Bee Science & Health

ABRC Proceedings, Part I

Abstracts from January 2015

American Bee Research Conference took place in Arizona this past January 2015. As part of an effort to introduce beekeepers to the science behind bee health, we have permission to reprint the abstracts for Kelley Beekeeping readership. Don't be afraid of the jargon- if a term is unfamiliar, you can always look it up. The abstract proceedings are divided into three parts. Part II will be published in the August 2015 KB issue; and part III will be published in the September 2015 KB issue.

1. Ahumada, F. - *VARROA* TREATMENTS: EFFICACY AND ECONOMIC IMPACT
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Varroa destructor continues to be a threat for the beekeeping industry despite the efforts by beekeepers to control it. Commercial and hobbyist beekeepers suffer tremendous colony losses throughout the year due to mite infestation. The repeated application and misuse of a variety of acaricides over the years led the mites to become resistant to these products and chemical residues have been found in brood combs as well as in apiculture products (Mullin,C.A et al; PLoS One 5: (3) e9754). The high levels of miticides and agrochemicals found in honey bee colonies have been suspected to cause honey bee losses as well as affecting brood development and adult longevity (Wu, J.Y et al; PLoS One 6: (2) e9754). Residues of such control agents in hives and their negative effects on bee health have become an important issue and need to be taken in consideration when making management decisions for Varroa control treatments.

The current field study was set up in Monterey County, CA and Mr. Gene Brandi provided forty-eight full size colonies. Pre-treatment mite population and colony strength were measured in all colonies. A set of twelve colonies with equalized mite levels was randomly assigned to each treatment group. Apiguard, Mite Away Quick Strips (MAQS) and Apivar were applied following manufacturer's instructions. Any adverse post-treatment effect on bees and/or brood was noted. All queens were marked at the beginning of the study and its presence was accounted for on every colony inspection. Queens were replaced as needed but subject to availability.

Mite levels were monitored from March through November 2014 and treatments were applied in Spring and Fall. The results show that mite levels started to increase in June achieving its highest peak in August. At this time, the first set of fall treatments were applied followed by consecutive treatments in late fall to reduce mite levels before the winter season. Mite levels in Apivar colonies remained low over time but increased in November. Mite levels in Apiguard and MAQS were higher than Apivar needing additional treatments. From March through November, Apivar colonies received 3 treatments, MAQS colonies 4 treatments and Apiguard colonies 5 treatments. Colony size was recorded and the number of frames of bees and brood was similar among the treatments and no adverse treatment effect on

colony size was observed. Colony losses were recorded for all treatments and the highest percentage was observed in September especially in MAQS colonies. Queen losses were also recorded and the highest percentage was observed in June on MAQS and Apivar colonies. Queen losses for Apiguard remained low until October where a second peak was observed in all treatments except for MAQS colonies. It needs to be mentioned that MAQS colonies received half of the recommended dose of 2 pads/colony due to the beekeeper's colony configuration.

Since this is an ongoing field study, the results obtained so far should be considered as preliminary, and further conclusive data will be presented later at the conclusion of the study on June 2015.

A complete statistical analysis will be performed at the end of the study to determine the efficacy on mite levels and the economic impact. This *Varroa* treatment comparison study and the complete economic impact evaluation will provide beekeepers with a detailed analysis of the cost of each treatment and help them make decisions about cost-effective treatments for their operation.

2. Andino, G.[a], Gribskov, M.[b], Anderson, D.[c], Roberts, J.[c], Hunt, G.[a] - SURVEY OF VIRUSES IN *VARROA JACOBSONI* MITES REPRODUCING IN *APIS CERANA* AND *APIS MELLIFERA* HOSTS
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[b]Department of Biological Sciences, Purdue University, West Lafayette, IN.
[c]CSIRO Ecosystem Sciences, Canberra, ACT 2601, Australia.

3. Borba, R. and Spivak, M. – EFFECTIVENESS OF A PROPOLIS ENVELOPE IN REDUCING AMERICAN FOULBROOD SYMPTOMS IN HONEY BEE COLONIES University of Minnesota, 1980 Folwell Ave. 219 Hodson Hall. St. Paul – MN 55117. Email: rsborba@umn.edu

Honey bees have immune defenses as individuals and as a colony (e.g., social immunity and social immunization). Social immunity describes colony level anti-parasitic and anti-pathogenic protection characterized by collective defensive behaviors, spatial organization, and regulation of contact among nestmates (Cremer and Sixt, 2009. *Philosophical Transactions of the Royal Society*, 364(1513), 129-142). Social immunization is a behavioral defense that promotes colony health by protecting naïve individuals through social contact with infected individuals.

One form of social immunity in honey bees is the collection and deposition of antimicrobial plant resins in the nest as a form of cement, called propolis. The presence of propolis on the inner walls of the nest, a propolis envelope, acts as an external antimicrobial layer that enshrouds the colony, which benefits individual bee immune defenses (Simone et al., 2009. *Evolution*, 63(11), 3016-3022.). The individual and social immunity benefits derived from propolis stem from the diverse composition of resins, each with their own complex mixtures of antimicrobial compounds. Colonies experimentally provided with a propolis envelope had significantly lower levels of a fungal pathogen, Ascosphaera apis, as compared to colonies without the propolis envelope (Simone-Finstrom and Spivak 2012. PloS one, 7(3), e34601). In this study, we tested whether clinical symptoms of American foulbrood (AFB), caused by the bacterial pathogen *Paenibacillus larvae*, were reduced in bee colonies with a natural

propolis envelope compared to colonies without the envelope. We also tested the effect of this bacterial infection on the immune response of individual bees. Our results indicated that colonies with a propolis envelope were able to significantly reduce the levels of AFB infection two months after the appearance of the first clinical symptoms ($F_{1,57} = 5.43$; $P = 0.001$). Additionally, bees from colonies with a propolis envelope mounted a stronger immune response after challenge with *P. larvae*, as indicated by significantly higher transcript levels of the antimicrobial peptides hymenoptaecin and apidaecin ($F_{1,147} = 12.31$; $P = 0.0006$ and $F_{1,146} = 15.10$; $P = 0.0002$, respectively).

We also tested if honey bees use social immunization as a behavioral defense, as do carpenter ants. Carpenter ant workers infected with a bacterial pathogen increased the frequency of trophallaxis with naïve nestmates sharing droplets of physiological immune elements (Hamilton et al., 2011. *Biology letters* 7.1: 89-92.). As a result, the naïve ant nestmates acquired some resistance to the pathogen and showed higher immune system activity upon subsequent contact with the pathogen, which increased individual and colony survivorship (Hamilton et al., 2011). Here, we tested the antimicrobial activity of larval food in colonies with and without a propolis envelope, and infected or not with AFB. The larval food in colonies with AFB infection had significantly higher antimicrobial activity against P. larvae compared to the two other treatments without AFB infection. Larval food from colonies without AFB infection and without a propolis envelope had the lowest inhibition of P. larvae while food from colonies without AFB infection and with a propolis envelope had intermediate levels of inhibition ($F_{3,321} = 9.26$; $P < 0.0001$). Thus, honey bees may display social immunization through feeding larvae food that is high in antimicrobial activity when the colony has symptoms of AFB. The source of the antimicrobial compounds put in larval food (from propolis, or from glandular secretions) requires further study.

4. Brutscher, L., Daughenbaugh, K., Cavigli, I., Garcia, E., Martin, M. and Flenniken, M. – HONEY BEE PATHOGENS AND COLONY HEALTH Department of Plant Sciences and Plant Pathology, Institute on Ecosystems, Montana State University, Bozeman MT

Honey bees are important pollinators of numerous crops (global economic value over $200 billion annually) and plant species that enhance the biodiversity of both agricultural and non-agricultural landscapes. Since 2006, honey bee populations in the U.S., Canada, and Europe have experienced high annual losses. To investigate the role of pathogens (viruses, bacteria, microsporidia, and a trypanosomatid) on honey bee health we longitudinally monitored honey bee colony health, using colony size as a proxy for health, and pathogen incidence (pathogen-specific PCR) and abundance (qPCR) in commercially-managed honey bee colonies. We determined that overall pathogen incidence in weak and strong colonies did not vary significantly, but that in some operations pathogen abundance (i.e., LSV2, BQCV, DWV) was greater in weak colonies. The majority of honey bee pathogens are (+)ssRNA viruses, which include the Lake Sinai viruses. LSVs were the most prevalent and abundant pathogens detected in bee samples obtained from colonies in Montana and California (2012-2014). To better understand the transmission and pathogenesis of LSVs we performed PCR, strand-specific PCR, and qPCR of individual dissected bees, and mites. We detected a greater abundance of LSV2 in the bee gut and abdomen as compared to the head and thorax, and detected LSV2 in *Varroa destructor*

mites. Together these results suggest that LSVs can be transmitted horizontally and that mites serve as vectors of LSVs. The prevalence and abundance of the LSV virus group underscores the importance of ongoing studies aimed at characterizing this virus group, and understanding virus-host interactions.

This research is supported by Project Apis m., Montana Department of Agriculture; MSU Institute on Ecosystems (IoE), NIH Idea Program Grant GM110732, Montana State Beekeepers Association, and USDA-NIFA Multi-State-Research Project NC-1173.

5. Carroll, M.J., Saunders, M., Goodall, C., Brown, N. - EFFECTS OF POLLEN DEPRIVATION ON QMP PHEROMONE COMMUNICATION AND QUEEN RETENTION
Carl Hayden Bee Research Center, USDA-ARS, 2000 E. Allen Rd., Tucson, AZ 85719

Honey bee queens signal their presence and reproductive viability to workers through release of Queen Mandibular Pheromone (QMP), a non-volatile pheromone mixture that is removed from the queen's body by retinue workers and maintains queenright status. Queens with weak or altered QMP signal profiles are often replaced by supersedure. We examined the effects of pollen deprivation on QMP emissions and queen retention during the end of a prolonged dearth period. We employed a novel, non-destructive method to measure emissions of QMP from the queen to attending retinue workers such that QMP emissions could be measured repeatedly from the same queen. Queens from pollen-deprived colonies experienced higher rates of supersedure attempts and smaller retinues than queens from pollen-fed colonies. QMP emissions of pollen-deprived queens were altered during colony pollen deprivation, with generally lower component emissions than observed from pollen-fed queens. In particular, pollen-deprived queens released lower amounts of HVA, a QMP component that is much lower in poorly-mated or virgin queens. The changes in QMP profiles may be due to reduced QMP production by pollen-deprived queens or altered interactions with the workers that disperse QMP pheromone to the colony as a whole.

Healthy honeycomb.

6. Corby-Harris, V., Snyder, L., Meador, C., Naldo, R., and Anderson, K.E. - PROBIOTIC USE OF ACETOBACTERACAE ALPHA 2.2 (*PARASACCHARIBACTER APIUM*) FOR IMPROVING HONEY BEE COLONY HEALTH
Carl Hayden Bee Research Center, USDA-ARS, Tucson, Arizona

7. DeGrandi-Hoffman, G.[a], Ahumada, F.[b], Zazueta, V.[a], Chambers, M.[a], Hidalgo, G.[a] - THE MIGRATION OF *VARROA* MITES AMONG HONEY BEE COLONIES AND THE EFFECTS ON MITE POPULATION GROWTH **[a]Carl Hayden Bee Research Center, USDA-ARS, Tucson, AZ 85719**
[b]AgScience Consulting LLC, Tucson, AZ, USA

We conducted a study on *Varroa* population growth in colonies established in May from packages. The colonies received miticide treatments, and mite numbers were kept at < 1-2 mites per 100 bees through August. However, by October mite populations ranged from 6.3 to 15.0 mites per 100 bees. We tracked *Varroa* and colony population growth from colony establishment in May to October and compared actual values to predictions from the VARROAPOP population dynamics model (DeGrandi-Hoffman and Curry, 2004 *Int J Acarol* 30:259–274). Though colony size and mite numbers did not differ from model predictions through the summer, in October mite numbers far exceeded predictions based on mite reproduction alone (DeGrandi-Hoffman et al. 2014, *Exp Appl Acarol* 64:171–186). One explanation for this rapid growth in *Varroa* populations is mite migration into colonies.

Others have reported migration of *Varroa* into colonies on foragers with phoretic mites drifting among colonies in apiaries or robbing colonies weakened by high *Varroa* populations (Sakofski et al. 1990, *J. Invertebr Pathol* 103:S96–S119; Kraus and Page 1995, *Apidologie* 26:149–157; Delaplane and Hood 1999, *Apidologie* 30:383–395; Kralj and Fuchs 2006, *Apidologie* 37:577–587; Frey et al. 201, *J Apic Res* 50:138–144; Frey and Rosenkrantz 2014, *Econ. Entomol.* 107(2): 508-515). The increase in mite numbers we detected in the late summer and fall could have been due to mite migration especially since mite numbers in colonies were low when they were established and increased little during the summer.

The purpose of the study reported here was to measure *Varroa* migration in summer and fall and evaluate its contribution to colony mite populations. In Europe, mite migration is reported to be low in spring, and then increase considerably during late summer through October (Frey and Rosenkrantz 2014, *Econ. Entomol.* 107(2): 508-515). Our colonies were started from packages in April. Initial mite counts using alcohol washes estimated phoretic populations to be 0.03 ± 0.02 mites per 100 bees. By November, estimates of phoretic mites increased to 4.3 ± 0.72 mites per 100 bees. Predictions from VARROAPOP estimated mite population to be about 1 mite per 100 bees in November. We consistently detected foragers with *Varroa* entering and leaving colonies beginning in September and continuing through November with a greater likelihood occurring in the afternoon. These mites could have contributed to the greater than expected mite population growth.

8. Downey, D.[a], Rusert, L.[a], Thomas, D.[b], Danka, B.[c] – DEVELOPING *VARROA*-RESISTANT STOCK IN HAWAII BY COMBINING VSH GERMPLASM AND RECURRENT SELECTION N A COMMERCIAL HONEY PRODUCTION OPERATION **[a]Hawaii Department of Agriculture, Hilo, HI**
[b]Hawaii Island Honey Company, Hilo, HI **[c]USDA-ARS Baton Rouge, LA**

9. Downey, D.[a], Chun, S.[a], Follett, P.[b] – RADIOBIOLOGY OF *AETHINA TUMIDA* AND PROSPECTS FOR MANAGEMENT USING STERILE INSECT RELEASES
[a]Hawaii Department of Agriculture, Hilo, HI
[b]USDA-ARS, U.S. Pacific Basin Agricultural Research Center, Hilo, HI

10. Drummond, F.[a], Eitzer, B.[b], Evans, J.D.[c] & Leblanc, L.[d] – EFFECT OF EXPOSURE IN HONEYBEES TO THE STEROL INHIBITING FUNGICIDE, PROPICONAZOLE, ON FLOWERS OF LOWBUSH BLUEBERRY
[a]School of Biology and Ecology, University of Maine, Orono, ME 04469, USA (email: frank.drummond@umit.maine.edu), [b]The Connecticut Agricultural Experiment Station, 123 Huntington St., New Haven, CT 06504, USA, [c]USDA-ARS, Beltsville, MD 20705, USA, [d]School of Food and Agriculture, University of Maine, Orono, ME 04469, USA

A field experiment conducted over a three-year period (2011 - 2013) was designed to assess honeybee colony level effects of propiconazole exposure when foragers visited contaminated wild blueberry flowers. This experiment was a whole field experiment (paired isolated fields: 1 field treated, 1 field not treated) testing residues of propiconazole on flowers under typical pest management applications. In all years, isolated non-sprayed fields and isolated treated fields were selected to place a set of newly established colonies (range 10-20 colonies per field in a year) in each field throughout bloom (period of 1 month). Colonies were monitored every 2-4 weeks both during and after bloom throughout the spring and summer. Propiconazole concentrations in pollen and flowers, colony worker population, brood population, queen presence and health, queen egg laying rate, larval survival, worker longevity, hypopharyngeal gland size, and evidence of disease (molecular markers and dissections) and parasitic mite prevalence were measured. We found that honeybee health affects of the commonly used fungicide, propiconazole, were not entirely consistent among years. Negative effects on bee health were documented. We found that exposure of honeybee foragers to residues on flowers does not reduce colony strength of worker or capped brood populations, nor colony overwintering success. Queen laying and brood survival also does not appear to be affected by exposure to sub-lethal doses of this fungicide. We did find evidence in all three years to suggest that workers reared as larvae during bloom (when contaminated pollen was brought into the hive) resulted in young nurse bees whose longevity is reduced, that neuroendocrine gland morphology is impacted, and that propiconazole residues are repellent to foraging bees.

Bee Science

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11. Gibson, J.[a], Kocher, S.[b], Tsuruda, J.[c], Arechavaleta-Velasco, M.[d], Hunt, G.[a] – NUCLEAR-MITOCHONDRIAL INTERACTIONS AND GENE EXPRESSION IN HONEY BEE HYBRIDS; LINKS BETWEEN AGGRESSION AND METABOLISM

[a]Department of Entomology, Purdue University, West Lafayette, IN.
[b]Department of Organismic and Evolutionary Biology, Harvard University, Cambridge, MA.
[c]Cooperative Extension and Public Service & Agriculture, Clemson University, Clemson, SC.
[d]Instituto Nacional de Investigaciones Forestales, Agricolas y Pecuarias, Ajuchitlan, Querétaro, Mexico

12. Given, K. – HOW TO SELECT FOR BEES THAT BITE VARROA MITES AND GROOM THEM FROM THEIR BODIES

Purdue University, West Lafayette IN

13. Heintz, C. – PROJECT APIS M.: THE RESEARCH WE FUND AND WOULD LIKE TO FUND
Project Apis m., 6775 Chardonnay Rd., Paso Robles, CA 93446, Cell: 520-834-2832, Email: christi@projectapism.org.

Project Apis m. (PAm) is a non-profit organization governed by a Board of Directors made up of nine beekeepers from across the nation representing major national and state beekeeping organizations. A group of four Scientific Advisors reviews all research funding proposals and provides recommendations to the Board.

PAm's Mission is **to fund and direct research to enhance the health and vitality of honey bee colonies while improving crop production.** Our name comes from *Apis mellifera*, the scientific name for the European honey bee. PAm has become the go-to organization at the interface of honeybees and pollinated crops. We've infused over $3 million into bee research and programs since our inception in 2006 to provide growers with healthier bees resulting in better pollination and increased crop yields. We do world-class research with very low overhead.

We have brought new technologies to honey bee health research, discovered new pathogens,

developed comprehensive Best Management Practices, enhanced honey bee health by initiating *Seeds for Bees* to plant thousands of acres of honey bee forage and habitat, and supported Tech Transfer Teams to provide rapid feedback from the field on bee health. We are the recipients of numerous state and federal grants, as well as corporate grants that support honey bee research.

PAm is the largest non-governmental, non-profit bee research funding organization in the U.S. We are working hard to not only create a more sustainable honey bee supply and agricultural system, but also to improve domestic honey production. PAm conducted well-planned research strategy meetings to focus research on the most critical research needs in honey bee health. PAm has solid relationships with not only the nation's commercial beekeepers, but also with top bee scientists in the country. We sponsor PhD scholarships and graduate student research cultivating new bee scientists, and brought new technology to the honey bee world by providing bee labs with equipment to conduct honey bee research.

PAm currently funds: (see table)

Project Apis m. Funded Research - 2014

Principal Investigator	*Institution*	*Project*	*Amt Funded*
Rangel	Texas A&M	Stratiolaelaps scimutus	$5,000
Flenniken	MSU	Virology and Immunology	$43,500
Martin	UK/HA	Virus-Pathogen Complex	$32,556
Wick	BVS, Inc.	Virus and Essential Oils	$15,000
Eischen	USDA	Colony Density Almonds	$16,200
Johnson, R	OH State	Dimilin and IGRs	$134,640
Tarpy	NCSU	Nexcelom System	$29,480
Engelsma	GWSU	Hive Tracker Network	$22,140
Seccomb	Bee Alert	Infra-red Imaging for Grading	$62,000
Johnson, B.	UC Davis	IVDS Utility	$15,000
Flenniken	MSU	Diagnostic Tools for Lake Sanai Viruses	$15,750
Brutscher	MSU	Host-Pathogen Interaction	$50,000
Borba	UMN	Benefits of Propolis	$12,000
Wagoner	UNOG	Hyglenic Bees/Varroa Resistance	$15,000
		TOTAL FUNDS 2014	$468,266

Project Apis m.'s next RFP will be distributed soon and will target research for Varroa mite control (so that there will be no 30-year birthday party for Varroa in U.S. honey bees). Varroa remains a critical priority and PAm is seeking innovative approaches to control this pest and will go outside our normal RFP distribution channels to solicit proposals from livestock and animal science departments as well. Another top priority project for PAm is building diverse nutrition sources for honey bees in two critical areas where commercial bees travel – California and the Upper Midwest. In California, the project is called *'Seeds for Bees.'* We are building forage resources for the 1.7 million colonies that come to California for almond pollination by planting pre- and post- almond bloom flowering plants. This past fall we distributed $100,000 in seed, free to landowners in the almond growing region, to 150 participants and covering 3,000 acres in bee-attractive pollen and nectar sources. Additionally, we are initiating a project with Pheasants Forever to build honey bee habitat in the Midwest.

The Varroa project and the honey bee forage projects are Project Apis m's highest priority projects at this time.

14. Hooven, L.A.[a], Son, J.[b], Harper, S.[b] & Sagili, R.R.[a] – NANOPESTICIDES AND HONEY BEES
[a]Department of Horticulture, [b]Department of Environmental and Molecular Toxicology, Oregon State University, Corvallis, OR

Honey bees have evolved specialized structures for gathering pollen grains. Much smaller particles of materials other than pollen may also be collected and stored within the colony, and honey bees are useful for sampling environmental contaminants. Pesticide active ingredients formulated into particles using nanotechnology have become ubiquitous in agriculture. Hydrophobic pesticides are shielded within various types of particles and polymeric shells, the surface chemistry of which can be manipulated to increase solubility, resist degradation, and adhere to the intended target.

Using electron microscopy, we are characterizing the size and shape of nanoengineered pesticide products (Figure 1), and their ability to adhere to bees (Figure 2). Of the pesticide products we have examined to date, scanning electron microscopy reveals sizes predominantly in the fine particle range (between 100 and 2,500 nanometers), as compared to pollen which generally ranges between 10,000-100,000 nm. We have found similar particles adhering to pollen grains collected in agricultural settings. We hypothesize that together with pollen, bees collect these nanotechnology-enabled pesticide particles, which may facilitate pesticide transport into the colony.

We are continuing to investigate whether nano-engineered pesticide formulations result in more or less accumulation of the pesticide within the colony, extended residual toxicity on foliage, or fugitive agricultural dust which may also adhere to bees. We are currently examining crop protection products which beekeepers, the literature, or our preliminary work suggest are of concern for bees. These include certain fungicides, which are applied when bees are in the act of pollination, and our data indicate exert delayed effects on larval development. We are also investigating encapsulated insecticides which have been reported to have extended residual toxicity to honey bees. Insect growth regulators, which may pose a particular risk to larval development if transported with pollen into the colony's

food stores, are also of interest. All of the pesticide active ingredients under investigation have been found in significant concentrations in pollen collected by bees while pollinating agricultural crops. Our work will provide new insights into pesticide exposure pathways for honey bee colonies, which may contribute to the protection of all pollinators.

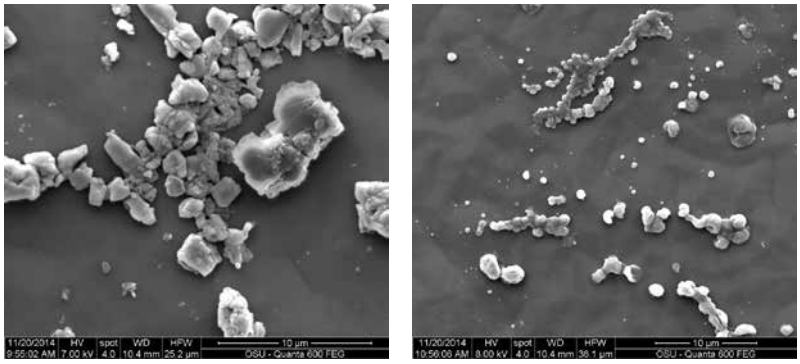

Figure 1. Examples of nanoengineered pesticide particles: Bravo (left) and Warrior II (right)

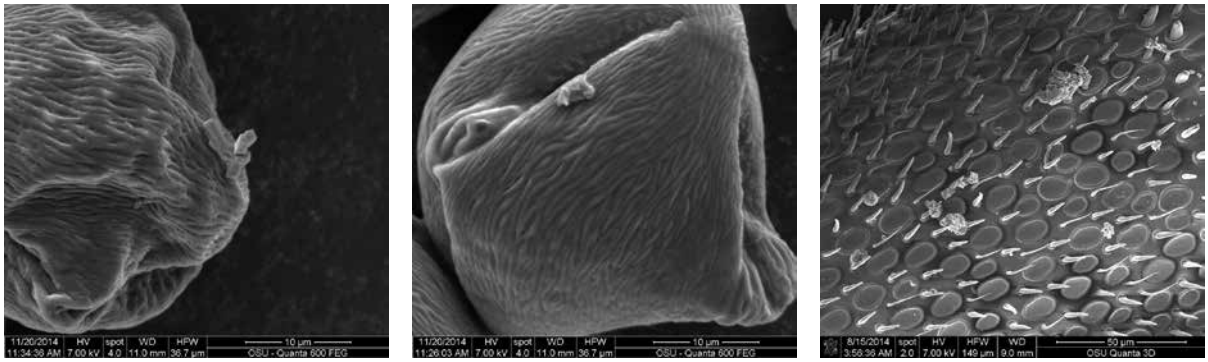

Figure 2. Possible synthetic particles found on almond pollen (left, center), and Beleaf particles transferred from leaf to bee antennae (right).

15. Huang, Z.Y. – EFFECT OF SIMULATED TRANSPORTATION ON CAGED HONEY BEES
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Honey bees are routinely transported in the United States for pollination and honey production purposes. Almond crop alone in California demands more than 50% of (about 1.3 million) all the colonies in US to be moved there for pollination and then back to their original location. In a previous study we showed that the size of hypopharyngeal glands was the most reliable indicator for transportation induced stress – in all three trials transported bees showed smaller gland sizes than stationary bees (Ahn et al. 2012. Psyche, doi:10.1155/2012/193029).

Transportation related stress can be further broken down into three parts. First is the transportation itself: long distance of it (>2000 miles) causes stress by reducing the hypopharyngeal gland sizes,

although the mechanism remains unknown. A second stress is the relocation as a result of the transportation: foragers will lose their familiar patch of nectar and pollen resources. Yet a third one is the monofloral diet bees have to feed on during the pollination service at the first transported location.

My study here focuses on the first process: can we find a proxy for transportation in the laboratory? If so this would allow us not necessary to truck bees 2000 miles for a study and easier to determine the actual mechanisms of transportation-induced stress.

In the first experiment I used an Eppendorf Thermomixer R (300 rpm) and put caged bees on this shake or not (control) and both groups of bees were inside the same incubator (34^{0}C and 50% RH). The shaking continued 24 hrs per day for 30 days. Sugar was provided as a paste (powdered sugar + 50% syrup) and pollen was also provided the same way (bee collected pollen + 50% syrup). Sugar and pollen pastes were each provided inside 10x75 mm glass tubes. Bees were sampled for hypopharyngeal gland size and hemolymph protein on day 10 and juvenile hormone on day 18. We had 2 cages per colonies per treatment (shaken vs. not). We found a significant effect of shaking on honey bee juvenile hormone levels. Shaken bees showed a significantly higher levels of juvenile hormone compared to the un-shaken bees. Higher juvenile hormone levels can be a sign that the workers are ageing faster and more stressed. However, we did not find a significant difference in hypopharyngeal gland sizes between the shaken and unshaken bees.

In the second experiment, I used a Thomas Scientific HybriShake at the maximum setting for speed. In this experiment I tried to determine if there is an interaction between being shaken and nosema infection. In other words, I wanted to see if shaken bees would show a reduced resistance to Nosema ceranae infection such that shaken and nosema-infected bees would show a higher mortality and perhaps higher juvenile hormone levels. For example infected and shaken bees might show the highest levels because a previous study showed higher hormone levels in nosema-infected bees (Goblirsch, M., Z.Y. Huang, M. Spivak. 2013. PLoS ONE 8(3): e58165). I thus had 4 treatments with 2 status of being shaken or not and 2 status of being infected or not. However, in this experiment, neither shaking nor nosema affected the hormonal titers, and there was no interactions either between the two factors (i.e. status of infection does not differ between shaken or not, in terms of hormonal titers). I am yet to analyze gland sizes, survival and nosema spore levels in the sampled bees.

Thus the two experiments were not consistent but I am not sure if it was caused by the two different shakers or by other factors. I will continue to search for a condition that emulates bees under transportation. This research was supported by a grant from the Department of Entomology, Michigan State University and I thank Logan Russell and Nick Zielinsky for help in measuring gland sizes and counting nosema spores.