1. Saraithong[a], P., Y. Li[b] & P. Chantawannakul[a,c] – BACTERIAL COMMUNITY STRUCTURE IN THE MIDGUT OF *APIS DORSATA* WORKERS IN THAILAND
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Asian giant honey bees are vital honey produces and pollinators of cultivated crops and wild plants. Relationships between gut microorganisms and honey bees are essential for maintaining proper nutrition and immunity. This study examined the bacterial community structures in the midgut of the Asian giant honey (*A. dorsata*) workers collected from two locations in Chiang Mai, Thailand. A total of 180 workers from six colonies were collected at different geographic sites in northern Thailand. Polymerase chain reaction-based denaturing gradient gel electrophoresis (PCR-DGGE) is a cultivation-independent molecular fingerprinting technique that allows the assessment of the predominant bacteria species present in bee midguts. The result showed the mean species richness and the Shannon index differed between colonies but nor by locations. Bacterial DNA profiles had similar patterns in individual colonies which differed amongst the replicate colonies, but was not affected by graphical location. Sequence analysis of DGGE products revealed evidence for core bacteria of the genera *Gammaproteobacteria* and *Firmicutes.* Although core bacteria existed in both populations, specific bacterial species were observed for each colony and site.

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2. Milbrath, M.O.[a], X.B. Xie[a, b], Zachary Y. Huang[a] CARBON DIOXIDE ANESTHESIA AFFECTS MORTALITY OF *NOSEMA CERANAE* INFECTED HONEY BEES [a]Department of Entomology, Natural Science Building, 288 Farm Lane Room 243, Michigan State University, East Lansing, MI 48824, USA, [b]Department of Laboratory Animal Science, 71 East Yangming Road, Nanchang University, Nanchang, Jiangxi 330006, China

We have known for almost a century that the microsporidia *Nosema apis* is a serious parasite of the Western honey bee (*Apis mellifera*). Only recently, we have identified that a related microsporida, *Nosema ceranae*, has transferred from its original host, the Eastern honey bee, and is causing serious infection in *A. mellifera* as well. The full effects of *Nosema ceranae* infection in this new host remain unknown. Numerous studies have examined mortality after experimental infection with *N. ceranae*, but they have had highly variable results. One reason for this variation may be differences in experimental techniques. We examined one technique, CO2 anesthesia, that may affect honey bee survival in the presence of nosema infection. We hypothesized that the use of CO2 anesthesia when infecting bees would reduce survival. We used four treatments (Control, Nosema only, CO2 anesthesia only, CO2 anesthesia /Nosema), repeating the experiment with three colonies. We found that bees infected with Nosema spores alone had significantly lower survival than control

bees (median survival = 21 days and 23 days, respectively), and that CO2 anesthesia had a greater effect on survival than nosema infection alone. Bees infected using CO2 anesthesia survived for significantly shorter times, regardless of their infection status (median survival = 18 days for both groups). Interestingly, bees infected using CO2 had significantly fewer spores than bees infected without anesthesia. These results indicate differences in honey bee mortality experiments may be due in part to experimental technique. Overall, our survival rates were higher than these previous nosema mortality experiments, indicating that variation in honey bee resistance to nosema may be an important factor in determining survival after being infected with this parasite.

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3. Xie, X.B.[a, b], G.W. Bian[b], Z. Xi[b], Z.Y. Huang[b]-USING RNAi TO HUNT FOR GENES IMPORTANT FOR *VARROA* SURVIVAL AND REPRODUCTION.[a]Laboratory Animal Science Department, Nanchang University, Nanchang, Jiangxi, 330006, China, [b]Department of Entomology, 243 Natural Science, Michigan State University, E. Lansing, MI, 48824, USA

The varroa mite, *Varroa destructor,* is the worst pest of the Western honey bee (*Apis mellifera*) and responsible for declines in honey bee populations worldwide. The issues of acaricide resistance and residues are of pressing concern to the U.S. beekeepers. In this study we used RNA interference (RNAi) technology to disrupt the life cycle of varroa mites by either causing death or causing a reduction in reproduction. We searched for gene orthologs in the newly established varroa mite genome (http://www.ncbi.nlm.nih.gov/genome/?term=varroa%20destructor). We tested the genes of Daughterless (*Da*), Proteasome 26S subunit 4 (*Pros26.4*), Ribosomal protein L8 (*RpL8*), Ribosomal protein L11 (*RpL11*), Ribosomal protein P0 (*RpP0*), and Ribosomal protein S13 (*RpS13*), all of which have shown to play roles in survival or reproduction in other tick species.

Results showed that our method of microinjection worked well because the survival of 48-h post injection (p.i.) was 85.51 ± 1.98 % (mean ± SE) for GFP injected groups. Gene suppression efficiency at 48-h pi was 62~84%. After microinjection, we assessed the effects on mite survival of 2 and reproduction of 4 candidate genes: Pts26.4 gene and Da gene caused a significantly reduction in mite survival compared to the GFP control; The mean (± SE) fecundities of mites that were injected with dsRNA of *RPL8, RPL11, RPP0,* and *RPS13* were 1.51 ± 0.20 (N=146), 0.20 ± 0.10 (N=94) and 1.05 ± 0.09 (N=90) and 1.30 ± 0.18 (N= 129) respectively, all statistically significantly lowered compared their own GPF injected controls (T-test, $P < 0.001$ for each gene). RPL8, RPL11, RPP0 and RPS13 therefore seem to be affecting reproduction in *Varroa destructor*.

In conclusion, we have discovered four genes important for mite reproduction and two genes important for mite survival. Future goals are to find ways to introduce these genes into varroa mites so that their survival or reproduction can be suppressed.

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4. Guzman-Novoa, E., G. Koleoglu, K. Reyes-Quintana & P.H. Goodwin – CELLULAR IMMUNE RESPONSE TO VARROA MITE INFESTATION IN EUROPEAN AND AFRICANIZED BEES School of Environmental Sciences, University of Guelph, Guelph, ON N1G 2W1, Canada

*Varroa destructor* feeds on the haemolymph of the honey bee, leaving an open wound in its host. Wound healing would be a consequence of a cellular immune response by the infested bee. However, not much is known about how *Varroa* affects the haemocyte response of bees to its infestation over time, and even less if different genotypes of bees have similar responses to the parasite.

Newly emerged Africanized and European honey bees were artificially infested with varroa mites, were punctured with an entomological pin, were injected with a macerate of varroa mites or with the buffer used for the macerate, and were compared with control, untreated bees, for their cellular immune response. Haemocyte counts were obtained from bees sampled at different time points. Piercing resulted in a rapid (2 hour) increase in number of haemocytes in the haemolymph of bees of both types, indicating a response to heal the wound. However, when bees were infested with mites or injected with varroa macerate, the response in haemocyte numbers decreased significantly within 12 hours relative to the control or buffer injection treatments in both types of bees. These results suggest that *Varroa* inoculates components that inhibit the cellular response of Africanized and European honey bees, possible through saliva secretions.

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5. Medrzycki, P.[a], S. Tosi[a,b], G. Bogo[a], C. Porrini[b] - INFLUENCE OF TEMPERATURE ON HONEY BEE SUSCEPTIBILITY TO PESTICIDES
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EPPO (*2010 EPPO Bulletin 40(3): 313-319*) and OECD (*1998*) are the European official guidelines that describe how to conduct trials for the evaluation of side-effects of plant protection products on honey bees. According to these guidelines, acute oral toxicity tests on adult honey bees should be carried out at 25±2°C.

In nature, adult forager bees may be exposed to a wide range of temperatures: from about 15°C (when foragers fly in spring) to 35°C (brood nest temperature) or even more (outside in hot climates). Since forager bees may also be exposed to pesticides, the purpose of this work was to investigate the influence of temperature on the susceptibility of forager bees to these substances.

Exiting forager bees from healthy and queen-right colonies were collected. Subsequently, acute oral $LD_{50}$ tests were carried out at three different temperatures: 25±0.5, 30±0.5 and 35±0.5°C. Three active ingredients (fipronil, clothianidin and thiamethoxam) were separately tested. Following the European official guidelines (EPPO and OECD), five different doses of each a.i. and a control were provided via bulk administration in 10µL 50% w/w of sucrose solution per bee. Three or four replicates in different seasons were carried out. Mortality at 24 hours was assessed and $LD_{50}$ with confidence intervals were calculated (Probit analysis, Polo LeOra software).

The results show that the $LD_{50}$ value depends on the test temperature. This relationship was confirmed statistically in all the replicates of fipronil and thiamethoxam and in 2 of 4 replicates of clothianidin. Furthermore, different substance groups have different $LD_{50}$ trends in relation to the temperature. In fact, with the increase of the temperature A) the toxicity of fipronil (phenylpyrazole) increases, while B) the toxicities of clothianidin and thiamethoxam (neonicotinoids) decrease.

To conclude, the toxicity of pesticides to forager bees is influenced by the temperature which the bees are exposed to. Interestingly, the strength and sign of this correlation depend on the characteristics of the a.i./substance group.

The European official guidelines used in the pesticide registration process (EPPO, OECD) allow to perform toxicity tests at a single temperature within 23-27°C: this wide range gives the interested parts the opportunity to carry out the tests at the temperature that will cause the preferred effect. Thus, to carefully evaluate the effects of an a.i., toxicity tests should be carried out at least at two different temperatures distant by 10°C (e.g. 25°C and 35°C). Otherwise, the hazard which the bees are exposed to could be underestimated.

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6. Tosi, S.[a,b], D. Bergamini[a], C. Porrini[a] & P. Medrzycki[b]- INFLUENCE OF POLLEN QUALITY ON HONEY BEE HEALTH [a]Department of Agricultural Sciences, University of Bologna, 44, Via Fanin, Bologna, 40127, Italy, [b]CRA-API (Consiglio per la Ricerca e la Sperimentazione in Agricoltura; Unità di ricerca di apicoltura e bachicoltura), 80, Via di Saliceto, Bologna, 40128, Italy

It is commonly agreed that the phenomenon named CCD (Colony Collapse Disorder), related to the recent honey bee colony losses, is multi-factorial. One of the factors, suspected of playing an important role in these losses, is the nutritional status of the colonies (Oldroyd, *2007 PLoS Biol. 5(6):e168*; vanEngelsdorp & Meixner, *2010 J Invertebr. Pathol. 103:80-95*).

Honey bees need to eat pollen to ensure the proper development and growth. Indeed, pollen is their main source of proteins. Forager bees tend to collect pollen from different plant species (Dimou & Thrasyvoulou, *2009 Apidologie 40:124-133*) and this behavior helps to completely satisfy the nutritional requirements of the colony through a balanced and varied diet (Brodschneider & Crailsheim, *2010 Apidologie, 41(3):278-294*). In fact, the relative proportion of the nutrients in the pollen can vary widely according to its botanical origin. Nevertheless, commercial colonies are often placed in agricultural landscapes, where usually there are few pollen-producing plant species available for the bees. For this reason, forager bees can collect pollen from only few different plant species available, according to the flowering time.

The aim of this work was to investigate if the quality of the pollen available to a colony can influence the health of the bees.

Fresh pollen loads from apiaries situated either in natural (NAT) or intensive agriculture (AGR) ecosystems were collected. The AGR pollen was characterized by lower diversity of botanical origin and lower protein content than NAT pollen. No insecticide residues were found in the tested pollen.

Newly emerged bees from the same healthy and queen-right colony were collected. Then, the bees were incubated in laboratory at 30°C and fed with water, organic *Robinia* honey and fresh pollen (AGR or NAT) *ad libitum*. Mortality and food consumption during incubation were assessed.
After 2 weeks of incubation, acute oral $LD_{50}$ tests were carried out. Two active ingredients (fipronil and thiamethoxam) were separately tested. Six test doses including control were administered to the bees, through bulk administration of an a.i. in 10µL 50% w/w sucrose solution per bee. Honey bee mortality was assessed at 24, 48, 72 hours. $LD_{50}$ and its confidence intervals were calculated (Probit analysis, Polo LeOra software).

The results showed that bees fed with AGR pollen, compared to those fed with NAT pollen, were characterized by: 1) higher mortality during the 2 weeks of incubation and 2) lower resistance to the intoxication by fipronil. No significant effect of the pollen quality on the susceptibility of the bees to thiamethoxam was found. In addition, more NAT pollen than AGR pollen was consumed by the bees during the incubation period.

To conclude, the survival of the bees and their susceptibility to pesticides may be influenced by the pollen nourishment. In this case study, Italian pollen with low diversity of botanical origin and low protein content (AGR) reduced the longevity of the bees and their resistance to fipronil (phenylpyrazole) but not to thiamethoxam (neonicotinoid). Thus, intensive agricultural landscapes may have negative impact on honey bee colonies through both the widespread presence of pesticides and the low nutritional quality of the pollen available.

Finally, it is assumed that the same stressor (e.g. intoxication) can cause lower or greater consequences to the colony in relation to the pollen supply/location of the colony.

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7. Mullin, C.A., J. Chen, W. Zhu, M.T. Frazier & J.L. Frazier - THE FORMULATION MAKES THE BEE POISON - Department of Entomology, Center for Pollinator Research, The Pennsylvania State University, University Park, PA 16802

Modern pesticide formulations, particularly when multiple active ingredients are blended, require proprietary adjuvants and ‘inerts’ to achieve high efficacy for targeted pests. Although numerous pesticides have been found in beehive samples, no individual pesticide amount correlates with recent bee declines. Formulations usually contain inerts at higher amounts than active ingredients, and these penetrating enhancers, surfactants and adjuvants can be more toxic on non-targets than the active ingredients. For example, we found that the miticide formulation Taktic® was four time more orally toxic to adult honey bees than the respective active ingredient amitraz. Impacts of 'inerts' in pollen and nectar alone or in combination with coincident pesticide residues on honey bee survival and behavior are unknown. An improved, automated version of the proboscis extension reflex assay with a high degree of trial-to-trial reproducibility was used to measure the olfactory learning ability of honey bees treated orally with sublethal doses of the most widely used spray adjuvants on almonds in the Central Valley of California. Three different adjuvant classes (nonionic surfactants, crop oil concentrates, and organosilicone surfactants)

were investigated. Learning was impaired after ingestion of 20 µg of any of the four tested organosilicone adjuvants, indicating harmful effects on honey bees caused by agrochemicals previously believed to be innocuous. Organosilicones were more active than the nonionic adjuvants, while the crop oil concentrates were inactive.

Monitoring methods are needed for major adjuvant residues so risks of formulation additives and their pesticide synergisms for pollinators can be assessed. Organosiloxane, nonyl- and octyl-phenol polyethoxylates are widely used as nonionic surfactants around honey bee hives or in their foraging areas as spray adjuvants or additives in agrochemical formulations. A method for analysis of organosiloxane, nonylphenol and octylphenol polyethoxylate surfactants in bee hive matrices was developed. A combined liquid-liquid extraction and solid phase extraction method was used. Less than 2 grams of honey, pollen or wax were extracted using the QuEChERS (Quick, Easy, Cheap, Effective, Rugged and Safe) approach. Identification and quantification were accomplished employing liquid chromatography coupled to electrospray ionization mass spectrometry (LC-ESI-MS). Nonylphenol more than organosiloxane and octylphenol polyethoxylates were found in wax samples, while pollen and particularly honey residues were lower. We will continue to focus on recent formulation technologies, including organosilicone surfactants and solvents like N-methylpyrrolidone (NMP), of unknown bee ecotoxicity, and to investigate the possibility of recent bee declines being associated with these ‘inerts’.

A larval rearing method was adapted to assess the chronic oral toxicity to honey bee larvae of the four most common pesticides detected in pollen and wax - fluvalinate, coumaphos, chlorothalonil, and chloropyrifos - tested alone and in all combinations. All pesticides at hive-residue levels triggered a significant increase in larval mortality compared to untreated larvae by over two fold, with a strong increase after 3 days of exposure. Among these four pesticides, honey bee larvae were most sensitive to chlorothalonil compared to adults. Synergistic toxicity was observed in the binary mixture of chlorothalonil with fluvalinate at the concentrations of 34 mg/L and 3 mg/L, respectively; whereas, when diluted by 10 fold, the interaction switched to antagonism. Chlorothalonil at 34 mg/L was also found to synergize the miticide coumaphos at 8 mg/L. The addition of coumaphos significantly reduced the toxicity of the fluvalinate and chlorothalonil mixture, the only significant effect in all tested ternary mixtures. We also tested the common ‘inert’ ingredient N-methyl-2-pyrrolidone at seven concentrations, and documented its high toxicity to larval bees. NMP was more orally toxic to larvae than adult honey bees. We have shown that chronic dietary exposure to a fungicide, pesticide mixtures, and a formulation ingredient have the potential to impact honey bee populations, and warrants further investigation.

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8. Reed Johnson and Eric Percel. The EFFECTS OF THE FUNGICIDE PRISTINE ON QUEEN REARING. The Ohio State University, Department of Entomology, Wooster, OH

There have been reports by commercial queen producers of occasional unexplained loss of large numbers of developing queens in the larval or pupal stage. Many of the

affected queen-rearing operations are situated among the almond orchards of California and report these losses in the weeks after almond trees bloom. Almond flowers are a rich foraging resource for bees, but are also commonly treated with fungicides, insecticides and spray adjuvants during bloom to control pests and pathogens. Queen producers have associated queen developmental problems with application of the fungicide Pristine, which contains the active incredients boscalid and pyraclostrobin, and the spray adjuvants containing organosilicone compounds. To test the effect of these pesticides queens were reared in closed swarm boxes for four days, until capping, with nurse bees fed pollen treated with four concentrations of Pristine (0.4, 4, 40 and 400 ppm), an organosilicone-containing spray adjuvant (Break-Thru, 200 ppm), the combination of Pristine and Break-Thru (400: 200 ppm), diflubenzuron (100 ppm) as a positive control or water as negative control. Low concentrations of pyraclostrobin (50 ppb), but no boscalid, was detectable in royal jelly fed to queens in the 400 ppm Pristine treatment. No significant difference in queen survival to capping or adult queen emergence was observed between any of the experimental treatments and the negative control. Only diflubenzuron, the positive control, caused a substantial reduction in queen cell capping. Interestingly, diflubenzuron use in almonds during bloom, at roughly the same time and scale as Pristine application, has seen on a steady increase over the last decade. Future work should focus on the role of diflubenzuron, possibly in combination with other pesticides, on queen development, survival and success.

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9. Villar, G., T. Baker, H. Patch, C.M. Grozinger – EXAMINING THE CAUSES OF DIFFERENTIAL RESPONSES TO THE QUEEN BY DRONES AND WORKERS. Pennsylvania State University, Chemical Ecology Lab – Room 101, University Park, PA 16802

In honey bees, the social interactions of workers and drones with the queen are primarily mediated by pheromones. Of these, a unique component of the queen mandibular gland pheromone, 9-ODA, has been found to function as both a social and sex pheromone. In workers, 9-ODA serves as an attractant, signaling the queen's presence in the hive and playing a role in the formation of the queen's retinue (Boch et al., 1975 *J Chem. Ecol.* 1:1:133-148). It also inhibits new queen rearing, slows worker maturation, and alters brain gene expression (Grozinger et al., 2003 *PNAS* 100:2). In drones, its effects are less well characterized, though we know it serves as a long range attractant, which allows drones to locate reproductively receptive queens at aerial congregation mating sites (Boch et al., 1975 *J Chem. Ecol.* 1:1:133-148).

The role that 9-ODA plays for the members of the hive is developmentally and spatially context dependent, however. Drones show no attraction to the queen while inside a hive, at any age. They also don't take mating flights outside of the hive before reaching maturity (Giray and Robinson, 1996 *PNAS* 93:21). Even after reaching sexual maturity, drones only attempt to find and mate with queens at specific times during the day. Workers, by contrast, are receptive to the queen soon after emerging; this is when they participate in queen tending and rearing and it is at this time that attraction to the queen and to 9-ODA is strongest. As workers age and transition from

nurses to foragers, exposure and receptivity to the queen decrease. Though we have a good sense of the contextual dependency of the behavioral interactions with the queen in workers and drones, our understanding of the physiological and molecular mechanisms underlying these differential behaviors is not well understood.

Here I discuss several studies which look at physiological and molecular phenomena that may be modulating worker and drone behavioral receptivity to the queen pheromone component, 9-ODA, at the level of the peripheral nervous system. Gene expression studies looking at expression levels for the recently characterized 9-ODA receptor,OR11 (Wanner et al., 2007 *PNAS* 104:36), in antennae shows receptor expression levels to be significantly higher in older mature drones and in young nurse bees as compared to immature drones and foragers, a pattern which coincides with periods of interaction and receptivity to the queen by the former groups. Though not significant, preliminary qualitative electrophysiological studies on workers show a consistent trend of increased sensitivity to the queen in the antennae of nurses vs. foragers, and a possible function of juvenile hormone in modulating olfactory sensitivity to 9-ODA. These findings may indicate a role for the peripheral nervous system in mediating, in part, the differential behavioral responses to the queen in workers and drones.

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10. Henderson, C.B. [a], J.J. Bromenshenk[a], D.L. Fischer[b]. Clothianidin exposure levels from bee-collected pollen and nectar in seed-treated corn and canola plantings. [a]Bee Alert Technology, Inc., 1620 Rogers Street, Suite 1, Missoula, MT 5902, [b]Bayer Crop Science, LP, Research Triangle Park, NC 27709

Field investigations of honey bee exposure to clothianidin from corn and canola grown from treated seed were conducted in 2010 and 2011. Fifty-three corn field sites, each consisting of 100+ acres of field corn grown from seeds treated clothianidin, were selected across three states, Illinois, Indiana and Nebraska. Pollen traps affixed to a single colony at each field were used to collect pollen gathered by foragers. A single bee-collected pollen sample was taken at each field, mid-tassel period in 2010. Three samples spanning the tasseling period were collected in 2011. Pollen for comparison was collected directly from tassels in 2010.

Seed treatment was at 0.5 mg clothianidin/seed in IL and IN; 1.25 mg /seed in NE. The former is the most common treatment rate in use in the US; the latter is maximum allowed by the label . Measured clothianidin residues in bee-collected pollen did not vary across the tasseling period, and the magnitude of residues were approximately proportional to the seed application rate. Consequently, values from 2010—treated at 0.5 mg/seed—were multiplied by 2.5 and pooled with those from 2011. For the pooled data set (N=53 field sites), the mean clothianidin concentration was 1.2 ppb with 95% of residue concentrations below 2.8 ppb. Thirty percent of bee-collected pollen samples were at or below LOD (0.44 ppb).

Corn pollen in bee-collected pollen averaged just 19 ± 22 percent whereas corn fields comprised 72 ± 14 percent of habitat within one mile of the study colonies. Clothianidin residue in tassel-collected pollen was higher than in bee-collected pollen averaging 4.4 ± 5.2 ppb with 95 percent below 11.9 ppb. There was a significant correlation between frequency of corn and clothianidin concentration in bee-collected

pollen, *r*=0.69, but there was no relationship between frequency of corn in bee-collected pollen and clothianidin concentration in tassel pollen, *r*=0.29.

Residues in canola were assessed in 2011 from thirty fields in southern Alberta, Canada. Bee-collected pollen averaged 1.7 ± 1 ppb and nectar averaged 0.8 ± 0.1 ppb clothianidin. Clothianidin concentration did not vary across the pollination period. Bees made heavy use of canola pollen; it comprised 72 ± 26 percent of pollen samples. Forty-four percent of bee-collected pollen samples were 100 percent canola. Unlike corn, there was no correlation between the concentration of clothianidin in bee-collected pollen and percentage canola pollen in the sample.

We conclude from these findings that clothianidin residues in food items collected by bees are greater for bees placed at canola field sites than at corn field sites. Even so, mean concentrations in pollen and nectar were 2 ppb or less and 95%tile levels were 4 ppb or less in these crop situations, levels that are not expected to pose a significant risk the honey bee colonies. Although residues in corn pollen sampled directly from tassels may reach 10 ppb or greater, preference by foraging bees for other kinds of pollen dilute their exposure to the lower levels we observed.

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11. Seccomb, R.A., C.B. Henderson, J.J. Bromenshenk. AUDIBLE CUES TO STRESS IN HONEY BEE COLONIES. Bee Alert Technology, Inc., 1620 Rogers Street Suite 1, Missoula, MT 59802

Investigation into honey bee response to sublethal exposure to airborne toxicants showed that honey bee colonies produce unique and characteristic sound profiles when exposed to different toxicants. Furthermore sonograms from different classes of toxicant were distinct and could be statistically differentiated at near 100% correct classification. Using these findings we explored whether other stressors of honey bee colonies induce similar identifiable sonographic profiles. We collected recordings of samples from free-flying colonies having verified conditions that included queenless and Africanized colonies as well as CCD, foul brood, small hive beetle, *Nosema*, and *Varroa* infections. Each of these conditions produced similar, unique sonographic profiles. We have developed an artificial neural network algorithm that uses these sonographic profiles to quickly assess the presence of these conditions. Using a microphone probe to make a 30 second recording, our instrument correctly identifies the presence of these conditions and the intensity of the infection with better than 85% reliability. Prototypes of our device are being tested in the field to further refine and improve the instrument's reliability in advance of its release for general use.

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12. Pernal[a], S.F., A. Ibrahim[a], S.E. Hoover[b], R.W. Currie[c], H.A. Higo[d], E. Huxter[e], M.M. Guarna[f] and L.J. Foster[f] - PROTEOMIC MARKER-ASSISTED SELECTION IN HONEY BEES: YEAR 2 UPDATE FROM THE BEEIPM PROJECT
[a]Agriculture & Agri-Food Canada, Beaverlodge Research Farm, P.O. Box 29, Beaverlodge, AB Canada T0H 0C0. [b]Alberta Agriculture and Rural Development, Lethbridge Agriculture Centre, 100-5401 - 1 Ave South, Lethbridge, AB, Canada T1J 4V6, [c]Department of Entomology, University of Manitoba, Winnipeg, MB, Canada R3T 2N2, [d]1077 237A St. Langley, BC, Canada V2Z 2Y2, [e]Kettle Valley

Queens, Grand Forks, BC, Canada V0H 1H5, [f]University of British Columbia, Department of Biochemistry & Molecular Biology and Centre of High-Throughput Biology, 2125 East Mall, Vancouver, BC, Canada V6T 1Z4

The Next Generation Integrated Pest Management Tools for Beekeeping (BeeIPM) Project aims to evaluate the efficacy of using proteomic marker-assisted selection for enhancing disease and *Varrroa destructor* resistance in honey bee populations. To evaluate the utility of this new tool for breeding, we embarked on a large-scale project. In 2011, 622 colonies were phenotyped across four Canadian provinces for hygienic behaviour (HB). A portion of these colonies was then randomly selected to establish an unselected benchmark population (*n*=83) while an $F_0$ population was established (*n*=110) from colonies most highly expressing HB. We successively tested, selected and propagated two generations from our $F_0$ during 2011 and 2012, in a parallel and direct comparison of proteomic-based marker-assisted selection (MAS) against traditional behaviorally-based phenotypic selection (FAS) on HB.

FAS-selected stock exhibited successive relative increases in hygienic behavior of 21.7 ± 2.4% and 45.7 ± 3.6% over benchmark populations in the $F_1$ and $F_2$ generations, respectively. Similar, though smaller, gains were observed for the MAS-selected stock where levels of HB increased 6.5 ± 2.8% and 29.2 ± 3.7 % over benchmark populations for the $F_1$ and $F_2$ generations. The $F_0$ and $F_1$ were also evaluated for *Varroa* Sensitive Hygiene (VSH) as described by Villa *et al., 2009* (*J. Apic. Res. 48: 162-167*). Though no significant differences were observed at one of two breeding sites in British Columbia, our Grand Forks $F_1$ FAS selected stock showed reductions in mite infestations of 40.9 ± 6.0% while MAS stock showed reductions of 50.8 ± 5.0%. $F_0$ performance was documented at 25.8 ± 3.0%.

Both FAS and MAS $F_1$ selected stocks were also evaluated via whole-colony challenge experiments with American foulbrood disease (AFB) (Pernal *et. al, 2008 J. Econ. Ent. 101:1095-1104*). Evidence of differences in colony-level resistance to AFB were observed for several parameters, including the numbers of clinical symptoms in colonies over time and the number of *P. larvae* spores in workers collected from the brood nest. At the end of the twelve week evaluation period, 100% of colonies of the unselected New Zealand stock and 83% of Western Canadian benchmark exhibited symptoms of AFB. In contrast, only 40% of MAS-selected and 15% of FAS-selected colonies exhibited symptoms.

*V. destructor* resistance was evaluated by examining changes in total colony mite populations after a ten week period, in September 2012, and again in November. Total mite levels in FAS and MAS selected colonies in the $F_1$ did not significantly differ in November (means ranging from 2004 ± 234 to 2680 ± 1240) however, differences in mean mite abundance (mites per 100 bees) and adult bee population sizes were found in September and November. Mean abundance of *V. destructor* was lower in FAS and MAS colonies than in New Zealand colonies for both sampling periods, though similar to Western Canadian benchmark colonies. Bee populations in FAS colonies were larger than in benchmark colonies in both time periods, and FAS colonies were also larger than New Zealand stock by November.

Based on results to date, we conclude that selection on proteomic markers as well as traditional phenotype has enriched HB over two generations and has

demonstrated initial proof of concept for proteomic selection in general. Detailed evaluations of the $F_3$ will be made during the summer of 2013.

13. Ingram, E.M., M.D. Ellis & B.D. Siegfried. TOXIC AND REPELLENT EFFECTS OF PYRETHROIDS USED IN ORCHARDS ON THE HONEY BEE, *APIS MELLIFERA L.* (HYMENOPTERA: APIDAE) Department of Entomology, Lincoln, NE 68583

Managed honey bee colonies are rented by fruit orchards to provide pollination services that improve fruit quality and yield. Placement of colonies in this agricultural setting increases the possibility of exposure to pyrethroids used for broad-spectrum pest control in orchards. Pyrethroids are highly toxic to bees (Smart and Stevenson, *1982 Bee World 63(4):150-152*), and studies have correlated their use with decreases in honey bee foraging after application (Reviewed in Thompson, *2003 Ecotoxicology 12:317-330*).

The goal of this study was to quantify sublethal behavioral effects associated with orchard-applied pyrethroid exposure in laboratory and semi-field situations. Quantification of sublethal effects may lead to more informed management decisions by growers and beekeepers. Development of video-tracking protocols may provide regulatory agencies with a risk assessment tool for measuring sublethal pesticide effects on pollinators.

Following sublethal topical treatment of esfenvalerate, *lambda*-cyhalothrin, or permethrin, honey bee locomotion, time spent in a food zone and social interaction were quantified using video-tracking software, Ethovision XT® and methods from Teeters *et al*. (*2012, Environ. Toxicol. Chem. 31:1349-1354*). Separate analyses were performed on experimental colonies (A and B) and responses differed between colonies. Moderate (12.98 ng/bee) and high (25.96 ng/bee) sublethal doses of esfenvalerate significantly decreased total distance moved in colony A (moderate: $p<0.0001$; high: $p<0.0001$) and colony B (moderate: $p=0.0195$; high: $p=0.0041$). Social interaction time in colony A was significantly decreased at the highest dose of esfenvalerate ($p<.0001$). The highest dose of permethrin (52.29 ng/bee) significantly decreased both total distance moved and social interaction time in colony A ($p<.0001$; $p<.0001$). These results suggest that video-tracking can detect sublethal effects of esfenvalerate and permethrin on locomotion and social interaction at these doses.

Repellencies of technical-grade esfenvalerate, *lambda*-cyhalothrin, and permethrin were measured at artificial feeders using methods adapted from Rieth (*1986, Doctoral dissertation, University of Arizona*). Control or treated filter paper was attached to polystyrene floats and placed in artificial feeders stocked with 20% sucrose solution and 30 ppm peppermint oil as an attractant. Contact pesticide exposure was simulated as foraging honey bees landed on the floats and the edge of the feeder in order to consume the sucrose syrup. Digital photos of the floats were taken every 10 minutes for 1.5 hours at each feeder. Using ImageJ software, digital images were examined to manually determine forager counts. Mean comparisons of forager counts over 10 time points were analyzed to assess repellency. Significantly fewer foragers were observed on permethrin-treated floats compared to control-treated floats at time points 3-10 (time point 3: $p=0.0019$; time point 4: $p=0.0037$; time point

5: p=0.0050; time point 6: p=0.0009; time point 7: p=0.0029; time point 8: p=0.0031; time point 9: p=0.0468; time point 10: p=0.0476)

14. Traver, B.E., N.G. Johnson, T.D. Anderson & R.D. Fell- EFFECTS OF PESTICIDE TREATMENTS ON PATHOGENS AND IMMUNITY IN HONEY BEE COLONIES - Virginia Tech Univeristy, Department of Entomology, Blacksburg, VA, 24061

Honey bee colony losses are still occurring. While initially thought to be due to one factor, it is now probable that losses are influenced by multiple factors. The goal of this project is to examine the effects of pesticide treatments on pathogen levels and immunity of honey bees. Here 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* and phenoloxidase levels. In the summer of 2012, colonies were established in apiaries that have not been treated with pesticides for five years. In the fall of 2012, colonies were either untreated (control) or treated with chlorothalonil (10 µg/L in sucrose solution), fumagillin (5 g/gallon in sucrose solution), or *tau*-fluvalinate (acaricide-impregnated strips; 10% w/w active ingredient). We collected samples of bees pre-treatment and 2 and 4 weeks post-treatment. For fall treatments, our results suggest that there was not a significant change in *N. ceranae* levels at any time point for the fumagillin-treated colonies compared to the untreated colonies. For chlorothalonil-treated colonies, there was a significant decrease in *N. ceranae* levels when comparing the pre-treatment and 4 weeks post-treatment ($p = 0.03$) time periods. *Nosema ceranae* levels also significantly decreased between the 2 and 4 weeks post-treatment ($p < 0.01$) time periods in these colonies. For *tau*-fluvalinate-treated colonies, there was a significant decrease in *N. ceranae* levels when comparing the pre-treatment and 4 weeks post-treatment ($p < 0.01$) time periods. *Nosema ceranae* levels also significantly decreased between the 2 and 4 weeks post-treatment ($p < 0.01$) time periods. Preliminary data for phenoloxidase activity suggest similar trends as *N. ceranae* levels for bees collected from the same pesticide-treated colonies.

15. Steinkampf, M.P[a], J. Hurst[b] & J Tew[c] – EFFECTS OF OPTIMIZING HIVE SOLAR ABSORPTION ON HONEY BEE HEALTH AND PRODUCTIVITY. [a]Sandhurst Bee Company, Mountain Brook, Alabama, [b]Rockhurst Farm, Birmingham, Alabama, [c]Auburn University, Auburn, Alabama

Ensuring that honey bee colonies maintain an optimal temperature remains one of the most debated and controversial topics of beekeeping. Although uncertainty about the benefit of conserving bee colony heat loss persists, wrapping hives in winter with tarpaper or insulation is still done by some beekeepers in colder climates. One potential reason for the inability to consistently document the benefit of hive insulation may be due to the fact that while insulation slows down the rate of energy exchange between the hive and the environment, it also limits penetration of heat generated via solar radiation. Preliminary experiments by our group indicated that honey bee hive temperature could be modulated using a hive coating that contained a

*thermochromic* pigment, which appears black at low temperatures, facilitating solar absorption, but becomes white when temperature exceeds a threshold value. The purpose of our study was to determine the effects of hive coatings that modulate solar radiation absorption on honey bee health and productivity.

Hive boxes and covers were primed with white latex primer followed by two coats of TC coating (black/colorless, transition temperature 86F [31C], LCR Hallcrest Corporation, Streamwood, IL) and topcoated with four coats of transparent UV-protective spar varnish. Other hives coated with white (W) or black (B) latex paint to serve as controls. In April 2011, we installed 13 three-pound honey bee packages from our local supplier after installing hives (5 TC, 5 W, 3 B) on single-beam platform scales. Brood development was assessed after the colonies were established and in March of the following year, with honey harvest accomplished three months later. During the study period, colonies were reestablished as needed so that all hives were occupied at the start of winter, and TC boxes were recoated in November to minimize the effect of pigment fading. .

Packages initially established colonies in all TC and black hive boxes, but in only 2 of 5 white hives (P=0.035). Total colony brood area 3.5 weeks after package introduction among established colonies was similar among the study groups (TC 561 $in^2$, W 539 $in^2$, B 674 $in^2$; P=0.819). Weight loss between November and the following January was also comparable (TC -5.2 lb, W -4.9 lb, B -1.9 lb; P=0.73), as was spring brood development (TC 604 $in^2$, W 612 $in^2$, B 701.8 $in^2$; P=0.85) and honey yield (TC 40.5 lb, W 32.4 lb, B 21.3 lb; P=0.95).

We conclude that hive coatings which increase the absorption of solar radiation in cool weather facilitate honey bee package installation, but otherwise they have no demonstrable effect on honey bee health or productivity.

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16. Yang, W.C.[a], H. Kuang[b], J. Wang[a], S.S. Wang[a], Z.H Wu[a], X.Q. Miao[a], Z. Y. Huang[c] COMPARATIVE SUCROSE SENSITIVITY IN *APIS MELLIFERA* AND *A. CERANA* FORAGERS. [a]College of Bee Science, Fujian Agriculture and Forestry University, Fujian, Fuzhou 350002, China, [b]Research Institute of Eastern Honeybee, Yunnan Agriculture University, Kunming, Yunnan 650201, China, [c]Department of Entomology, Michigan State University, East Lansing, MI 48824, USA

Previous studies in the Western honey bee, *Apis mellifera*, have shown that pollen foragers have a lower sucrose threshold when tested using a proboscis extension response (PER) assay. Based on the biology of the Eastern honey bee, *A. cerana*, we hypothesized that *A. cerana* should have a lower threshold for sucrose. We compared the sucrose thresholds between pollen foragers and nonpollen foragers for *A. cerana* and *A. mellifera* in Fujian Province, China. Pollen foragers were more responsive to sucrose than nonpollen foragers in both species. Across the two species, *A. mellifera* was more sensitive than *A. cerana* in both types of foragers. In mixed species colonies where both species shared the same colony environment, *A. mellifera* also showed a higher PER score than *A. cerana*, so the higher sensitivity of *A. mellifera* was not due to a different colony environment. Based on these data, we predicted that nectar foragers in *A. mellifera* should bring in lower concentration nectar compared to that of *A. cerana*. We determined the nectar concentrations at each hour of seven-paired

colonies of the two species of bees for seven days but found that the concentration of nectar foraged *A. mellifera* was not significantly higher than that of *A. cerana.* There might be other mechanisms to enable *A. cerana* to perform well in areas with sparse nectar resources.