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Proceedings of the 2017 American Bee Research Conference

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Introduction

The 2017 American Bee Research Conference (ABRC) was held January 12-13, 2017 at the Galveston Island Convention Center in Galveston, TX. This was a special joint conference between the American Association of Professional Apiculturists, Canadian Association of Professional Apiculturists, and Apiary Inspectors of America. ABRC was held concurrently with the North American Beekeeping Conference, a joint meeting of the beekeeping organizations American Beekeeping Federation, American Honey Producers Association and the Canadian Honey Council. The following are the submitted abstracts for presentations given at the 2017 American Bee Research Conference.

Keywords: *Apis mellifera*, honey bee biology, apiculture

Abstracts of Oral Presentations

1. Israeli acute bee paralysis virus and the health of honey bee queens

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Among the many factors causing honeybee colony loss, failure or loss of the queen is considered an important issue. It is believed that the queen is well protected by nurse worker bees leading to lower exposure to infectious diseases in the colony. Nevertheless, existing colony pathogens including viruses can infect the queen. In this project, we used Israeli acute bee paralysis virus (IAPV) as a model, since this virus has been linked to colony loss. IAPV along with Acute bee paralysis virus (ABPV) and Kashmir bee virus (KBV) are part of a complex of closely-related single stranded viruses from the family *Dicistroviridae*. IAPV often exists in the honey bee colony as a covert, low-titer infection but can become extremely virulent and kill its hosts quickly. In a series of laboratory experiments, we studied queen-worker interactions to determine whether behavioral adjustments can protect the queen from the virus. We found that queens generally reduce their contact with infected workers, presumably to protect themselves. In a second experiment, we studied the horizontal virus transmission route among workers, as well as between workers and the queen as a potential route for IAPV transmission in the colony (see Figure 1). Our data identify oral-oral transmission pathways of IAPV between colony members. However, restricting physical contact between infected workers and queens lowers the queen virus infection, suggesting that IAPV can also be transmitted by close bodily contact between queens and infected bees. Generally, the queens exhibited lower IAPV titers than surrounding workers, which may indicate that they are better protected, but this observation could also be explained by a time-lag of several days for infections to build up during the experiment. Overall, it can be concluded that queens might be better protected against IAPV than workers but they experience infection with IAPV by trophallaxis and physical contact with infected workers.

Figure 1. Queen receiving food from IAPV-infected worker bees.

2. Evaluation of synthetic miticides efficacy in *Varroa* mites' control

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The development of pest resistance to a control product is not a new phenomenon. For control of the varroa mite (*Varroa destructor*) replacement of a single miticide (e.g. Apistan®) with another single miticide (e.g. CheckMite+™) has resulted in development and acceleration of resistance to these miticides. Once again by relying on Apivar® for 8 or more consecutive years in Canada and the USA, varroa mites will also eventually develop resistance to this product. In order to provide alternative miticides with different modes of action to manage resistance and enhance varroa mite control, the effects of 3 active ingredients and formulated synthetic miticides on varroa mites and honey bees (*Apis mellifera*) were evaluated in laboratory bioassays using the vial test (Elzen *et. al. 1999 Apidologie 30: 13-17*). Eight to ten mites were exposed to serial dilutions (0.0%, 0.0001%, 0.001%, 0.01%, 0.1 and 1%) of active ingredients (Bifenazate, Spiromesifen, and Acequinocyl). Amitraz was used as a positive control. Acetone was used as a solvent. The formulated products including Amitraz as a positive control were also tested using serial dilutions (0.0%, 0.01%, 0.1, 1% and

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10%) and water as a solvent. Five replicates were used in each tested concentration. Varroa mites were exposed to the tested material covering the inner surface of glass vials for 6 h at 25 °C. Mite mortality was counted and the surviving mites were then transferred to clean vials containing pink-eyed pupae for feeding. Vials were then incubated at 25 °C for 18h. Mite mortality was counted to determine 24 h post-treatment kill.

Varroa-infested worker bees (average 108 ± 0.98 worker bees/ jar) were exposed to the same tested concentrations of active ingredients or formulated products in Mason jars. Plastic strips coated with tested concentrations of active ingredients or formulated products were placed in Mason jars for bee and mite exposure. All jars were incubated at 25 °C. The mortality rates of mites and bees were then assessed after a 24h-period. Higher mortality was observed in mites exposed to Amitraz® after 6h ($p<0.0001$) and 24h ($p= 0.0003$) post-treatment. Bifenazate and Acequinocyl ($p=0.0007$) and their formulated products ($p=0.009$) caused significantly higher mite mortality after 24 h in comparison to the control (solvent) excluding Amitraz®. The rate of bee mortality was similar within formulated products, active ingredients and controls averaged overall 1.53%, but it was significantly higher in Bifenazate ($p=0.0053$). The higher doses of Amitraz® 1% and 10% increased the rate of bee death by 37% and 96%, respectively. These findings suggest that tested active ingredients that showed higher mite mortality under laboratory conditions with lower side-effects on bees should be assessed in the field on full size honey bee colonies for further development of an effective miticide to be used in varroa mite control.

Funding

Research was funded by Growing Forward 2 (a federal-provincial- territorial initiative) and Alberta Crop Industry Development Fund (ACIDF) Ltd, and Alberta Beekeepers.

3. Small hive beetle: exploration of a screening method via DNA analysis of hive debris and scraps

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The small hive beetle (SHB or *Aethina tumida* Murray) originated from Africa and is now found in numerous countries since 1998 (USA, Canada, Australia, Portugal, Mexico, Italy), suggesting it will likely spread to other regions of the world in the future. Detection of SHB in hives, typically made by visual inspection and traps, is time-consuming, needs trained inspectors and SHBs may not be detected if present at low levels of infestation. There is the need to find a fast and reliable way to assess the status of colonies of newly infested areas. PCR analysis is a promising method to detect particles of SHB retrieved from hive debris (Ward et al., 2007 *Apidologie 38:272-280*). Different haplotypes of SHB are successfully distinguished from honey bees, varroa mites and other Coleoptera. The objective of this research was to assess the sensitivity of conventional PCR to detect SHB in colonies infested at low levels. Bottom board debris was collected in 4 apiaries from (1) non-infested colonies from Quebec (negative control) and (2) infested colonies from southern Ontario. Each hive was visually inspected to assess the status of the colony. Zero to 19 SHB adults were found in the 118 colonies inspected. No larvae were found during the inspection. DNA from *A. tumida* cytochrome oxidase I (COI) gene was amplified using two primers with different length: (1) a short primer (109 bp) (Ward et al., 2007 *Apidologie 38:272-280*) and (2) a long primer (1080 bp) (Lounsberry et al., 2010 *Ann. Entomol. Soc. Am. 103:671-677*), as well as honey bee actin as a positive control for DNA. Several false negative results were found. The primers used might not be suitable for debris analysis. DNA from debris might also be damaged as it stays in the colony for several weeks before collection. The extraction technique for hive debris has to be improved and further testing developed to find a more appropriate primer for SHB debris analysis with conventional PCR.

4. Sustaining and securing Canada's honey bees using 'omics approaches

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Overwintering mortality of honey bee colonies in Canada has been continuously greater than the acceptable range of 0% to 15% since the winter of 2006/2007 (Leboeuf *et. al*., 2016 http://capabees.org/capa-statement-on-honey-bees/). The main causes of colony death, as reported by Canadian beekeepers, include high pathogen/parasite infestation levels (e.g. *Varroa* mites, *Nosema* spp.), poor quality queens and severe weather conditions. Every year, Canadian beekeepers import hundreds of thousands of queens, mainly from the U.S.A. The importation of foreign queens has the potential to introduce undesirable pathogens or genetics and supply bees that have not been selected to survive in northern temperate climates (Parker *et al*., 2010 *PLoS One* 5(6):e11096). The goal of our project is to measure 12 economically-valuable traits of honey bees (colony phenotypes) and develop genomic and proteomic markers for each trait that will enable beekeepers to rapidly select and breed healthy and productive colonies that are well adapted to the Canadian climate. In 2016, 1,045 colonies across Canada (British Columbia, Alberta, Manitoba, Ontario and Quebec) were sampled and phenotypic data was collected for the following colony-level traits: 1) *Varroa* mite population growth 2) grooming behaviour; 3) hygienic behaviour; 4) defensive behaviour; 5) honey production; 6) sealed brood population; 7-9) pathogen abundance (viruses, *Nosema* spp., Trypanosomatids); 10) innate immunity factors; 11) gut microbiota; and 12) overwintering success. Pre-wintering measurements for colony overwintering success were taken in the fall of 2016 and will be concluded in the spring of 2017. Here, we will present a sub-set of the phenotypic data collected during 2016. The measurement of colony-level traits and the identification of bio-markers for each trait comprise the first step of this novel research. In the summer of 2017, putative markers will be validated against a test population, with the end goal of having this technology transferred to end-users, such as the National Bee Diagnostic Centre (Beaverlodge, AB), where it will be made available to beekeepers. This is the first large-scale study for marker assisted selection in honey bees using integrated genomics and proteomics tools. Our innovative research will promote a healthier honey bee population and support the sustainability of the Canadian beekeeping industry.

5. Honey bee transcriptional response to virus infection

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Honey bees are significant plant pollinators in both agricultural and non-agricultural landscapes. Since 2006, annual losses of managed honey bee colonies in parts of North America and Europe have been high (e.g., US 33% loss). Colony losses are influenced by abiotic and biotic factors, including (+) single stranded RNA (ssRNA) virus infections. Honey bees antiviral defense mechanisms include RNA interference (RNAi) and additional immune pathways, but their relative roles in antiviral defense are not well understood. To better understand honey bee double stranded RNA (dsRNA) triggered immune responses, bees were infected with a model virus (Sindbis-GFP) with or without dsRNA. In our experiments, dsRNA, regardless of sequence specificity, reduced virus production. To investigate the mechanisms of dsRNA-mediated immune responses in honey bees, we utilized RNA sequencing to examine transcriptional responses triggered by virus +/- dsRNA. Virus-infected and dsRNA-treated bees had greater expression of genes involved in canonical insect immune pathways, but the majority of genes with increased expression are not well characterized in the context of the immune response. Further investigation of these genes will yield a better understanding of dsRNA on bee physiology and antiviral defense and may lead to identification of evolutionarily conserved sequence-independent dsRNA-mediated immune pathways in other organisms.

Funding

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6. Canadian national honey bee health survey

Carlos Castillo[1], Patricia Wolf Veiga[1], Jamie Lee Martin[1], Christine Curran[1] and Stephen F. Pernal[2].
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In 2016 Canadian beekeepers managed 750,155 colonies and produced 42,000 tons of honey valued at CD$ 157 million. Pollination services to farmers are estimated to contribute an additional 4 to 5 billion dollars to the agriculture sector. The beekeeping industry reported winter loses of 16.8%, with Provincial averages ranging from 7.7% in Newfoundland and Labrador to 24.4% in Prince Edward Island. Beekeepers cited several culprits including biotic and abiotic factors. The National Honey Bee Health Survey is a four year (2014 - 2018) study to evaluate the health of honey bee colonies in Canada. The aim of the project is to document the prevalence, intensity and distribution of most common pests and pathogens in Canadian apiaries, targeting beekeeping operations distributed throughout all provinces. Samples are collected in the summer (July and August) when hives are strong; bee populations reach their peak and before fall treatments. Results are obtained from the individual analyses of samples and reported as averages per provincial region and totals per province.

The data generated during the first two years of the project show that: 1) *Nosema* infection was detected in 16 of the 19 provincial regions in British Columbia, Alberta, Manitoba and Ontario; 2) *Nosema ceranae* was the most prevalent species within and between provinces; 3) *Varroa* was detected in all regions sampled in 2015, with provincial infestation levels ranging from 0.8% in Alberta to 3.2% in British Columbia; 4) Upon visual inspection, provincial AFB incidence ranged from 0% in Ontario to 0.7% in British Columbia. When cultivated in the lab, AFB was detected in samples from 9 of the 19 regions and was absent from all Ontario samples; 5) The most prevalent viruses detected in the survey were Black Queen Cell Virus (BQCV) and Sacbrood Virus (SBV). Conversely, Acute Bee Paralysis Virus (ABPV) was entirely absent in British Columbia, Alberta, and Manitoba- only identified in samples from southwest Ontario; 6) *Tropilalaeps* was not identified in any of the 212 composite samples collected. Preliminary data from the 3rd year sampling (summer 2016) comprising 9 Canadian provinces and 1 territory was presented.

7. Viruses in unexpected places: new transmission routes of European honey bee (*Apis mellifera*) viruses

Megan J. Colwell[1], Robert W. Currie[1], and Stephen F. Pernal[2].
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Although there are many insect pollinators, European honey bees (*Apis mellifera*) are arguably the most economically important and recognizable pollinators. However, higher than normal losses in the past decade have put the honey bee industry at risk. Viruses are one of the key factors in honey bee health. Little work has been done to explore the possible role of wax, the substrate on which all hive activities take place, as an element in virus transmission. Additionally, no work has been done on the possibility of the inquiline *Braula coeca* (Diptera) as a carrier of honey bee viruses. This study explores various routes of transmission of viruses between honey bees and wax, and the presence of viruses in *Braula* compared to *Varroa destructor* (see Figure 2). Potential transmission routes, contact and airborne, were tested in a cage experiment. Bees used in cages originated from two sources, high *Varroa* (high virus, n=8) and low *Varroa* (low virus, n=8) colonies. There were also cages with no bees (no virus, n=8), and also two types of wax (light and dark) that were not exposed to the incubator. Bees were taken from six colonies per treatment, mixed together separately, and bee cages each contained 300 bees. All cages contained a

Figure 2. *Varroa* mite (left) and *Braula coeca* (right) specimens (photo credit: Megan J. Colwell).

sheet of wax foundation. Cages were maintained at 75% RH and 30°C in an incubator for seven days. Both *Braula* and *Varroa* were collected with sticky boards from colonies with high *Varroa* levels. Wax and organisms were tested for viruses (Black Queen Cell Virus and Israeli Acute Paralysis Virus) using quantitative PCR. Viruses were found to be introduced to wax by worker bee contact and by aerosolization within the incubator. Viruses were found in *Braula* at lower levels than in *Varroa*. This provides evidence for the possibility of wax as a transmission route, as well as the possibility of airborne transmission of viruses. Additionally, *Braula* carry viruses, and may serve as vectors within honey bee colonies. Further work is underway to determine if waxborne and airborne viruses are infective to honey bees.

8. Environmental consultancy: dancing bees bioindicate the landscape's profitability for pollinators

Margaret J. Couvillon. Department of Entomology, Virginia Tech.

Although insect-pollinated crops are an increasing proportion of our diet, pollinating insects, including honey bees (*Apis mellifera*), continue to decline in North America and Europe. Honey bees face many challenges including pests, pathogens, and pesticides. However, independent of these is another issue affecting wildlife in general: landscape changes in the last century, such as agricultural intensification, have reduced flowers and flower-rich habitats that provide nectar and pollen for insects. Here I will present work in which I demonstrate that the honey bee waggle dance, a naturally occurring behavior where a returning forager indicates the direction and distance from the hive to a good food source, may represent an untapped tool for ecology. By "eavesdropping" on these communication dances, we may obtain biologically-realistic information about temporal and spatial variation in forage availability. These data may then be used to better direct efforts to help honey bees.

9. Manipulating varroa mite and virus levels on a colony scale to quantify their impact on honey bee colony winter survival

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The varroa mite and the viruses it vectors are considered to be among the most critical factors affecting honey bee colony loss. We conducted a long-term field study to attempt to uncouple the effects of mites and viruses and elucidate the effects of long term exposure to viruses on winter survival by manipulating mite levels through combinations of spring and fall treatments over a period of 1.5 years.

Forty colonies of honey bees (*Apis mellifera*) with equal numbers of workers, mites (*Varroa destructor)* and a queen were established in standard Langstroth equipment in spring of 2014. Colonies were continuously monitored from the summer of 2014 to the spring of 2016 and varroa mite infestation level and concentrations of seven economically important viruses were quantified by qRT-PCR.

Colonies were randomly assigned to one of four treatments (n=10) as follows: 1) No acaricide treatment; (2) Fall only treatment (2014, 2015); (3) Spring only treatment (2015); Fall and Spring treatment (2014-F, 2015-S+F). Following these long term treatment combinations which were designed to produce different combinations mite and viral infestations, the effects on colony performance and survival were assessed.

In the first winter, the treatments resulted in two combinations of mites and virus with untreated colonies (N=20) having moderate mite infestations and moderate virus concentrations. Fall-treated colonies had low varroa and also had moderate virus concentrations. In the first winter colony survival was not affected by different varroa mite and virus combination levels. As predicted, in the second winter, the different treatment combinations generated different mite and virus combinations ("low mite"-"low virus", "low mite-high virus", "high mite-low virus" and "high mite-high virus") and these combinations had significant effects on winter survival and spring population size in the second winter (Figure 3).

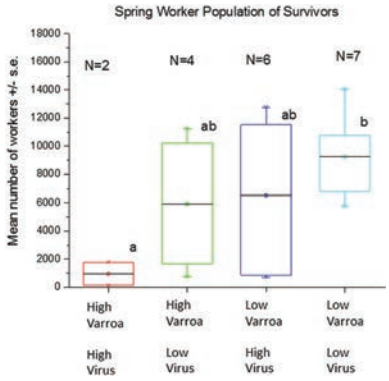

Figure 3. Spring population size of surviving colonies and number of colonies of colonies surviving (n= number surviving) after the second winter following manipulative treatments of varroa mite and viruses levels. Means followed by the same letter are not significantly different (P<0.05).

10. Detection of imidacloprid residues in pollen and nectar collected from annual and perennial bedding plants purchased from selected retail garden stores

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In the spring of 2015, a study was initiated to determine the levels of total imidacloprid residues in the pollen and nectar of annual or perennial flowering bedding plants purchased from selected retail garden stores. The study sites were select stores in metropolitan areas in Florida, California, Kansas/Missouri, and North Carolina. The study was conducted in two phases: 1) sampling plant matrices at purchase time at 5 stores in each of the four areas, and 2) sampling of plant matrices after purchased plants were grown in the ground for approximately 4 weeks to allow for re-bloom in two locations: Kansas/Missouri and North Carolina. Results indicated that the majority of the plant species sampled after purchase was below the level of concern for risk to honey bee colonies for either pollen or nectar (98 and 93%, respectively). Results also indicated that residues in plant matrices significantly decreased and were all below the level of concern 4 weeks after planting. Based on the results of this study, the plants sold in the selected retail garden centers represent a minimal risk to honey bees when grown in residential landscapes.

11. Determining relative attractiveness of reconstructed native prairies to honey bee foragers

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Recent concern over honey bee colony mortality rates, caused in part by poor nutrition, has led to efforts to increase the availability and diversity of food sources for bees (Spivak *et al., 2011 Environ. Sci. Technol. 45:34-38;* Smart *et al., 2016 Agr. Ecosyst. Environ. 230:139-149*). Current agricultural practices establish large areas of monoculture crops, which may be a "food desert" for honey bees (Otto *et al., 2016 PNAS 113: 10430–10435*). One potential motivation for increasing the area of native prairie ecosystems is to increase diversity in bee diets. However, it is unclear whether honey bees prefer to forage on restored/reconstructed native prairie plants or the predominantly non-native, weedy flowers found adjacent to these areas.

Three observation hives of honey bees were located between large reconstructed prairies in eastern Minnesota. To determine which sites in their foraging radius were seen as high-quality and worth the recruitment of other bees, waggle dances of foragers in these hives were recorded over the course of the summer. By decoding these dances—measuring the direction, distance, and relative attractiveness of the site being advertised—maps were created to determine the most attractive areas to foragers (Schürch *et al., 2013 J. Comp. Physiol. A 199:1143–1152*; Couvillon *et al., 2014 Curr. Biol. 24:1212-1215*). To link these locations with the flower species that honey bees advertised throughout the season, pollen loads were collected from a subset of dancing pollen foragers for identification by microscopy.

Preliminary results indicated that honey bees foraged primarily outside the prairies, especially on *Trifolium, Lotus, and Melilotus*. However, foragers did visit native species within the prairies, with the highest percentage of foragers visiting sites inside the prairie during August and September when *Asteraceae* flowers (goldenrods, other asters) are in bloom. This study provides insight into whether current prairie planting practices can provide honey bees with particularly attractive food sources relative to prevalent non-native weedy flowers.

12. BeeProbio: Sustaining honeybee health with probiotics

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[1]Département de biologie, Université Laval, Quebec. [2]Agriculture & Agri-Food Canada, Beaverlodge, Alberta, Canada.

Maintaining health status of honeybee colonies is a major concern for beekeepers. Various parasites and pathogens are responsible for unsustainable declines of honeybee populations over the past decade. Homologated treatments against disease and pests rely on chemicals and antibiotics that can be harmful for honeybees. For example, antibiotics allow resistant opportunistic pathogens to trigger secondary infections as their natural antagonistic gut bacteria are killed or weakened. Therefore, there is an urgent need to develop new tools to treat and prevent honeybee diseases that are efficient, sustainable and safe for honeybee micro flora. The nutritional probiotic approach is straightforward and it has proved its efficiency in improving health for various farm animals.

Our research team is currently developing bee specific probiotic strains that improve significantly colony survival, performance and disease resistance. Furthermore, we are developing metagenomic tools to monitor gut microbiota homeostasis. Such tools will enable beekeepers to rapidly and cost-effectively monitor health status of their colonies in order (1) to identify healthy colonies and (2) to target those that need personalized probiotic treatments to restore gut microbiota homeostasis, and thus disease resistance.

We have tested survival of caged bees (20 cages per experimental group, 20 bees per cage) with various probiotic candiates/supplements in 1:1 sucrose syrup. Our current results demonstrate that four probiotic candidates improve honeybee survival in caged bee trials, both in prophylactic and curative contexts with experimental infections of the microsporidian parasite *Nosema ceranae*.

13. Pheromonal control of overwintering in honey bee colonies

Mehmet Ali Döke and Christina M Grozinger.
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Overwintering represents a unique state during the annual honey bee life cycle, with distinct behavioral (i.e. decreased individual activity, cessation of brood rearing and formation of a thermoregulating cluster) and physiological (i.e. altered endocrine profiles, increased nutrient stores and longevity) features (Döke *et al., 2015 Current Opinion in Insect Science 10:185–193*). Overwintering is also a very stressful period for honey bee colonies in temperate regions, with ~30% average winter losses reported by US beekeepers in the last decade (Lee *et al., 2015* BIP). Several factors including varroa mites, viruses, geographic location, and genotype are correlated with winter colony losses. Reviewing the published literature on honey bee overwintering, maturation, and longevity, we developed a model which hypothesizes that interacting environmental and social (pheromonal) cues regulate the timing of winter bee production and therefore overwintering success (see Figure 4). Based on this model, we predict that the application of forager pheromone (ethyl oleate) in the fall will stimulate the production of a winter bees, and thus early application of this pheromone should trigger early production of winter bees, thereby increasing the size of the overwintering colony. As we found earlier,

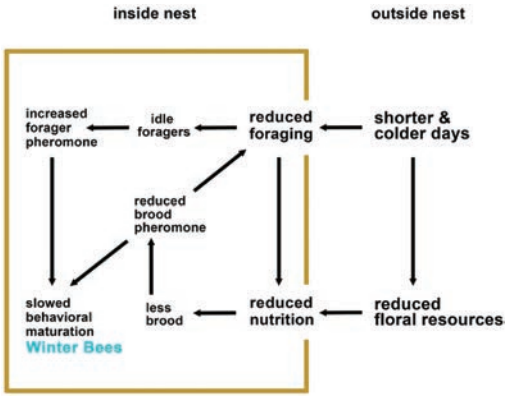

Figure 4. Proposed model for regulation of production of winter bees. The production of winter bees is likely regulated by Interactions among temperature and photoperiod cues, environmental nutritional resources, brood/brood pheromone levels, and forager pheromone (ethyl oleate). *Graphical design by Harland Patch, Penn State.

increasing colony size should lead to significant improvements in the overwintering success of honey bees (Döke *et al.,* in prep).

With funding from a NE SARE Graduate Research Award, we initiated a pheromone treatment study in Summer 2016. Pheromone treatments were applied from September through late November. Every 2 weeks we introduced 100 paint-marked one-day-old workers into each colony and censused the marked bees (that were introduced 2 weeks earlier) to determine the percentage of each cohort that remains in each colony (since increased longevity is a signature trait for winter bees) to determine the timing and rate of winter bee production. Colonies were also monitored for number of worker bees, brood, weight, food storage, and varroa mites. This study will conclude in early Spring 2017, when colonies will be inspected for overwintering survival. Using the results of this controlled trial, we aim to develop a "pheromone treatment plan" that can be used by beekeepers in the field for improving their colonies' overwintering survival. For the second phase of this study, we will work with beekeepers and provide them pheromone treatments to test in their own apiaries in fall. Collaborating with 10+ beekeepers (200+ colonies) will give us a field relevant, realistic evaluation for the potential of the pheromone treatment for improving overwintering success in honey bees in Northeastern US.

14. Evaluating the risk pesticide use at ornamental nurseries pose to honey bees

Brian D. Eitzer, Kimberly A. Stoner and Richard S. Cowles. The Connecticut Agricultural Experiment Station.

The risk posed to honey bees from pesticide use by the ornamental horticulture industry requires an understanding on the routes of exposure. Which of the many plants grown are being visited by pollinators? How does the timing and mode of application of pesticides translate to concentrations of those pesticides in pollen and nectar from those plants? Do the observed concentrations pose a significant risk? To answer these questions we have begun to study pesticide residues in honey bee collected pollen at nurseries in Connecticut, and also residues in pollen and nectar taken directly from plants grown under differing pesticide application practices.

Figure 5. Pollen from one sample collected from a hive at an ornamental nursery, the diversity of colors indicates that the honey bees were collecting from multiple floral sources each of which would have a different amount of pesticides present.

Honey bee pollen was collected throughout the growing season from three nurseries, each of which had three honey bee hives located on site. Pollen was collected using traps and analyzed by LC/MS using a broad pesticide screen. A hazard quotient (HQ) for each sample was determined based on the concentration of each pesticide divided by the oral LD_{50} for honey bees and then summed for all pesticides found in each sample. A pollen HQ of 1000 indicates that the pesticide concentrations are at 1% of the LD_{50}, assuming acute toxicity is additive. Almost 90% were less than 1,000 indicating that they are relatively safe though a few were higher with one sample with an HQ of 70,000 (70% of LD_{50}). The pollen collections of the highest hazard samples were sorted by color and these samples were reanalyzed to determine which specific colors contained which particular pesticides (see Figure 5 for an example of a color sorted pollen sample). A subset of each of these sorted pollens was prepared for pollen analysis so that the particular plants from which that pollen was collected could be determined. We have also been examining agricultural practices in model plants to determine how timing or pesticide application rate can affect pollen and nectar concentration. Collection of the pollen and/or nectar directly from plants can be quite laborious, and concentrations can be in the parts per billion range, so it is important to have analytical methods that are sensitive and specific even for small sample sizes.

15. Intensity of grooming behavior and resistance to *Varroa* in honey bees

Berna Emsen, Peter Unger, Laura G. Espinoza-Montaño, Tatiana Petukhova, Mollah M. Hamiduzzaman, Greg J. Hunt, and Ernesto Guzman-Novoa. School of Environmental Sciences, University of Guelph.

Results of studies conducted to analyze the relationship between infestation levels of the parasitic mite *Varroa destructor* in honey bee colonies, rates of damage of fallen mites, intensity with which bees of different genotypes groom themselves to remove mites from their bodies and expression of associated genes will be discussed. Genotypes that are presumably susceptible and resistant to the varroa mite were compared at the colony level for number of mites falling and for proportion of damaged mites. They were also compared at the individual level for intensity of grooming and mite removal success. Bees from the "resistant" colonies had lower mite population rates (up to 15 fold) and higher percentages of damaged mites (up to 9 fold) than bees from the "susceptible" genotypes. At the individual level, bees from the "resistant" genotypes performed significantly more instances of intense grooming (up to 4 fold), and a significantly higher number of mites were dislodged from the bees' bodies by intense grooming than by light grooming (up to 7 fold) in all genotypes. The odds of mite removal were high and significant for all "resistant" genotypes when compared with the "susceptible" genotypes. Additionally, the expression of sets of genes presumably associated to this behavior was affected. The results of these studies suggest that grooming behavior and the intensity with which bees perform it, is an important component in the resistance of honey bee genotypes to *V. destructor* and that this behavior is at least in part, regulated by genetic effects.

16. The negative effects of in-hive pesticides on honey bee (*Apis mellifera*) drone sperm viability

Adrian Fisher II, Dr. Juliana Rangel. Department of Entomology, Texas A&M University.

Honey bee (*Apis mellifera*) drones are produced seasonally for the sole purpose of mating with virgin queens from nearby colonies. Because drones do not contribute to other colony tasks such as food collection, brood rearing, or defense, they are often overlooked in honey bee

research. However, a recent examination of drone spermatozoa viability (i.e., proportion of total spermatozoa in a drone's seminal vesicles that are viable and can fertilize an ovule) found significant variation in viability among drones from apiaries in different locations in Central Texas. This observed variation may be influenced by the contamination of the lipophilic beeswax with agrochemicals, as well as the beekeeper-applied miticides used in the treatment of the ectoparasitic mite *Varroa destructor*. Both pesticide groups have been found in high concentrations in wax samples across the United States and seem to be contributing to the decline of honey bee populations nationwide. To assess the potential effect of exposure to in-hive pesticides on drone spermatozoa viability, we compared the viability of spermatozoa collected from drones reared in pesticide-free wax to that of drones reared in wax contaminated with field-relevant doses of select pesticides. Using a standard sperm staining technique, live spermatozoa were stained with Sybr-14 while unviable spermatozoa were dyed with propidium iodide. The samples were then run through a cell counter (Cellometer Vision CBA, Nexelcom®), which identified viable and non-viable spermatozoa and provided relative cell counts in a sample. Our results suggest a significant negative effect of in-hive pesticide exposure during development on spermatozoa viability.

17. Beyond the gut: *Nosema* parasitism in honey bees impacts neurochemistry and olfactory learning and memory

Stephanie L. Gage[1,2], Catherine Kramer[3], Samantha Calle[1], Mark Carroll[1], Michael Heien[3] and Gloria DeGrandi-Hoffmann [1,2].
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Nosema parasitism is one of the most pressing concerns in managing healthy honey bee colonies. *Nosema* is a parasite of the midgut that has profound consequences in several areas of physiology. Infected bees are more likely to die prematurely, while persistent infection can result in imbalanced division of labor and collapse of the hive. In our research, we ask whether there are brain-specific consequences to *Nosema* infection. *Nosema*-infected bees have been reported to be poor foragers that are unable to return to the hive. This suggests that there may be deficits in the underlying neurobiology that support these behaviors.

Figure 6. A bee performing a proboscis extension reflex in response to an odorant stimulus presentation.

In this study, we examine whether *Nosema* affects odor learning and memory—a task essential to successful foraging—and whether the brains of parasitized bees show differences in amino acids and neurotransmitters that may account for behavioral changes. To do so, we took newly emerged bees and fed them with a *Nosema ceranae* inoculum. At approximate nurse and forager ages, we employed an odor-associative conditioning assay using the proboscis extension reflex (see Figure 6) to assess learning and memory, and two bioanalytical techniques to measure changes in brain chemistry. We find nurse-aged bees infected with *Nosema* significantly outperform their control counterparts in odor learning and memory, but by forager age, they are slower to learn and show memory impairment. We also see concentration differences in amino acids and neurotransmitters in the brains of *Nosema* bees. These findings suggest that the effects of *Nosema* parasitism extend to the brain and cognitive tasks essential for foraging may be compromised.

18. Exposing queens to high temperature affects their performance

Marta Guarna[1], Katie Joselow[2], Jeffery Pettis[2,3], Steve Pernal[1].
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The health and performance of honey bee queens is an important factor determining colony productivity and survival. Recent studies have shown that queens can be exposed to temperature extremes during transport and these temperature events can decrease the viability of the sperm stored in the queen's spermatheca. We have initiated colony level studies to assess the effect of a temperature-induced reduction in queen sperm viability on queen and colony performance. We confirmed that queens exposed to high/low temperature have reduced sperm viability while showing no visible indication of poor health status. We then introduced treated and control queens to experimental colonies and observed a clear difference between groups. Treated queens showed a decrease in their performance as assessed by analysis of brood pattern and brood quantity. In addition, the productivity of colonies headed by treated queens showed lower adult population and honey production. We will discuss how these results can guide queen producers and beekeepers on queen handling and management decisions to reduce the need for frequent queen replacement and improve colony productivity and survival.

19. Evaluating the effects of mosquito control adulticides on honey bees

Kristen Healy[1], Jim Ottea[1], Todd Walker[2], Frank Rinkevich[1,3], Joseph Margotta[1], Vivek Pokhrel[1], Randy Vaeth[2], Clint Hoffman[4], Bradley Fritz[4], and Robert Danka[3].
[1]Department of Entomology, Louisiana State University. [2]East Baton Rouge Mosquito and Rodent Control, Baton Rouge, LA. [3]USDA-ARS, Baton Rouge, LA. [4]USDA Aerial Applications Technology Lab, College Station, TX.

While mosquito control adulticides can be effective in rapidly reducing mosquito populations during times of high arbovirus transmission, the impacts of these control measures on pollinators has been of recent interest. The purpose of our study was to evaluate mosquito and honey

bee mortality using laboratory, semi-field and field based experiments. In semi-field studies, honey bee mortality was significantly lower than mosquito mortality for all products, distances, and application rates tested, except the low rate of Scourge, which had low mortality for both bees and mosquitoes. Field studies with sentinel beekeepers showed no significant differences in bee mortality and health indicators in hives within mosquito control areas versus those outside of treatment areas. Results from our studies have suggested that when done correctly and according to label instructions, mosquito control adulticides should have minimal effects on honey bees. However, improved communication between mosquito control and beekeepers should be done to ensure reduced non-target effects.

20. Overwintering strategies and their effects on honey bee nutrition

Brandon K. Hopkins and Walter S. Sheppard. Department of Entomology, Washington State University.

Over-wintering honey bee colonies in California "holding yards" can be a challenging place to keep colonies alive and healthy during the winter months. Beekeepers need a place to stage bees that are easy to access for transport to almond orchards at the start of the pollination season. An increasing number of commercial beekeepers are turning to indoor storage of their colonies in potatoes sheds, fruit storage warehouses, and purpose built facilities to increase winter survival and still have access to move bees when needed. There remains little research on the effects that different overwintering storage conditions have on honey bee heath/survival. We investigated the effects of wintering locations/conditions on honey bee lipids and hypopharyngeal head protein. Bees were emerged in incubators and painted. Painted bees were equally distributed among 24 hives. Those hives were distributed between 4 different over wintering environments (6 colonies each): California holding yard, outdoors in Washington, cold room (4 °C) and a controlled atmosphere room (4 °C). Bees were recaptured 64 days later as colonies were prepared for almond pollination. Heads were removed and analyzed for protein content and abdomens were processed for lipid content. Both indoor environments resulted in recaptured bees with significantly greater head protein content than the other treatments. Colonies wintered in the controlled atmosphere facility had significantly greater average lipid weight/bee than bees held in the California holding yard. This provides some evidence of the potential benefit to using indoor wintering instead of wintering colonies in California holding yards.

21. An update on parental effects on aggression and gene expression in African-European hybrid honey bees

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European € and Africanized (A) hybrid honey bees differ in their aggressive behavior depending on the direction of the cross. Hybrids with European maternity (EA hybrids) have significantly higher propensity to sting than AE hybrids, whether it is measured at the colony or individual level (Guzmán-Novoa et al. 2005 J Hered 96:376-380; Gibson et al. 2015 Frontiers Genet 6:343). Sequencing RNA from hybrids showed that about eight percent of genes were highly biased towards the maternal allele in EA hybrids, but not AE hybrids. Interestingly, the genes involved were enriched for mitochondrial proteins and genes involved in metabolism, which fits with recent findings that aggressive bees show a shift towards aerobic glycolysis in brain tissues (Alaux et al., 2009 PNAS 106:15400-15405; Chandrasekaran et al., 2015 Genes Brain Behavior 14:158-166). We hypothesized that the asymmetry we observed in maternally biased gene expression was caused by inappropriate signaling between the mitochondria and the nucleus. We also hypothesize that small interfering RNA from either the egg or sperm may be involved, probably piwi-interacting RNA (piRNA) or transfer RNA fragments (tRF). Both piRNA and tRF have been shown to silence gene expression in some circumstances. Precursor piRNA are processed at mitochondria and they are mainly known for their role in silencing transposons. Very recently tRFs have been linked to trans-generational inheritance patterns in mice.

We sequenced small RNAs from sperm and eggs of European and Africanized bees related to the crosses that showed asymmetric maternal effects and found that eggs of both European and Africanized bees had similar amounts of RNA showing a peak in the piRNA size range (27-30 bp), but Africanized sperm had more than twice the level of RNA of tRF size (31-34 bp) compared to European sperm (Figure 7). We discussed the significance of this result and the possible involvement of African nuclear alleles in these novel phenomena.

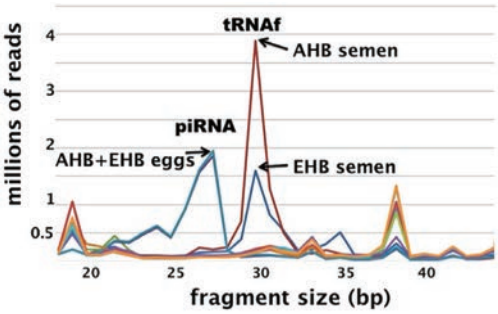

Figure 7. The number of times sequences appear in the data corresponds to their expression level. The size of these sequences are plotted on the X-axis. RNA from eggs or semen of both European and Africanized bees are shown. piRNA = piwi interacting RNA. tRF = transfer RNA fragments.

22. The efficacy of artificial brood interruption and oxalic acid treatments in controlling the honey bee pest *Varroa destructor*

Cameron Jack and Jamie Ellis. Department of Entomology and Nematology, University of Florida, Gainesville.

A successful Integrated Pest Management (IPM) approach to *Varroa destructor* control must be an improvement over existing control methods and include new cost-effective treatments that can be employed by beekeepers readily. Herein, we tested the ability of oxalic acid (OA) vaporization as well as the common European beekeeper practice of artificial brood interruption to control *Varroa*. These methods have not been tested together in the U.S. A full-factorial design was used as 60 experimental colonies were randomly assigned to one of six treatment

groups with 10 colonies composing each group. The six treatments were: (1) OA applied once, brood interruption, (2) OA applied three times, brood interruption, (3) No OA, brood interruption, (4) OA applied once, no brood interruption, (5) OA applied three times, no brood interruption, (6) No OA, no brood interruption (negative control). The OA was applied via vaporization. Artificial brood interruption was accomplished by caging the respective colonies' queens in a plastic queen cage for a period of 24 days to halt all brood rearing activities within the colonies. An additional 10 colonies served as positive controls and were treated with the common commercial miticide amitraz (Apivar). *Varroa* populations were estimated before, during, and after treatment applications by measuring the number of *Varroa* on sticky screens. Our data suggest that queen caging to achieve artificial brood interruption negatively impacts colony strength and survival (see Figure 8). We observed high colony mortality in some treatments, despite diligent colony management to alleviate the side effects of the treatments. Colonies receiving the standard amitraz treatment were generally healthier and had better survival than those treated with OA vaporization. Our results will provide information on these new treatments, thus aiding beekeepers as part of an IPM approach to control *Varroa*.

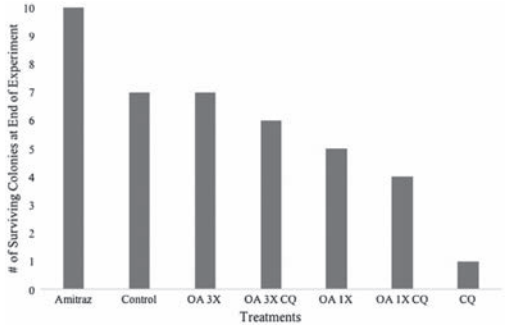

Figure 8. The number of surviving colonies at the end of the experiment across all treatments. The x-axis labels are as follows: OA – oxalic acid, 1X – oxalic acid was applied via vaporization one time, 3X – oxalic acid was applied via vaporization three times, CQ – artificial brood interruption achieved by caging the queen, Amitraz – amitraz applied via Apivar strips.

23. Effect of pesticide combinations applied to almonds during bloom on honey bee workers

Reed M. Johnson, Andrea Wade, Bridget Gross, Ashley Cordle and Chia-Hua Lin. Department of Entomology, Ohio State University, Wooster, OH.

Beekeepers providing honey bee colonies for almond pollination have reported unexplained losses of bees during pollination and the subsequent weeks. The California Pesticide Use Reporting Database shows that application of a suite of insecticides is widespread during the blooming period (Figure 9). In 2014, the most recent year for which use data is available, insecticides tank-mixed with fungicides were applied to approximately 180,000 acres of almonds during bloom. To examine the effects of a range of pesticide combinations on adult bee survival, we fed field-relevant ratios of insecticide-fungicide combinations in pollen and assessed mortality over the following 10 days in the laboratory. Overall we found that most insecticides and insecticide-fungicide combinations did not kill bees over the 10 day period, however the combination of the insecticide chlorantraniliprole with the fungicide propiconazole resulted in increased adult mortality. Addition of a spray adjuvant to the pollen mix did not affect the toxicity of insecticides or insecticide-fungicide combinations.

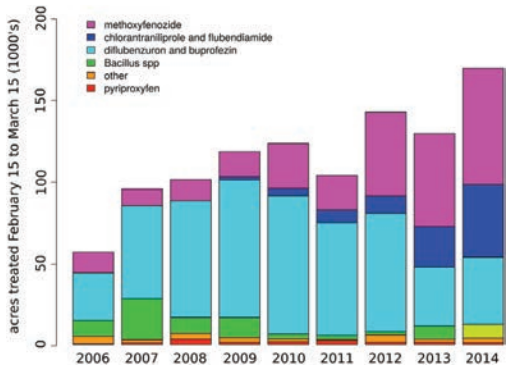

Figure 9. Acreage of almonds s to which insecticides were applied during the blooming period (February 15 to March 15) over 2006-2014.

24. The Sentinel Apiary Program: Collaborating with beekeepers to improve colony health and management

Kelly Kulhanek, Nathalie Steinhauer, Dan Reynolds, Rachel Fahey, Anthony Nearman, and Dennis vanEngelsdorp. Department of Entomology, University of Maryland, College Park, MD.

The Sentinel Apiary Program, piloted in 2015, provides beekeepers and beekeeping groups with real-time information about the health of their colonies. Sentinel Apiaries are meant to act as an early warning system to alert beekeepers of potentially escalating health problems.

Figure 10. A map of 2016 Sentinel Apiaries. All scale data viewable at beeinformed.org/programs/sentinel-hive-scale-program/.

Through monthly disease testing, hive scale monitoring and pollen sample collection, we are able to inform beekeepers of what management their apiaries may need. In 2015, the program included 21 participants sampling 176 colonies. 857 samples were processed for *Varroa* (alcohol wash) and *Nosema* (microscope counting). 38 pollen samples were tested for pesticide residues by the Maryland Department of Agriculture. Number of *Varroa* mites/100 bees for 2015 Sentinel participants was consistently lower than the national average for each month (USDA-APHIS Honey Bee Disease Survey, 2015). Counts ranged from a low of 1.59 in May to a high of 7.64 in October. By November, counts were down to 3.43, indicating successful control techniques implemented in response to high counts in October. *Nosema* spores ranged from 0.15 million spores/bee in July to 2.56 in March. Pollen analysis resulted in 24 detections of 13 pesticides, with 60.5% (n= 23) of samples zero pesticides detected. The Sentinel Apiary Program grew by 43% in 2016 (Figure 10), indicating beekeepers find this type of monitoring and data collection meaningful and useful.

25. Palynological analysis of pollen collected by honey bees (*Apis mellifera*) in developed areas in four regions of the United States

Pierre Lau, Juliana Rangel, Vaughn Bryant, Dan Schmehl, Joseph Sullivan, Zachary Huang, Jamie Ellis, and Ana Cabrera. Department of Entomology, Texas A&M University.

Honey bee (*Apis mellifera*) colony maintenance depends on foraging workers to obtain resources from flowering plants year round. Floral nectar provides the carbohydrates needed for the colony's energetic needs, while pollen is consumed as the main source of protein, providing a colony with essential amino acids and proteins critical for growth and development. Studies indicate that a polyfloral diet directly improves colony immunocompetence. Thus, access to diverse floral sources can greatly improve colony health. Because urban development has drastically altered resource availability and diversity for pollinators, understanding the floral resources collected by honey bee colonies in urbanized areas is critical to assess the variety and type of resources that are being consumed in developed areas.

The goal of this project is to identify the floral sources collected by bees in urban settings to better understand the floral ecology and general bee floral preferences in four developed areas in the United States. Pollen and nectar samples are being collected from urban sites in Florida, Michigan, California, and Texas. Pollen is acetolyzed and identified to the family, genus, or species level using palynological techniques. Relative abundance of each type of pollen grain is also conducted. Pollen types vary temporally and over 40 taxonomic families of plants have been identified so far, with important pollen types belonging to the families Aceraceae, Arecaceae, Fabaceae, Rosaceae, Saliaceae, Rutaceae, Lythraceae, Anacardiaceae, Fagaceae, Apiaceae, Myrtaceae, Asteraceae, Arecaceae, Lythraceae. This study is the foundation for future studies looking at honey bee nutrition and floral preferences in urban environments.

26. RNA interference (RNAi) as a novel treatment for *Nosema ceranae* infection in European honey bees *Apis mellifera*

Wenfeng Li[1], Jay D. Evans[1], Qiang Huang[1, 2], Cristina Rodríguez-García[3], Jie Liu[1], Michele Hamilton[1], Christina M. Grozinger[4], Thomas C. Webster[5], Songkun Su[6], Yan Ping Chen[1].

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Nosema ceranae is a new and emerging microsporidian parasite of European honey bees, *Apis mellifera* that has been implicated in colony losses worldwide. RNA interference (RNAi), a post-transcriptional gene silencing mechanism, has emerged as a potent and specific strategy for controlling infections of parasites and pathogens in honey bees. While previous studies have focused on the silencing of parasite/pathogen virulence factors, here we explore the possibility of silencing a host factor as a mechanism for reducing parasite load (Figure 11). Specifically, we use an RNAi strategy to reduce the expression of a honey bee gene, *naked cuticle* (*nkd*) which is a negative regulator of host immune function. Our studies found that *nkd* mRNA levels in adult bees were upregulated by *N. ceranae* infection (and thus the parasite may use this mechanism to suppress host immune function), and ingestion of dsRNA specific to *nkd* efficiently silenced its expression. Furthermore, we found that RNAi-mediated knockdown of *nkd* transcripts in *Nosema*-infected bees resulted in upregulation of expression of several immune genes (*Abaecin*, *Apidaecin*, *Defensin-1*, and *PGRP-S2*), reduction of *Nosema* spore loads, and extension of honey bee lifespan. The results of our studies clearly indicate that silencing the host *nkd* gene can activate honey bee immune responses, suppress the reproduction of *N. ceranae* and improve the overall health of honey bees. This study represents a novel host-derived therapeutic for honey bee disease treatment and will have positive implications for honey bee disease management practices.

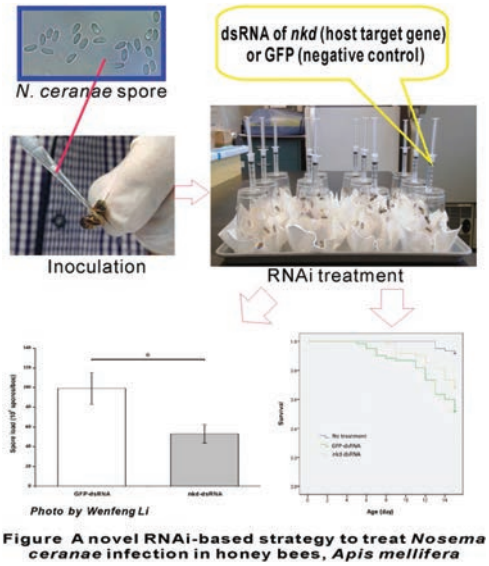

Figure 11. A novel RNAi-based strategy to treat *Nosema ceranae* infection in honey bees, *Apis mellifera*.

27. Glucose oxidase production does not increase after colony infection: Testing its role in honey bee social immunity

Margarita M. López-Uribe[1] & Michael Simone-Finstrom[2].

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Honey bees rely on a variety of defense mechanisms to reduce disease infection and spread throughout the colony. Hygienic behavior, resin collection, and antimicrobial peptide production are some examples of defenses that bees use against parasites (Evans & Spivak, *2010 J Invertebr Pathol 103:S62*). Many of these defenses rely on the collective action of multiple individuals to prevent, reduce or eradicate pathogens—often referred as 'social immunity' (Cremer *et al.*, *2007 Curr Biol 17:R693*). Glucose oxidase (GOX) is an enzyme that produces hydrogen peroxide, a compound with antiseptic properties. GOX and some antimicrobial peptides (e.g. Defensin-1) are secreted by the hypopharyngeal gland of bees to help sterilize food (e.g., honey) and are present as part of the glandular secretions fed to developing larvae. Because of their antiseptic properties and presence in larval food and colony food stores, GOX, in particular, has been used as a "biomarker"

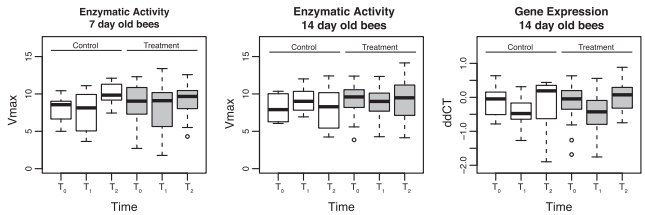

Figure 12. Levels of GOX enzymatic activity (quantified as Vmax) and gene expression (quantified as ddCT) in 7 day and 14 day old bees. White and grey boxplots depict control and AFB treatment colonies, respectively.

for social immunity (Alaux *et al.*, *2010 Environ Microbiol 12:774*). However, the direct role that GOX has as a colony-level immune mechanism has not previously been studied. The aim of this study was to investigate if GOX production increases at the colony level after pathogen exposure to determine if they are compounds that can be induced in worker bees to protect larvae from infection. Specifically, we tested whether GOX activity changes after a colony-level bacterial infection by American Foulbrood (AFB, *Paenibacillus larvae*). We quantified GOX activity in 7 day old and 14 day old honey bee adults before and after bacterial infection using enzymatic assays and transcript expression quantification. We did not detect a change in GOX enzymatic activity between control and AFB infected bees 7 day old (F= 0.01, P= 0.923) or 14 day old (F= 0.116, P= 0.752) bees (see Figure 12). These results were corroborated by the gene expression data (F= 0.007, P= 0.936). Overall, our results indicate that (1) level of GOX production is not induced by exogenous infections in honey bees and may be an example of how mechanisms of individual immune defense can be co-opted to function as an immune mechanism at the colony level, and (2) this lack of inducibility should be considered when using GOX assays as a general biomarker of social immunity.

28. Behavioral and molecular mechanisms of pheromone transmission in honey bees (*Apis mellifera*)

Rong Ma, Gabriel Villar, Christina M. Grozinger, Juliana Rangel. Department of Integrative Biology, University of Texas Austin.

Chemical cues are ubiquitous and are fundamental to the understanding of a diverse range of biological processes. Honey bees have a complex, nuanced pheromonal language for coordinating changes in physiology and behavior. In the context of cooperative brood care, honey bee larvae produce two sets of pheromones—brood pheromone and (E)-beta-ocimene—that elicit an increase in brood care and foraging activity. Since brood care and foraging behaviors are performed by mutually exclusive groups of workers, we tested how these two pheromones are transmitted throughout the colony and processed in the worker brain.

Combining neurobiology and behavior, this series of experiments provides evidence for the transmission mode of two distinct sets of pheromones in honey bee colonies. Our data support the hypothesis that larval signals target multiple worker types and reveal molecular mechanisms involved in pheromone-influenced food preference.

29. *Nosema ceranae*: A sweet surprise? Investigating the viability and infectivity of *N. ceranae* spores

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Nosema disease is a prominent malady among adult honey bees (*Apis mellifera* L.), caused by the microsporidian parasites *Nosema apis* and *N. ceranae*. The biology of *N. apis* is well understood (Fries, *1993 Bee World 74(1):5-19*), as this parasite was first described over a century ago. Unlike *N. apis*, *N. ceranae* is an emerging parasite of the honey bee (Fries, *2010 J Invertebr. Pathol. 103:S73-S79*, Higes *et al., 2010 Apidologie 41:375-392*, Higes *et al., 2013 Environ. Microbiol. Rep. 5:17-19*), and consequently, we do not yet understand how long spores of this parasite survive in honey bee colonies, or how they are transmitted among bees. We have investigated the viability and infectivity (see Figure 13) of the infectious (spore) stage of *N. ceranae* in substrates associated with honey bee colonies after exposure to 20, 33, -12, and -20°C, over various time intervals. Spores stored in honey and sugar syrup survived freezing temperatures for up to one year, considerably longer than those stored in water or on wax comb. Honey and sugar syrup appear to provide a reservoir of viable and infective spores that can initiate or perpetuate *N. ceranae* infections within and between honey bee colonies. These results may help guide current management recommendations to minimize the spread of *N. ceranae* infections.

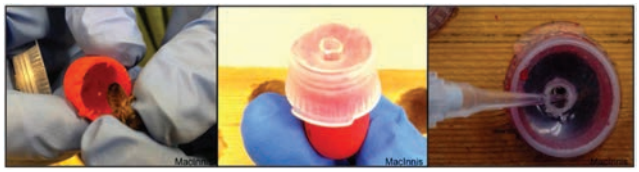

Figure 13. Infectivity was assessed by hand-inoculating newly-emerged *Nosema* spp.-free bees with treated *N. ceranae* spores and estimating number of spores/bee in surviving bees 14 days post inoculation.

30. Feeding strategies to shift honey bee foraging behavior during cranberry pollination and the impact on colony development

Georges Martin[1], and Pierre Giovenazzo[2].
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Over the past years, many beekeepers reported that their colonies weakened during cranberry pollination. The idea of weakening their colonies through cranberry pollination refrains beekeepers from renting them, which in turn reduces the growing potential of the cranberry industry in Canada. The first part of this study had 3 objectives : 1)Evaluate the impact on honey bee colonies of participating in cranberry pollination; 2)Determine if feeding pollen supplement and/or sugar syrup would prevent the weakening of the colonies; and 3) Verify if feeding the bees has a negative impact on cranberry pollination. Different feeding strategies were used with 5 experimental groups of 9 colonies: 1) no feeding, no pollination; 2) no feeding; 3) 15L of 1:1 syrup; 4) 2.25kg of pollen supplement; 5) 15L of 1:1 syrup and 2.25 kg of pollen supplement. Results showed that sugar syrup feeding enhanced cranberry pollen foraging; the ratio of pollen collected coming from cranberry was 19% for the control group and rose to 57% for the syrup fed group. The second part of this study is to determine the

most cost efficient syrup feeding method to enhance cranberry pollen foraging. The following feeding strategies are used with 5 experimental groups of 10 colonies: 1) no feeding; 2) 1 feeding of 15L of 1:1 syrup; 3) 1 feeding of 5L of 1:1 syrup. 4) 2 feedings of 5L of 1:1 syrup 5) 3 feedings of 5L of 1:1 syrup. Preliminary results were presented.

31. Mechanical disturbance as a non-chemical control of varroa mites in honey bee colonies

Meghan E. McConnell and Dennis vanEngelsdorp. Department of Entomology, University of Maryland, College Park, MD

Many factors contribute to honey bee declines (Potts et al., 2010, vanEngelsdorp & Meixner, 2010); however, varroa mites (*Varroa destructor*), and the viruses associated with them, are arguably the primary driving force behind the high rates of loss suffered by beekeepers in the United States (Boecking and Genersch 2008, Guzmán-Novoa et al., 2010). This problem is confounded by the fact that many chemical treatment options fail to work, as mite populations have become tolerant to them (i.e. Amitraz: Elzen et al., 2000, Maggi et al., 2010; Coumaphos: Spreafico et al., 2001; Apistan: Lodesani et al., 1995). Additionally, some treatments require specific temperature ranges for effective mite control to be obtained (i.e. formic acid), while others cannot be applied until after honey flows (i.e. Apiguard), resulting in treatment delays, often well after varroa mite levels surpass economic thresholds. Therefore an effective chemical-free alternative treatment for varroa mites is imperative.

The primary objective of this multi-year study is to determine if nightly, repeated, regular, long-term mechanical disturbance (knocking) of hives will have a measurable impact on varroa mite population growth over the course of a production season. Daily sticky board mite drops were counted and defensive reactions were recorded. Varroa mite and *Nosema* samples were taken monthly in alcohol, as well as brood disease evaluation. Viral and survivorship were analyzed throughout the experiment. The initial analysis shows that more mites are in the brood area of the control (non-knocked) colonies; while the daily sticky board counts showed more mites had fallen out of the knocked colonies than the control, suggesting increased dislodgement.

32. Pollinator protection in Oregon: Getting beyond best management practices?

Andony Melathopoulos and Ramesh Sagili. Department of Horticulture, Oregon State University, Corvallis, OR.

Managed pollinator protection plans (MP3s) are initiatives led by State departments of agriculture to reduce honey bee pesticide exposure. Key to these initiatives are best management practices (BMPs), which are voluntary guidelines for beekeepers, pesticide applicators and growers that, if followed, would reduce exposure. The major obstacles to finalizing these plans, is developing meaningful BMPs that can actually work in the world to reduce exposure on the ground – going beyond best management practices to practices that are simply routine. For BMPs to be meaningful they must: 1) be specific to the context of their State, 2) have a means to set priorities (i.e., to tackle practices of highest risk first), 3) result in goals that can be acted on by extension (i.e., be "extension-able") and 4) have measurable outcomes. We describe efforts in Oregon to deal with these four challenges. The *specific* context for pollinator protection in Oregon revolves around three features, namely the state is constituted by minor/specialty crops (i.e., the path to better outcomes for honey bees has to pass through a shift in practices distributed out across a wide range of crops) and concern for pollinators in the state is not entirely focused on agriculture, but also to a considerable extent around the urban use of pesticides following the 2013 poisoning of bumble bees in suburban Portland. Finally, a peculiarity of Oregon's context is that pollinator protection has overlapping mandates from the Oregon Department of Agriculture (charged with MP3s through EPA) and Oregon State University (which was charged by the Oregon Legislature to develop a pollinator health education and outreach plan).

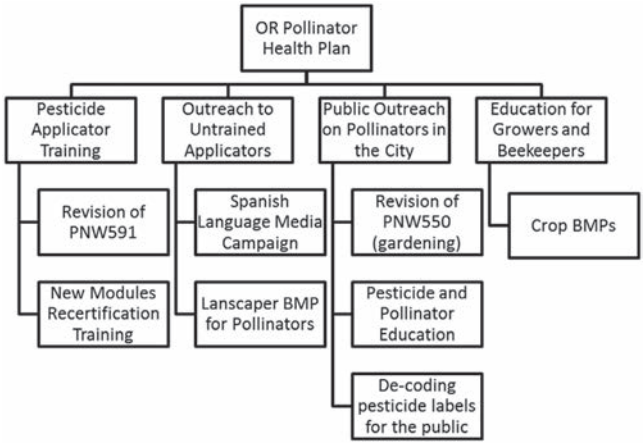

Figure 14. Organizational chart describing the extension and outreach efforts that are part of the Oregon Pollinator Health Plan.

The challenge of *prioritization* and *success metrics* is currently being addressed in Oregon working through a pilot project around the development of Pest Management Strategic Plans (PMSPs). In this process pesticide applicators not only identify current patterns of pesticide usage, allowing for the calculation of actual pesticide exposure hazard, but also reveal the most difficult to control pest complexes associated with exposure, allowing extension to gain better insight into the pest management challenges growers face. The combination of pest management and pesticide hazard could then help inform extension to the highest priority for an "extension-able" plan. Through this process the Oregon Pollinator Health Plan has identified four key groups, around which extension and outreach efforts are being focused (see Figure 14).

33. Sublethal effects associated with supplemental feeding and other stressors in honey bees

Christina Mogren, Jim Ottea and Kristen Healy. Department of Entomology, Louisiana State University.

During periods of pollen and nectar dearth, beekeepers provide supplemental food sources to honey bee colonies in the form of sucrose (SS), high-fructose corn syrup (HFCS), and artificial pollen. However, these artificial substitutes lack many critical nutrients present in pollen, nectar, and honey. We evaluated how honey bees fed these artificial food sources responded to heat, cold, and pesticide stress by measuring survival, food consumption, worker longevity, and levels of protective enzymes (heat-shock protein 70, esterase, and superoxide dismutase) in laboratory and semi-field experiments. While mortality did not differ between feeding treatments in the lab, hive bees fed SS and HFCS consumed significantly

less than those fed honey and HFCS + pollen. Variable differences were observed in enzyme production across treatments with bees fed SS and HFCS generally expressing lower levels, and there was an interaction between feeding treatment and stress exposure. In semi-field experiments, colonies with newly emerged bees fed SS experienced higher rates of worker mortality compared to the other feeding treatments for three weeks after emergence, after which mortality did not differ between feeding treatments. Together, these results suggest that supplemental feeding may have harmful effects on honey bees that are simultaneously experiencing other stressors in the environment.

34. Efficacy of novel bio-pesticides on varroa mites in honey bee colonies

Bernardo D. Nino, Patricia Bohls and Elina L. Nino. Department of Entomology, University of California, Davis.

The ectoparasitic mite *Varroa desctructor* is highly associated with honey bee colony losses in the United States. Synthetic and organic chemicals, also referred to as bio-pesticides, have been used to mitigate their detrimental effect on honey bee colonies by reducing mite infestations within the colonies to below an economic threshold. However, varroa mites have developed resistance to several commercially available synthetic miticides that beekeepers have heavily relied upon in the past. Development of resistance has limited chemical control options for the beekeeping industry and has underlined the need for developing and utilizing novel, preferably "soft" miticides for varroa mite control. Here we evaluated the efficacy of several bio-pesticides against varroa mites within honey bee colonies. Field studies were completed at the University of California Davis in the summer and fall 2016. We evaluated mite infestation levels pre-treatment and every two weeks after that until the completion of the study. Overall, we found there was a significant reduction in total percent change of mite infestation for several tested products.

One novel active ingredient in particular has shown great potential in suppressing *Varroa* infestation. With further investigation and determination of optimal dosage and application timing, this product has the potential to become a valuable additional product for use by beekeepers. We also conducted a longitudinal evaluation of colony strength, stored food resources and survivorship in order to determine product safety. We observed significant differences in colony survivorship between treatment groups as well as hive strength throughout the study. However, we did not record significant differences in the percent change of colony weights between different treatments. Our results point to several novel and promising products that will benefit the beekeeping industry, as well as highlight the importance of implementing a varroa mite management strategy in beekeeping operations.

35. New insights into seminal fluid regulation of post-mating changes in honey bee queens

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Characterizing which specific factors modulate molecular mechanisms regulating the post-mating changes in honey bee queens which could have implications for advancing breeding efforts of beneficial insects such as honey bees. The results of our previous research suggest a complex interplay of various factors triggering and maintaining post-mating changes in queens. For example, the act of copulation itself and seminal fluid volume both play an important role in triggering and maintaining ovary activation, while seminal fluid components are crucial for maintenance of transcriptional changes in various reproductively-important tissues as well as mandibular gland pheromone production. Our current research is focused on further identification of specific seminal components in regulating specific behavioral and physiological processes associated with mating and reproduction. Preliminary data suggest that semen-associated and not sperm-associated proteins are involved in regulating sexual receptivity and possibly pheromone production.

RNA-sequence analysis of transcriptional changes in brains and fat bodies has revealed the regulation of several reproduction-associated pathways. For example, we found expression differences in brain tissue of genes associated with phototransduction and flight, while fat body analysis revealed expression differences in genes associated with juvenile hormone processing. These results will allow us to identify genes for potentially a more targeted manipulation. Identifying individual proteins or protein complexes that support queen reproduction will undoubtedly lead to improved breeding protocols necessary for breeding more resilient honey bee stock.

36. Chronic toxicity of clothianidin supplied via nectar substitute to honey bee colonies

Allen W. Olmstead. Bayer CropScience, Research Triangle Park, NC.

Clothianidin is a systemic, neonicotinoid insecticide that elicits effects in target organisms by interaction with the nicotinic acetylcholine receptor. As part of ongoing registration review activities in the US and Canada, two separate colony feeding field studies were carried out with honey bee colonies in 2014 and 2016. Colonies were exposed to one of five treatment levels of clothianidin (10 to 160 ppb) in 24-26.5 L of sucrose solution which was supplied to colonies inside the hive over six weeks. Control colonies received untreated sucrose. Hives were placed in one of twelve separate apiaries in a randomized block design. Honey bees were allowed to forage freely throughout the duration of the study. Exposure occurred during the summer in which a dearth of floral resources was expected in order to increase consumption of treated solution. Colony condition assessments were performed at various time points before, during the exposure, and after the exposure until October. Two final assessments after overwintering were performed the next year in March. Significant decreases were observed in pollen stores and capped brood during and immediately after exposure at treatment levels of 30 ppb clothianidin and above. These effects were highly consistent between the two studies and exhibited well-characterized concentration-response curves. Additional effects were observed on other endpoints such as adult bee counts; however, these endpoints were generally less sensitive and effects occurred later suggesting these were downstream effects. Overall, these study results indicate that chronic clothianidin exposure of ≥30 ppb results in decreased pollen stores, potentially due to reduced foraging activity. The reduced pollen levels would be expected to result in lower amounts of brood which then translate into effects on other aspects of colony dynamics at later time points as observed in this study. The no observable adverse effect concentration for this study was 20 ppb, which is above residue levels in nectar typically measured in the field for most agricultural uses.

37. The effects of honey bee (*Apis mellifera*) queen insemination volume on colony growth

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A honey bee queen's insemination volume has been demonstrated in previous studies to impact a number of physiological and behavioral traits in both queens and workers including queen pheromone production (Niño et al., 2012) and queen-worker interactions (Richard, Tarpy, & Grozinger, 2007). This study, however, aims to determine how the insemination volume of honey bee queens affects the physiology and productivity of the superorganism colony by comparing the colony growth of hives led by high-quality queens (those instrumentally inseminated with 9 µL of semen) to hives led by low-quality queens (those instrumentally inseminated with 1.5 µL of semen). Virgin queens will naturally mate with an average of 12 drones (7 µL of semen) from diverse genetic sources (Tarpy, Nielsen, & Nielsen, 2004). The results of this study concluded that there were no statistical differences between the two queen types for any of the measured parameters that comprised colony growth. These parameters were recorded during the months of May to October of 2015 and consisted of the hive's amount of worker and drone comb built, sealed worker and drone brood produced, the total amount of food stored (honey, nectar, and pollen), and the total adult population. Survivorship was also measured and compared between the two queen types, but no statistically significant difference was found as well. The results of this study demonstrated that the insemination volume of a honey bee queen does not appear to have any effect on the growth of a colony as a whole for the measured parameters outlined above.

38. Progress in marker-assisted selection for honey bee breeding

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Most economically desirable traits in honey bees show considerable levels of heritability and thus can be improved via artificial selection. Indeed, cross-based genetic analyses have identified broad regions of the honey bee genome (quantitative trait loci or QTLs) that causally affect aggression, hygienic behavior, and several aspects of worker foraging behavior. Although previous honey bee QTL studies have demonstrated the strong genetic basis of many economically desirable traits, they have not been successful at providing stable and robust markers for assisted selection. The honey bee's high recombination rate necessitates new approaches for identifying markers for selective breeding.

Our team developed a novel approach to marker identification, notably the discovery of protein expression patterns that were highly correlated with the specific behavioral traits. We identified 9 putative biomarkers for hygienic behavior (HB), isolated from the antennae of nurse bees, that survived stringent control for multiple hypothesis testing (Guarna e*t al*., 2015, BMC Genomics 16:63). These proteins were further determined to be involved in semiochemical sensing, nerve signal transmission or signal decay. Our data suggested that protein expression patterns were heritable and could be used to selectively breed bees to enrich HB. We then used a panel of protein expression biomarkers to successively test, select and breed several hundred colonies over three generations across western Canada, in a direct comparison of proteomic-based marker-assisted selection versus traditional behaviorally-based phenotypic selection on HB. Selected stock was shown to have improved resistance to American foulbrood disease, improved overwintering survival with *Varroa destructor* infestations as well as favorable economic performance.

Based on the success of HB trait enrichment using protein expression biomarkers, we are currently embarking on a large-scale study to combine proteomics and genome-wide association as these have the greatest potential for identifying highly discriminant markers for bee breeding. Full genome sequencing has the ability to leverage the bee's high recombination rate for identifying single nucleotide polymorphisms (SNP's) that are casually linked to a trait of interest. Progress in identifying proteomic and SNP markers for twelve economically desirable traits, measured in 1,000 colonies across Canada, will be reviewed along with implications for improved methods for trait selection in honey bees.

39. *Varroa destructor* feed primarily on honey bee fat body not hemolymph

Samuel D. Ramsey and Dennis vanEngelsdorp. Department of Entomology, University of Maryland, College Park.

Efforts to mitigate the elevated losses of honey bee colonies have reached a global context as one of the primary drivers of these losses, *Varroa destructor*, has achieved a nearly ubiquitous distribution. Better understanding of the association of this parasite and its host is integral to developing sustainable management practices but very little study, if any, exists as support for the heretofore uncontested conclusion that *Varroa* feed exclusively on the hemolymph of adult and immature honey bees. This study was conducted to determine the primary host tissue composing *Varroa*'s diet. Findings in a preliminary study suggest that the mites may feed on fat body. To test this hypothesis, honey bees were reared to specific ages corresponding to the development of the fat body and fed one of two fluorescent biostains *ad libitum*. The Uranine O biostain persisted in the hemolymph and Nile Red biostain persisted in the fat body. Mites were allowed to feed on these bees for 24 hours and were then crushed and placed in a spectrophotometer. The biostain associated with the fat tissue was present in *Varroa* in significantly greater proportions than the hemolymph biostain in all 4 honey bee age treatment groups. *Varroa* consumed 3 times as much fat body as hemolymph when allowed to feed on the age group associated with nurse bees, at which time fat body is at peak development. To determine the importance of each host tissue in *Varroa*'s diet, adult female mites were collected from uncapped brood in several untreated colonies. These mites were then placed in queen rearing cups lined with beeswax and fed fat body, hemolymph, or a combination of the two through an artificial membrane. Fecundity was measured and analyzed. *Varroa* fed hemolymph produced no eggs while *Varroa* produced eggs in all treatments containing fat body. We are currently conducting studies of survivorship of mites fed on these two host tissues. Preliminary data shows *Varroa* fed only fat body have greater survivorship as well, which suggests that the ingestion of hemolymph may not be integral to growth and development of this mite.

40. Morphological and functional characterization of honey bee hemocytes

Rodney T. Richardson, Megan N. Ballinger, Feng Qian, John W. Christman and Reed M. Johnson. Department of Entomology, The Ohio State University.

Circulatory insect immune cells, known as hemocytes, are specialized in the detection and eradication of pathogens from endogenous tissue. This is accomplished through myriad functional capacities including the production of reactive oxygen species (ROS) and phagocytosis. Here, we describe an optimal method for collecting honey bee hemolymph which consistently yields 4 µL of hemolymph from young nurse bees (see Figure 15). We also differentiate the hemocyte types of honey bees using differential cell staining, characterize the abundances of hemocyte types across multiple ages and assess the phagocytic capacity of these cells. Overall, two cell types were identified, granulocytes and plasmatocytes. Granulocytes were most abundant in developing larvae while plasmatocytes were predominant in adults. Additionally, we observed clear instances of mitotic cell division in circulating granulocytes, suggesting that the fat body is not be the sole origin of hematopoiesis in the honey bee. Using a neutral red staining assay, plasmatocytes were found to exhibit decreased phagocytic capacity relative to granulocytes.

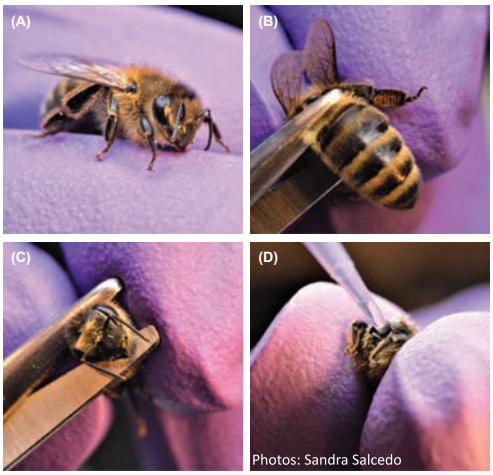

Figure 15. Images showing optimal method of collecting honey bee hemolymph. Honey bees are anesthetized on ice (A), the abdomen is removed (B), followed by the head (C), and a micropipette is used to collect hemolymph from the anterior opening of the thorax (D).

41. Influence of varroa mite (*Varroa destructor*) infestation levels and management practices on insecticide sensitivity in the honey bee (*Apis mellifera*)

Frank D. Rinkevich[1], Robert Danka[1] and Kristen Healy[2].
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Because varroa mites may cause devastating losses of honey bees through direct feeding, transmitting diseases, and increasing pathogen susceptibility, chemical and mechanical practices commonly are used to reduce mite infestation. While miticide applications are typically the most consistent and efficacious varroa mite management method, miticide-induced insecticide synergism in honey bees and evolution of resistance in varroa mites are reasonable concerns. We treated colonies with the miticide amitraz (Apivar®) or used non-chemical management techniques (screened bottom boards, powdered sugar grooming stimulation, and drone brood mite trapping), and left some colonies untreated and then measured the effect of different mite infestations on the sensitivity of bees to phenothrin, amitraz, and clothianidin. Amitraz treatment significantly reduced mite populations compared to the control or non-chemical management methods. Sensitivity to all insecticides varied throughout the 5 month test among and within treatment groups. Clothianidin sensitivity decreased with increasing mite levels, but no such trend was seen with phenothrin or amitraz. In-hive amitraz treatment according to the labeled use did not synergize sensitivity to the pesticides tested; this finding should alleviate concern over potential synergistic effects. Non-chemical mite management methods were largely ineffective at reducing varroa mite infestation in our tests for unknown reasons. These data demonstrate the complex and dynamic variables that contribute to honey bee colony health. The results underscore the importance of controlling for as many of these variables as possible in order to accurately determine the effects of each of these factors as they act alone or in concert with others.

42. Healthy Colony Checklist: A practical system to quickly assess, record, understand, and plan management of honey bee colony health

Richard E.L. (Dick) Rogers. Bayer Bee Care Center, Bayer, Research Triangle Park, NC.

Over the past three decades, *Varroa destructor*, *Acarapis woodi*, *Aethina tumida*, numerous viruses, and a variety of other stressors, have presented increasing challenges to honey bee colony health. The result, in recent years, is that colony health can change dramatically over a short period of time. Therefore, monitoring honey bee colonies is essential, and is a key component to the practice of Integrated Pest Management and Integrated Apiculture (see Figure 16). However, there are many ways to monitor and inspect honey bee colonies, ranging from infrequent casual entrance examinations, to opening hives for detailed quantitative assessments on a regular schedule. Inspections may, or may not, include collection of samples for microscopic, chemical, or molecular analyses. There is also a range of recordkeeping forms and methods, as well as many versions of how inspection observations are processed and used. This all makes for a very complicated and variable approach to monitoring and management of honey bee colony health.

Figure 16. Colony inspections in a California holding yard in January 2013.

To efficiently and effectively protect and improve colony health, it is now essential to monitor colonies more frequently, even as often as weekly. A method for weekly colony assessments would have to be easy to use, fast, thorough, and yield observations that are meaningful and easy to interpret for practical management decision-making by apiarists, apiculturists, and apiologists.

The starting point for the development of a practical colony assessment system was the following simple, high-level description which captured the basics of a healthy colony:

During an assessment, a managed honey bee colony can be considered "healthy" if it does not have any apparent pests and diseases, and seasonally appropriate strength and health sustainability factors are present or can be managed with a reasonable amount of inputs by the beekeeper as needed.

From this description, a more detailed description was crafted which identified six key assessable conditions of a healthy colony. This presentation outlines and discusses the six key conditions used in the Healthy Colony Checklist (HCC), reviews the checklist design and guidelines for use, demonstrates a relational database for the HCC that includes sub-conditions and fatal conditions, and proposes how the HCC system might be used effectively for practical colony inspections and management planning. How the HCC system might be used for identifying beekeeper knowledge gaps, and as a training tool, will also be mentioned. The HCC system goes a long way to making more frequent colony assessments and better management of honey bee colony health achievable.

43. New insights into *Nosema ceranae* infection: Colony level infection dynamics and effects of pollen nutrition

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Nosema ceranae is a widely prevalent microsporidian parasite in the western honey bee. There is significant uncertainty regarding infection dynamics of this important pathogen in honey bee colonies. Understanding the infection dynamics at the colony level may aid in development of a reliable sampling protocol for *N. ceranae* diagnosis, and provide insights into efficient treatment strategies. In the first study our objective was to characterize the prevalence (proportion of the sampled bees found infected) and intensity (number of spores per bee) of *N. ceranae* infection in bees from various age cohorts in a colony. We examined *N. ceranae* infection in both overwintered colonies that were naturally infected with *N. ceranae* and also in quadruple cohort nucleus colonies that were established and artificially inoculated with *N. ceranae*. We also examined and quantified effects of *N. ceranae* infection on hypopharyngeal gland protein content and gut pH. There was no correlation between the prevalence and intensity of *N. ceranae* infection in composite samples. Our results suggest that the prevalence and intensity of *N. ceranae* infection is significantly influenced by honey bee age. The *N. ceranae* infection prevalence values from composite samples of background bees were not significantly different from those pertaining to marked-bee age cohorts specific to each sampling date. The foraging-aged bees had a higher prevalence of *N. ceranae* infection when compared to nurse-aged bees. *N. ceranae* did not have a significant effect on hypopharyngeal gland protein content. Our results suggest that analyzing individual bees in a composite sample (mixed age bees) for *Nosema* prevalence appears to provide the most accurate diagnosis of a colony's infection. This study provides comprehensive insights into *N. ceranae* infection dynamics at the colony level.

Honey bee colonies are fed pollen or protein substitute during pollen dearth to boost colony growth and immunity against pests and pathogens. In the second study we hypothesized that *N. ceranae* intensity and prevalence will be low in bees receiving high pollen diets, and that honey bees on high pollen diets will have higher survival and/or increased longevity. We examined the effects of different quantities of pollen on (a) the intensity and prevalence of *N. ceranae* and (b) longevity and nutritional physiology of bees inoculated with *N. ceranae*. Significantly higher spore intensities were observed in treatments that received higher pollen quantities (1:0 and 1:1 pollen:cellulose) when compared to treatments that received relatively lower pollen quantities. There were no significant differences in *N. ceranae* prevalence among different pollen diet treatments. Interestingly, the bees in higher pollen quantity treatments also had significantly higher survival despite higher intensities of *N. ceranae*. Here we demonstrate that diet with higher pollen quantity increases *N. ceranae* intensity, but also enhances the survival or longevity of honey bees. The information from this study could potentially help beekeepers formulate appropriate protein feeding regimens for their colonies to mitigate *N. ceranae* problems.

44. The temporal and spatial distribution of the honey bee pest *Nosema* spp. in the United States

Daniel R. Schmehl, Kim Huntzinger and Dick Rogers. Bayer Crop Science, Research Triangle Park, NC.

Nosema (*Nosema* spp.) is a microsporidian parasite of the Western honey bee (*Apis mellifera* L.) and is implicated as a contributing factor for colony loss. Nosema germinates inside the honey bee midgut and is known to destroy the epithelial cell membrane and is quickly spread among individuals and within the colony through trophallactic feeding and defecation. Nosema has a global distribution and spans multiple climatic zones, but it is not well understood how Nosema spread and intensity changes among different regions and times of year. Our project goal was to characterize the spatial and temporal spread and intensity of Nosema in the United States.

Apiaries were identified in four states (Florida, Indiana, North Carolina, and Utah) and approximately 12 hives were selected within each state. Returning foragers were collected each month (depending on environmental and climatic conditions) from the same hives and stored frozen until processing. The resulting foragers were processed at the Bee Care Center (Research Triangle Park, North Carolina, USA) and spores quantified. We did not find any significant differences in Nosema infection among states, but there is a significant difference by month. The greatest intensity of spore loads is during the late winter; however Florida has higher Nosema infections in the summer when compared with the other locations. The data do not suggest a

correlation between pre-wintering Nosema loads and peak intensity, suggesting that pre-winter monitoring or treatments will not provide a benefit for reducing overwintering colony losses. Our data can be used to further our understanding of when colonies are most at risk from Nosema infection and how these findings can be used to reduce annual honey bee colony losses.

45. Noisy dancers: information and individuality in honey bee waggle dance communications

Roger Schuerch. Department of Entomology, Virginia Tech, Blacksburg, VA.

When a honey bee forager has found a good resource, usually the nectar or pollen that is her food, she recruits her nestmates by communicating the location of the forage with the waggle dance. This unique behavior may therefore provide valuable data to researchers studying honey bee foraging because it conveys biologically relevant information about where bees go to collect food. However, this information is noisy: waggle dances possess high amounts of intra-individual variation, or imprecision, which must be considered in the decoding, analysis, and mapping of honey bee waggle dances. Here I additionally report that honey bee waggle dances possess high amounts of inter-individual variation, or inaccuracy, between individuals, even from the same colony. These data provide a fascinating glimpse into how communication signals may vary, even when one expects selection to favor accuracy and precision, and opens future investigations into how variation may incur a communication cost (or benefit).

46. Re-evaluating pesticide risk by mode of action

Kirsten S. Traynor, Jeffery S. Pettis, David R. Tarpy, Christopher A. Mullin, James L. Frazier, Maryann Frazier & Dennis vanEngelsdorp.
Department of Entomology, University of Maryland, College Park.

Honey bees interact with their environment, traversing a wide range of habitat. During their foraging flights they are exposed to a large range of toxins. Some of these they bring back to the colony, which are then transferred into the in-hive matrix. Because of the social nature of honey bees colonies and the wide territory they cover, understanding their true exposure is a very difficult problem. We are still struggling to understand and quantify true risk. Multiple pesticide residues often combine in the colony environment, complicating risk assessment. In the 147 samples of stored pollen analyzed, we found 61 different pesticide products or degradants, with 1,061 total detects, a mean of 7.22 ± 0.30 per sample. However, when we exclude detects that contribute less than 50 points to the Hazard Quotient (HQ) score, this drops to 13 different products, 156 detects, with a mean of 1.06 products per sample. 15% of all pollen samples exceeded the HQ safety threshold of 1,000 points (Figure 17).

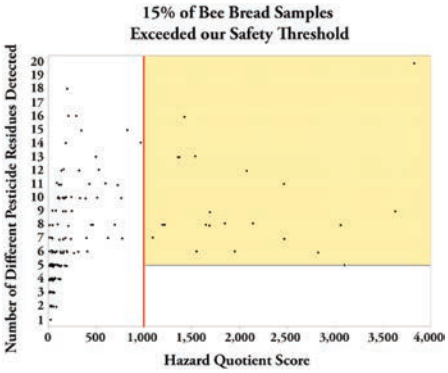

Figure 17. Pollen samples exceeding the hazard quotient threshold of 1,000 points in the shaded region, indicating a nurse bee would consume 10% of her LD_{50} during her 10-day nursing phase if toxicity were calculated cumulatively and no detoxification occurred.

Honey bees must detoxify foreign substances, thus grouping pesticide contamination by their mode of action can provide insight into which groups may be detrimental to honey bee health. While fungicides are currently considered bee safe, we found that fungicides with particular modes of action increased disproportionally in wax within colonies that died during the beekeeping season. These same G. sterol and M. multisite fungicides were also correlated with increased queen events, as were pesticides classified as 3. NaCh, sodium channel modulators. This work is published as Traynor *et al*., 2016 *Scientific Reports* **6**: 33207.

47. Expert-based best management practices for US beekeepers

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[1]Department of Entomology, University of Maryland, College Park, MD. [2]Faculty of Veterinary Medicine, Université de Liège, Liège, Belgium.
[3]Department of Entomology and Plant Pathology, University of Tennessee, Knoxville, TN.

Over the past 10 years, a survey of honey bee (*Apis mellifera*) mortalities in the US revealed an average of one in three colonies dying over the winter (Seitz et al., 2016 J. Apic.Res. 54(4): 292-304; Kulhanek et al. *in prep*). We successfully developed a scoring system, based on expert opinions, able to aggregate complex management information into a simple index that correlates with increased survivorship of colonies over the winter. Sensitivity analysis was used to identify the core management criteria driving the correlation. The top management criteria were identified in various subsets of respondents, resulting in different sets of recommendations based on region and operation-size. In particular, we will develop the topic of *Varroa* management and how it differed between small-scale and commercial operations. The disparity of the top influencing criteria between operation types illustrates the divergence in the beekeeping industry and the need to develop extension programs that address backyard and commercial beekeepers independently.

48. Insecticide fungicide interaction and increased toxicity to honey bees

Andrea Wade and Reed Johnson. Department of Entomology, The Ohio State University.

Honey bees are a crucial part of modern agriculture and are heavily relied upon for their pollination services. The California almond industry uses 2.12 million hives each year during bloom for pollination services (Nat. Res. Coun., 2007 PNAS). Recently beekeepers have

Figure 18. Photo of topical dosing of three day old adult worker honey bees with 5ul of chlorantraniliprole solution.

been observing abnormally high die offs in their colonies used for almond pollination. Many beekeepers and researchers are looking to pesticides as a potential cause (Inskeep, 2014 NPR). Honey bees have a natural detoxification mechanism using P450 enzymes to facilitate metabolism and excretion of foreign compounds that might otherwise be toxic. It has been discovered that a certain class of fungicides, sterol biosynthesis inhibitors (SBI) can inhibit honey bee P450 enzyme activity. Without P450 enzyme activity, toxicity of some simultaneously exposed insecticides increases, resulting in honey bee mortality (Colin et al., 1992 Pestic. Sci.; Iwasa et al., 2004 Crop Prot.; Johnson et al., 2013 PLOS ONE; Pilling et. al, 1993 Pestic. Sci.; Thompson et al., 2003 Bull. Insectology).

Dose response bioassays using three day old worker honey bees topically dosed with chlorantraniliprole or chlorantraniliprole and propiconazole were performed (see Figure 18). Chlorantraniliprole is an anthranilic diamide class insecticide and propiconazole is an SBI fungicide. Alone, propiconazole did not result in significant honey bee mortality. When combined with chlorantraniliprole, propiconazole decreased the lethal dose 50 value of chlorantraniliprole from 0.706 µg bee^{-1} to 0.098 µg bee^{-1}. Larval rearing bioassays were performed using young larvae grafted into tissue culture plates and kept in incubators. Larvae were given a single exposure of either 1.00 µg bee^{-1} chlorantraniliprole or 2.25 µg bee^{-1} propiconazole alone or a combination of the two through their diet. The chlorantraniliprole and propiconazole treatments had 73.9% and 78.3% survival to adult emergence respectively, which were not significantly different from the solvent control group, while the combined chlorantraniliprole propiconazole treatment had 4.3% survival. The results from this study demonstrate that in both adult and larval honey bees, propiconazole increases chlorantraniliprole toxicity in a synergistic manner. The findings of this study suggest that combinations of agricultural chemicals honey bees might encounter while foraging could pose higher risks than previously assumed from testing of compounds independent from each other.

49. The effects of pesticides on honey bee (*Apis mellifera*, L.) queen reproductive physiology and behavior

Elizabeth Walsh and Juliana Rangel. Department of Entomology, Texas A&M University.

Honey bee (*Apis mellifera*) populations continue to decline, partially due to the ectoparasite *Varroa destructor*, which often causes colonies to collapse and die. Varroa mites were initially controlled with two in-hive miticides: the organophosphate coumaphos and the pyrethroid tau-fluvalinate. Although neither are currently used (due to the development of mite resistance to both products), coumaphos and fluvalinate as well as other agrochemicals are still found together at high concentrations in commercial colonies across the country, likely due to their long half-life and their absorption into the lipophilic beeswax. Sublethal in-hive levels of these pesticides have been shown to cause colony-wide health problems. To date, most studies on the effects of pesticides on colony health have either not used field-relevant concentrations of pesticides, or have not explored the effects of pesticide combinations.

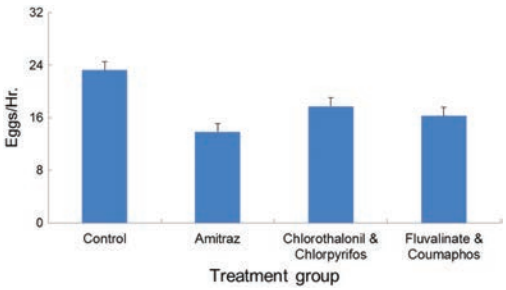

Figure 19. Queens reared in pesticide-free wax had higher egg laying rates than queens reared in pesticide-contaminated wax. Control mean: 23.32+/-0.02; Amitraz mean: 13.85+/-0.01; C+C mean: 17.72+/- 0.02; F+C mean: 16.26+/-0.01 (Tukey-Kramer, $p<0.0001$).

In this study, we formed three experimental groups exploring whether (1) the combined presence of coumaphos and fluvalinate, as well as the currently used (2) amitraz, and (3) chlorothalonil and chlorpyrifos in the queen-rearing beeswax environment has an effect on queen reproductive health by raising queens in pesticide-free beeswax or beeswax containing known concentrations of pesticides. We measured queen attractiveness to workers, a proxy measurement for queen health, as well as egg-laying rate as measured in eggs laid/min (Figure 19), sperm viability in the spermathecae, and mating frequency.

Our results indicate that exposure to pesticides during queen development severely alters the reproductive health of honey bee queens by impacting the queen pheromones, which are what the queens use to attract caretakers, and the queen reproductive physiology. Our results have important implications regarding the potential synergistic effects of beekeeper-applied miticides and agrochemicals on colony health. In light of our findings, it seems clear that the beekeeping industry needs to adopt an integrated pest management (IPM) approach to *Varroa* control where cultural and physical-mechanical methods of pest control are utilized before pesticides. To beekeepers, this means carefully monitoring and recording *Varroa* infestation levels inside of colonies in order to keep below the economic injury threshold and culling old frames out of operations in order to keep pesticide concentrations as low as possible.

50. Effects of neonicotinoid pesticides on male honey bee reproduction

Geoffrey Williams[1,2,3], Lars Straub[2], Laura Villamar-Bouza[2,4], Selina Bruckner[2], Panuwan Chantawannakul[5], Laurent Gauthier[3], Kitiphong Khongphinitbunjong[6], Gina Retschnig[2], Aline Troxler[2], Beatriz Vidondo[7], Peter Neumann[2,3].

[1]Department of Entomology and Plant Pathology, Auburn University, Auburn, AL. [2]Institute of Bee Health, Vetsuisse Faculty, University of Bern, Bern, Switzerland. [3]Agroscope, Swiss Bee Research Centre, Bern, Switzerland. [4]Environmental Science Department, University of Koblenz-Landau, Landau, Germany. [5]Bee Protection Laboratory (BeeP), Department of Biology, Faculty of Science, Chiang Mai University, Chiang Mai, Thailand. [6]School of Science, Mae Fah Luang University, Chiang Rai, Thailand. [7]Veterinary Public Health Institute, Vetsuisse Faculty, University of Bern, Bern, Switzerland.

Sub-lethal effects of neonicotinoid pesticides on honey bee lifespan, behavior and physiology are relatively well-documented; however, their possible impact on reproduction is less so. Here we reveal that neonicotinoids can affect the reproductive health of male honey bees (see Figure 20). Drones reared using queenright colonies were randomly allocated to either (1) neonicotinoid or (2) control groups (N=10 colonies per group). Pollen supplements were provided to colonies ad libitum for 8 weeks; those fed to the neonicotinoid group were spiked with 4.5 ppb thiamethoxam and 1.5 ppb clothianidin. Emerged drones were maintained in laboratory cages for 10 days to sexually mature prior to assessment. While no significant effects were observed for drone emergence body mass (Three-level General Linear Model, P=0.804) and sperm quantity (Three-level Negative Binomial Model, P=0.1375), the data clearly showed reduced drone lifespan (Three-level Survival Model, P<0.0001), as well as reduced sperm viability (proportion living vs. dead) (Three-level Ordered Logistic Model, P=0.0277) and living sperm quantity (Three-level Negative Binomial Model, P=0.049) in the neonicotinoid group. Our results demonstrate for the first time that neonicotinoid pesticides can negatively affect male insect reproduction, and provide one possible explanation for managed honey bee queen failure and wild insect pollinator declines. This work is published as Straub *et al.*, 2016 *Proc. R. Soc. B* 283: 20160506.

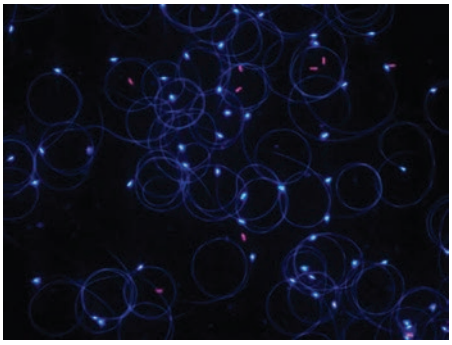

Figure 20. Stained drone honey bee *Apis mellifera* sperm viewed using fluorescent light microscopy. Those colored red and bluish-green are dead and alive, respectively.

51. Enhancing turf lawns to benefit honey bees and other pollinators

James Wolfin, Marla Spivak, Eric Watkins, Ian Lane. Department of Entomology, University of Minnesota, Twin Cities.

Turf lawns currently account for nearly 2% of the area of the continental United States, with this number expected to grow as a greater percentage of the country's population moves to urban and suburban areas. While the main function of a traditional lawn is to provide aesthetic appeal around one's property, it may be possible to redesign the turf lawn to increase the biodiversity of pollinators, including honey bees. Enhancing a turf lawn with low growing flowers can provide these pollinators with the floral rewards that are essential to their diet. We evaluated which species of grass and forbs were best suited for a bee lawn, and examined the effect of different pre-seeding disruption treatments on forb establishment. In addition, we sampled bees on white clover in Minneapolis parks to define the communities of bees present before turflawn enhancement. We found that hard fescue (*Festuce brevipila*) and Kentucky bluegrass (*Poa pratensis*) result in higher rates of forb establishment, and four of eight forbs tested were able to establish in Minnesota soils. Additionally, scalping and aeration improved forb establishment. Our surveys found that at least 37 species of bees visit white clover. Our results help to determine the best methods for establishing a bee lawn, and provide a basis for comparing bee diversity and abundance in enhanced and unmodified lawns in the future (see Figure 21).

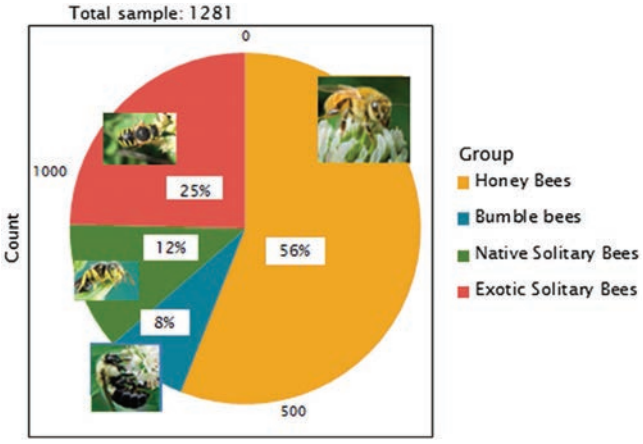

Figure 21. Community composition of bee pollinators in Minneapolis parks, 2016.

Abstracts from Poster Presentations

52. Data standardization: The first step to building an intelligent hive management system

Joseph Cazier. Center for Analytics Research and Education, Appalachian State University.

This presentation focuses on the data standardization process of a larger study designed to build an intelligent hive management systems for beekeepers and engage citizen scientists in bee data collection to help optimize bee health and pollination services. The data platform will be used and designed by our data partners, which include commercial beekeepers, some smaller beekeepers,

those already using various smart hardware and software systems willing to share their data with this project as well as others partners who volunteer via the Hive Tracks commercial software platform.

(1) The first step is data standardization. In this phase of the project we go through an open process of engaging key stakeholders, including researchers, the commercial beekeeping community, pollination dependent farmers and others. Additionally this step will include scientific benchmarking to assist and validate that the right data is being collected in the right way. The goal of this process is to present and get feedback on the data that matters the most for the project and trying to define a process and procedure for what it means. This way there is a common and clear definition for all of the data going into the data platform. (2) The second step is the modification and expansion of Hive Tracks current data platform to collect, store and process the standardized data needed for this project in a way that will allow the application of advanced data analytics to generate key insights to build into improved software models. (3) The third step is data management and integration. (4) The fourth step in the process is the application of advanced analytical techniques to the data collected and managed in the previous techniques. (5) The fifth step of data dissemination involves the sharing of knowledge and insights gained with key stakeholders.

The result of these 5 steps will be a comprehensive data platform that collects hive management and natural world data that can be managed, aggregated and analyzed to optimize commercial hive management.

53. Molecular investigation of honey bee foraging on soybean, Glycine max, in Ohio, USA

Hailey Curtis, Rodney Richardson, Chia-Hua Lin, Reed Johnson. Department of Entomology, The Ohio State University.

In a previous study, we surveyed Ohio honey samples and quantified soybean content using palynology. We found the proportion of soy pollen to be related to the amount of soybean cultivation surrounding an apiary. However, given the difficulty of identifying pollen to the species level using palynology, we applied a molecular method to confirm the presence of soy pollen. We extracted and analyzed pollen DNA from eight honey samples and a positive control sample of Glycine max leaf tissue. Using PCR with soybean-specific primers and gel electrophoresis, we confirmed the presence of Glycine max in Ohio honey samples.

54. Honey bees in Brazil do not require treatment for *Varroa* infestations

David De Jong, Aline Patty Turcatto, Joyce Mayra Volpini de Almeida, Elisa Cimitan Mendes. Genetics Department, University of São Paulo, Brazil.

The honey bees in Brazil survive infestations with the mite *Varroa destructor* without treatment. They are therefore considered tolerant of *Varroa*. Though infestations were initially high, with up to 50% of bees infested in colonies in our university apiaries in 1981 (first found in Brazil in 1978), they soon decreased to a mean of less than 5%. The large population of freely-breeding wild colonies, freely mating with the bees in apiaries in which less than 1% of queens are artificially substituted per year, allowed natural selection to act unimpaired. Initially we were concerned about the effects of the mites in the cooler regions of the country. Some beekeepers in the southernmost states of Santa Catarina and Rio Grande do Sul indicated that they had very high infestation levels. Infestation levels increased with latitude as we sampled apiaries from north to south, reaching critical levels in the Pampas region of Argentina. We decided to compare infestations in a warm region of Brazil (Ribeirao Preto, Sao Paulo state) and in one of the coolest regions of the country (Sao Joaquim, Santa Catarina state, 1600 m altitude), where it sometimes snows. We found that the infestation levels were much higher in the cool region, even though we had reared and mated all the queens in the same apiary in Sao Paulo state. We can still find *Varroa* in all in Brazil, but at very low infestation levels, that do not cause detectable colony-level damage.

Africanized bees are more adept at removing the mites from their bodies than are European bees, and this behavior is heritable. Africanized honey bees are more hygienic than are European bees, which means that they will more efficiently remove varroa-damaged brood. Other factors that influence the effect of these mites on Africanized honey bees include cell size, comb age, colony size, nutrition, climate, distance between colonies, differential reproduction and progeny mortality. One of the possible reasons for the lack of serious problems with *Varroa* in Brazil was that the *Varroa* in Brazil was somehow different. However, this did not seem likely, given that varroa was introduced initially into Paraguay (from Japan) and then spread to Brazil and south to Argentina. Supposedly, this was a contiguous population, yet the mites killed the colonies in Argentina. Later, two types of *V. destructor* were discovered, a more virulent Korean/Russian type, common in Europe and North America, and a less virulent Thai/Japanese type found in Brazil. This could help explain the tolerance of the Africanized honey bees. However, later collections in the 1990s revealed that Brazil also had the Korean/Russian type, and more recently, only the Korean/Russian type has been found on the continent. The bees in Brazil still survive the mites without treatment, so apparently they have also adapted to this new more virulent mite, though there have been some reports of higher infestations in more recent years. It is possible that the bees initially adapted to a less-virulent type of mite and consequently were partially pre-adapted to the Korean/Russian type, subsequently adapting to the new form of *Varroa*.

55. Antioxidative enzymes expression in honey bee (*Apis mellifera*) queens as an assessment of reproductive quality

Alejandra N. Gonzalez Rojos and Juliana Rangel-Posada. Department of Entomology, Texas A&M University.

Honey bee (*Apis mellifera*) queens mate at the beginning of their lives with 12 to 15 drones. After mating, a subset of the spermatozoa travels to the spermatheca, the spermatozoa storage organ of queens. After mating, the spermatazoa should remain viable for 2-5 years, which is the queen's life span. Previous studies suggested that spermatozoa remain metabolically active inside the spermathecae.

Metabolism produces the energy needed for long term storage, but also generates reactive oxygen species (ROS) which can damage spermatozoa viability. We hypothesize that antioxidative enzymes in the spermathecae of mated queens and semen of drones are necessary to eliminate ROS and maintain spermatozoa viability. Therefore, we measured relative gene expression of Catalase, Glutathione-S-transferase (GST), SOD1, Thioredoxin reductase 1 (TrxR-1), Thioredoxin 2 (Trx-2), SRP16 and a predicted Glyoxalase domain-containing protein 4-like in the spermathecae of mated and unmated queens. To analyze conservation of the antioxidative enzyme's gene expression, we measured and compared gene expression in two different subspecies of honey bees. We found conservation along all measured parameters in both subspecies. Expression of TrxR-1, Trx-2 and Catalase was elevated in mated queens when compared to virgin queens. In addition, Glyoxalase domain-containing protein 4-like and SOD1 were highly expressed in the spermathecae of mated and virgin queens. Our findings suggest that antioxidative enzymes might play a role in spermatozoa viability inside the spermathecae of mated honey bee queens.

56. Assessing *Monarda fistulosa* var. *menthifolia* for possible honeybee health and habitat enhancement

Robert Heyduck[1], Melanie Kirby[2], Todd Bates[3].
[1]New Mexico State University, Alcalde, NM. [2]Zia Queen Bees, Truchas, NM. [3]New Mexico Native Plant Recyclers, Embudo, NM.

Extracts of oregano (*Origanum vulgare*) have been shown to positively affect animal health and production in such varied organisms as poultry (Giannenas, et al. 2003 Archives of Animal Nutrition 57.2: 99-106), fish (Zheng, 2009 Aquaculture 292.3: 214-218), and rabbits (Botsoglou, et al. 2004 Archives of Animal Nutrition 58.3: 209-218). The majority of this effect is attributed to carvacrol and thymol, two main constituents in the essential oil of oregano which have shown bactericidal action. Specific to bee health, thymol has been used to successfully control varroa mites and prevent fermentation and the growth of mold in bee colonies (Calderone, 1999 J Econ Entomol 92.2: 253-260; Floris, 2004 J of Econ Entomol 97.2: 187-191), and thymol based formulations are already commercially available. In addition, essential oils of oregano have been tested as a supplement to realize the same effects. *Monarda fistulosa* var. *menthifolia*, is a widespread North American native plant (alternately known as bee-balm, wild bergamot, or *oregano de la sierra*) that possesses a similar chemical profile to oregano including carvacrol, thymol, α-pinene, β-pinene, sabinene hydrate, α-terpinene, citronellyl acetate, and β-caryophyllene (Zamureenko, et al. 1989 Chem Nat Compd 25.5: 549-551). We seek to evaluate *Monarada* as a habitat enhancing plant by assessing the presence and relative concentration of thymol and carvacrol in nectar, honey, and hive architecture while *Monarda* is flowering and afterwards to determine the persistence of the chemical constituents and evaluate the effect on mite populations (see Figure 22).

Figure 22. A blooming stand of *Monarda fistulosa* var. *menthifolia*, which is also known as bee-balm, wild bergamot, or *oregano de la sierra*.

57. Pollen collection, honey production, and paid pollination services

Shelley Hoover and Lynae Ovinge. Alberta Agriculture and Forestry, Alberta, Canada.

The provision of pollination services is becoming an increasingly important part of the beekeeping industry in North America. We discuss the production of hive products (honey and pollen) as part of the management of hives used to pollinate hybrid canola seed production fields in Southern Alberta. The addition of pollen collection to pollination management offers beekeepers a more diverse income stream, as colonies produced an average of 3.98 lbs trapped pollen during the bloom. However, there was a negative impact of the addition of pollen traps on honey production. Hives without a pollen trap produced 44.4 lbs honey, whereas those with a pollen trap produced 30.1 lbs over the month long pollination contract. There was, however, no trade-off between honey production and pollen collection among hives with a pollen trap in place, as honey production and pollen collection were positively correlated. There was also no negative impact of trapping pollen on brood production.

Overall, at current prices for either bulk or farmers' market sales (bulk sales $1.10 / lb honey, $14 clean dried pollen; farmers' market $8/lb honey, $45 / lb pollen), the addition of targeted pollen trapping during pollination service provision can increase the per-colony profit of beekeepers, without negatively affecting colony health or crop pollination (see Figure 23).

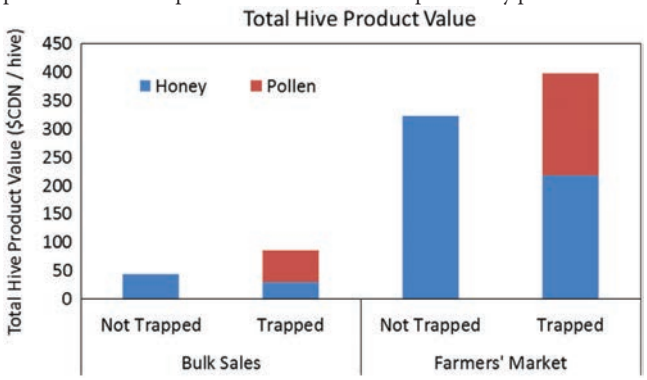

Figure 23. Proportions of the value of honey (blue) and pollen (red) sold either in bulk or at farmers' markets from colonies where pollen was trapped or not.

58. Trans-regional survivor stock and longevity-based breeding program: A reflection of living laboratory case studies (2000-2017) from the shores of Lake Superior to the banks of the Rio Grande

Melanie Kirby[1], Mark Spitzig[2].
[1]Zia Queenbees Farm & Field Institute, New Mexico. [2]Superior Honey Farms, Michigan.

The bee industry has long relied on stock lines from a dwindling genetic pool; and in some cases, stock propagated in compromised settings or in overly stressful circumstances. And while selective pressures for testing quality stock lines is needed to ensure "conditioning" of the bees and to activate genetic stories for coping and adapting, the current onslaught of environmental and social implications does make the task of finding bees and queens that can endure daunting. Zia Queenbees Farm (ZQB, see Figure 24) has been on a mission since its inception to define survivor stock and establish the OLT (Overall Lifetime Merit) which not only has site specific implications; but also the potential to transcend regional boundaries by testing and exchanging stock in multiple regions of the U.S. (McNeil, 2014, *Amer. Bee J.* 154 (10): 1087-1091)

Figure 24. Colonies located in a meadow in Truchas, New Mexico.

ZQB has been collaborating with beekeepers in MI, FL, CO, VT, OR, PA, NC, CA & HI for the past 17 years. ZQB has helped to nurture strain diversification of the bottle necked genetic pool; and support honey bees chosen by beekeepers for beekeepers through a *Father Time Tested-Mother Nature Approved* paradigm (McNeil, 2009. *Amer. Bee J.* 194 (4): 354-355).

- 2000-2009: Superior Honey Farms (MI). Original stock: NWC; SMR/VSH Italian; Cordovan. Crosses selected for overwintering and productivity under a no-treatment approach.
- 2005: ZQB established (NM). Incorporation of additional VSH; NWC; Cordovan strains.
- 2006: ZQB queen production (NM & CO).
- 2007: WSARE grant for establishing The Southwest Survivor Queenbee Project (Kirby, 2008. *Amer. Bee J.* 148 (93) 233-236).
- 2008: Integration of 85% Russian crosses from Vermont: Champlain Valley Queens & Bees- (K. Webster); Oregon: Old Sol Apiaries (J. Jacob)
- 2010: Initiation of California "Surf-ivor Bees" with central valley & bay area beekeepers: ZQB survivor queens found in CA of 2, 3, and 4 years of age that endured 7 annual pollination migrations throughout CA (Kirby, 2011. *Amer. Bee J.* 601-606).
- 2011: Scientific Beekeeping- R. Oliver conducts random mite counts on ZQB crossstocks (<.03%)
- 2012: ZQB mentors Bonnie Bee & Co. (CA), Marin Adapted Survivors. 2*nd* WSARE grant for The Rocky Mountain Survivor Queenbee Cooperative educational network and stock exchange program with 9 beekeepers from Santa Fe, NM to Fort Collins, CO (477 miles; 7 counties; 5000-9000') (Kirby, 2013. *Amer. Bee J.* 175-177).
- 2013: ZQB mentors Wings of Nature Bees (CA- Bay Peninsula Preserve). ZQB Recipient of NMDA Ag Advance & Product Promotion Grant.
- 2014: Mitotype DNA testing of ZQB breeding stock by Dr. Juliana Rangel (Texas A&M). Old World Strains found in isolated New Mexico canyon and ZQB mating apiary. Survivor stock virgin queen exchanges with PA queen breeders: (S. Repasky; V. Aloyo – EAS Master Beekeepers).
- 2015-2016: Collaborations with northern CA breeders: Wings of Nature Bees; Can-Am Apiaries; Heitkam's Honeybees. Integration of ZQB breeding stock for large scale commercial queen production.
- 2017+: Continued collaborations in CA, CO (Dr. Jose Villa), HI (Big Island Queens); FL (Wonderful Bees) for further testing, propagation and distribution.

59. Teachers, gardeners and small-scale farmers: A hands-on educational approach to talking about honey bees

Martin A. Matisoff[1], S. Kivikko[2], S. Kivikko[2], L. Deem[3] and Tom C. Webster[1].
[1]College of Agriculture, Kentucky State University, Frankfort, KY. [2]Ag in Progress Partnership and Illinois Department of Agriculture, Springfield, IL. [3]Director of the UIUC Pollinatarium, University of Illinois, Urbana-Champaign, IL.

Reading books is the traditional method for teaching educators, gardeners, small farmers, and students about honey bees; however, books do not provide the full picture. The only way to learn about any topic is through interactive learning, and honey bees are no different. One way to learn about bees is to link life to memory, i.e., draw a conclusion based on analysis and visual aids such as illustrations. Working with bees requires a basic knowledge about beekeeping: what is a bee? how do I prevent swarms, when should I re-queen, why are my bees acting in a particular way, what is a waggle dance, or why do queens pipe? At the July 2016 Heartland Apicultural Society meeting in Bowling Green, Kentucky, a group of educators met to talk about the most beneficial methods for teaching students about biology, specifically, apiculture. We agreed that "field books" provide the best method for teaching students about biology, and apiculture, in particular. When used in tandem with hands-on laboratories, students can learn to think in broader terms, i.e., cause and effect. By using illustrations, farmers, gardeners, and students can "actually" see what is happening because drawing creates visual and memory links that help us understand complex topics. Illustrations allow us to imprint information in memory. By using a rubric, educators can successfully measure how well individuals understand and can apply what they learn from in workshops and field days.

60. Optimizing drone fertility with nutritional supplements to honey bee colonies during spring

Andrée Rousseau[1], Pierre Giovenazzo[2].
[1]Centre de recherche en sciences animales de Deschambault. [2]Biology Department, Laval University.

Supplemental feeding of honeybee colonies in spring is essential for colony buildup in northern beekeeping regions. The impact of this feeding on drone production and sperm quality is not well-documented, but may be essential to optimize fecundation of early-bred queens. In this study, the impact of feeding sucrose syrup and/or protein supplements to 20 colonies in early spring in Quebec (Canada) was evaluated on drones. Drones were reared under different nutritional regimes: a group receiving a sugar solution made up in the proportion of 1 kg sugar dissolved in 1 liter of water (1:1) (group S, N=5), a group receiving protein (pollen substitute) supplement (group P, N=5), a group receiving both: sugar solution (1:1) and protein (pollen substitute) supplement (group PS, N=5) and a control group with no supplemental feeding (group C, N=5). A total of 917 mature drones were evaluated for weight, thorax and abdomen measurements. Manual eversion was realized on those drones (7 pools of 5 drones per colony) and semen quality was evaluated for each pool (semen volume, sperm count and viability). Results showed significant increases in drone weight and abdomen size when colonies were fed sucrose and a protein supplement. Colonies receiving no additional nourishment had significantly less semen volume per drone and lower sperm viability. Our study demonstrates that feeding honeybee colonies in spring with sucrose syrup and a protein supplement can enhance drone quality and could ultimately lead to optimal queen bee mating (see Figure 25; Rousseau & Giovenazzo 2016. *J. Econ. Ent.* 109(3): 1009-1114).

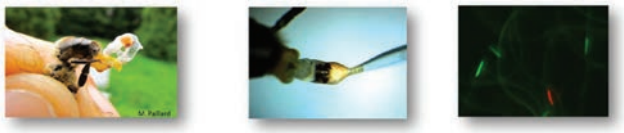

Figure 25. A photographic depiction of the process of assessing honey bee drone fertility. From left to right: (1) Eversion of the drone endophallus; (2) collection of semen; (3) staining of live (green) versus dead (red) sperm for determination of viability.

61. Soybean as a major nectar source for honey bees

Sreelakshmi Suresh, Chia-Hua Lin, Emma Matcham, Douglas Sponsler and Reed Johnson. Department of Entomology, The Ohio State University.

Honey bees depend on floral nectar sources to create the honey they rely on as food. In turn, humans benefit directly from the pollination of insect-dependent crops and other important plants; bees' work is valued at $11.68 billion a year. However, due to seasonal variations in bloom times, floral resources are not always readily available, especially during late summer in the Midwest. One of the few plants that bloom during this period are soybeans, which covered 84.1 million acres in the U.S. in 2014. While in bloom, honey bees may find soybean nectar a viable food source for honey production. However soybean flower attractiveness likely depends on cultivar, weather conditions, soil nutrients, and competition from other nectar sources. By analyzing the bees' waggle dance language, I aim to examine whether honey bees forage on soybean flowers. Bee dances were recorded with a standard video camera trained on a glass-walled observation hive, and the videos were processed with FIJI image processing software. Finally, I used R based methods in combination with GIS satellite data to determine where bees are foraging. This information will be used to determine the attractiveness of soybean flowers to bees.

62. *Apis cerana ceranae* less sensitive to neonicotinoids than *Apis mellifera L*

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Pollinators play an essential role in ecosystem services and global food security. With the development in agriculture and diet change in humans, the pollinator dependent crops have increased approximately 3-fold in the past 50 years. However, evidence shows that the population of honey bees decreased worldwide in recent decades, this decline may be attributed to many stressors, including parasites, habitat losses, pesticides and their interactions. Among them, pesticides, especially neonicotinoids, have arguably received the most attention.

Neonicotinoids are neurotoxins that target the insect central nervous system, causing overstimulation, paralysis, and death. For the high efficiency, wide spectrum, low vertebrate toxicity and systemic of neonicotinoids, seven neonicotinoid insecticides, imidacloprid, acetamiprid, nitenpyram, thiamethoxam, thiacloprid, clothianidin and dinotefuran were commercially marketed. It has also been reported that neonicotinoids occupied more than 25% of the pesticide market in 2014. Residues of these neonicotinoid pesticides have been found in pollen, nectar, soil, guttation droplets and water, and many postulate that these residues are one of the main causes for the decline of the bees in worldwide.

There are over 9.02 million colonies in China in 2013, among them, more than one third are Chinese indigenous honey bees (*Apis cerana* Fabricius, *Ac*), and the rest are *Apis mellifera* Linnaeus (*Am*). It is well known that these two species differ in their morphological, biochemical, physiological and behavioral traits. For example, *Ac* has a better olfactory sense than *Am*, and is more efficient in finding and pollinating the flowering plants scattered in the forest region, while *Am* hardly visits the sporadic plants growing in a secluded place. In addition, the low and high limits of foraging temperature for *Ac* foragers is wider than *Am*, so that *Ac* can spend more time foraging and pollinating plants, it can also pollinate effectively at higher latitude with lower temperature. Despite its larger body and colony size, *Am* is not a more effective pollinator than *Ac*. These two managed species thus play their unique roles in maintaining the ecosystem balance and agriculture economic development. The

wide use of neonicotinoid pesticides on crops and in forests had a negative impact on both species, yet the comparative sensitivity of these bees to neonicotinoids are not known. In this study, we evaluated the toxicity of 5 neonicotinoids to these two species of honey bees.

We found that there is a significant difference in size between these two species, as expected. However, the pattern of toxicity differences between the two species is not consistent with their respective sizes. The two species showed the same sensitivity to dinotefuran,. *A. mellifera* L. was less sensitive for only one pesticide, acetamiprid. *A. cerana* F. showed more resistance for the other two pesticides, imidacloprid and thiamethoxam. These results suggest that the sensitivity of honey bees to neonicotinoids is closely associated with the structure of pesticide, but not with body size of the bees. The results also suggest that the hazard risk from pesticides to different pollinators can not be inferred from one species to another.