



Kelley Beekeeping

SERVING THE BEEKEEPER SINCE 1924

ISSUE 45: MARCH 2014



**Spring Has Sprung!
Let's Have Some Fun!**

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Bee Science

Abstracts from 2014 American Bee Research Conference Meeting

IN EARLY JANUARY 2014, THE AMERICAN ASSOCIATION OF PROFESSIONAL APICULTURISTS HELD THEIR ANNUAL AMERICAN BEE RESEARCH CONFERENCE. THIS YEAR'S HOST CITY WAS SAN ANTONIO, TEXAS, SAME STATE OF AAPA PRESIDENT DR. JULIANA POSADA-RANGEL, WHO IS ALSO AN ASSOCIATE PROFESSOR AT TEXAS A&M UNIVERSITY. THE ABRC BRINGS SOME OF THE FINEST AND FITTEST RESEARCHING SCIENTISTS TOGETHER TO SHARE THEIR RESEARCH FINDINGS THROUGH PEER-REVIEWED SUPPORT.

THROUGH THESE CHALLENGING TIMES OF AMERICAN BEEKEEPING, IT IS IMPERATIVE THAT DIVERSE INSTITUTIONS, BEEKEEPERS OF VARYING PRACTICES AND CAPACITIES, AND STEWARDSHIP ORGANIZATIONS SUPPORT MULTI-DISCIPLINARY EFFORTS TO BETTER UNDERSTAND AND FURTHER PROMOTE POSITIVE POLLINATOR MANAGEMENT. IN AN EFFORT TO INTRODUCE MANY OF YOU TO THE BROADER SCOPE OF BEE HEALTH AND SCIENCE RESEARCH, KELLEY BEEKEEPING NEWSLETTER WILL BE RUNNING THE ABRC ABSTRACT PROCEEDINGS OF SUBMITTED RESEARCH FROM ACROSS THE UNITED STATES OVER THE NEXT FEW ISSUES. HERE IS THE FIRST BATCH PRESENTING PLENTY OF FOOD FOR THOUGHT.

Compiled and Edited by Drs. Juliana Rangel, Associate Professor and Rose-Anne Meissner, Research Associate, Dept. of Entomology, Texas A&M University, College Station, TX 77843

1. Burand, J.^a, S. Zheng^a, E. Ramos^b, N. Reich^b & A. De^a - VIRUS INCIDENCE IN MASSACHUSETTS BEEHIVES ^aDepartment of Microbiology, University of Massachusetts Amherst, Amherst, MA 01003 (e-mail: jburand@microbio.umass.edu), ^bDepartment of Public Health, University of Massachusetts Amherst, Department of Public Health, Amherst, MA 01003

Individual honey bees (*Apis mellifera*), from hives in University of Massachusetts apiary were analyzed over a two year period for the presence of viral pathogens. Bees from these hives were found to be infected with one or more of the 3 honey bee viruses: Black Queen Cell Virus (BQCV), Deformed Wing Virus (DWV), and Sacbrood Virus (SBV). During this time period (August 2010 to August 2011) the prevalence of each of these viruses varied both within a hive and between hives throughout both seasons. In both years DWV was the most prevalent of the viruses being present in 37% and 44% of the bees in 2010 and 2011 respectively. BQCV and SBV levels were high in 2010 bees (23.6% and 32.4% respectively) with the levels of both these viruses dropping to ~11% in 2011. Throughout this study we found a significant number of bees that were infected with more than one virus. We are currently examining the rates at which bees are infected with single and multiple viruses to determine if virus infection can lead to immune activation or suppression in individual bees.

2. Burand, J.P.^a, S. Zheng^a & K. Stoner^b. - Epizootiology of Honey Bee Viruses in Bee Populations Pollinating Cucurbits ^aDepartment of Microbiology, University of Massachusetts Amherst, Amherst, MA 01003 (e-mail: jburand@microbio.umass.edu), and ^bConnecticut Agricultural

Bee Science *continued*

Experiment Station, New Haven, CT 06504

Several honey bee viruses including Black Queen Cell Virus (BQCV), and Deformed Wing Virus (DWV) have been found to infect other bees including several species of bumble bees. These findings have led us to examine the possibility that these viruses move from honey bees to other bees during foraging on common floral resources. To investigate this possibility we used a molecular approach to detect the presence and prevalence of both of these viruses in bee pollinators found foraging on pumpkins (*Cucurbita sp.*) on four farms in Connecticut. The three main groups foraging on pumpkins, *Apis mellifera*, *Bombus sp.* and *Peponapis pruinosa*, were sampled 5 times at each site from early June to late September. Sampling included approximately 20 bees of each group when available. Our initial analysis has focused on BQCV which appears to be the most prevalent of the viruses in bees we have examined to date. Of the ~ 300 bees we have analyzed to date, 47% were found to be infected with BQCV. This virus was the most prevalent in *Apis* with 58% being infected, while the *Bombus* and *Peponapis* were infected at 39% and 3% respectively. The level of virus-positive bees from the different farms ranged between 4% and 58% and overall our results suggest a correlation between the level of this virus in honey bees and the level of infection of other bee species. On the two farm sites where we found honey bees infected with BQCV at 93% and 75%, *Bombus* bees were at 77% and 40% respectively. At the site where we found only 10% of *Apis* infected with BQCV we were able to detect only 5% BQCV infected *Bombus*, suggesting that the infection of *Apis* and *Bombus* is clustered and may be connected in some way.

3. Catalfamo, K.M., B.E. Traver, H.K. Feazel-Orr, N.G. Johnson, T.D. Anderson & R.D. Fell - VIRUS PREVALENCE IN HONEY BEES FOLLOWING COLONY TREATMENT WITH CHLOROTHALONIL, FUMAGILLIN, AND TAU-FLUVALINATE
Virginia Tech University, Department of Entomology, Blacksburg, VA, 24061
(e-mail: kmcat92@vt.edu)

Increased colony loss has been a rising concern for European honey bee, *Apis mellifera*, populations. Although the causes of increased losses are unknown, researchers have hypothesized that various factors are to blame. Two factors believed to strongly correlate with colony losses are viral infections and exposure to pesticides. Viruses often lead to cell death and can cause the host to die. Pesticides are known to reduce immune system responses and make organisms more vulnerable to disease. This study focused on exposing honey bee colonies to three different pesticides to determine whether black queen cell virus (BQCV) and deformed wing virus (DWV) infection levels were impacted in honey bee colonies. The three pesticides used were the miticide *tau*-fluvalinate, the antibiotic fumagillin used for *Nosema* control, and chlorothalonil, a commonly encountered fungicide outside of the hive. The presence of DWV and/or BQCV in randomly sampled female workers was determined using RT-PCR. For samples positive for either virus, a semi-quantitative approach using band density was used to measure virus levels. All virus levels were normalized using each sample's level of β -actin. For DWV, we did not see significant differences in levels across time or across treatments for fall, winter, and spring. There were no significant differences in levels



Bee Science *continued*

of BQCV in the fall or winter, but there was a significant difference in the infection levels of BQCV across time during the spring ($p < 0.01$). As a trend, the levels of BQCV decreased after the fall, persisted at low levels in the winter, and then increased in the spring. The levels of DWV decreased from fall through spring. We also saw a significant decrease in varroa mite levels in the fall with *tau*-fluvalinate treatment ($p < 0.01$). The data from this study show low incidence of BQCV and DWV in colonies in both the absence and presence of pesticides. Our data suggest that chlorothalonil, fumagillin, and *tau*-fluvalinate do not have a direct impact on the prevalence of BQCV or DWV in honey bee colonies.

4. Coulson, R.N., M.D. Tchakerian, J. Rangel & W. Baxter – THE TEXAS APIARY INSPECTION SERVICE: INFORMATION MANAGEMENT SYSTEM (TAIS/IMS) Texas A&M University, Department of Entomology, College Station, TX 77843

The Texas Apiary Inspection Service Information Management System (TAIS/IMS) is an INTERNET-based application designed to standardize and automate apiary inspection in Texas. The Texas Apiary Inspection Service is charged with regulating the honey bee industry so that colonies with diseases and parasites are restricted from movement or are destroyed. The new program employs a mobile mapping/data collection system that captures, displays, and archives spatial and tabular information on the distribution, abundance, and location of honey bee diseases, parasites, and associated organisms. TAIS/IMS will expedite the apiary inspection process and facilitate timely reporting of results.

5. Eiri, D.M.^a, G. Suwannapong^b, M. Endler^c & J.C. Nieh^c – NOSEMA CERANAE CAN INFECT HONEY BEE LARVAE AND REDUCE SUBSEQUENT ADULT LONGEVITY – ^aDepartment of Integrative Biology, University of Texas, Austin, TX 78712 (e-mail: eiri@utexas.edu), ^bDept of Biology, Burapha University, Chon Buri 20131, Thailand, ^cDivision of Biological Sciences, Section of Ecology, Behavior, and Evolution, University of California, San Diego, La Jolla, CA 92093

The microsporidian *Nosema ceranae* is a global problem that decreases honey bee fitness. It is thought to only infect adult honey bees and thus its potential to infect a key life stage, larvae, has been neglected. We reared honey bee (*Apis mellifera*) larvae *in vitro* and demonstrate that *N. ceranae* can infect larvae with subsequent detrimental effects. Three-day-old larvae were given a single dose 0 (0K), 10,000 (10K), or 40,000 (40K) spores. After larvae had defecated and become pre-pupae, we dissected out their midguts and counted the number of *N. ceranae* spores. Significantly more spores were present in pre-pupae from the 10K and 40K treatments as compared to the control. Separately, we reared larvae to adulthood and counted midgut spores at adult death. Larval infection significantly decreased 40K-treated adult longevity. However, the low dose (10K), unexpectedly led to significantly more infection than the high dose (40K). Adults infected as larvae contained an average of $16,021 \pm 3,085$ spores (maximum of 215,000 spores), and $12,454 \pm 3,647$ spores (maximum of 295,000 spores) for the 10K and 40K doses respectively. Control bees were minimally contaminated. Differential immune activation may account for these results if the higher dose triggered a stronger response. Immune defense can be costly and could result in 40K-treated bees having lower infection levels and longevity as compared to 10K-treated bees. Honey bee larvae can thus be a reservoir of *N. ceranae* infection whose

Bee Science *continued*

effects may be masked by relatively low levels of adult infection but nonetheless decrease lifespan.

6. Eitzer, B. D.^a, C.H. Krupke^b, E.Y. Long^b & J.D. Holland^b – ANALYSIS OF PESTICIDES IN PLANTER EXHAUST DUST AND DOSIMETERS SURROUNDING FIELDS DURING PLANTING ^aDepartment of Analytical Chemistry, The Connecticut Agricultural Experiment Station, New Haven, CT 06504 (e-mail: brian.eitzer@ct.gov), ^bDepartment of Entomology, Purdue University, West Lafayette, IN 47907

One possible route through which honey bees can be exposed to pesticides is the dust that is produced and exhausted by pneumatic planters during planting. Seed coated with systemic pesticides will transfer some of those pesticides to the lubricants added to keep the seed flowing smoothly. The resulting concentrations of pesticide residues in exhaust materials can be extremely high, in the parts per thousand range (Krupke et al. 2012 PLoS ONE 7(1): e29268). We are studying how pesticides are transported away from fields along with the dust that arises during the planting of pesticide-treated seed.

We conduct these studies by using dosimeters to collect the dust. The dosimeters are composed of glass microscope slides coated with a sticky material. These are placed in vertical and horizontal positions surrounding fields at varying distances from the field edge (0 -100 meters) just prior to planting. Immediately after planting they are collected and then frozen until laboratory analysis is conducted. The slides are extracted with acetonitrile and analyzed by liquid chromatography/mass spectrometry.

We have found that the pesticides can be transported off the field during planting. The amount of pesticide found can vary with many factors including distance from the field, lubricant used to keep the seed flowing, wind speed and direction. The amounts found on the slides range from below detection limits (approximately 0.1 ng active ingredient/slide) to over 100 ng active ingredient/slide.

This work has been supported by the USDA-NCIPM program, award #2012-01993, the Indiana Corn Management Council, and the NIMSS-Multi-State-Research Project NC-1173.

7. Feazel-Orr, H.K., B.E. Traver, K.M. Catalfamo, C.C. Brewster, T.D. Anderson & R.D. Fell - NUTRIENT LEVELS IN INDIVIDUAL HONEY BEES FOLLOWING COLONY EXPOSURE TO DIFFERENT PESTICIDES
Department of Entomology, Virginia Tech University, Blacksburg, VA, 24061

The use of pesticides, including fungicides and miticides, has placed a stress on honey bees which could contribute to weaker hives and increased colony losses. Twenty colonies were studied during fall 2012 through summer 2013 in Blacksburg, VA to examine the effects of pesticides on honey bee nutrient levels. A total of five colonies each received either a treatment of fumagillin, *tau*-fluvalinate, or chlorothalonil in the fall, while 5 colonies received sugar syrup as a control. Samples were taken at pre-treatment, 2 weeks post-treatment, 4 weeks post-treatment and in the following winter. Fifteen individual bees randomly selected from each colony were analyzed for total lipid, protein, and carbohydrate levels. Average bee weight was significantly affected by treatment ($p < 0.01$) and treatment period ($p < 0.05$). Bees from control and fumagillin-treated colonies did not differ significantly, but

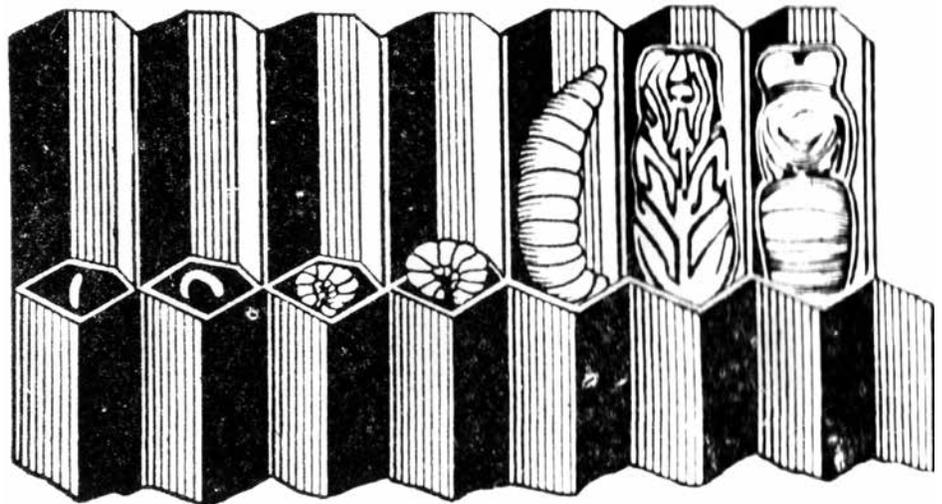
Bee Science *continued*

weighed significantly less than bees treated with chlorothalonil and *tau*-fluvalinate ($p < 0.01$). Overall, bees from colonies treated with *tau*-fluvalinate had significantly higher mean protein levels compared with bees from control, chlorothalonil, and fumagillin-treated colonies ($p < 0.01$). A significant increase in protein levels across time was observed when the data from all treatments were considered; however, average protein levels decreased in all pesticide-treated colonies in the winter, while protein levels from bees in control colonies continued to increase. Average lipid levels in bees did not differ significantly among treatments ($p = 0.59$), although lipid levels among sampling periods differed significantly ($p < 0.01$). Overall, lipid levels decreased from pre-treatment to 2 weeks post-treatment, and then increased through 4 weeks post-treatment and during the winter. Carbohydrate levels in bees were not significantly different between the control colonies and *tau*-fluvalinate-treated colonies; however, chlorothalonil and fumagillin-treated bees had significantly lower carbohydrate levels compared with the controls ($p < 0.05$). Macromolecule levels followed similar trends in the fall and winter, regardless of pesticide treatment, leading to the conclusion that *tau*-fluvalinate, chlorothalonil and fumagillin do not have significant effects on macromolecule levels, and that the differences in protein, carbohydrate and lipid levels among bees within the colonies were most likely due to other environmental factors.

8. Frisbie, R., A. Birt, M. Tchakerian & R. Coulson – TEXAS CROP REGISTRY SYSTEM Department of Entomology, Texas A&M University, College Station, TX 77843

The Texas Crop Registry System (TCRS) was developed to address pesticide drift from target to non-target crops. This INTERNET-based system consists of three main components or interfaces: one for the crop producers, one for the pesticide applicators, and one for the general public. The producers interface allows the crop producers to register and map their farm location and enter crop information related to their site into the website. The applicators interface allows the pesticide applicators to register and enter their preferences for notifications of registered crops based on location at three different levels: statewide, countywide, and specific locations of interest to the applicator. The public interface displays information and location of crops within Texas. This website enables Texas crop producers to map their farm locations and post relevant information regarding their pesticide-sensitivity. The

information is used by pesticide applicators to modify treatments in order to minimize damage to registered crops located nearby the application area. TCRS was designed to accommodate protection of honey bee colonies and apiaries from pesticide exposure. Beekeepers can identify apiary and colony sites and place a buffer around the location to alert pesticide applicators.





Kelley Beekeeping

SERVING THE BEEKEEPER SINCE 1924

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Thumbs Up for Queens!

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Bee Thinking About

It Takes a Community to Raise Bees...& Beekeepers

by Kaat Byrd

The future generations of beekeepers are in our midst. Those inspired today and tomorrow will help to carry the torch in coming seasons as honeybee and pollinator stewardship evolves to overcome the challenges faced. They are seeking to develop their skills and to share with others; as we all work together learning to adapt to varied circumstances and topographies.



I had the great pleasure of meeting a vibrant and insightful beekeeper by the name of Kaat Byrd while I volunteered for the USAID Partners of the Americas Farmer to Farmer program at the end of last year. Kaat has been travelling and interning at bee farms near and far—learning more about bees and beekeepers at each of her stops along the way as she journeys on the path of creating her own beekeeping experiences.

Kaat has quite a unique perspective in that she is also a deaf beekeeper. Her interactions with bees and beekeepers she encounters share a distinct perspective. I have encouraged Kaat to share her unique perspective with Kelley Beekeeping readers. For indeed, though one may not hear the buzz, it is felt... an emotion that all beekeepers can relate to.

For four moons I lived on the enchanted island of Jamaica as a student of the Yerba Buena Farm and Apicultural School.

Through the Farmer-to-Farmer project, which is funded by USAID Partners of America, the Yerba Buena Farm and Apiculture School has been able to host professional beekeepers from all over the globe. These trainers volunteer to teach hundreds of beekeepers through trainings and workshops to explore solutions for the various circumstances and challenges that Jamaican beekeepers face. Each trainer brings their own expertise, whether it be treatment-free beekeeping, top-bar hive construction and management, survivor queen rearing, DIY equipment construction, habitat conservation, value-added products, marketing, and anything else that is important to know for practical and conscientious beekeeping.

Behind Yerba Buena Farms is Agape and Kwao Adams; who, so far, are the only known beekeepers in Jamaica implementing a treatment-free system in their apiaries. Their stock consists mostly of small dark bees from feral colonies gathered from local swarms and tree cut-outs. Agape and Kwao mainly work with top-bar hives, which, compared to the conventional Langstroth hives, has proven to be a cheaper and more accessible alternative for Jamaica. The training apiary of Yerba Buena Farm incorporates top-bar hives constructed with various materials such as woven wicker and bamboo, cloth, hemp bags, particle board as well as locally-sourced wood.

Bee Thinking About *continued*

While traveling all over the island with three trainers, the impact of the exchange of knowledge was unmistakable. The eyes of Jamaican beekeepers, who have been struggling with hives filled with strong mites and weak bees, sparked as the trainers guided them through the methods of beekeeping that fosters naturally healthy bees. The island's beekeepers are understanding the concept that, by using chemical treatments, they are using an un-sustainable solution that creates a weak bee. They are eagerly learning how to breed for a strong survivor stock that is disease and pest resistant. The vast potential and market of value-added products made from the hive's medicines was also taught.

During the trainings there are lively discussions of experiences, challenges, solutions, and experiments that are shared for all to benefit from. There is a saying that if you ask ten beekeepers a question, you will get eleven different answers. I came to Jamaica with the intention of exploring these diverse perspectives and ideas of simple, humble, and pure honeybee stewardship.

Some beekeepers prefer to puff billowing amounts of smoke directly into the hive, some smoke the air around the hives yet others rarely touch their smokers. Some like to inspect every comb and manipulate the brood-nest in attempts to maximize strength and size and minimize swarming; others only look for eggs before closing up and refuse to manipulate the brood-nest. Beekeepers have to be mindful of their resources, biospheres, and needs so various philosophies and techniques develop.

There is no one 'right' way to beekeep—because circumstances vary constantly. Therefore, every beekeeper is continuously developing their own style and system that (hopefully) works for their relationship with their bees. By sharing our techniques and staying open to all the different perspectives and ideas of bee tending, no matter how absurd or negative some manipulations and interventions may seem, we can learn from them and decide for ourselves whether or not it may be appropriate for our own systems.

This internship helped me explore the diverse relationships that can

be found between bee and keeper and taught me how to observe carefully, question everything, and try to put myself in the beekeeper's shoes as well as the bee's wings. While meeting beekeepers and visiting apiaries, I experienced some ideas and practices that made me question whether it was an appropriate approach for the situation since it seemed to be working against the bees rather than with them. The



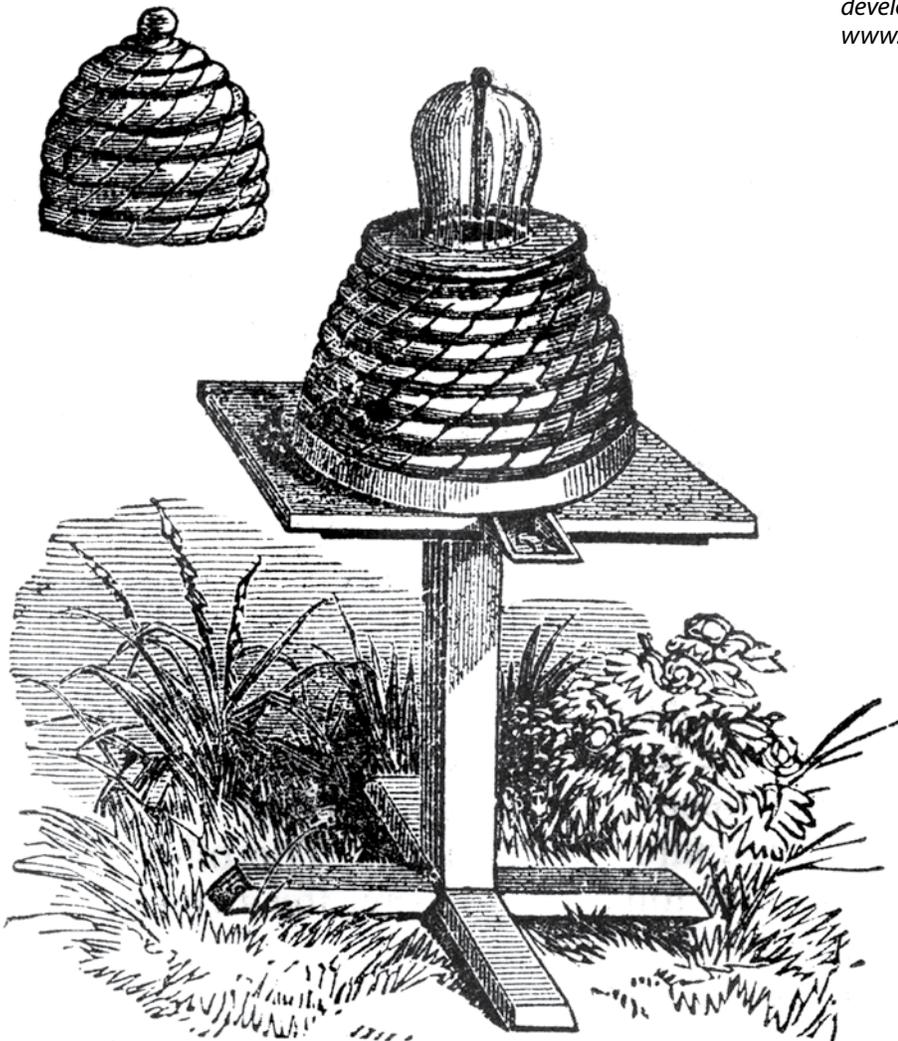
Kwao Adams talking to Jamaican beekeepers.

Bee Thinking About *continued*

actions that appeared to be negative ended up giving me lessons as valuable as the positive actions did. By working in cooperation (rather than competition) to advocate healthier bees and a cleaner environment through sustainable solutions, we can better care for these humming super-creatures that provide us with an abundance of fruiting plants, hive medicines and an ancient wisdom.

Agape and Kwao dedicate countless hours of their time, energy, and focus to provide Jamaican beekeepers with beekeeping alternatives that are environmentally and economically sustainable. It is safe to say that this duo is jump-starting a sustainable beekeeping movement in Jamaica by blazing trails towards naturally healthy bees. It has been a blessing to learn and work with them. There are internships available for anyone who is interested.

Kaat Byrd is a Deaf traveling beekeeper who signs for the bees. She is focusing on educating and empowering the Deaf community with American Sign Language (ASL) friendly honeybee workshops and presentations. Since her four-month beekeeping internship in Jamaica, Kaat Byrd has been focusing on exploring sustainable beekeeping techniques with a DIY approach. To keep up with Kaat's developing beekeeping experiences, visit her blog at: www.rootflux.com Email: kaatbyrd@gmail.com



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Bee Science

Abstracts from 2014 American Bee Research Conference Meeting

Through these challenging times of American beekeeping, it is imperative that diverse institutions, beekeepers of varying practices and capacities, and stewardship organizations support multi-disciplinary efforts to better understand and further promote positive pollinator management. In an effort to introduce many of you to the broader scope of bee health and science research, Kelley Beekeeping newsletter will be running the 2014 American Bee Research Conference abstract proceedings of submitted research from across the United States.

This is the second batch of proceedings (Abstracts #9-12). For first batch (Abstracts #1-8) please see March 2014 issue of Kelley Beekeeping. We'll run batch 3 (Abstracts #13-18) in May 2014 issue and batch 4 (Abstracts #19-25) in June 2014 issue of Kelley Beekeeping. The ABRC brings some of the finest and fittest researching scientists together to share their research work and to support each other through the peer-reviewed process.

Compiled and Edited by Drs. Juliana Rangel, Associate Professor and Rose-Anne Meissner, Research Associate, Dept. of Entomology, Texas A&M University, College Station, TX 77843

9. Hooven, L.A. - THE FUNGICIDE IPRADIONE REDUCES DEVELOPMENT OF LARVAE AND CAPPED BROOD IN SEMI-FIELD EXPERIMENTS Department of Horticulture, Oregon State University, Corvallis, OR 97331 (e-mail: hoovenl@hort.oregonstate.edu)

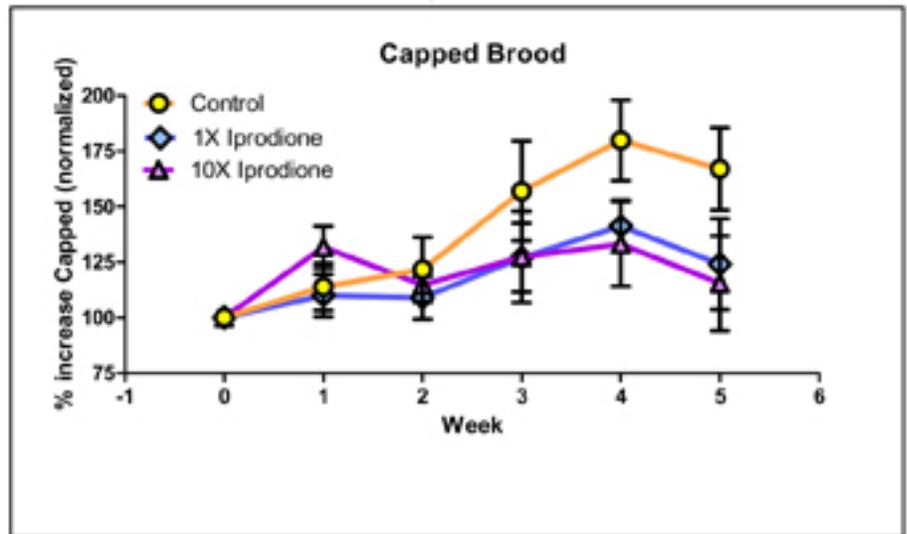
Results from conventional toxicity protocols modeling short-term exposure suggest fungicides have little or no effect on adult bees (Adaskaveg et al, 2011 UC Davis IPM Program). As a result, most fungicide products do not display a precautionary statement for bees on the label, and are freely applied during bloom while bees are actively foraging. However, in addition to direct spray exposure to adult bees, multiple fungicides are transported with pollen into the colony, persist in hive materials (Mullin et al., 2010, PLoSOne e9754), and may affect the microbial ecology of the colony (Yoder et al., 2013, Journal of Toxicol Environ Health A. 76:10). Nurse bees and larvae would be expected to consume the resulting contaminated pollen or bee bread.

Despite their low toxicity rating, beekeepers suspect that fungicide applications may result in delayed effects on honey bee development (Mussen, 2008, Apiculture News, Nov/Dec), although these reports are difficult to link to a specific pesticide. Certain fungicide treatments exhibit adverse effects on bee larvae in laboratory tests (Mussen et al., 2004, Journal of Environ. Entomol 33:5), and may affect susceptibility to *Nosema* (Pettis et al., 2013, PLoSOne e70182). Field tests are needed to validate the relevance of lab studies, and determine whether specific fungicides are related to beekeeper reports.

Bee Science *continued*

In semi-field studies, we fed pollen spiked with fungicides to honey bee colonies, and evaluated colonies weekly for six weeks. In a preliminary study, we observed that capped brood in controls increased over several weeks, while capped brood in chlorothalonil and iprodione exposed colonies did not increase. We did not observe any significant effects in response to boscalid/pyracostrobin treatments.

In an expanded study, we exposed honey bees to pollen spiked with increasing concentrations of iprodione, and found similar results, with increasing larvae and capped brood in controls, with little increase in treatment groups. Together, these results suggest that ingestion of iprodione in pollen may target larval development, possibly through toxicity to colony microflora, toxicity to nurse bees, or direct toxicity to larvae. Additional studies are needed to confirm similar preliminary results from chlorothalonil treatment, and determine whether other fungicides, fungicide mixtures, or repeated exposures have detrimental effects on honey bee colonies.



Together, these results suggest that ingestion of iprodione in pollen may target larval development, possibly through toxicity to colony microflora, toxicity to nurse bees, or direct toxicity to larvae. Additional studies are needed to confirm similar preliminary results from chlorothalonil treatment, and determine whether other fungicides, fungicide mixtures, or repeated exposures have detrimental effects on honey bee colonies.

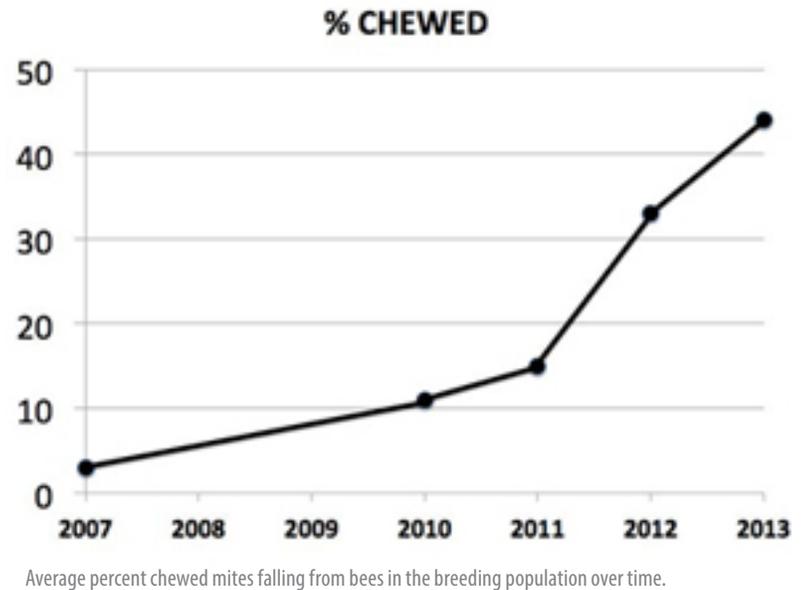
10. Hunt, G.J.a, K. Givena, J.M. Tsurudaa & M.E. Arechavaleta-Velasco - PROGRESS TOWARDS BREEDING FOR INCREASED MITE-GROOMING BEHAVIOR IN HONEY BEES Department of Entomology, Purdue University, West Lafayette, IN 47907 (e-mail: ghunt@purdue.edu), b Agricultores y Pecuarias, Apiculture, Ajuchitlan, Instituto Nacional de Investigaciones Forestales, Ajuchitlan, Querétaro, 76280, Mexico

Varroa destructor mites and the virus complex associated with them are generally believed to be the greatest health threat to honey bees worldwide. One trait that reduces *Varroa* population growth in colonies is grooming behavior (Arechavaleta-Velasco & Guzmán-Novoa, 2001 *Apidologie* 32:157-174). Genetic studies identified a chromosomal region and candidate genes influencing grooming behavior, which demonstrated that the trait is heritable (Arechavaleta-Velasco et al., 2012 *PLoS One* 7:e47269). However, there has been little effort to incorporate this trait into breeding lines. We began selecting for increased grooming behavior in based on the proportion of chewed mites that fall onto sticky sampling sheets because colonies with relatively high levels of chewed mites are better at removing mites from themselves (Andino & Hunt, 2011 *Apidologie* 42:481-484). Mites from sticky boards are placed on glass slides with legs up (usually only legs are bitten) and



Bee Science *continued*

examined at 15X magnification. A fairly strong response to selection has been observed in the breeding population, resulting in an increase in the average proportion of chewed mites from 3% to 44% last year (Figure). Preliminary data indicates that colonies with higher grooming rates have relatively fewer mites on adult bees. Because of the variability of this trait (apparently grooming activity is variable seasonally) we recommend that breeders assess the trait more than once a year.



11. Johnson, R.M. & E.G. Percel - EFFECT OF THE IGR INSECTICIDE DIMILIN ON QUEEN DEVELOPMENT Department of Entomology, The Ohio State University, Wooster, OH 44691

Over a million honey bee queens are reared in California's Central Valley each year, with many queen rearing operations situated among the state's 800,000+ acres of almond orchards. While almonds provide a rich foraging resource for bees when they are in bloom, bees foraging on almonds may be exposed to high doses of pesticides applied during almond flowering.

In recent years queen rearing operations have experienced unexplained mortality of immature queens, as high as 80% in some reports, in the weeks after almond bloom. Many have attributed these losses to the bees' exposure to bloom-time fungicide sprays, but it has not been possible to replicate the reported effects on queen survival through experimental application of fungicides. However, analysis of pesticide use data maintained by the California Department of Pesticide Regulation reveals that insecticides may be blame. In 2011 insecticides were applied to 130,000 acres of almonds

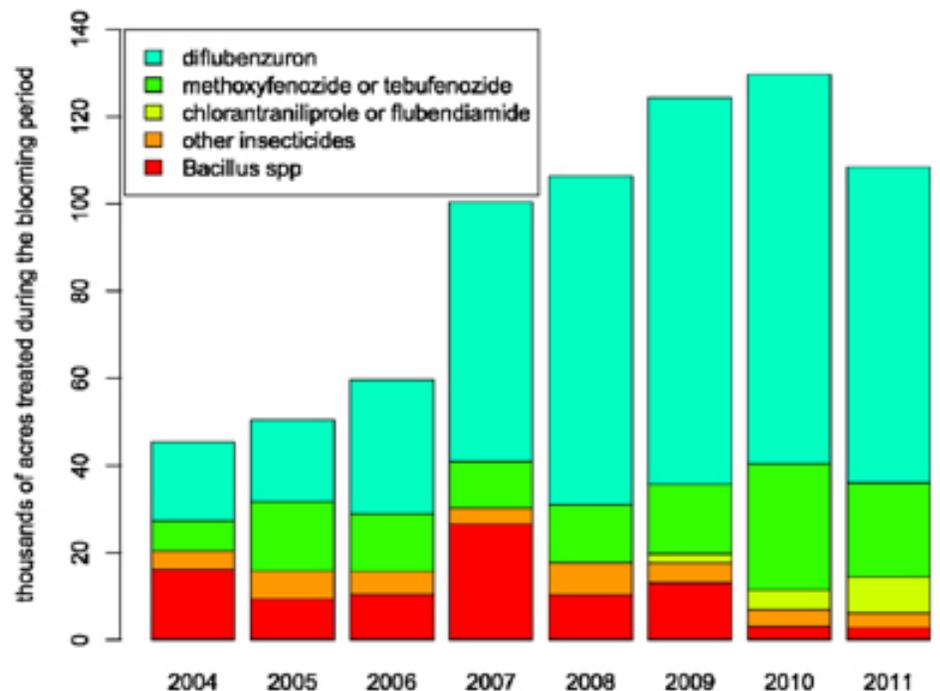


Figure. Area of almonds to which insecticides were applied during the bloom period (February 15 - March 15) in California.

Bee Science *continued*

during the almond bloom period (see figure), and these insecticides were tank-mixed with fungicides 98% of the time. To determine the effect that the most popular insecticides have on queen development we reared queens in closed “swarm boxes” provisioned with pollen artificially contaminated with 100 ppm chlorantraniliprole (Altacor), 100 ppm methoxyfenozide (Intrepid 2F) or one of three concentrations of diflubenzuron (Dimilin 2L): 1, 10, or 100 ppm. None of the queens in the 100 ppm diflubenzuron treatment emerged as adults, and significantly fewer queens emerged as adults in the 10 ppm diflubenzuron and 100 ppm chlorantraniliprole treatments compared to the control treatment. The widespread use of insecticides on almonds during bloom should be reconsidered in light of the potential for these insecticides to harm immature bee development.

12. Kirby, M., A. Lewis & M. McGee - THE ROCKY MOUNTAIN SURVIVOR QUEENBEE COOPERATIVE Zia Queenbee Co., Truchas, NM 87578 (e-mail: rmsqbcoop@gmail.com)

The Rocky Mountain Survivor Queenbee Cooperative is continuing into its fourth year of treatment-free stock exchange (see Table). New Mexico experiences 7/8 climactic zones and thus proposes challenging topographical influences that readily test honeybee stock. The concept that Nature vs. Nurture is derisive no longer reveals contradictory forces; but rather, a symbiotic relationship of nature nurturing that allows bees and their genetic story to unfold and display itself (Maleska 2010, PLOS). Reality and research based methodologies (Tarpay et al. 2013, *Naturwissenschaften*, 100: 723-728; Rangel et al. 2012, *ABRC/Amer. Bee J*, 152: 405; Wilson-Rich et al. 2012, *J of Insect Phys.*, 58: 402-407; Delaney et al. 2011, *Apidologie*, 42: 1-13; Kocher et al. 2009, *Behavioral Ecology*, 20: 1007-1014; Seeley & Tarpay 2007, *Proc. of the Royal Soc. of London, B.*, 274: 67-72; Tarpay & Seeley 2006, *Naturwissenschaften*, 93: 195-199; Tarpay 2003, *Proc. of the Royal Soc. of London, B.*, 270: 99-103) have been applied to better develop a breeding and production protocol founded on observations from within our living laboratory as it nurtures the cycles of life and longevity (Kirby 11/2012 *Bee Culture*; Kirby 6/2011 *Amer. Bee J*) and tests our bees on the rugged topography and fluctuating microclimates that abound in the Land of Enchantment and the Rockies.

Participating beekeepers of various hive design practices spread across high desert, riparian and alpine landscapes—experiencing diverse and adverse conditions at elevation maintain regionally fortified stock lines, minimize importation threats, and support rural entrepreneurship. Objectives include promotion of survivor (longevity-based) cross-stock queen breeding through treatment-free management, extended timeline for rearing, and professional development exercises. These objectives enhance individual and collective apiary managements and offered services benefiting the surrounding communities of each beekeeper.

The Rocky Mountain Survivor Queenbee Cooperative initially began as an out of pocket pilot program between NM beekeepers located in the Pecos, Carson and Santa Fe National Forests. In 2012, The Cooperative was granted funding from Western Sustainable Agriculture Research Education (Kirby, 2012 www.sare.org #FW12-096; Kirby 2/2013 *Amer. Bee J*) and included 9 beekeepers, spanning close to 500 miles, through 2 states and 7

Bee Science *continued*

counties from Santa Fe, NM to Fort Collins, CO (Kirby, 2/2013 Amer. Bee J). In 2013-2014, the New Mexico Department of Agriculture: Ag Advancement and Product Promotion grant has been awarded for focus on NM beekeepers.

The cooperative's roots are based on the "Southwest Survivor Queenbee Project" (Kirby & Spitzig, 2007 www.sare.org #FW07-032; Kirby 11/2007 Amer. Bee J). In the summer of 2013, The RMSQB Cooperative was awarded the Sustainable Santa Fe Award for Climate Adaptability. And in the fall of 2013, the RMSQB Cooperative participated at Apimondia- Ukraine, sharing their concepts and case studies with beekeepers from around the world.

As with grass roots efforts, the crux of ensuring a steady and sustainable pace to success lies in consilience of funding and multi-disciplinary collaborations. The RMSQB Cooperative seeks research collaborations with various individuals and scientists to further promote regional fortification and production while also supporting local, regional and national food production and security. The Rocky Mountain Survivor Queenbee Cooperative objectives for 2014 include publication of a survivor stock rearing manual and outreach workshops. For info on breeding stock, email rmsqcoop@gmail.com.



TABLE: Rocky Mountain Survivor Queenbee Cooperative Mating Apiary Sites 2013-2014



COMMUNITY COUNTY ELEVATION (ft)/(meters)
2013-2014 New Mexico

Truchas	- Rio Arriba	8300'	2530m
Mora-	Mora	7179'	2188m
Arroyo Hondo-	Taos	6798'	2072m
El Prado-	Taos	7123'	2171m
2013 Crosses: TxAH, TxM, TxEP, MxAH, MxT, AHxEP, AHxT, AHxM			

2012 New Mexico

Truchas-	Rio Arriba	8300'	2,530m
Mora-	Mora	7,179'	2,188m
Buena Vista-	Mora	6,998'	2,133m
Arroyo Hondo-	Taos	6,798'	2,072m
Santa Fe-	Santa Fe	7,260'	2,213m

2012 Colorado

Walsenburg-	Huerfano	6,182'	1,881m
Denver-	Arapaho	5,183'	1,580m
Ft. Collins-	Larimer	5,003'	1,523m

2012 Crosses: TxM, TxAS, TxAH, TxSF, TxFCx, AHxT, MxAS, ASxT, ASxM, ASxFC, DxX,

2011 New Mexico

Truchas, Mora, Buena Vista
2011 Crosses: TxM, TxBV, BVxM, BVxT, MxT, MxBV
Santa Fe, Pecos, Carson National Forests



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Bee Science

Abstracts from 2014 American Bee Research Conference Meeting

In an effort to introduce many of you to the broader scope of bee health and science research, Kelley Beekeeping newsletter is running the 2014 American Bee Research Conference (ABRC) abstract proceedings of submitted research from across the United States. This is the third batch of proceedings (Abstracts #13-18) from the 2014 ABRC that was held in January in San Antonio, TX. The ABRC brings some of the finest and fittest scientists together to share their research work and to support each other through the peer-reviewed process.

For first batch (Abstracts #1-8) please see March 2014 issue of Kelley Beekeeping; 2nd batch (Abstracts #9-12) in April 2014 issue. We'll run the fourth and final batch in next month's June 2014 issue (Abstracts #19-25). Through these challenging times of American beekeeping, it is imperative that diverse institutions, beekeepers of varying practices and capacities, and stewardship organizations support multi-disciplinary efforts to better understand and further promote positive pollinator management.

Compiled and Edited by Drs. Juliana Rangel, Associate Professor and Rose-Anne Meissner, Research Associate, Dept. of Entomology, Texas A&M University, College Station, TX 77843

13. Lin, C.-H., D. B. Sponsler, J. O. Quijia Pillajo & R. Johnson - Identifying pollen sources foraged by honey bees as a route of corn seed treatment dust exposure in central Ohio's agricultural landscape Department of Entomology, The Ohio State University, Wooster, OH 44691 (e-mail: Lin.724@osu.edu)

Conventional large-scale corn planting using pneumatic planters and seeds coated with insecticide treatment could generate airborne dust particles contaminated with insecticides that are highly toxic to bees. Therefore, seed treatment dust deposited on flowers poses a threat to bees foraging near corn fields. The risk of exposure to seed treatment dust via pollen depends on the types of pollen sources preferred by bees and spatial proximity of corn fields to these bee-attractive flowers at planting. Understanding honey bees' pollen preferences in an agricultural landscape could provide guidelines for minimizing seed treatment dust exposure by vegetation management. In 2013, we surveyed pollen sources utilized by honey bees during spring corn planting at three apiaries, each consisted of 6 colonies, in Central Ohio. Fields of rotating corn and soybean crops comprised over 80% of the surrounding landscape, followed by semi-natural (forests and uncultivated lands) and residential areas (Figure). Pollen collected by bees was sampled twice weekly from two colonies at each apiary using entrance pollen traps. Sampling was focused on the interval of April 29 – May 16, four days prior to the start of intense local planting until planting was complete. During this period, over 70% of bee-collected pollen originated from trees and shrubs (Figure) while the remaining pollen originated predominantly from weeds including dandelions (*Taraxacum officinale*) and wild mustards (family Brassicaceae) found in fields, field margins, roadsides,

Bee Science *continued*

and residential yards. Rosaceous plants, including apples (*Malus* spp.) and hawthorns (*Crataegus* spp.), were the dominating pollen sources, comprising over 50% of woody plant pollen and 40% of total samples. Our results suggested that the small amounts of forests, semi-natural habitats, and residential areas supported the bulk of pollen sources for honey bees during corn planting in a landscape dominated by conventional crop fields. Determining the correlation between distance to corn planting sites and risks of contamination in the identified bee-attractive pollen sources would be an important proceeding step toward providing recommendations for mitigating the impact of seed treatment dust on bees via the management of pollen forages.

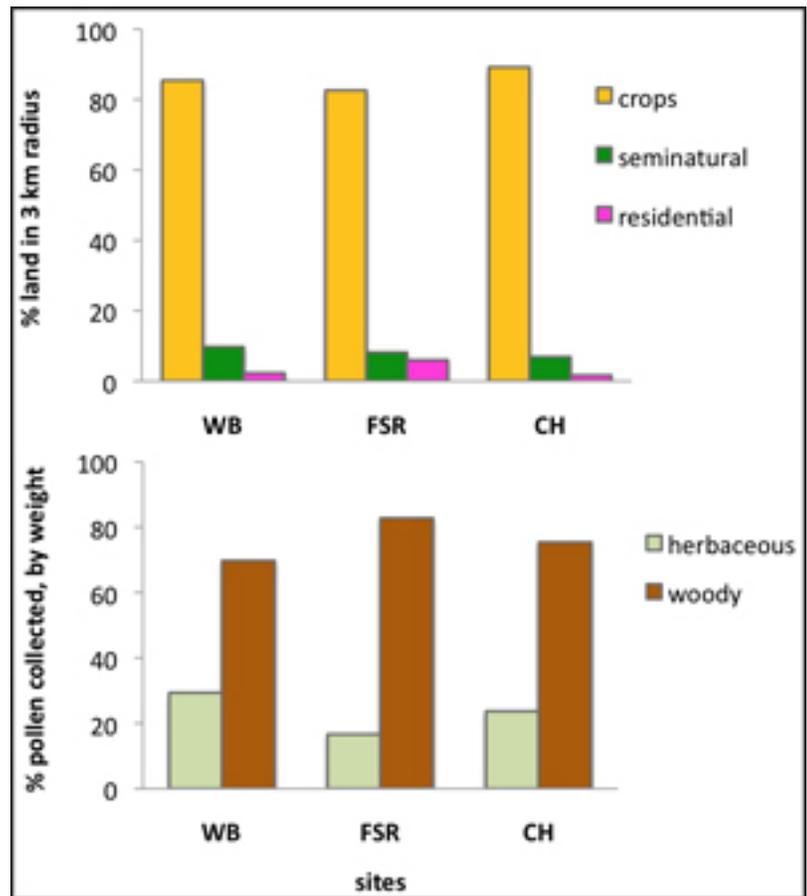


Figure. Landscape composition and pollen sources collected by honey bees.

14. Matisoff, M., C. Butler & T. Webster

- HONEY BEE MIDGUT IMAGES USING HISTOLOGICAL TECHNIQUES College of Agriculture, Kentucky State University, Frankfort KY 40601

We prepared honey bee midguts for imaging by light microscopy in order to understand the morphology of this organ and how it responds to *Nosema ceranae* infection. Worker honey bees were killed by submersion in soapy water, and dissected soon afterward. The midgut, often including parts of the crop and ileum, was lifted from the abdomen after immobilizing the killed bee in paraffin and removing the dorsal sclerites. This tissue was fixed in formalin, and then dehydrated in a series of ethanol dilutions, xylene and paraffin. Three-micron sections were then re-hydrated and stained with either hematoxylin and eosin for bright field observations, or calcofluor (also known as fluorescent brightener, for fluorescence microscopy). Micrographs were taken with an Olympus BX-41 fluorescence microscope and DP72 digital camera, at 400x and 1000x.

These images clearly show the proventricular valve as it protrudes into the crop and a constriction at the posterior of the midgut which might be caused by accumulated peritrophic matrix (PM) material. Also, bristles at the posterior midgut are apparent by vivid auto-fluorescence, although very little scientific literature refers to these structures. Their function is unknown. Close clusters of midgut epithelial cells seen secreting at least four layers of PM. The chitin in the PM stains vividly with calcofluor, and the cells auto-fluoresce. Possibly, we can use the number of PM layers as an index of the health and nutritional status of the bee. The possibility that the PM protects a worker bee from *N. ceranae* spores that germinate in the midgut lumen is not supported by our observations so far.

15. Olmstead, A.W., J.L. Louque & D.L. Fischer - IMPACT OF CHRONIC IMIDACLOPRID EXPOSURE ON HONEY BEE COLONIES EXPOSED VIA SUCROSE SOLUTION Bayer CropScience, RTP, NC 27709

Imidacloprid is a systemic, neonicotinoid insecticide that elicits effects in target organisms by interaction with the nicotinic acetylcholine receptor. As part of a continued evaluation of the chronic hazard of imidacloprid to honey bees (*Apis mellifera*), a colony feeding field study was carried out with honey bee colonies. These colonies were exposed to either control or one of five treatment levels of imidacloprid in 12 L of sucrose solution which was supplied to colonies inside the hive over 6 six weeks. Treatment levels ranged from 12.5 to 200 ppb imidacloprid. 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. Colony condition and hive weights were assessed at various time points starting in May, during the exposure, and after exposure until October. Exposure occurred during the summer in which a dearth of floral resources was expected in order to increase consumption of treated solution. Significant decreases were observed in pollen stores and capped brood during and immediately after exposure at treatment levels of 50 ppb imidacloprid and above. Additional effects were observed on hive weight, honey stores, and adult bee counts; however, these endpoints were generally less sensitive. Overall the results indicate that chronic imidacloprid exposure results of ≥ 50 ppb results in decreased pollen stores, potentially due to reduced foraging activity. The reduced pollen levels likely results in lower amounts of brood which then translate into effects on other aspects of colony dynamics at later time points. The no observable effect concentration for this study was 25 ppb, which is above residue levels in nectar typically measured in the field for most agricultural uses.

16. Pernal, S.F.a, A. Ibrahima, S.E. Hooverb, R.W. Curriec, M.M. Guarnad & L.J. Fosterd - NEXT GENERATION IPM TOOLS FOR BEEKEEPING: PROGRESS AFTER THREE GENERATIONS a Beaverlodge Research Farm, Agriculture & Agri-Food Canada, Beaverlodge, AB Canada T0H 0C0 (e-mail: Steve.Pernal@agr.gc.ca) b Lethbridge Agriculture Centre, Alberta Agriculture and Rural Development, Lethbridge, AB, Canada T1J 4V6, c Department of Entomology, University of Manitoba, Winnipeg, MB, Canada R3T 2N2, d Department of Biochemistry & Molecular Biology and Centre of High-Throughput Biology, University of British Columbia, Vancouver, BC, Canada V6T 1Z4

The Next-generation Integrated Pest Management Tools for Beekeeping (BeelPM) project aims to demonstrate the efficacy of proteomic marker-assisted selection for enhancing disease and mite resistance. In 2011, 622 colonies were tested for hygienic behavior (HB) across four Canadian provinces. A portion of these colonies was then randomly selected to establish an unselected benchmark population ($n=83$) as well as an F0 population ($n=110$). We successively tested, selected and propagated three generations from our F0 from 2011 to 2013, in a comparison of proteomic-based marker-assisted selection (MAS) against traditional behaviorally-based phenotypic selection (FAS) on HB. Here we present some results from the third field season of the project, in which 597 FAS and MAS queens were propagated and distributed for evaluation. FAS-selected stock exhibited relative increases in hygienic behavior of 28.6, 19.9 and 31.7% over

Bee Science *continued*

benchmark populations in the F1- F3 generations, respectively. Similar, though smaller, gains were observed for the MAS-selected stock where levels of HB increased 10.6, 13.0, and 20.1 % over benchmark populations for the F1 through the F3. Progeny for each generation were also evaluated for Varroa Sensitive Hygiene using the 7-day “quick test” as described by Villa et al., 2009 (J. Apic. Res. 48: 162-167). Overall, F3 colonies showed a reduction in Varroa-infested brood of $38.8 \pm 5.6\%$ for FAS-selected stock and $40.2 \pm 4.2\%$ for MAS-selected stock, compared with benchmark stock at $21.9 \pm 3.9\%$. Nevertheless, the fertility of mites remaining in the brood remained unchanged for FAS and MAS selected stock over three generations (combined mean of $89.8 \pm 1.1\%$), and similar to the benchmark population.

V. destructor resistance was also evaluated by examining changes in colony mite populations over a ten week period. For the F1 generation, overall differences in phoretic mite abundance ($P < 0.040$) and adult bee populations ($P < 0.003$) were observed among stocks. Prior to winter, the abundance of V. destructor on adult bees was lower in both FAS and MAS colonies than in benchmark colonies headed by New Zealand or Western Canadian queens, though the MAS stock was not statistically different from the latter group. F1 FAS colonies also had larger cluster sizes than benchmark stocks prior to wintering, whereas MAS colony population sizes were intermediate between FAS and benchmark stocks. After continued exposure to high V. destructor levels without treatment, 7 of 18 FAS and 3 of 18 MAS F1 colonies survived the winter of 2012-13, while no benchmark colonies survived. In 2013, F3 colonies inoculated with V. destructor were assessed for the proportion of recapped brood cells among stocks ($P < 0.010$). Colonies headed by MAS queens had higher proportions recapped cells than the benchmark stocks, with FAS colonies being intermediate.

Both FAS and MAS selected stocks were also evaluated via whole-colony challenge experiments with American foulbrood disease (AFB). Clear evidence of improved colony-level resistance to AFB was observed for FAS and MAS stocks in the F1 and F3 generations for several parameters, including the numbers of clinical symptoms in colonies over time, proportion of colonies infected, as well as overwintering survival.

Our data are first to show the enrichment of disease and mite resistance using proteomic markers and the utility of this novel technology in bee breeding.

17. Rangel, J. - THE EFFECTS OF HONEY BEE (APIS MELLIFERA) QUEEN INSEMINATION VOLUME ON COLONY GROWTH - Department of Entomology, Texas A&M University, College Station, TX 77840. Fax: 979-845-6705. (e-mail: jrangel@tamu.edu)

Reproductive division of labor in the honey bee (*Apis mellifera*) is characterized by the presence of a queen that monopolizes reproduction, in part through the production of a blend of pheromones that alter worker behavior and physiology. Previous studies have shown that queen insemination volume and quality alter queen mandibular pheromone profiles (Richard et al., 2007 PLoS ONE 2(10): e980; Kocher et al., 2008 BMC Genomics 9: 232-247; Kocher et al., 2009 Behav. Ecol. 20: 1007-1014) and affects queen survival, worker retinue response, and Dufour's gland composition (Niño et al., 2012 J. Ins. Physiol. 58: 1082-1089). To date, however, no one has looked at the effect of queen insemination volume on colony growth and queen supersedure. In this 1-year preliminary study, we

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investigated the effects of queen insemination volume on colony growth and queen supersedure.

We did so by comparing the growth of nine colonies headed by “high-volume” insemination queens (i.e., those instrumentally inseminated with 9.0 μ L of semen) to nine colonies headed by “low-volume” insemination queens (i.e., those instrumentally inseminated with 1.5 μ L of semen). Experimental queens were raised by grafting, and each virgin queen was instrumentally inseminated with a set volume of mixed semen from a pool of drones from 6 different colonies. On 13 May 2013, experimental colonies were established by introducing a caged experimental queen into a nucleus hive containing 2 lbs of bees (or approximately 7,000 workers) and new frames with alternating full and partial wax foundation. Every 2 to 4 weeks we measured from each colony the amount of worker and drone comb built, the amount of worker and drone sealed brood produced, the amount of food stored and all supersedure events.

We found a significant positive effect of queen insemination volume on a colony’s production of drone brood, as well as honey and pollen production. We found no significant effect of insemination volume on the amount of comb or worker brood produced, however. We observed 10 supersedure events, all occurring within 90 days after colony establishment, although supersedure rate was not associated with queen insemination volume. Our preliminary results provide evidence that in honey bees, queen insemination volume affects some key aspects of colony growth. However, more studies are needed to determine whether queen insemination volume clearly affects worker productivity and potentially, colony fitness.

18. Seeley, T.D. - SWARM INTELLIGENCE IN HONEY BEES Department of Neurobiology and Behavior, Cornell University, Ithaca, NY 14850 (e-mail: tds5@cornell.edu)

Swarm intelligence is the solving of a cognitive problem by two or more individuals who independently collect information and process it through social interactions. With the right organization, a group can overcome the cognitive limitations of its members and achieve a high collective IQ. To understand how to endow groups with swarm intelligence, it is useful to examine natural systems that have evolved this ability. An excellent example is a swarm of honey bees solving the life-or-death problem of finding a new home. A bee swarm accomplishes this through a process that was discovered at the Zoological Institute of the University of Munich in the 1940s, and that has been analyzed more deeply in recent years. It includes collective fact-finding, open sharing of information, vigorous debating, and fair voting by the hundreds of bees in a swarm that function as nest-site scouts. We will see how these incredible insects have much to teach us when it comes to achieving collective wisdom and effective group decision making.





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Ready for the June Bloom

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Bee Science

Abstracts from 2014 American Bee Research Conference Meeting

In an effort to introduce many of you to the broader scope of bee health and science research, Kelley Beekeeping newsletter is running the 2014 American Bee Research Conference (ABRC) abstract proceedings of submitted research from across the United States. This is the fourth and final batch (Abstracts #19-25) from the 2014 ABRC that was held in January in San Antonio, TX. The ABRC brings some of the finest and fittest scientists together to share their research work and to support each other through the peer-reviewed process.

Compiled and Edited by Drs. Juliana Rangel and Rose-Anne Meissner, Research Associates, Department of Entomology, Texas A&M University, College Station, TX 77843

19. Steinhauer, N.A., K. Rennicha, M.E. Wilson & D. vanEngelsdorpa - A NATIONAL SURVEY OF MANAGED HONEY BEE 2012-2013 ANNUAL COLONY LOSSES IN THE USA: RESULTS FROM THE BEE INFORMED PARTNERSHIP Department of Entomology University of Maryland, College Park, MD 20742 (e-mail: nsteinha@umd.edu), Department of Entomology and Plant Pathology, University of Tennessee, Knoxville, TN 37996

For the past 6 years in which overwintering mortality of honey bee colonies has been surveyed in the US, estimates of colony loss have fluctuated around one-third of the national population (vanEngelsdorp et al., 2007 Am Bee J 147(7): 599–603, 2008 PLoS One 3(12), 2010 J.Apic.Res.49(1): 7–14, 2011 J.Apic.Res.50(1): 1–10 and 2012 J.Apic.Res. 51(1): 115–124; Spleen et al., 2013J.Apic.Res. 52(2): 44–53).

In April 2013, we collected data from 6,482 US beekeepers (6,114 backyard, 233 sideline, and 135 commercial beekeepers) to document the 2012-2013 overwintering mortality rates of honey bee colonies for the United States. Responding beekeepers reported an overall 30.6% (95% CI: 30.16 – 31.13%) loss of colonies over the winter, with each beekeeper losing on average 44.8% (95% CI: 43.88 – 45.66%) of their colonies. The self-reported level of acceptable winter loss was 14.6%, and 73.2% of the respondents had mortality rates greater than this level. The leading self-identified causes of overwintering mortality were different according to the operation type; backyard beekeepers generally self-identified “manageable” factors (e.g., starvation, weak colony in the fall), while commercial beekeepers generally identified non-manageable factors (e.g., queen failure, pesticides) as the main cause of losses. For the first time in this survey series, we estimated mortality during the summer (total loss = 25.3% (95% CI: 24.80 – 25.74%), average loss = 12.5% (95% CI: 11.92 – 13.06%) (n=4,181 respondents). The entire 12-months period (April 2012 to April 2013) yielded a total loss of 45.2% (95% CI: 44.58 – 45.75%), and an average loss of 49.4% (95% CI: 48.46 – 50.43%) (n=4,429 respondents).

The total seasonal losses varied across states (range: 11.0% to 54.7% for winter loss; 4.0% to 59.8% for summer loss and 18.8% to 73.5% for annual loss (see figure)). While we found that commercial beekeepers lost fewer colonies than backyard beekeepers in the winter (30.2% (95% CI: 26.54 – 33.93%) vs 45.4% (44.46 – 46.32%) respectively, p-value < 0.001), the situation was reversed in the summer where

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commercial beekeepers reported higher average losses than backyard beekeepers (21.6% (95% CI: 18.4 – 24.79%) vs 12.1% (11.46 – 12.65%), p-value < 0.001). For all operation types, the winter period brought about a higher mortality than the preceding summer (Mann Whitney U test: p-value < 0.001 for backyard beekeepers; p-value < 0.001 for sideline beekeepers and p-value < 0.05 for commercial beekeepers).

We did not detect an overwintering mortality difference between commercial and sideline beekeepers who indicated they moved at least part of their colonies to California almond orchards for pollination in 2012 compared to those who did not. We also did not detect a difference in overwintering mortality between those who indicated that they moved their colonies at least once during the last year (“migratory”) and those who did not. These findings demonstrate the ongoing difficulties of US beekeepers in maintaining overall colony health and survival and suggest

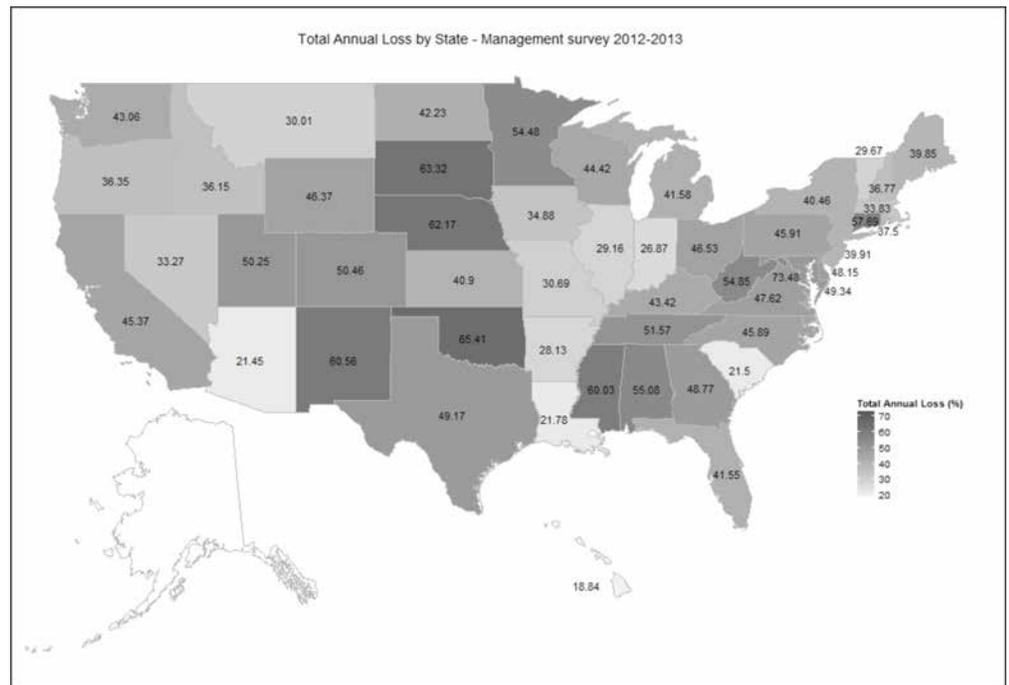


Figure. Total annual loss (%) by state

that to capture a more complete picture of honey bee colony mortality and understand its drivers, survey studies documenting colony losses should report annual losses rather than winter losses only.

Respondents who managed colonies in more than one state had all of their colonies counted in each state in which they reported managing colonies. Data for states with fewer than five respondents are withheld.

20. Tapy, D.R., M. Simone-Finstroma, M. Huang, M. Strand & O. Rueppell – EFFECTS OF MIGRATORY BEEKEEPING ON WORKER LONGEVITY AND OXIDATIVE STRESS Department of Entomology, North Carolina State University, Raleigh, NC 27695, US Army Research Laboratory, Research Triangle Park, NC 27709, Department of Biology, University of North Carolina at Greensboro, Greensboro, NC 27403

Given current health issues plaguing honey bees, understanding the effects of possible stressors at the individual, cellular, and molecular levels are of increasing importance. Stress and aging are often highly related. Honey bees are an excellent model to simultaneously investigate this relationship between stress and longevity, as well as how it impacts both individual and group-level phenotypes. The aim of this study was to determine potential effects migratory beekeeping practices might have on levels of oxidative stress in individual honey bees and subsequent effects on lifespan. Foragers and in-hive bees were collected at known ages to quantify cellular and molecular measures of oxidative

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stress from two different sets of colonies: 1) “stationary colonies” maintained at one agricultural site and 2) “migratory colonies” that were moved every 21 days to different agricultural landscapes for 3 months. Another subset of bees were used to assess any effects on individual lifespan in laboratory conditions. Our findings indicate that bees collected from “migratory” hives have slightly but significantly shorter lifespans on average than bees from stationary hives. Results will also be presented on measures of oxidative stress, including lipid peroxidation and protein oxidation.

21. Traver, B.E., N.G. Johnson, T.D. Anderson & R.D. Fell- NOSEMA CERANAE LEVELS AND IMMUNE RESPONSES IN BEES FOLLOWING HIVE TREATMENT WITH PESTICIDES Department of Entomology, Virginia Tech University, Blacksburg, VA, 24061 (e-mail:traverb@vt.edu)

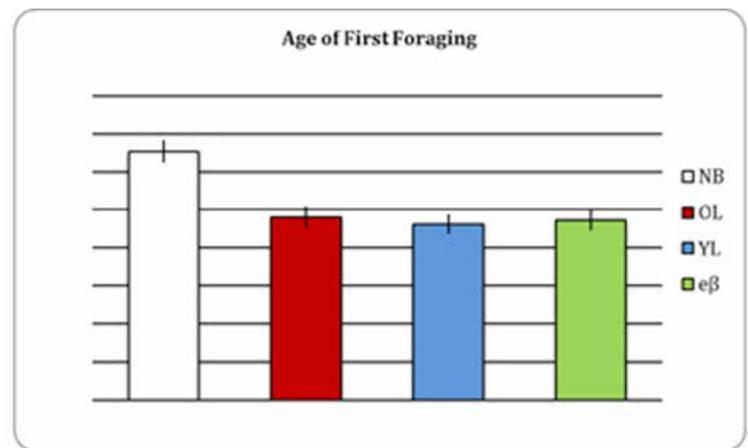
Honey bee colony losses have been a concern for the past several years. Many efforts have been put forth to investigate factors that could be involved in increased losses. Most researchers and beekeepers now agree that there is no “silver bullet” and losses are most likely due to multiple factors. For this project, we sought to determine whether three different pesticides, two used by beekeepers and one frequently encountered in the environment by honey bees, impact pathogen levels and immunity in honey bees. We report the effect of 1) chlorothalonil, a commonly used fungicide, 2) fumagillin, the antibiotic used for *Nosema* control, and 3) tau-fluvalinate, an acaricide used for varroa mite control on *Nosema ceranae* levels, glucose oxidase (GOX) and phenoloxidase (POX) levels. One story colonies were established in the end of summer/early fall of 2012. Five colonies were then treated with one of the three pesticides in the fall 2012 (October), the spring 2013 (April), and the summer of 2013 (July) for a total of 20 colonies. Samples of bees were taken pre-treatment and 2 and 4 weeks post-treatment. Colonies were also sampled, but not treated with anything, in January 2013 to observe a baseline level of each parameter during the winter. For *N. ceranae* levels in the fall and winter, and summer, there were no significant differences in levels between treatments or sampling period (for the fall and summer only). In the spring, chlorothalonil-treated colonies had a significant increase in *N. ceranae* levels from pre-treatment to 2 weeks post-treatment ($p < 0.05$), however, these data should be interpreted carefully as the sample size for the spring was based on reduced sample size ($n=2$) due to colony losses from starvation. There was no difference in POX activity in the winter and spring. Conversely, in the fall, overall regardless of treatment, there was significantly higher activity at 2 weeks post-treatment versus pre-treatment ($p < 0.01$) and significantly lower POX activity at 2 and 4 weeks post-treatment compared to pre-treatment levels in the summer ($p < 0.01$). There was no significant difference in GOX activity across time or treatments for the fall, winter, and spring. However, in the summer, GOX activity was the same across treatments, but across time, in the control and fumagillin-treated colonies, GOX activity was significantly lower in the 2 and 4 week post-treatment compared to the pre-treatment levels ($p < 0.05$). Overall, colonies treated with tau-fluvalinate (Apistan strips) or fumagillin, or those exposed to chlorothalonil in the fields, do not seem to impact *N. ceranae* levels, POX activity, or GOX activity in a negative way.

22. Traynor, K.S., Y. Wang, C. Brent, G. Amdama, & R.E. Pagea - THE PRIMING INFLUENCE OF HONEY BEE LARVAE ON AGE OF FIRST FORAGING AND FORAGING BIAS Arizona State University, Tempe, AZ 85004, U.S. Arid Land Agricultural Research Center, U.S. Department of Agriculture, Maricopa, AZ 85138, Department of Chemistry, Biotechnology, and Food Science, Norwegian University of Life Sciences, Aas, Norway

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Current models predict that honey bee larvae influence age of first foraging via their pheromones; young larvae accelerate behavioral maturation, while old larvae delay maturation. Newly emerged bees were raised in four different brood priming environments for ten days: 1) young larvae (YL), 2) old larvae (OL), 3) e-beta ocimene (e β), the pheromone of YL or 4) no brood (NB) as a control. At 10 days of age, samples were collected and examined for physiological characteristics associated with behavioral maturation. The focal bees were then combined into a common garden environment and monitored for age of first foraging. The early priming environment of different brood treatments had a significant effect on age of first foraging. Bees that experienced a no brood environment foraged significantly later than any of the other three brood treatments (See Fig). Bees raised in both the YL and e β environments biased their first foraging trip toward pollen collection.

The brood priming environments also had significant effects on all three physiological characteristics examined that are associated with the transition from nursing to foraging: HPG development, vitellogenin and juvenile hormone. HPG development, an indicator of nurse status, significantly differed across treatments in bees after 10 days of priming. Bees in the NB treatment had less developed glands compared to the three brood treatments. Vitellogenin (VG), an egg-yolk precursor, and juvenile hormone (JH) are correlated in a double repressor relationship. In nurse bees VG is typically elevated and JH suppressed. As bees mature, VG declines and JH increases; this change in hemolymph titers corresponds with a transition from nursing to foraging. Brood treatments significantly influenced both VG and JH titers.



23. Tsuruda, J.M., S. Subramanyam, C.E. Williams, M.M. Hamiduzzaman, B. Emsen, E. Guzman-Novoa, G.J. Hunt –BEHAVIORAL RESISTANCE TO VARROA MITES – GROOMING AND NEUREXIN GENE EXPRESSION Clemson University, Clemson, SC 29634, Department of Entomology, Purdue University, West Lafayette, IN 47907, Department of Agronomy, Purdue University, West Lafayette, IN 47907, Agricultural Research Service Crop Production and Pest Control Research, United States Department of Agriculture, West Lafayette, IN 47907, University of Guelph, School of Environmental Sciences, Guelph, Ontario, N1G 2W1 Canada

Grooming is a behavioral trait of honey bees that can reduce Varroa mite populations. Adult bees attempt to dislodge mites from their bodies by swiping movements of their legs and may bite and damage the mite. The identification of the genes involved in this behavior could allow for marker-assisted selection and facilitate the breeding of mite resistant bees.

We previously conducted a study to look for associations between grooming behavior and genotype by mapping quantitative trait loci (QTL). We used a behavioral assay that measured the time for a bee to respond to a mite placed on her thorax. One QTL was identified that contained 27 candidate genes, one of which, neurexin, is associated with neurological processes that could play a role in honey bee

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grooming. Neurexin has been shown to be involved in autism spectrum disorder in humans, which can be characterized by repetitive and agitated movements and hypersensitivity to stimuli, and in mice it is known to affect grooming behavior. This is a relatively large gene of 28 exons and two major forms (A and B), with at least 12 different isoforms due to alternative splicing. Preliminary tests showed similar expression patterns for the A and B forms so we investigated the relationship between the expression of B forms and grooming behavior in worker honey bees. Two behavioral studies were performed – one measuring grooming intensity (use of multiple legs or one leg, fast movements or slow movements, removal or mite or no attempt to groom), and one measuring response time. Individuals from each study were selected for qPCR to evaluate expression of the B forms of neurexin relative to a housekeeping gene.

We found significant differences in the relative transcription levels of neurexin between bees that exhibited intense grooming or mite removal and the those that exhibited slow or no grooming. Our results also show a correlation between grooming response time and neurexin expression and a significant difference in neurexin between bees that responded in less than 8 seconds and bees that responded in more than 8 seconds. These results suggest a possibility for using neurexin in a marker assisted selection program for Varroa resistance. Future directions include RNAi to validate gene function and characterization of neurexin in *A. cerana*. Ultimately, we aim to use this gene and genes involved in Varroa sensitive hygiene to breed for behavioral resistance to Varroa and provide beekeepers with more resilient stocks of bees.

24. Webster, T., M. Matisoff & C. Butler - MIDGUT MORPHOLOGICAL CHANGES THAT ACCOMPANY NOSEMA CERANAE INFECTION IN HONEY BEES College of Agriculture, Kentucky State University, Frankfort KY 40601

We sought to answer three questions pertaining to the effects of the fungal pathogen *Nosema ceranae* infecting honey bees: Does *N. ceranae* infection cause midgut / peritrophic matrix deterioration? Does the peritrophic matrix retain *Nosema ceranae* spores? Is there a means by which *N. ceranae* infection of midgut epithelial cells can later lead to infection of organs far from the midgut (hypopharyngeal glands, fat bodies)? Worker honey bees were fed *N. ceranae* spores and observed later for changes in midgut morphology. We used a method similar to that of Rinderer (1976, *J. Econ. Entomol.*) by which individual worker bees were placed into capsules with small tubes that could be filled with diet. We found that 80 – 95% of the bees we captured consumed their diet within several hours. By this method, approximately 200 bees were each fed 50,000 *N. ceranae* spores in 10 uL of 50% sucrose. They were then caged collectively and sustained on 50% sucrose for the following 14 days. Worker honey bees were killed by submersion in soapy water, and dissected soon afterward. The midgut, often including parts of the crop and ileum, was lifted from the abdomen after immobilizing the killed bee in paraffin and removing the dorsal sclerites. This tissue was fixed in formalin, and then dehydrated in a series of ethanol dilutions, xylene and paraffin. Three-micron sections were then re-hydrated and stained with either hematoxylin and eosin for bright field observations, or calcofluor (also known as fluorescent brightener, for fluorescence microscopy). Micrographs were taken with an Olympus BX-41 fluorescence microscope and DP72 digital camera, at 400x and 1000x. Calcofluor staining and observation by bright field and fluorescence microscopy showed little degradation of midgut tissues, compared to control bees fed sucrose solution without spores. The peritrophic matrix retained many spores, but heavy infections of midgut cells developed nevertheless.

Our observations of midgut infection showed that *N. ceranae* was present even in those midgut cells which were outermost, and in contact with the hemolymph. Consequently, it is possible to

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visualize the release of *N. ceranae* spores into the hemolymph of a heavily infected bee and the passage of the spores to other organs in the body of the bee. This supports the findings of those who found *N. ceranae* DNA in the hypopharyngeal glands, fat bodies and other organs of infected bees (Chen et al 2009, *J. Eukaryotic Microbiol.*; Gisder et al 2010, *Appl. and Environ. Entomol.*)

25. Zhu, X., S. Zhou & Z.Y. Huang - TRANSPORTATION AND POLLINATION SERVICE INCREASE ABUNDANCE AND PREVALENCE OF NOSEMA CERANAE IN HONEY BEES (APIS MELLIFERA) Bee Science College, Fujian Agriculture and Forestry University, Fuzhou, Fujian, China, Department of Entomology, Ecology, Evolutionary Biology, and Behavior, Michigan State University, East Lansing, MI, 48824

Nosema ceranae affects honey bee physiology and behavior adversely and is also recently shown to increase its infection probability when bees were exposed to pollen with fungicides in a lab study (Pettis et al. 2013, *journal.pone. 0070182*). However it is not clear if transportation stress plus the subsequent pollination service would increase *N. ceranae* prevalence and abundance.

Fourteen colonies were randomly divided into two groups. A group of “transported and pollinating colonies” (TP) was moved to provide pollination for blueberry for 11 days, 138km away. A group of “stationary colonies” (S) was not moved (at East Lansing, MI) and served as the control during the same time. Fifteen foragers were sampled per colony from all 14 colonies using a bee vacuum, 1 day before transportation took place (TP0 and S0) and then 7 days after the bees were moved back (TP1 and S1). Spore loads in the midgut in individual bees were then determined and we also ran PCR to verify the spores to be *N. ceranae*. There was no significant difference in infection rates between TP0 and S0 ($T=0.013$, 2-tailed test, $P=0.99$) and between S0 and S1 ($T=0.43$, 2-tailed test, $P=0.67$) (Fig. 1A). This suggests that TP and S groups had the same *Nosema* prevalence at the beginning of the experiment. In addition, prevalence of S group did not change before and after transportation and pollination. However, we found a significantly higher *Nosema* prevalence in TP1 than S1 ($T=2.14$, 1-tailed test, $P=0.027$), suggesting that transportation and/or pollination caused a higher infection rate in workers.

Nosema abundance followed a similar pattern (Fig. 1B). There was no significant difference between the TP0 and S0 ($T=0.44$, 2-tailed test, $P=0.67$), and between S0 and S1 ($T=0.37$, 2-tailed test, $P=0.71$). Therefore TP and S had the same *Nosema* abundance before bees were moved for pollination service and S colonies did not change their *Nosema* abundance during the same period. However *Nosema* abundance was significantly higher in TP1 than TP0 ($T=1.72$, 1-tailed test, $P=0.05$). Our small experiment suggests that transportation and pollination affects *N. ceranae* prevalence and abundance, perhaps by affecting honey bees’ immune system. It is not clear if these effects were due to transportation alone, pollination alone, or both combined. This research was supported by a Managed Pollinator CAP USDA NIFA Grant #20098521805718.

