential benefits and trade-offs associated with adoption of cover crops in annual grain cropping systems for native bee conservation. With the current issues facing managed honeybee colonies, the conservation of native bee communities is critical to maintaining 'free' pollination services. However, native bees are negatively affected by agricultural intensification. Cover crops are a technique employed in sustainable agriculture for soil quality maintenance, but little data exists on the potential of flowering cover crops as a resource for native bees in annual cropping systems where springtime flowers may be uncommon or sporadic over space and time. This project evaluates canola (*Brassica napus*), a commonly used overwintering cover crop, grown in monoculture and polyculture at achieving optimal use by bees. Visitation to each crop mixture was compared to landscape-level trapping to evaluate actual versus potential use over time. Additionally, we monitored onset and density of canola bloom as it related to bee visitation. We hypothesize that crops planted in monoculture will attract a greater abundance and diversity of bees than polycultures. This data will supplement other research projects in determining the value of cover crops as a native bee conservation strategy.

## Do bumblebees match their foraging movements to the scale of resource patchiness?

**Carla J. Essenberg, Daniel R. Papaj**

University of Arizona

Resource distributions are patchy, and animals will forage most efficiently if they concentrate their efforts in high-quality locations. Common methods for achieving this are departing from a patch with greater probability and traveling farther after experiencing low foraging success. These tactics will be most effective if the animal's movements match the scale of patchiness: for instance, if patches are larger, a forager needs to travel farther to exit a low-quality patch. Little is known about how the scale of animal foraging movements is determined, and in particular, the extent to which it is fixed or acquired. Using laboratory experiments, we are testing whether bumblebees (*Bombus impatiens*) alter their patch departure behavior to match the scale of patchiness in nectar distributions. Preliminary results suggest that the distances traveled after encountering unrewarding flowers are relatively insensitive to the scale of patchiness experienced.

## **A New Stage-Structured Model of Honey Bee Colony Population Dynamics Assessing Impacts of Pesticides and Other Stressors.**

**Wanyi Zhu, Tim Reluga <sup>1</sup>, Chris Mullin, James Frazier**

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A healthy honey bee colony is a population of closely interacting individuals that form a highly complex society. As an aid to testing hypotheses for the causes of recent colony failure and providing suggestions for management actions to promote recovery of honey bee population, we developed a worker-based, stage-structured model of honey bee population dynamics. This model was formulated with difference equations consisting of six discrete stages based on the temporal polytheism: egg, larva, pupa, nurse, house bee and forager stage. Numerical simulation of a healthy colony exhibited seasonal patterns typical of colonies in the US, and produces colony level dynamics and end of season honey stores that match 5 previous studies from the literature, as well as our own field experiments. The model thus has high biological validation.

Sensitivity analysis suggested the critical threshold of stage-based survival rate beneath which colony size decreases gradually, but if social factors such as brood care, transition rate and foraging behavior, and especially precocious foraging, is interrupted beyond the critical threshold rapid population decline is predicted and colony failure is inevitable. This model suggested that a disrupted colony by varying social regulation factor in the colony might be able to produce sudden collapse symptoms similar to colony collapse disorder.

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# Elucidating the Role of Transcriptional and Epigenetic Mechanisms in Antiviral Immunity in Honey Bees (*Apis mellifera*)

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In the United States, beekeepers lose an average of 30% of their colonies each winter. These losses are thought to be the result of numerous factors, but viruses undoubtedly play a major role. One pathogen in particular, Israeli acute paralysis virus (IAPV), has received increased attention, due to its hypothesized link to Colony Collapse Disorder (CCD). However, information about the molecular mechanisms mediating antiviral immunity in honey bees is surprisingly limited. To determine the molecular and physiological responses to acute viral infection, callow honey bee workers were infected with IAPV, and control bees and infected, symptomatic bees were collected ~15 hours later. We performed transcriptome- and bisulfite-sequencing of the worker fat bodies to identify genome-wide gene expression and DNA methylation patterns associated with viral infection. Several hundred genes were differentially expressed in infected versus control bees, including several genes involved in epigenetic pathways and RNAi pathway. Understanding the antiviral responses employed by honey bees will provide important information for improved colony management strategies.

## Monitoring of environmental health by local community using pollinators in Western Kenya

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Tropical forests facilitate key ecosystem services whose benefits to mankind are invaluable. Unfortunately, the “tragedy of the commons” remains a big challenge in reference to conservation of tropical forests biodiversity and their associated ecosystem services. Moreover, the delivery and importance of pollination service by pollen vectors in many forest-agricultural mosaics remains poorly understood by local communities in various species-rich tropical ecosystems. Kakamega Forest is one of such ecosystems in Kenya despite its richness in both plant and animal species. In order to empower the local community living around the forest to monitor the health of the environment, two community groups, that is, Kakamega Environmental Education Program (KEEP) and Muliro Farmers Conservation Group (MFCG) were chosen for training and collection of bioindicators data. Pollinators especially bees have been identified as excellent indicators of habitat quality. About four sites were selected based on the level of disturbance and data on bee diversity was collected seasonally using pan traps and sweep nets in plots of 100 x 100 m from 2011 to 2012. During the monitoring period, over 80 species of bees were collected in cooperation with the community members. The highest bee diversity was recorded in natural forest sites, moderately disturbed forest followed by bush lands. The least diverse site was the farming zone outside the forest characterized by intensive farming activities. Following the low level of pollinator knowledge among the community members as well as policy makers within the region, a pollinator garden was established during the monitoring exercise for increased public awareness. The results of the this project reveals the need to empower the custodians of biodiversity with monitoring skills in order to conserve, monitor and sustainably utilize the ecosystem services provided by nature freely.

Key words: Ecosystem service, tropical forests, pollinators, bioindicators

# A Cell Line Tool to Improve Honey Bee Health

**Michael Goblirsch, Marla S. Spivak, and Timothy J. Kurtti**

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It has become an annual occurrence for beekeepers to sustain heavy colony losses. The cause of these atypical levels of colony death has not been resolved, but it is likely the outcome of exposure to a suite of detractors to bee health such as pests, pathogens, pesticides, and poor nutrition. A major hindrance to the study of honey bee pathogens or the effects of pesticides and nutritional deficiencies is the lack of controlled in vitro systems derived from honey bee cells. We have developed a method incorporating traditional techniques that supports long-term growth of honey bee cells in culture. We used honey bee eggs late in their embryogenesis to establish primary cultures, as these eggs contain cells that are progressively dividing. Serial transfer of material from several primary cultures was initiated and has led to the isolation of a cell line. This cell line is composed mainly of fibroblast-type cells that form an adherent monolayer with a population doubling time of approximately 4 days. Importantly, the cell line is subcultured every 10-14 days and has survived cryopreservation in liquid nitrogen. The cell culture system we developed will be useful for studies aimed at honey bee development, pathogenesis, transgenesis, and toxicology.

## Exposure to pesticides alters expression of genes involved in detoxification, immunity and nutrition in honey bee workers

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Honey bee populations are in decline worldwide. Numerous stressors have been implicated, including pesticides, pathogens, malnutrition, environmental changes, and management practices. Pesticides, in particular, are almost ubiquitous in the hive environment, with over 130 different pesticides and pesticide metabolites detected in honey bee colonies in the US. Here, we explore the impact of sublethal pesticide exposure on honey bee gene expression to determine the molecular and physiological responses to pesticides. We found that bees exposed to two of the most abundant pesticides detected in US colonies, coumaphos and *tau*-fluvalinate, had a distinct gene expression profiles compared to control bees. Expression levels of 814 transcripts were significantly upregulated by pesticide exposure, representing multiple functional categories such as drug metabolism (including several p450s), transport, and other metabolic processes. Comparisons with previous microarray studies in honey bees revealed a significant overlap with genes involved in nutrition, immune response, and behavioral maturation. Additional studies demonstrated that diet can significantly alter the impacts of pesticide exposure. Overall, our findings suggest that honey bees mount a strong molecular and physiological response to sublethal doses of pesticides, and this information can be used to improve honey bees' ability to cope with pesticide stress.

# Seed Treatment Stewardship: Protecting Pollinators

## Bill Hairston

Director Seed Treatment Product Development, Bayer CropScience

Potential exposure to Neonicotinoids via dust exhausted from planters during planting of treated corn seeds has been highlighted as a potential risk to honey bees. Numerous new scientific publications have been issued on this topic and triggered a debate as to the relationship between the exposure of dust from planters and bee health.

The importance and impact of stewardship measures taken by the industry such as high quality clean seed, accurate product application, proper seed treatment equipment, use of high quality seed coatings, quality control as well as good practices for planting treated seed will be discussed.

This poster/presentation is not only focused on existing and new technologies (i.e., potential new seed lubricant for planting) for seed treatment but also on the communication and cooperation between stakeholders regarding best management practices for seed treatment applicators growers and beekeepers.

## Using larval *in vitro* bioassays to determine the toxicity of various forms of pollutants on *Apis mellifera* (Hymenoptera:Apidae)

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*Apis mellifera* L. (Hymenoptera: Apidae) is an important agricultural pollinator in the United States and throughout the world. In areas of selenium (Se) contamination, honey bees may be at risk due to the biotransfer of Se from foraged plant products such as nectar and pollen. Several forms of Se can occur in accumulating plants, and these compounds (selenate, selenite, methylselenocysteine and selenocystine) were assessed using artificial diet bioassays. Honey bee larvae were chronically exposed over a 12 day period. The inorganic forms were more toxic than the organic forms (LC<sub>50</sub> selenate = 0.72 µg g<sup>-1</sup>, LC<sub>50</sub> selenite = 1.03 µg g<sup>-1</sup>, LC50 methylselenocysteine = 4.74 µg g<sup>-1</sup>, LC<sub>50</sub> selenocystine = 4.41 µg g<sup>-1</sup>). Overall, inorganic forms of Se caused rapid mortality, but the organic forms had substantial sublethal effects on development. Larvae accumulated substantial amounts of Se, but only at the highest doses. Previous research showed foragers from honey bee hives within or adjacent to Se-contaminated areas will collect contaminated pollen and nectar, and with larval LC<sub>50</sub> values for Se being so low, even modest transfer to brood will likely cause increased development times and mortality.

## Limited impact of the microsporidian parasite, *Nosema ceranae*, on drone (*Apis mellifera*) gene expression, physiology and behavior

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*Nosema ceranae* is an important microsporidian pathogen of honey bees (*Apis mellifera*). A number of deleterious effects of *Nosema* infection have been documented in workers, where infection is energetically costly, shortens life-span, alters expression of immune genes and accelerates the normal transition of workers from nursing to foraging behavior. Few studies, however, have investigated whether predicted energetic costs of infection impact drone gene expression, physiology and behavior. To characterize drone response to infection, we measured expression of candidate genes in drone fat body tissue across a time course, quantified CO<sub>2</sub> production in flying drones, counted sperm stored in seminal vesicles and recorded duration of drone mating flights. *Nosema* infection only significantly affected expression of candidate genes in drone fat body tissue but had no impact on other physiological or behavioral markers of drone energetic status or maturation. Together, these results suggest that drones may manage *Nosema* infection differently than workers.

## Effect of “Bee-Safe” Insecticides and Fungicides on Honey Bee Queen Development and Survival

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“Bee-safe” insecticides and fungicides are sometimes applied to crops that are attractive to bees while in bloom. While these pesticides are not acutely toxic to the adult bees foraging during application, there is the potential for these compounds to harm developing bees within the colony. Many insecticides available for application during bloom are members of the Insect Growth Regulator class and specifically target immature insects. While exposure to developmental toxicants has the potential to harm worker larvae, their effects can be especially damaging in commercial queen rearing operations. We looked for effects on queen development when queen-rearing “swarm boxes” were supplied with pollen artificially contaminated with insecticides (Dimilin 2L, diflubenzuron; Intrepid 2F, methoxfenozide; Altacor, chlorantraniliprole) and fungicides (Pristine, pyraclostrobin and boscalid; Rovral, iprodione; Tilt, propiconazole) commonly applied to almond orchards during bloom. Queen survival to capping and adult emergence were recorded, as was the size of the emerged adult queens. While most pesticides tested appeared to have no effect on immature queens, the insecticide diflubenzuron significantly reduced queen survival.

# Apple Pollination Practices, Knowledge and Attitudes of Fruit Growers in Pennsylvania

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Pollination is important in apple production system. There are several species of insects that help in pollination in apples. Native/Alternative pollinators such as orchard mason bees could be important in apple pollination in Pennsylvania. This survey was conducted with the main objective to collect information related to apple growers' pollinators uses, pesticide/pest management practices and consideration for the use of alternative pollinators in apple production system in Pennsylvania. A survey questionnaire with 25 questions was distributed to apple growers in various extension meetings and field days during Fall 2010 to determine apple growers pollinator and pest management practices, knowledge and attitudes. The questionnaire was also made available online. This study reveals that the most of the surveyed growers (~ 50 %) do not rent honey bee hives for apple pollination. Apple growers considered that the alternative /native pollinators provide on an average ~ 50 % of pollination, and consider them as valuable pollinators in apple orchards. Additionally, the survey results show that the majority of apple growers (~ 79.5%) follow integrated pest management practices in their apple orchards, and most of the growers have not used commercially available orchard mason bees or bumble bees for apple pollination.

## Foraging patterns of Japanese orchard bee, *Osmia cornifrons* (Radoszkowski) in tree fruit orchards

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Foraging patterns of bee species in tree fruits such as apple and cherry should be understood for proper placement of nest sites and to ensure adequate pollination. Field and laboratory studies were conducted to quantify the foraging patterns of *Osmia cornifrons* (Radoszkowski) (Megachilidae, Hymenoptera) with an immunomarking method in commercial cherry and apple orchards in Pennsylvania. Adults of *O. cornifrons* were self-marked with chicken egg-white protein powder from a dispenser nest placed at the center of the study orchards at early bloom. Flower samples were collected from randomly selected trees (n= 30) located at different distances from the nest. Flowers were analyzed for the presence of immunomarker protein with enzyme-linked immunosorbent assay. Foraging patterns were determined by measuring the distance and direction of marked flowers from the nest. While marked flowers were found out to 55 m, most marked flowers were found within 35-40 m from the nest and the percentage of marked flowers declined rapidly beyond that distance. *O. cornifrons* adults are efficient tree fruit pollinators, but their foraging range is limited requiring strategic nest placement to ensure proper orchard pollination.

## Abundance and ecosystem services of some pollinator bees in apple orchards in Pennsylvania

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Recently, concern about declining bee populations in the United States and other countries has led researchers to investigate the impact of potential causes such as pesticide residue, pathogens, adjuvant chemicals, parasites, landscape diversity as it relates to forage and nest sites, floral resource availability and diversity etc. on the health, abundance and diversity of different species of bees and pollinators. Though most of the past research has focused on the impact on mainly honey bee and bumble bees foraging in different agricultural crops, investigation of factors affecting non-*Apis* native pollinators' abundance and resulting fruit set and yield in commercial apple orchards (with cultivars such as 'Honeycrisp,' 'York' and Golden Delicious') has not been investigated. We investigated bee species abundance, diversity, and seasonality and impact on pollination and fruit set in relation to orchard type and landscape factors in Pennsylvania, USA. The result of this study reveals that the abundance of native solitary bees was higher in orchard regions adjacent to bee habitats. In addition, abundance of other pollinators such as honey bee and bumble will be presented, and pollination services (in terms of fruit set and yield) of pollinator bees in apple orchard ecosystem will be discussed.

## Diversity of wild bees (Apoidea: Hymenoptera) in the Suez Canal and its allied areas, Egypt

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Suez Canal region one of the most important part of Egypt for agriculture development. The region includes five important governorates in Egypt Port Said, Ismailia, Suez, North Sinai and South Sinai. Therefore, the diversity of flora and fauna is interesting in particular solitary bees, which are essential for obtaining a good harvest of seeds, vegetable and fruit productions. The objective of this study was to evaluate the biodiversity of solitary bee populations and their nesting habitats around the Canal region. About 900 to 1000 specimens of bees were collected from different locations of Ismailia, Suez and Sinai. Fifty five species of bees are identified. With the exception of family Melittidae, all bee families are present at the Canal region. Total species number for each family as follows 7, 9, 11, 13 and 15 species for Andrenidae, Colletidae, Apidae, Halictidae and Megachilidae respectively. The most abundant species were found with a great numbers at the Suez Canal Region are *Andrena ovatula* ssp. *ovatula* (Kirby, 1802), *Ceratina tarsata* Morawitz, 1872 and *Colletes lacunatus* Dours 1872.

# The Role of Local Plant Communities in Supporting Native Bees in Pennsylvania Apple Orchards

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Weedy plants in and around agricultural fields increase the agroecosystem plant diversity, and it is hypothesized that a diverse plant community will support an abundant and diverse pollinator community. Therefore, we describe the plant communities in and around Pennsylvania apple orchards and explore the correlation between local plant communities and wild bee diversity and abundance.

We measured bee abundance and diversity in six apple orchards in 2011 and 2012 from mid-April to October using bowl traps. At the same time, plant diversity and abundance was assessed in 0.1 ha plots in the orchard floor, adjacent forested parcels, and the orchard-forest edge ecotone. We found that the edge plant community was the most species rich, with a mean of 48.6 species, but highly variable, ranging from 27 to 69 species. Orchard and forested communities had an average of 24.5 and 32.3 plant species, respectively. In 2011, there were 24 bee species, ranging from 14 to 18 species per orchard. Unexpectedly, plant community diversity in the edge was negatively correlated with total bee abundance and richness in the orchard. However, redundancy analysis showed species-specific responses of bees to local plant community metrics which merit further research and inclusion in conservation and management strategies.

## **Trends in the Use of Pesticides Having Acute and Sub-lethal Effects on Pollinators in California and the U.S.**

**Susan E. Kegley and Joshua R. Pepper,**

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Pesticides are a contributing factor to the uncharacteristically high hive losses observed over the last decade in the U.S. Honey bee exposure to pesticides results in both acute kills and sublethal effects such as impaired reproduction, homing behavior and navigation, immune function, and hive communications. Several classes of insecticides are under scrutiny for their potential effects on pollinators, primarily systemic insecticides (neonicotinoids, phenylpyrazoles, keto-enols and spinosyns), which are incorporated into plant tissue, pollen and nectar; and the pyrethroid insecticides, which are not systemic because they are not sufficiently water soluble, but are frequently found in the wax in beehives. Relatively little data on the use patterns of systemic insecticides and pyrethroids is available, making it difficult to assess the relationship between increased pesticide use and loss of honey bee populations. Since the 1994 introduction of the first of the systemic pesticides, imidacloprid, the number of acres that can legally be treated with these systemic insecticides in the U.S. has increased, as pesticide manufacturers have added new crops to the label-approved uses. This presentation will provide an analysis of trends in use of systemic and pyrethroid insecticides at the national level and in California, and correlations with observed colony losses.

# Bee Friendly Farming®

## Kathy Kellison

Partners for Sustainable Pollination (PFSP)

## Kimberley Fellows

Pollination Canada

Partners for Sustainable Pollination's (PFSP) poster profiles its Bee Friendly Farming (BFF®) initiative, launched in 2010. The lack of natural forage for bees is commonly understood to be a limiting factor in bee health. To encourage more forage for bees and other pollinators through a cooperative approach, caring citizens from all walks of life are empowered to help—including farmers and ranchers, school groups, local governments, nonprofits, businesses and beekeepers. The BFF® initiative helps raise consumer recognition and support for helping bees by (1) recognizing producers who provide bee habitat; (2) providing cost-share assistance to growers wanting to plant for bees; and (3) encouraging consumers and businesses to purchase farm products and local honey bearing the BFF® logo. Canada recognized the importance of this program and expanded it into their country in 2012. Interested participants can self-certify on line at <http://pfspsbees.org/bee-friendly-farming/certification>. There are now over 250 BFF® certifications in 34 states and 6 provinces, in a growing wave across the U.S. and Canada. PFSP is dedicated to improving the health of both honey bees and native pollinators, with a primary focus on identifying, increasing, and enhancing bee forage in the U.S. and Canada.

## Multiple infections in bees: chalkbrood in managed *Megachile rotundata*

**E. G. Klinger** and **R. R. James**

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The alfalfa leafcutting bee (ALCB), *Megachile rotundata* (Hymenoptera: Megachilidae) is used commercially in North America to pollinate alfalfa for seed. Chalkbrood is a disease of bee larvae caused by several fungi in the genus *Ascosphaera*, and is one of the top three causes of ALCB larval mortality. Using PCR markers to identify infections in larvae, we found *A. aggregata* to be the main pathogen present in commercial populations of the ALCB throughout the western US. Less common *Ascosphaera* usually occurred as co-infections with other species, especially with *A. aggregata*. We conducted laboratory bioassays to determine the virulence of *A. aggregata* and the second most common pathogen, *A. proliperda*, both in isolation and in combination. Although mortality did not significantly differ when *A. aggregata* occurred alone or with the *A. proliperda*, temporally-spaced exposures to the pathogens resulted in significantly higher survival than concurrent exposures, but the level of survival depended upon the order in which the pathogens were given to the developing larvae. The effects of pathogen co-infections on bee health has previously received very little experimental evaluation.

# Conservation of Melittophilic Plant Species Diversity as the Essential Environmental Function of Bee Pollinators

**E.N. Kostyleva**

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Melittophilic plants are recognized as a vital part of biological diversity and global environmental sustainability. Diversity of melittophilic plant species can serve as the main indicator of the essential environmental function of bee pollinators.

Therefore, we studied melittophilic plant species diversity in the state of Mississippi.

In total, more than 180 species of honey and pollen plants from 41 genera of 99 families were found. About half of the registered species are common for the region. Of these, 10 species were rare, 35 were distributed sporadically, and the remaining species were distributed almost throughout the study area.

We also found the species endemic to North American Atlantic Region (NAAR), which are conserved largely due to bee pollination. Many of these endemics are threatened or endangered species.

For the Appalachian Province of NAAR (northern part of the state) endemic species include *Magnolia acuminata*, *M. macrophylla*, *Claytonia virginica*, *Tilia americana*, *Gleditsia triacanthos*, *Robinia pseudacacia*, *Acer saccharum*, *Aesculus glabra*, *Catalpa speciosa*, species of *Polygonum*, *Viola*, *Salix*, *Clethra*, *Rubus*, *Prunus*, *Crataegus*, *Penstemon*, *Solidago*, *Aster*, and *Eupatorium*.

For the Atlantic and Gulf Coastal Plain Province (southern part) endemics are *Magnolia grandiflora*, *M. virginiana*, *Nyssa biflora*, species of *Asclepias*.

## **Molecular modeling study on a metalloprotease from *Paenibacillus larvae* and its peptide based inhibitors**

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The metalloproteases from *Paenibacillus larvae* have been described as an important virulence factor for American foulbrood disease. The three dimensional structure of a metalloprotease from *P. larvae* was generated by using computational modeling method and also validated by molecular simulation with AMBER 09. The 3D structure of the metalloprotease showed a similar structure to the known metalloprotease, Thermolysin. Hexapeptides were also chosen to form a binding complex with the metalloprotease. The C and N terminal of hexapeptide sequences were tagged with hydroxamic acid and hydrazide analogues, which have been reported to be potent and specific inhibitors for thermolysin, a closely related enzyme. These functional groups had a positive effect in improving potency and stabilizing the enzyme-inhibitor binding complex. This could be a novel inhibitor for *P. larvae* metalloprotease.

## Characterisation of the interaction partners of secreted *Nosema ceranae* proteins

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The microsporidian *Nosema ceranae* has been identified as one of the factors contributing to colony collapse disorder (CCD). It has switched its host from the Asian honey bee (*Apis cerana*) to the Western honey bee (*Apis mellifera*) and there has been an increase incident of infection in recent years. It has been recently postulated that *N. ceranae* infection rates have overtaken that of the other major honey bee pathogen – *Nosema apis*. Both species are obligate intracellular parasites infecting the bee gut wall entirely relying on metabolites and proteins supplied by the host. As a result, microsporidian genomes are highly reduced (7.86 Mb in *N. ceranae*).

In spite of the reduced number of genes (2678), *N. ceranae* has retained ORFs with signal peptides, hypothesized to be destined for secretion. Since the publication of the *N. ceranae* genome (Cornman *et al.*, 2009) we can now further investigate potential roles for those genes.

Using bioinformatics tools, a list of 88 putative secreted proteins was identified. In order to investigate the mechanisms of virulence and to elucidate interactions of these proteins in the host, a number of experiments were designed applying a wide range of molecular biology techniques.

## Pollinator cognition and floral complexity

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### **Rainee Kaczorowski**

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Flowers are multisensory, but most research on pollinator learning and memory focuses on responses to unimodal stimuli (e.g. color or scent alone). Thus, there remain many open questions regarding how pollinators process multimodal stimuli and untested hypotheses regarding the function of floral signal complexity. Do sensorially complex displays benefit plants and pollinators? In experiments on nectar-foraging bumblebees, we found that the presence of both color and scent enhances bees' ability to discriminate between flowers that differ in reward value. This effect occurs in two contexts, (1) as an inter-signal interaction whereby scent enhances recall of color (2) in a variable environment, floral scent can compensate for the loss of distinguishing visual information. We present a framework for testing functional hypotheses relevant to floral signal complexity, highlighting cases in which the complexity of the floral display promotes both pollen transfer and bees' foraging performance, as well as cases when it primarily benefits the plant. We discuss the implications of this research for understanding the causes and consequences of pollinators' responses to novel floral displays.

# Population genomics of stable and declining North American bumble bees

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Conservation geneticists have long used molecular markers to study genetic diversity of wild populations, but the limited information associated with traditional approaches is increasingly being recognized. New technologies are making it feasible to analyze genome-wide data from non-model organisms, providing unprecedented opportunities to estimate genetic parameters relevant to conservation. Bumble bees have emerged as indicators of the global pollinator crisis. Genetic diversity has been proposed as one factor determining why certain species are declining while others remain abundant. Here we compare traditional conservation genetics approaches with emerging population genomic technologies to investigate how genetic variation relates to the conservation status of two North American bumble bees: *Bombus pensylvanicus*, which is in decline across its range, and *B. impatiens*, which remains abundant. Analyses of microsatellite markers have previously revealed substantially reduced levels of diversity in *B. pensylvanicus*. My recent analysis of nearly 10,000 single nucleotide polymorphisms in each species suggest that earlier results may be premature, with *B. pensylvanicus* and *B. impatiens* having nearly indistinguishable levels of genome-wide heterozygosities. Results have implications for understanding of genetic diversity in native pollinator populations, and also for our ability to make inter-species inferences about relative diversity from small numbers of genetic markers.

## Microsatellite analysis of museum specimens reveals historical differences in genetic diversity in declining versus stable *Bombus* species

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There is worldwide concern that pollinators like bumblebees undergo worldwide decline, where loss of genetic diversity can play an essential role. In this project, we investigated the level of genetic diversity of declining and stable species with use of 10 microsatellite loci. Hereto we genotyped 74 museum specimens of seven declining *Bombus* species and 85 specimens of four stable species. These specimens were collected between 1918-1929, from 6 provinces in the Netherlands. For the stable species *B. pascuorum*, we also selected populations from two additional time periods: 1949-1955 and 1975-1990. Furthermore, to assess the generality of our findings, we performed a meta-analysis on available data for genetic diversity from our study and literature. The results indicate that there were no changes in genetic diversity and population structure in *B. pascuorum* over the three time periods, and that populations of declining bumblebee species showed a significantly lower genetic diversity than co-occurring stable species, before their major declines, indicating that populations of declining species were more vulnerable for the major drivers of bumblebee decline. The results are discussed in relation to current conservation strategies, which should pay more attention to promoting gene flow and creating large, high quality, habitats for bumblebees.

Key words: genetic diversity, bumblebee decline, microsatellite loci, museum collections, conservation

# Pollination Services to Apples as a Function of Landscape Context

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Declines in commercialized honeybees have incited research into alternative pollinators derived freely from landscapes surrounding fruit farms. This study investigated pollination services provided by indigenous bees to apple orchards along a space-for-time urbanization gradient. A total of 12 orchards in 2011, and 21 orchards in 2012 were sampled in southern Quebec, Canada, for bee diversity, bee pollinator efficacy, as well as fruit and seed set. Native bee diversity and abundance decreased logarithmically with increasing orchard area, and was positively correlated with the proportion of surrounding meadowland and degree of urbanization. A cutoff value of orchard size for which it would be inappropriate to depend solely on native bees was identified, as was a prescriptive amount of semi-natural area for land conversion. Seed set was positively associated with the functional dispersion of the bee guild, a diversity metric incorporating blossom visitation rates, orchard flight patterns and the likelihood of contacting the apple stigma. Findings provide incentive for the protection of native bees, as seed set is a known predictor of apple fruit quality. Regression functions modeling native bee diversity were projected across the orchard extent to identify areas more vulnerable to honey bee declines, in the absence of a compensatory pollinators.

## **Bees and Neonicotinoids: Outline of the Latest Developments in Europe – An Industry Perspective**

**Christian Maus**

In the last months, bee safety of neonicotinoid insecticides has been a subject of intensive debate in Europe. Numerous new scientific publications have been issued on this topic. In a recent re-evaluation of seed-treatment and granular product, the European Food Safety Authority EFSA has flagged up concerns regarding systemic residues in treated crops and dust released during planting of treated seeds, and identified further areas where additional information may be required for a risk assessment, e.g. on wild pollinators. This re-evaluation has triggered proposals of the EU Commission for far-reaching restrictions of the use of neonicotinoids in Europe, although extensive monitoring data from several European countries do not support the hypothesis of a correlation between the use of neonicotinoids and colony mortalities. Here we give an overview about the latest developments from an industry perspective. Results of recent research activities on neonicotinoids and bees are outlined and reviewed, and their implications for the risk evaluation are discussed. New approaches and technologies to minimize the exposure of bees to dust of seed treatment products during planting are presented and discussed. On the basis of the data presented, future prospects for further optimization of the bee-safe use of neonicotinoids are discussed.

# Effects of pest management intensity on wild bee communities in highbush blueberry

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Wild bees provide essential pollination services to highbush blueberry (*Vaccinium corymbosum* L.), which requires insect pollination for economically viable yields. Over 100 wild bee species have been recorded in Michigan blueberry fields during bloom, including ~10 species exhibiting high abundance and/or fidelity to *Vaccinium* flowers. However, management of insect pests following bloom generally necessitates several insecticide applications per season. While short-lived bees that are tightly linked with blueberry bloom may not be affected by post-bloom insecticides, the sprays may have fitness consequences for bees with longer life cycles, including bumble bees (*Bombus* sp.), some of which are experiencing significant population declines in the eastern US. A 3-year pan trapping study conducted from 2004-6 in 15 blueberry fields in western Michigan spanning a gradient of pest management intensity found declining wild bee abundance and richness to be significantly related to increasing risk of the insecticide programs applied to those fields in the prior year, though effects tended to be masked by interyear variability in bee community composition. Using the same methods as the previous study, we resurveyed the wild bee communities at these sites to determine the long-term effects of insecticide program risk on wild bee abundance during bloom.

## Floral metal accumulation alters plant-pollinator interactions

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The abiotic environment often alters biotic interactions, particularly plant-animal interactions, as plants are heavily dependent upon their abiotic surroundings for nutrient acquisition. Several species of plants are known to accumulate soil metals into vegetative tissues, which in turn can alter plant-herbivore interactions. However, less is known regarding whether these metal-accumulating plants sequester metals into floral tissues, including anthers and nectar, and whether metal accumulation can affect plant-pollinator interactions. We conducted a manipulative experiment where soils of *Streptanthus polygaloides*, a nickel (Ni) hyperaccumulator, were supplemented with Ni to determine if the soil environment altered floral chemistry and pollinator visitation. We found soil Ni was accumulated into floral tissues, including anthers and nectar. Experimental plants grown in Ni-rich soils received fewer pollinator visits compared to plants grown in Ni-free soils, though the probability of pollinator visitation was unaffected by Ni treatment to soils. Our results suggest that metal-rich soils can have far-reaching effects on plant and pollinator health. Metals accumulated into floral tissues can decrease pollinator visitation, which may influence plant reproductive output on both naturally metal-rich and human-polluted soils. Furthermore, pollinators foraging in polluted landscapes may be subjected to toxic floral rewards.

# Pollinators in Peril: Effect of Chronic Clothianidin Consumption on Bumblebee Survival

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Bumblebees and other insect pollinators carry tremendous biological, social, and economic value due to the irreplaceable ecosystem service that they provide. Over the past decade, worldwide pollinator populations, including several North American bumblebee species, have declined at unprecedented rates. Although the cause of these declines is currently unknown, one significant contributing factor is thought to be exposure to pesticides. Here, we examined the effect of chronic consumption of the common systemic neonicotinoid pesticide, clothianidin, on the survival of *Bombus impatiens* workers, males, and queens. Alarmingly, we found that consumption of an environmentally-realistic dose of 10ppb over just a few days significantly reduced survival in all three bee castes, particularly males. To our knowledge, this is the first study showing that male bees are more sensitive to the negative effects of oral exposure to clothianidin than queens and workers. Moreover, our results suggest that chronic oral exposure to clothianidin in floral nectar poses a serious threat to the health of wild bumblebee populations, and help explain current population declines.

## The Impacts of Traditional Beekeeping Practices on Honey Bee Health in East Africa

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In Africa, thousands of years of attracting native honey bee (*Apis mellifera*) swarms to traditional log hives for honey production has resulted in a kind of “beekeeping” that is in tune with the seasons, tough environmental conditions and economic circumstances of local people. Attempts to improve the livelihoods of rural people in East Africa through beekeeping development projects have been largely unsuccessful for more than 50 years. These projects primarily focus on the introduction of equipment, including movable frame hives and management techniques developed for European honey bees in temperate climates based on economies of scale. Based on our preliminary research data and beekeeper interviews, minimal management, practiced when keeping bees in log hives, may be responsible for lower honey yields but has likely resulted in bee populations that are resistant to diseases and pests. Evolved resistance potentially extends to newly introduced parasitic *Varroa* mites and viruses. Drought and pesticide use are additional factors that may impact honey bee health. Only a small number of pesticides at low concentrations were found in pollen and wax samples collected from 26 sites across the country, but nearly all tested locations had some level of contamination and several pesticides at two locations are considered highly toxic to bees.

# Genetic and pathogen spillover from commercial to wild populations of bumblebees

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Worldwide, wild bumblebees are experiencing marked declines, with up to 11% of species currently under threat of extinction. In parallel, the increasing demand for pollinator-dependent crops has led to the year-round production and global trade of bumblebee hives for pollination. In Europe, companies rear and export the native species *Bombus terrestris*, but nine subspecies are recognised across its geographic range. Given the widespread production and transport of these commercial bumblebee hives across the EU since the early 1980s, data on genetic and pathogen transmission or 'spillover' between commercial and wild conspecific populations is lacking. To investigate the potential for genetic and pathogen spillover, we sampled 64 commercial hives and 877 wild-caught *B. terrestris* at increasing distances from sites of bumblebee importation in Ireland. Bees were genotyped at 11 microsatellite loci, the number and origin (native, putative hybrid, and non-native) of colonies ascertained and the prevalence of parasites quantified. Our data suggest extensive introgression between commercial and native populations, and sex-specific differences in declining pathogen prevalences up to 10 km from sites of importation. Despite the benefits of commercial bumblebee pollination, the potential genetic impact on, and pathogen transmission to, wild conspecifics should be mitigated at local, national and international levels.

## Changes in honey bee queen pheromone bouquet communicate mating status and quality

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Mating is a complex process that causes many changes on behavioral, physiological, and molecular level in females of sexually-reproducing species. In insects, various mating factors are involved in regulating these post-mating changes. For example, previous studies on honey bees suggest that both insemination volume and seminal fluid components play a role in triggering early changes in queens. However, their long-term effects have not been previously characterized. By manipulating the mating process we found that instrumental insemination (presumably mimicking copulation) and insemination volume cause early behavioral changes, while seminal fluid components play a role in maintaining long-term physiological and transcriptional changes in queens that reached their final reproductive state of high ovary activation. Interestingly, these factors also have distinct effects on two different pheromone-releasing glands in queens and, more importantly, workers can detect these subtle differences in pheromones and adjust their behavior and physiology accordingly. On the other hand, stressors such as viral infection and pesticide exposure do not seem to alter queen pheromone profiles suggesting that the queen has adapted her pheromone production to communicate information specifically about her mating state and mating quality. In conjunction with previous studies, we develop a model of the factors and processes regulating progressive post-mating changes in honey bee queens. These results also lay the groundwork for future detailed studies of social insect reproduction which could lead to improved breeding techniques of agriculturally important insects including honey bees.

# Investigating the role of pheromones in mediating social behavior in bumble bees (*Bombus impatiens*)

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Reproductive division of labor is a hallmark of eusociality, but proximate mechanisms establishing this have only been identified in some species. Bumble bees are widely used models for social behavior, but surprisingly mechanisms regulating queen dominance over reproduction are unknown. When isolated from the queen workers become reproductively active and lay haploid eggs that develop into males. We investigated if volatile compounds produced by the queen play a role in the competition for reproduction. We developed a “reduced colony” system in which three callow (one-day old) workers received airflow from a cage containing a queen, brood and three callow workers. Within this chamber the queen inhibited worker reproduction as in a full sized colony. However, workers exposed to airflow from the reduced colonies were able to fully activate their ovaries and lay eggs. A mesh experiment was then developed to determine if a queen could inhibit reproduction through limited contact with workers. A wooden cage was separated into two compartments by a mesh screen that allowed antennal contact between bees on each side. Workers in direct contact with the queen were again completely reproductively inhibited. Workers that had limited contact with the queen were able to fully activate their ovaries and lay eggs. These data suggest that reproductive inhibition is dependent on direct queen contact. Workers in full contact with the queen were always reproductively inhibited while workers exposed to air from reduced colonies and with limited queen contact (antennation) became reproductively active.

## Honey Bee Health in East Africa

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In East Africa, honey bees provide critical pollination services and income for small holder farmers and rural families. While honey bee populations in North America and Europe are clearly in decline, little is known about the health of honey bees in Africa. We initiated a nationwide survey encompassing 24 locations across Kenya in 2010 to evaluate the numbers and sizes of honey bee colonies, assess the presence of key parasites (*Varroa* mites and *Nosema* microsporidia) and viruses, identify and quantify pesticide contaminants in hives, and assay for levels of hygienic behavior. *Varroa* mites were present throughout Kenya, except in the remote north. Levels of *Varroa* were positively correlated with elevation and significantly impacted by geographic location, suggesting that landscape ecology may play a role in honey bee host-parasite interaction. However, while *Varroa* infestation dramatically reduces honey bee colony survival in the US and Europe, in Kenya *Varroa* presence alone does not appear to impact colony health. *Nosema apis* was found at three sites along the coast and one site on Mt Elgon. Only a small number of pesticides at low concentrations were found in colonies. Of the seven common US/European honey bee viruses surveyed, only deformed wing virus (DWV), black queen cell virus (BQCV), and acute bee paralysis virus (ABPV) were identified in Kenyan bee populations; DWV and BQCV were widespread, but absent from northern Kenya. Overall, our results suggest several factors thought to undermine bee health in North America and Europe are not yet directly impacting Kenyan bee populations, and thus chemical control methods for *Varroa* and *Nosema* are not necessary for Kenyan bees at this time. These studies highlight the importance of viruses and landscape ecology; these two parameters undoubtedly similarly impact honey bee health globally, but their effects have been challenging to elucidate due to the presence of multitude synergistically acting stressors.

## Topical Toxicity of Four Pesticides Against Africanized Honeybee

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Honeybees are important pollinators and their activity increases genetic variability contributing to the maintenance of plant biodiversity. *Apis mellifera* also stand out as efficient pollinators in crops ensuring higher yields. Pesticides are often taken as the first line of defense against pests. In this scenario, honeybees can get in contact with such substances. The aim of this study was to determine the lethal dose (LD50) of four different pesticides (imidacloprid, thiametoxan, acetamiprid and fipronil) used in many Brazilian crops against Africanized honeybees. After all tests the species sensitivity distribution were carried out. To determine the lethal dose, adult workers were anesthetized with CO<sub>2</sub> (ten seconds) and exposed to a range of doses of each pesticide dissolved in acetone. For application of 1 µL/bee on the pronoto region, a microsyringe coupled with a repetitive dispenser was used. After 24 h, the number of dead bees to perform the statistics were recorded. The fipronil was the most toxic to the honeybee in laboratory studies with the lowest LD50 value followed by thiametoxan, imidacloprid and acetamiprid, respectively. These results can be useful in field studies and guide registration processes for these pesticides and their modes of use.

## Relative influences of the landscape and bees on crop yield

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Declines in bee populations have stimulated interest in identifying landscape-level impacts on bee-mediated pollination services. Previous studies have qualitatively concluded that certain landscape features increase bee populations and this translates to greater crop yields. However, these linkages have not been determined quantitatively. Using pumpkin as a model system and conditional process modeling, we analytically identified the causal mechanisms of how landscape factors either directly or indirectly through bee visits to pumpkin flowers impact pumpkin fruit yield. Results indicated that direct effects of landscape diversity and percent grassland significantly predicted yield, while indirect effects of honey bee and bumble bee visits to flowers did not. By probing the interactions between landscape factors and bee visits to flowers, results indicated that in highly diverse landscapes there was a positive indirect effect of bumble bee visits to flowers on yield. No conditional indirect effects were identified for honey bees. Bumble bees benefited from a diverse landscape and their visits to flowers positively impacted pumpkin production. Growers can use this information to make decisions on where to plant pumpkins based on surrounding landscape, identify scenarios where landscape diversity could be increased and where supplementation with bees might be beneficial.

## **Development of “L-TIP”: A New Delivery System Comprising Microspheres, Nanospheres and/or Pico spheres Made With Beeswax And Natural Fumigant Compounds For Function as A Low-Toxicity Integrated Pest Management Product**

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Feral and commercial honey bee populations in the USA are at great risk of dying due to parasite infestations by trachea mites, varroa mites, AFB/EFB (American/European Foul Brood Disease) and more recently in southeastern states, the (bee) Hive Beetle. Other risk factors, e.g., exposure to neurotoxins found in man-made, chemical pesticides, present additional concerns. Recalcitrant problems for beekeepers is attack on bee hives by large and small Wax Moths, rodentia and other consumers, resulting in the loss of individuals (larvae) and decreases honey flows/output and harvests. Current pesticide products, although marginally effective, are costly with some containing chemicals and compounds that have been found to have migrated into the human food chain with additional concern that these may traverse blood-bell barriers and gain entry to the human genome. Too, these products may be lethal to other beneficial pollinators, to symbiotic insects, ants, for example, which also play a role in pollination. Some products are found to be cumbersome, bulky, difficult to use, costly, and not readily available in more remote regions of the USA. Expense of present products to small/large producers, alike, is also a major consideration. Of further concern is overburden/use of chemical pesticides to remedy honey bee infestations resulting in possible negative genetic impact and consequences to targeted individuals, target-vectors and future apis phylogeny/ontogeny.

This paper discusses recent advancements in development of a novel delivery system wherein conventional treatment modalities, e.g., bee cakes, wafer-type sheets and products placed on paper (strips), are replaced with ‘microcapsules’ configured to enable delivery of low-toxicity adjuvants at the macro, micro, nano and pico scales. Unique features of microsphere-manufactures, e.g., ability to configure new delivery modalities in the macro, micro, nano and pico scales-ranges, is discussed with attention to affording the new delivery modulus with specificity for 1) targeted treatment vectors; 2) elimination of lethal chemical components and replacement with low-toxicity, natural, organic compounds; and, 3) reduction of physical, ergonomic and logistical stressors to the colony by eliminating present treatment barriers, i.e., worker bees having to access present treatment products, e.g., densely-formed bee-cakes, wafers and wafer-strips. A proposal to configure next-generation adjuvants for delivery at the pico-scale enabling delivery of low-toxicity countermeasure to residual neurotoxins, e.g., nicotinamide, is presented.

## Developing Floral Provisioning Plantings for Enhancement of Pollinators: A Case Study in Pennsylvania Apple Orchards

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Bees are important pollinators within a number of crop systems and are dependent on floral resources both within and outside of the crop for survival. Floral provisioning plantings have potential to enhance bee populations by providing valuable floral resources outside of crop bloom. The impact of floral provisioning plantings on bee diversity and abundance was assessed within apple orchards in Pennsylvania. Bee abundance and diversity was assessed in both floral provisioning plantings and apple orchards through net collecting and trapping methods. Plant diversity and abundance was assessed using a modified Whitaker plot design. Pollinator plant abundance and diversity differed dramatically by site and was impacted by both site preparation and management practices. Pollinator plants accounted for only 0.9-24% of the total plant community and had a maximum species richness of 5. A total of 58 bee species were documented within the floral provisioning plantings from March-October compared to 147 species within apple orchards. Of these species, 44 were found to utilize both floral provisioning plantings and apple orchards but only 15 of these species are pollinators of apples. Pollinator provisioning plantings seem to promote overall bee diversity within the landscape but are not specifically benefiting pollinators of apples within Pennsylvania.

## Proteomic analysis of the brain of newly-emerged Africanized honeybees (*Apis mellifera*) exposed to fipronil

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Several synthetic substances are used in agricultural areas to combat insect pests; however, the indiscriminate use of these products may affect non-target insects, such as bees. In Brazil, one of the most widely used insecticides is fipronil, a phenylpyrazole insecticide which targets the nervous system of insects. To assess the effects of fipronil in the brain of Africanized *Apis mellifera* workers, this study focused on the toxicoproteome profiling of the brain of newly emerged honeybee workers that were exposed to a sub-lethal dose (1/100 of LD50) of the insecticide. Proteomic analysis identified 20 proteins that were differentially up-regulated or down-regulated when the fipronil-exposed and non-exposed groups were compared. These proteins were related to pathogen susceptibility, neuronal chemical stress, neuronal protein misfolding, a higher occurrence of apoptosis, ischemia, visual impairment, damaged synapse formation, brain degeneration, memory and learning impairment. The exposure of honeybees to a very low dose of fipronil, even for a short period of time (five days), was sufficient to cause a series of important neuroproteomic changes in the brains of honeybees.

Key words: brain proteome, phenylpyrazole, insecticide, proteomic analysis, 2-DE, mass spectrometry, brain damage

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## Bee Health Research

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In its recent comprehensive assessment on honey bee health, the United States Department of Agriculture (USDA) and Environmental Protection Agency (EPA) supported the generally accepted concept that bees are suffering from a complex set of stressors, including parasites and diseases, genetic characteristics, and nutritional issues.

For more than 25 years Bayer has been actively involved in finding solutions to improve honey bee health and is opening two bee care centers in Germany and the United States to further collaboration and understanding of the health of bees.

Ongoing research in the areas of bee health will be discussed including: new research into solutions for varroa mite control, small hive beetle IPM, honey bee health monitoring and diagnosis, (incl. thresholds, tools, methods & treatments, early warning and prediction systems such as sentinel hives programs, evaluation of process for purification of beeswax, stewardship, best management practices and biodiversity (habitat and nutrition).

Future research will also be presented, planned for the new North American Bee Care Center, which will include a full laboratory and research apiary; honey extraction and workshop space; interactive learning center; and meeting, training and presentation facilities; all with the objective to coordinate and extend research projects directed toward bee health.

## Floral provisioning resources from an invasive thistle

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Land managers invest considerable time and resources to control the populations of invasive weeds in agroecosystems. At the same time, they are increasingly searching for ways to support wild and domesticated bees that provide the ecosystem service of crop pollination. To ensure sufficient pollination services for crop species dependent on insect pollination, land managers may require hives of honeybees or colonies of other domesticated bee species, and floral resource supplementation with native habitat, all of which can incur large economic costs. With a two year field experiment, we demonstrate that an invasive thistle (*Carduus acanthoides*) is both highly visited and strongly preferred by bees relative to ten other flowering annual species. The overall abundance and species richness of bees was significantly increased in habitat patches where the thistle was present. In addition, bees of the genus *Bombus*, often cited as effective crop pollinators, responded especially strongly to the presence of the thistle in habitat patches. Our results suggest that the invasion of a non-native species in an agroecosystem may have the potential to provide benefits to pollinators by provisioning them with floral resources.

## The impact of neonicotinoid-coated seeds on bees during corn planting period in Quebec

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Sowing of neonicotinoid-coated seeds, with a pneumatic drilling machine, produces atmospheric emission of particulate matter containing the insecticide. Pollinators may be exposed through the wind current transporting fragments of insecticides or by collecting contaminated nectar and pollen from exposed nearby vegetation. In this 2-year study, 12 commercial apiaries, located in Quebec (Canada), were monitored during the 2012 and 2013 corn sowing period (early May to mid-June). Control sites were located 3 km from fields with coated seeds. In each apiary, five colonies were targeted in front of which dead honeybees were counted, collected every other day and analyzed by liquid chromatography tandem mass spectrometry to detect the presence of insecticide. Water samples were also collected from cornfields and analyzed for pesticide residues. In addition, one colony of bumblebees (*Bombus impatiens*) was installed at each study site and foragers were captured alive and analyzed with Real Time qPCR to determine the expression level of a biological marker (AChE). Results showed an increase in honeybee mortality in sites exposed to coated seeds. Clothianidin was found in all samples of surface water and thiamethoxam in 60% of them. Finally, qPCR analyses of AChE did not reveal treatment effect for bumblebees.

## Yeast community associated with Brazilian Honeybees

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It is well recognized that bees carry a microbiota that may play a relevant role in health maintenance and colony homeostasis, therefore representing an intriguing source for undiscovered microbial species. The association between yeasts and honeybees was the aim of this research. Three sampling events were performed in 2011 in Carmo da Mata, Minas Gerais, Brazil, in five nests of Africanized *Apis mellifera*. Pollen, bee bread, hive wastes, body surfaces of nurse and forager bees and intestinal contents were evaluated by inoculation on Yeast-Extract Malt-Extract (YM) agar plates. The isolates were identified by the sequencing of the D1/D2 region. To date, 76.6% of 141 isolates were identified. The most prevalent species were *Aurobasidium pullulans* (29.6%), *Candida orthopsilosis* (25%) and *Rhodotorula mucilaginosa* (14.8%), followed by *Kwoniella mangroviensis* (5.5%), *Exophiala dermatitidis* (4.6%), *Candida sorbosivorans* (3.7%) and *Cryptococcus neoformans* (2.8%). Species also observed were: *Candida hawaiiiana*, *C. parapsilosis*, *Cryptococcus laurentii*, *C. albidosimilis*, *Metschnikowia koreensis*, *Meyerozyma guilliermondi*, *Rhodotorula minuta*, *R. slooffiae*, *Occultifur aff. externus*, *Sporidiobolus ruineniae* and *Sporobolomyces poonsookiae*. It is important to highlight the occurrence of *E. dermatitidis* and *C. neoformans*, two opportunistic yeasts species in association with honeybees. This result establishes the preliminary yeast community associated with Brazilian honeybees.

## Bumble bee nest density in agroecosystems of small diversified farms

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Wild bees can provide pollination services within agroecosystems. These populations are sustained by the availability and accessibility of resources within the environment. Understanding resources requirements, such as nesting habitat, will contribute to the development of better land management and farming practices, which will sustain and promote wild bee populations.

In this study, we relate the estimated number of nests represented by foragers visiting a crop to the surrounding farmscape (up to 500m) and the wider landscape (up to 2000m). Using microsatellite markers, we determined sib-ships between *Bombus impatiens* foragers collected in pumpkin patches on small, diversified farms over two years. We used this information to estimate the number of nests using this floral resource at each farm site, and also to estimate the nest density in the surrounding landscape. We related estimated nest density to landscape metrics to assess if variations in land cover at different distances (farmscape or landscape) affected the number of nests present in agroecosystems.

## Interaction of fipronil and dimethoate cause abnormalities along the development of africanized honeybee in laboratorial conditions

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Honeybee larvae can be exposed to insecticides during their feeding phase by means of food and wax, which could contain residues of these agrochemicals. The aim of this work was evaluate the effect of the interaction between fipronil and dimethoate on the development of africanized honeybee under toxicity bioassays rearing larvae to imago. Larvae were individually exposed by contact on the dorsal region of each larvae, at the day 4 of the bioassay, according to experimental groups: I) Control- 1µL of solvent; II) Fipronil- 0.5 ng/µL/larvae ( $LD_{50/10}$ ); III) Dimethoate- 200 ng/µL/larvae ( $LD_{50/10}$ ); III) Synergism- 0.5 ng/µL/larvae and 200 ng/µL/larvae applied simultaneously. Although the larval mortality tax was not statistically significant among the groups, the results showed at 0.5 ng/µL/larvae of fipronil and 200 ng/µL/larvae of dimethoate (synergism group) the morphological abnormalities on pupae increased in relation to the control group, as well as to the fipronil-exposed and the dimethoate-esposed groups. In the synergism group, the presence of pupae with pigmented head and thorax, but without pigmented abdomen, was the most common abnormality observed during the pupal stage. The results showed the association of fipronil insecticide dimethoate and causes serious disturbance on the honeybee larval development, even on lower concentrations.

## **Influence of land use on honey bee health and wild bee diversity**

**Matthew Smart, Elaine Evans, and Marla Spivak**

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Our goals are to 1) relate landscape to individual and colony-level honey bee health and 2) correlate wild bee diversity and abundance with specific landscape features. Observations took place in or near six apiaries, three of which were “non-bee friendly”, surrounded primarily by soybeans, corn, and wheat while the other three sites were “bee-friendly”, with greater amounts pasture, CRP, flowering trees, grassland and hay land.

Honey bee health was assessed at the colony level (size, pollen stores, honey production, pesticide exposure, disease and parasite incidence, virus levels, queen status/events) and individual bee level (lipids, vitellogenin, hemocyte counts, production of anti-microbial peptides) and was correlated with surrounding land-use. We found that honey bee health, indicated by individual bee measures (vitellogenin levels, immune responses) and colony survivorship, is affected by landscape composition and that certain types of land use are associated with better (e.g. pasture) or worse (e.g. soybeans) health outcomes for honey bees.

Wild bees were collected using sweep nets, bowl traps, and trap nests. Over 8,400 bees representing 148 species have been identified to date. Preliminary analyses indicate that wetlands and wooded areas influence bee abundance and diversity respectively. Inclusion of the complete data set is needed before drawing conclusions.

## Bee-Wise Philly: West Parkside Habitat Project

### **Julie Snell**

Pennsylvania Horticultural Society

### **Marjorie H. Ogilvie**

Philadelphia Business & Technology Center

### **Don Shump**

Philadelphia Bee Company

### **Liz Garbor**

Philadelphia Industrial Development Corporation

Last year, 22 beehives on the 10,000-square-foot roof of a former textile factory in the West Parkside neighborhood of Philadelphia produced 300 pounds of honey. The hives were the brainchild of the building's current owner, the Philadelphia Business & Technology Center, and the Philadelphia Bee Company. The label on a jar of West Parkside Honey reads:

*The honey bee population is in crisis. We at the Philadelphia Business & Technology Center believe all of us must do our part to preserve Earth's life-sustaining ecosystem for future generations. One hundred percent of your donations for this honey will be used to promote urban beekeeping.*

Motivated to make a difference, the two organizations have produced a video series called "Don the Beekeeper" to teach children and their parents about the importance of bees and native pollinators to human health and the environment. They have also enlisted the help of the Philadelphia Industrial Development Corporation and the Pennsylvania Horticultural Society to develop ways to use nearby land—a combination of public and private open spaces—to expand summer forage and habitat for both honey bees and native pollinators.

## **Bee Populations and Pollination Services in Washington County, Ohio**

**MaLisa Spring and Dr. Katy Lustofin**

Marietta College, Department of Biology and Environmental Science

This project examined the bee populations and diversity in Washington County, Ohio as well as the pollination services contributed by each species as denoted by the pollen found on the scopa. Our lack of information about bee populations makes it difficult to notice change in populations that could be detrimental to agricultural and natural ecosystems. Furthermore, not many studies had examined the pollen collected by each individual bee. This project looked at bees in three sites in Washington County to get a better idea of the diversity present. Bees were collected using the standardized pan method as well as some supplemental sweepnetting. The bees were identified to genus and species if possible in the lab and individually coded so pollen samples could be directly attributed to each individual. The pollen was identified to the family or lower if possible. This project provides a baseline understanding of our current bee populations in SE Ohio as well as insight into the pollination services of each bee species. This data will also contribute to nationwide research about the current status of bee populations.

## **Analysis of the relationship between the honey bee, *Apis mellifera* and the ectoparasite *Varroa destructor*.**

**Carla Surlis, Kevin Kavanagh**

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The honey bee, *Apis mellifera*, is an extremely important economic insect, responsible for around 80% of the global agricultural pollination services. The closely interacting and densely populated colonies of up to 50,000 individuals provide an optimum environment for the proliferation of various pathogens and parasites, and the implications of this are extremely important for the survival of the honeybee.

One of the most serious threats to the survival of honey bee colonies is the hemophagous mite, *Varroa destructor*. It is an ectoparasite that attaches itself to the cuticle of both the larval and adult stage of the bee, feeding on the haemolymph. The effects of parasitization by *V.destructor* are detrimental, with a negative effect on the physical health of the bee due to loss of haemolymph, but also on the immune system of the larvae, due to immune suppression and the transmission of viral particles around and between colonies. Also, in recent years, *V.destructor* has developed resistance to one of the two products licensed for use in Europe for the treatment of colonies.

Through proteomic analysis, the relationship between *A.mellifera* and *V.destructor* was examined, with emphasis on the effect of parasitization on the immune system of *A.mellifera* larvae. The possibility of transmission of viral particles from the feeding of haemolymph by *V.destructor* was also examined, with RT-PCR analysis of six bee viruses to indicate the presence or absence of viruses in the mites and the honeybee.

This work described here is funded by the Irish Research Council (IRC)

## Larval mortality of africanized honey bee *Apis mellifera* after exposition to thiamethoxam

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Honey bees contribute to pollination and maintenance of native and cultivated areas. These bees can be affected by insecticides through contaminated pollen and nectar, which can be stored inside the colony, affecting adults and larvae. Thus, the present study aimed to evaluate the larval mortality of africanized *Apis mellifera* after topical application of thiamethoxam. For this, we performed dose-response bioassays and larvae were kept under controlled laboratory conditions. On the 4th day, larvae were exposed to acute doses ranging from 0.05 to 250ng of thiametoxan/ $\mu$ L acetone, 48 larva/concentration and mortality were assessed 24, 48 and 72 hours after exposure. After 24h of exposure the results showed higher mortality rate in exposed groups than in control groups, mainly from the concentration 5ng/ $\mu$ L, showing that thiamethoxam is toxic to larvae of africanized honey bees. Besides, the results demonstrate that larvae can be tolerant for this insecticide, because for concentration higher tested the mortality didn't reach 80%. Thus, this study adds important information about the toxicity of thiamethoxam to bee larvae and can be used as a background for future investigations.

# Bee diversity and functional composition in grassland habitats of the tallgrass prairie

**Bethany Teeters<sup>1</sup>, Craig R. Allen<sup>1</sup>, and Chris Helzer<sup>2</sup>**

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2. The Nature Conservancy

This study was a comparison of the bee assemblages of different grassland habitats in the tallgrass prairie ecosystem. The goals were to identify correlations between bee diversity and abundance and floral resource availability, and to examine the functional composition of the pollinator community between grasslands of different quality. Three habitat types were compared: remnant prairie, grazed pasture, and properties enrolled in the conservation reserve program (CRP). Bee species richness, species diversity, and species abundances were measured. Additionally, species were divided into functional groups according to foraging range, degree of plant specialization, nesting behavior, and sociality.

Preliminary results suggest that the correlation of bee abundance and species richness to floral resource availability is not strong in these types of bee habitat, although the assemblage of species differs in functional composition. While CRP habitats had the greatest bee species richness and abundance, contrary to expectations as these are the least florally-rich habitats, they were not as diverse as the remnant prairies. The latter had both the greatest bee diversity and forb diversity of the grasslands examined. This has important implications for pollinator conservation planning and raises questions of how various functional groups of pollinators use habitats of different quality in fragmented landscapes.

## Identification of some viruses and pathogens among *Apis mellifera* honey bee samples from different regions of Turkey

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*A.m. anatoliaca*, *caucasica*, *meda*, *syriaca*, and an ecotype from the *carnica* subspecies of *Apis mellifera* exist in different climactic zones and habitats of Turkey. Sudden and excessive colony losses were first reported in Turkey almost simultaneously with those in the USA in 2007. These mysterious losses had economical impacts in terms of honey bee colonies and crops. Our purpose here was to quantify levels of viruses and other pathogens in honey bees from 6 different regions of Turkey. RNA extracts were screened for American foulbrood (AFB), Sacbrood virus (SBV), Acute bee paralysis virus (ABPV), Deformed wing virus (DWV), Black queen cell virus (BQCV), Israel acute bee paralysis virus (IAPV), Kashmir Bee Virus (KBV), *Nosema apis* and *Nosema ceranae*. Among the samples, mixed infections of Acute bee paralysis virus (ABPV), Deformed wing virus (DWV), Black queen cell virus (BQCV) and *Nosema ceranae* were detected. Virus and pathogen frequency in samples from migratory beekeepers were higher than in samples from stationary colonies. No differences were noted in this survey among honey bee races, although work is ongoing to better sample each of the diverse races in Turkey.

## ***Nosema bombi* prevalence in spring bumble bee queens in Kansas**

**Amber D. Tripodi and Allen L. Szalanski**

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*Nosema bombi* is associated with bumble bee decline in the US. This pathogen overwinters within host queens and is transmitted vertically. Colonies founded by surviving, infected queens are smaller and produce fewer reproductives. Using a molecular diagnostic technique, we surveyed foraging spring queens (n = 119) at three sites in Kansas. Six *Bombus* species were present, *Bombus auricomus* (n = 63), *B. bimaculatus* (n = 12), *B. fraternus* (n = 1), *B. griseocollis* (n = 23), *B. impatiens* (n = 8) and *B. pensylvanicus* (n = 10). Overall, 12% of sampled queens were positive for *N. bombi*, but prevalence was not evenly distributed among species or sites (Fisher's exact, both  $P < 0.001$ ). Only *B. auricomus* and *B. pensylvanicus* were positive (n = 8, n = 6), and most (n = 12) were from one site in Barber county. Prevalence of *Nosema bombi* in queens may indicate local population decline.

## Microbial Gut Diversity of Honey Bee Larval Instars

**Svjetlana "Lana" Vojvodic** <sup>1\*</sup>, **Sandra M. Rehan** <sup>2</sup>, **Kirk E. Anderson** <sup>1,3</sup>

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We demonstrate for the first time that the larval honey bee gut is colonized by bacterial groups previously described from adult honey bees or other pollinators. Bacterial diversity changes with larval age; where first and second larval instars contained almost exclusively Alpha 2.2, a core Acetobacteraceae, later instars were dominated by one of two very different *Lactobacillus* spp., depending on the sampled site. Royal jelly, the main source of larval nutrition, has known antimicrobial properties. The control strains *Staphylococcus* and *L. kunkeei* originating from flowers were strongly inhibited by royal jelly, but only larval-isolated bacteria from the Neisseriaceae clade were inhibited. *L. kunkeei* was found in both larval guts and flowers, but only strains isolated from flowers were inhibited, suggesting strain adaptation to different environments.

## **Pesticide impacts on a non-*Apis* species: Broadening the data sets on sub-lethal effects of neonicotinoid insecticides and pollinators**

**Keith Walters**

In recent years conventional pesticides, particularly neonicotinoids, have been a significant focus of European research into the causes of pollinator decline, with the majority addressing effects on honeybees. Relatively few studies address the sub-lethal effects on non-*Apis* pollinators, many have used unrealistically high exposure rates, few consider avoidance behaviour mediating exposure, and most investigate single-product responses (mainly imidacloprid). Extrapolation from data with such limited focus has contributed to the current polarised/unresolved debate on the actual environmental impact of neonicotinoids, causing difficulties for policy makers arising from contradictory interpretation of the evidence, and leading to a 2-year moratorium on defined uses of neonicotinoids.

This paper explores some of these data gaps, identifying the key non-*Apis* pollinator groups/species, and for one (*Bombus terrestris*), sub-lethal responses to field-realistic exposure to thiamethoxam, a DMI-fungicide (propiconazole), and potential synergism. To support correct interpretation, evidence of a *B. terrestris* behavioural response to pesticide contaminated plants and its likely impact on exposure rates in field crops is also described. Colony level responses were obtained only at the highest exposure rates tested. Factors leading to the current polarised debate and the consequences of the moratorium for environmental protection are discussed in the light of the results.

## **How to build it so they come – identifying key floral resources and designing effective seed mixes for pollinator habitat in agricultural landscapes of California**

**Kimiora Ward, Claire Brittain, Neal Williams**

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Recognition of the importance of diverse floral resources in supporting both wild pollinator communities and honey bee nutritional health has led to a surge of interest in developing plant materials and methods for establishing wildflower habitat in agricultural landscapes. We are developing and testing mixes of herbaceous flowering plants for their establishment success, attractiveness to bees, and compatibility with agricultural practices in California. Surveys of the diversity and abundance of insects attracted to test mixes have identified particular plant species that stand out in terms of their attractiveness to bees. We designed a seed mix of these best-performing plants for use on conventional watermelon farms, a diverse mixture of both annuals and perennials that provides season-long resources and that consistently attracts larger numbers of both managed and wild bees compared to naturally vegetated controls. We are currently exploring additional plant mixtures tailored for use with almond orchard systems. Preliminary surveys suggest enhancements attract bees before and after almond bloom and do not compete with the crop. Patterns of bee use of individual plant species reveal their reliance on multiple plant species at any given time, as well as the importance of testing plants for their utility to pollinators before investing in their establishment on farms.

## Investigation of the bee community in sunn hemp, a cover crop for mitigating fall armyworm spring populations

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Sunn hemp (*Crotalaria juncea*) is a fast-growing nitrogen-fixing legume used as a green manure cover crop, nutrient rich forage, and fiber species. We are developing sunn hemp as a potential cover crop to control spring populations of fall armyworm (*Spodoptera frugiperda*: Noctuidae) in the southeastern U.S. prior to their northward migration, as it increases larval development time and mortality when used as a host. Widespread usage of sunn hemp is currently limited by the availability of affordable, locally sourced seed. Previous work investigating seed production potential of sunn hemp in Florida suggested that pollination might be a limiting factor. Toward identifying an effective pollination strategy for sunn hemp, we surveyed the bee community present in sunn hemp and adjacent crops (i.e. sorghum-sudangrass, iron-clay cowpea, and corn) grown in Florida in 2011 and 2012. We collected 980 bees, approximately 70% of which are *Melissodes* (mining bees). This is in contrast to previous work in Florida suggesting that *Xylocopa* (carpenter bees) and large *Megachile* (leafcutter bees) are the most likely pollinators of sunn hemp. Interestingly, only two of the bees we sampled were *Xylocopa*. This season we will determine which bee species actually visit sunn hemp and assess their effectiveness in pollination.

## **Artificial nesting sites for native bees: a critical step toward improving the landscape for pollinators.**

**John W. Wenzel**

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I report a large-scale experiment to provide artificial nesting sites for native, wild bees in a location where they did not already live. Four hundred tons of commercially available sand was shaped into dunes, and inoculated with native bees caught 20km away. In a few days, more than 2,000 free flying bees colonized the dunes. The experiment demonstrates that it is possible to use ordinary construction methods to produce favorable wild bee nesting habitat.

## Susceptibility of the Small Hive Beetle (*Aethina tumida* Murray) to Fungal Pathogens

**S. Wheeler \***, L. H. B. Kanga

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The small hive beetle (SHB) *Aethina tumida*, an invasive species from sub-Saharan Africa, entered the United States through Florida in 1998 and has become a serious pest of honey bee. This destructive insect has already invaded more than 30 states, and threaten the honey bee industry in southern US. The chemicals currently used for control of SHB are not effective and leave residues in honey products. This study was designed to identify potential microbial control agents. Two potential microbial control agents, *M. anisopliae* 3020 and *M. anisopliae* 5680 were tested against the small hive beetle under field conditions in an apiary located in Quincy, FL. Four pounds of sand was mixed with dried fungal spores and 43.0 grams of Crisco® vegetable shortening in a metallic pan and placed under each treatment hive; mortality was observed for 73 days. Results indicated that soils treated with both fungal spores (*M. anisopliae* 3020 or *M. anisopliae* 5680) significantly reduced small hive beetle populations in bee colonies as compared to the controls. However, the percentage of survival of honey bee colonies was significantly higher in colonies treated with *M. anisopliae* 5680 spores than in those treated with *M. anisopliae* 3020 spores. Thus, these fungal pathogens offer new and environmentally sound avenues for the successful control of SHB in honey bee colonies.

Key Words: Small hive beetle, honey bee, microbial control, fungal pathogens

## **Operation Pollinator: Evaluation of flowering plant mixes for supporting pollinator biodiversity in agricultural systems**

**Neal M. Williams<sup>1</sup>, Rufus Isaacs<sup>2</sup>**

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Wild bees face challenges from agricultural intensification, but can contribute to crop pollination where their habitat needs are met. These opposing points call for proactive strategies to promote robust wild bee populations and increase the sustainability of pollination service they can provide in agricultural systems. Provision of pollinator habitat in proximity to cropped areas is an appealing conservation approach, but effective implementation requires an understanding of the plant species attractive to bees, how to best establish and manage such plantings, where to locate them relative to the crop, and demonstration of their ability to support bees. As part of the Operation Pollinator™ initiative researchers in California, Florida and Michigan evaluated regionally-adapted flowering plant mixes identified based on two previous seasons of field testing. Mixes were planted using standard agronomic practices in large plots on conventional farms growing a target crop (CA watermelon, FL squash, MI blueberry), and assessed for their flowering performance and attractiveness to wild insect pollinators. Pollinator plantings produced significantly more flowers than un-enhanced field margins. Bloom within the enhancements persisted throughout the growing season in each region, supporting a diverse and more abundant community of wild bees than unenhanced field margins in all regions.

# Constraints of diet specialization in pollinators: influences of sociality and body size

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We describe patterns in diet specialization among bird and insect pollinators and examine potential constraints of diet specialization. Here, we focus on two common pollinators, hummingbirds and bees. These groups, which in addition to playing important ecological roles, also demonstrate diverse feeding habits, social structure, habitat requirements and geographic distribution. We redefine generalist and specialist in a dietary context that takes into consideration foraging adaptations. Thus species are placed in a multi-dimensional diet landscape that incorporates gradients of diet breadth and foraging strategy breadth. We then examine how diet specialization may be further constrained by social structure and pollinator biomass.

## Bees, a Prairie, and G.I.S.!

**Jennifer J. Zentmyer**

University of Wisconsin Parkside

Bees are essential pollinators for agriculture and ecosystems. According to records from 2000 the value of bee pollination to U.S. agriculture alone was \$14.6 billion. Pollination services are one major reason why bee conservation is essential. However, long-term research shows bees are experiencing widespread declines, threatening this vital ecosystem service. Bees are threatened from many anthropogenic sources including habitat fragmentation and land use conversion. As a University of Wisconsin Parkside (UWP) undergrad with the Center for Environmental Studies, I am using the campus' outdoor laboratories to address the general question of how we can promote bee conservation through classroom activities, undergraduate research, and community service. Specifically, I will test research theory that proposes bloom size and seed production increase bee visitation and diversity. I will quantify and identify bee populations by species within major campus habitat types, focusing on the effects of floristic diversity (species richness, flower size, reward types, etc) on bee diversity and visitation rates. I want to understand which plants we can add to the degraded prairies and old-field habitats to improve bee habitat. I will use interactive Geographic Information System (G.I.S.) mapping to show and track site data and promote bee conservation in the community.







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