Proceedings of the American Bee Research Conference

The 2009 American Bee Research Conference was held February 4-6, 2009 at the University of Florida in Gainesville, Florida. The twenty-third American Bee Research Conference will be held in conjunction with the American Beekeeping Federation in Orlando, Florida in 2010. The following are abstracts from the 2009 Conference.

1. Afik, O.[a], A. Dag[b], & S. Shafir[a] – THE CAUSE FOR HONEY BEE'S AVOIDANCE OF AVOCADO FLOWERS AND THE POTENTIAL FOR SELECTION OF NON-AVOIDING GENETIC LINES - Intensive activity of honey bees (*Apis mellifera* L.) is essential for a high fruit set in avocado orchards, but even when hives are located inside the orchard, many bees still search for alternative blooms, probably due to the high mineral concentration of avocado nectar (Afik *et al. 2006 J. Chem. Ecol. 32: 1949-1963*). We tested for a possible genetic component for a preference of avocado bloom relative to competing blooms. Bee hives were placed in two avocado orchards in Israel, and the honey from each hive was extracted separately at the end of the avocado bloom. The concentration of perseitol, a carbohydrate that is unique to avocado, was analyzed as a measure for avocado foraging (Dag *et al. 2003 Apidologie 34: 299-309*).

During the first year five bee strains were compared. Significant differences were found between strains in honey perseitol concentrations, indicating differences in their efficiency as avocado pollinators. Colonies with the highest and lowest perseitol concentrations were selected to parent 'high' and 'low' lines, respectively. Queens were raised from the selected hives and were instrumentally inseminated with sperm of drones from corresponding hives. During the second and third years, hives with inseminated queens were introduced to the avocado orchards, together with the surviving previous year hives. Hives of high-line inseminated queens had greater perseitol concentrations than those of the low line, though differences were significant only during the second year. Both years, selected hives that survived from the previous year again had outstanding, high or low concentrations, respectively, during the following year.

Five colonies from each line, selected due to their performance in the field during the second year, were tested for their sensitivity to minerals in a follow up experiment. Each of these colonies was kept in a separate enclosure provided with five feeders of sucrose solution enriched with minerals at increasing concentrations. Two sets of cafeteria style experiments were conducted. The first set tested the sensitivity of the bees to potassium and phosphorus (K_2HPO_4), and the second tested their sensitivity to magnesium and sulfur ($MgSO_4$). All of these minerals were detected in avocado nectar. Both combinations of minerals repelled bees at concentrations similar to these in avocado nectar, but did not repel them at concentrations similar to those in citrus nectar, a common competing nectar source. Magnesium and sulfur were the more repulsive combination, but their concentration in the nectar was also lower. Nevertheless, no differences were detected between colonies from the different genetic lines in their preference among the mineral enriched solutions.

The results reveal a genetic component for foraging upon avocado flowers. This genetic component may be used to improve avocado pollination by selecting the most efficient bee strain. We might even be able to achieve further improvement in pollination efficiency through a long-term breeding program. The results also demonstrate the important role of nectar minerals in deterring bees from avocado flowers. The similar sensitivity of the two bee lines to minerals may indicate that there are additional traits, other than mineral sensitivity, that affect the colony level preference for avocado nectar.

2. Carroll, M. J.[c], A. Duehl[c], S. Willms[c], & P. E. A. Teal[c] – ATTRACTION OF THE VARROA MITE TO HOST VOLATILES FROM HONEY BEES - Varroa mites initiate cell invasion of capping brood cells by detection of volatile cues from the target host/host cell. We compared volatiles collected off brood of different castes and ages to identify odor compounds specifically associated with attractive larval hosts. Headspace volatiles were collected *in situ* off active brood comb enclosed within glass and aluminum observation frames; volatiles were removed via a push-pull volatile collection system and trapped on Super Q absorbant packing. Collected volatiles were extracted from the Super Q filter, identified by GC-MS, and quantified by GC-FID. Two unnamed compounds (encoded CA and CB) were emitted at higher rates from attractive brood castes (drones > workers >> queens) and developmental stages (capping and capped brood) than less attractive castes and brood stages (younger larvae). Both phoretic mites and free-roaming mites responded to volatile CA and CB with greatly increased activity and attraction toward the odor source. 29% to 37% of phoretic mites exposed to CA and CB volatiles in an inverted jar-bottom screen bioassay moved off their hosts into a water trap within 20 minutes. In a four-choice diffusion bioassay, free-roaming mites were attracted to synthetic CA and CB odors released from capillary vials under the arena floor. These compounds appear to be effective as attractants at relatively high concentrations and over short distances, as might be expected for a phoretic mite responding to host cues embedded in a complex and conflicting hive odor environment.

3. Chapon, L.[d], M.D. Ellis[d] & A. Szalanski[e] - *NOSEMA* AND TRACHEAL MITES IN THE NORTH CENTRAL REGION - 2008 SURVEY - We surveyed honey bee colonies in four North Central Region states to determine the prevalence and abundance of *Nosema* and tracheal mites during the summer of 2008. Both organisms have historically caused severe losses, and this survey was undertaken to investigate their potential role in unexplained colony losses that occurred in 2007 and 2008. We examined 177 colonies for tracheal mites. Tracheal mites were detected in only 9 colonies, and the mean infestation in the nine colonies was 6.4%. We surveyed 332 colonies for *Nosema*. We found a mean of 1.26 (± .13) million spores per bee in all colonies surveyed. Among the positive

colonies we found 2.11 (± .17) million spores per bee. Some colonies included in the survey had been treated with Fumagillin-B during the previous 6 months. Treated colonies averaged .10 (± .32) million spores per bee. Seven of the samples were examined for molecular markers to determine which *Nosema* species were present. All 7 samples were found to have molecular markers for *Nosema ceranae*. None of the 7 samples had markers for *Nosema apis*. Our results suggest that: (1) *Nosema ceranae* was present at levels that warrant concern, (2) *Nosema ceranae* was the only *Nosema* species detected, (3) *Nosema ceranae* pathology studies should be a priority, (4) Fumagillin-B is effective at suppressing *Nosema ceranae*, and (5) tracheal mites were not present at levels that warrant concern.

4. De Jong, D.[f], T. M. Francoy[f], & L. S. Gonçalves[g] – NEW MORPHOMETRICS TECHNIQUES PERMIT DIFFERENTIATION OF BEE SPECIES AND SUBSPECIES BASED ON WING VENATION PATTERNS - As the Africanized honey bees spread throughout the Americas, their identification became a matter of high priority. Traditional morphometry has played an important role in helping with identifications (Daly *et al. 1982 Ann. Entomol. Soc. Am. 75: 591-594*). However, the preparation of specimens is time consuming, which increases costs and greatly limits the number of samples that can be processed. Currently, advances in computational techniques have lead to a great improvement in the accuracy of these analyses and has reduced the time needed to identify bee species and subspecies (Francoy *et al. 2006 Apidologie 37:91–97*; Mendes *et al. 2007 Biosci. J. 23: 147–152*; Francisco *et al. 2008 Insectes Sociaux 55: 231–237*). One of these new techniques is the Automatic Bee Identification System (ABIS), which was developed at the University of Bonn in Germany. ABIS is a fully automated system that uses features extracted from forewing images to discriminate bee species. It was able to discriminate European members of the genera *Colletes*, *Andrena* and *Bombus* to the species level with a precision of 99.8% (Steinhage *et al.* 2001. BIOLOG Workshop, German Programme on Biodiversity and Global Change, Status Report, pp. 194–195), and it correctly identified 94% of several honey bee subspecies (Francoy *et al. Genet. Mol. Res.* in press). When it was used to identify Africanized honey bees in comparison with other honey bee subspecies, ABIS achieved a success of 99.98%, needing no more than two minutes to identify each specimen (Francoy *et al. 2008 Apidologie 39: 488–494*). Another computer-assisted methodology to study shape variation among organisms is called relative warp analysis; it is based on wing vein landmarks and the variation of these points as Cartesian coordinates (Bookstein, 1991, *Morphometric tools for landmark data*, Cambridge University Press). The advantage over traditional morphometrics is that Cartesian coordinates maintain the information on the relative position of the point, allowing us to reconstruct the shape of the analyzed structure and to clearly locate the variation in the points. We have used this method for the identification of bee species, subspecies and it has aided in the discovery of new bee species (Francoy *et al. Genet. Mol. Res.* in press). When we applied it to the differentiation of Africanized bees from individual bee subspecies, we achieved 99.2% success; it also has the advantage that all the software needed is freely available on the Internet (Francoy *et al. 2008 Apidologie 39: 488–494*). Subpopulations and bee gender have also been identified with this methodology. These new techniques are very useful to help resolve biological questions and for regulatory needs, with the advantage of being cheap and fast, and they need no specialized labs and personal; this makes morphometrics a very attractive alternative to other modern methods, such as DNA analysis, which are much more expensive and require a sophisticated lab.

5. Delaplane, K. S.[h] & J. A. Berry[h] - A TEST FOR SUBLETHAL EFFECTS OF SOME COMMONLY USED HIVE CHEMICALS - There is evidence that some of the chemicals used routinely in beekeeping are hazardous to bees and contribute to bee decline. Residues of these chemicals are pervasive (Frazier *et al. 2008 Am Bee J 148(6): 521-523*), and their effects occur at sublethal levels which are not easily detected by casual observation (Desneux *et al. 2007 Ann Rev Entomol 52: 81-106*). Understanding this piece of the CCD puzzle will help beekeepers move toward less chemical-oriented management. We are involved in a two-year, two-state (GA, SC) experiment examining sub-lethal effects of selected bee hive chemicals; the list included registered products at label rates, as well as two off-label formulations. Here are the results for one year from Georgia. Compared to non-treated controls, significantly negative effects on brood area were found for Maverik (fluvalinate) and CheckMite (coumaphos), on bee learning for copper naphthenate wood preservative and Maverik, and bee memory for copper naphthenate and Taktic (amitraz).

Table - Sub-lethal effects of some common bee hive chemicals on honey bees. Values are mean ± standard error. Numbers in parentheses = *n.* Column values followed by the same letter are not significantly different (α = 0.05). Where no letters are shown there were no column differences. Maverik and Taktic are not labeled for use in bee hives.

Chemical	*Nosema* spp. (% bees +)	Brood viability (% open brood alive after 3 d)	Brood (cm^2)	Learning[1]	Memory[2]
Non-treated	2.8 ± 1.4 (3)	98.4 ± 0.8 (11)	5810 ± 809 (16)a	1.1 ± 0.2 (75)ab	35.5 ± 6.7 (8)a
Cu naphthenate[3]	14.3 ± 6.0 (3)	91.8 ± 2.7 (10)	5398 ± 717 (14)ab	0.7 ± 0.2 (65)b	8.7 ± 3.9 (4)b
Apistan (fluvalinate)	8.0 ± 3.4 (7)	91.8 ± 2.1 (14)	4735 ± 532 (16)ab	1.4 ± 0.2 (61)a	58.7 ± 5.5 (8)a
CheckMite (coumaphos)	2.9 ± 2.9 (3)	89.6 ± 3.5 (10)	4315 ± 667 (15)b	1.0 ± 0.2 (72)ab	44.8 ± 5.9 (8)a
Maverik (fluvalinate)	8.2 ± 2.6 (6)	92.8 ± 2.4 (10)	4504 ± 546 (14)b	0.6 ± 0.1 (71)b	36.8 ± 10.8 (8)a
Taktic (amitraz)	7.2 ± 1.5 (3)	91.2 ± 1.8 (11)	4667 ± 682 (15)ab	1.2 ± 0.2 (78)ab	8.1 ± 3.4 (8)b

[1] Learning was measured as sum of bees responding to a series of 6-sec learning trials with the proboscis extension reflex assay (Malone & Pham-Delègue 2001, *Apidologie* 32: 287-304.)

[2] Memory was measured as % of bees conditioned above responding to stimulus after a lapse of 56 min.

[3] Copper naphthenate wood preservative was applied as a 2% water-soluble solution on a plywood panel which was allowed to air-dry and placed on hive floor.

6. Desai, S.[i], Y. J. Eu[i] & R. Currie[i] - INHIBITION OF DEFORMED WING VIRUS (DWV) GENE EXPRESSION AND REPLICATION IN HONEY BEES BY RNA INTERFERENCE - Honey bees are important crop pollinators and play a critical role contributing to the production of crops with a market value that exceeds $217 billion dollars on a global basis (Science Daily, 2008). Among honey bee pathogens, viruses are one of the major threats to the health and their interactions with varroa mites have likely caused serious problems for the beekeeping industry.

The ability to control viruses directly could be of considerable benefit as it could allow beekeepers to tolerate higher mite levels without experiencing economic loss. The objective of our research is inhibition of deformed wing virus gene expression and replication in honey bees by RNA interference.

RNA Interference (RNAi) is a simple, rapid and specified method for silencing gene function. RNAi reduces gene expression by causing degradation of the target mRNA or viral RNA. RNA interference has recently been utilized in a number of species including human beings, plants, animals and insects (*Drosophila*) to suppress viruses. In this study, we have cloned the 700bp region of RNA dependent RNA polymerase (RdRp) gene into a plasmid with T7 promoter in inverse and forward directions (Figure). Later we cloned inverted repeat of the above 700bp region into a plasmid with T7 promoter. The dsRNA targeting to RdRp mRNA was made using in vitro transcription with T7 RNA polymerase.

Feeding dsRNA to honey bees (or topical applications) should result in a reduction in DWV titer. This in turn should lengthen life span of bees with DWV infection relative to infected untreated bees.

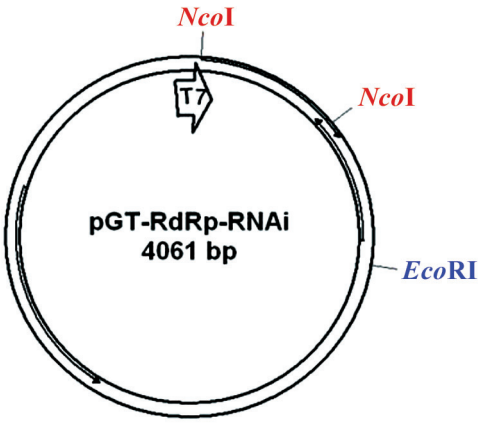


Figure - DNA construct for RNAi. Two copies of the 700 bp of RdRp of DWV are inserted into the restriction enzyme sites of NcoI and EcoRI in inverse direction. Double-stranded RNA is made using T7 RNA polymerase *in vitro*.

The experiment outlined above will increase our understanding of how viruses affect life span of honey bees and will use recent advances in molecular biology to develop methods to control viruses. If successful, the results will help beekeepers to manage honey bees with higher thresholds for varroa mites while reducing the probability of colony loss.

7. Duehl, A.[c], M. Carroll[c] and P. E. A. Teal[c] – RESPONSES OF THE VARROA MITE TO HOST VOLATILES FROM HONEY BEES -Varroa mites are able to successfully find and enter cells with brood that are close to capping. We found that volatile cues are sufficient to entice mites to enter cells. In order to show that Varroa mites can detect the specific chemical components that we found in various ages of brood, we used electrophysiological techniques. When testing a standard of synthetic colony volatiles on a preparation of mite forelegs with GC-EAD, we found a clear response to one peak and slight responses to two additional peaks (compounds CA and CB; see Carroll abstract above). We further analyzed these responses by challenging forelegs with EAG puff detection and found that these chemicals indeed produce clear responses from mite chemosensillae. Other common hive odors such as the alarm pheromone component isopentyl acetate did not elicit a response from the forelegs. This indicates that the mites only detect certain types of compounds, and that these compounds include some that we detect in increasing amounts as larvae approach capping.

8. Eischen, F.A.[j], R. H. Graham[j], and R. Rivera[j] - OPTIMUM TIME FOR FEEDING PROTEIN TO WINTERING HONEY BEE COLONIES IN PREPARATION FOR ALMOND POLLINATION - This study examined the time of protein feeding on over wintering honey bee colonies in South (Escondido) and Central (Modesto) California. In each location, groups of 30 colonies were randomly assigned to a start time beginning in September 2007 and continuing through, Oct., Nov., Dec. and Jan. 2008. Once started, colonies were fed BeePro+4% pollen continuously until evaluated in late Jan. 2008. Colonies were evaluated by estimating the quantities of brood and adult bees prior to starting the trial and at its end (Table).

Colony performance in both locations was similar with respect to feeding start times. Unfed (control) colonies performed poorest by losing about 31 – 40% of their starting adult bee strength (Table). Colonies fed continuously from September through January gained on average 31.9% in strength, which was the best performance. Colonies fed starting in October and November gained adult bee strength, but colonies fed starting in Dec. and Jan. lost strength. We conclude that feeding early was superior to feeding late during the winter season.

Table - Feeding start time and adult bee strength of colonies near Escondido, CA.

Month feeding started	n	Beginning bee strength[1]	Ending bee strength	% Change
Sept. 2007	30	6.8	8.4	+31.9
Oct.	28	6.3	8.0	+27.3
Nov.	28	6.1	6.7	+10.3
Dec.	28	7.0	6.3	-9.1
Jan. 2008	25	6.8	5.6	-13.5
Check (not fed)	28	6.3	4.3	-31.1

[1]All colonies measurement in tenths of a standard Langstroth frame.

9. Esaias, W.E.[k], R. Wolfe[k], C. Jarnevich[l] & T. Stohlgren[l] - CHANGING NECTAR FLOWS, CLIMATE, AND AHB'S: NASA'S HONEYBEENET - Climate and land cover/land use have a significant impact on nectar flows, and presumably on the distribution of the Africanized Honey Bee (AHB). The timing of nectar flows is changing in response to both climate and land cover in parts of the US, and the ultimate range of the AHB in the US may be impacted as well. Trends in the phenology (seasonality) of nectar flows derived from volunteer scale hive records shows a high correlation to trends in satellite vegetation phenology in the Mid-Atlantic, both advancing by about 0.5 d/yr since the early 1980's. Relating these trends to other floral and climate regions requires sample scale hive records to validate the satellite relationships. The number of scale hive records has been doubling annually (14 and 30 sites in 2007 and 2008) and over 60 volunteers have expressed their intent in 2009 so far.

Additionally we use historical records, including many with replicates by E. Oertel from the Baton Rouge ARS site, to assess regional variations in metrics of the nectar flow. The scale hive records will be related to satellite data throughout the US and Canada within the context of bee forage regions as defined by Ayers & Harman (1992, *The Hive and the Honey Bee*, 437-535). Their table is now available as an interactive map (http://honeybeenet.gsfc.nasa.gov), giving coarse temporal (±2 mo) but continuous spatial coverage of blooming periods for 267 plants. This will be refined based on correlations between satellite, climate, and scale-hive records to produce vegetation fields optimized to bee forage characteristics. One record per state per region (total 119) would give a tremendous advance in knowledge of nectar flows, so there is a clear need for more volunteers. The long-range goal is to develop an improved map of time dependent bee forage for North America.

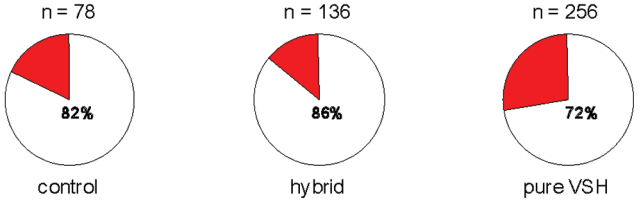
Distribution models based on the locations of the AHB, combined with climate GIS layers and seasonal satellite vegetation information provide better understanding of the suitable habitat of the AHB, and how it may change. Initial MaxEnt model results for the US using USDA county-wide data are consistent with current and some predicted ranges of the AHB and show significant improvement when annual bulk vegetation data are included. The runs show a negative relationship between the AHB habitat and fractional tree cover. However, their coarseness underscores the need for precise AHB location points (latitude-longitude). Preliminary runs using occurrence points supplied by officials and scientists from several states are encouraging, but are potentially misleading until all presence states are included. The next stage will include more point location data, followed by inclusion of vegetation phenology derived from satellite data at 1 km and 8 day resolution from the NASA MODIS sensor (http://accweb.nascom.nasa.gov). Improvements in bee forage distribution and variability will directly benefit our understanding of the suitable habitat of the AHB and honey bee nutrition in general.

We would like to acknowledge the many individuals who have contributed to this work, including the scale-hive volunteer 'citizen scientists'.

10. Harris, J. W.[m], R. G. Danka[m] & J. D. Villa[m] – HYGIENIC ACTIVITY TOWARD VARROA MITES IN CAPPED BROOD IS NOT DEPENDENT ON MITE REPRODUCTIVE STATUS - The varroa resistance of bees selectively bred for high levels of varroa sensitive hygiene (VSH) is characterized by a reduction of

(1) the mite infestation rate (Harris *2007 J. Apic. Res. / Bee World 46: 134-139*) and (2) the percentage of fertile mites (Harris and Harbo *1999 J. Econ. Entomol. 92: 83-90*) after naturally infested capped brood is exposed to the bees. Selective removal of pupae that are infested with fertile mites (those with offspring) could explain both results (Harbo and Harris *2005 J. Apic. Res. 44: 21-23*).

This experiment tested for a bias by VSH bees for chewing pupae infested with fertile mites. Combs of naturally infested worker brood were put into control (n=12), hybrid VSH (n=7), and pure VSH (n=8) colonies for 3 hours. Half of the capped brood on each comb was protected by a screen to prevent hygienic manipulations by the bees. The percentage of fertile mites in protected brood was compared to the percentage fertile mites from chewed pupae at the end. Biased removal of pupae with fertile mites should increase the fertility (*i.e.* above background levels of protected brood) of mites on chewed pupae that were being removed from capped brood by bees. Exposure of brood to bees was limited to 3 hours so that hygienic bees could uncap mite-infested pupae and begin chewing some of them, but the interval was too short to allow complete removal of most targeted pupae. The percentage of fertile mites on chewed pupae was not significantly different from that of mites from protected brood ($\alpha = 0.05$). There were no significant differences in percentage of fertile mites on chewed pupae among the three types of bees despite significant differences in overall hygienic activity (hybrid and pure VSH bees chewed more pupae than control bees). These results suggest that VSH bees removed mite-infested pupae independent of the presence of mite offspring (Figure). Thus, other processes related to hygienic behavior of VSH bees must decrease the frequency of fertile mites from capped brood.



Figure—Comparison of the fertility of varroa mites from chewed pupae among 3 types of honey bees after capped brood was exposed to each colony for 3 hours. The percentage of fertile mites (white slice) being removed from capped brood by hybrid or pure VSH bees did not differ—and clearly was not greater—than that in protected brood (84±5%) on the same combs. The number above each pie chart is the total number of chewed pupae from all colonies within a bee type.

11. Hood, W.M.[n] & M.P. Nolan IV[h] – TRAPPING SMALL HIVE BEETLES IN THE TOP AND BOTTOM OF HONEY BEE COLONIES – Trapping small hive beetles (SHB) is one alternative to controlling this hive pest. Most SHB traps that are currently marketed in the US are designed for use in the bottom of beehives. Possible reasoning for trapping beetles near the bottom of a beehive is to trap the beetles when they first enter the colony which is through the hive entrance. Also, the brood chamber is where pollen and bee brood is located which provide the necessary protein for beetle nutrition and reproduction. However, large numbers of adult beetles are often observed in the top of beehives on a regular basis.

Torto *et al.* (*2007 Envir. Entomol. 36[5]: 1018-1024*) reported a greater number of SHB in traps located in the bottom of beehives versus traps placed in the top of beehives. However, they used differently designed traps in the bottom versus the top which may have contributed to their results during 4-week and 7-week trials. We report here results of a 7-month investigation of trapping SHB in the top and bottom of beehives using one trap design.

Twenty-five honey bee colonies were established in 10-frame Langstroth beehives from 31 March – 2 April 2007 using 0.9 kg (2 lb.) packages of honey bees and queens purchased from Wilbanks Apiaries, Inc. (Claxton, Georgia). Five apiaries were setup containing five test colonies each in the Clemson University Experimental Forest, Clemson, South Carolina. Each apiary was located in partial shade and was separated by a minimum 2.4 km and colonies were spaced approximately 0.76 m. apart in each apiary. Natural SHB immigration occurred from nearby beetle-infested colonies.

On 1 May, two Hood beetle traps (Brushy Mountain Bee Farm, Inc., Moravian Falls, North Carolina) were installed in each colony, one randomly placed in frame position one or ten in each of the brood chamber and the top honey super. The one-way beetle traps were attached to the bottom bar of new frames with two pan-head sheet metal screws (#6 x 12.7mm). The trap middle compartment was filled 80% capacity with apple cider vinegar and the two outer compartments were filled 40% with food grade mineral oil as the lethal agent (Nolan & Hood *2008 J. Apic. Res. & Bee World 47[3]: 229-233*). Test colonies were serviced at 3-week intervals till 19 November. Trapped adult beetles were counted during each service visit and new vinegar and mineral oil were loaded into traps.

There was no significant difference in number of dead SHB counted in traps in the top honey supers versus the traps in the brood chambers for each of the 10 trapping periods. Over the 7-month SHB trapping investigation, a total of 12,705 beetles were trapped in the top honey supers and 12,505 beetles in the brood chambers. Twenty-three of the 25 test colonies survived the investigation and the two colony losses were apparently not a result of SHB activity.

The results of our investigation indicate that SHB trapping in the top super of a honey bee colony can be just as effective as trapping beetles in the brood chamber when using the Hood beetle trap. However, placement of traps in both the top and bottom of a beehive may be a better option to maximize the number of beetles removed from a SHB-infested colony.

12. Johnson, R. M.[o], H. S. Pollock[o] & M. R. Berenbaum[o] – SYNERGISTIC INTERACTIONS BETWEEN IN-HIVE MITICIDES IN *APIS MELLIFERA* - The in-hive miticides Checkmite+® (coumaphos) and Apistan® (tau-fluvalinate) have been used over the last 15 years to control infestations of parasitic varroa mites (*Varroa destructor*) in honey bee colonies. The active ingredients in both miticides are lipophilic compounds that are rapidly absorbed by the wax of the hive. Both are stable in wax and concentrations can build up in the hive over repeated treatments such that bees can be exposed to both compounds simultaneously. Coumaphos and tau-fluvalinate were chosen as in-hive miticides due to their low toxicity to honey bees, but that low toxicity depends, at least in part, on rapid metabolism by cytochrome P450 monooxygenase enzymes (P450). P450s play a vital role in the metabolism of pharmaceuticals in humans, and administration of multiple drugs to humans can lead to adverse drug-drug interactions as the efficiency of P450-mediated drug metabolism is altered. In a laboratory study a large increase in the toxicity of tau-fluvalinate was observed in three-day-old bees that had been previously treated with a sublethal dose of coumaphos. A moderate increase in the toxicity of coumaphos was also observed in bees previously treated with a sublethal dose of tau-fluvalinate. This apparent synergism may be the result of competition between coumaphos and tau-fluvalinate for access to detoxicative P450s. These results suggest that honey bee mortality may occur when otherwise sublethal doses of tau-fluvalinate or coumaphos are simultaneously present in the hive.

13. Stoner, K.A.[p] & B.D. Eitzer[q] – MEASURING PESTICIDE IN POLLEN TRAPPED FROM HONEY BEE HIVES IN CONNECTICUT - There are many unanswered questions about pesticides and how they may be affecting honey bees. In this study, we chose to look at this question: **What pesticides are found, and in what quantities, in pollen collected from honey bee colonies in a few representative locations in Connecticut?**

We studied pollen collected by healthy honey bee colonies, and thus collected baseline data. We cannot say anything about Colony Collapse Disorder or other bee die-offs because none of the colonies in this study died, and there have been no documented cases of Colony Collapse Disorder in Connecticut.

Methods: In 2007, we collected pollen pellets twice weekly from May through September using a bottom-mounted pollen trap on hives in the following four Connecticut locations:

1. our offices in New Haven on the edge of the city,
2. our experimental farm surrounded mostly by suburbs,

3. an orchard on the edge of a suburb only during the blooming season of apples and blueberries, and
4. another suburban site on the edge of a large agricultural area growing vegetable crops.

Pollen samples were analyzed by a multi-residue technique developed by Steven J. Lehotay at the USDA, which has had a multi-laboratory validation that included imidacloprid residues. The samples were extracted with acetonitrile and cleaned up with solid phase dispersion to separate the pesticides from the rest of the pollen, and the extracts were analyzed by high performance liquid chromatography/mass spectrometry (HPLC/MS). To enhance the specificity of analysis, the ion yielded by HPLC/MS is fragmented again in a technique known as MS/MS. This allows us to measure imidacloprid to a detection limit of 0.1 parts per billion (PPB), other neonicotinoids to a detection limit of 0.5 – 2 PPB, and most other pesticides we will be reporting here to 1 – 10 PPB.

Detections: In the 102 samples analyzed, we detected 37 pesticides: 15 insecticide/acaricides, 11 fungicides, 10 herbicides, and 1 plant growth regulator. All samples had at least one pesticide detected. The mean number of detections was 4.25 pesticides per sample. The most commonly detected pesticide was coumaphos in 96 of 102 total samples. Carbaryl (66 detections) and phosmet (38) were the most commonly detected field pesticides. Imidacloprid was detected 30 times, mostly at low levels, as will be discussed further below.

Maximum levels: The pesticides found at the highest concentrations were both fungicides, myclobutanil (1460 ppb) and boscalid (848 ppb). Carbaryl was the only pesticide found both frequently and at relatively high concentrations (>50 ppb) at all sites (maximum from 55-227 ppb at the 4 sites). Imidacloprid was found at a high level (70 ppb) in a single sample – all other samples in 2007 were at 3.4 ppb or below. We are in the process of separating this sample into components and analyzing each component further.

Conclusions: Imidacloprid was found frequently, but, with only one exception, at levels below 3.5 ppb. Carbaryl was also detected frequently and occasionally at high levels (>50 PPB). Coumaphos was also found frequently in our samples. Our levels of coumaphos were relatively low, but we were measuring pollen as bees brought it in from outside the hive. We are currently analyzing pollen collected in 2008.

14. Villa, J. D.[m] – BROOD FROM DIFFERENT COLONIES AFFECTS THE REPRODUCTION OF VARROA MITES – A mechanism of resistance to varroa mites derived from brood may be useful to complement traits dependent on adult behavior. Some evidence exists for a genetically determined influence of brood on mite reproduction. Most notably, only 50% of mites reproduced in Africanized brood compared to 75% in European brood (Camazine *1986 Ann. Ent. Soc. Amer. 79: 801-803*). Colonies with VSH (varroa sensitive hygiene) as the primary resistance mechanism also have a weaker brood effect which reduces mite reproduction (Harbo & Harris *1999 Apidologie 30: 183-196*; Ibrahim & Spivak *2006 Apidologie 37: 31-40*).

Thirty-five colonies from six sources in the U.S. were tested in 2007 and 2008 for effects of brood source on mites. Four to nine colonies were tested at a time in a total of 11 tests. For each test, queens were confined on comb for one to three days to obtain equally aged brood. Larvae were either reared in their own colonies (tests in 2007) or in common nurse colonies (tests in 2008). Combs from each test were moved to a common infested colony (more than 10% of adults infested) when larvae were less than two days from capping and then to an incubator four to five days after capping. After two to four days of incubation, pupae were examined for foundress females, progeny mites, developmental stage of pupae and anomalies in mite development. On average, 47 infested cells were recorded for each colony. Differences between colonies for the variable "progeny mites/female mite" were analyzed by analyses of variance using test and development stage of pupae as factors.

Brood from some VSH colonies had reduced mite reproduction compared to that in Italian colonies in the earlier tests (see Table). In later tests, VSH colonies did not reduce mite reproduction consistently (Table). Other sources also had high variability between colonies. Statistical analyses indicated that five colonies with the lowest reproduction differed significantly from five colonies with the highest ($P > 0.05$). Crosses among colonies with extreme phenotypes should clarify the genetic contribution to these brood effects.

Table – Range in the number of progeny mites per foundress female in infested pupal cells in VSH and other source colonies. Number of colonies and number of genetic sources are indicated for each category. Statistical analyses indicate that colony means differing by more than 1 progeny mite per foundress female are significant.

Test (Year)	VSH (n colonies)	Other (n colonies, n sources)
1 (2007)	1.41 - 1.54 (3)	2.80 - 3.14 (3, 1)
2, 3 (2007)	1.90 - 3.00 (4)	2.52 - 3.04 (3, 1)
4-11 (2008)	1.97 - 3.05 (5)	1.71 - 3.46 (18, 5)

15. Wilson, M. W.[r], J. Skinner[r], & L. Chadwell[s] – MEASURING THE EFFECTS OF FOUNDATION ON HONEY BEE COLONIES: A SARE PRODUCER GRANT PROJECT - Some beekeepers have proposed that 5.4mm foundation alters the biology of honey bees in a way that increases *Varroa* mite populations (Beesource.com). As a part of the idea, termed 'natural cell beekeeping', it is reported that bees build smaller cells when managed without foundation. To test this idea, beehives were managed with starter strips by turning the wedge of a 'wedge top' frame on its side and applying a bead of wax.

In 2007, ten colonies were started from splits of natural cell colonies. Five control colonies used standard wax foundation and five natural cell colonies used wooden starter strips. In 2008, these ten colonies were observed another year, while ten new colonies were made from splits. Five control and natural cell colonies were split from their respective groups. The ten group 2007 hives and ten group 2008 hives were allowed to build up to 3 medium boxes. When applicable, honey supers were provided above a queen excluder with drawn comb and foundation.

Mite populations were recorded as 24hr natural mite fall averaged over 3 days. During colonies' first year, mite levels did not significantly differ. However, during the second year of group 2007 colonies, mite levels were significantly lower in natural cell hives (60 ± 11, mean ± s.e.) than in control hives (114 ± 22; $P = 0.0004$). Despite these lower numbers, hives in both groups surpassed economic thresholds and experienced colony death.

The reason for the lower mite levels appears unrelated to worker cell size. Control colonies had a worker cell size of 5.3mm ± .004 (mean ± s.e., $n = 493$), while natural cell colonies had a worker cell size of 5.4mm ± .008 ($n = 381$, $P \leq 0.0001$). Cells of natural cell colonies did not decrease in size between 2 years (2007) and 3 years (2008) of management without foundation.

The average strength of group 2007 colonies did not significantly differ when measuring the rate of comb building in spring 2007, the hive weight in summer 2007, and the area of bees, brood, pollen and honey in the brood chambers during spring, summer, and fall 2008. However, there was significantly more surplus honey produced by control colonies (25.4 frames ± 3.9, mean ± s.e.) over natural cell colonies (5.4 frames ± 3.5; $P = 0.0052$). This difference may be related to the greater amount of drone comb produced by natural cell colonies (33% ± 3.5%, mean ± s.e.) as opposed to control colonies (1% ± 0.2; $P \leq 0.0001$). Plentiful drone production was evident in the second year of group 2007 natural cell colonies, as opposed to controls. In first year natural cell colonies, drone production was not as evident. In a previous study, (Seeley *2002 Apidologie 33: 75-86*) colonies with 20% drone comb were found to gain half the weight of control colonies. Seeley proposed the reduction was due to energy costs associated with raising drones, along with possible increased *Varroa* reproduction. This study suggests that an increased mite reproduction rate in drone cells (Martin *1994 Exp Appl Acarol 18: 87-100)* may not increase total *Varroa* reproduction in drone-producing colonies.

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