# CENTER FOR POLLINATOR RESEARCH



## 1st International Conference on Pollinator Biology, Health and Policy

July 24 - 28 2010 University Park, Pennsylvania, USA





The conference organizers would like to thank Harland M. Patch for the graphics used on the front cover of the program booklet and the conference bags. Images on the bags are copyrighted by: Z. Huang and M. Tuttle

## 1<sup>st</sup> International Conference on Pollinator Biology, Health and Policy July 24-28, 2010

## Agenda

## Saturday, July 24, 2010

12:00 (noon) Registration check-in begins, Rotunda, Nittany Lion Inn

#### Welcome Reception (5:00-7:00pm)

Assembly Room, lower level

5:40 - 5:50	Opening remarks (Christina Grozinger, Penn State University)
5:50 - 6:00	Bruce McPheron, Dean, College of Agricultural Sciences, Penn State University
6:00 - 7:00	Keynote Address: May Berenbaum, Professor, Dept of Entomology, University of Illinois, Urbana-Champaign

## Sunday, July 25, 2010

Ballroom

#### **Behavioral Ecology**

Symposium organizers: C. Grozinger, J. Tumlinson

- 8:30 9:10 Abraham Hefetz, Tel Aviv University, Israel Pheromone involvement in reproductive competition in the bumblebee Bombus terrestris.
- 9:10 9:35 Robert Gegear, University of Massachusetts, Worcester. *Chasing migration genes in the monarch butterfly.*
- 9:35 10:00 Theresa Pitts-Singer, Pollinating-Insect Biology, Management, and Systematics Research Unit, US Department of Agriculture, Logan, UT Nesting and reproductive success of alfalfa leafcutting bees used for U. S. alfalfa seed production.

10:00 - 10:20 Break

10:20 - 10:45	Heather Mattila, Wellesley College
	Colony productivity depends on queen promiscuity.

- 10:45 11:10 Christina Grozinger, Penn State University Elucidating the role of social communication in honey bee health.
- 11:10 11:35 Peter Teal, Chemical Research Unit, USDA-ARS, Gainesville, FL. Development of Semiochemical Based Control Programs for Arthropod Pests of Honeybees.

#### Pollinator Biology, Health and Conservation

Symposium organizers: S. Fleischer, N. Ostiguy, H. Patch, A. Surcica

1:30 - 1:45	Graham Thompson, University of Western Ontario, Canada Genome-wide analysis of genes related to reproduction in worker honey bees.
1:45 - 2:00	Amy Toth, Penn State University Transcriptomic and chemical signatures of reproductive dominance in Polistes paper wasps.
2:00 - 2:15	George Yocum, USDA-ARS Fluctuating thermal regimes improve survival of the alfalfa leafcutting bee during cold storage.
2:15 - 2:30	Veerle Mommaets, Free University of Brussels, Belgium Side-effects on bumblebee foraging behaviour: A laboratory bioassay.
2:30 - 2:45	Coffee Break
2:45 - 3:00	Geoff Williams, Dalhousie University, Canada Colony Collapse Disorder in context.
3:00 - 3:15	Bach Kim Nguyen, University of Liege, Belgium Honey bee colony losses in Belgium: a multifactorial approach.
3:15 - 3:30	Nancy Ostiguy, Penn State University Honey Bee Epidemiology: Disease Prevalence, Morbidity and Mortality in Seven Stationary Apiaries across the United States.
3:30 - 3:45	Benjamin Dainat, Agroscope Liebefeld Posieux Research Station ALP, Switzerland Pathogen markers for honey bee colony collapse and life expectancy of infected workers.

3:45 - 4:00 Break

4:00 - 4:15	Amber Renee Sciligo, Bio-Protection Research Centre, Lincoln
	University, New Zealand
	Pollinator declines in New Zealand.

- 4:15 4:30 Mary Gikungu, National Museums of Kenya, Kenya Urban areas as bee refugia: A case study of Nairobi City Park, Kenya.
- 4:30 4:45 Alexandra Harmon-Threatt, University of California, Berkeley *The role nativity, abundance, and nutrition play in pollen preference of a native pollinator.*
- 4:45 5:00 Suann Yang, Penn State University Stability in plant-pollinator networks.
- 5:00 5:15 Sarina Jepsen, The Xerces Society Bumble bee Policy in the United States: History and Future Directions.

#### Plenary Lecture (6:00-7:00 pm)

Boardroom, lower level

6:00 - 7:00 Claire Kremen, University of California, Berkeley Pollination services and agro-ecosystems: searching for sustainability.

### Monday, July 26

Ballroom

#### **Evolving Policies on Pollinator Risk Assessment and Conservation** Symposium organizers: J. Frazier and E. Rajotte

8:30 - 8:50	Stuart Roberts, Reading University, UK The European Response to Pollinator Threats.
8:50 - 9:10	Doug Holy, USDA- NRCS Responses of the U.S. Federal Government to Pollinator Population Declines.
9:10 - 9:30	Thomas Moriarty, Environmental Protection Agency The Role and Activities of the USEPA in Response to Pollinator Decline.
9:30 - 9:50	Robyn Rose, APHIS APHIS' Responsibilities and Response to Pollinator Decline.
9:50 - 10:10	Jim Rauh/Kristin Brugger, DuPont Development of Rynaxypyr®: Assessment of Impact to Nontarget Organisms including Pollinators in the Insecticide Discovery Process.

- 10:10 10:30 Steve Ellis, National Honey Bee Advisory Board The National Honey Bee Advisory Board works to promote honey bee sustainability through balance.
- 10:30 10:50 Break
- 10:50 11:10 Mace Vaughan, Pollinator Conservation Program, Xerces Society *Putting Federal Pollinator Conservation Policies into Practice.*
- 11:10 11:30 R. Thomas Van Arsdall, Pollinator Partnership Evolving Policies on Pollinator Risk Assessment and Conservation.
- 11:30 11:45 Mary Purcell-Miramontes, Competitive Programs, USDA-NIFA USDA-NIFA Programs Addressing Pollinator Decline and CCD.
- 11:45 12:00 Mark Sharer, Penn State University Development *Philanthropic funding of pollinator programs.*

#### **Press conference (12:00-1:00 pm; lunch provided)**

#### **Status of Pollinators World Wide**

Symposium organizers: J. Pettis and D. van Engelsdorp

1:30 - 1:35	Jeff Pettis Opening remarks
1:35 - 2:00	Dennis vanEngelsdorp, Penn State University Historical review of colony losses in the US.
2:00 - 2:25	Peter Neumann, Honeybee Pathology Section Swiss Bee Research Centre, Bern, Switzerland <i>Honey bee decline: the need for a COLOSS.</i>
2:25 - 2:50	Keith Delaplane, University of Georgia Pollinator Decline: Getting a Handle on its Consequences.
2:50 - 3:10	Break
3:10 - 3:35	David De Jong, University of São Paulo, Brazil
3:35 - 4:00	Stuart Roberts, Reading University, UK
4:00 - 4:25	Yves Le Conte, Institut National de la Recherche Agronomique, Avignon- France Disease ecology and honey bee losses.

#### Reception at the Penn State Arboretum (5:30-8:00 pm)

6:00 Kim Steiner, Director, The Arboretum at Penn State Short presentations from conference sponsors

### Tuesday, July 27

#### Ballroom

#### **Impacts of Environmental Toxins**

Symposium organizers: M. Frazier and C. Mullin

- 8:30 9:10 Jean-Marc Bonmatin, Centre de Biophysique Moleculaire, Centre national de la Recherche Scientifique (CNRS), Orleans, France Impact of Environmental Toxins: Bioavailability of Systemic Insecticides in Pollen and Interactions with Bee Colonies.
- 9:10 9:35 Andreas Thrasyvoulou, Aristotle University of Thessaloniki, Greece *Residues in Honey and Beeswax Caused by Beekeeping Treatments.*
- 9:35 10:00 Chris Mullin, Penn State University Pesticides and Pollinators: Assessing Residues and Multiple Interactions in Honey Bees.
- 10:00 10:20 Break
- 10:20 10:45 Reed Johnson, University of Nebraska Drug Interactions between Miticides and Fungicides in Honey Bees.
- 10:45 11:10 Gloria DeGrandi-Hoffman, Carl Hayden Bee Research Center, USDA ARS, Tucson, Arizona The Impact of Fungicides on Symbiotic Microbes in Honey Bee Colonies.
- 11:10 11:35 Jeff Pettis, USDA-ARS Bee Research Laboratory, Beltsville, Maryland The Role of Pesticides in Colony Health and Increased Pathogen Loads in Adult Bees.

#### **Disease Ecology**

Symposium organizer: D. Cox-Foster

1:30 - 1:55 Diana Cox-Foster, Rajwinder Singh, and Abby Kalkstein, Penn State Disease ecology of RNA-viruses in Honey bees and native insect pollinators: impacts of parasites and environmental chemicals.

1:55 - 2:20	Ben Sadd and Paul Schmid-Hempel, Institute of Integrative Biology (IBZ), ETH Zurich, Zurich, Switzerland <i>The influence of the environment on immunity and parasite resistance</i> <i>in humblebees</i>
	in bumblebees.

- 2:20 2:45 Marla Spivak, Department of Entomology, University of Minnesota *Disease Ecology of Honey Bees.*
- 2:45 3:10 Rosalind James and Junhuan Xu, USDA-ARS, Logan, Utah The impact of a warmer climate on disease transmission in a solitary bee: immune response isn't everything.
- 3:10 3:30 Break
- 3:30 3:55 Michael Otterstatter and James Thomson, University of Toronto, Toronto, Canada *Evidence for pathogen spillover between introduced and native bumble bee species.*
- 3:55 4:20 Thomas H. Kunz, Boston University Disease ecology of white-nose syndrome in brown bats and possible impact on pollinator species.
- 4:20 4:45 Dick Rogers, Bayer Crop Science How monitoring contributes to understanding honey bee health and colony losses: a summary from a decade of experience.

#### Posters with Reception (5:30-7:30 pm)

Boardroom, lower level

### Wednesday, July 28

Morning Sessions in Ballroom Lunch and Afternoon Sessions in Assembly room, lower level

#### **Conservation and Ecological Applications of Native Pollinators**

Symposium organizers: C. Grozinger, A. Hefetz, D. Cox-Foster, Y. Mandelik

- 8:30 8:55 Carol Boggs, Stanford University Butterfly Population Responses to Floral Resource Availability
- 8:55 9:20 Sydney Cameron, University of Illinois, Urbana-Champaign Status of bumble bee decline across North America.

- 9:20 9:45 Baldwyn Torto, Behavioral and Chemical Ecology Department, International Centre of Insect Physiology and Ecology, Nairobi, Kenya. *The Importance of Bee Health for the African Pollinator Initiative: the icipe contribution.*
- 9:45 10:10 Julianna Tuell, Keith Mason, Rufus Isaacs, Michigan State University University; Jeff Peters, Syngenta Crop Protection Neal Williams and Katharina Ullmann, University of California Davis; James D. Ellis, Jaret C. Daniels and J. Akers Pence, University of Florida Operation Pollinator- Habitat Restoration in Agricultural Landscapes.
- 10:10 10:30 Break
- 10:30 10:55 Yael Mandelik, Hebrew University of Jerusalem, Israel Diversity patterns and crop pollination services by bees in a Mediterranean and an arid agro-ecosystem.
- 10:55 11: 20 Lora Morandin and Claire Kremen, University of California, Berkeley Native pollinator diversity, abundance, and forage preference in native plant hedgerow restorations.
- 11:20 11:45 David Mortensen and John Tooker, Penn State University A threat to beneficial-insect habitat from new herbicide programs.
- 11:45 12:10 Jim Cane, Pollinating-Insect Biology, Management, and Systematics Research Unit, US Department of Agriculture, Logan, UT *Pollination and pollinators for wildflower seed farming and post-fire wildland rehabilitation.*

#### Lunch (provided)

1:30 - 1:55 David Biddinger, Penn State University Strategies to improve native pollinator biodiversity and ecosystems services in orchards.
1:55 - 2:20 Dolores A. Savignano, U.S. Fish and Wildlife Service U.S. Fish and Wildlife Service Pollinator Conservation and Education Activities.
2:20 - 2:45 Nicholas W. Calderone, Cornell University The contributions of insect pollinators to US agriculture: 1992-2008.
2:45 Ed Rajotte, closing remarks

#### Invited Speaker Abstracts (Listed in order by the program schedule)

Pheromone involvement in reproductive competition in the bumblebee <i>Bombus terrestris</i> Abraham Hefetz
Chasing migration genes in the monarch butterfly Robert J. Gegear
Nesting and reproductive success of alfalfa leafcutting bees used for U.S. alfalfa seed production Theresa L. Pitts-Singer
Colony productivity depends on queen promiscuity Heather R. Matilla
Elucidating the role of social communication in honey bee health Christina M. Grozinger
Development of Semiochemical Based Control Programs for Arthropod Pests of Honeybees Peter E. A. Teal
Genome-wide analysis of genes related to reproduction in worker honey bees Graham J. Thompson
Transcriptomic and chemical signatures of reproductive dominance in <i>Polistes</i> paper wasps Amy L. Toth
Fluctuating thermal regimes improve survival of the alfalfa leafcutting bee during cold storage G.D. Yocum
Side-effects on Bumblebee Foraging Behaviour: A Laboratory Bioassay Veerle Mommaerts
Colony Collapse Disorder in context Geoffrey R. Williams
Honey Bee Epidemiology: Disease Prevalence, Morbidity and Mortality in Seven Stationary Apiaries across the United States N. Ostiguy
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Drug interactions between miticides and fungicides in honey bees ( <i>Apis mellifera</i> ) Reed M. Johnson
The effects of fungicides on the diversity of microbes in stored pollen and the physiological repercussions on worker and queen honey bees Gloria DeGrandi-Hoffman
Sub-lethal pesticide exposure in honey bees: Chronic pesticide exposure at the colony level results in increased susceptibility of workers to pathogen infection Jeffery S. Pettis
Contingency and the outcome of host-parasite interactions Ben M. Sadd
Propolis and Social Immunity in Honey Bee Colonies Marla Spivak
The impact of a warmer climate on disease transmission in a solitary bee: Immune response isn't everything Rosalind James
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## Pheromone involvement in reproductive competition in the bumblebee Bombus terrestris

Abraham Hefetz, Department of Zoology, Tel Aviv University, Tel Aviv 69978, Israel

*Bombus terrestris* forms monogyne colonies with singly inseminated queens. Kin selection therefore predicts a queen-worker and worker-worker competition for male production, which is deferred to the end of the colony annual cycle after gynes have been produced. Accordingly, colony development is first typified by queen reproductive monopoly and harmonious worker behavior, followed by worker reproduction and all-out aggressive interactions. Behavioral evidence suggests that queens may have a suite of pheromones that regulates both worker behavior and caste determination.

Chemical analysis of Dufour's gland exudates revealed pheromone caste specificity, sterile workers possess in addition to typical to all castes hydrocarbons, a series of octyl esters that are absent in queens as well as reproductive workers. Behavioral and chemical analyses revealed that the workers that contain the octyl esters are less molested by dominant nestmates during the turbulent competition phase. We therefore suggest that these esters comprise sterility signal and their function is to appease dominant females they may encounter. Such a signal facilitates the reinstatement of a reproductive division of labor even at the competition phase or under queenless conditions and through that insures good colony reproductive output, and thence higher inclusive fitness to all colony members

#### Chasing migration genes in the monarch butterfly

Robert J. Gegear, Haisun Zhu, and Steven M. Reppert, Department of Neurobiology, University of Massachusetts Medical School

Each fall, millions of monarch butterflies (*Danaus plexippus*) in eastern North American undergo a spectacular migration to their overwintering sites in central Mexico. The migratory state in monarchs is characterized by several physiological and behavioral traits; arguably the most notable is the ability to maintain long-distance flight in a south/southwesterly direction using a time-compensated sun compass. Although some aspects of this navigational ability in monarchs have been well-studied, its hormonal and genetic regulations remain largely unknown. By increasing juvenile hormone (JH) activity in normally JH-deficient migrants, we could initiate reproductive development in migrants, while not affecting proper sun compass navigation. We then defined molecular correlates of the migratory state using microarray analysis to compare gene expression profiles in brain between fall migrants and summer butterflies. We identified a suite of 40 genes whose differential expression was correlated with proper migratory flight behavior. These results provide insights into the genetic basis of the navigational system used by monarchs during their migratory state.

## Nesting and reproductive success of alfalfa leafcutting bees used for U.S. alfalfa seed production

<u>Theresa L. Pitts-Singer</u>, USDA ARS Bee Biology & Systematic Laboratory, Utah State University, Logan, Utah 84322

The alfalfa leafcutting bee, *Megachile rotundata* (Hymenoptera: Megachilidae), provides pollination services to produce an estimated 35,000 tons of certified alfalfa seed in the U.S. Canada is the primary producer of these bees, sending bees across the border on a one-way street to U.S. seed producers who are very dependent on this supply. If a catastrophic event were to negatively impact this bee supply, there is no readily available alternative pollinator supply to replace it. Current research in the behavioral, environmental, and chemical ecology of *M. rotundata* can lead to ways in which its management in the U.S. can be improved for sustainability.

#### Colony productivity depends on queen promiscuity

Heather R. Mattila, Department of Biological Sciences, Wellesley College, Wellesley, MA, 02481

The productivity and health of honey bee colonies is integral to their efficacy as pollinators, yet we are only beginning to understand how fundamental aspects of honey bee biology contribute to the success of colonies. The extremely polyandrous mating behavior of honey bee queens is an example of a derived trait that has clear benefits for colonies. The ancestral state for Hymenoptera is presumed to be monandry; however, queens of every honey bee species are highly promiscuous. While extreme polyandry drives down levels of relatedness within colonies, it simultaneously boosts levels of genetic diversity by introducing into each work force multiple subfamilies of workers who carry genes from many drone fathers. Why should the commercial beekeeping industry pay attention to this strange quirk of honey bee biology? Honey bee colonies with extremely promiscuous queens are substantially more active as foragers than colonies that lack genetic diversity because of single drone paternity. Enhanced foraging effort in genetically diverse colonies is supported in part by a greater exchange of foraging-related signals, such as waggle dances and shaking signals, between senders and receivers. These findings have important consequences for the impact of queen mating status on the performance of honey bees as pollinators.

#### Elucidating the role of social communication in honey bee health

<u>Christina M. Grozinger</u>, Department of Entomology, Center for Pollinator Research, Penn State University, University Park, PA

Pheromonal communication is critical for organizing worker behavior, physiology, and colony social structure in honey bees. Pheromone production and response are highly variable, and depend on an individual's physiological state, behavioral state, or the environmental context. Pheromones released by honey bee queens regulates many aspects of honey bee social behavior and division of labor. We have demonstrated that queen pheromone production is linked to the mating quality and reproductive state of the queen, and this has consequences on worker behavior and physiology, as well as queen longevity. Recognition pheromones are used by workers to distinguish between nestmates and nonnestmates. We have demonstrated that production of these recognition pheromones is altered by immunostimulation, and results in a change in nestmate social interactions. Thus, pheromone communication in honey bees appears to be a highly sophisticated system for conveying information about individual and colony status, and perturbations in this system may have consequences on colony social structure and overall health and productivity. These studies were conducted by Elina Lastro Niño, Sarah Kocher, and Freddie-Jeanne Richard, in collaboration with David Tarpy (NCSU) and Abraham Hefetz (Tel Aviv University).

#### **Development of Semiochemical Based Control Programs for Arthropod Pests of Honeybees**

<u>Peter E. A. Teal</u>, Adrian J. Duehl, Center for Medical, Agricultural and Veterinary Entomology. USDA-ARS, 1700 SW 23 Dr., Gainesville, FL

Mark J. Caroll, Carl Haden Bee Research Center, USDA-ARS, 2000 East Allen Road, Tucson, AZ 85719

Baldwin Torto, International Centre of Insect Physiology and Ecology, P.O. Box 20772-00100, Nairobi, Kenya

In recent years the apiculture industry has experienced serious problems created by exotic pests including Varroa destructor and the Small hive beetle, Aethina tumida, arthropods that, if left untreated, lead to a decrease in colony health and possible collapse. Control of these pests is problematic because Honey bees are extremely sensitive to pesticides and the public is becoming increasingly concerned about food containing pesticide residues. Most pesticides have modes of action specifically targeted to arthropods and so have difficulty managing arthropod pests of other arthropods while sparing beneficial species like Honey bees. An effective control program for these pests requires the development of alternative approaches to classical pesticide control. One alternative approach, to turn the semiochemicals used by the pests to survive in hives against themselves, is highly attractive. We have studied the semiochemical mechanisms associated with invasion of honey bee brood by Varroa mites and hive invasion by the Small hive beetle. These studies have identified chemicals responsible for these behavioral events for both pests, and, in the case of small hive beetle, identifying a more attractive alternate host. Here we report on the biological and chemical research that led to the identification of these compounds and on our efforts to develop these semiochemicals into control methods for these pests.

#### Genome-wide analysis of genes related to reproduction in worker honey bees

<u>Graham J. Thompson</u>, Department of Biology, University of Western Ontario, London, Canada N6A 5B7

A defining characteristic of eusocial animals is their division of labour into reproductive and non-reproductive specialists. Here, we use a microarray study to identify genes associated with functional sterility in the worker honey bee Apis mellifera. We contrasted gene expression in workers from a functionally sterile wild type strain with that in a mutant (anarchist) strain selected for high rates of ovary activation. We identified a small set of genes from the brain (n = 7) and from the abdomen (n = 5) that are correlated in their expression with early stages of ovary activation. Sterile wild type workers up-regulated unknown genes. By contrast, reproductive anarchist workers up-regulated genes for venom peptides and the yolk protein vitellogenin, among others. The differentially expressed genes identified are involved in early differentiation into sterile and reproductive worker phenotypes and may therefore form part of the gene networks associated with the regulation of honey bee worker sterility and division of reproductive labour. The up expression of vitellogenin, which has previously been linked to reproductive status and division of labour in honey bees, suggests that expression of this gene in particular is important in the regulation of worker fertility.

#### Transcriptomic and chemical signatures of reproductive dominance in *Polistes* paper wasps

<u>Amy L. Toth</u>, Department of Entomology, Penn State University, University Park, PA Department of Ecology, Evolution, and Organismal Biology, Iowa State University, Ames, IA

John F. Tooker and Christina M. Grozinger, Department of Entomology, Penn State University, University Park, PA

Dominance behavior in *Polistes* wasps plays a key role in the organization of these primitively eusocial societies. We investigated brain and ovary gene expression patterns associated with caste and reproductive status within the social dominance hierarchy of Polistes metricus. Via microarray analysis of dominant and subordinate foundresses, workers, and queens, we found differences in brain expression of several hundred genes related to metabolism, stress response, and neurotransmitter synthesis, including genes with roles in aggression in Drosophila and juvenile hormone signaling. In addition, a large subset of the transcriptome showed differential expression in the ovaries, with many genes exhibiting an expression pattern that reflected ovarian physiology, rather than behavioral state. In addition, we investigated the potential role of pheromonal modulation of *P. metricus* social organization by surveying the chemical composition of cuticular hydrocarbons and three exocrine glands. Using the same individuals used for transcriptomic analysis, we found differences in chemical profiles related to seasonality (cuticular hydrocarbons), dominance during colony founding (mandibular glands), and mating status (Dufour's glands). This wealth of both chemical and transcriptomic data on the same set of individual wasps was also used to look for novel connections between chemical profiles and gene expression within and between individuals.

Abstracts

## Fluctuating thermal regimes improve survival of the alfalfa leafcutting bee during cold storage

<u>G.D. Yocum</u>, R.P Rinehart and W.P. Kemp, Insect Genetics & Biochemistry Research Unit, USDA-ARS-RRVARC, Fargo, ND

The alfalfa leafcutting bee, *Megachile rotundata* is commonly held at low temperatures for overwintering the prepupae or for interrupting spring incubation to synchronize adult emergence with the peak alfalfa bloom. However, static low temperature exposures can be stressful depending on the temperature, duration of exposure, and the developmental stage exposed. Short high-temperature pulses have been demonstrated to alleviate the harmful effects of prolonged low temperature exposure. We determined the effect of a pulse at 20°C as well as the duration and frequency of the pulse on the survival of developing *M. rotundata* stored at 6°C. Finally, we examined the effect of high-temperature pulses on the survival of dormant prepupae to determine the feasibility of multi-year storage. This investigation clearly established that high-temperature pulses are important factors affecting survival of developing *M. rotundata* stored at low temperature stored at 6°C.

#### Side-effects on bumblebee foraging behaviour: a laboratory bioassay

<u>Veerle Mommaerts</u>, Laboratory of Cellular Genetics, Department of Biology, Faculty of Sciences, Free University of Brussels, Brussels, Belgium

Guido Sterk, Biobest NV, Westerlo, Belgium

Guy Smagghe, Laboratory of Cellular Genetics, Department of Biology, Faculty of Sciences, Free University of Brussels, Brussels, Belgium and Laboratory of Agrozoology, Department of Crop Protection, Faculty of Bioscience Engineering, Ghent University, Ghent, Belgium

Bumblebees are important pollinators in agriculture/horticulture over the world and are likely exposed to pesticides while foraging. Nowadays risks assessments are evident for all plant protection products and in this context several tests exist to assess lethal and sublethal side-effects of pesticides on bee survival, growth/development and reproduction. Here we report on the development a new bioassay to assess the impact of sublethal concentrations on the bumblebee foraging behaviour under laboratory conditions. In brief, the experimental setup of this test consists of two plastic containers (15 x 15 x 10 cm) connected with a tube of about 20 cm and use of queenless micro-colonies of 5 workers. In one container the bumblebee workers started to develop their nest (brood), and in the other food (sugar and pollen) was provided. Before exposure, the worker bees were allowed a training to forage for untreated food; afterwards this was replaced by treated food. Bumblebee workers were orally exposed via drinking sugar water treated with the neonicotinoid imidacloprid (200, 20, 2 and 0.2 ppm; and 20 and 10 ppb) that is expected to impair the foraging behaviour.

The median lethal concentration was lower in the test including foraging behaviour as compared with the classic toxicity test: the respective LC50-values were 20 ppb and 59 ppb. Similarly, the median sublethal effect concentration was 3 to 10 times lower in the test including foraging behaviour (EC<sub>50</sub> 3.7 ppb and NOEC <2.5 ppb) than in the classical toxicity test (EC<sub>50</sub> 37 ppb and NOEC 20 ppb). Finally, we validated our new laboratory bioassay by exposing queenright colonies of *B. terrestris* to sublethal concentrations of imidacloprid (20, 10 and 2 ppb) in a greenhouse. Workers needed to forage/fly for food as pollen and sugar water was placed at a distance of 3 meter from their hives. Under these greenhouse conditions the NOEC for the

sublethal effects on colony performance was 2 ppb which agreed with the value (< 2.5 ppb) obtained with our new laboratory bioassay.

#### **Colony Collapse Disorder in context**

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Recent large-scale die-offs of western honey bees (*Apis mellifera*) around the world are of extreme concern because of humanity's increasing reliance on pollinator-dependent crops. Although declines in honey bee populations have occurred in the past, the magnitude and speed of these recent declines are unprecedented. Often in the media, and sometimes in the scientific literature, these losses are inappropriately attributed to "Colony Collapse Disorder" or CCD, which is characterized by rapid disappearance of adult bees from colonies containing brood and food stores but lacking damaging levels of parasitic *Varroa destructor* mites or *Nosema* microsporidians. In reality, US beekeepers self-diagnosed CCD as only the 8<sup>th</sup> most important contributor to colony mortality during winter 2008/09, behind starvation, queen-related issues, and parasites. There is a growing consensus that colony mortality is the product of multiple factors, known and unknown, acting singly or synergistically. Although we agree that CCD is a cause for concern, we believe that it is imperative to appropriately order CCD within the context

of other honey bee morbidities occurring worldwide. This will allow for more informed and appropriate allocation of research efforts into CCD specifically and other causes of mortality in general.

#### Honey Bee Epidemiology: Disease Prevalence, Morbidity and Mortality in Seven Stationary Apiaries across the United States

Aronstein, K., A. Averill, F. Drummond, B. Eitzer, J. Ellis, <u>N. Ostiguy</u>, S. Sheppard, M. Spivak, K. Visscher

A four-year, seven location stationary apiary study was initiated in spring 2009 to study the disease prevalence, morbidity and mortality of honey bee colonies over time. The apiary locations are scattered across the United States and are located in California, Florida, Maine, Minnesota, Pennsylvania, Texas, and Washington. Thirty colonies were established in each apiary using local packages that were requeened in April or May using queens from Kohnen apiary. Bees were established in new wooden equipment and Perco plastic foundation was coated with wax from the same source. A sample of this wax was analyzed for the presence of pesticides. Each month the health of each colony is evaluated. The data collected include samples of bees for Nosema, tracheal mite, a varroa mite and virus identification and determination, number of adult bees, quantity of brood, and presence and number of small hive beetles. Additionally samples of pollen are collected each month for pesticide analysis.

Several apiaries obtained packages of bees from the same source. Interestingly the type and prevalence of virus was not the same in the packages. Viruses type and prevalence also differed by geographic location.

Abstracts

#### Pathogen markers for honey bee colony collapse and life expectancy of infected workers

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Yan Ping Chen, Jay D. Evans, USDA-ARS Bee Research Laboratory, Beltsville, MD 20705

Pathogens can cause honey bee colony collapse, creating demand to identify predictive markers and underlying mechanisms. Long term monitoring of colonies appears crucial, because pathogens causing death may have disappeared leaving room for secondary infections. Here we aim to identify markers at the colony level and shortened life span of infected bees as one mechanism. From September 2007 to spring 2008 100 pooled workers were sampled from each of 29 colonies in summer, fall and winter. In September, freshly emerged workers (N=500 per colony) were colony specific labeled and reintroduced into their maternal colonies, which were equipped with traps in order to monitor bee mortality. Colonies were surveyed for losses and *Varroa destructor* infestation using bottom board counts. Moreover, pooled and single workers were analyzed for 6 pathogens using RT-qPCR. The data for pooled bees show that *V. destructor, Nosema ceranae* and DWV can be predictive markers for colony death in different seasons. Moreover, data for single bees confirmed that *N. ceranae* loads predict colony death and also revealed that DWV infections have a significant negative effect on life expectancy. Our long term monitoring over 1 year points towards testable mechanism for colony collapse in the honey bees.

#### Pollinator declines in New Zealand

<u>Amber Sciligo</u>, Bio-Protection Research Centre, Lincoln University, Canterbury, New Zealand Linda Newstrom-Lloyd, Landcare Research, New Zealand

Preventing and reversing pollinator declines on small remote oceanic islands requires balancing native and non-native pollinator populations across natural and agricultural ecosystems. Islands, like New Zealand, typically have a subset of the types of pollinators found on continents and therefore have different dynamics in pollination systems. For example, New Zealand lacks large social bees, so honey bees and bumble bees were introduced to develop agriculture since all native bee species are small and solitary. Honey bees and bumble bees have both naturalized, however, feral honey bees are in total decline due to Varroa's arrival in 2000. Vertebrate pollinators have significantly declined particularly on mainland islands. Thus, honey bees and bumble bees could be important pollinators for some native plants where birds are absent. In agriculture, options for alternative pollinators for management. Three imported managed solitary bees have not been widely used. An overarching concern for using imported pollinators is the issue of naturalization, invasiveness and competitive displacement of native pollinators. On islands, it is necessary to use a landscape scale approach for managing pollinators due to extremely low pollinator diversity and high vulnerability of native species to invasions.

#### Urban areas as bee refugia: A case study of Nairobi City park, Kenya

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Pollinators such as bees, butterflies and birds are to some degree compatible with urbanization and may be able to survive in urban and peri-urban areas in diverse assemblages. Unfortunately, data on pollinator diversity in the urban areas has been lacking yet many of them are surrounding by agricultural systems. The current study aimed at documenting the first record of bee fauna in Nairobi City Park and comparing the species richness with that of Kakamega forest, a tropical rain forest in the Kenya. The bee survey was conducted in three microhabitats of Nairobi City Park which included open grasslands, bush land and understory community. From a sample of 860 individuals, we documented 68 species of which 90 % percent are found in Kakamega forest. The most diverse bee family was Apidae (50%) followed by Halictidae (26%) and Megachilidae (22%). The family Colletidae was the least diverse with only one species from the genus Hyleus. The key drivers of high bee diversity within the park were identified as habitat heterogeneity and plant diversity. The results of this study suggest that urban parks and green open areas in East Africa host unique and diverse native bee assemblages and can act as pollinator refugia.

#### The role nativity, abundance, and nutrition play in pollen preference of a native pollinator

<u>Alexandra Harmon-Threatt</u> and Claire Kremen, UC Berkeley, Department of Environmental Science, Policy and Management

Pollinator diet and preference has recently garnered increasing amounts of attention. Despite this, no general trends have been found on the interaction between native bees and native or nonnative plants. Using *Bombus vosnesenskii*, a generalist and widespread pollinator, as a model species we examined pollen usage to determine the preference of native and non-native plants. By directly examining pollen collection with respect to pollen availability we are able to determine direct preference for pollen resources, a more sensitive and selective resource for bees. We also examined the nutritional quality, protein and amino acid content, the available plant species offer to better understand the preference of each species. All pollen loads included both native and non-native plant species. *B. vosnesenskii* does not uniformly prefer native species over invasive, nor does it simply collect pollen in proportion to its availability. Nutrition and seasonality appear to be the most important factors for bee preference.

#### Stability in plant-pollinator community networks

<u>Yang, Suann</u> and Shea, Katriona, Biology, Penn State University, University Park, PA Campbell, Colin and Albert, Réka, Physics Departments, Penn State University, University Park, PA

Plant-pollinator mutualisms are key components of terrestrial ecosystems. However, local and widespread changes in plant-pollinator communities are being documented more frequently. Because of the interconnectedness of the plant-pollinator community, the introduction or extinction of one species may have cascading effects on the whole community. In this study, we consider the response of plant-pollinator communities to changes in species composition, using a dynamic network model. We simulate the assembly of stable communities from a regional source pool of species, and then perturb them by removing or adding species. In general, we find that stability is regained quickly, and that most of the post-disturbance communities are identical to, or closely resemble, the original stable communities. However, the size of the regional source pool has an important effect on the robustness of a community to perturbations. Communities occasionally undergo a complete loss of biodiversity after perturbation, and the risk of this collapse is higher for communities assembling from smaller regional species pools. Together, these results suggest that plant-pollinator mutualisms are somewhat resistant to changes in local species composition, but that this resistance is dependent on regional species biodiversity. Our work emphasizes the importance of large-scale maintenance of biodiversity in plant-pollinator communities

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#### Bumble bee Policy in the United States: History and Future Directions: How changes in policy can mediate threats to bumble bees

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Bumble bees are among the most important native pollinators of numerous crops, including hothouse tomatoes, blueberries and cranberries, and have been commercially produced for use in agriculture since the early 1990s. Current policy allows the common eastern bumble bee (*Bombus impatiens*) to be shipped to most western states, outside of the species' native range. Recent declines in multiple North American bumble bees species have sparked concern over the spillover of pathogens from commercial bumble bees to wild bumble bees, establishment of nonnative species in new areas, and hybridization and competition with native species. We reviewed existing U.S. laws that govern the movement of insect pollinators, disease, and plant protection, and found that the USDA's Animal and Plant Health Inspection Service (APHIS) has the authority under three separate laws to regulate the interstate movement of bumble bees: the Plant Protection Act, the Honeybee Act and the Animal Health Protection Act. To conserve wild bumble bees, The Xerces Society recommends that APHIS create rules prohibiting the movement of bumble bees outside of their native ranges, and that the agency regulate movement of bumble bees within their native ranges by requiring that bumble bees are certified as disease-free prior to movement.

#### **Responses of the U.S. Federal Government to Pollinator Population Declines**

<u>Doug Holy</u>, National Invasive Species and Pollinator Specialist, USDA-Natural Resources Conservation Service

Since the publication of the National Academy of Sciences, National Research Council's "Status of Pollinators in North America," the discovery of what has been described in the United States as "Colony Collapse Disorder," new legislation directly and indirectly affecting pollinators and their habitats (e.g., "2008 Farm Bill," State s wildlife action plans), and increased pollinator-focused energies from various non-governmental organization (NGO) partners, the U.S. Federal government has greatly increased its attention to honeybees and other pollinators that, thanks to their generally unnoticed but critical pollination services, facilitate reproduction of about 70% of the world's flowering plants which includes more than two-thirds of the world's crop species. The speaker, who is actively-involved with both Federal government and NGO research-related and conservation-related aspects of pollinators and their habitats, will present actions of the U.S. Federal government and its partners in addressing research and habitat needs of honeybees and other pollinators.

#### Development of Rynaxypyr®: Assessment of Impact to Nontarget Organisms including Pollinators in the Insecticide Discovery Process

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Axel Dinter, DuPont de Nemours, Deutschland, Neu Isenberg, Germany

DuPont's Crop Protection R & D process has undergone substantial changes over the last decade in response to meeting grower needs for products having improved efficacy and safety profiles. This presentation will provide an overview of how this process has changed, emphasizing efforts to move characterization work previously done at the time of Development, earlier in the Discovery process. Early identification of regulatory issues in a new area can influence the direction of optimization efforts and the final selection criteria for the candidate molecule to be taken into Development. Such changes can increase the probability of successful commercialization for Development candidates. An example from this process is the recent introduction of Rynaxypyr®, an anthranilic diamide insecticide which is an excellent tool for integrated pest management programs and has an excellent profile with respect to conserving important pollinators. The active ingredient in Rynaxypyr®, chlorantraniliprole, has a novel mode of action. It has low acute toxicity to honey bees as well as low risk of systemic exposure via pollen and nectar. The profile of Rynaxypyr® with respect to pollinators will be summarized. Continuing efforts to incorporate early testing with various non-target organisms, including pollinators, into the Discovery process will be discussed.
## The National Honey Bee Advisory Board

Steve Ellis, National Honey Bee Advisory Board

The National Honey Bee Advisory Board works to promote honey bee sustainability through balanced pesticide policy, evidence based decisions, and proactive education. Working collaboratively with other organizations, we seek to protect pollinators from the dangers of pesticides. The NHBAB works to ensure that pollinator protection receives proper attention at a National policy level.

### **Putting Federal Pollinator Conservation Policies into Practice**

<u>Mace Vaughan</u>, Director, Pollinator Conservation Program, and Eric Mader, National Outreach Coordinator, Pollinator Conservation Program, Xerces Society for Invertebrate Conservation

The 2008 Farm Bill contains significant and specific language that makes pollinators a national priority for the USDA-administered conservation programs. These programs include over \$1 billion per year to support conservation projects on private lands. This presentation will highlight state and regional efforts across the country to create the on-the-ground infrastructure and knowledge needed to successfully implement the pollinator conservation provisions of the Farm Bill. These efforts include the development of (a) demonstration projects and robust vetted plant lists from across the country in collaboration with the NRCS Plant Material Program and farmers, (b) clear guidelines that address the need for adequate and robust weed control, (c) accurate landowner reimbursement rates that reflect the true cost of implementing projects, and (d) communication of a clear demand for pollinator project support from NRCS field offices and from farmers.

The 2008 Farm Bill also authorized a new \$20 million per year research budget and made pollinators a priority of the USDA Specialty Crops Research Initiative grant program. To date, the new funding has not been appropriated and requires significant outreach from farmers, beekeepers, scientists and conservationists to educate lawmakers on the value and need for increased study into problems facing crop pollinators.

### **Evolving Policies on Pollinator Risk Assessment and Conservation**

R. Thomas Van Arsdall, Pollinator Partnership, Director of Public Affairs

In recent years, increased attention has been paid to the needs of managed and native pollinators in the national policy arena. With increased awareness about the importance of our pollinating partners, multiple threats, and the need to take action to protect pollinators and their habitat, long-neglected pollinator policy is expected to continue to receive attention. Efforts have been most productive when based on "pollinating" existing policies and programs. Pollinator-beneficial actions have been taken in the Congress, such as through the 2008 farm bill and the annual appropriations process, with other possibilities ahead such as through transportation reauthorization. Pollinator protection has proven to have strong bipartisan support in the Congress and among diverse stakeholder groups. Federal agencies that manage or influence landscapes have been working to incorporate pollinator conservation into their programs and practices, in part through collaborative efforts facilitated by the North American Pollinator Protection Campaign. USDA, Interior and EPA are current leading examples. It is important to base pollinator policy outreach and initiatives on sound science and research. Opportunities exist for researchers and other pollinator stakeholders to participate in and influence the policy process.

### **USDA-NIFA Programs Addressing Pollinator Decline and CCD**

Mary Purcell-Miramontes, National Program Leader, Competitive Programs, USDA-NIFA

Pollinators are suffering major losses worldwide. In 2006, the phenomenon known as Colony Collapse Disorder (CCD) became a significant threat to the honeybee pollination industry in the USA and several other countries. CCD continues to be of paramount concern and honeybee losses are unsustainable to the beekeeping industry. Also at risk, are increased costs to growers for pollination services and potential crop losses. USDA's National Institute of Food and Agriculture provides significant extramural funding for fundamental and applied research and outreach projects relevant to pollinator protection and honeybee health. Current and future opportunities will be highlighted in this presentation.

### A Historical review of honey bee populations in the US

Dennis van Engelsdorp, Penn State University, Department of Entomology, 501 ASI Building, University Park., PA 16802

Jeff Pettis, USDA-ARS Bee Research Laboratory, Beltsville, MD USA 20705

Honey bee colonies have been in decline in the US for over 60 years. Many factors – pesticides, pathogens, parasites, changes in land use, and socio-economic reasons – have likely played a role. Here we critically explore the historical evidence for colony declines, placing the plethora of proposed putative causes into perspective. Focusing on our winter loss surveys of the last 4 years we will examine beekeeper self identified reasons for loss, and evaluate longitudinal data which supports and refutes these claims.

#### Honey bee decline: the need for a COLOSS

<u>Peter Neumann</u>, Swiss Bee Research Centre, Agroscope Liebefeld-Posieux Research Station ALP, Schwarzenburgstrasse 161, CH-3003 Bern, Switzerland

The drivers of honey bee decline are numerous as well as the approaches to study them. In order to eliminate this latter variability, an international standardisation of methods for both monitoring and bee research is urgently required. Moreover, inevitable complex interactions between individual drivers of decline (due to *Varroa destructor*) and the high number of factors exceed research facilities of individual laboratories and entire countries. Therefore, local efforts to limit honey bee decline are probably doomed and internationally coordinated ones are required. For that purpose, the COLOSS network (Prevention of honey bee COlony LOSSes) has been initiated to concert efforts to explain and prevent large scale losses at a global scale. International standards will be developed for both monitoring and research in the form of an online BEE BOOK. The present organisations do not fulfil all required networking aspects. Finally, socioeconomic and political factors are also drivers of decline for beekeeping and coordinated international efforts will certainly be helpful in this regard. Opportunities for sustainable financial support will be discussed. In conclusion, it seems as if only a global network on honey bee health will successfully enable collaborative large scale international efforts to limit honey bee decline.

#### Pollinator Decline: Getting a Handle on its Consequences

#### Keith S. Delaplane, University of Georgia

If it is true that bee decline is a matter of societal priority, then it is important to characterize the extent to which agriculture, and by extension human well-being, depend on bees and other pollinators. It seems the public conversation is frequently over-heated with hyperbole, and as an antidote I propose a conceptual model that provides a starting point for designing experiments on the importance of agricultural pollinators. The relevance of a candidate pollinator is highly contextual – in terms of (a) the reproductive responsiveness of a plant to the flower visitor and (b) the pollen vectoring competence of the visitor. These components, in turn, are each the product of at least two interacting dynamics, the plant's response to the flower visitor being the product of the plant's obligation to out-crossing and the morphological or temporal specializations of its flower which may invite or exclude pollinators, and the pollen vectoring competence of the product of its potency to set fruit (% fruit set per single visit) and its availability (visits per minute). This framework accommodates related considerations such as the global increase in pollinator-dependent crops and the effects of parasites on bee foraging behavior.

#### Disease ecology and honey bee losses

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Massive honeybees losses have been reported in the world, but the specific causes are still unknown. Single factors have not yet explained this global decline, leading to the hypothesis of a multifactorial syndrome. Consequently, testing the integrative effects of more than one stress is an interesting approach to understand colony losses. We tested the effects of an infectious organism and an insecticide on honeybee health. We demonstrated that a synergistic effect between both agents, at concentrations encountered in nature, significantly weakened honeybees. *Nosema* in combination with imidacloprid caused in the short term a higher rate of mortality and energetic stress than either agent alone. A measure of colony level immunity, glucose oxidase activity, was significantly decreased only by the combined treatments, emphasizing their synergistic effects. We demonstrated an effect of *Nosema* on worker pheromone production, which shows that a pheromonal disruption related to different stress could be involved in weakening colonies. We also showed that the quality and diversity of pollen can affect honey bee health. We, thus, provide evidence for integrative effects of different agents and stress on honeybee health, both in the short and long term.

# Impact of environmental toxins: Bioavailability of systemic insecticides in pollen and interactions with bee colonies

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New pesticides are suspected to contribute to the decline of pollinators worldwide, especially where farming is intensive. Systemic insecticides are mainly used at 50-170 g/ha (0.04-0.15 lb/acre) for seed-dressing of crops and for fruit trees. Nicotinoids and pyrazoles are neurotoxicants blocking the central nervous system of target-insects. But very low doses have deleterious effects on bees and colonies. Thus, contamination of pollen is a crucial point for risk assessment.

We developed analytical methods (GC/LC-MS) to quantify such insecticides in pollen from fields. Methods were fully validated and limits of detection were below 1 ng/g. The mean level for imidacloprid was 2-3 ng/g in pollen (corn, sunflower). Fipronil (often with its active sulfone metabolite) was found in 50% of pollen (corn). Here, the mean level was 0.7 ng/g. Thiamethoxam (often with its clothianidin insecticide metabolite) was detected in a very large majority of pollen (corn) at an average level of 0.5 ng/g.

Data should be integrated into risk assessment, not only in terms of acute effects ( $LD_{50}$ ), but also in terms of i) chronic exposure (subletal effects, chronic mortality) and ii) synergies between toxicants, pathogens and parasites; this to better understand weakness (or collapse) of bee colonies.

This research program was equally supported by UE and CNRS.

### **Residues in Honey and Beeswax Caused by Beekeeping Treatments**

<u>Andreas Thrasyvoulou</u>, Karazafiris Emmanouel and Chrysoula Tananaki, Beekeeping Lab., Aristotle University Thessaloniki Greece

We review the acaricide residue levels found in honey and beeswax in Greece and other European countries. We developed and validated in full methods for determining residues in products of the hive. We analyzed 728 samples of honey from the Greek market and the beekeepers and recorded their contamination. We examined also the impact of the application technique in beehives, the effect of different treatment procedures on the presence of residues, the distribution of acaricides within the combs of treated colonies and the fate of acaricide residues in honey.

Furthermore, we collected and analyzed 1320 samples of greek honey during four–year surveillance program for residues of the chemicals 1,4-dichlorobenzene, 1,2 –dibromoethane and naphthalene used to protect honey-bee combs from wax-moth. The presence of pesticide residues in honey, originating from plants in adjacent crops was investigated by transferring colonies in heavy treated areas. Samples from beekeepers are also collected and analyzed for the presence of 25 different active pesticide substances that may find their way to bee products.

Abstracts

#### Pesticides and Pollinators: Assessing Residues and Multiple Interactions in Honey Bees

<u>Chris Mullin</u>, James L. Frazier and Maryann Frazier, Department of Entomology, The Pennsylvania State University, University Park, PA 16802

Honey bees encounter numerous toxic chemicals through hive treatments and on foraging trips to acquire essential pollen and nectar foods. Recently, a broad survey for pesticide residues was conducted in pollen, wax, bee and associated hive samples in proximity to diverse fruit, nut, vegetable and seed cropping sites in US and Canada. We found 121 different pesticides and metabolites up to 214 ppm using a modified QuEChERS method and LC/MS-MS and GC/MS. The fungicide chlorothalonil and both in-hive miticides fluvalinate and coumaphos co-occurred in 47% of wax and pollen samples, while the 3 were combined with a systemic pesticide (e.g. aldicarb, boscalid, imidacloprid, and myclobutanil) in 32% of samples. There were fewer pesticides, particularly systemics, found in bees except for those linked to bee kills. Almost all comb and foundation wax was contaminated with miticides and other pesticides, averaging 8 detections with a high of 39 per sample. Synergistic effects on bees were established for the contact fungicide chlorothalonil in combination with miticides and systemic insecticides and fungicides frequently coincident in pollen. Potential consequences to bee health of pesticide combinations in their food and comb, including pro-systemicides and their toxic degradates, will be discussed.

#### Drug interactions between miticides and fungicides in honey bees (Apis mellifera)

<u>Reed M. Johnson</u>, Department of Entomology, University of Nebraska-Lincoln, Lincoln, NE 68583

The ectoparasitic Varroa mite (Varroa destructor) is one of the most serious pests of honey bees today. Beekeepers often suppress Varroa infestations using pesticides applied at therapuetic doses as anti-parasitic drugs. Three commonly used synthetic miticidal drugs – coumaphos (Checkmite+<sup>TM</sup>), fenpyroximate (Hivastan<sup>TM</sup>) and tau-fluvalinate (Apistan<sup>TM</sup>) – appear to be tolerated through cytochrome P450 (P450) mediated detoxification in bees. Just as metabolic interactions can occur between drugs in humans, drug interactions can also occur between miticides detoxified by P450s in bees. Simultaneous exposure to multiple miticides is likely to occur given the high levels of miticide contamination reported in beeswax. Bees are also likely to be be exposed to high doses of fungicides applied to bee-pollinated crops. Fungicides are generally considered safe for bees and there are few restrictions on their application during bloom. However, some fungicides may affect bees' ability to tolerate miticides. Chlorothalonil (Bravo<sup>TM</sup>), a common fungicide found in pollen stores and wax, decreases bees' tolerance of mitides. Prochloraz, an inhibitor of cytochrome P450 activity in fungi, increases the toxicity of coumaphos, fenpyroximate, and greatly increased the toxicity of tau-fluvalinate. Based on these findings it would be prudent for beekeepers to avoid repeated use of P450-interacting miticides and to avoid using these miticides when bees are likely to come into contact with these or other potentially interacting fungicides.

# The effects of fungicides on the diversity of microbes in stored pollen and the physiological repercussions on worker and queen honey bees

<u>Gloria DeGrandi-Hoffman</u>, Kirk Anderson, Mark Carroll, Bruce Eckholm, and Diana Sammataro, Carl Hayden Bee Research Center, USDA-ARS, 2000 East Allen Road, Tucson, AZ 85719

Honey bee colonies harbor a wide range of microbes, many of which play vital roles in the preservation and digestion of pollen. Bees store pollen in comb cells and it is there that the pollen is fermented through the action of microbes and converted into bee bread. There are numerous bacteria and fungi present in bee bread that pre-digest the pollen grains and make the nutrients inside more accessible to the bees. The microbes also supply essential nutrients through their metabolic processes. The action of symbiotic microbes might be compromised if they are exposed to pollen contaminated with fungicides. To test this, we collected pollen from colonies in almond orchards during pollination. In this pollen we detected >6000 ppb of Boscalid, 1700 ppb of Pyraclostrobin, >2800 ppb of Propiconazole, and >12500ppb of Iprodione. Bee bread sampled from colonies in the same orchard had >9000 ppb of Boscalid, >2000ppb of Pyraclostrobin, and 7700 ppb of Iprodione. Propiconazole was not detected in the bee bread samples. From our pilot studies, we found that bee bread made from pollen contaminated with fungicides has a lower diversity of microbes compared with bee bread made from uncontaminated pollen. In our current work, we are investigating the effects of the reduction in microbial diversity on the ability of bees to process pollen into worker jelly. Whether there are effects on the ability of the queen to lay eggs and generate volatile signals communicating her egg laying activity also is being determined.

## Sub-lethal pesticide exposure in honey bees: Chronic pesticide exposure at the colony level results in increased susceptibility of workers to pathogen infection

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Dennis vanEngelsdorp, Penn State University, Department of Entomology, University Park, PA

Josephine Johnson, University of Maryland Baltimore, Department of Toxicology, Baltimore, MD

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Global pollinator declines have been attributed to habitat destruction, pesticide use and climate change or some combination of these factors and managed honey bees, *Apis mellifera*, are part of worldwide pollinator declines. We exposed honey bee colonies during three brood generations to sub-lethal doses of a widely used pesticide, imidacloprid, and then subsequently challenged newly-emerged bees with the gut parasite, *Nosema ceranae*. The pesticide dosages used were below levels that are thought to cause harm to honey bees. We demonstrated an increase in pathogen growth within individual bees reared in colonies exposed to the pesticide. Interactions between pesticides and pathogens could be a major contributor to increased mortality of honey bee colonies and other pollinators worldwide. Other sub-lethal pesticides studies will also be discussed in trying to understand the role of pesticides in declining bee health.

### Contingency and the outcome of host-parasite interactions

Dr. Ben M. Sadd, ETH Zürich, Institute of Integrative Biology (IBZ), Universitätsstrasse 16, 8092 Zürich, Switzerland, <u>ben.sadd@env.ethz.ch</u>

The environment encountered by organisms, whether it is the abiotic or biotic environment, is rarely likely to be stable over both spatial and temporal scales. This variation has the potential to have profound effects on the immune defence of individuals, and in turn their resistance to parasite attack. In bumblebees, contingency can have a large role to play in determining levels of immune defence and the outcome of host-parasite interactions. Examples include the presence of specific immune priming following pathogen exposure, trans-generational immunity that is dependent on a mother's immune experience, and condition dependence in the outcome of natural host-parasite interactions. The occurrence of these environmental dependent effects in bumblebees has implications for the understanding of immune defence in social insects, and the role that environmental heterogeneity has in the determining the outcome and evolutionary dynamics of host-parasite associations.

#### **Propolis and Social Immunity in Honey Bee Colonies**

Marla Spivak and Mike Simone-Finstrom, University of Minnesota

Social immunity describes the collective physiological and behavioral defenses of individuals against pathogens and parasites within a social insect colony. Behavioral defenses, or antiseptic behaviors, include grooming, hygienic behavior, undertaking, avoidance, glandular secretions, and use of resins in the nest. Resins are complex plant secretions with diverse antimicrobial properties. The harvesting and incorporation of antimicrobial compounds in social nest architecture is a relatively unexplored behavior. In natural nest cavities such as tree boles, honey bees deposit resin (called propolis) along the inner walls, forming a protective envelope around the colony. Our research is revealing the benefits of the propolis envelope to honey bees' individual cellular and humoral immunity, and thus to colony-level social immunity. Propolis reduces in-hive microbes and thus lowers a bee's physiological investment in its immune system. We also are exploring if propolis has more direct effects on bee health by testing its activity against the parasitic mite, *Varroa destructor*, in field colonies, and against bee viruses in a laboratory assay.

# The impact of a warmer climate on disease transmission in a solitary bee: immune response isn't everything

Rosalind James, USDA-ARS Pollinating Insects Research Unit, Logan, UT

Junhuan Xu, Dept. Biology, Utah State University, Logan, UT

Amphibian populations crashed during the 1990s due to a fungal disease that spread across the world, probably aided by global warming. Could diseases coupled with climate change also play a role in bee declines? The fungus *Ascosphaera aggregata* causes chalkbrood outbreaks in the alfalfa leafcutting bee (*Megachile rotundata*) in the U. S., but is more readily controlled in Canada. Bee larvae are most susceptible to this disease at 25-30°C, yet immune response genes typically associated with fungal invasions are highly active at these temperatures. Other genes may play a more important role in the susceptibility seen at these temperatures, such as those associated with metabolism, cell communication, and protein synthesis. In addition to temperature-sensitive immune response systems, we found that pathogen transmission was greatly increased by the onset of bivoltinism in this bee, and the proportion of the population that is bivoltine (vs. univoltine) typically increases with warmer climates. Thus climate affects not only host immune response, but also the transmission capabilities of the pathogen.

# How monitoring contributes to understanding honey bee health and colony losses: a summary from a decade of experience

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Abstracts

#### The Importance of Bee Health for the African Pollinator Initiative: the *icipe* contribution

<u>Baldwyn Torto</u>, International Centre of Insect Physiology and Ecology (icipe), P.O. Box 30772-00100, Nairobi, Kenya

Pollinators are invaluable to global food production and ecological balance of nature. Their conservation and management are therefore of the utmost importance in a changing global climate. At *icipe*, our pollinator programme focuses on research on honeybee interactions with pests and the effects of these interactions on bee health. In Kenya surveys of honeybee colonies have revealed the presence of four species of coleopteran pests, including three scarabs and one nitidulid. The distribution of one of these scarabs Oplostomus haroldi and nitidulid Aethina tumida within honeybee colonies and behavioral interactions of these two pests with bees have been studied. More than 90% of O. haroldi and A. tumida were found in the hot, wet and humid (coastal) area of Kenya. While A. tumida was found mostly on the bottom board of the hives, O. haroldi was found on the frames. Because of the size of O. haroldi, larger than bees, this allows the beetle to access food resources in a greater area and to interact more frequently with bees in the colony than A. tumida. In laboratory bioassays, adults of both O. haroldi and A. tumida responded strongly to bee volatiles and fermented pollen. However, these two beetle species complete their life cycles in different substrates, O. haroldi in cow dung pads, with A. tumida in soil. These findings have important implications for bee health, which will be discussed in this presentation.

## Diversity patterns and crop pollination services by bees in a Mediterranean and an arid agro-ecosystem

<u>Yael Mandelik</u>, Dept. of Entomology, Faculty of Agriculture, Food and Environment, The Hebrew University, POB 12, Rehovot 76100, Israel

Bees provide vital pollination services to crops and wild plants. Modern agriculture relies on a few commercially grown bee species for pollination, mainly honey bees and bumblebees, even though wild bees can provide significant pollination services. The reliance on honey bees as the main agriculture pollinator is inherently risky especially in light of sharp declines in their colonies across the world, including Israel. Furthermore, honey bees can negatively affect the wild bee fauna. Little is known about the potential contribution of wild bee communities to crop pollination in the different ecosystems of Israel and the how it is affected by land-use practices. In two study systems in Israel, a Mediterranean and an arid agro-ecosystems, abundant and diverse wild bee communities were found in the open landscape surrounding agriculture fields and orchards. In both study systems the activity of wild bees in the agriculture fields and orchards was significantly affected by land-use practices at the field and landscape scales. In the arid ecosystem we found that wild bees were the main visitor to watermelon flowers, but only under traditional (extensive) agricultural practices. Under intensive agriculture regime, commercially grown honey bees were almost the sole visitor to crops. In the Mediterranean system we found that the distance from the surrounding open landscape and the proportion of natural vs. agricultural land surrounding agriculture fields and orchards significantly affects wild bee abundance and activity on crop flowers. Nevertheless, commercially grown honey bees were the main visitor in all the Mediterranean sites studied. No correlation was found between visitation rates of honey bees vs. wild bees. Overall, we found that wild bee communities can significantly contribute to crop pollination in both the Mediterranean and the arid agroecosystems of Israel, but their contribution is greatly affected by agriculture practices and surrounding land-use. Under the intensive agriculture practices common in many parts of Israel, the contribution of wild bees to crop pollination, especially in the arid zone, might be limited.

# Native pollinator diversity, abundance, and forage preference in native plant hedgerow restorations

Lora Morandin and Claire Kremen, University of California, Berkeley, Department of Environmental Science Policy and Management, Berkeley, CA

Intense agricultural systems are lacking in pollinators and hence pollination services to crops. Restoration of unmanaged, weedy field edges with native shrubs and forbs is a realistic way, in working farmlands, of increasing biodiversity, pollinators, and possibly pollination services. While this is a practical way of creating semi-natural habitat in intense agricultural landscapes, few studies have examined whether restored hedgerows are able to increase pollinator abundance, diversity, and crop services, and how native bees are using resources in hedgerow restorations. We compared native bee abundance and diversity in native plant hedgerow restorations with paired, relatively unmanaged weedy edges, in Northern California. In mature and younger restoration sites, we assessed native and honey bee preference for native and exotic plants. In addition, we have conducted preliminary studies assessing if hedgerows are acting as a source for pollinators and pollination services to adjacent crops or if they are simply concentrating existing populations of pollinators from the surrounding landscape. Knowledge of how native bees are using resources in hedgerow restorations and information on the impact of restoration on pollinator communities will advance our ability to enhance pollination services in intense agricultural landscapes.

#### A threat to beneficial-insect habitat from new herbicide programs

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New herbicide technologies may pose a direct threat to floristic biodiversity in agroecosystems and an indirect threat to the pollinator communities reliant on non-crop habitats. Widespread adoption of glyphosate-tolerant soybeans has increased the number of weedy species resistant to this herbicide. To address this problem, the industry is commercializing soybeans that are resistant to both glyphosate and dicamba. Dicamba, a broadleaf herbicide, is highly volatile and extremely active on many broadleaf plant species, including both crop and non-crop species. The high risk of injuring soybeans not carrying the dicamba trait is likely to drive adoption of these new cultivars. Extensive use of dicamba in agroecosystems has high potential to stress plant communities in field edges and nearby non-crop habitats, significantly diminishing floristic biodiversity and associated arthropod communities, including pollinators and the services they provide.

# Pollination and pollinators for wildflower seed farming and post-fire wildland rehabilitation

James H. Cane, USDA-ARS Pollinating Insect Research Unit, Utah State University, Logan, UT 84322 USA

Public land managers in the US Intermountain West buy seed of grasses and shrubs, and more recently perennial native wildflowers, to rehabilitate millions of acres of sage-steppe, pinyon-juniper and aspen communities degraded by altered wildfire regimes, poor grazing practices, and exotic invasives. For 15 candidate, prevalent wildflower species starting to be farmed to satisfy this anticipated 100+ ton annual seed market, we found that most all need pollinators, typically bees. A minority can be pollinated with currently managed bees (*Apis, Megachile rotundata*), others by managed populations of cavity-nesting native *Osmia* bees now under development. The remainder will require simple on-farm stewardship of wild species where practical (esp. *Bombus*, but also some specialists like *Diadasia* for globemallows). In the wild, some floral guilds are dominated by oligolectic specialists (e.g. *Andrena, Osmia, Diadasia*), others by rich diversities of non-social ground-nesting generalists, especially *Osmia* (1-34 spp) and *Eucera*. We are finding that these guilds survive wildfires – mostly a function of limited heat penetration into their nesting soils – with the result that recovering floras are amply visited, provided that there is natural or provided bloom in the year that follows burning.

#### Strategies to Improve Native Pollinator Biodiversity and Ecosystems Services in Orchards

David Biddinger & Ed Rajotte, Penn State University, University Park, PA

James Gillis - USDA-NRCS, Adams County

Tim Leslie - Long Island University

Of the approximately 450 species of bees found in Pennsylvania, over 150 species have been found in Pennsylvania fruit orchards, with at least 50 species contributing to fruit pollination. A 3-fold increase in honey bee hive rental fees has forced fruit growers to utilize hives at lower densities than the 1-2 hives/acre recommended by Penn State University or to look to alternative pollinators. Efforts to quantify the contribution of non-*Apis* pollen bees to orchard crop pollination and to conserve and manage established populations of *Osmia cornifrons* and *O. lignaria* will be discussed. Results of a 3-year ecotoxicology study examining the effects of reduced risk IPM programs on bee and syrphid biodiversity and abundance will be presented. The development and grower implementation of USDA-NRCS pollinator conservation programs with Penn State University and the Xerces Society will also be discussed.

### U.S. Fish and Wildlife Service Pollinator Conservation and Education Activities

Dolores A. Savignano, U.S. Fish and Wildlife Service

The mission of the U.S. Fish and Wildlife Service is working with others to conserve, protect, and enhance fish, wildlife, plants, and their habitats for the continuing benefit of the American people. Activities are focused on conserving the lands and resources that are part of the National Wildlife Refuge System, conserving and managing migratory birds, recovering threatened and endangered species, working with others on landscape conservation cooperatives, and connecting children with nature. Pollinators are included in these focus areas. This paper/poster will provide a sampling of Fish and Wildlife pollinator conservation activities, such as: land management practices that promote pollinators, bee inventories, investigation of herbicide effects on rare butterflies, participation in the Monarch Joint Venture, recovery actions for listed pollinators, and technical assistance to land owners conserving pollinator habitat. I also will provide a sampling of outreach activities, such as a kid's clubhouse (Neighborhood Explorers), pollinator demonstration gardens, conferences and training, website, and a Museum Exhibit, done to raise awareness of the need for and methods of pollinator conservation.

#### Bee Pollinators of Southwest Virginia Crops (Video for Poster Session)

Nancy Adamson, Richard Fell, Donald Mullins, Entomology Dept., 216A Price Hall (0319), Blacksburg, VA 24061

Video montage of bee behavior (primarily native bees) while pollinating peach, apple, blueberry, blackberry, and various types of cucurbits (squash, melon, cucumber) and nesting. Highlights diversity (*Apis, Andrena, Bombus, Ceratina, Eucera, Melissodes, Osmia, Peponapis, Triepeolus, Xylocopa,* and a few sweat bees), buzz pollination, mating of squash bees, nectar robbing by carpenter bees, the change in behavior of bumble bees through the growing season (skittish queens in early spring to relaxed, probably workers, in late summer), pollen-packing, and grooming behaviors. Also shows Dr. T'ai Roulston marking a tomatillo specialist bee, *Colletes latitarsis*, after cooling the bee on ice, a technique that allows for capture, release, and recapture, without harm to the bees. One clip shows worker honey bees forcing drones (males) out of the hive at the end of the season, somewhat like thoughtful advisors might push their graduate students out when it is time.

#### Sustainable Landscape Designs Utilizing Native Species to Increase Pollinator Habitats

Pamela Bailey, Research Botanist, US Army Engineer Research and Development Center Mary Anderson, Natural Infrastructure Manager, Air Force Space Command, Asset Management

Plant-pollinator relationships are one of the keystones of healthy ecosystems. Loss of species from within networks of co-dependent plants and pollinators can exacerbate decreases in pollination services by triggering vortexes of linked extinctions. Due to the rapid decline in pollinators and loss of biodiversity of native habitats, it is critical to provide islands and linkages of native species habitats. The use of native plant species in military installation landscaping can provide our native pollinators with appropriate habitat on military lands. Our focus in this project was to provide a booklet entitled "Sustainable Landscape Designs Utilizing Native Species to Increase Pollinator Habitats, A Design Manual" for DOD land managers as a tool. Resource managers and maintenance managers can use it as a guide in establishing planting areas as habitat for our native pollinator species, therefore increasing pollinator interactions.

This project was funded by the Department of Defense Legacy program.

# A Comparison of Natural and Manmade Fragmentation Effects within Three Pollination Networks of *Erigeron*

<u>Pamela Bailey</u>, Dr. Richard Lance, and Denise Lindsay, U.S. Army Engineer Research and Development Center, Environmental Laboratory, Vicksburg, MS

The loss and fragmentation of natural habitats by human activities are pervasive in terrestrial ecosystems and the main cause behind current biodiversity loss. Understanding how fragmentation affects pollination mechanisms and gene flow in plant species is a critical issue to further limit the "island effect" with which many of our military bases are challenged. This can have wide application for resource management on military bases and other refuges that are islands, surrounded by growth and development. Few studies have investigated how plants evolve with pollination networks in fragmented habitats - one of the most influential interactions affecting plant demography and genetic viability. This on-going study, funded by the Basic Army Research Program, is a pioneering effort to study network properties of pollination networks in relationship to a genetic analysis for 3 species of Erigeron affected by fragmentation. We will be applying network science approaches to understanding how plant species subjected to fragmented habitat have adapted pollination network strategies to ensure adequate gene flow. Our study will focus on three species of *Erigeron* (Fleabanes); one endemic plant adapted to naturally sparse cliff wall habitat (*E. lemmonii*) compared to two others (*E. arisolius and E. neomexicanus*) adapted to more diverse habitat conditions.

#### The Conservation Status of Eastern North American Bumble Bees

Sheila R. Colla and Laurence Packer, York University, Toronto, Ontario, Canada

Pollinator declines have recently become a significant conservation issue globally. The potential cascading effects of these declines in agriculture and native ecosystems has brought much attention from the public, policy makers and the media. However, in North America our baseline data on native pollinator populations has thus far been lacking. Here, I will summarize what is known on the decline of *Bombus affinis (*The rusty-patched bumble bee) using museum records, recent surveys and GIS analyses. This species was previously one of the most common bees throughout eastern North America and is currently up for listing under Canada's Species At Rick Act due to a dramatic decline over the past 20 years. The decline of this species is likely due to multiple stressors including pathogen spillover, habitat loss and climate change. Recommendations for the conservation of this and other bumble bee species based on these findings will be discussed.

#### Mutualistic Relationship Between Small Hive Beetle and Associated Yeast

Tracy Conklin, Penn State University, University Park PA

The small hive beetle (Aethina tumida) is a pest of honeybee hives introduced to the United States from Africa in 1996. Recently, researchers have identified a yeast (Kodamaea ohmeri) associated with the small hive beetle which may serve as a host-finding cue, emitting honeybee alarm-pheromone-like components when growing on pollen to attract the beetles. We report the characterization of the relationship between the beetle and the yeast. Experimental evidence corroborates the hypothesis that the relationship is mutualistic.

#### **Supporting Scientific Observation of Pollination in the Garden**

#### Catherine Eberbach, Rutgers University

How do children make the transition from *seeing* the natural world to *scientifically observing* the natural world? This study explored how differences in parent disciplinary knowledge of pollination and parent conversational style impact children's experience observing biological phenomena during shared informal learning. 79 parent-child pairs with children aged 6-10 participated in a controlled study in which half the parents used their natural conversational style and the other half were trained to use four conversational strategies during family observations of pollination in a botanical garden. Parents were also assigned to high and low knowledge groups according to their knowledge of pollination biology. Findings suggest that parents who received training used the conversational strategies more than parents who used their natural conversational style. Parents and children who knew more about pollination at the start of the study exhibited higher levels of disciplinary talk in the garden. However, the use of the conversational strategies also increased the amount of disciplinary talk in the garden, independent of what families knew about pollination. The extent to which families engaged in disciplinary talk in the garden predicted significant variance in what children learned from the experience.

# Implications of Climate-mediated Changes in Nectar Flow Phenology in eastern N. America for Honey Bee Health, Behavior, and Management

<u>Wayne E. Esaias</u>, Robert Wolfe, NASA Goddard Space Flight Center, Greenbelt MD 20771 Jaime Nickeson, Peter Ma, GSFC/Sigma Space, Greenbelt, MD 20771

Catherine Jarnevich, USGS, National Institute for Invasive Species Science, Fort Collins, CO

Using scale hive records together with satellite vegetation observations, species distribution models, and nectar plant distributions, we show evidence for relative shifts in spring nectar flows up to 40-60 days between the northern and southern regions of the eastern U. S. consistent with decadal scale climate, satellite vegetation trends, and blooming data. Nectar is becoming available later in the south, and earlier in the north. These relations can serve to assess impacts of potential future climatic change on bee forage and honey bee nutritional status, and for native pollinators. We present evidence that climate-mediated nectar flow phenology in the southeast is a controlling factor in the distribution of the Africanized Honey Bee, due to a mismatch of its behavior and nectar availability.

These results have several implications related to honey bee health and regional management to ameliorate climate impacts. These relate to potential infringement of feral AHB in southern queen breeding regions; timeliness of availability of southern EHB queens to replace northern colonies lost to disease; risks to nutritional status of bees in late summer due to longer dearth and effects on overwintering; genetic diversity; and the need for re-establishing a more extensive and permanent bee forage monitoring network.

## Native bees respond to landscape composition and configuration in a cranberry agroecosystem (Wisconsin, USA)

Hannah R. Gaines and Claudio Gratton, University of Wisconsin, Madison

Native bees are important crop pollinators and have been shown to provide the majority of pollination services in some agricultural systems. Habitat loss, changes in land use, and intensive agricultural practices may be causing native bee populations to decline. The purpose of this study is to determine if and how native bees respond to the composition and configuration of the surrounding landscape in Wisconsin cranberry bogs. To address our hypotheses we pan trapped bees in 15 commercial cranberry bogs in central Wisconsin. Sites were chosen to cover a gradient of surrounding landscape from 15-82% woodland and 10-76% agriculture within one kilometer. We found that native bee abundance was negatively correlated with agriculture (i.e. cranberry) and positively correlated to forest edge density in the surrounding landscape. Species richness was also negatively correlated with cranberry, positively correlated with forest cover, and positively correlated with forest edge density in the surrounding landscape. These results show that native bees respond to the type (e.g., forest), availability (i.e. percent cover) and distribution (as measured by edge density) of habitat on the landscape. This suggests that both composition and configuration are important factors affecting bee abundance and richness.

### Customized Native Bee Habitat Assessments for Delaware Farms: a Model Approach

<u>Heather Harmon Disque</u>, Faith B. Kuehn, and Bonnie MacCulloch, Delaware Department of Agriculture

The assessment framework is a guidance tool designed to analyze an individual farm and its farming practices to develop a recommended set of improvements that will benefit bees and other insect pollinators. With background information such as current farm practices, land use, plant diversity, native bee abundance and diversity, and crop pollination requirements, a current state and needs profile was created for each individual farm.

A three-tiered approach to improved bee conservation was developed. Tier 1 offered simple changes, such as maintaining existing native vegetation and improved control of invasive plants. Basic low-cost improvements such as the provision of nesting habitats and the allowance of untilled areas near crops were suggested in tier 2. Tier 3 improvements involved a higher level of investment or time commitment, such as the installation of native wildflower and shrub buffer strips and the reduction or modification of insecticide use.

The assessment reports were discussed with each grower in a round-table meeting. Following this meeting, a Pollinator Habitat Improvement contract between the Department of Agriculture and the grower was drafted and signed. This document detailed at least three changes that the grower was willing to make in the coming year.

# Native Bee Diversity in Fragmented Landscapes is Influenced by Local and Landscape Features

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Habitat fragmentation of tall grass prairies is thought to reduce floral and nesting resources available to native, solitary bees with the possible consequence of reducing diversity at small remnants of native prairie habitat. We have examined bee diversity in large, undisturbed prairies preserves (>30 ha), small, high quality remnants (<5 ha), ruderal grasslands, and naturally small, high quality hill prairies in Iowa. Our results show that regardless of size, high quality prairies attract equivalent diversity of bees regardless of size, but that diversity and abundance at sites with poor resources also have relatively poor bee richness. Analysis of landscapes surrounding preserves indicate that floral resources in the landscape have a significant effect on bee diversity with poor landscapes comprised of row-crop agriculture and low floral resources resulting in low diversity of native bees at prairie remnants. Landscape effects are seen up to 2 km from the center of prairie remnants and grasslands are the most important landscape element influencing diversity at a site. Our results indicate that preservation of bee communities at small prairie remnants will need to address changes in landscapes surrounding sites as well as preservation of local on site resources.

## Effects of the pollutant selenate on the gustatory and foraging behaviors of the honey bee (*Apis mellifera*): Implications for plant-pollinator interactions

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Brian H. Smith and Julie A. Mustard, Arizona State University, School of Life Sciences, Tempe, AZ 85287

Selenium (Se) from agricultural runoff has contaminated some areas of the central valley California where insect pollination can be critical to the functioning of both agricultural and natural ecosystems, yet we know very little about how soil-borne pollutants such as Se can impact pollinators. In greenhouse studies using plants, a phytoremediator species (*Brassica juncea*) as well as a weed species (*Raphanus sativus*) accumulated up to 900  $\mu$ g Se g<sup>-1</sup> in floral tissues. In the laboratory, we used proboscis extension response (PER) to test whether honey bees (*Apis mellifera*) can detect Se. Antennae and proboscises were stimulated with 1) selenate in sucrose solution, 2) sucrose alone (control). There were no significant differences in PER or total consumption between treatments, suggesting bees may not be able to detect Se. We conducted a semi-field experiment using Se-treated radish to quantify honey bee foraging visits. We also measured Se concentrations in plant tissues, bee bodies and pollen sacs. If pollinators cannot detect and avoid toxic Se compounds in the plant tissues they are foraging upon, they may suffer similar adverse effects to those reported in other insect guilds. Alternatively, Se is a micronutrient when ingested in low quantities and may be a beneficial antioxidant to pollinators.
# Genomic analysis of the effects of *Nosema apis* infection on honey bee (*Apis mellifera*) workers

<u>Holly L. Holt<sup>a</sup></u>, and Christina M. Grozinger, Department of Entomology, Penn State University, State College, PA, USA

Katherine Aronstein, Honey Bee Research Unit, USDA, Welasco, TX, USA

Pathogens represent a major threat to pollinator populations. Globally, honey bees are declining, and loss of their pollination services would have severe consequences for both agricultural and natural landscapes. *Nosema apis*, a microsporidian pathogen of honey bees, causes commercial losses due to reduced hive productivity. *N. apis* spores are spread via the fecal/oral route and infections are initiated in the midgut. Infected bees suffer from diarrhea, mature at behaviorally accelerated rates and have shortened life spans. Using microarrays, we characterized gene expression in worker bees with *N. apis* infections to better understand molecular pathways underlying the physiological and behavioral symptoms associated with infection. We compared midgut gene expression in healthy and infected bees at 2 days post-infection and fat body gene expression at 2 and 7 days post-infection. We hypothesize that genes regulating metabolism, behavioral maturation and immunity will differ between control and infected bees. This study will contribute to our basic understanding of bee immunology and lay the groundwork for future studies of the impact of *Nosema apis* and an additional pathogenic microsporidian, *Nosema ceranae*, at the molecular, physiological, behavioral and colony levels.

# Potential pollinator exposure to imidacloprid in red maple trees treated for Asian longhorned beetle

Josephine D. Johnson, doctoral candidate at the University of Maryland, Baltimore. My research is overseen by Jeff Pettis, Ph.D., USDA/ARS Bee Research Lab, Beltsville, MD

Animal & Plant Health Inspection Service (APHIS) is working to eradicate the infestation of the invasive Asian Long Horned Beetle (*Anoplophora glabripennis*) in New York City that began in 1996. To protect those tree species that are beetle susceptible, one of which is red maple (*Acer rubrum*), trees are treated by soil or trunk injection with imidacloprid. This systemic neonicotinoid pesticide may disrupt foraging and other normal behaviors in honeybees as it has the potential to be found in pollen and nectar of treated trees. Samples of red maple flowers and leaves from treated trees are being analyzed for imidacloprid and compared to control trees nearby. Since honey bees favor the nectar and pollen of red maples in early spring, hives were placed on the ground during the red maple bloom and returning foragers and pollen trapped at the hive entrance are analyzed for imidacloprid in an effort to determine if the pesticide is transmitted to pollinating honey bees. The data from two years of a three year study will be presented.

# Impact of immune stress on viral incidence and evolution, including generation of quasispecies of Deformed Wing Virus in newly emerged honeybees (Apis mellifera)

<u>Abby L. Kalkstein</u>, Intercollege Graduate Program in Genetics, Penn State University, University Park, PA and The Department of Entomology, Penn State University, University Park, PA

Diana Cox-Foster, The Department of Entomology, Penn State University, University Park, PA

Viral epidemics, caused by single stranded positive sense RNA viruses belonging to the order *Picornavirales*, have been found in the honeybees *Apis mellifera* since the first honeybee viral symptoms were described in 1913. Deformed wing virus (DWV), an Iflavirus, is a serious pathogen of honeybees, resulting in decreased survivorship in newly emerged honeybees and wing deformity if the bee has been parasitized by the mite *Varroa destructor*. This research addresses the impact of immune stress on viral incidence and intra-host diversity of DWV in newly emerged worker bees. RT-PCR confirmed our previous finding that varroa parasitization was linked to high levels of DWV. Eight-hours after challenge with heat-killed *Escherichia coli*, the viral titers of mite-parasitized bees is higher than mite-free bees. Preliminary evidence indicated an increase in viral diversity within bees infected with varroa mites and a phylogenetic distinction in the DWV of the treatment groups. Our research indicates that the interaction of immune incompetency in bee hosts (via mite parasitization), DWV infection and microbial challenge leads increased in viral diversity

#### **Bee Friendly Farming**

Kathy Kellison, Executive Director, Partners for Sustainable Pollination (PFSP)

The lack of natural forage for bees is commonly understood to be a limiting factor in bee health. This can be address through bee friendly conservation practices, including planting for bees. To encourage more forage for bees and other pollinators, the "Bee Friendly Farming<sup>TM</sup>" (BFF) initiative empowers caring citizens from all walks of life to help—including farmers and ranchers, school groups, local governments, nonprofits, businesses and beekeepers—at all scales of landscapes—including farms, orchards, ranches, gardens, schools, parks and other private and public landscapes. 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. Interested participants can self-certify on line, with a \$25 fee used to augment cost-share assistance provided to farmers and ranchers through USDA and other programs. The questionnaire can be accessed at http://www.pfspbees.org/selfcert.htm. PFSP is an organization dedicated to improving the health of honey bees in pollination services, with a primary focus on identifying, increasing, and enhancing bee forage in the U.S. http://www.pfspbees.org.

# Pollination services provided to small and large highbush blueberry fields by wild and managed bees

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Pollination of many crops is achieved by a combination of indigenous native bees and by managed bees brought to fields during bloom. To pollinate fields of highbush blueberry, *Vaccinium corymbosum*, large commercial farms rent colonies of *Apis mellifera* honey bees whereas most small isolated blueberry fields are not stocked with bees and are typically dependent on native pollinators. We measured bee communities and components of crop yield in blueberry farms of these contrasting types to determine the relative role of wild bees and honey bees across the Michigan blueberry industry.

Observational samples in large commercial fields and small isolated fields during bloom revealed contrasting communities of pollinators in which large fields were dominated by *A*. *mellifera* (over 95% of bees) while more than 50% of bees in the small fields were wild species. By combining berry weight increases attributable to honey bees and wild bees in blueberry fields with measurements of the bee community adjusted for pollinator efficiency, we calculated the relative contributions of honey bees and wild bees to pollination of Michigan blueberries. We estimate that wild bees provide 82% of the pollination in small fields but only 12% of the total pollination services across this system.

# Establishing and maintaining productive package bee colonies using synthetic brood pheromone (SuperBoost).

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We evaluated a year-long treatment regime using synthetic, 10-component, honeybee brood pheromone (SuperBoost) on the productivity and vigor of package bee colonies. Fifty nine newly-established colonies treated three times with SuperBoost at 5 week intervals starting 30 April 2009 were compared with 54 untreated control colonies. Both groups were used in commercial pollination of blueberries, raspberries and cranberries. Treated colonies produced more than twice as much honey as controls. Net survival of treated and control colonies by fall of 2009 was 79% and 73%, respectively, and treated colonies had 30% more adults than controls. Of the surviving treated colonies, 21 were treated with SuperBoost during a four-week long fall feeding period using pollen substitute, and again during a 6-week spring build-up feeding period started 25 February 2010. Of these 76% survived, and produced 8 splits, for a net colony gain of 22%. In contrast, 40% of 15 untreated control colonies survived and produced one split, for a net colony loss of 53%. We conclude that SuperBoost helped improve the productivity and survival of package bee colonies, and hypothesize that similar results could be achieved with established colonies.

# Long-Term Effects of Honey Bee Queen (Hymenoptera: APIS Melliffera L.) Insemination Volume on Queen-Worker Interactions

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As the only reproductive female, the health of the honey bee queen is critical for colony fitness. The queen produces a pheromone blend that maintains colony organization and regulates worker physiology and behavior. Previous studies indicate that the insemination quantity alters queen mandibular pheromone production in recently inseminated queens, thereby modulating worker attraction to the queen. Here we show that the queen insemination volume significantly affects the survival of laying queens in a colony, worker retinue response, and the number of queen cups and cells built by workers in preparation for potential supersedure. Workers exposed to low-volume inseminated queens initiated production of queen-like esters in their Dufour's glands, which typically occurs in queenless, laying workers. Our results suggest that the queen

insemination volume has long term effects on worker behavior and physiology, which impacts queen longevity and ultimately could affect the colony health and productivity.

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#### Effects of landscape and planting date on native pollinators in pickling cucumber

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The negative impacts on ecosystem services from agricultural intensification and the honey bee losses from Colony Collapse Disorder have increased awareness about the pollination services provided by native bees. Recent studies have confirmed the importance of incidental pollination services by native bees in several crops. There is a scarcity of research regarding native bee communities in cucumber, an open-pollinated vine crop that requires multiple insect visits for marketable fruit set, and how bees are impacted by adjoining landscapes. We examined if proximity of cucumber fields to natural areas and if planting date affected abundance and diversity of native pollinators. Native pollinator communities were sampled on fair weather days through pan traps and sweep netting on open flowers. Over 700 bees were collected, and native bee abundance was highest at sites surrounded by a high proportion of non-crop, natural areas. Ongoing research will investigate if a stronger relationship exists between planting date and bee abundance and if native bees are the dominant visitors to flowers under certain environmental conditions. Once the factors affecting native bees are known, efforts should focus on conserving and enhancing the native bee communities with a more stable supply of floral resources and nesting sites.

#### Current and Future Pollination Services Provided by Native Bees in Vegetable Crops

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The European honey bee, *Apis melifera*, is an important pollinator of many vegetable crops, but several issues including colony collapse disorder have negatively affected the US honey bee industry. Consequently, hive rental fees have doubled over the past three years and vegetable growers want to reduce costs by taking advantage of pollination services from native bees that commonly occur near their fields. Yet, it is not known how frequently native bees visit vegetable fields, especially large fields, nor it is known how efficient they may be in pollinating vegetable crops relative to *A. melifera*. In New York in 2008 and 2009, these questions were addressed using pumpkin as a model crop. The common eastern bumble bee, *Bombus impatiens*, and the squash bee, *Peponapis pruinosa*, were the most prevalent native bees visiting pumpkin flowers. Both species visited flowers in large fields equally well, but *B. impatiens* was distributed throughout the field, whereas *P. pruinosa* tended to concentrate along field edges. *B. impatiens* was a much better pollinator of pumpkin than *P. pruinosa* and was better than *A. melifera* in one of two years. The role of *B. impatiens* in future pollination services of vegetable crops is discussed.

#### A framework for comparing pollinator performance: effectiveness and efficiency

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Measuring pollinator performance has become increasingly important with emerging needs for risk assessment in conservation and sustainable agriculture that require multi-year and multi-site comparisons across studies. However, comparing pollinator performance across studies is difficult because of the diversity of concepts and disparate methods in use. Two different assessment concepts predominate: the first estimates stigmatic pollen deposition and pollinator behavior parameters, while the second estimates the pollinator's contribution to plant reproductive success. Both concepts include a number of parameters named under a diversity of names and synonyms. However, these concepts are overlapping because pollen deposition success is the most frequently used proxy for assessing the pollinator's contribution to plant reproductive success. We analyzed the diverse concepts and methods in the context of a new proposed conceptual framework with a modular approach based on pollen deposition, visit frequency, and contribution to seed set relative to the plant's maximum female reproductive potential. A system of equations is proposed to optimize the balance between idealized theoretical concepts and practical operational methods. Our framework permits comparisons over a range of floral phenotypes, and spatial and temporal scales, because scaling up is based on the same fundamental unit of analysis, the single visit.

# The effect of distance from arable field margin in driving insect pollination and pest control in faba beans in England

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Edge habitats surrounding intensively managed agricultural fields are of considerable conservation value for insect communities, which provide key ecosystem services such as pollination and natural pest control to crops. We examine how plant position within a faba bean field affects the amount of pollination and natural pest control it receives. Sampling was done at increasing distances from the field edge (wild flower rich field margin and hedgerow) along transects in a typical bean crop in South East England. Results indicate significant declines in pollinator abundance and species richness with increasing distance from field edge, emphasizing the importance of non cropped areas outside of the agricultural fields as sources of pollinators to crops. Overall, insect pest predators'(mainly coccinelid beetles) abundance decreased significantly with an increase in distance from the field margin, possibly as a response to availability of diverse food resources at the edge than in the crop. Distance from a flower margin and hedgerow is a key driver of both floral visitors and natural enemies of pests' abundance and species richness in field beans agricultural systems.

#### A Multi-Year Field Study Investigating the Potential Effects of Winter Oilseed Rape Treated as Seed with Thiamethoxam to Honey Bees

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This study was conducted in the Alsace region of France to determine the effects of annual exposure to winter oilseed rape, treated as seed with thiamethoxam and two fungicides, on colonies of honey bees , *Apis mellifera* L., under field conditions over a four-year period. Every spring, at the start of winter oilseed rape flowering, honey bee colonies were placed at the treated and at the control field plots. Mortality, foraging activity and behavior of adults, and brood development were assessed during this period. At the end of flowering in each year, the colonies were relocated to an area without extensive agricultural crops that were attractive to bees and with minimal pesticide usage for over-wintering. Over the observation period from spring 2005 to March 2009, annual exposure of honey bees to winter oilseed rape grown from thiamethoxam-treated seeds had no effect on honey bee colony development. Further, no test item-related differences on honey bee mortality, flight intensity in the test fields, and behavior of the bees during the time of exposure to the winter oilseed rape fields were observed.

#### Diversity and per-visit effectiveness of native bees in apple pollination

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Recent declines in honey bee health and increasing demand for pollination services highlight a need for enhancing wild pollinators in agriculture. Case in point is apple, an economically important crop in the U.S. that requires cross pollination by bees to set fruit. Though commercial growers rent honey bees to insure adequate pollination, the phenology of apple bloom corresponds to an important period of native bee activity in the Northeast. Little is known about wild bees visiting apple or their relative contribution to pollination. In 2009 and 2010, we net surveyed bees visiting apple blossoms in orchards of Central and Western New York. Studywide, native bees were abundant and diverse, with more than 70 bee species visiting apple. In 2010, we compared pollinator effectiveness of honey bees with that of an abundant native *Andrena* subgenus, *Melandrena*, by recording the number of pollen grains deposited by one visit to a virgin, emasculated apple flower. The effect of farm-scale variables such as spray regime (EIQ), number of honey bee rentals and field size on native bee diversity and abundance, as well as the relative pollinator contribution of native *A. (melandrena*) will be discussed.

#### Pheromonal modulation of foraging behaviour in the honey bee, Apis mellifera

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Honey bee workers have two potential life histories: normally in a healthy colony workers are sterile and help the queen by nursing her offspring, but if a colony becomes queenless or is failing workers can develop their ovaries and produce male brood of their own. Clearly worker behavioural and physiological development is influenced by the condition of its colony, but what information signals colony condition to workers, and how do bees acquire and weight this information? Queen and brood pheromones are two key colony signals that influence worker development. Here we explore how these signals work independently and in combination to influence the behavioural and physiological state of workers. We examined how brood pheromone, queen pheromone and supplemental feeding interact to influence foraging behaviour and colony growth by experimentally varying the amount of brood pheromone, queen presence and protein supplementation between colonies established from packages at the start of the season. Later we focused on the effects of brood pheromone on foraging behaviour, worker ovary development and colony productivity on established field colonies. Our work will reveal how pheromone systems interact to modulate bee societies, and help improve management of the honey bee for the honey and pollination industries.

#### **Operation Pollinator: Positive Action for Pollinators**

Jeff Peters, Syngenta Crop Protection

Julianna Tuell, Michigan State University

Neal Williams and Katharina Ullmann, University of California Davis

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Pollinating insects are essential for the production of many food crops and the health of the natural environment, but their numbers have declined dramatically over recent years. In response, Syngenta launched Operation Pollinator<sup>™</sup>, a global initiative that includes research over the next five years in California, Florida and Michigan. This collaborative effort involves a diverse group of stakeholders whose goal is to develop a conservation program which growers can integrate into their farm landscapes. The Program will evaluate the provision of additional foraging habitat and nesting sites for native pollinators on marginal land or edge of field in an effort to enhance biodiversity and promote sustainable agricultural practices. The Program will also consider the economical and ecological benefits of conservation buffers. Through the research, a set of economically viable management/conservation practices will be developed and provided to the agricultural community. The goal is to develop land-use management practices that are practical and can also improve farm productivity.

# Pollination requirement of Clementine (*Citrus clementina* Hort. ex Tan) in South Sardinia (Italy) and the effects of landscape context on pollinator community

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Citrus fruit species show a great variation in terms of pollination needs among and within species due to a wide range in breeding systems, thereby generalization are unsuitable and species-focused studies are more appropriate. A two year study on Clementine (*Citrus clementina* Hort. ex Tan) has been carried out in South Sardinia (Italy), to improve the knowledge on pollination requirement of this self-incompatible species. During the blooming season two different treatment were performed: in the first, flowers were left for a natural insect pollination and in the second, insect visitation was precluded. At the same time a pollinator survey was carried out. The percentage of ripened fruit was recorded as well as other fruit quality parameters as fruit size, weight and seeds number. Landscape context was also considered, as potentially effecting insect pollinators presence.

Results show that pollination positively affects fruit development and size, though the more commercially valuable parthenocarpic seedless fruit may increase in number under favorable weather condition during the blooming season. Honey bees are the most frequent visitors (89%) followed by dipterans (8%). Landscape context do not seem to affect fruit production in this circumstance, even though insect abundance is inversely correlated with surrounding semi-natural areas.

#### Morphometric Study of Honey Bee Population in District Khairpur, Pakistan

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In this study, District Khairpur, Pakistan was chosen as the sampling area owing to the spatial variation of soil, climate and natural flora. One hundred worker bees from each of the seven randomly selected villages were chosen for the study. Thirteen morphological characters were measured on each sample. Sample mean and standard deviation were computed for each morphometric character. Analysis of variance (ANOVA) displayed statistically significant differences among the characters (P << 0.05) but the ratio of error variance was > 4, therefore to find out the true difference, non parametric analyses were performed. Principal Component Analysis (PCA) showed that the most of the variation (61%) is caused by the variable length of leg, wing and tongue. Results of Discriminant Function Analysis (DFA) were corroborative to the conclusion of PCA. Cluster Analysis (CA) showed that one village is not only distant from the rest of the villages but also geographically different from other six villages of Khairpur Pakistan. It may be inferred from this study that there is a consistant morphometric variation between Khairpur honey bee samples. This variation needs to be explored extensively, therefore, further investigation taking molecular markers in consideration will be helpful to delineate the true picture.

#### NSF REU at OSU: Training a new generation of pollination researchers

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The worldwide decline in native bees has resulted in international initiatives promoting collaborative research for addressing the 'pollination crisis'. Students trained in interdisciplinary research are needed to understand dynamic interactions between native bees, the flowers they pollinate, and the surrounding landscapes, to accomplish the goals of these initiatives. This need is being addressed at Oregon State University (OSU) through the NSF-funded REU (Research Experiences for Undergraduates) on Pollination Biology. This novel program provides undergraduates with a unique 10 week summer experience in cross-disciplinary, comparative research. Initially, students learn about the scientific process, and about relationships between plants and bees while becoming skilled in identification of local bees and flowers. Subsequently, each student rotates between three sites: a dryland prairie in the northeast, a forest ecosystem in the foothills of the Cascade Mountains, and agricultural landscapes in western Oregon. At each site, participants assist with existing projects, and gain first-hand experience in comparing native bee pollinator diversity and dynamic mutualistic interactions with local flora. In the tenth week, they compile and analyze results, and make individual presentations in a Pollination Biology Conference. Program impact, assessed through Pre- and Post-quizzes, indicated a 10 to 50% increase in scores on questions related to biology, the scientific method, pollination and conservation in the first year. Thus, through the program, a new generation of pollination researchers is being trained with enhanced awareness of the global pollination crisis, the critical need for protecting and preserving native pollinators, and the role of native bees in crop pollination.

#### Taxonomic resolution and plant-pollinator networks

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Network theory is increasingly being used as a tool to describe the complexity of mutualistic interactions. To maximize the amount of information obtained, networks require species level identification, but for insect pollinators this process is time consuming and logistically challenging. Because failure to identify specimens to the species level could result in the loss of critical information, identification remains a significant roadblock in the study of plant-pollinator communities. We constructed networks at different taxonomic resolutions to ask what level of identification is required to fully understand these communities. In our system, all community members, both plants and visiting bee pollinators (Apoidea), were identified to the species level. We then constructed networks at the species, genus, and family level of bees. We also created a network with common categories for field identification: honeybee, bumblebee, and solitary bee. We compared the structure of each network to the species level network. The family level network retains similar values for some measures, such as average degree and nestedness, and may be sufficient if the researcher is interested in these characteristics. However, structural information is lost as resolution decreases and low resolution is likely inadequate to understand the full complexity of interactions within these communities.

# Molecular identification reveals highly selective foraging behavior in solitary Hawaiian Bees

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Pollination is a critical and valuable ecosystem service, and understanding plant-pollinator interactions is vital for both conservation efforts, and enhancing agricultural systems. Using molecular methods in an ecological context, we identified pollen in the crops (internal storage organs) of *Hylaeus* bees (Hymenoptera: Colletidae) from Hawaiian national parks. Molecular analyses of crop pollen indicate *Hylaeus* species exhibit high floral fidelity in pollen foraging despite the availability of pollen from numerous other native and non-native plant species. Furthermore, analyses of *Hylaeus* crops found only pollen from native plants, indicating that these solitary bees are important pollinators of Hawaiian plants. This molecular approach provided species-level floral visitation information without requiring the palynological expertise needed for high-throughput visual pollen identification. Ultimately, this novel approach helped to elucidate plant-pollinator interactions that would have been missed by using traditional observational studies alone.

# **RNA** viruses in the pollinator community: Their distribution, inter-species transmission and health impact on bees

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RNA viruses are suspected as one of the major contributors to the recent honey bee (Apis *mellifera*) decline. Since honey bees interact with several other pollinators, it is vital to study the virus ecology and epidemiology in the context of whole pollinator community. Sampling wild pollinator populations and subsequent virus analysis using reverse transcriptase-PCR and sequencing, showed wide distribution of these viruses among hymenopteran pollinators. Further, phylogenetic analysis indicated that these viruses are freely circulating in the pollinator community, mediated in part by pollen as the route of inter-species transmission. To study their impact on native pollinators and further understand the intricate complexities of inter-species virus transmission, controlled greenhouse experiments were conducted. Israeli acute paralysis virus (IAPV) successfully moved between honey bees and bumble bees (*Bombus impatiens*) within a week of foraging together on common flowers. IAPV infection negatively impacted the colony survival and foraging activity in bumble bees. Detection of virus in different bee tissues, suggests both horizontal (via infected feces and food) and vertical (transovarial) routes of transmission for IAPV. This study suggests broader impact of RNA viruses on the pollinator community and highlights the risk of potential disease outbreaks from the movement and importation of pollinators.

#### Nectar production, composition and pollinator diversity in some local species

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Present investigations were carried out during the year 2004-2006 at Akot (Latitude 21<sup>°</sup> 06' N and Longitude 77<sup>°</sup> 06'E) situated in Akola district of Maharashtra State (India). The observations on different aspects of nectar dynamics of selected plants were undertaken at different study sites. Eight plant species namely; *Butea monosperma* (Lamk.), *Erythrina variegata* L., *Mucuna pruriens* Hook., *Ipomoea hederifolia* L., *Pyrostegia venusta* (ker. Gawl.), *Adhatoda zeylanica* Medic., *Peristrophe bicalyculata* (Retz.), and *Vitex negundo* L. were selected for the investigations.

Plant species were observed for nectar dynamics and visitors census. Time of anthesis, anther dehiscence, nectar secretion and nectar secretion span were significantly differing in studied plant species. In all species nectaries are either around ovary or staminal.

Average nectar volume 85.31µL was found to be maximum in *Erythrina variegata*. Maximum average nectar concentration and average amount of sugar was found to be 43.53% and 15.7mg in *Adhatoda zeylanica*. Nectar-sugar composition represents glucose and fructose in all plants and sucrose was found to be present in *Mucuna pruriens*, *Ipomoea hederifolia*, *Pyrostegia venusta* and *Vitex negundo* only. Nectar-amino acids were found to be varied in different species. Maximum number of amino acids was recorded in *E.variegata*.

Birds were found to be very frequent visitors in *Butea monosperma* and *Erythrina variegata*. Amongst the bees *Apis cerana indica, Apis dorsata* and *Apis florea* were the common bee foragers. In *Vitex negundo* very frequent butterfly visitors were *C. pyranthe, C. pomona* and *E. cnejus*.

#### Key Words: Nectar Composition, Pollinator Diversity

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# *Nosema ceranae* infection levels for honey bee colonies during the fall and winter in Virginia

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Over the past 10 years, honey bee losses have averaged 30 percent in Virginia. Reasons for these losses are partially attributed to increased stresses from new or unidentified pathogens. After a statewide survey in Virginia for *Nosema spp.*, we found that more than 70% of colonies were infected with *Nosema ceranae* while *N. apis* was very rare (2.7%) and only found as a low-level co-infection with high infection levels of *N. ceranae*. Traditional diagnostic approaches utilize spore counts while we utilize a multiplex real-time PCR assay to determine infection levels simultaneously for *N. apis* and *N. ceranae*. Here we report on infection levels in 10 colonies sampled monthly from fall through early spring. As part of the sampling process, bees were collected from different areas of the hive, or winter cluster, and examined for difference in infection levels. The results show infection levels ranging from high to low levels with average  $C_T$  values ranging from 26 to 36.

#### Genetic Diversity of Bumble Bees in the South Central United States

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Although there is increased interest in native pollinators due to CCD in *Apis mellifera*, the genetic diversity of native *Bombus* species in the US is largely unknown. A preliminary study on the genetic diversity of four species of bumble bees in Arkansas, Missouri and Tennessee was conducted using DNA sequences of a portion of the mtDNA 16S rRNA gene. Specimens from both museum collections and contemporary sampling were analyzed to compare genetic diversity. The highest level of genetic diversity was observed in *Bombus bimaculatus*, followed by *B. griseocollis*, *B. impatiens* and *B. pensylvanicus*. Analysis of the DNA sequences provides information on the amount of genetic diversity within each species and may indicate if any species have low levels of genetic diversity. Low levels of genetic diversity could indicate that the species has undergone a genetic bottleneck and may be at risk. Population genetic analysis was conducted on the DNA sequences to determine if there are any populations of bumble bees that are genetically distinct. These populations may be locally adapted and movement of commercial bumble bees of this species into this area may displace these locally adapted populations.

# Measuring the risk to native bees of pest management programs in a pollinator-dependent crop

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Native bees are essential to sustainable pollinator-dependent crop production, yet little is known about the effects of typical insect pest management programs on native bee communities. We developed an Insecticide Program Risk (IPR) index to quantify the relative risk to native bees of insecticides applied to blueberry fields and determine the relationship between IPR and native bee communities over three seasons. In two of three years, bee abundance and species richness declined with increasing IPR. Bee diversity declined with IPR in one of three years. These results indicate that native bee communities are negatively affected by increasingly intensive chemical pest management activities in crop fields, and that inter-year variability in bee populations has the potential to mask such effects in short-term studies. When several species were analyzed separately, two out of three solitary and one out of three social blueberry-foraging species declined with increasing IPR values suggesting that different life histories and nesting habits may help some bee populations escape the negative effects of insecticides applied post crop bloom. The IPR index provides a standard method to compare pest management programs for their potential effect on native bee communities, with broad application for use in other agricultural systems.

#### Soil Substrate Preferences of Female Ground-Nesting Bees in Northern Virginia

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Two thirds of the  $\sim 4000$  native North American bee species are ground-nesting species. Ground-nesting bees if provided substantial floral and nesting resources can enhance the pollination services of honey bees and potentially substitute honey bee services. The reproductive success of ground-nesting bees depends on adequate brood cell conditions that provide structural integrity and protect the young from predation (parasites) and unfavorable climatic conditions throughout development. When developing conservation techniques it is important to understand what type of habitat conditions are preferred by a nesting female bee. This study experimentally tested vegetation, moisture and disturbance preferences of the female ground-nesting bee community in Northern Virginia during nest site selection. The abundance and diversity of ground-nesting bees were higher in bareground and revegetated plots than those with existing vegetation. There were differences in the response to vegetation and moisture treatments in and among the most commonly collected taxa, *Lasioglossum (Dialictus) spp.*, Halictus ligatus, and Calliopsis andreniformis.. Response of nest site initiation was also correlated with moderate disturbance, soil hardness and floral resources. This study has identified a useful method in looking at further questions about the edaphic conditions that ground-nesting bees respond to and how conservationists might manage habitat for bees.

## Evaluation of pollinator manageability for agricultural use on a native Great Basin legume <u>Kristal M. Watrous</u>, Utah State University, Department of Biology, Logan, UT James H. Cane, USDA-ARS Bee Biology and Systematics Lab, Logan, UT

Native wildflowers are increasingly being used in wildland restoration projects and water-wise landscaping. Only farming can typically deliver abundant clean affordable supplies of native seed. Adequate pollination is vital on-farm to ensure consistent good seed production, especially for self-incompatible species. We surveyed the floral visitors of *Astragalus filipes*, a native Great Basin legume, in 22 natural populations and in two agricultural settings to identify promising pollinator species. We chose nine bee species common on *A. filipes* and evaluated each of them for overall practicality for on-farm pollination, considering the floral host preference, flight seasonality, nesting needs, and several indicators of pollination promise for each species. We measured foraging tempo and counted frequency of stigmatic contact while foraging. Bees in the genus *Osmia* dominate this pollinator guild in the wild. *Bombus nevadensis* queens were the fastest foragers on *A. filipes*. Honey bees tended to rob flowers for nectar, contacting stigmas least often. We recommend cavity-nesting *Osmia bruneri* and *Hoplitis hypocrita* as promising bees to manage as pollinators of *Astragalus filipes* and other Great Basin legumes in cultivation.

#### Landscape features affect bumblebee abundance in Vermont

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Since their introduction to the United States over 250 years ago, honeybees have become important pollinators of many plant species. The recent decline in native pollinator species diversity could be due, in part, to habitat degradation and/or fragmentation, as well as competition with non-native honeybees. I studied the relationship between habitat fragmentation and bumblebee abundance in Vermont in two summers. I studied bees at the flowers they visited in meadows and old-fields of varying sizes and degrees of isolation from both other meadows and from forests. Agricultural fields (corn, alfalfa, and pasture) and urbanization adds to the fragmented landscape that these pollinators navigate. Using GIS, I examined the effects of land-use cover on bumblebee abundance. The proportion of visits to flowers by bumblebees was positively correlated with percent forest cover and negatively correlated with percent pasture cover. In addition, bumblebee abundance increased with increasing distance to honeybee colonies and with the proportion of the plant species in the fields that are native. These results suggest that bumblebee abundance is affected by many factors: density of honeybees (competition), floral composition in fields (floral morphology), and forest cover (nesting habitat).

#### Study on the pollinators of Mangrove flowers at Sementa, Selangor, Malaysia

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This study was conducted from 2007 to 2009 at Sementa and Kapar mangrove forest, Klang, Malaysia. Field observation to locate insects on mangrove flower, branch and leaves were done. Fresh flower and fresh leaf, flower volatiles, extracts and control were tested in no choice and dual choice olfactometer against insects collected from light traps under flowering *Rhizophora apiculata*, *Rhizophora mucronata*, *Ceriop tagal*, *Bruguiera gymnorrhiza*, and *Avicennia*. This study evidenced that *Vespa affinis* (Hymenoptera: Vespidae) was attracted to *Ceriop tagal* flower. *Bruguiera gymnorrhiza* flower attracted beetles *Minastra violaceipennis* and *Metrogaleruca obscura*. *Trigona* sp (Hymenoptera: Apidae), beetles *Adoretus sinicus*, *Mimastra violaceipennis* and *Amarygmus micans* were attracted to calyx of *Rhizophora mucronata*. Fragrance of *Avicennia alba* flower attracted *Trigona* sp and cerambycid beetle *Diorthus* sp. This finding is important for conservation and management of mangrove a buffer against tsunami.

Keyword: mangrove, flower, pollinator

#### Contrary perspective to bumble bee declines: The Oregon Experience

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Agricultural landscapes are believed to be poor in foraging resources required for sustaining bumble bees since their colonies live for several months while crop bloom lasts for just a few weeks. However, bumble bee studies in cropping systems have largely focused on a single crop. In the current study, we determined bumble bee diversity and abundance in two crops that bloom in sequence, namely, blueberries in spring and red clover seed crops in summer. We estimated populations using blue vane traps and through timed visual observations of foragers on crop bloom. The study was conducted in commercial fields in the Willamette Valley, an agriculture dominated region in western Oregon on the west coast of the United States. At least six bumble bee species were observed both in traps and during visual counts in both crops. The number of bees captured/day increased from 6/trap in blueberries to >30/trap in red clover. Based on visual count data, an average of 1 bumble bee/min foraged on blueberries and 15-30 bees/min on red clover during peak bloom. Thus, contrary to reports from elsewhere, a rich fauna of bumble bees is flourishing in western Oregon. Bumble bee colony development in the area is likely sustained through the practice of farming bee-pollinated crops that bloom in sequence, and in synchrony with foraging by queens and workers. Based on our studies, we recommend integration of multiple agro-ecosystems or native plants that bloom in sequence for sustaining and building bumble bee populations.

#### SPEAKER ABSTRACTS ADDENDUM

#### **Pollinator Protection in EPA's Office of Pesticide Programs**

#### Tom Moriarty, Office of Pesticide Programs

# EPA will present a brief overview of its regulatory frame work and actions it is taking to ensure that pollinators are not subject to unreasonable harm from the use of pesticides.

As directed by the Federal Insecticide, Fungicide, Rodenticide Act (FIFRA), the EPA Office of Pesticide Programs evaluates the potential risk to non-target organisms from pesticide products. FIFRA directs EPA to balance risks and benefits when making decisions with regard to non-target animals and plants. EPA's current ecological risk assessment paradigm incorporates a qualitative assessment of hazard to terrestrial invertebrates, for which honey bees (A*pis mellifera*) are used as a surrogate. OPP employs label language to mitigate potential risk from use of a compound and provide direction on ways to reduce potential exposure.

EPA's Pesticide Program has set up a multi-disciplinary team to evaluate its tools to assess and manage potential risk to terrestrial invertebrates from pesticides. Because regulatory actions to manage potential risk are predicated on sound science, a focus of this team is to ensure that data used to inform risk assessment for pollinators adequately characterize both the potential hazard and potential exposure. OPP is currently engaged in several activities to develop the information necessary to support risk assessment for pollinators, such as: identifying and in some cases supporting current research efforts; engaging in cooperative efforts with state and federal partners; and, participating in the development of a global science conference on risk assessment for pollinators.

As the Agency engages in different actions to further develop our risk assessment process, and to identify and review data needed to inform that process, it is also beginning to consider how best to enhance/develop appropriate risk management options/tools for beneficial insects such as pollinators. The Agency intends to engage its state and federal partners along with other stake holders, in the development of these tools and to further awareness of the options and challenges to protect terrestrial invertebrates. Risk management should aim to protect insect pollinators but must also provide flexibility for growers and be cost effective. The protection of pollinators is an integral part of the Agency's mission to protect and enhancing the environment.

#### Honey bee colony losses in Belgium: a multifactorial approach.

<u>Bach Kim Nguyen</u>, Claude Saegerman, Jacques Mignon, Edwin De Pauw, Eric Haubruge, University of Liege; Magali Ribière, AFSSA Sophia-Antipolis; Dennis vanEngelsdorp, Pennsylvania Department of Agriculture, Pennsylvania State University; Dirk de Graaf, Ghent University, Laboratory of Zoophysiology

Since 1999, abnormally high honey bees colony losses have been observed in Belgium. Many studies carried out in Europe clearly highlight the impact of several factors including parasites, diseases, climate, food availability and low farmland biodiversity. In this context, a multifactorial study was set up in order to discover the factors of risks which are likely to influence the vitality of the bees in Belgium. This project began in 2004 and since then, we have been studying the effect of pesticides, pathologies and environment. A list of pesticides including both product types used in apiculture and in the surrounding crop fields has been set up and quantification of residues were carried out. The pathologies were studied combining field observations, microscopic, classic microbiological and molecular approaches. Finally, the correlation between the colony mortality and the presence of pesticides, pathologies and the environment were analyzed. Eighteen pesticides were detected in the hives but no relationship between these chemicals and the honeybee mortality was observed. Concerning the pathologies, the mite Varroa destructor, American foulbrood and Acute Bee Paralysis virus were linked with increased colony mortality. Co-infection with more than two viruses before the overwintering also had an appreciable negative effect on colony survivorship.

#### Robyn Rose, USDA APHIS Honey Bee Health Program

Protecting American agriculture is the basic charge of the U.S. Department of Agriculture's (USDA) Animal and Plant Health Inspection Service (APHIS). APHIS provides leadership in ensuring the health and care of animals and plants. The agency improves agricultural productivity and competitiveness and contributes to the national economy and the public health. Declining honey bee health has been documented for years. The known negative honey bee health challenges are attributable to parasites, diseases and environmental toxins. APHIS is involved with several initiatives to assess potential impacts and improve upon honey bee health through collaborations with states, universities, the Environmental Protection Agency (EPA) and USDA Agricultural Research Service (ARS). Through these collaborations APHIS is doing the following: 1) working toward determining residue levels of imidacloprid in nectar and pollen from maple trees treated for the Asian longhorn beetle program, 2) has convened a Technical Working Group to consider pesticide toxicity testing, 3)is working with Hawaii on the Varroa mite and small hive beetle infestations on the Big Island.

In addition, a limited national survey of honey bee pests and diseases has been funded by APHIS. This survey is being conducted in an attempt to document which bee diseases, parasites, or pests of honey bees are currently present in the U.S. The primary focus of this survey is to verify the absence of the parasitic mite Tropilaelaps and other exotic species (e.g., Apis cerana) and subspecies of honey bees in the U.S. Current international trade law prevents the U.S. from banning imports of bees from another nation unless the exporting nation has a disease, parasite, or pest of honey bees that is not found in the U.S. To maximize the information gained from this survey effort, samples will be analyzed for other honey bee diseases and parasites known to be present in the U.S. This cross-country survey will be the most comprehensive honey bee pest and health survey to date. Coordination of this survey is in collaboration with the ARS Bee Research Lab in Beltsville, Pennsylvania State University, and the Florida Department of Agriculture and Consumer Services. The conference organizers would like to thank the following sponsors for their generous support of the 1st International Conference on Pollinator Biology, Health and Policy



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