**NC\_TEMP1173: Sustainable Solutions to Problems Affecting Bee Health**

**Statement of Issues and Justification**

Insect pollinators provide essential pollination services to growers of U.S. fruits, vegetables, nuts and seeds. Honey bees are the premier managed pollinator and account for $11.7 billion of the $15 billion of agricultural output attributable to insect-mediated pollination (Calderone, 2012). To satisfy the demand for pollination about 2 million of the 2.6 million managed honey bee colonies in the U.S. are rented and placed in nearly 100 different crops each year.

Efficient delivery of managed pollination services is threatened by the poor state of U.S. honey bees. Since the mid-2000’s beekeepers have consistently witnessed the loss of 30-35% of colonies over winter (Spleen et al., 2013). While beekeepers can often make up for these losses through intensive management of surviving colonies, current management tools are costly and may not be sufficient to indefinitely sustain the honey bee colony numbers or colony strength needed for pollination.

Wild pollinators also contribute substantially to agricultural pollination in many crops (Garibaldi et al., 2013). Unfortunately, the long-term health and abundance wild pollinators is under threat as well.

The causes of honey bee and pollinator declines in the U.S. are varied, complex, and defy a simplistic explanation, as multiple stressors are almost certainly involved. Significant progress in identifying contributing factors to bee declines has been made by many current members of the NC1173 multi-state project through a $4.1M, 4-year USDA CAP project that was funded in 2008 to study the causes of Colony Collapse Disorder (CCD) and other factors affecting bee populations. Current members are also part of the $5M CAP through the USDA Global Food Security program to establish the Bee Informed Partnership, an extension-only effort to collect and disseminate information about the health of the managed bee population.

Many of the findings from these large collaborative projects were presented and synthesized at the Stakeholders Conference on Honey Bee Health convened by the USDA and the U.S. Environmental Protection Agency in October 2012. The summary of this conference provides a roadmap for future research to be addressed by members of the NC1173 multi-state project:

**1. Parasites and pathogens -**

The Varroa mite and the viruses it helps to transmit remain a top concern for beekeepers. The gut parasite Nosema has been implicated some honey bee colony losses and other species of Nosema affect managed and wild bumble bees. A range of other bacteria, fungi, and animals negatively affect bee health. Improved understanding of the interaction between bees and their parasites and pathogens will yield better management and control strategies.

**2. Breeding and genetic diversity -**

Breeding resistance to parasites and pathogens in bees is a viable means to mitigate colony losses, but stock improvement through breeding is in no way complete and success will only be achieved through constant incremental improvement. Additionally, the genetic diversity and mating success of honey bees also plays a role in colony success.

**3. Forage availability and nutritional stress** -

The nutritional requirements of honey bees and other pollinators are not met by the floral landscape in some parts of the U.S. Research is needed into land- and farm-management practices associated with high levels of colony and pollinator success.

**4. Pesticides and environmental contaminants -**

Insecticides designed to kill insects may harm pollinating insects as well. Other pesticides and environmental contaminants also have the potential to affect bees. Additionally, drugs used to control pests and pathogens may have unintended side-effects. Work is needed to determine the effects of pesticide exposure on colony health, honey production and delivery of pollination services.

The consensus is that these multiple stressors, working in concert, are together responsible for the honey bee and pollinator health issues manifest in the U.S. While advances are currently being made in all four of these key research areas, a real solution to honey bee and pollinator health will only come by taking them all together a task that is too big and too complex to be managed by any researcher working independently. As such, the collaborative work fostered by the NC1173 multi-state research project is critical to building a holistic understanding of honey bee and pollinator health.

As such, there is a clear need defined by the stakeholders to mitigate the continued decline of honey bees and other insect pollinators. The consequences of inaction are a further destabilized food-production system, decreased yields and quality of fruits and vegetables, and potentially higher produce prices. The technical feasibility of the proposed working group is greatly facilitated by the existing practice of adjoining the American Bee Research Conference (ABRC) the annual professional meeting of the American Association of Professional Apiculturists (AAPA) with one of the three national apiculture associations in the US in alternating years: the American Beekeeping Federation (ABF), the American Honey Producers of America (AHPA), and the Apiary Inspectors of America (AIA). This tradition of interfacing with the clientele and other professional groups concerning beekeeping is ideally suited to collaboration, interaction, and discussion of current problems that face the industry. Thus there is a clear advantage of fostering this multi-state effort, because there is great similarity in the threats to American beekeeping across all regions. The impacts from these ongoing interactions have been significant (see above), and therefore a continuation of the NC1173 working group will advance these successes going forward.

**Objectives**

1) To evaluate the role and causative mechanisms of parasitic mites, viruses, and microbes in pollinator abundance and honey bee colony success

2) To facilitate the development of honey bee stock selection, maintenance and production programs that promote genetic diversity and incorporate traits conferring resistance to parasites and pathogens

3) To determine how land management practices affect pollinator nutrition and how nutrition affects honey bee colony productivity and success

4) To assess the effects of exposure to pesticides and other xenobiotics on the survival, health and productivity of honey bee colonies and pollinator abundance and diversity

5) To determine the effects of interactions among various factors affecting pollinator and honey bee colony health

6) To develop and recommend "best practices" for beekeepers, growers, land managers and homeowners to promote honey bee and pollinator health

**Related, Current and Previous Work**

*Leveraging Collaborative Grants*

Members have used the NC1173 multi-state project as a springboard to leverage $21.3 million in large collaborative multi-year grants from USDA-NIFA, in addition to $1.2 million in grants in 2012 from other federal agencies, commodity groups, beekeeping organizations and non-profit groups.

A majority of the committee members (19), representing 15 Universities and Agricultural Experiment Stations, were participants in the $4.1 million 4-year USDA Managed Pollinator Coordinated Agriculture Project (CAP) that ran from 2008 to 2012 (http://www.beeccdcap.uga.edu/ ). Meetings facilitated by the NC1173 project, and the NC508 project that came before it, were absolutely essential in making this project possible.

Four current committee members (Spivak, Skinner, Tarpy, Delaplane), and one incoming member (vanEnglesdorp), are Project Directors or Collaborators on the Bee Informed Partnership (http:// http://beeinformed.org/), a $5.7 million 5-year Extension-focused project funded by USDA to gather information from beekeepers and use that knowledge to improve the practice of beekeeping in the U.S. Additional members have worked to connect this project with local beekeepers in the various states and to facilitate the collection of bee samples. The goal of this project is to reduce the number of honey bee colonies that die over winter and the ongoing results will serve to inform and focus all aspects of practical research on beekeeping.

Members are also involved in two multi-institution USDA-funded grants focused on pollination from more of a grower’s perspective: the ‘Integrated Crop Pollination” project (Ellis and Winfree) and the “Pollination Security for Fruit and Vegetable Crops in the Northeast” (Drummond, Stoner, Burand, Eitzer and Skinner). While NC1173 has historically been focused on bee management, it is becoming increasingly clear that bee nutrition is heavily influence by landscape management and that the actions of growers, land managers and homeowners are together is an underappreciated component in overall bee health.

*Deliverables to Stakeholders*

The scientific forum for the NC1173 group, the American Bee Research Conference (ABRC), is open for attendance by beekeepers. In most years this meeting is scheduled during one of the two national beekeepers meetings (ABF or AHPA) and participants registered for the larger trade meeting are invited to attend the NC1173-affiliated scientific meeting without further expense. Additionally, the proceedings of the ABRC meeting are published in one of the national beekeeping trade journals, “American Bee Journal” or “Bee Culture” and several are available through the eXtension web site (<http://www.extension.org/pages/58650/proceedings-of-the-american-bee-research-conference-2011#.UqnJrSfWtYU> )

Best Management Practices for a number of practical beekeeping topics have been developed and are available online: <http://www.extension.org/pages/33379/best-management-practices-for-beekeepers-and-growers#.UqiY0bSA3L9>

Additionally, more than 30 research-based articles have been written for the beekeeping community by members as part of the Managed Pollinator CAP: <http://www.extension.org/category/bee_cap_updates>. These articles were also published monthly in both nation beekeeping tradejournals. These articles form a core or the Bee Health Community of Practice (<http://www.extension.org/bee_health> ) that currently includes over 300 pages of research-based information bee biology, native bees and honey bee management practices.

***Articles available on eXtension organized by current project objectives.*** [http://www.extension.org/pages/24315/managed-pollinator-cap:-coordinated-agricultural-project#.UqjnRdJDsrV](http://www.extension.org/pages/24315/managed-pollinator-cap%3A-coordinated-agricultural-project#.UqjnRdJDsrV)

**Objective 1 Pests and Pathogens**

Varroa Mite Reproductive Biology (Huang)

Impacts of Varroa Parasitism on Honey Bee Health (Aronstein)

Sunlight, Water, and Nosema Spores (Webster)

Effects of Nosema on Honey Bee Behavior and Physiology (Huang)

Genetic Toolkits for Bee Health (Evans)

Microsporidia: Friend, Foe (and Intriguing Creatures) (Solter)

Wild Bee Status and Evidence for Pathogen Spillover with Honey Bees (Averill)

Nosema ceranae - The Inside Story (Webster)

Detect Nosema Parasite in Time to Save Bee Colonies (Aronstein)

**Objective 2 Stock selection and genetics:**

Recollection of European Apis Mellifera Germplasm for Honey Bee Breeding (Sheppard)

Honey Bee Genetic Diversity and Breeding: Towards the Reintroduction of European Germplasm (Sheppard)

An Update on Bee Breeding Efforts in Indiana: Breeding for Resistance to Israeli Acute Paralysis Virus (Hunt)

Laying Groundwork for a Sustainable Market of Genetically-Improved Queens (Spivak)

Breeding Bees for Resistance to Parasites and Diseases (Hunt)

**Objective 3 Nutrition**

Honey Bee Nutrition (Huang)

**Objective 4 Pesticides**

Assessing varroacide toxicity to queens and workers (M. Ellis)

Neonicotinoid Seed Treatments and Honey Bee Health (Hunt)

Nest Location in Bumble Bees: Effect of Landscapes and Insecticides (Averill)

Miticide and Fungicide Interactions (Johnson)

Pesticides and Their Involvement in Colony Collapse Disorder (Frazier, Mullin)

Assessing the Risks of Honey Bee Exposure to Pesticides (M. Ellis)

Pesticides Applied to Crops and Honey Bee Toxicity (M. Ellis)

When Varroacides Interact (Johnson)

**Objective 5 Combinations**

The First Two Years of the Stationary Hive Project: Abiotic Site Effects (Drummond, Aronstein, Ellis, Evans, Chen, Ostiguy, Sheppard, Spivak, Vissher)

The Managed Pollinator CAP after Three Years: Highlights and Emerging Trends (Delaplane)

Best Management Practices (BMPs) For Beekeepers Pollinating California’s Agricultural Crops (M. Ellis, Delaplane)

Honey Bee Medical Records: The Stationary Apiary Monitoring Project (Spivak)

Sustainable Beekeeping (Ostiguy)

*Publications*

In 2012 members of NC1173 reported publishing 67 scientific journal articles, book chapters and trade publications. Nearly half of these (27) were the product of collaborative research among members of the multi-state project at different institutions.

**METHODS**

***Objective 1 -- To evaluate the role and causative mechanisms of parasitic mites, viruses, and microbes in pollinator abundance and honey bee colony success***

**Rationale and Significance**

Honey bee colonies are constantly under threat from existing pest and disease complexes and also from other new species invasions. Recently, some honey bee colonies in the U.S have become infested with *Nosema ceranae* a gut parasite which is prevalent in the Asian honey bee. *Nosema ceranae* is a newly found fungal parasite that affects *Apis mellifera* worldwide (Evans and Schwartz, 2011; Higes et al., 2013). Currently little is known about biology and epidemiology of this relatively new species of *Nosema* and the effects of this species on honey bees at both the individual and colony level vary from study to study. Understanding the basis of this variation is important for recommending a course of action for beekeepers through BMPs (http://www.extension.org/pages/33379/best-management-practices-for-beekeepers-and-growers#.UqnxdifWtYU)

**Methods**

**Colony-level distribution of pathogens:** Within colony prevalence and intensity of *Nosema ceranae* and viral infection will be determined. Experimental colonies with four different age cohorts will be established and inoculated with known concentrations of *Nosema* spores. Two weeks after spore inoculation the experimental colonies will be killed by freezing and the prevalence and intensity of *Nosema* infection in each age cohort will be determined by light microscopy. Results from this study will provide insights on prevalence and intensity of *Nosema cerana*e. This information regarding prevalence and intensity will help better formulate *Nosema* sampling protocol that will help beekeepers assess realistic need for colony treatment.

QT-PCR analysis will be used for determining infection levels of bees with IAPV, DWV, BQCV, SBV and other viral pathogens. Eggs, larvae, pupae or adults will be placed in a 1.5 ml tube containing RNALater and frozen at -80o C until analysis. Standard extraction protocols using RNAlater will be followed and QT-PCR using SYBR green will be performed using validated published primer sequences for bee pathogens (de Miranda et al., 2013; Evans et al., 2013).

**Individual-level consequences of pathogen infection:** For cage studies, we use the following protocol to determine consequences of pathogen infection (Goblirsch et al., 2013): 1. Obtain newly emerged bees by incubating mature brood overnight inside cages at 35 degrees C and 50% RH. 2. Fresh *Nosema* spores (within 24 hrs) are obtained from live bees, cleaned of debris by centrifuging in water (5000xg, 10 ming), verified by PCR to be mono-specific. 3. Individual workers are starved for 2 hrs and then hand fed with a calibrated dose of *Nosema* spores in 2 microliter 50% sucrose syrup. Control bees are fed syrup only. 4. Workers are isolated in 20 ml glass scintillation vials for 30 min at 35C to reduce transfer of spores among bees. 5. Workers are then caged together and mortality observed daily. Sugar syrup (50%) and pollen are changed every 5 days. 6. Pollen are frozen (-20C) and heated (60C) at 12 hr minimum for three cycles to inactivate potential spores of either N. ceranae or N. apis. 7. We then use survival analysis to compare different effects of different treatments, either using SAS or R package. For colony study, bees are paint-marked or tagged with numbers and inoculated with Nosema spores (or sugar only) and released to colonies. Survival is determined once every 5 days by noting the presence of numbered bees on each frame (2x). Age of first foraging is observed by recording the ID of returning bees at least 2 hours per day.

**Collaborators:** Huang, Solter, Sagili, Cox-Foster, Ostiguy

***Objective 2 -- To facilitate the development of honey bee stock selection, maintenance and production programs that promote genetic diversity and incorporate traits conferring resistance to parasites and pathogens***

**Rationale and Significance**

The many problems that currently face the U.S. honey bee population has underscored the need for sufficient genetic diversity at the colony, breeding, and population levels. Genetic diversity has been reduced by three distinct bottleneck events, namely the limited historical importation of subspecies and queens, the selection pressure of parasites and pathogens (particularly parasitic mites), and the consolidated commercial queen-production practices that have reduced the number of queen mothers in the breeding population. An additional goal of this research is to measure the genetic impact of stock importation and release on domestic stocks.

**Methods**

**Analysis of diversity:** We will use a meta-analysis approach to compare the pedigree relationships of honey bee reproductives (queens and their mates) across five different studies and to quantify the overall genetic diversity of breeding populations. We will compare the inferred genotypes of queens and their mates from microsatellite analyses of worker offspring from a feral Africanized honey bee population (which serves as a negative control for inbreeding), an experimentally derived population of sister queens (which serves as a positive control for inbreeding), and three separate commercially managed populations. We will also use microsatellite analysis to compare allelic diversity these New World populations of honey bees with populations of Old World bees where *Apis mellifera* is endemic.

We will then compare the relatedness of all drones mated to each queen (mate-mate), all queens within each population (queen-queen), each queen with each of her mates (queen-mate), and all drones within each population (drone-drone). This will enable us to quantify the levels of genetic similarity among the managed honey bee populations compared to the two ends of that continuum.

**Preservation of favorable genetics and augmentation of diversity using imported honey bee semen**.  Since 2010 we have developed practical methods for the cryogenic storage and recovery of honey bee germplasm and maintained aliquots of imported material in a genetic repository.  In 2013, we expanded this cryogenetic program to include conservation of “top-tier” genetics of US domestic stocks of honey bees.  The goal of this program is to allow queen producers the future ability to “breed through time” via backcrossing to extant lines.

Effective cyropreservation techniques have also made possible the importation of honey bee germplasm for evaluation and breeding purposes. Annual collection and importation of honey bee semen from three Old World subspecies of beekeeping interest (*ligustica, carnica and caucasica*). Since 2008, we have collected and imported honey bee germplasm, managed it through USDA-APHIS quarantine. The genetic material has also been incorporated into commercial stocks of honey bees through various collaborating queen producers in California.

Collaborators: Tarpy, Sheppard, Delaney, Spivak, vanEngelsdorp

***Objective 3 -- To determine how land management practices affect pollinator nutrition and how nutrition affects honey bee colony productivity and success***

**Rationale and Significance**

Flowering plant species differ considerably in the nutritional content of their pollen, and this can have important ramifications for bee health (Levin and Haydak 1957, Standifer 1967). Nectar is also important for bee nutrition because, as the primary carbohydrate source for bees, sufficient quantities are essential for larval growth and meeting the energetic demands of bee activity (Brodschneider and Crailsheim 2010). The diversity and quality of floral resources at the landscape level may therefore have a significant impact on the nutrition of bees, particularly those with large foraging ranges such as honey bees. We will investigate the role of nutrition on colony physiology, growth and immunocompetence, and recommend both bee and land management practices to improve bee nutrition, in several ways. These data can be incorporated into models used to support quantitative analyses and qualitative assessments for pollinator habitat enhancement, and to determine pollen preferences among *Apis*  and non-*Apis* bees.

**Methods**

1) We will study how land use and the diversity of foraging resources affect the growth, development, and health of honey bee colonies by experimentally placing hives into landscapes that vary in floral resource quality and diversity, and subsequently measuring variables related to hive health. For example, we could use three land types (three treatments): 1) florally diverse natural land, 2) heterogeneous cropland containing a variety of flowering crop types, including crops depending on bee pollination, as well as non-crop vegetation, and 3) monoculture cropland. We will use a monoculture flowering crop system that relies on honey bee pollination, such as blueberry or cranberry, and will select only fields that are managed without the use of pesticides. We will use ArcGIS to find 5 replicate sites within each of the 3 landcover types for a total of 15 sites in all. Nested within each site we will place 3 honey bee hives in different places. Due to sharing the same landscape, these 3 will not be considered independent replicates, but they will serve to reduce error and produce a better measure of the mean colony health for each landscape. Experimental hives will be developed using 45 identical packages of bees (Italian variety) established into single deep 10 frame hive bodies with drawn comb. After placing hives in early May, we will inspect hives every two weeks until late September. Pollen traps, which collect pollen from honey bee pollen foragers when they enter hives, will be placed on all hives three days prior to inspection and removed on the day of inspection, in order to determine the dominant pollen type brought into each hive. We will assess colony growth and development by weighing hives and measuring proportional brood cover on frames. From within each hive, we will select 20 workers at random to measure and weigh to determine differences in worker size and mass between treatments. We will assess colony health by measuring colony load of two common honey bee pathogens: *Nosema ceranae* and *Varroa destructor* mites. *Nosema* levels will be determined by spore counts of groups of 20 bees (Cantwell 1970) and mite loads will be determined through the use of the powdered sugar method (Macedo et al. 2002). Hypopharyngeal gland protein content of nurse bees will be estimated using Bradford protein assay and colony growth will be measured each week. Phenoloxidase, prophenoloxidase and glucose oxidase enzyme activities will also be analyzed that are indicators of honey bee immune system function (Di Pasquale et al., 2013). The effect of the landscape and thus floral variety treatments on the various outcome measures related to honey bee health will be analyzed with Generalized Linear Mixed Models (for repeated measures) with landscape treatment as a fixed effect and site as a random effect. Pollen collected in pollen traps will be analyzed as both outcome of landscape treatment, and predictor for colony growth and health.

2) Analogous studies of how land use and the diversity of foraging resources affect the growth, development, and health of native bees could be done using a commercially available Bombus or Osmia species, as in Williams et al. (2012) and Williams and Kremen (2007).

3) The value of specific floral (nutritional) resources for multiple species of wild, native pollinators can be inferred by collecting data on which flower species native pollinators prefer to forage on while collecting pollen and/or nectar. Such data can be obtained through an observational design, in which researchers collect pollinator from plants at multiple sites while also collecting data on the relative abundance of each flowering plant species. An index of pollinator preference can then be calculated (Johnson 1980). {Johnson, 1980 #2562}Alternatively, an experimental design can be used, in which replicate single-species plots of native plant species are sampled over the entire growing season to determine which wild pollinator species collect pollen and/or nectar at each. One method currently being undertaken is to identify pollen collected by bees from on and off-crop flowers and bee-specific at 12 cranberry farms. Pollen grains are counted and identified for each pollen load with the goal of determining pollen preferences as well as site-specific bee diversity.

Using either the observational or experimental design, the preferred plant species can be determined and this information can be used in land use planning and pollinator restoration work. For example, in restoring habitat to support native pollinator species, it is important to include preferred forage plants such that some plant species are in bloom at all times in the growing season.

**Collaborators:** Winfree, Spivak, Ellis, Johnson, Toth, Nault, Sagili, Huang, Averill

***Objective 4 -- To assess the effects of exposure to pesticides and other xenobiotics on the survival, health and productivity of honey bee colonies and pollinator abundance and diversity***

**Rationale and significance**

Bees are exposed to an array of xenobiotics in the course of foraging in a landscape – natural plant-derived toxins, metals, pollutants, pesticides and spray adjuvants (Johnson et al., 2010). In high concentration these xenobiotics may have direct effects on bees, either through acute mortality at the larval or adult stage, or more subtly, through colony-level effects that harm a colony’s chances of surviving over the course of a season. Combinations of xenobiotics may increase bees’ susceptibility to exposure to a particular pesticide, or the cocktail of xenobiotics may produce unexpected synergistic interactions (Glavan and Bozic, 2013). Further work is needed to determine relevant xenobiotic exposures in bees as well as establish exposure levels above which xenobiotic exposure directly harms bees’ ability to produce hive products and deliver pollination services. Insights gained can be provided to beekeepers, growers, manufacturers and regulators to mitigate any effect that xenobiotic exposure has on honey bee health and productivity.

**Methods**

**Seed treatment insecticides in agronomic crops:** Most of the corn and soybean seed planted in this country has been coated with systemic pesticides.  During planting of that seed a dust cloud can be created with very high levels of pesticide which can transport off site and potentially expose bees either directly to the cloud, or, indirectly by landing on bee forage (Krupke et al., 2012).

A survey of apiaries will be done that will represent areas of intensive row-crop production, areas of commercial cucurbit vegetable production where honey bees are used for pollination purposes, urban areas, and areas where honey bee exposure to neonicotinoid insecticides is unlikely. Satellite imagery and other land-use databases will be used to assess potential food sources around each apiary.

Transport processes affecting seed treatment insecticides will be examined by placing dosimeters at various distances around fields during planting and then analyzing those dosimeters for pesticides by liquid chromatography/mass spectrometry.  Different fluency agents added to the seed will be compared to determine practices that will minimize this potential exposure route.

**Assessing exposure and effects of spray adjuvants:** Assessing effects of Impacts of co-formulants and their degradates, individually and corporately at sub-lethal levels, on key honey bee behaviors/physiology including memory and learning will be investigated. Toxic or sublethal effects on honey bees of pesticide and inert combinations relative to formulation controls, including interference with associative learning, will be determined by direct feeding or incorporation in artificial nectar or uncontaminated pollen or wax, or by topical application of extracts to bees or brood. Colony-level impacts of formulation ingredients will be determined in field experiments.

 Frequently found co-formulants in pesticides and spray tank adjuvants will be characterized and their identity confirmed. Hive samples of stored pollen, comb wax, nectar and bees or field floral samples with known or suspected high levels of frequently occurring fungicides, insecticides and other pesticides will be analyzed for active ingredients and inerts on our LCMS-2020 at primarily the > 5 ppb limit of detection (LOD). Portions of priority samples will be preserved and sent to the USDA-AMS-NSL in Gastonia for follow-up residue analysis at a more sensitive 1 ppb LOD. Remaining portions of each sample will be used in toxicity and behavioral studies.

After identification of key inert ingredients in agrochemicals used frequently around bees, we will develop an appropriate sensitive method for their analysis, similar to a recent methods developed in our lab for analyzing three trisiloxane surfactants and nonylphenol polyethoxylates. We will use these analytical methods to study the environmental fate of trisiloxane, nonyl- and octylphenol surfactants and other key inerts, including their degradates, in and around beehives.

Metabolism of free or formulated inerts and pesticides within bee bioassays (including excreta) or in pollen, wax, nectar and other matrices will be addressed through analysis over time of residues relative to the treatment or dose using the appropriate LC/MS-MS method based on chromatographic, spectral and mass transition comparisons with authentic standards.

To assess potential toxicity or other negative impacts of formulation components, inerts alone or in combination with active ingredients will be fed at dose levels detected in hive samples in artificial nectar, royal jelly diet or pollen-substitute cakes to adult bees, queens, drones, and brood, or topically applied, and other factors such as bee behavior and colony longevity evaluated. Mortality and other toxicity symptoms as well as altered behaviors will be scored over the course of the bioassay, and regressed relative to pesticide treatment dosages. Chronic feeding of bioactive formulation ingredients and combinations will also be conducted. Altered behaviors will be investigated further through proboscis extension reflex (PER) bioassays. A tier approach will be used where significant impacts at the larval and adult toxicity bioassay and sublethal PER levels will proceed into semi-field (nuke) or field level studies when priority effects are observed.

**Field monitoring for pesticide exposure:** An important aspect of assessing the effects of pesticides on bees is to identify routes of exposure and measure concentrations of different pesticides to which the bees would be exposed over time.  By trapping pollen as it is brought into the hive by honey bees, collecting it on a regular basis and analyzing it for a range of pesticides using liquid chromatography/mass spectrometry, we can monitor exposure by this route over the long term and quantify “field realistic” levels.  We plan to monitor hives in both urban and agricultural environments, and to evaluate toxicity using a Pollen Hazard Quotient (concentration in ppb/LD50 in ug/bee) (Stoner and Eitzer, 2013).

**Field effects of pesticide exposure:** Hives will either receive xenobiotic exposure either through direct application or through direct field exposure when pesticides are used on nearby crops. For direct application exposure the xenobiotics will be applied through contaminated wax sprayed on foundation or contaminated pollen patties or sugar syrup fed to the colonies. Uncontaminated wax, pollen patties or syrup will serve as the control for direct application experiments. For field exposure hives half of the experimental hives will be relocated to the agricultural site(s) at the start of the growing season while half are maintained in areas with less pesticide use.

Hives will be given sucrose syrup and protein patties as needed for establishment. All the hive components (box, bottom board, cover and frames with foundation comb), hereafter referred to as the “non-colony” component, will be weighed individually. Bee colonies will be monitored for at least two months prior to deployment in field studies. Research sites in agricultural fields planted with crops that are exposed to at least some bee activity and with moderate to heavy pesticide application will be identified through consultation with growers and beekeepers. During the initial monitoring period, all hives will be kept on stainless steel electronic balances (TEKFA® model B-2418, Galten, Denmark) with an overall precision of ±20 g. Two temperature probes will be placed between the center frames of the brood box. The balance and probes will be linked to 12-bit dataloggers (Hobo® U-12 External Channel, Onset Computer Corp.). The balance and datalogger systems for hives at the agricultural site will be solar- and battery-powered. The dates and locations of pesticide applications and other row crop management practices will be recorded and correlated with changes observed in the continuous datasets. Ambient temperature and rainfall will be recorded throughout the experiments.

At least 50 adult workers will be sampled from frames within each hive two weeks prior to the start of the experiment, and 10 bee bread samples will be taken per colony and mixed for a pooled bee bread sample per colony. Samples will be collected into coolers and transferred to freezers for storage prior to shipment. A subsample of the bees will be examined for disease causal agents, another subsample of bees and the bee bread samples will be forwarded to a professional laboratory for pesticide residue analysis (USDA-AMS-NSL, Gastonia, NC), and the remaining bees will be weighed with a precision electronic balance to estimate average bee weight.

Hives will be inspected monthly and additional samples of 25 nurse bees taken and processed as described above. At each inspection the hive will be examined for signs of disease or parasites and treatment provided as necessary to all the hives. Also at each inspection, each brood box frame, and the super box if present, of each hive will be weighed separately on a portable electronic balance after shaking off the bees. Digital photographs will be taken of each side of each frame using a high resolution camera, and the area of brood, capped honey and pollen per frame will be estimated using image analysis software (J. Byers, U.S. Arid-Land Agricultural Research Center, USDA-ARS, Maricopa, AZ). Weights of brood and food stores will be estimated from these data using techniques described by Meikle et al. (2008). Weight of the “colony” component consisting of adult bees, brood, honey, pollen and wax will be estimated by subtracting the non-colony weight from the observed hive weight. The weight of empty drawn frames of comb will be estimated by weighing at least 20 frames of empty drawn comb and subtracted from the frame weight data. Adult bee mass will be calculated as the difference between the total hive weight and the sum of the weights of the colony and non-colony components. Total adult population will be estimated by dividing the adult bee mass by the average worker weight determined from the bee samples. Brood weight per frame will be estimated by counting brood cells using the imaging software and calculating weight based on either published brood density values or by weighing frames containing only brood and converting those values to density by regressing weight (total observed frame weight less weight of empty drawn comb) on surface area. Food weight will be estimated by subtracting brood weight from total frame weight for a given frame.

**Collaborators**: Mullin, Eitzer, Stoner, Skinner, Hunt, Johnson, Spivak

***Objective 5 -- To determine the effects of interactions among various factors affecting pollinator and honey bee colony health***

***Objective 5a – Determine whether there is an interaction between nutritional status and Nosema disease***

**Rationale and significance**

Nutritional status can affect the functioning of immune system in insects (Siva-Jothy and Thompson, 2002). In honey bees, foragers have inferior immuno-capability compared to nurses, mainly because the high vitellogenin concentration in nurses protects nurses from oxidative and other stresses (Seehuus et al 2006). Because pollen is the only source of protein for honey bees, we hypothesize that pollen nutrition can play an important role in the development of the disease because poor nutrition might result in a less robust defense system, resulting in stronger negative effects by *N. ceranae* and bee viruses in both caged studies and in whole colonies.

**Methods**

Newly emerged workers will be individually inoculated with 10,000 spores on day 0 and provided with no pollen, mixed-bee pollen or “monocultural” pollen from a few representative plants important for honey bee pollination (cherry, almond, corn, etc). Bees are provided with 50% sugar syrup (changed every 3 days) and distilled water *ad libitum*. One hundred bees per cage (cage size: 14 × 12 × 16 cm), 4 cages per treatment will be used for each colony. Longevity of workers will be compared among the treatments by survival analysis using SAS 9.1.3.

Using small, experimental colonies, we will examine the effects of the interaction between diet (no pollen, monofloral pollen, and polyfloral pollen) and IAPV infection (in bees that already possess DWV and BQCV) on colony population, number of bees exiting/returning to the hive, nutritional state (protein, lipid, micronutrients), and the gut transcriptome.  This will allow us to fully characterize the effects of poor nutrition and viral infection, and observe whether they lead to CCD-like symptoms such as reduced population and colony abandonment by workers.

We are infecting bees that already harbor background levels of ubiquitous viruses (such as DWV and BQCV) with high levels of a viral cocktail (containing IAPV and SBV) under three different diets: no pollen, monofloral pollen, and polyfloral pollen.  We are testing whether bees under monofloral and no pollen diets show increased viral infection rates, increased mortality, and increased viral replication as compared to bees fed polyfloral pollen diets, as well as documenting effects on nutritional physiology.

**Collaborators:** Toth, Huang, Sagili

**Objective 5b – *Determine whether there is an interaction between landscape characterization and viral infection***

**Methods**

We are measuring the incidence of viruses in *A. mellifera* and 10 native bees across landscapes encompassing different types of habitats: prairie (remnant and restored), crop-dominated (>70% croplands within 2km), and limited-crop habitat (<30% croplands within 2km). We used nets to collect samples of 10 common wild bee species (families Apidae, Halictidae, Colletidae, and Megachilidae) from several replicates of:  (1) remnant and restored prairie, (2) soybean fields in crop-dominated landscapes and (3) soybean fields in limited crop landscapes.  Also, sampled *A. mellifera* from over 40 apiaries across the state of Iowa with variation in the extent of surrounding crop and urban habitat. Bees (n>10/species) were flash frozen and stored at -80°C for species identification and real time qRT-PCR, which will provide quantitative estimates of the levels of 8 common *A. mellifera* viruses-of-concern.  Using GIS (Geographical Information System) landscape characterization methods we will test for correlations between surrounding land use and virus diversity.

**Collaborators:** Toth, O’Neal, Cox-Foster

***Objective 6 -- To develop and recommend "best practices" for beekeepers, growers, land managers and homeowners to promote honey bee and pollinator health***

**Background:** Members have developed a series of 7 Best Management Practices for the eXtension.org Bee Health Community of Practice (<http://www.extension.org/pages/33379/best-management-practices-for-beekeepers-and-growers#.UqhtXSfWtYU>). These include BMPs on “Nutrition”, “Pest/Varroa Control”, “Disease Control/Nosema”, “Hive Equipment”, “Colony Management”, “Business Management” and “Almond Growers Renting Bees”.

**Methods**:

Research teams are comprehensively addressing causes of bee decline in **Objectives 1-5**. As new insights are gained from these research objectives, and from the survey results generated by the Bee Informed Project, these updated recommendations will be formulated incorporated into the BMPs through a biennial process of review and comment (every two years starting in 2015) as a part of the annual NC1173 meeting.

While we have one BMP aimed at almond growers, additional BMPs directed at growers, land managers and homeowners are needed. These BMPs will provide a concise resource to guide these stakeholders in pesticide use, planting for pollinators, provision of nesting habitat and weed management.

**Collaborators:**

These BMPs were developed in cooperation with the 18 members participating in the CAP project and Project *Apis m.*, a non-profit California-based group. BMPs will be reviewed and updated by all participating members as a part of the annual NC1173 meeting in 2015. The grower/land manager BMPs will be developed in coordination with research teams in the USDA-funded “Integrated Crop Pollination Project” and the “Pollination Security for Fruit and Vegetable Crops in the Northeast” projects, of which 7 members are participants.

**Outputs:**

1. Improved knowledge about the role of *Nosema* in bee health problems and methods to ameliorate its effects on colony health and productivity
2. An understanding of the role that nutrition plays, at the individual, colony and landscape level, in the prevalence and virulence of pests and pathogens
3. Measures of honey bee genetic diversity in the U.S. and a comparison of this diversity with other areas in which beekeeping occurs
4. Establishment of the range of exposure concentrations, routes of exposure, and colony effects for pesticides and other xenobiotics used in agriculture, particularly those compounds used in seed treatments and those applied to bee-attractive crops during bloom.
5. Consistently updated advice to stakeholders through “Best Management Practices” published to the Bee Health community of practice at the eXtension web site.
6. Continued annual delivery of research updates to the beekeeper and stakeholder community through open meetings, published proceedings and reports published in trade journals and on the eXtension web site.

**Outcomes or Projected Impacts:**

1. Guidelines for beekeepers to reduce harmful effects of *Nosema* in honey bee colonies
2. Recommendations for landscape modifications, including plantings, that are likely to improve the health of honey bees and other pollinators
3. A strategy for beekeepers to improve honey bee stocks in the U.S. in regards to innate tolerance of biotic and abiotic stress, fitness for honey production and pollination, and overall genetic diversity
4. Changes in the patterns of pesticide use and application methods to reduce the exposure of pollinators to pesticides
5. Improved bee husbandry in the U.S. that is more cost-effective and better satisfies the nation’s need for hive products and pollination services

**Milestones**

**2015 –**

1. Review and update *Nosema* and *Varroa* BMPs listed on eXtension’s Bee Health Community of Practice (Obj. 1 and 6)

2. Discuss grower, land manager and homeowner BMPs and identify members to contribute to drafting these guides (Obj. 3 and 6)

3. Progress reports on all objectives due to be presented in ABRC talks and published in Proceedings

4. Publish reports on *Nosema* (Obj. 1), seed treatment insecticides (Obj. 4) and honey bee genetic diversity (Obj. 2) on eXtension site and trade journals

**2016 –**

1. Draft BMPs for growers and land managers and review and update BMPs for (Obj. 1 and 6)

2. Review and update Nutrition BMP listed on eXtension’s Bee Health Community of Practice (Obj. 2 and 6)

3. Progress reports on all objectives due to be presented in ABRC talks and published in Proceedings

4. Publish reports on spray adjuvants (Obj. 4), bee nutrition (Obj. 3) and the interaction between *Nosema* and nutrition (Obj. 5) on eXtension site and trade journals

5. Prepare report for mid-term review

**2017 –**

1. Review and update Hive Equipment and Colony Management BMPs listed on eXtension’s Bee Health Community of Practice (Obj. 6)

2. Progress reports on all objectives due to be presented in ABRC talks and published in Proceedings

3. Publish reports on viral prevalence and effects in honey bees (Obj 1.), landscape effects on honey bees and other pollinators (Obj. 3) and honey bee breeding (Obj. 3)

**2018 –**

1. Review and update *Nosema* and *Varroa* BMPs listed on eXtension’s Bee Health Community of Practice (Obj. 1 and 6)

2. Progress reports on all objectives due to be presented in ABRC talks and published in Proceedings

3. Publish three reports on emerging issues on eXtension site and trade journals

4. Discuss renewal of multi-state project and plan for submission of renewal

**2019 –**

1. Review and update Nutrition and Grower BMPs listed on eXtension’s Bee Health Community of Practice (Obj. 2 and 6)

2. Progress reports on all objectives due to be presented in ABRC talks and published in Proceedings

3. Publish three reports on emerging issues on eXtension site and trade journals

4. Prepare termination report for expiring project

**Outreach Plan**

Many results from NC1173-affiliated projects have already been published on the eXtension website in the Bee Health community of practice (http://www.extension.org/bee\_health). This has provided a central repository of up-to-date research-based information relevant to beekeepers and growers of insect pollinated crops. Members feel that this has been a successful method to disseminate research results to the stakeholder community and we will continue to make Proceedings of the meeting, BMPs and research reports available through the eXtension site. Committee members are also occasional or regular contributors to the two beekeeping trade publications, American Bee Journal and Bee Culture.

Members of the project will also conduct workshops with beekeepers, speak at local, state, regional and national beekeeper meetings. The annual NC1173 meeting is often co-located with an annual meeting of one of the national beekeeping organizations at which many NC1173 members are presenters.

**Organization and Governance**

The committee is lead by a chairperson and a secretary. The secretary is responsible for meeting minutes and annual reports. The chair is responsible for planning and running the annual meeting and coordinating proposal writing. The timing and location for the annual meeting will be established the previous year and, unless otherwise agreed upon, will be coincident with the annual meeting of the American Association of Professional Apiculturists (AAPA) and the scientific program and research discussion for the multi-state project will be a substantial component of the American Bee Research Conference (ABRC) organized by the AAPA.

Candidates for secretary are nominated by the members, and elected to a two-year term. After serving as secretary it is custom for that the secretary be nominated for the chair position and, if elected by the members, that person will serve for an additional two-year term as chair.

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