Proceedings of the American Bee Research Conference

The 2012 American Bee Research Conference was held February 7-8 at APHIS Headquarters in Greenbelt, MD in conjunction with the annual meeting of the Apiary Inspectors of America. The twenty sixth American Bee Research Conference will be held in Hershey, PA in conjunction with the annual meeting of the American Honey Producers Association in January 2013. The following are abstracts from the 2012 Conference.

1. **Burand[a,b], J.P., A. De[a] & R. Zheng[c] - PREVALENCE OF VIRAL SEQUENCES IN HONEY BEES FROM THE UMASS APIARY-** Honey bees (*Apis mellifera*), from hives maintained in an apiary on the University of Massachusetts-Amherst campus were analyzed over a two year period for the presence of viral pathogens. Individual bees from these hives were found to be infected with one or more of the 4 honey bee viruses: Black queen cell virus (BQCV), Deformed wing virus (DWV), Sacbrood virus (SBV) and Lake Sinai virus (LSV-1). The prevalence of each of these viruses varied both within a hive and between hives throughout the season. In both years DWV was the most prevalent of the viruses and, as expected, was found in all the hives. Generally, the level of DWV was low or not detected early in the season rising to levels as high as 80% of the bees in a hive being infected by this virus at the end of season. BQCV was the second most prevalent virus and was also found in all the hives in both seasons. Although generally present at low levels, BQCV did reach levels as high as 50% in one hive. SBV was also found in all of the hives sampled in 2010 and 2011 and was found in almost half the bees sampled from one hive in August in 2010 and in a different hive in 2011. LSV-1 was found at low levels in almost all the hives sampled.

2. **Butzloff[d], P.R. - NEXT STEPS IN MICRO-CT X-RAY IMAGING OF MICROBES IN HONEY BEE ORGANS AND *VARROA* MITES USING SILVER NANOPARTICLES AND IODINE -** Metallic, uncharged silver nanoparticles are highly toxic to viruses, fungi, and bacteria. Ionic silver, rather than silver nanoparticles, probably maximizes the capture of silver by viruses. Ionic silver is able to diffuse and migrate throughout an insect. Digestion and metabolism are not required for this diffusion to take place in solution. Light exposure using 2-propanol as a mild reducing agent is sufficient to carry out the development of silver nanoparticles from ionic silver inside honey bee tissues for excellent resolution in micro-CT imaging with x-rays (Butzloff 2011, *PLoS ONE 6: e27448).*

   Mites are implicated as potential disease vectors to honey bees. The progression of chemical or microbial damage may be different in the host compared with the parasite. The ability to image both insects at once provides an internal reference for relative damage effects that may be related to insect pathology in both insects. The micro-CT X-ray technique using silver nano-particles is expected to help compare the apparent efficacy of varrocacides by use of the *in-situ* silver staining reaction.

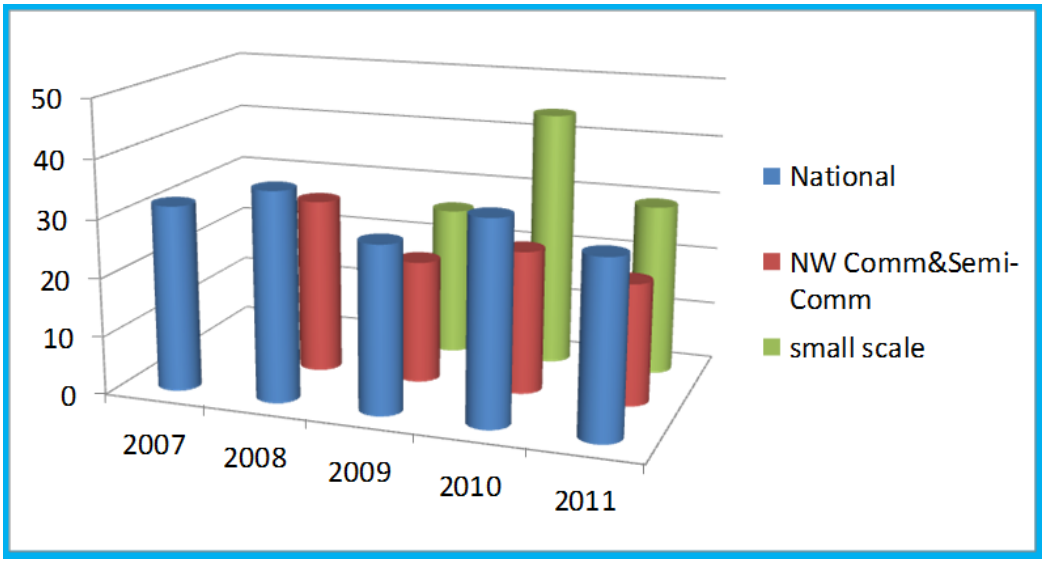
The use of two image contrast enhancement atoms in the staining treatment allows for one of these, preferably the iodine as the generic or diffuse agent, to be effectively "switched off" by reducing the x-ray irradiation energy on the treated insect in a second scan. This leaves the reacted silver pattern to be examined by subtraction, while the combined patterns allow a three-dimensional view of the context of surrounding organ structures. The sequence of chemical introduction of the iodine staining agent compared with silver, as well as the use of ionic silver versus silver nanoparticles, are key factors expected improve the selectivity of the structures and the microbes imaged.

3. **Caron[e], D.M., & R. Sagili[e] - SURVEYING PNW BEEKEEPERS - OVERWINTER LOSSES & POLLINATION RENTALS -** We have a tradition of surveying Pacific Northwest (PNW) beekeepers on both bee losses and pollination economics. Traditional annual colony losses (10-15%) increased to over 20% in PNW commercial and semi-commercial bee operations after arrival of bee mites (Burgett 1998). National surveys since the spring of 2007 have averaged 30% plus overwintering losses (vanEngelsdorp *et al.* 2012). Oregon overwintering losses included in the 2 most recent national surveys were less but in accord with regional surveys. Loss levels display a pattern of higher one year followed by lower level of losses the next in PNW and national surveys (Figure).

Mail surveys of PNW commercial and semi-commercial beekeeper overwinter losses were conducted for the last 4 years, and also surveys were conducted at association meetings of small scale beekeepers for the past 3 years. Losses of commercial semi-commercial beekeepers were lower (24.2%) than the national losses (33%). Small scale beekeeper losses were nearly the same (33.3%) as the national level for the last 3 years (Caron & Sagili ABJ 2012), reflecting the predominant small scale beekeeper participation in the national surveys.

PNW commercial and semi-commercial beekeepers have provided information on a one-page mail survey of pollination economics for the past 25 years (Burgett 2011). We extended and expanded the survey in 2011, receiving 63 responses representing 70% of estimated beekeepers with 5 or more honey producing colonies (USDA, NASS). Weighted average pollination fee was $90.62 returning the trend to annual increase following a drastic increase in 2010. There were 4.2 average rentals reported by the 63 individuals, which included almond rentals for all but 4 semi-commercial individuals and 12 other crops.

Figure – Patterns of colony losses through time.



4. **Chen[f], J., J.L. Frazier[f], M.T. Frazier[f] & C.A. Mullin[f] - IDENTIFICATION OF ORGANOSILOXANE SURFACTANTS IN AGROCHEMICAL SPRAY ADJUVANTS USING LIQUID CHROMATOGRAPHY COUPLED TO MASS SPECTROMETRY-** Agrochemical formulations are generally mixtures of active and inert ingredients. Recently, the residues of active ingredients in honey bee, honey, pollen, beebread and wax have been intensely studied worldwide. Presently, no individual pesticide level appears to correlate with recent bee declines. Thus, more generic formulation 'inerts' that co-occur across classes of pesticides may be involved.

Organosiloxane surfactants, the most novel class of non-ionic surfactants in agrochemical formulations, have been widely used around hives or in honey bee foraging areas as pesticide spray adjuvants. They increase the uptake of active ingredients into plants or target organisms due to their super wetting, super spreading, and super penetrating abilities. Previous study in our lab (T. Ciarlo et al.) showed that Silwet L-77 (a commercial organosiloxane adjuvant) at 0.1% oral dose in artificial nectar significantly reduced the learning performance of forager honey bees, and at 1% was highly lethal to adult honey bees.

The structure of the organosiloxane surfactant plays a vital role in their potent detergent action and extreme ability to reduce the surface tension of water. The high density of methyl groups characteristic of siloxane backbones accounts for their non-polar character, while the polyalkylene oxide chain serves the hydrophilic role. Structural diversity in commercial organosiloxane surfactant derivatives is achieved through building from trisiloxane or tetrasiloxane backbones by addition of single or double polyalkylene oxide chains capped by methoxyl, hydroxyl, or acetoxyl groups.

Detailed component information is not given in most commercial adjuvants. Hydrolyzed products or alternative degradates of organosiloxane surfactants also can be produced during storage or usage. To fully understand and assess risk of organosiloxane surfactants to honey bee health requires disclosure of structural identities and amounts of individual components, including synthetic impurities and degradates, in commercial adjuvants.

Here a LC-MS (Liquid Chromatogram coupled to Mass Spectrometry) strategy for structural identification of organosiloxanes in commercial adjuvants is introduced. The structure of the siloxane moiety is identified using an isotope ratio rule. A single charged/double charged mass ion ratio is calculated to distinguish single or double polyalkylene oxide chains in the hydrophilic portion of the molecule. Using a database developed for this study, which includes the mass ion information of all potential structures, allows assignment of the organosiloxane surfactant structure including its side-chain links and cap-groups.

By this LC-MS method individual organosiloxane surfactant compounds present in five commonly used commercial adjuvants have been identified by integrating their chromatographic and mass spectrometric characteristics. As a result, *hydroxy(polyethyleneoxy)propyl-heptamethyltrisiloxane, methoxy(polyethyleneoxy)propyl-heptamethyltrisiloxane, acetoxy(polyethyleneoxy)propyl-heptamethyltrisiloxane* were found as the most common components. The identifications were validated by ^{1}HNMR, ^{13}CNMR, and HMQC (heteronuclear multiple bond coherence) spectrums of respective HPLC fractions that were pooled from multiple adjuvant injections. Moreover, hydrolyzed products of organsilicone surfactants were also identified. This information will allow for the first time

the development of an analytical method to quantify organosiloxane residues in bees and other hive samples and environmental matrices.

5. **Chen[g], Y.P., J.S. Pettis[g], Y. Zhao[h], R.S. Cornman[g] & J. D. Evans[g] - SEQUENCING AND GENOME ANNOTATION OF HONEY BEE MICROSPORIDIAN PARASITE, *NOSEMA APIS* AND COMPARATIVE GENOME ANALYSIS WITH ITS SYMPATRIC CONGENER, *N. CERANAE*-** The microsporidian parasite *Nosema* is one of the several suspected factors contributing to the current collapse of honey bee colonies. *Nosema apis* is one of two *Nosema* species that causes a serious honey bee adult disease, Nosemosis. Here we report analysis of the completed *N. apis* genome sequence and annotation as well as comparative analysis with *N. ceranae*, a recent emerging microsporidian parasite of honey bees. Sequencing and annotation of the *N. apis* genome provide a comprehensive overview of the genetic content, structure, and organization of the parasite and give some interesting insights into the complex biological and molecular processes of the parasite. The comparative genomic analysis led to the identification of genes that are conserved between *N. apis* and *N. ceranae*, and genes that are unique characteristics of the individual species, thereby providing a list of virulence factors that are associated with virulence of the parasites in honey bees. There genes are potential targets for innovative therapeutics to break down the life cycle of the parasite.

6. **Corman[g], R.S., A. Zimin[i], G.J. Hunt[j], L. Bourgeois[k], C. Elsik[l] & J.D. Evans[g] – GENOMIC STUDIES OF *VARROA DESTRUCTOR*, A MAJOR PEST OF THE HONEY BEES, *APIS MELLIFERA* -** Technological advances have enabled the cost-effective sequencing of virtually any organism of interest. Genome sequencing and the associated technologies of RNA sequencing and proteomics can rapidly improve our understanding of the ecology and evolutionary history of a species. It also provides immediate practical tools for biologists to design experiments and test hypotheses. As bee researchers are particularly concerned about the damaging parasite *Varroa destructor*, this mite has become the target of a major genome sequencing effort led by the *Varroa Destructor* Sequencing Consortium. Approximately ~90-fold coverage of the genome has been achieved through single and paired-end Illumina sequencing, and 454 and Illumina scaffolding runs have been performed. An assembly of the ~565 MB genome is currently being optimized. Initial analyses of *Varroa* DNA and RNA sequencing have already yielded important discoveries, enabled new methods of manipulating *Varroa*, and identified unexpected avenues for future work. For example, RNAi has been demonstrated to be effective in mites, and several novel viruses have been identified that are present in mites as well as bees. In our samples, the sequence of Deformed Wing Virus circulating in mites is not distinct from that detected in bees. Sequence polymorphisms identified through this initial effort, and more importantly, through re-sequencing of different *Varroa* lineages, will improve our understanding of the mechanisms of host specificity. Finally, we have found evidence that some *Varroa* transcripts are being transferred to honey bees during feeding, the functional significance and generality of which remains to be determined. As annotation of the *Varroa* genome proceeds, we expect more experimental avenues for *Varroa* control to be revealed.

7. **Cox, R.[m] & K. Aronstein[m] – *NOSEMA APIS* IN SMALL NUCLEUS COLONIES -** In a small scale, preliminary field trial nucleus colonies were fed *Nosema apis* spores in protein diet to determine the effect of nosematosis on colony growth and productivity. A stock solution of fresh *Nosema apis* spores was prepared for inoculating small nucleus colonies. Pure *Nosema apis* spore samples from single bees were identified by multiplex-PCR. These spores were amplified in laboratory cages to obtain enough spores to inoculate 6 small nucleus colonies (nucs) of honey bees. Laboratory methods are described in Aronstein *et al.*, Am. Bee J. 151(5):507. Nine small colonies in 5 medium frame nuc boxes were established in May with approximately 2,300 package bees each. The nucs were provided with Italian queens from the same queen producer. At the time colonies were established they were provided protein diet containing *Nosema* spores (ca. 50,000 spores/bee). Six nucs were fed protein diet with *Nosema apis* spores and 3 nucs were fed diet without spores. At six and ten weeks post inoculation colony populations were estimated by counting the frames covered with adult bees and measuring the area of comb containing sealed brood. We also weighed each colony at the same times. Bee samples were taken periodically, starting two weeks post inoculation, for 10 weeks. Starting at four weeks samples of both nurse bees and forager bees were collected and analyzed separately. The number of spores was counted in each of 10 bees with a Neubauer hemocytometer under a phase contrast microscope at 200x magnification.

   Adult bee populations of control colonies more than doubled in six weeks while nosema treated colonies were only 25% larger on average than at the beginning of the study (statistically different $p<.05$). *Nosema apis* treated colonies had an average of 28% (range = 0-69%) fewer sealed brood cells than the control colonies, but the difference was not statistically significant. Two out of six nosema treated colonies appeared to be queenless six weeks P.I. *Nosema apis* treated colonies on average gained 2.66 kg (35%) less weight than did control colonies ($p=0.0523$). If these results apply to full size honey bee colonies, then nosematosis could result in colonies with smaller populations which do not pollinate or produce honey as well as larger colonies.

   There was no significant difference in the mean number of spores in forager bees versus nurse bees in this study. The mean number of spores/bee decreased during the study and by 4 weeks P.I. there was no statistical difference in the number of spores between inoculated and control colonies. The number of spores/bee significantly decreased from two weeks (2.38×10^7) to four weeks P.I. (1.87×10^6). However, in a preliminary laboratory cage study the spore levels increased from 2 weeks (1.52×10^6) to 4 weeks P.I. (6.47×10^7). However, this is not what we observed in our field study. Apparently, there is a different dynamic between pathogen and its host in a field setting.

   Further research should focus on long term effects of both species of *Nosema* in full size colonies and interactions with other stress factors such as the time of year, other diseases, pests and nutrition.

8. **Dainat[n], B., J.D. Evans[g], Y.P. Chen[g], L. Gauthier[n] & P. Neumann[n,o] – DEAD OR ALIVE? PATHOGENS AND LIFE SPAN OF WINTER HONEY BEES -** Honey bees, *Apis mellifera*, suffer numerous biotic and abiotic stresses and have recently faced large repeated losses. Although the scientific community agrees that losses may result from several factors acting synergistically, it is not clear yet what causes this phenomenon. It has

been proposed that pathogens are involved in winter losses. We hypothesized that pathogens may reduce the life expectancy of individual winter bees to a point that the critical size threshold of a cluster to survive winter would be reached leading to the collapse of the colony. To test this hypothesis, a monitoring experiment on 29 colonies has been performed in winter 2007/2008 in Switzerland where levels of three pathogens including *Deformed wing virus* (DWV), *Acute bee paralysis virus* (ABPV) and *Nosema ceranae*, *Varroa destructor* (Vd) infestation, and life expectancy of individuals workers were measured. The results show that neither ABPV nor *Nosema ceranae* could be incriminated as a deadly factor in this study. Conversely, both DWV and Vd, in this order, reduced significantly life expectancy of winter workers. Furthermore, the number of DWV-infected bees in colonies, which didn`t survive winter, was significantly higher than in colonies which survived. The data of this study are in contrast with the intriguing Colony Collapse Disorder in the U.S, for which *Varroa destructor* and DWV were poor correlates with CCD risk. Finally, the results suggest that reduced life expectancy of individual bees is one proximate mechanism of colony winter losses and *Varroa destructor* remains one of the main culprits.

9. **Eischen[m], F.A., R.H. Graham[m] & R. Rivera[m] - IMPACT OF *NOSEMA CERANAE* ON HONEY BEE COLONIES: A 14 MONTH STUDY -**This study monitored the performance of colonies infected with Nosema ceranae for 14 months (October 2009 – December 2010) near Ville Platte, Louisiana, USA. Eight groups colonies (n = 50) were involved, i.e., 1) negative control, 2) fed only, 3) not fed, fumagillin-treated, 4) fed, fumagillin-treated, 5) not fed, amitraz-treated, 6) fed, amitraz-treated, 7) not fed, fumagillin- and amitraz-treated, 8) fed, fumagillin- and amitraz-treated. Protein patty feeding was stopped in late January 2010 and the colonies were transported to California for almond pollination. In late March 2010 they were returned to Louisiana for honey production.

   Colonies infected with >500,000 spores/bee at the start of the trial had significantly fewer colonies meet a 6-frame minimum size criterion for pollination in February 2010, irrespective of treatment received. Infections in untreated colonies peaked during winter and fell dramatically during spring and summer. Colonies treated with fumagillin exhibited significantly reduced soluble protein levels for two-three months after treatment, suggesting that this treatment has a short term negative impact. Fumagillin-treated colonies produced significantly more honey than untreated colonies or those treated only with amitraz. Colonies receiving both varroa and nosema treatments produced more honey than any other group.

   At the end of the trial, colonies that had been treated with fumagillin were significantly larger than those not treated for nosema (Table). The group that received treatment for varroa, nosema and were fed had significantly more colonies survive than the other groups. We conclude that controlling nosema is critical for long term health.

Table - Survival and strength of colonies after 14 months (October 2009 – December 2010)

Treatment	n[1]	Strength[2] (x ± SD)
Amitraz, fumagillin	20 b	14.4 ± 2.6 a
Amitraz, fumagillin, diet[3]	33 a	12.9 ± 3.0 b
Fumagillin	22 b	10.3 ± 2.7 c
Fumagillin, diet	18 b	7.5 ± 2.1 d
Amitraz	23 b	3.8 ± 1.2 e
Negative Control	21 b	3.2 ± 1.2 e
Amitraz, diet	21 b	3.2 ± 1.2 ef
Diet	17 b	1.9 ± 0.8 f

[1]Fifty colonies started the trial.
[2]Frames of adult bees as measured by the California almond pollination criterion. Means followed by the same letter are not significantly different.
[3]BeePro + 4% Pro-Len (Mann Lake Ltd.)

10. **Goblirsch[p], M., Z. Huang[q] & M. Spivak[p] – PHYSIOLOGICAL EFFECTS OF *NOSEMA CERANAE* ON WORKER HONEY BEES -** The condition of the pollinating capacity and overall health of honey bees in the U.S. has eroded in recent years. Since 2006, it has been an annual occurrence for a significant number of U.S. beekeepers to loose over one-third of their colonies. Interestingly, the incidence of any one factor (e.g., mite pests, poor nutrition, pesticides, or pathogens) as an explanation for colony losses has not been substantiated. The obligate intracellular fungus, *Nosema ceranae,* is likely a contributing factor to these losses; therefore, it is important to understand how this emerging pathogen affects bees physiologically. Our research examines how *N. ceranae* may disrupt levels of vitellogenin and juvenile hormone, endocrine signaling factors that are fundamental to regulating adult development and lifespan in honey bees. We also present evidence from field studies showing the effects on the onset of foraging and life span in honey bees infected with *N. ceranae*. Findings from this research will aid in the development of management strategies to counter the negative effects of *N. ceranae* infection on honey bee health.

11. **Grozinger[f], C.M, H.L. Holt[f], F.J. Richard[r] & K.A. Aronstein[m] - GENOMICS ANALYSIS OF SOCIAL IMMUNITY IN HONEY BEES -** Honey bees use molecular, physiological and behavioral responses to combat pathogens and parasites. The honey bee genome contains all of the canonical insect immune response genes, and several studies have demonstrated that pathogens can activate expression of these genes. Honey bees also use behavioral responses, termed social immunity, to remove parasites, pathogens and diseased

individuals from the hive. These behavioral responses include hygienic behavior (to remove diseased larvae), grooming (to remove ectoparasites), and accelerated behavior maturation of diseased workers, which effectively removes them from the brood nest. We have previously demonstrated that immunostimulation causes changes in the cuticular hydrocarbon profiles of workers, which results in altered worker-worker social interactions. Here, we examine the behavioral, chemical and genomic responses of worker honey bees to a panel of general immune stimulants. We also examine genomic responses to *Nosema* infection across a time-course in specific tissues. We demonstrate that gene expression responses to immunostimulation and parasitization are quite broad, and include substantially more genes than those found in the canonical immune response pathways. Furthermore, gene expression patterns are closely associated with the physiological and behavioral changes triggered by immunostimulation and parasitization. For example, injection with bacteria causes expression changes in several genes that may play a role in cuticular hydrocarbon biosynthesis, while *Nosema* parasitization results in significant expression changes in genes associated with metabolism, nutrition and behavioral maturation. These studies suggest that genomic responses to pathogens and parasites are substantially broader than expected, and mediate the behavioral changes associated with social immunity. This research was funded by a USDA-NIFA Managed Pollinator CAP grant (PI Delaplane) and USDA-NIFA-AFRI grant to CMG and FJR.

12. **Guarna[s], M.M., S. Hoover[t], A. Melathopoulus[t], S.F. Pernal[t] & L.J. Foster[s] - USING QUANTITATIVE PROTEOMICS TO DEVELOP IPM TOOLS FOR MANAGING BEE PATHOGENS -** We are using proteomic approaches to develop integrated pest management tools for managing bee pathogens, and for increasing our understanding of the pathogenesis of bee diseases. In one application, we have used a shotgun quantitative proteomics approach to identify protein markers of resistance to honey bee pathogens. We correlated estimates of social immunity behavior, hygienic behavior and varroa sensitive hygiene (VSH) with the relative levels of proteins in antennal samples collected over a period of 3 years. Both statistical significance and the magnitude of the change were used to identify the top markers for validation.

    To confirm the association of these putative protein markers with social immunity traits, we sampled and tested 500 colonies from 38 beekeeping operations in 12 geographical areas within three Canadian provinces: British Columbia, Alberta and Manitoba. In this confirmatory phase of the project we are using a targeted proteomic approach, Multiple Reaction Monitoring (MRM), using stable isotope-labeled standard (SIS) peptides, selected based on MS- observed peptides during the discovery phase of the project and established criteria based on amino acid sequence and peptide length. Using these MRM results, we will select the markers with higher predictive value for high hygienic behaviour to select queens in a selective breeding program that aims to demonstrate marker assisted selection (MAS).

    We are also using proteomics to investigate the molecular mechanism of pathogenesis of viral and fungal diseases and to identify potential targets for the development of RNA interference tools. Within a larger project, we aim to implement integrated approaches that include MAS and RNAi towards a decrease in colony losses, increased honey production and greater availability of bees for pollination.

13. **Huang[u], W.-F., P.M. Yau[v], B. Imai[v], L.F. Solter[u]-DIMINISHING FUMAGILLIN LEVELS RESULT IN HYPERPROLIFERATION OF *NOSEMA CERANAE* -** Fumagillin is the only approved antibiotic drug to control nosema disease in honey bees and has been extensively used in United States apiculture for more than 50 years. It is known to be toxic to mammals and must be applied periodically and with caution to avoid residues in honey. We show that the current seasonal application protocol for fumagillin may benefit microsporidia, especially *Nosema ceranae*, allowing hyperproliferation of the pathogens when the drug is diluted to low levels as occurs in hives over the spring and summer. Further investigations suggest that fumagillin continues to alter proteins in the honey bee midgut under very low dosages. *N. ceranae* is apparently released from the suppressive effects of fumagillin at dosages that continue to impact the bee midgut tissues, resulting in spore production that is significantly higher than in untreated bees. *N. apis* is likewise released to produce more spores, although not significantly more than in untreated bees, and release occurs at lower dosages than for *N. ceranae.* Diminishing fumagillin levels in hives may further compromise bees to *Nosema* spp. infection and proliferation.

14. **Huang[q], Z.Y., J. Adam[q], & A. Jiang[q] - EFFECT OF SINGLE AND MIXED SPECIES INFECTION OF *NOSEMA CERANAE* AND *NOSEMA APIS* ON WORKER FORAGING BEHAVIOR AND LONGEVITY -** *Nosema ceranae* is an emerging intra-cellular fungal parasite that was suggested to play a major role in honey bee colony collapse, especially in Spain (Higes *et al. 2008 Environ. Microbiol. 10, 2659–2669*). Earlier studies also suggested that *N. ceranae* is more virulent than *N. apis* and bees in cages died 8 days after inoculation. Our 2001 data showed clearly that in cage studies, both *N. apis* (30,000 spores/bee) and *N. ceranae* (30,000 spores/bee) caused a significant reduction in survival compared to the control (no spores) but the two species did not show any difference in virulence. Furthermore we saw that *N. apis* and *N. ceranae,* when mixed at various proportions (1:5, 1:2, 1:1, 2:1, 5:1 *ceranae:apis*, with a total of 30,000 spores), all bees died significantly earlier than the single species infected bees.

In this study we tried to determine if this is true also under field conditions. We individually inoculated bees with either 50,000 spores of *N. ceranae,* 50,000 spores of *N. apis*, or a combination of both species (25,000 spores of each species) and a group of control. Fifty bees were tagged and 50 bees were paint marked and they were combined with 500 foragers from a *Nosema* free colony and a queen in a nucleus colony. Bees were surveyed for survival every 4-5 days and foraging observations were done daily for 2 hours per day when bees started foraging. In both trials, mixed infected bees foraged and died earlier than others, with the exception of trial 2, where *N. apis* infected bees died similarly to mixed infected bees with *N. ceranae* infected bees showing similar survival as the control. Strangely, juvenile hormone (JH) titers were not significantly higher in 14 day old mixed infected bees even though these bees foraged earlier. *Nosema apis* infected bees showed the highest JH titers followed by *N. ceranae* infected bees, with mixed infected bees showing almost identical levels as the control which received no spores (Figure). Previous studies in our laboratory has shown that *N. apis* can increase JH biosynthesis resulting in higher JH titer in bees, which then causes earlier foraging (cited by Chen and Huang, *2010 Apidologie 41:364-374*).

Our results here show that mixed infected bees with equal spores of both *N. apis* and *N. ceranae*, not only caused earlier death inside cages, but also did so in field colonies. It is not clear whether this increased virulence is due to a "division of labor" (i.e. totally different pathology) of the two *Nosema* species, or due to an immune-suppression by *N. ceranae*, which then enabled *N. apis* to produce more spores resulting in increased mortality. It is also not clear why mixed infected bees failed to show higher juvenile hormone, which has been shown to be associated with foraging behavior in many studies.

This research was supported by a USDA NIFA AFRI grant "Managed Pollinator Coordinated Agricultural Project" (2009-85118-05718) and a GREEEN grant from the Michigan State University.

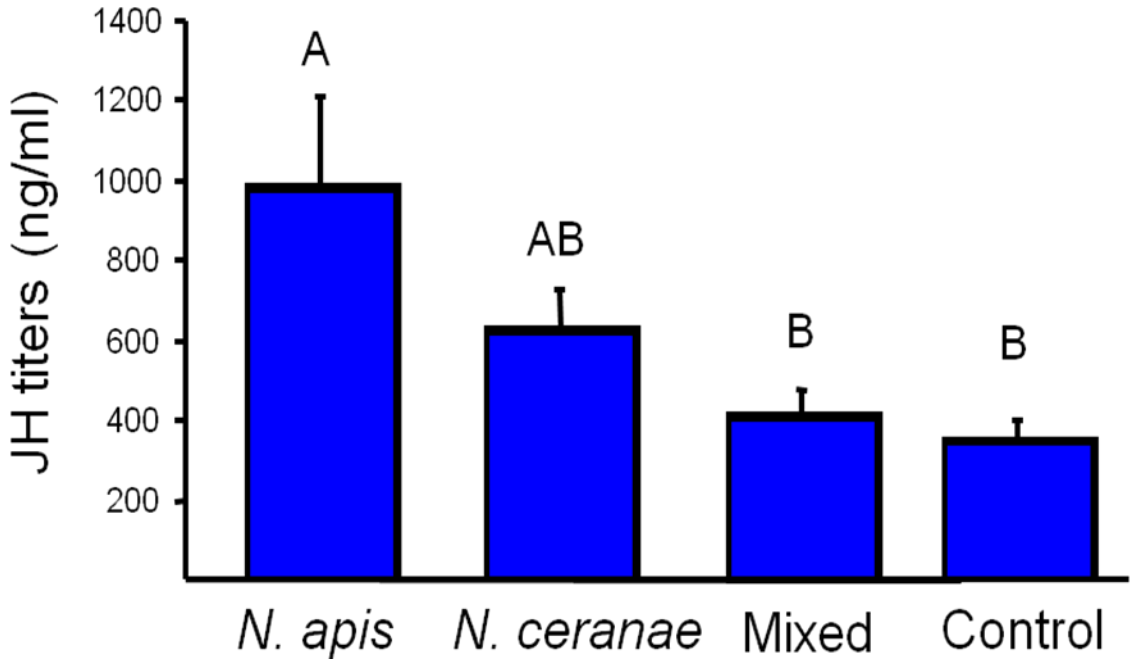


Figure. Juvenile hormone titers of bees infected with single species of *Nosema apis* or *N. ceranae* (50,000 spores/bee) or mixed infections (25,000 spores of each species) and control. Bars with different letters signify significant difference (Fishes Protected Least Significant Difference, $P<0.05$) after analysis of variance. Data transformed by taking the logarithm of JH data but presented here without transformation.

15. **Ingram[w], E.M., M.D. Ellis[w] & B.D. Siegfried[w] - PROPOSED RESEARCH ON TOXIC AND REPELLENT EFFECTS OF PYRETHROIDS USED IN ORCHARDS ON THE HONEY BEE, *APIS MELLIFERA* -** Fruit orchards frequently rent managed honey bee colonies for pollination, and honey bee visits improve fruit yield and quality. Orchards may also use insecticides to control insect pests. Pyrethroids are a class of insecticides that can be used in fruit crops. They 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). In order to secure the most effective pollination for growers and to protect honey bee colonies, it is important to establish if foraging is decreased due to contact repellency and if the repellency is sufficient to prevent honey bee losses.

    Preliminary testing in 2011 used video-tracking software, Ethovision® XT, to examine sub-lethal behavioral effects due to imidacloprid and *tau*-fluvalinate. A decrease in locomotion was detected after bees were exposed to all dose-levels of *tau*-fluvalinate and differences were significant from the control in the two higher doses (figure). Other response variables were noticeably different but did not show significance when pair-wise comparisons were made. These results suggest that video-tracking has the sensitivity necessary to measure sub-lethal effects of this pyrethroid.

    The purpose of this study is to investigate sub-lethal behavioral effects due to orchard-applied pyrethroids and to evaluate their repellent properties under both laboratory and field conditions. After treatment, bee locomotion, time spent feeding and social interactions will be quantified using Ethovision® XT and methods established in preliminary testing (Teeters et al. 2012 Environ. Toxicol. Chem. In press.). The goal of this study is to develop quantifiable screening methods that will provide regulatory agencies with a risk assessment tool for assessing repellency and toxicity when reviewing pesticide registration requests.

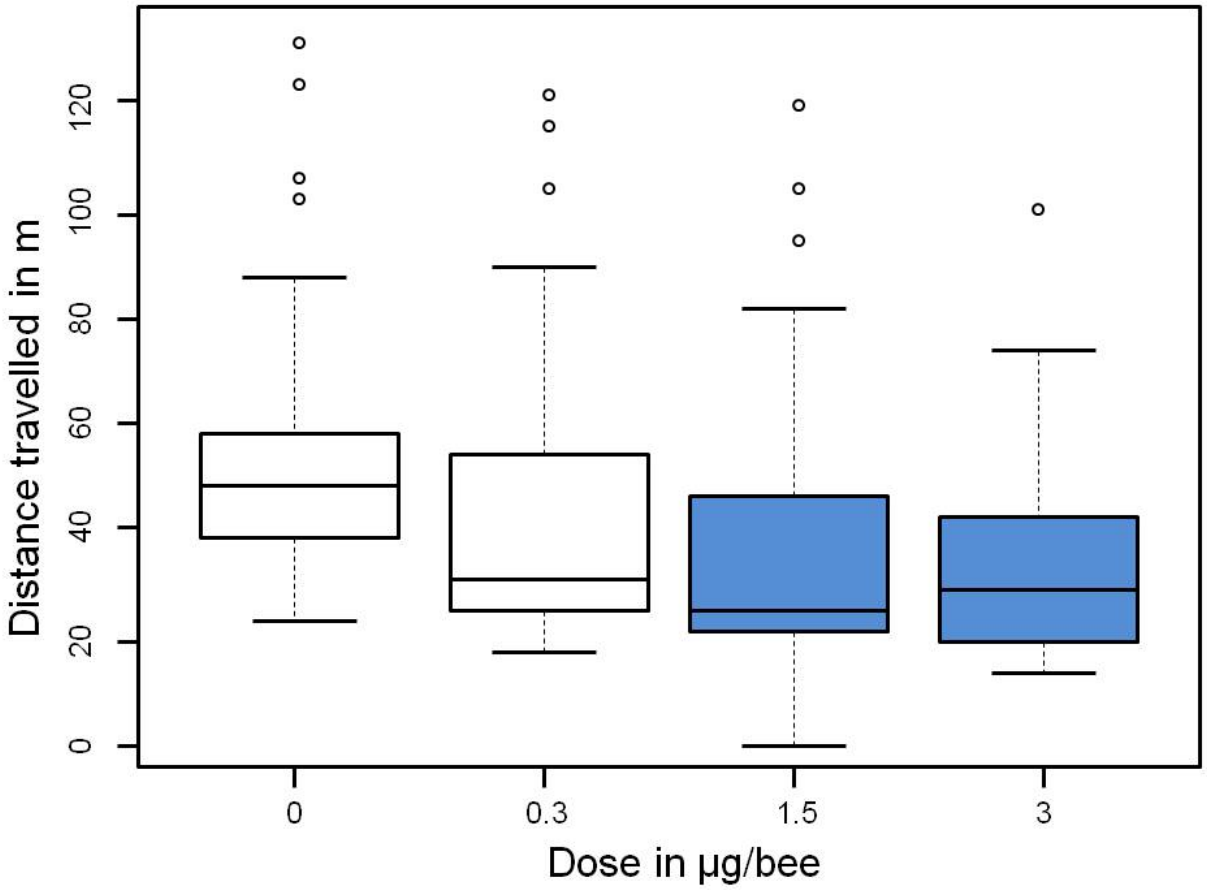


Figure. Analysis of the effect of a single topical application of *tau*-fluvalinate on distance travelled. Doses included: vehicle control (n=12), 0.3 (n=12), 1.5 (n=10), and 3 µg/bee (n=10). Shaded boxes indicate sublethal doses in which distance travelled was significantly reduced.

16. **Johnson[x,w], R.M., E Siegfried[w] & M.D. Ellis[w] – SYNERGISTIC PESTICIDE INTERACTIONS IN WHOLE HONEY BEE COLONIES -** Potent and potentially deadly interactions between tau-fluvalinate, the active ingredient in Apistan, and the sterol biosynthesis inhibiting (SBI) fungicides have been observed in laboratory bioassays on caged adult honey bees. We tested the potential for this combination of compounds to harm whole honey bee colonies at field-relevant doses. Forty-seven 2-frame nucleus colonies were treated with one of five treatments in 50% sucrose syrup: syrup alone as a negative control, Dimethoate 4E (16 ppm a.i.) as a positive control, Rally 40WSP (32 ppm a.i.), one Apistan strip, and Rally 40WSP plus one Apistan strip. All colonies received 1 liter of sucrose syrup over 8 days of treatment. Dead bees were collected from colonies every 2 days throughout the 26-day experiment and colonies were weighed at 4-day intervals. Digital photos of three 120-cell areas filled with eggs were taken at 2-day intervals and scored using a web-based system (http://broodmapper.com). The positive control treatment, dimethoate, decreased colony survival, decreased both total bee mass and colony weight, increased the number of dead bees collected, and greatly reduced brood survival from 70% (n=3162) in control colonies to 36% (n=1389). Neither Rally alone nor Apistan alone had any measurable effect on colonies. The combination of Rally and Apistan marginally decreased brood survival

(62%, n=2567), but colonies receiving treatment with both compounds were otherwise similar to control colonies. These results suggest that potent synergistic pesticide interactions observed in lab studies may be much less harmful in whole honey bee colonies at field-relevant doses. Beekeepers should continue to avoid fungicide exposure while treating their bees for *Varroa* mites, but, in some circumstances, the benefits of mite suppression may exceed the hazard posed by any interaction between tau-fluvalinate and SBI fungicides.

17. **Krupke[j], C.H., B. Eitzer[y], G. Andino[j], K. Given[j], G.J. Hunt[j] – ASSESSING THE ROUTES AND LEVELS OF EXPOSURE OF HONEY BEES TO NEONICOTINOID SEED TREATMENTS IN THE MIDWEST -** Neonicotinoid seed treatments have become widely used in annual crops during the past decade and are highly toxic to honey bees. Virtually all corn planted in the U.S. is treated. Neonicotinoids used on corn seed have been found in previous analyses of honey bee pollen and comb material. However, the routes of exposure have remained largely undefined. We used LC/MS-MS to analyze samples of honey bees, pollen stored in the hive and several potential exposure routes associated with plantings of neonicotinoid treated corn seed. Our results demonstrate that bees are exposed to these compounds and several other agricultural pesticides in several ways throughout the foraging period (Krupke *et al.* 2012; doi:10.1371/journal.pone.0029268). During spring, extremely high levels of clothianidin and thiamethoxam were found in planter talc material that is exhausted to the atmosphere during the planting of treated corn seed. We also found neonicotinoids in the soil of each field we sampled, including unplanted fields that had not had treated seed present for 2 growing seasons. Plants visited by foraging bees (dandelions) growing near these fields were found to contain neonicotinoids, as were dead bees collected near hive entrances during the spring planting period, although whether exposure was oral (consuming pollen) or by contact (soil/planter dust) is unclear. We also detected clothianidin in pollen collected by bees and stored in the hive. When corn plants in our research fields reached anthesis, corn pollen from treated seed was found to contain clothianidin and other pesticides; and honey bees in our study readily collected this pollen. Overall, 44% of the pollen bees collected (by volume) during this time was corn pollen. Half of the bee-collected pollen samples contained neonicotinoids; all samples contained strobilurin fungicides. These findings clarify some of the mechanisms by which honey bees may be exposed to agricultural pesticides throughout the growing season. These results have implications for a wide range of large-scale annual cropping systems that utilize neonicotinoid seed treatments.

18. **Kuster[z],R,D, H. Boncristiani[z] & O. Rueppell[z] – IMMUNE GENE EXPRESSION IN RESPONSE TO *VARROA* IN HONEY BEE WORKER LARVAE -** The parasitic mite *Varroa destructor* is one of the biggest health problems of the Western Honey Bee, *Apis mellifera*. It feeds from the bees' hemolymph, compromising energetic supplies, and it vectors several honey bee pathogens, including some widespread viruses. *V. destructor* has also been reported to compromise honey bee immunity, lowering expression of antimicrobial peptides, but available data are somewhat contradictory and measured during different development stages of the host. It is hypothesized that mites actively suppress immune-gene responses to increase the fitness of their offspring, which share the same feeding site initiated by their mother on the developing host. This makes the reproductive phase in the mite's life cycle that takes place entirely within individual brood cells the relevant time period. Developing honey bees are essentially defenseless during this time period, and thus the

degree of immune-gene response at the individual level is a very relevant approach when considering honey bee health.

This study investigates the contribution of host age as well as the number of foundress mites as factors affecting honey bee immunity. Frames of experimental brood cells were established in three colonies during the peak months of brood production. Immediately after cell-capping, a portion of available cells received either manually-introduced mites varying from 1 to 4 per host bee or served as mite-free controls. Additionally, a control for the initial feeding site was created by piercing larval pre-thoracic segments with a glass capillary needle. In-hive sampling methods were performed on randomized brood cells over every subsequent 24 hour time interval for a total of 10 days to capture the variable of host development. Samples were analyzed for expression levels of a wide range of immune response targets from several well-studied immune pathways using quantitative RT-PCR.

Preliminary findings show a positive association between increasing mite number and expression of several target genes, contradicting the previously hypothesized general immunosuppression. Replication of deformed wing virus was significantly higher in the mite-infested groups than in the non-treated groups. The artificial wounding group was also found to have a similar, increased viral load, suggesting that wounding may trigger or enable virus replication. The wound control group showed the highest level of immune-gene expression in the days immediately after manipulation. These expression levels significantly exceeded those of the mite-infested groups during the initial days of the experiment but not towards the end of bee pupal development. This might be explained by a weakening effect of the initial wounding relative to the mites. Alternatively, the mites might temporarily suppress the honey bees' normal response to cuticle wounding but cannot do so with increasing mite feeding or accumulation of higher virus titers. Thus, our results indicate the importance of physical trauma caused by wounding and suggest complex temporal dynamics in the relationships between bee host, mite parasite, and vectored pathogens. They highlight the need for further studies of these relationships.

19. **Matisoff[aa], M.A. & T.C. Webster[aa] - EFFECTS OF OSMOTIC PRESSURE, IONIC COMPOSITION, SUGARS, TEMPERATURE, AND TIME ON *NOSEMA CERANAE* POLAR FILAMENT EVERSION -** Microsporidia belong to a diverse group of obligate, intracellular, eukaryotic parasites that grow and divide inside the honey bee's midgut epithelial cells. Microsporidia have a unique method for infecting their host's cells that involves the release of a polar filament under extreme pressure. Oftentimes, the filament can attain lengths as much as 100 times the length of the spore body. After the filament pierces the host cell, it transmits it sporoplasm, containing its DNA, through the polar filament into the host's cell. Once the spore's DNA is inside the epithelial cell, it hijacks the host cell's mitochondria and nutrients so that it can complete its life cycle. In this study, we examined some of the factors that influence spore germination and filament release. We found that polar filament release is a function of osmotic pressure, ion composition, temperature cycles, micromolar sugar concentrations, and time. Our results also show that germination fluid that consist of ions and micromolar concentrations of five- and six-carbon sugars had the greatest effect on polar filament eversion. At this time, the actual mechanism of action that leads to polar filament eversion is unknown. Our data, however, suggest that it may be possible to interrupt or inhibit the spore life cycle before the infection reaches a critical load and spreads throughout the hive or leads to colony death.

20. **Mullin[f] C.A., T.J. Ciarlo[f], W. Zhu[f] & J. Chen[f] - PESTICIDE FORMULATION ADJUVANTS MAY IMPACT HONEY BEE HEALTH -** Modern pesticide formulations and seed treatments, particularly when multiple active ingredients are blended, require proprietary adjuvants and inert ingredients to achieve high efficacy for targeted pests. An adjuvant such as a surfactant, penetrant enhancer, activator, spreader, sticker, wetting agent, buffer, antifoaming agent, drift retardant, etc. is much less expensive than the active ingredient, but can reduce the effective pesticide dose as much as 10-fold. Typical formulations contain less than 50% active ingredients with the remainder surfactants and solvents. Adjuvant use has evolved from focus on alkylphenol, alcohol, fatty acid and sorbitan ethoxylates in combination with sulfonates to new technologies comprising fatty (tallow) amine and organosilicone ethoxylates and co-solvents like N-methylpyrrolidone (NMP). Although we have found more than 130 different pesticides and metabolites in beehive samples, no individual pesticide amount correlates with recent bee declines. Here we examine if more generic formulation 'inerts' that co-occur across classes of pesticides may be involved.

Given the synergistic nature of certain chemicals, we are concerned that active ingredients may affect honey bees differently depending on the formulation ingredients in agrochemicals used around apiaries or where bees forage. In our work, the nonionic Triton X-100 and organosilicone Silwet L-77 were moderately toxic to adult honey bees at an oral dose of 1% in artificial nectar while NMP was less, but still significantly, acutely toxic. Moreover, we demonstrated much higher toxicity of NMP to honey bee larvae than to adults. A 1% oral dose of NMP can kill all the reared larvae during the first day of exposure. Doses down to 0.01% were lethal to larvae over a 4 day treatment period. A Bravo® formulation was at least 4X more toxic orally to adult honey bees than the active ingredient chlorothalonil, while Taktic® was about 4X more toxic than amitraz. This indicates that 'inert' ingredients can have crucial impacts on survival of both honey bee larvae and adults.

Organosiloxane surfactants have super-penetrant and super-spreading activities compared to all other classes of surfactants and greatly increase pesticide efficacy, and thus expectedly are more ecotoxic. Hundreds of thousands of pounds of organosilicone surfactants are used every year on almonds in California alone. The sublethal proboscis extension reflex (PER) assay was used to measure the olfactory learning ability of honey bees treated orally with the most widely used spray adjuvants from each of three different adjuvant classes. Organosilicones were more active than the nonionic adjuvants, while the crop oil concentrates were inactive. Silwet L-77 at an oral dose of 5 µg/bee or above significantly reduced learning using PER, and all organosilicone adjuvants tested (Dyne-Amic, Syl-Tac, Silwet L-77, Sylgard 309) at 20 µg/bee, equivalent to a 2 second feeding at 1 µl/sec on a 1% adjuvant solution, induced learning impairment. Honey bees treated with the nonionic surfactant Activator 90 experienced a similar reduction in learning ability. Since there is no environmental monitoring method established for the organosilicone adjuvants, we are developing an LC-ESI-MS method to resolve and sensitively analyze by selective ion monitoring the various trisiloxane and tetrasiloxane polymeric components commonly used. The impact of pesticidal blends on bees, which depend on plant nectars and pollens readily contaminated by toxicants, cannot be fully understood without identification and risk assessment of inert residues and their agrochemical interactions.

21. **Pernal[1], S.F., S.E. Hoover[1], A. Masson[1], M. M. Guarna[2] & L.J Foster[2] – NEXT GENERATION IPM TOOLS FOR BEEKEEPING: PROJECT PLANS AND COLONY SURVEYS -** The Next-generation Integrated Pest Management Project has three main aims: (1) to demonstrate the efficacy of proteomic marker-assisted selection (MAS) for enhancing disease and mite resistance, based on selection for hygienic behaviour (HB) and *Varroa*-sensitive hygiene (VSH), (2) to evaluate new RNAi reagents for controlling honey bee pathogens, and (3) to develop economic models of beekeeping management and production. Here we present some of the results of the first field season of the project, including a survey of HB, VSH and pathogen levels of colonies across western Canada.

Average levels of HB in were 64, 67 and 54% for the provinces of British Columbia, Alberta and Manitoba, respectively, based on complete removal of freeze-killed brood within 24 h (n=635). A portion of colonies were then randomly selected to establish a benchmark population (n=83) against which breeding advances will be assessed, and an F_0 population was established (n=110) from colonies most highly expressing HB, on which we will unidirectionally breed. Within these groups, 1 and 16% of colonies, respectively, were found to completely remove 95% of frozen pupae within 24 hr. The F_0 was also evaluated for VSH using the 7-day "quick test" as described by Villa et al., 2009 (*Journal of Apicultural Research* 48: 162-167). Overall, these colonies showed a reduction in *Varroa*-infested cells of 26.7± 3.7% (mean ± SE) with 71.0 ± 1.4% mites remaining in the brood being fertile. Collectively, these values represent an F_0 relatively non-selected for both VSH traits.

In order to provide an economic baseline against which to evaluate future gains in stock selected by MAS, twelve western Canadian producers were intensively surveyed for productivity parameters, management practices as well as for diseases and pests during the spring, summer and fall of 2011. *Nosema* spp. were found to be prevalent across western Canada, with the parasite detected in 66% of colonies, containing an average of 1.5 million spores per bee over spring and summer sampling periods. Numbers of spores per bees increased slightly during summer over spring sampling periods. *Nosema ceranae* was the predominant species, with *N. apis* only being detected from two producers in Manitoba. Levels of *Varroa destructor* were variable in British Columbia and low across the prairie provinces. Across all provinces and time periods sampled, *V. destructor* was found in 30% of colonies, with an average 0.8 mites per 100 bees. Data on levels *Paenibacillus larvae* spore loads in adult workers and honey bee viruses are still being analyzed.

We plan to successively select and propagate three generations from our F_0 during 2012 and 2013, in a parallel and direct comparison of proteomic-based MAS against traditional behaviorally-based phenotypic selection on HB and VSH. Detailed comparisons of the F_1 and F_3 generations will be made to evaluate resistance to *P. larvae* and *V. destructor* as well as evaluating economic parameters.

22. **Peterson[cc], S.M. & W.M. Hood[cc] – INVESTIGATIONS INTO "TRAPPING SINKS" TO CONTROL SMALL HIVE BEETLES, *AETHINA TUMIDA*, IN APIARIES OF HONEY BEES, *APIS MELLIFERA* -** The small hive beetle, *Aethina tumida* Murray, is one of the most recent pests of European Honey Bees, *Apis mellifera* Linneaus, and has become a serious issue for beekeepers in the United States. Since its initial discovery in 1996 and its subsequent identification in 1998, several methods have been developed to control this pest. Comparing several traps for their effectiveness at capturing the small hive beetle adults led to the observation of lower numbers of beetles being trapped in control colonies than expected.

This observation led to the development of the "trapping sink" theory that the small hive beetles accumulating in the traps of the treated colonies could be attracting other beetles, which would decrease the number of beetles at the control colonies. To test this hypothesis, investigations were performed in 2010 and 2011 from April through November. Fifteen colonies were established with #2 package bees in 2010 in five apiaries of three colonies and an additional forty-five colonies were established with #2 package bees in 2011 in fifteen apiaries of three colonies. The 2010 study had two apiaries with one colony having traps as a sink, two apiaries with two colonies as sink traps, and one apiary with no traps as a control. The 2011 study had six apiaries with one colony as a sink trap, six apiaries with two sink trap colonies, and three control apiaries with no traps. Colonies were established in the Clemson University Forest and were isolated by a distance of at least 0.4km. The small hive beetles captured were counted every two weeks, and the traps were emptied and restored. Surveys of the small hive beetle adult numbers were done in every colony in a twenty-four hour trap survey every six weeks following a three day varroa mite survey. Colony strength measurements were monitored by estimating percent of frame coverage of capped brood, honey, and adult bees every eight weeks. At the end of the project, in November, each colony was shaken out and all the adult beetles killed and counted.

The results from both years were combined to give a dataset of sixty colonies to investigate the "trapping sink" theory. The end-of-project shakeout of every colony showed no significant difference ($P>0.05$) in the number of beetles remaining in the colony in each apiary treatment of either one sink trap, two sink traps, or no sink traps. This indicates that there is no apparent "trapping sink" effect, since there were not a significant number of beetles being drawn either toward or away from the treated apiaries when compared to the controls. There was no significant difference found in either the varroa mite numbers or the colony measurements between the treated and non-treated apiaries. The two-week surveys showed a significant difference ($P<0.05$) between the apiaries with one trapped colonies and the apiaries with two trapped colonies on one date comparison, but there was no significant difference overall. The twenty-four hour surveys showed no significant difference ($P>0.05$) in the number of small hive beetles trapped in each apiary treatment. These results indicate that although installing traps in a colony will help to control the small hive beetle population, a trap must be installed in every colony in order to have an observable effect.

23. **Rangel[dd], J., J.J. Keller[dd] & D.R. Tarpy[dd] - THE EFFECTS OF HONEY BEE (*APIS MELLIFERA* L.) QUEEN REPRODUCTIVE POTENTIAL ON COLONY GROWTH** - Reproduction in most species of eusocial insects is monopolized by one or a few individuals, while the remaining colony tasks are performed by the worker caste. This reproductive division of labor is exemplified by honey bees (*Apis mellifera* L.), in which a single, polyandrous queen is the sole colony member that lays female eggs. Previous work has revealed that the developmental fate of honey bee queens is highly plastic, and this plasticity is strongly associated with the reproductive potential of queens. In this study, we investigated the effects of queen reproductive potential ("quality") on the growth of newly established honey bee colonies. We did so by comparing the growth of colonies headed by "high-quality" queens (i.e., those raised from young worker larvae) to those headed by "low-quality" queens (i.e., those raised from older worker larvae). We confirmed that queens reared from young worker larvae were significantly larger in size than queens reared from old worker larvae. We also found a significant positive effect of queen quality on a colony's

production of worker comb, worker brood, worker population, and stored food (honey and pollen). Our results demonstrate that in honey bees, queen reproductive quality significantly influences several important measures of colony fitness. We therefore argue that a honey bee colony can be viewed as the extended phenotype of its queen (and her mates), and thus selection acting predominantly at the colony level can be congruent with that at the individual level.

24. **Rivera[m], R., F.A. Eischen[m], R.H. Graham[m] & J. Patt[m] - VOLATILE COMPONENTS IN HONEYBEE COLLECTED POLLEN ENHANCE POLLEN PATTY CONSUMPTION -** Commercial beekeepers feed pollen supplements to honey bees in fall and winter in preparation for almond pollination. Eischen, *et al.*, 2008, have shown feeding protein patties increases adult bee populations and may also alleviate stresses caused by varroa, nosema, disease, and transport. Good nutrition is essential to maintain healthy colonies for almond pollination and for honey production.

    This trial was conducted in South Texas to determine the number of adult bees produced from feeding different supplemental patties. About 100 nucleus honeybee colonies were established in South Texas. After one month, the colonies were evaluated for frames of adult bees, brood, sealed brood and stored pollen, then every 21 days as before, including amount of pollen patties consumed. Two pounds of patties were maintained on colony dried patties were removed. Four formulations of pollen patties were brewer's yeast and soy protein (supplement), supplement plus 30% almond pollen, supplement plus 30% Chinese pollen and almond pollen with no supplement. All formulations contain 20% sugar in water (50:50). Almond pollen patties produced the most total bees (106,000), almond+supplement (87,000), Chinese+supplement (78,000) and supplement (69,000). As patties were introduced into colonies, the honeybees swarmed the almond pollen patty but were not attracted to other patties. The honeybees consumed more of the almond patties.

    The honeybees were attracted either by odor or taste. This honeybee behavior led to investigating pollen volatiles. Supplements and pollens were analyzed for volatiles using solid phase micro extraction (SPME). SPME technique captures volatiles and for analyses by gas chromatography (GC). The GC gives a chromatogram or fingerprint profile of odor and flavor of many volatile compounds in pollen. The GC Mass Spectrometer (GC/MS) determines chemical structures. Analysis of bee collected almond pollen, Chinese pollen and commercially purchased pollen showed differences in volatiles. About 40 volatile components in almond pollen were identified as possibly attractive to honeybees. These individual volatile compounds in almond pollen are being tested for attractiveness to honeybees.

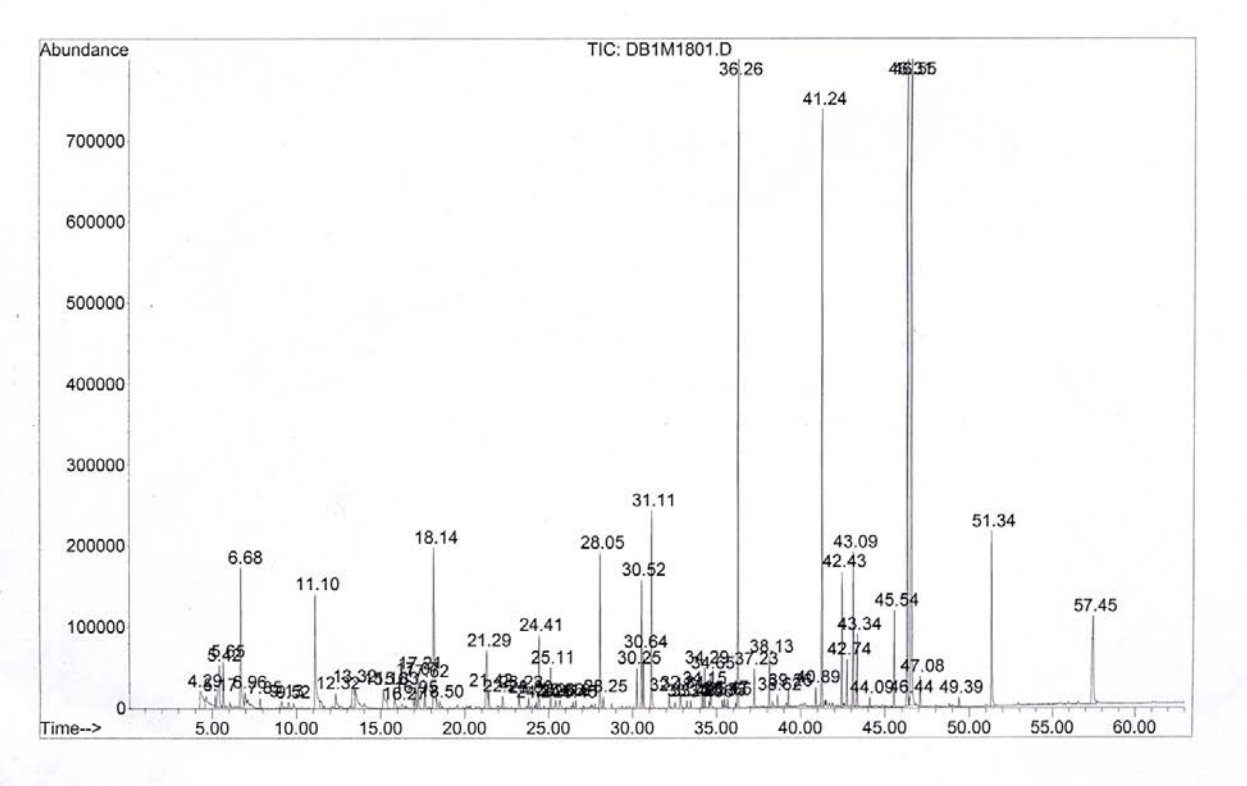


Figure. GC/MS SPME – Profile of Volatiles in Honeybee Collected Almond Pollen

25. **Sagili[e], R.R., & C. Breece[e] - EFFECTS OF POLLEN QUALITY (DIVERSITY) ON HONEY BEE PHYSIOLOGY, IMMUNOCOMPETENCE AND COLONY GROWTH -** Poor nutrition or nutritional stress is one among several potential factors attributed for colony collapse disorder. Improved bee nutrition is key in dealing with most of the stress factors affecting honey bee health. Honey bee colonies that are poorly nourished are more susceptible to the gut parasite *Nosema ceranae* when compared to colonies that receive adequate nutrition (Eischen and Graham 2008). Protein deficiencies might compromise immune responses of an organism such as encapsulation (Siva-Jothy *et al.* 2005). Large monocultures and destruction of pollinator habitat have resulted in restricted choice of pollen diet in honey bees. Very little is known about effects of single-source pollen consumption for extended periods on honey bees. In this study we investigate and compare the effects of single source pollen consumption versus a multi-source pollen on honey bee physiology, immunocompetence and colony growth.

A large flight cage partitioned in to eighteen segments was used for this experiment. There were two treatments a) Single-source pollen and b) Multiple-source pollen. Each week comb area occupied by egg, larvae, pupae, honey, pollen and empty space was measured in each colony with a metered grid (Pankiw 2004). Also, each week 20 paint marked bees that were 7 days old were collected from each of the experimental colonies for hypopharyngeal gland protein estimation. Phenoloxidase and prophenoloxidase enzyme activity levels that are indicators of immunocompetence in honey bees were measured following the method of Laughton & Siva-Jothy 2010).

Nurse bee hypopharyngeal gland protein content was significantly lower in single-source pollen treatments when compared to multi-source pollen treatments ($P < 0.01$). Multi-source pollen treatments had significantly higher colony growth in week's 4 and 5 ($P < 0.01$)

when compared to single-source pollen treatments. Both phenoloxidase and prophenoloxidase enzyme activities were significantly higher in multi-source pollen treatments when compared to single-source pollen treatments ($P < 0.001$ and $P < 0.01$ respectively) (Figure).

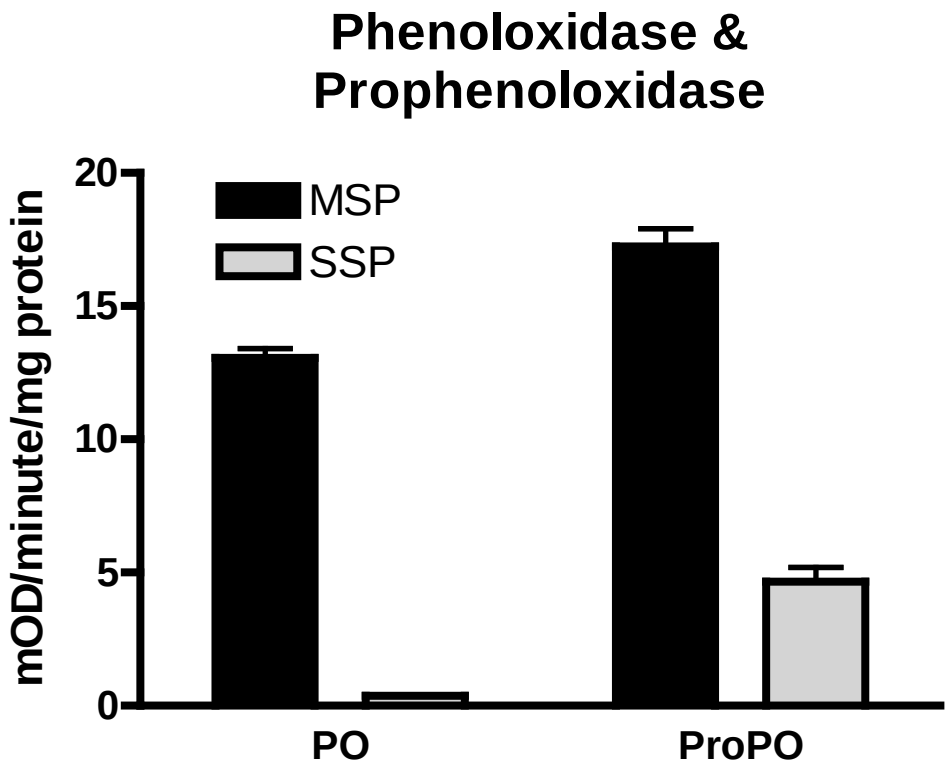


Figure. Phenoloxidase and Prophenoloxidase activities expressed in mOD/minutes/mg protein in Multi-source and Single-source pollen treatments.

26. **Schwarz[g], R.S. & J. D. Evans[g] – RE-EXAMINING *SPIROPLASMA* IN HONEY BEES - MOLECULAR DETECTION AND SEASONAL DISTRIBUTION -** While bacterial agents of disease are of prominent concern in honey bee brood, adult honey bees also incur bacterial pathogens, including the mollicute bacteria genus *Spiroplasma* that can cause disease such as 'May disease' and 'spiroplasmosis' noted by neurological disorders and mortality. Two distinct species isolated from *Apis mellifera* were first described in the 1980's as infectious agents, *Spiroplasma apis* and *Spiroplasma melliferum*. At the time, species identification of spiroplasmas required specialized and laborious serological and electrophoretic protein separation techniques. Both species were found commonly in Spring but rarely or absent at other times of year. Spiroplasmas are also known to occur on plant materials, including pollen and nectar, which have been suggested as an important reservoir for passive transmission. However, their ability to persist during non-flowering and winter periods in temperate climates were unknown. Thus, a clear understanding of the ecology of these spiroplasmas remains to be determined.

Our objective was to monitor and describe current seasonal prevalence of both *S. apis* and *S. melliferum* populations using specific and relatively simple molecular techniques in a large sampling of *A. mellifera* colonies. In 2011, we monitored hives from 7 apiaries located in Beltsville, MD from March through October beginning with 55 colonies that dwindled to 39 by the end of the survey. Fifty adult workers were used for pooled RNA extraction followed by cDNA synthesis and rapid analysis with species-specific primers via quantitative PCR. Here we present prevalence data for *S. apis* and *S. melliferum* from these colonies with the following conclusions: 1) hives incurred two peaks of spiroplasma infections, one in the Spring (83.7% of hives) and one in the late Summer (65% of hives); 2) hives maintained spiroplasma infections throughout the year at a minimum frequency of 20%; 3) *S. melliferum* predominated during the Spring and late Summer peaks while *S. apis* predominated during non-peak periods; 4) hives had mixed and single species infections during spiroplasma peaks; 5) during non-peak periods, hives were infected by either *S. apis* or *S. melliferum* but never both. This is the first description of seasonal *S. apis* and *S. melliferum* prevalence in honey bees using molecular techniques in North America and our data implicate honey bee colonies as reservoirs for these species and identify bi-modal prevalence peaks.

27. **Tsuruda[j], J.M., M.E. Arechavaleta-Velasco[ee], K.I. Alcala-Escamilla[ee], C.A. Robles-Rios[ee] & G. J. Hunt[j] - INVESTIGATING THE ROLE OF *NEUREXIN I* IN HONEY BEE MITE-GROOMING BEHAVIOR -** *Varroa* parasitism of honey bees is widely considered to be the greatest threat to beekeeping. Grooming is one of two behaviors identified as important traits to reduce *Varroa* mite populations. Bees exhibiting this behavior sweep their legs over their bodies in attempts to dislodge the mite from their bodies or they may bite the mite with their mandibles.

We conducted a study to look for associations between phenotype (grooming behavior) and genotype in order to map quantitative trait loci (QTL) and find candidate genes that influence mite-grooming behavior. Honey bee mite-grooming behavior was evaluated with a laboratory assay. A mite was placed on the thorax of a young adult worker bee and the time that elapsed before the bee engaged in grooming behavior (swiping at the mite with her legs or biting) was recorded. A high mite-grooming colony was crossed with a low mite-grooming colony to produce a hybrid F1 queen, which was then backcrossed to a drone from the high mite-grooming colony to produce a population of workers that was assayed for grooming behavior and used to map quantitative trait loci (QTL) that affect grooming behavior.

The genomic DNA of the F1 queen was sequenced with the ABI SOLiD sequencing system and the sequences were then aligned with the honey bee genome to identify single-nucleotide polymorphisms (SNPs) that would differ among her offspring. We identified over 9000 SNPs that were heterozygous and would therefore be informative for following the segregation of alleles in the backcross mapping population. Probes were designed for 1536 SNPs and the GoldenGate genotyping assay (Illumina) was used to genotype 120 fast-grooming and 120 slow-grooming backcross workers. A high-resolution genetic map was constructed and one major QTL was identified by interval mapping. The 95% confidence interval for the major QTL region contains relatively few candidate genes (around 25) and includes *neurexin 1*, *ataxin*, and *atlastin*. N*eurexin 1* is a particularly promising candidate since it is involved in Autism Spectrum Disorder and Schizophrenia in humans (which are associated with repetitive and agitated movements) and grooming behavior in mice. This is a

relatively large gene with 28 exons and at least 12 different isoforms, due to alternative splicing. We hypothesize that these isoforms, or relative amounts of these isoforms, affect the level of grooming behavior in worker honey bees and use conventional PCR and qRT-PCR to look for the presence/absence and expression of different isoforms.

Once we confirm the involvement of *neurexin 1* in honey bee grooming behavior, we aim to use marker-assisted selection to facilitate and speed up breeding programs for grooming behavior. This may also allow for the development of bees that have high tendencies for both grooming behavior and other mite-resistance traits, such as *Varroa* sensitive hygiene, reducing mite levels and the dependence on chemical treatments in honey bee hives.

28. **Webster[aa], T.C., E. Hogue[bb] & M.A. Matisoff[aa] *NOSEMA CERANAE* SPORE VIABILITY IS AFFECTED BY FECAL MATTER -** *Nosema ceranae* spores exposed to bee feces respond differently to ultraviolet light, compared to spores not exposed to feces. This was determined by exposing spores, with or without bee feces, to sunlight. Spore preparations were allowed to dry on microscope slides. During three experimental periods in October and November, 2011, these slides were exposed from 20 to 120 minutes of sunlight on a clear day. After exposure, spores were stained with trypan blue to determine which suffered ruptured membranes. Those which absorbed this stain fluoresced vividly, as a rose-red color with a maximum of 650nm. In each of the trials, spores which had been treated with feces suffered greater membrane breakage. (See figure. Red dashed lines represent spores exposed to feces. Blue solid lines represent spores without feces.) These observations suggest that the normal life cycle of *N. ceranae*, in which mature spores pass through the bee rectum, must be considered when this pathogen is studied and bees are experimentally inoculated.

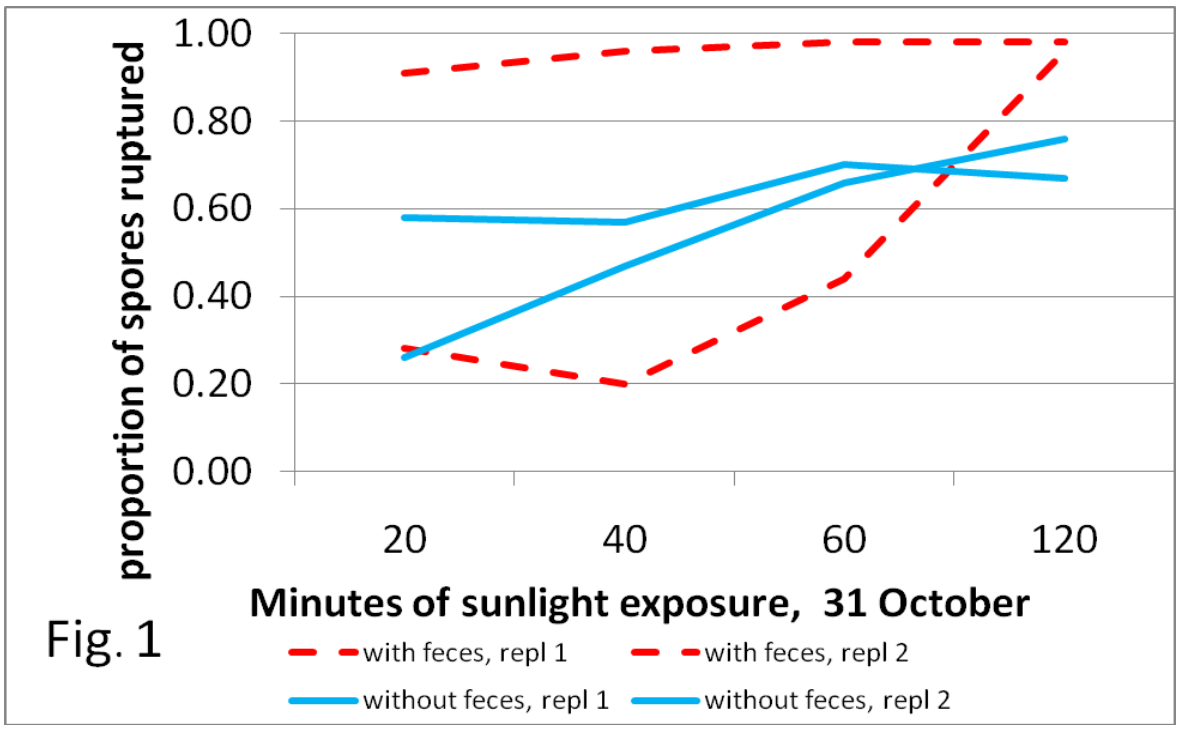

1.00
0.80
0.60
0.40
0.20
0.00
proportion of spores ruptured
20
40
60
120
Minutes of sunlight exposure, 31 October
with feces, repl 1
with feces, repl 2
without feces, repl 1
without feces, repl 2

Fig. 1

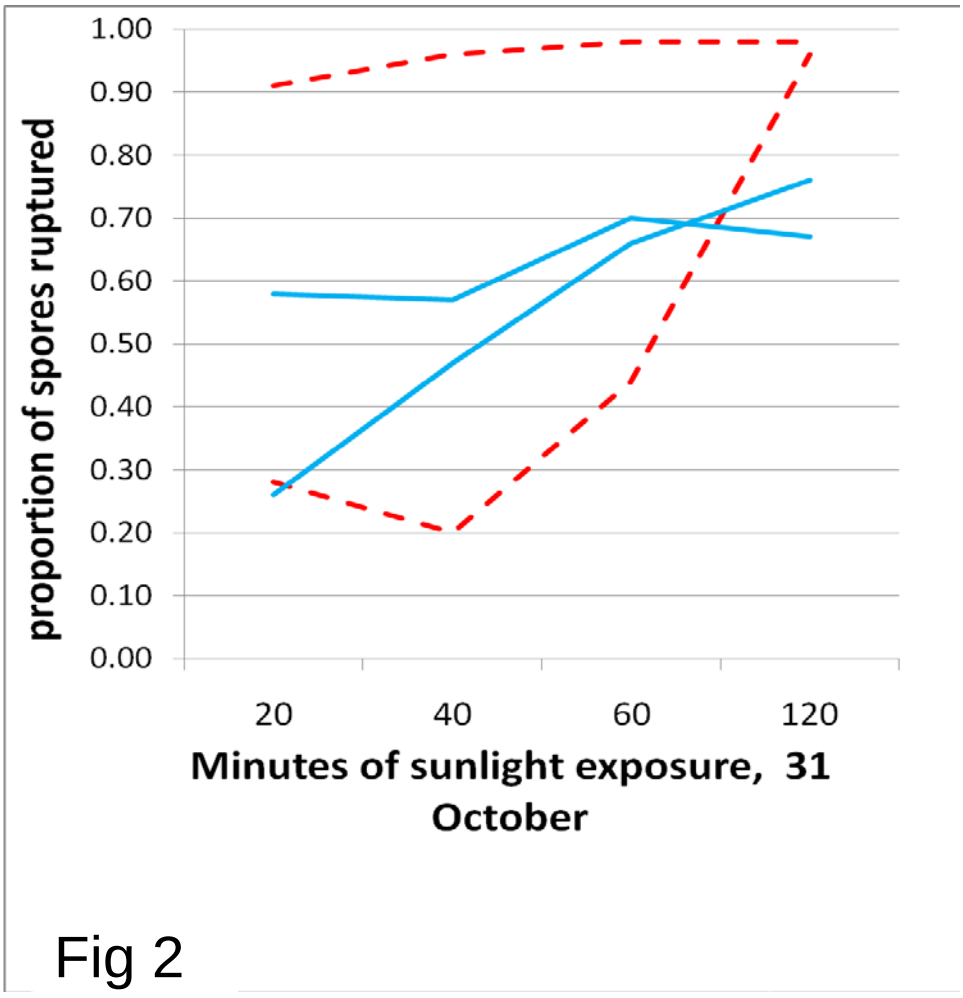

1.00
0.90
0.80
0.70
0.60
0.50
0.40
0.30
0.20
0.10
0.00
proportion of spores ruptured
20
40
60
120
Minutes of sunlight exposure, 31 October

Fig 2

29. **Wu[p], J., V. Krischik[p], M. Spivak[p] – SUB-LETHAL EFFECTS OF IMIDACLOPRID EXPOSURE ON HONEY BEE QUEEN EGG-LAYING AND ACTIVITY -** Pesticides such as neonicotinoid insecticides have been implicated as a contributing factor to honey bee losses. The objective of this study is to examine sub-lethal effects of imidacloprid on honey bee queen egg-laying and activity. Observation hives, containing about 1500 bees and a laying queen on newly drawn comb, were given 80 ml of sugar syrup with various imidacloprid treatments (0, 20, 50, 100 ppb) every other day. A total of 16 observation hives, or 4 colonies per treatment, were set-up in July and August of 2011. Queen egg-laying rate and activity were recorded in 15-minute intervals and quantified over 3 weeks. After 3 weeks, colonies were quantified for total adult and brood population, nectar and pollen stores, presence of disease, and weight of newly emerged bees. Preliminary results show that queen laying rates were affected at each imidacloprid treatment dose (20, 50, and 100 ppb). This study will be repeated in the summer of 2012. The findings will improve our understanding of known imidacloprid studies on honey bee colonies and workers. In addition, this study will highlight the need to focus future risk assessment studies on sub-lethal effects of neonicotinyl insecticides on honey bee queen health and behavior.

30. **Zhang[ff], X., J.D. Evans[g], J.S. Pettis[g] & Y. P. Chen[g] - NEW EVIDENCE THAT DEFORMED WING VIRUS AND BLACK QUEEN CELL VIRUS ARE MULTI-HOST PATHOGENS -** The host-range breadth of pathogens can have important consequences for pathogens' long term evolution and virulence, and play critical roles in the emergence and spread of the new diseases. *Black queen cell virus* (BQCV) and *Deformed wing virus* (DWV) are the two most common and prevalent viruses in European honey bees, *Apis mellifera*. Here, we provide the evidence that BQCV and DWV attack wild species of honey bees, *Apis florea* and *A. dorsata*. Phylogenetic analyses suggest that these viruses might have moved from *A. mellifera* to wild bee species and that genetic relatedness as well as the geographical proximity of host species likely play an important role in host range of the viruses. The information obtained from our study can have important implication for understanding the population structure of bee virus as well as host-virus interactions and emphasizes the importance of viral disease control as an integrated part of biodiversity conservation efforts.

31. **Zhu[gg], W., T. Reluga[hh], C. Mullin[gg], J. Frazier[gg] - A STAGE-STRUCTURED MODEL OF HONEY BEE COLONY POPULATION DYNAMICS ASSESSING IMPACTS OF PESTICIDES AND OTHER STRESSORS -** A healthy honey bee colony is a population of closely interacting individuals that form a highly complex society. As an aid to testing hypotheses for the causes of recent colony failure and providing suggestions for management actions to promote recovery of honey bee population, we developed a worker-based, stage-structured model of honey bee population dynamics. This model was formulated with difference equations consisting of six discrete stages based on the temporal polytheism: egg, larva, pupa, nurse, house bee and forager stage. Numerical simulation of a healthy colony exhibited seasonal patterns (see figure) similar to published field data (McLellan, *1978 J Appl. Ecol. 15:155-161).*

Sensitivity analysis suggested the critical threshold of stage-based survival rate beneath which colony size decrease gradually. Also, if the social factor (brood care, transition rate and foraging behavior), especially precocious foraging, is interrupted beyond the critical

threshold rapid population decline is predicted and colony failure is inevitable. This model suggested that a disrupted colony by varying social regulation factor in the colony might be able to produce sudden collapse symptoms similar to colony collapse disorder.

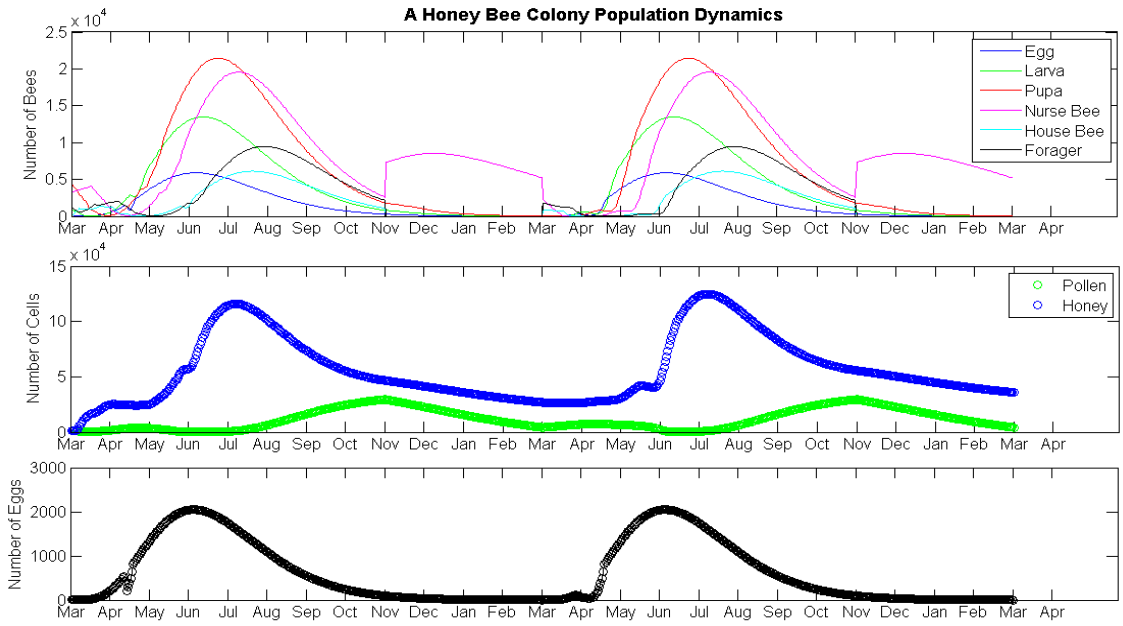


Figure. Stage-structured Model for Honey Bee Population Dynamics.

ADDRESSES OF AUTHORS AND FOOTNOTES

[a]Departments of Plant Soil and Insect Science, University of Massachusetts - Amherst, MA 01003; Fax: 413-545-2115, email: jburand@microbio.umass.edu

[b]Department of Microbiology, University of Massachusetts - Amherst, MA 01003

[c]Department ofBiochemistry, University of Massachusetts - Amherst, MA 01003

[d]Honey Bee Research Institute and Nature Center, Saint David, Maine; Ph.714-222-7122, email: Pbutzloff@gmail.com

[e]Department of Horticulture, Oregon State University, Corvallis, OR-97331-7304. Caron's contact information: Ph. 302-353-9914, Fax: 541-737-3479, email: carond@hort.oregonstate.edu; Sagili's contact informationPh. 541-737-5460, Fax: 541-737-3479, email: sagilir@hort.oregonstate.edu

[f] Department of Entomology, Center for Pollinator Research, The Pennsylvania State University, University Park, PA 16802. Chen contact information: Ph. 814-954-2556, email: jzc16@psu.edu; Grozinger contact information: cmg25@psu.edu; Mullin contact information: Ph. 814-865-2435, email: camullin@psu.edu

[g]U.S.D.A. Bee Research Lab, BARC East, Bldg. 476, 10300 Baltimore Ave. Beltsville, MD 20705. Schwarz's contact information: Ph. 301-974-1234, FAX: 301-504-8736, email: schwarz.ryan@gmail.com

[h]USDA-ARS Molecular Plant Pathology Laboratory, Beltsville, MD 20705

[i]University of Maryland, College Park, MD

[j]Department of Entomology, Purdue University, West Lafayette IN 47907

[k] USDA-ARS Honey Bee Breeding, Genetics & Physiology Laboratory, 1157 Ben Hur Road, Baton Rouge, LA 70820

[l]Georgetown University, Washington, D.C.

[m]Honey Bee Research Unit, USDA-ARS-SARC, 2413 E. Hwy 83 Weslaco, TX 78596. Eischen contact information: Ph. 956-969-5007, Fax: 956-969-5033, email: Frank.Eischen@ars.usda.gov

[n]Swiss Bee Research Centre, Agroscope Liebefeld-Posieux Research Station ALP, Bern, Switzerland

[o]Department of Zoology and Entomology, Rhodes University, Grahamstown, South Africa

[p]Department of Entomology, University of Minnesota; St. Paul, MN 55108

[q] 243 Natural Science, Department of Entomology, Michigan State University, E. Lansing, MI, 48824

[r]Laboratoire Ecologie Evolution Symbiose, University of Poitiers, Poitiers, Cedex, France, F-86022

[s]University of British Columbia, Department of Biochemistry & Molecular Biology and Centre of High-Throughput Biology, 2125 East Mall, Vancouver, BC Canada V6T 1Z4

[t]Agriculture & Agri-Food Canada, Beaverlodge Research Farm, P.O. Box 29, Beaverlodge, AB Canada T0H 0C0. Ph. 780-354-5135, Fax: 780-354-5150, email: Steve.Pernal@agr.gc.ca

[u]Illinois Natural History, Prairie Research Institute, University of Illinois, 1816 S. Oak St., Champaign, IL 61820

[v]Roy J. Carver Biotechnology Center, Protein Sciences Immunological Resource Center, 307 Noyes Laboratory, 505 S. Mathews St., Urbana, IL 61801

[w]Department of Entomology, 103 Entomology Hall, Lincoln, NE 68583. Ph. (402) 472-2123, Fax: (402) 472-4687, email: erin.michelle.ingram@gmail.com, mellis3@unl.edu

[x]Department of Entomology, The Ohio State University – OARDC, Wooster, OH

[y]Department of Analytical Chemistry, The Connecticut Agricultural Experiment Station, PO Box 1106, New Haven, CT 06504

[z]Department of Biology, University of North Carolina at Greensboro, 321 McIver Street, Greensboro, NC, 27412. Ph. 336-2562591, email: olav_rueppell@uncg.edu

[aa]College of Agriculture, Food Science and Sustainable Systems, Kentucky State University, Frankfort KY 40601. Ph. 502-597-6351, email: thomas.webster@kysu.edu

[bb]Kentucky Department of Fish and Wildlife, Frankfort KY 40601

[cc]School of Agricultural, Forest, & Environmental Sciences, Clemson University, Clemson, SC 29634, Ph. 864-656-0346, Fax 864-656-0274, email: mhood@clemson.edu

[dd]Department of Entomology, North Carolina State University, Raleigh, NC 27695-7613

[ee]Instituto Nacional de Investigaciones Forestales, Agricolas y Pecuarias CENID-FyMA; Ajuchitlan; Queretaro, Mexico

[ff]Eastern Bee Research Institute, Yunnan Agricultural University, China

[gg]Department of Entomology, Pennsylvania State University. email: jfrazier@psu.edu

[hh]Department of Mathematics, Pennsylvania State University