Accomplishments and Impacts of NC1173:
Presented January 14, 2010 in Orlando, FL

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Brian D. Eitzer and Kimberly Stoner, The Connecticut Agricultural Experiment Station

1. Purpose

1. Determine the baseline exposure of honey bees to pesticides while foraging for pollen
2. Determine how pesticide exposure varies with time and sample location
3. Relate systemic pesticide use techniques to the amount of the pesticide that translocates into the pollen

2. Current Activities

In 2009 a total of 166 pollen samples were taken from honey bee hives from 5 locations in Connecticut and grouped into 59 composite samples. The locations represent urban, suburban, and rural locations including an orchard during the apple and blueberry blooming season. These samples are being screened for pesticide residues using a multi-residue procedure. In brief, extraction with acetonitrile, dispersive SPE cleanup followed by HPLC/MS/MS analysis. We also have examined the movement of neonicotinoid insecticides into plants treated at labeled rates using techniques and timing that would be realistic in a production system. These include studies of movement of: imidacloprid into Maine blueberry flowers; acetamiprid, thiamethoxam, and spirotetramat into Massachusetts cranberry flowers; and imidacloprid and thiamethoxam into summer squash plants and pollen in Connecticut.

3. What We Have Learned

Our study of pollen trapped from honey bee hives has shown that there is a large amount of variability in which pesticides and what concentration of pesticides honey bees are exposed to. This variability occurs both between hives from different locations sampled at the same time as well as over the course of time within a single hive. For example, in 2009 samples of pollen taken from an orchard had an average of 12.4 residues per sample as compared to 5.9 residues per sample for all the other samples of this year analyzed to date. In addition, at the orchard site all 8 composite samples had difenconazole and 7 of the 8 had myclobutanil; neither of these compounds were seen in any of the other 44 composite samples analyzed this year. There is also a difference between high frequency of detection and high concentration when detected; some of the compounds found most frequently were only observed at low concentrations while the compounds found at the highest concentrations often were often only seen in a couple of samples. Our studies on the translocation of systemic pesticides show that these pesticides do move into the flowers and pollen of plants where they are applied, and therefore bees foraging on the plants could be exposed to the pesticide well after the pesticide was applied.

4. Why is This Important?

These studies are important as they will help us to learn about how honey bees are exposed to pesticides, and how this may be affected by timing or application methods. This knowledge can then be used to help devise strategies that will minimize that pesticide exposure which could hopefully lead to healthier honey bee colonies.

5. For More Information

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David R. Tarpy, North Carolina State University

1. **Purpose**

- Assess the reproductive quality of commercial honey bee queens.
- Determine the relationship between colony genetic diversity and productivity.
- Instruct beekeepers on basic queen-rearing and advanced bee-breeding techniques.

2. **Current Activities**

Research 30%: We have conducted a large-scale assessment of queen reproductive quality, funded by USDA-NRI. We are also characterizing the feral honey bee population using a molecular ecology approach. We are also initiating research into the collective decision-making process of queen supersedure, as well as pollination ecology of small fruits (particularly blueberries). Finally, in collaboration with Tufts University, we are engaged in ongoing research of the health benefits of intracolony genetic diversity.

Extension 50%: we are conducting many large-scale workshops on queen rearing for beekeepers, then selectively choosing individuals to conduct small scale clinics on bee breeding. We will also be hosting the annual conference of the Eastern Apicultural Society in August in Boone, NC. Our ongoing Master Beekeeper program has 5400 participants, which will be conducting several dozen bee schools across the state to educate approximately 1200 new people in bee management. We are also developing a web-based distance education course for Honey Bee Biology and Management.

3. **What We Have Learned?**

We have demonstrated that commercial queens mate with an adequate number of drones and store an expected number of sperm. Preliminary data also suggests that there is a well-established feral population of honey bees with significant levels of genetic diversity. Finally, we have established several links of colony genetic diversity—as a consequence of the queen mating multiply—to colony health (e.g., disease prevalence) and productivity (e.g., worker population).

4. **Why is This Important?**

A focus on honey bee queens is a priority for apiculture research, as surveys of beekeepers self-identify problems associated with queens. Determining that commercial queens are adequately mated rules that factor out as a major determinant of colony ill-health, which therefore enables us to focus on other, less-direct factors associated with queen problems. This will enable us to bolster colony productivity and minimize colony loss in a tangible manner.

5. For More Information –

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Diana Cox-Foster, reporting for Penn State

1. Purpose –

1. To develop and recommend to beekeepers "best practices" for Varroa mite control based on currently available methods and strategies for mite management, (Ostiguy, MT Frazier, D vanEngelsdorp).
2. To evaluate the role and causative mechanisms of parasitic mites and pathogens such as viruses, protozoa and bacteria in honey bee colony deaths. (Grozinger, D vanEngelsdorp, Cox-Foster, Rajotte, Tumlinson, Ostiguy, MT Frazier).
    A. Determine if volatile organic compounds can be used as diagnostics of colony presence of pathogens and organisms affecting bee health.
        1. Identify the VOC diagnostic for diseases and organisms infesting bee hives, including the small hive beetle, and other pathogens and organisms to be determined.
        2. Develop diagnostic methods based on analysis of VOC from bee hives to allow the health of hives to be monitored and maladies to be detected and diagnosed early before the health of the hives deteriorates beyond recovery.
    B. Examine the causal basis of gross pathology of "new" parasites found in CCD bees and determine correlation to colony health.
        1. Examine predictive value of certain "gross" symptoms (i.e pyloric melanization and flagellate infections).
        2. Document the life cycle and pathogenicity of organisms.
        3. Demonstrate Koch postulate of organisms.
        4. Initiate histological study of organisms in host.
        5. Complete a preliminary survey for organisms in samples from the US and internationally.
    C. Determine if particular pathogens are linked to colony deaths and interact with particular triggers to result in Colony collapse Disorder.
        1. Survey for new viral species found in CCD colonies in queen breeding operations and Pennsylvania apiaries.
        2. Determine if new viral species impact colony health through controlled infections and how these viral species interact with pesticides and nutritional stresses.
        3. Determine how pathogen levels correlate with colony health under different stress levels (nutrition, chemical exposure, and parasite loads).
    D. Determine how pathogens are being transmitted between colonies and potentially among pollinator species to affect bee colony health and other pollinator health.
        1. Determine the extent to which different bee pathogens are present on pollen being brought into colonies.
        2. Determine route by which pollen is infected, on the plants themselves and the role of the nectar and pollen foragers.
        3. Determine if pathogens are moving among pollinator species and affecting both bee and other pollinator species health.
3. To determine the effects of environmental chemicals and miticides on honey bee colony

health. (Mullin, Cox-Foster, JL Frazier, Ostiguy, MT Frazier, D vanEngelsdorp).

   A. Investigate if chemical pesticides are a key factor in declining health of honey bees and/or a possible "triggering" factor for CCD.
      1. Quantify the pesticide load in honey bee colonies and track the health of these colonies over time.
      2. Conduct toxicity tests of individual pesticides and their combinations to assess their causative association with CCD.
      3. Determine sublethal effects of pesticides and selected combinations of pesticides on physiological and behavioral systems of insects, including immune system suppression, interference with associative learning, and detection and/or alteration of the chemical senses of honey bees.
      4. Determine how registered in-hive use of miticides affects disease loads and overall colony health over time.

4. To determine how environmental factors, including nutrition and management practices affect honey bee colony health. (Cox-Foster, D vanEngelsdorp, MT Frazier, Ostiguy).
5. To determine the effects of interactions among various factors affecting honey bee colony health. (Mullin, Grozinger, D vanEngelsdorp, Cox-Foster, JL Frazier, JH Tumlinson, Ostiguy, MT Frazier).
6. To coordinate research and extension efforts related to bee colony health. (Ostiguy, MT Frazier).
7. To facilitate, through research and extension activities, the development of industry-based honey bee stock selection, maintenance and production programs that demonstrably incorporate traits that confer resistance to pests, parasites and pathogens. (Grozinger, Cox-Foster, Ostiguy).
8. To focus on non-*Apis* bees, their conservation, pathology, susceptibility to pesticides, and their contribution to crop pollination including economic value. (Cox-Foster, Rajotte, Mullin, JL Frazier, MT Frazier).

2. **Current Activities –**

Nine different researchers (see below) are involved in multiple research avenues, directed at determining key factors in bee biology and factors that determine colony health. Areas being researched include the following: bee and colony behavior as regulated by pheromones; disease ecology in the hive; impacts of stress upon bee immunity; elucidating the roles and impacts of pesticides present in healthy and diseased colonies on bee biology and health; mitigating the impacts of mites, pests, and diseases on colony health of honey bees and also on native bee species. Outreach to the public, students, media, and beekeepers are being made on a regular basis by all participants.

3. What We Have Learned –

Via epidemiological study, 61 variables were compared between individual control and diseased colonies associated with CCD, with no single causal agent being found. CCD colonies had higher pathogen loads and bees were co-infected with more pathogens, suggesting compromised immunity in CCD bees. At the operational level, IAPV was still found to be associated with CCD. Cage studies with adult bees of known ages indicate that younger adult bees are more highly susceptible to IAPV and exhibit greater mortality as compared to older bees. Via common floral sources in a greenhouse, IAPV moved readily between honey bees and bumble bees, and vice-versa. Bumble bee colonies infected with IAPV had decreased colony life and decreased foraging strength. Field studies suggested that stress results in increased IAPV titers, with nutrition and pesticides hypothesized to be the likely suspects. Ostiguy initiated studies to investigate the impact of viruses on survivorship of brood; preliminary findings support the viral infections as causing significant loss of eggs and larvae. Analysis of initial viral infections in colonies in the CAPS sentinel colonies indicate that DWV, SBV, and BQCV are commonly found in the packages of all the apiaries at low prevalence, with IAPV and ABPV found less often among the colonies. No KBV or CBPV were detected. In Kenya, varroa was found to be widely present in colonies with multiple subspecies. To date, 121 different pesticides and metabolites have been detected in 887 wax, pollen, bee and associated hive samples (average of 6.2 detections per sample) from 23 states and one Canadian province. Chorothalonil, a contact fungicide, was a marker for pollen entombed in wax in colonies with poor health. Entombing may be a defensive behavior of bees faced with large amounts of potentially toxic food stores. Currently, the association of any one pesticide with CCD remains unclear; although, higher coumaphos levels may benefit the colony presumably via mite control. Pesticide interactions with other stressors including Varroa, IAPV, and Nosema require further study. The Grozinger lab in studies of the effects of pheromones demonstrated that brood pheromone significant affects behaviorally-relevant, brain gene expression in workers. The effects are age-dependent and distinct from those produced by queen pheromone. In studies on the reproductive state versus queen pheromone and queen-worker signaling, pheromone production and worker responses were linked to ovarian activation. Pheromone production may serve as an honest signal of queen quality. Finally, studies on the chemical communication of disease among honey bee workers demonstrated that immunostimulation alters chemical profiles and nestmate interactions. With coupled gas chromatography-electroantennographic detection and gas chromatography-mass spectrometry, the Tumlinson lab in collaborative research identified three volatile compounds, collected from larvae infected with chalkbrood and detected by adult honey bees. Field bioassays revealed that phenethyl acetate is a key compound associated with chalkbrood infection that induces hygienic behavior.

4. Why is This Important? –

Honey bees are essential for pollination of over 90 fruit and vegetable crops worldwide, with the pollination valued at more than $14.6 billion in the U.S. Since 2006, deaths in bee colonies with unique symptoms (termed Colony Collapse Disorder (CCD)) have severely impacted their number and threaten diverse pollination needs of fruit and vegetable producers. In the U.S., the losses due to CCD are combined with major losses due to the parasitic Varroa mite, creating major concern for beekeepers in the U.S. and their economic sustainability. It is essential for both agriculture and natural ecosystems that this be resolved. The honey bee is the primary pollinator of agricultural crops dependent upon insect pollination. Other pollinators are also at risk and declining. Determining how pathogens and pesticides impact bees is needed to ensure healthy ecosystems, dependent upon pollination of flowering plants. CCD has continued to plague the United States, along with increased colony deaths due to other causes. Our research indicates that the underlying causes of CCD are complex as revealed by an epidemiological analysis. CCD colonies have high pathogen prevalence, in particular picorna-like viruses; and data indicate that additional stresses such as chemical exposure and lack of adequate nutrition are playing a role. How the interaction among pathogens relates to CCD and bee colony loss is still at question. Environmental chemicals may be part of the problem in colony health. Over 121 different pesticides have been found, representing almost all classes of pesticides. How these chemicals interact with each other and affect the bee biology and health is unknown and is of major importance for understanding impacts on colony health and on native pollinators. It is clear that multiple factors are present that impact colonies. How these factors interact and how the combinatorial impacts determine colony health is key to overcoming honey bee colony loss and is essential for bee keepers. Volatile organic compounds (VOC) may be useful in disease diagnosis, since every aspect of colony function is regulated by chemical signals. VOC emitting from a colony may be potentially specific to particular pathogens and used for diagnosis. VOC may also reflect stress from either pesticide exposure or nutritional stress. Specific pheromones may also be affected by pathogen infections or pesticide exposures and affect the colony health and strength. Learning how the pheromone signals in the colony are being controlled and modulated under the different stresses may be essential to understanding colony health. Are other pollinators being affected by common pathogens or by environmental chemicals? Movement of viruses via the pollen itself allows viruses to move among pollinator species that can also become infected. It is essential to determine if pathogens and/or exposure to environmental chemicals are impacting the native pollinator survivorship and population levels.

5. For More Information –

Contact the researchers below at Department of Entomology, 501 ASI Bldg, Penn State University, University Park, PA 16802. Dept. Phone number—814-865-1022; email to either Diana Cox-Foster (dxc12@psu.edu) or Christina Grozinger (cmgrozinger@psu.edu).

Cox-Foster Diana L: Lead researcher on NC-1173 project. Involved in determining the role of diseases in colony collapse disorder and overall bee health and in determining how stress and other factors interact.

Frazier James L: Researcher involved in determining the role of pesticides in colony health.

Frazier Maryann T: Senior extension associate responsible for dissemination of information and involved in research determining the role of pesticides in colony health.

Grozinger, Christina: Researcher involved in examining the interactions of genetics, behavior, diseases, and stress on honey bees and colony health and traits associated with disease resistance.

Mullin Christopher A: Researcher involved in determining the role of pesticides in colony health.
Ostiguy Nancy M: Researcher involved in examining the interactions of mites, chemicals, and diseases in colony health, using epidemiological methods.
Rajotte Edwin G: Researcher involved in determining the role of bee diseases and their transmission among honey bees and other pollinators.
Tumlinson James H: Researcher involved in determining the chemical ecology of small hive beetle, varroa, and bees and also in diagnosis of diseased bees.
VanEngelsdorp Dennis: Senior extension associate and Acting State Apiarist, responsible for education, regulation, and research on bee diseases, mite control, and Colony Collapse Disorder

Francis A. Drummond, University of Maine

1. Purpose –

1. Assess effects of non-lethal doses of insecticides on honey bee health
2. Present Extension talks to Maine honeybee keepers about new research findings associated with honey bee health

2. Current Activities –

A field trial designed to expose honey bee colonies to either uncontaminated blueberry flowers or imidacloprid contaminated flowers was conducted during the spring of 2009. The treated lowbush blueberry fields were sprayed a week prior to bloom.

3. What We Have Learned –

Results up to late June show that by comparing the number of capped brood in the pre-treatment (May 15, 2009) assessment to the third sample (July 28, 2009) that worker buildup appeared to be suppressed in colonies in the imidacloprid treated field compared to the non-treated field, however, the fourth sample (Aug 25, 2009) suggests that this decline in buildup may be reversed. Worker bee population levels appear to be suppressed in the hives exposed to imidacloprid in the first three sample dates, but by the fourth sample date this trend appears to be reversed. We did observe more chalkbrood in the treated hives, but the levels were still quite low (<0.1 % of brood). The hives will be inspected in the spring after overwintering.

4. Why is This Important? –

Imidacloprid and other neonicotinoid insecticides are becoming registered for lowbush blueberry pest management in Maine. Currently, both imidacloprid and acetamiprid are registered. Before blueberry growers begin to use these insecticides to a great extent the potential danger to honey bees that forage in treated fields should be assessed in field studies.

5. For More Information –

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Greg J. Hunt, Purdue University

1. Purpose

- Identify genes that confer resistance to Varroa and pathogens, and genes that respond to biotic challenges.

- Use knowledge of genetics to improve bee breeding.

- Conduct technology transfer to foster local queen breeders.

2. Current Activities

Dr. Hunt's NRI project is ready to map quantitative trait loci (QTL) and identify candidate genes that influence grooming behavior that confers resistance to Varroa mites. This is being coordinated with the CAP-funded initiative that will identify genes for another resistance trait, Varroa sensitive hygiene (VSH). Mapping genes for VSH is being led by the Baton Rouge USDA Bee Lab, with molecular and statistical analyses to be performed by the Hunt lab at Purdue University. All of the crosses and phenotypic analyses have been accomplished for both VSH and grooming behavior. DNA has been extracted from individual bees in the mapping families. We have sequenced the genomic DNA of the F1 queen used for mapping grooming behavior with the ABI SOLiD sequencing machine and identified about 4,000 useful SNPs (those single-nucleotide polymorphisms that are heterozygous in the queen and will be informative for following the segregation of genes in the backcross family). We are analyzing the sequence of the F1 queen from the VSH study and will identify SNPs that are informative for both traits. The molecular and statistical analyses will be performed in the spring of 2010.

A laboratory assay was developed to speed the selection for an important trait that confers resistance to Varroa mites – grooming behavior.

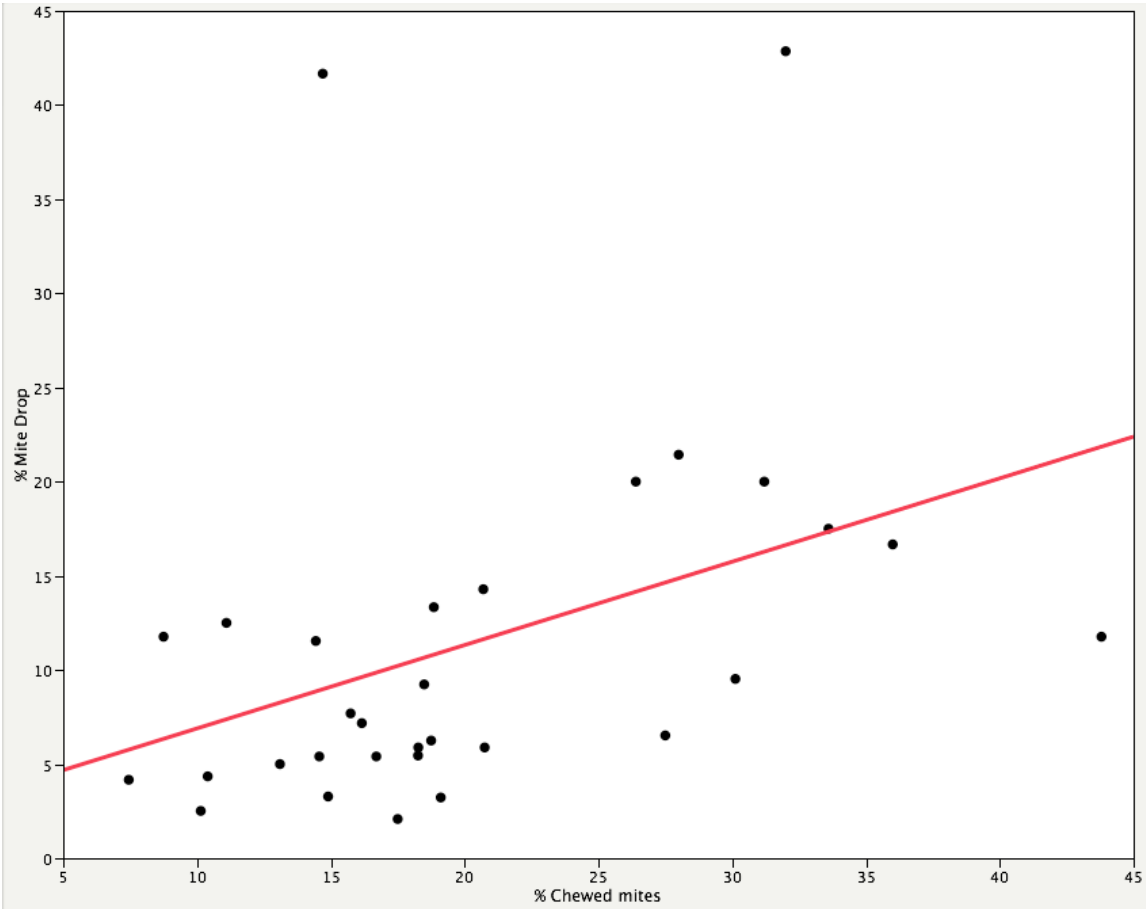
Preliminary cage studies used *Nosema ceranae* inoculation of bees from different source colonies.

A three-day queen rearing course was conducted at Purdue University in 2009. Twenty-seven participants learned how to successfully rear their own queens and were taught the basic principles of selection for stock improvement.

3. What We Have Learned

Results of the lab assay for mite-grooming behavior correlated with more tedious methods of examining the proportion of mites chewed by the bees and also correlated inversely with mite infestation levels, indicating grooming behavior reduces mite infestation.

Preliminary cage studies in which bees were inoculated with *Nosema ceranae* were conducted, using bees from about twenty sources.



Correlation of data from a lab assay for mite grooming behavior with colony results. The proportion of mites removed by the bees during three days in a cage assay correlated with the proportion of mites that were chewed on "sticky boards" at the bottom of the hives from which the bees were sampled ($p<0.01$).

4. Why is This Important?

Mapping QTL for resistance traits that impact Varroa mites will allow us to eventually identify genes that influence resistance and possibly lead to marker-assisted selection to speed breeding for resistance.

Grooming behavior was one of two traits (grooming and VSH) shown to be important when testing diverse queen sources for resistance to Varroa mites. The new assay will speed selection and underlines the importance of the trait.

Demonstrating variability of survival in response to *N. ceranae* is the first step in selecting for resistance and mapping genes that influence resistance.

Annual queen rearing workshops will help to foster "micro breeders" and maintain diversity of stocks adapted to northern conditions.

5. **For More Information** – Greg J. Hunt, ghunt@purdue.edu, Purdue University

John Burand, University of Massachusetts Amherst

Project Title: Development and Implementation of Diagnostic Tools to Assess Bee Colony Health
Project Director: John P. Burand, University of Massachusetts - Amherst
Co-PDs: Anne Averill, University of Massachusetts – Amherst, and Frances Drummond, University of Maine

1. **Purpose**

Develop and implement molecular methods to monitor the presence and abundance of pathogens and parasites in the major commercially-available pollinators (honeybees and bumble bees) used in the Northeast region.

Examine the impact of the use of anti-microbial/anti-pathogen treatments on these pathogens and on bee health. Our efforts focus on bees in cranberry and blueberry, two of the most economically important crops in the region.

2. **Current Activities**

Local and migratory bees collected in Eastern Massachusetts were analyzed individually for the presence of seven viruses. Only three of the seven were detected: 3 black queen cell virus (BQCV), deformed wing virus (DWV), and sacbrood virus (SBV). DWV was most common, followed closely by BQCV and then by SBV. BQCV and SBV were present at significantly higher rates in the migratory bees assayed.

3. **What We Have Learned**

The presence and level of viruses is different in migratory honey bees brought into Eastern Massachusetts compared to stationary hives in this area of the state.

4. **Why is This Important?**

These results bringing into question the impact that the importation of bees has on the health of local bee populations.

5. **For More Information –**

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Additional –
John Burand is collaborating with Anne Averill through the CAP and with Frank Drummond on imidicloprid work. He is conducting pathogen survey in honey bees and bumble bees. Northeast Regional IPM work involved with survey of both stationary hives with honey producers in the state, comparing pathogens in stationary, resident hives to commercial migratory, out of state hives for pollination of cranberry. He is also looking at Virus interactions in bumble bee cell lines on the molecular level. He interacts with county beekeeping associations and honey producers. They are extremely interested in what's going on. He is also educating average college student about bees in a general education course.

John Skinner, University of Tennessee

1. **Purpose –**

   1. Establish a managed pollinator Community of Practice with eXtension.org and populate web site with new literature on Best Management Practices and Bee Conservation.
   2. Document beekeeper adoption of sustainable bee management practices.
   3. Use knowledge of genetics to improve bee breeding.
   4. Better understand the pollination ecology of *Cornus florida* and *Cornus kousa*
   5. Survey fruit and vegetable farms for bee diversity.
   6. Explore alternative treatments for Nosema, a disease of honey bees.
   7. Provide beekeepers and the public with information about bees through workshops, field days, beekeeper meetings, and personnel communication.

2. **Current Activities –**

The eXtension.org website www.extension.org/bee_health has been developed over the past year with 123 articles and 119 FAQs. Content from the Community of Practice is solicited, modified, and uploaded by the University of Tennessee, but comes from many participants at other institutions. Funding comes from the AFRI, NRI (CAP) for Managed Bees and the USDA-ARS Areawide Program. Also through the CAP program, we helped develop an online beekeeper survey and are co-coordinating collection of bee and comb samples in Tennessee as part of above objective 2. Progress is underway to establish a bee breeding program at the University of Tennessee centered on the VSH line produced by the USDA-ARS, which will provide instruction for the public through workshops and bee breeding information through eXtension.org.

A survey of native pollinators to *Cornus florida* and *Cornus cusua* was conducted which included pollen flow analysis to determine how far insects move the pollen in an orchard setting. In a separate pollinator survey, funded by SARE, crops and wildflower plantings at farm sites are monitored for differences in diversity of bees to see if information can be extracted to better inform pollinator management decisions.

In a new SARE project, preparations are underway to examine alternative treatments for Nosema and evaluate their effectiveness using a histological approach. Bees will be infected with *Nosema spp*. and treated with 3 different compounds. The ability of the parasite to invade and reproduce in cells at will be examined at different temperatures.

The Tennessee Beemaster Program provides extensive classes and demonstrations with 5 classes offered in 2009. These classes emphasize how to manage mite and disease populations using integrated pest management strategies.

3. What We Have Learned? –

The web-initiative on eXtension.org has proven to be a very effective delivery method for information about bees, and a useful mechanism to collaborate with other Research and Extension personnel. Numerous tools, strategies, and staff support are offered by eXtension.org to the Bee Health group that has allowed us to quickly organize and make new information widely available, such as monthly progress articles from the CAP project on the latest research findings about bees.

Native bees in the families Andrenidae and Halictidae are the most important pollinators of flowering dogwood, *Cornus florida*. At some sites, beetles may also be important pollinators. Flies visit the flowers but are of secondary importance. Beetles (the rose chafer and soldier beetles) are the most important pollinators of Cornus kousa. Halictid bees are also very important. *C. florida* pollen tends to move 15 meters or less in an open pollinated orchard setting. This distance, however short, is much farther than suggested by a previous study. Differences in the species of insects visiting the flowers at different sites may be responsible for differences in pollen movement. Differences in relative bloom time between trees may also dictate how far pollen moves.

At farm sites, native bees, particularly bumble bees, are contributing a large number of visits to crop flowers. However, the diversity of bees differs depending on the crop. Some crops, and beneficial wildflower plantings, are preferred more by native bees while other crops and flower plantings are preferred by honey bees.

4. Why is This Important? –

The eXtension.org project will be a legacy of the CAP and Areawide project which will provide an organizational framework for future outreach collaborations. Accurate, credible information about bees will provide consistent deliverables throughout the project which will produce improved sustainable bee management decisions.

Information about the insects that pollinate flowering dogwood may aid in the cross breeding of these important ornamental landscape trees. Additionally this information helps better understand the ecology of Andrenid and Halictid bees in eastern Tennessee.

Small vegetable and fruit farms can easily monitor pollinating bees on their crops. Recognition of the contribution provided by native bees places value on the surrounding natural habitat which supports these non-managed bees. Beneficial flower plantings can be used to supplement food sources for wild and managed bees, but selection of flower types may be desirable to target wild bumble bees, the most frequent and constant native visitor.

5. For More Information –

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Kate Aronstein, USDA/ARS

1. **Purpose**

Determine the role of pests, pathogens and pesticides in causing disease in stationary honey bee colonies across the United States

2. **Current Activities**

7 CAP Stationary Apiaries across the US were established in 2009 to determine the role of different factors or the interactive effect between these factors (pests, pathogens and pesticides) in causing disease in honey bee colonies. This is a very large and complex study that will be replicated twice in the duration of the CAP Project. Scientists involved in Stationary Apiaries project have met on the regular basis and, as a result of these meetings and monthly conference calls, developed a unified set of documents that allowed all locations in this multi-state study to produce a meaningful and compatible data. First season (April-October, 2009) data reports will presented at the ABF/ABRC conference in Orlando, FL.

3. **What We Have Learned**

Preliminary data analysis (April-October, 2009) revealed that:

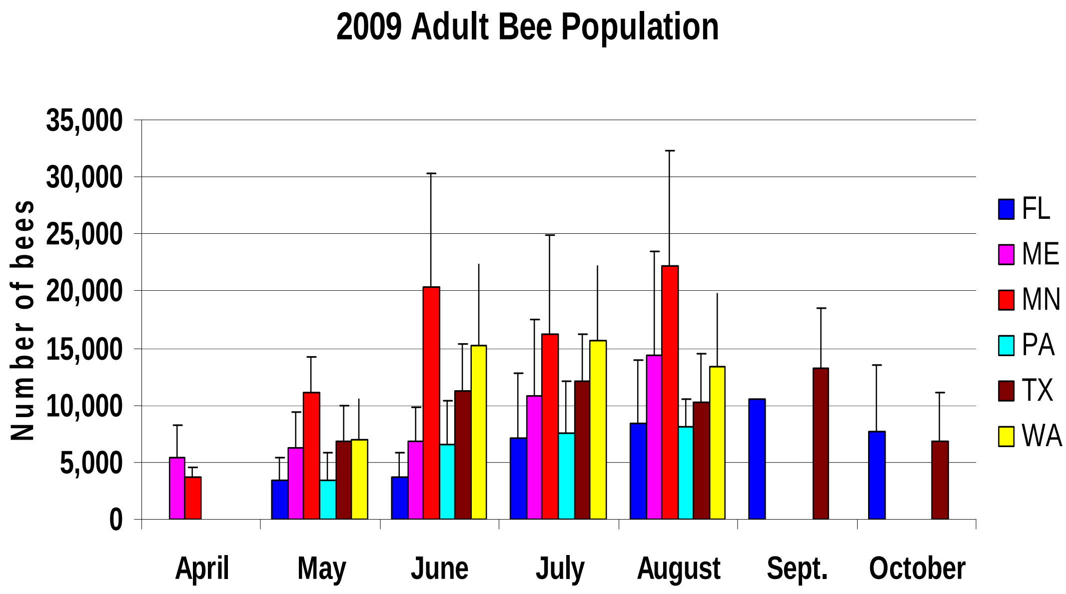
- The highest colony strength (estimated as a numbers of adult bees and brood, and colony growth rate) was recorded in two states, MN and WA.

- TX and FL had the highest level of *Varroa* infestation. These were also the only two states that reported a double infestation of HB colonies with *Varroa* mites and the Small Hive Beetle (SHB) in Stationary apiaries.

- The highest colony losses were recorded in TX and PA. Statistical analysis showed that colony losses in TX were highly correlated with *Varroa* infestation (RSQ = 0.93) and presence of SHB (RSQ=0.73). Though, these correlations do not explain real courses of colony losses, they point to a critical effect of pest infestation on honey bee health and survival.

CAP Stationary Apiary, MN (August 2009).

CAP Stationary Apiary, TX (November, 2009).



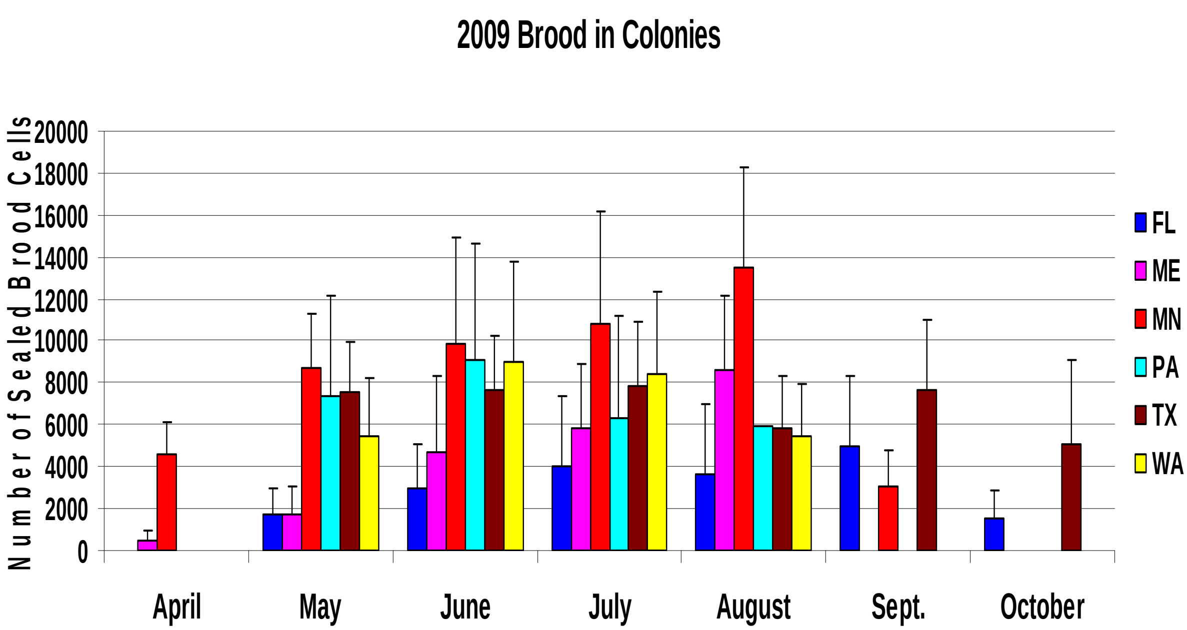


Fig. 1. Strength of honey bee colonies in CAP Stationary Apiaries across the US, 2009 season.

4. Why is This Important? –

Direct or indirect effects of pest infestation (e.g., *Varroa* mite feeding, *Varroa* transmitted viral diseases, inhibition of HB immune defenses, additional pressure by the secondary pest) have been for a long time suspected as key factors effecting strength of bee colonies. This and additional data generated by CAP study will help us to better understand the interactions between all these factors (e.g., pest infestation, microbial diseases and pesticide residues) and survival of honey bee colonies.

5. For More Information – Dr. Kate Aronstein, USDA/ARS, Kate.Aronstein@ars.usda.gov

Marian Frazier, Penn State, Extension

Local beekeepers have a strong demand for courses, which are being held. Disseminating info to new beekeepers through the MAAREC website http://maarec.psu.edu . A new Center for Pollinator Research has been established with an international conference coming up this summer at Penn State. Currently 22 Faculty members from numerous departments at Penn State are interested in center and coming together with it to participate. Policy a focus of the center. Numerous people coming from Europe and one from Nepal. Info is being shared between eXtension.org with MAAREC. Possible collaboration with MAAREC webinars through eXtension.org may be pursued.

Marion Ellis, University of Nebraska

1. **Purpose –**

- To determine if synergistic interactions exist among the various drugs used to control bee diseases and pests.
- To determine if synergistic interactions exist between selected fungicides (boscalid, pyraclostrobin, chlorothalonil and prochloraz) used on crops and drugs used in beehives.
- To determine if drugs used in beehives to control diseases and pests affect drone survival, or sperm viability.
- To determine if drugs used in beehives to control diseases and pests have sublethal effects on honey bee queen survival, egg laying, egg viability or brood production.

    - Drugs used in beehives include: miticides (fluvalinate, coumaphos, fenproximate, thymol, amitraz, oxalic acid, formic acid), antibiotics (terramycin, tylosin, fumagillin), and insecticides (coumaphos).

2. **Current Activities –**

- We have completed bioassays for synergistic interactions among the various varroacidal drugs. We have also completed bioassays for interactions between varroacidal drugs and selected fungicides used on crops.
- We have completed experiments to determine if varroacidal drugs affect drone survival, or sperm viability.
- We have completed experiments to determine if oxalic acid affects queen survival, egg laying, egg viability or brood production. Experiments to determine these parameters for other varroacidal drugs used in beehives to control bee diseases and pests are ongoing and will be completed by the fall of 2010.

3. **What We Have Learned –**

- Combinations of varroacides can be more toxic than individual varroacides.
- Bees exposed to low, sublethal doses of coumaphos (Checkmite+®) were 14 times more susceptible to tau-fluvalinate (Apistan®).
- Bees exposed to low, sublethal doses of fenproximate (Hivistan®) were 7.6 times more susceptible to coumaphos (Checkmite+®).
- Bees exposed to low, sublethal doses of fenproximate (Hivistan®) were 5.6 times more susceptible to tau-fluvalinate (Apistan®).
- Three varroacides, tau-fluvalinate, coumaphos and fenpyroximate, all appear to be tolerated by bees by means of cytochrome P450-mediated detoxification.
- Competition between varroacides for detoxification by cytochrome P450 may be the basis for the observed interactions.

- Some agricultural fungicides, known as "group 3" fungicides (egrosterol biosynthesis inhibitors) are designed to inhibit fungal cytochrome P450, and it appears that these fungicides are also effective inhibitors of honey bee cytochrome P450 as they can greatly increase the bee toxicity of varroacides that are detoxified by this group of enzymes.
- Pretreatment with prochloraz increased the toxicity of tau-fluvalinate 1118 fold.
- No changes in sperm viability was detected in drone honey bees exposed to a single sub-lethal dose of the varroacides amitraz, tau –fluvalinate, coumaphos, fenproximate, oxalic acid or thymol.
- Drones do not appear to be markedly more susceptible to these varroacides than worker bees.

4. Why is This Important? –

- Care should be taken with the 3 varroacides that are detoxified by cytochrome P450s, tau-fluvalinate, coumaphos and fenproximate, because of the potential for increased toxicity when present in combination.
- Multiple P450 detoxified varroacides should never be used in combination, and our results suggest that they should be alternated with a non P450 detoxified varroacide or a non chemical control technique (drone comb trapping, powdered sugar, resistant stock) as part of a resistance management plan.

P450 detoxified varroacides	Not P450 detoxified varroacides
tau-fluvalinate (Apistan®)	formic acid (Miteaway II®)
coumaphos (Checkmite+®)	thymol (ApiGuard® & ApiLife Var(®)
Fenpyroximate (Hivastan®)	amitraz (not available in U.S.)
	oxalic acid (not available in U.S.)

- Bees receiving coumaphos, fenproximate, or particularly, tau-fluvalinate treatment should not be exposed to "group 3" agricultural fungicides, as these fungicides may make these varroacide treatments much more toxic to bees.
- A single low dose exposure of adult drones to most varroacides does not appear to harm sperm drone survival or sperm viability, however, chronic exposure during development may affect survival and sperm viability.

5. For More Information –

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Additional –

Marion Ellis conducts beginning beekeeping programs and workshops, regional participants and recently hired a student, funded by nature conservancy, to do some work along the Platte River.

Reed Johnson works in Marion Ellis's lab, currently testing bees with pesticides and miticides using the whole genome microarray.

Marla Spivak, University of Minnesota

1. **Purpose –**

- Obj 1. To develop and recommend to beekeepers "best practices" for varroa mite control based on currently available methods and strategies for mite management.
- Obj 2. To evaluate the role and causative mechanisms of parasitic mites and pathogens such as viruses, protozoa and bacteria in honey bee colony deaths.
- Obj 4. To determine how environmental factors, including nutrition and management practices affect honey bee colony health.
- Obj 6. To coordinate research and extension efforts related to bee colony health.
- Obj 7. To facilitate, through research and extension activities, the development of industry-based honey bee stock selection, maintenance and production programs that demonstrably incorporate traits that confer resistance to pests, parasites and pathogens.

2. **Current Activities –**

Objective 1. Student Katie Lee developed the first efficient and rapid sampling plan to help beekeepers monitor mite levels, which will enable them to make educated treatment decisions and reduce pesticide treatments for the mites.

Objectives 2 and 4: Graduate student Mike Goblrisch is characterizing the pathogenicity and effects of Nosema ceranae on honey bee physiology and behavior. His hypothesis is that infection by *N. ceranae* provides a mechanism that promotes premature and pathological onset of foraging in workers, leading to a shortened life span at the individual level and an absence of adult bees at the colony level. He will test if *N. ceranae* causes JH titer to rise prematurely in haemolymph, and if Vg transcript and protein levels are simultaneously repressed in fat body and haemolymph. In addition, Mike will test if *N. ceranae* causes premature onset of foraging and result in reduced longevity for infected bees.

As part of the USDA-CAP stationary apiary project, we established one of six replicate apiary sites (MN, WA, TX, FL, ME, PA) to evaluate the factors that lead to colony death. The data for 2009 is being analyzed by collaborating labs across the country, and will include analyses of nutrition, levels of parasitic mites (Varroa and tracheal mites), Nosema disease, viruses, Small Hive Beetles, analysis of pesticide residues in comb and in pollen, and assessments of colony strength over 2 years time.

Objective 6. Our on-line course entitled, “Healthy Bees,” developed and sold by Univ MN Extension Store, is now available through the Bee Health eXtension site. Additionally, we provided information on breeding bees for hygienic behavior, and strategies for keeping bees healthy.

Objective 7. Through a unique technology transfer effort, we are assisting honey bee breeders in Minnesota and California select honey bee colonies for traits that help bees defend themselves against pathogens and parasites. These producers, particularly those in CA, are the source of most of the genetics (queen bees) for the beekeeping industry in the U.S. We visited 3 operations based in MN in February and in June 2009, and 18 operations in CA in March over two years (2008 and 2009). We spent 1-3 days at each operation each visit, helping them test their breeder colonies for hygienic behavior, a mechanism of resistance to diseases and parasitic mites. The three queen producers in Minnesota have effectively fixed the hygienic trait in their breeding line so we do not need to provide them inseminated breeders anymore. We continue to evaluate their stock on-site to ensure quality and degree of hygienic behavior. This hands-on service is the most highly effective method for helping producers adopt new techniques, despite the prevalence of on-line information. The bee breeders uniformly responded that they learned more in these on-site visits than they learned in over 10 years of listening to researchers and extension personnel at meetings. This assistance is the fist step toward the goal of implementing a permanent Tech Transfer Team (much like professional crop consultants) to help with stock selection and certification.

3. What We Have Learned –

Varroa Sampling Plan: We developed sampling plans for use by beekeepers and researchers to estimate the density of mites (per 100 bees) in individual colonies or whole apiaries. The recommendation to beekeepers is to collect ~300 adult bees from a single frame to estimate colony mite density. For sampling whole apiaries, beekeepers should examine ~300 bees from a frame in each of 8 colonies, to achieve $C = 0.25$. Beekeepers can extrapolate to colony mite density by multiplying the number of mites on 300 bees by a correction factor of 2. Researchers requiring greater precision in estimating colony mite density should examine three, 300-bee sample units to achieve $h = 0.5$ or $C = 0.1$. Researchers may need to estimate adult bee and brood numbers and their respective mite densities to estimate total number of mites per colony. These practical sampling plans will allow beekeepers and researchers to quantify mite density in individual colonies and apiaries to enhance understanding and management of *V. destructor*. The results will be submitted for publication in January 2010.

4. Why is This Important? –

The practical Varroa sampling plans will allow beekeepers and researchers to quantify mite density in individual colonies and apiaries to enhance understanding and management of this devastating mite parasite. The sampling plan will facilitate the development of regional treatment thresholds for *Varroa.*

Through technology transfer, we are making measurable gains in helping U.S. queen bee breeders incorporate traits that help honey bees resist pathogens and parasitic mites in commercially available stocks. A long-range goal is to develop a national stock certification and breeding program for honey bees. Toward this goal, we have published the results of our evaluations of stock from the bee breeders in MN in two U.S. beekeeping trade journals. We are applying for funds from the Almond Board in CA, and from other industry groups, to support the formation of a permanent Tech Transfer Team in CA to work with the bee breeders. This team would be similar to professional crop consultants common to other commodity groups.

5. **For More Information –**

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Publications

Spivak M, Reuter GS, Lee K, Ranum B. 2009. The future of the MN hygienic stock is in good hands! Amer. Bee J. 149(10): 965-967

Spivak M. 2009. News from the Ivory Tower: Pesticides, Nutrition, and Bees. Quarterly Newsletter of the Minnesota Honey Producers Association.
February.

Spivak M. 2009. News from the Ivory Tower: Conserving Pollinators: A Primer for Gardeners. Quarterly Newsletter of the Minnesota Honey Producers Association.
May.

Spivak M. 2009. News from the Ivory Tower: Bee-Friendly Lawns. Quarterly Newsletter of the Minnesota Honey Producers Association.
October.

Participants

Marla Spivak, PI.
Gary Reuter, Scientist, assists with all aspects of bee breeding, data collection for field experiments, routine maintenance of honey bee colonies. He co-teaches all extension courses and co-authors and delivers extension publications and talks.
Mike Goblirsch, PhD Graduate Student, Investigating Nosema disease of honey bees and its impact on colony health and Colony Collapse Disorder of honey bees.
Drs. Keith Delaplane (Univ GA), J. Ellis (Univ FL), N. Ostiguy (Penn State U), Brian Etzer (USDA Pesticides CT), Anne Averill (U Mass), Frank Drummond (U Maine), W Sheppard (WSU), Z. Huang (MSU), S Cobey (UC-Davis), Kate Aronstein (USDA Weslaco TX); J. Skinner (U Tenn): Research and Extensnion Collaborators on USDA-CAP grant
Darrel Rufer, Jeff Hull, Mark Sundberg: MN bee breeders propagating the MN Hygienic Line
California Bee Breeders Association, 18 commercial bee breeders in northern CA.

Target Audience

Beekeepers, bee breeders, fruit and vegetable growers that depend on bees for pollination and ecosystem services, master gardeners, general public

Nancy Ostiguy, Penn State

She is doing collaborative work on pesticides in honey extracted in fall and honey from the same colonies next spring. A paper is in process, which examines the effects of coumaphos and fluvalinate on survivorship of eggs.

Rachael Winfree, Rutgers University

1. **Purpose –**

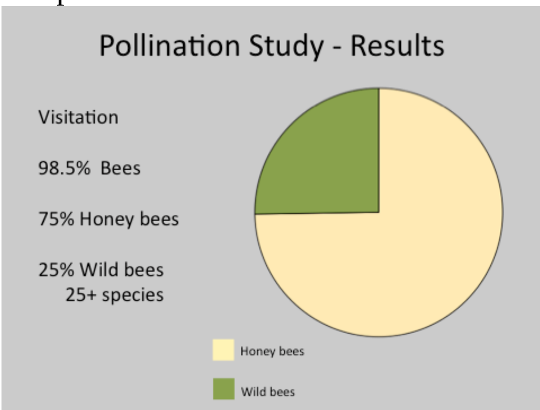
- Measure the extent of native bee and honey bee visitation to cranberry crop flowers
- Assess the role of farm field size and distance from the farm field edge in determining native bee visitation rates
- Measure pollen grain deposition per flower visit for each bee species

2. **Current Activities –**

In 2009 we measured bee visitation to cranberry crop flowers at 16 farms in New Jersey. We made over 150 hours worth of observations of bee visits. We collected 1700 specimens of native bees foraging on cranberry. These specimens have been identified to >25 species. In 2010 we will measure pollen deposition per flower visit.

3. **What We Have Learned –**

We found that honey bees provided 75% of the visits and native bees 25%. Most (67%) of the native bee visits were from bumble bees, although overall there were 25 native bee species recorded on cranberry. Field size did not have a significant effect on native bee visitation. However native bees were significantly more abundance on crops near the farm field edge, as compared to the farm field interior.



4. **Why is This Important? –**

Native bees are providing more pollination than expected to cranberry crops. Most of the farms in our study grow large monocultures of cranberry and New Jersey is one of the top 3 cranberry producing states in the US. Furthermore, all farms in the study stocked their fields with honey bees. Nonetheless native bees are still present and are likely making an important contribution to pollination.

5. **For More Information –** Rachael Winfree rwinfree@rutgers.edu Rutgers University

Ramesh Sagili, Oregon State
With a 70% research and 30% Extension appointment, Ramesh is starting up a new lab. A major focus of research is in nutrition comparing single source pollen and multi source pollen. No There is no baseline for hive health in Oregon, so he is doing bee diagnostics with commercial hives. Also, he is studying brood pheromones and methods for increasing pollination of carrot seed. He organized 6-7 bee schools, while trying to start a small masters bee program. Beekeepers are interested and want to do it.

Steve Sheppard, Washington State University
He is doing breeding and semen importation work, which includes distribution of imported stock, by giving queens to local associations and letting them breed from them. There are monetary challenges in doing more of this. Working with education on how introduce queens and other items to make distribution of selected genetics more successful. He has a student working on semen cryopreservation, which would improve preservation for travel and long term storage. He runs a Colony Health Diagnostic center that processes samples from all over the west. Currently, this service is free to beekeepers. Beekeepers have donated money for hiring staff to process about 3000 samples this year. The legislature gave one time money to hire an extension associate to upgrade their website.

Tom Webster, Kentucky State University
Extension: A state grant to extract honey has helped beekeeper to extract their honey and generate income. Grant paid for itself. Conducing a nosema survey with a transect of states from Minn. to Florida to understand the distribution of *N. cerana* and *N. apis*. He conducted a study with infrared spectoscopy looking at germination of spore in the gut and possible resistance to Nosema.

Wei-Fone, University of Illinois
CAP participant and Post-Doc researcher is studying the temperate effect in caged bees for *N. cerana.* In co-operation with others adding virus infection to caged studies in next year with temperature differences

William M Hood, Clemson University
Extension, 70% Master beekeeper program, biggest year every coming up for training new beekeepers, lots of people interested. 11 local associations hosting local courses, 250-300 people involved. Seemed to be growing.
Research, small hive beetle, cooperating with Keith Delaplane testing subleathald does effects of on hives, fluvalinate and comofus.

Comments from group: many states getting these massive requests

Zachary Huang, Michigan State University

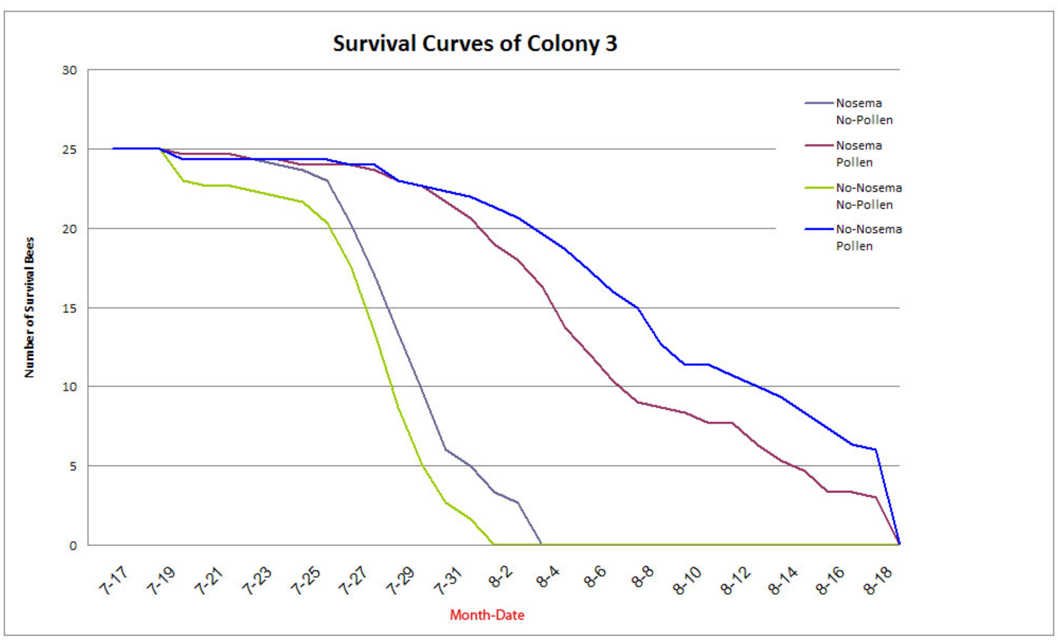
1. Purpose –

- To determine the effect of transportation on honey bee physiology
- To determine if *Nosema ceranae* spore production is dependent on pollen nutrition
- To determine the comparative virulence of *Nosema apis* and *N. ceranae*

2. Current Activities –

We studied (as a third replicate, with the two replicates from summer of 2008) how long distance transportation affected honey bee physiology. Two cohorts of bees from the same source colony were color marked upon emergence, split and introduced into two colonies of similarly strength. One colony was assigned to be transported (T) and one stationary (S). One group of bees experienced the migration as young bees (4-6 days old) and one cohort as old bees (14-16 day old bees). Bees were transported around for about 800 miles per day for 3 days but returned to E. Lansing each night. We then sampled aged marked bees for their juvenile hormone titer, total protein in the head, and size of hypopharyngeal glands. Size of hypopharyngeal glands showed consistent differences between the stationary and migrated bees, in both age groups. Juvenile hormone titers and head proteins did not show consistent differences.

We studied possible interaction of pollen nutrition and Nosema infection status using N. ceranae (50,000 spores per bee, with or without pollen provided). We observed a much stronger effect of pollen on worker bee survival, but nosema still exerting an effect (Fig. 1). However, we did not see any sign of interaction between nosema infection and the presence or absence of pollen.



We infected bees with the various dose (0, 3,000, 10,000, 30,000 and 100,000 spores per bee) of either *N. apis* or *N. ceranae* species to worker bees from three different colonies (2 cages per dose, 3 colonies), one group of bees were co-infected with 30,000 spores of both species. We did not observe a strong relationship between spore dose and worker survival in either species. However there is a trend that co-infected workers showing higher mortality compared to single species infections of the same dose. These results are preliminary because the spores of both species were stored in a refrigerator which may have affected *N. ceranae* viability more than *N. apis*, since the former is more sensitive to being stored.

3. What We Have Learned –

Transportation can have an adverse effect on honey bees, especially the development of workers hypopharyngeal glands. The adverse effect could not be compensated even after the bees were "rested" for about one week. Supply of pollen seems to be more important to the longevity of caged bees than the nosema infection status. There might be strain differences of *N. ceranae*, since in our study bees did not show high mortality even at the 100,000 per bee dose.

4. Why is This Important? –

Honey bees are the most important pollinators for our fruits and vegetables. The effect of long distance transportation was rarely studied even about 50% of the colonies in the nation are moved for almond pollination each year. Nosema ceranae is a newly discovered pathogen to affect the Western honey bees and may play a role in the recently occurring CCD (colony collapse disorder).

5. For More Information –

Zachary Huang, Department of Entomology, Michigan State University, East Lansing, Okemos MI 48814. bees@msu.edu, ph: 517-351-8136.

Additional –

Zach Huang has recently done some work in Australia (Nosema related work), Spain and China. He also helped with large bee meeting in Michigan, with much interest from beekeepers. He is conducting Tech Transfer through a in vitro lab for the EPA to teach commercial lab companies to do bee related assays. He is also doing a transportation study on its effect of bees.