Proceedings of the American Bee Research Conference

The 2008 American Bee Research Conference was held January 8-13 at the DoubleTree Hotel in Sacramento, California. The twenty-second American Bee Research Conference will be held in conjunction with the Apiary Inspectors of America in Gainesville, Florida during February 4-6, 2009. The following are abstracts from the 2008 Conference.

1. Benda, N.D.[a], M.J. Carroll[a] & P.E.A. Teal[a] – FALLEN FRUIT AS A PUTATIVE ALTERNATIVE FOOD SOURCE OF THE SMALL HIVE BEETLE - The small hive beetle (*Aethina tumida*) (SHB) is reputed to occasionally use fallen fruit as an alternative food source in the absence of honey bee hives. However, attraction to fruit has not been adequately documented, nor has the effect of fruit age or microbial infection on attraction been considered. We first investigated the ability of fruits to support growth of the yeast *Kodamaea ohmeri*, a SHB symbiont that produces volatile cues that strongly attract SHB adults to beetle-infested honey bee comb. The yeast spread as a creamy white growth within 72 hours across the surface of inoculated oranges and blackberries, but not apples. We also evaluated the orientation responses of individual SHB adults to volatiles from exposed oranges 1) in various stages of senescence and 2) variably inoculated with *K. ohmeri* in a four-arm olfactometer bioassay. SHB adults were attracted more often to odors from oranges in advanced stages of senescence (15 days of ripening) over less senescent fruits. By contrast, SHB adults did not significantly differentiate between odors from yeast-inoculated and sham-inoculated oranges. Headspace volatile analysis revealed that apples, oranges and blackberries infected by *K. ohmeri* emit far lower amounts of isopentyl acetate (IPA) and 2-heptanone (two bee alarm pheromone mimics that are known to strongly attract SHB) than yeast-infested hive materials. Host preferences of SHB adults for honey bee hive materials over fruits may be driven by the low production of these important volatiles by its yeast symbiont. Use of fruit by SHB may be limited to older, more senescent fruit.

2. Berry, J.A.[b], B. Owens[c] & K.S. Delaplane[d] - A TEST OF "SMALL CELL" FOUNDATION AS AN AID TO VARROA CONTROL - The chief interest in small-cell technology resides in its potential as a non-chemical limiter of *Varroa* population growth. Brood cells are down-sized from conventional foundation to smaller foundation in hopes of culturally controlling mites. In a two-season field study, we compared biometrics of *Varroa* mite and honey bee populations in bee colonies housed on one of two foundation types: small-cell (4.9 ± 0.08 mm cell width, walls inclusive) or conventional-cell (5.3 ± 0.04 mm).

Test colonies were started in August 2006 or March 2007 and dependent variables measured October 2006 and June 2007. June colony bee populations were significantly higher in small-cell colonies (Table). However, October mites per 100 brood cells were also significantly higher in small-cell colonies. Moreover, with five of six remaining *Varroa* population metrics, mean trends for small-cell were unfavorable (Table). This study failed to demonstrate a benefit of small-cell foundation for *Varroa* management.

Variable	Conventional cell	Small cell
Oct mites per 24 h sticky sheet	18.5 ± 5.4 (7)	15.9 ± 1.8 (7)
Jun mites per 24 h sticky sheet	20.6 ± 7.4 (9)	34.9 ± 7.5 (15)
Oct mites per 100 brood cells	0.9 ± 0.5 (7)	3.3 ± 0.8 (7)*
Jun mites per 100 brood cells	2.7 ± 1.8 (9)	3.1 ± 1.0 (15)
Oct live bee weight (mg)	136.2 ± 4.4 (7)	134.1 ± 3.4 (7)
Jun colony bee population	9,149.8 ± 1672.2 (9)	13,222.1 ± 1077.9 (15)*
Jun cells of brood	18,765.8 ± 4681.0 (9)	25,161.6 ± 2335.1 (15)
Jun colony mite population	453.5 ± 113.0 (9)	1,267.4 ± 339.7 (15)
Percent Jun mite population in brood	**41.0** ± 12.7 (9)	**50.7** ± 9.0 (15)
Percent change in mite population ((end-beginning)/beginning)×100	**85.0** ± 92.5 (9)	**159.2** ± 61.0 (15)

Table - Mean values for bee and *Varroa* population metrics in bee colonies housed on conventional-sized cell foundation or small-cell foundation. Colonies of both cell types were set up in August 2006 (15,966 bees) or March 2007 (11,612 bees). Data were collected in October 2006 (Aug 2006 colonies only) and June 2007. Numbers in parentheses = *n*. The occurrence of significant treatment effects ($P < 0.05$) is indicated by *.

3. Calderone, N.W.[e] & J.P. Strange[f] – ESTIMATES OF MATING FREQUENCIES BY QUEENS IN COMMERCIAL POPULATIONS OF THE HONEY BEE, *APIS MELLIFERA* L. - The longevity and quality of commercial honey bee queens and the colonies they produce have declined over the past two decades. This decline is seen in the form of increased levels of queen supersedure and decreased colony performance. A number of explanations have been proposed to explain this decline, including exposure of immature queens to miticides during the rearing process, inadequate nursing of queen larvae, decreased genetic diversity among mates and an inadequate number of matings by queens. Each of these factors may impair colony health and the attractiveness of queens to workers. We examined mating frequencies in commercial queens using microsatellite analysis. Preliminary results based on an analysis of progeny from 13 queens (Table) revealed a high degree of variation in the number of times that queens mate and suggest a correlation between mating frequency and long-term survival.

Colony	Race/Line	Number of offspring genotyped	Estimated number of matings	Alive spring 2007
C04	BSMART	83	22	Y
C05	Unspecified	95	21*/22	N
C08	Russian	80	38	Y
C09	Italian	79	30	Y
C10	Italian	90	25	Y
C11	Unspecified	83	38	Y
C15	Golden Italian	90	12	N
C20	All-Star	77	9	N
C24	Russian	89	20	N
C25	Caucasian*SMR*Russian	91	9*/12	N
C27	Caucasian* SMR*Russian	87	8*/9/11/12	N
C28	SMR*Carniolan	91	16/16*/18/19	N
C30	Hygienic	88	18	Y
	Average values Mean ± SD	86 ± 5.47 (n=1,123 workers)	21.0 ± 10.50	

*best estimate

Table - Estimates of mating frequencies by commercial queens.

4. Chan, Q.W.T.[g] & L.J. Foster[g] – LARVAL IMMUNITY PROTEINS: INSIGHTS FROM HONEY BEE DEVELOPMENT – The bacterium *Paenibacillus larvae* is the causative agent of American Foulbrood (AFB), a disease which threatens the domesticated honey bee. This deadly infection is highly contagious and can easily wipe out an entire apiary in several weeks, resulting in tremendous economic burden on individual beekeepers and the beekeeping community. The antibiotic oxytetracycline is used to prevent outbreaks, but resistance to this drug is emerging (Miyagi, T., et al., *2000 J Invertebr Pathol 75: 95-6*; Evans, J.D., *2003 J Invertebr Pathol 83: 46-50*), driving the scientific community to find more effective treatment or prevention methods. This effort is hampered by the severe lack of information about the molecular biology of AFB, an area that we hope to address by understanding the molecular mechanism of this disease.

Honey bee larvae are vulnerable to AFB infection within 1-2 days after the egg hatches, but become resistant by day 3, which prompted us to examine the changes in abundance of individual proteins throughout the larval developmental stage. Previously, we showed that adult workers express significantly higher levels of immunity-related enzymes compared to larvae, suggesting that the lack of these key defense molecules in young larvae may be the underlying reason for the correlation of disease resistance to age.

For unbiased relative quantification of all detectable proteins between samples ("quantitative proteomics"), we employed mass spectrometry technology (reviewed in Ahn, N.G., et al., *2007 ACS Chem Biol 2: 39-52*). Using this method, we have found three proteins with known functions in the immune system to be upregulated in AFB-infected hemolymph of fifth-instar worker larvae compared to healthy controls, namely: lysozyme, hymenoptaecin, and prophenoloxidase (Figure).

We have also tracked the relative changes in concentration of 180 proteins in hemolymph over the larval developmental stage, most of which are previously unreported in bees. The data suggests that indeed, higher levels of immunity-related proteins are observed in older larvae, which may explain why younger ones are susceptible to AFB while older ones are not. We also observed, by a parallel spectrophotometric enzyme assay, that phenoloxidase activity increased over the course of larval development. Phenoloxidase activity is as an indicator of melanization capability, a key defensive strategy against pathogens used by insects. We were unable to detect phenoloxidase activity in 1- to 2-day old larvae and the levels spiked dramatically by days 4 and 5. It is not known whether resistance against *P. larvae* infection is directly conferred by melanization or a combination of several immune pathways; however, our data show a very strong correlation between susceptibility to infection and phenoloxidase activity. This information, together with the identities of several proteins implicated in larval immune resistance, allows us to start modeling the host-pathogen interactions underlying AFB.

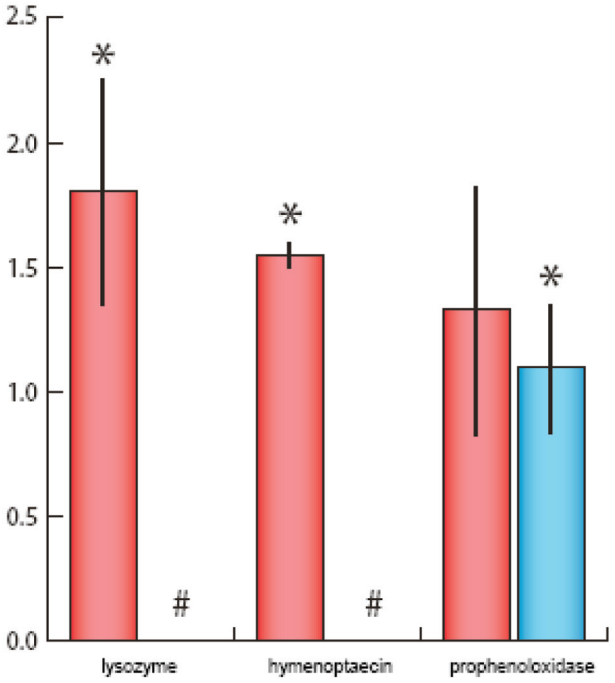

Figure - Concentration of three overexpressed immunity-related proteins in *P. larvae*-infected hemolymph from five-day old honey bee larvae compared to healthy controls. Infections to hives were induced in two ways: spraying with a laboratory strain of *P. larvae* (red bars) and spraying with a homogenate of scales of naturally infected colonies. Fold-regulation is expressed on a natural log scale (log_e). (* p < 0.05, # - not quantified).

5. de Guzman, L.I.[h] & A.M. Frake[h] - INFLUENCE OF *VARROA* INFESTATION AND COLONY PARAMETERS ON SMALL HIVE BEETLE POPULATION - Colonies of Italian (n =15) and Russian bees (n = 15) were monitored for small hive beetle (SHB) population, varroa mite infestation, adult bee population, number of capped brood cells and availability of pollen from June to October 2007. Colony divisions were made in April 2007 using the tower technique.

Our results showed that the Italian colonies had significantly higher populations of SHB (P = 0.011; Italian = 9.92 ± 1.39, Russian = 4.83 ± 0.56 beetles) and varroa mites (P = 0.011; Italian = 4,365 ± 741, Russian = 1,937 ± 366 mites) than the Russian honey bee colonies. A linear regression analysis between these two variables showed a weak but significant relationship, explaining 29% of the variation (Figure, A). The Italian bees (r^2 = 0.2656, P = 0.0002) showed a stronger relationship than the Russian bees (r^2 = 0.1587, P = 0.013).

Italian bees (17,783 ± 1,547) also had more capped brood cells than the Russian bees (12,663 ± 575 cells) (P = 0.025). Regression analysis also showed a weak but significant relationship between capped brood number and SHB (Figure, B). Similarly, Italian colonies were more populous (11,057 ± 625) than the Russian colonies (8,447 ± 569 bees) (P = 0.015). Both stocks had similar number of pollen cells (P = 0.601). No relationship between the number of adult bees (r^2 = 0.0249, P = 0.152) or number of pollen cells (r^2 = 0.0237, P = 0.152) and the number of beetles were detected. Elzen et al. (*1999 Apidologie 30: 361-366*) showed that SHB are only attracted to bucket traps baited with adult bees alone or combined with pollen and honey, but not with brood. Similarly, Suazo et al. (*2003 Apidologie 34: 525-533*) found that beetles

responded strongly to volatiles of worker bees. In contrast, we found that adult bee population and pollen cells did not influence SHB population. Our results showed that SHB infestation was associated with varroa infestation and the number of capped brood. These observations suggest that the amount of brood influences SHB invasion.

We also assumed that the beetles were cuing more on the volatiles from varroa-infested brood since the Italian colonies had more brood, varroa mites and SHB than the Russian colonies. Increased SHB invasion and reproduction are perhaps hastened when dead brood caused by parasitic mite syndrome (PMS) are present accompanied by the decline in adult bees. The first Italian colony that collapsed in October (with hundreds of SHB larvae) had the highest varroa (32%) and SHB (36 beetles) infestations in August. Another component that may affect invasion success involves the bees' ability to defend their colonies. Russian bees are more aggressive to adult beetles (de Guzman et al., *2006 Am Bee J 146: 618-620*) and they can prevent SHB invasion better than Italian colonies (Frake et al., *2006 Am Bee J 146: 447*).

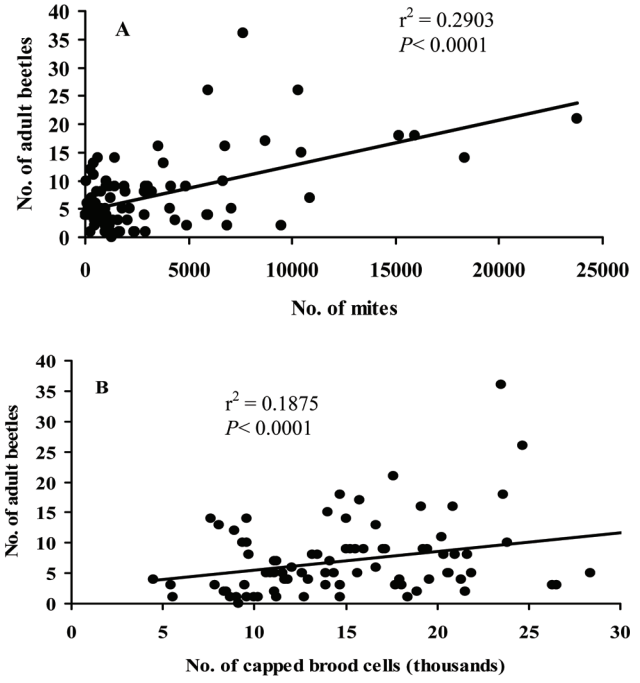

Figure - Relationships between the number of varroa mites (A), or the number of capped brood cells (B) with the number of SHB in Italian and Russian honey bee colonies.

6. Delaplane, K.S.[d] & A.M. Ellis[i] - EFFECTS OF VARROA AND SMALL HIVE BEETLES ON HONEY BEE POLLINATION EFFICACY - These experiments are the first to examine directly the impacts of honey bee nest invaders on plant pollination. A cost to pollination could occur either: (1) at the colony level where nest invaders compromise the health of foragers and reduce their efficacy as pollinators or (2) at the community level where invaders simply kill bee colonies and reduce the local population of pollinators. Honey bee colonies were manipulated to achieve different levels of *Varroa destructor* or *Aethina tumida* and tented under mature bushes of rabbiteye blueberry (*Vaccinium ashei*) with potted pollenizers. On the basis of single-bee flower visits, fruit-set was reduced in blueberry with bees from varroa-parasitized colonies. However, on the basis of colonies (tent averages), there were no differences in blueberry fruit-set and number of blueberry pollen grains deposited on the stigma among colonies with different levels of nest invaders or no-invader controls (Table). Thus, within the range of nest invader densities used in this study, individual inefficiencies were erased by compensatory multiple flower visits by this colonial pollinator. By failing to affirm the functionality of scenario (1), this study indirectly supports scenario (2): the major contribution of nest invaders toward a pollinator deficit is the simple eradication of colonies.

treatment	% fruit-set from single-bee flower visit	% fruit-set at colony level	no. pollen grains per stigma
VM	2.2 ± 1.5 (15) b	54.5 ± 0.03 (11) a	61.2 ± 11.8 (5) a
SHB	44.8 ± 10.0 (13) a	57.9 ± 0.04 (11) a	48.3 ± 6.4 (5) ab
NIC	33.2 ± 7.5 (14) a	43.9 ± 0.04 (8) ab	57.1 ± 7.9 (5) a
OC	.	35.7 ± 0.03 (8) b	74.8 ± 15.3 (5) a
NBC	.	17.6 ± 0.03 (8) c	12.0 ± 3.6 (5) b

Table - Mature blueberry plants were tented during bloom with honey bee colonies manipulated to have the following treatments: varroa mite (VM), small hive beetle (SHB), or no-invader control (NIC). The experiment included open-plot controls (OC) and no-bee controls (NBC, plants tented without bees). Values are mean ± SE (*n*). Column means with different letters are different at α = 0.05. Least square means were separated by Tukey's test.

7. Eischen, F.A.[j] & R.H. Graham[j] - FEEDING OVERWINTERING HONEY BEE COLONIES INFECTED WITH *NOSEMA CERANAE* - We hypothesized that high levels of nosema disease might be involved in the recent phenomenon of colony collapse disorder (CCD). Further, we suspected that colonies weakened by poor nutrition or other stress-related events would be more affected by *Nosema ceranae*. This study examined the value of feeding overwintering honey bee colonies infected with *N. ceranae*.

Overwintering honey bee colonies (n = 28) infected with *N. ceranae* ($\bar{x}$ = 2.4 million spores/bee) were either 1) fed pollen supplement, 2) treated with fumagillin (Fumagilin-B) + fed pollen supplement or 3) given nothing. The two fed groups had significantly larger adult bee populations than unfed control colonies. Colonies treated with fumagillin and fed were not (on average) larger than the fed, untreated colonies. However, when colonies within the two groups not treated with fumagillin were sorted by infection level, those with infections ≤ 1.5 million spores/bee had larger ending bee populations than those with >3 million spores/bee. This suggests that colonies under similar conditions and with infections of about 3 million spores/bee or greater should be treated annually.

At the end of the trial, the two fed treatment groups had 82 and 85% of their colonies meet or exceed six frames of adult bees, i.e. the criteria by which many colonies are accepted for almond pollination in California. Only 35.7% of unfed control colonies met this standard. The similarity of colony strength between the two fed groups (i.e., pollen supplement; pollen supplement + fumilligin) suggests that feeding colonies through the winter may be more valuable than treating *N. ceranae* infections when infections are ≤ 1.5 million spores/bee.

The data strongly indicate that overwintering colonies with scant pollen stores and low protein levels should be fed repeatedly if they are being prepared for almond pollination. The disturbance of feeding, preparing colonies for pollination during cool weather, and transporting them about 3000km did not cause *N. ceranae* populations to rise significantly during the time of observation.

8. Eischen, F.A.[j], R.H. Graham[j] & R. Rivera[j] - OVERWINTERING NUTRITIONALLY STRESSED HONEY BEE COLONIES INFESTED WITH *VARROA DESTRUCTOR* - This study examined the dual impact of poor nutrition and infestation by the parasitic mite *Varroa destructor* on wintering honey bee colonies in southern California during the winter of 2006-2007. An extended drought probably caused colonies to start the trial with low protein reserves. Colonies were selected for low

mite (0-5 mites/200 bees) and high mite (5-30 mites/200 bees) populations. Half the colonies in each group were then randomly assigned to receive nothing or a continuous supply of BeePro® + 4% pollen.

Fed, low-mite colonies had a 77.4% success rate in meeting the size criterion (≥ 6 frames of bees) for almond pollination, while unfed, low-mite; fed, high-mite, and unfed, high-mite colonies met this criterion only 45.2, 48.4, and 19.4% of the time, respectively. Under our conditions, the data indicate that varroa and diet were additive factors in colony strength maintenance during winter.

Winter feeding increased brood production, protein levels of adult bees and weight of newly emerged adults. Varroa populations did not increase significantly in fed colonies. Winter feeding is a viable option in many areas of the U.S. where colonies are overwintered and awaiting almond pollination.

9. Esaias, W. E.[k] – HONEY BEES, SATELLITES, AND CLIMATE CHANGE - A NASA project will apply satellite-derived information on vegetation distribution and phenology to improve estimates of the range of the Africanized Honey Bee in North America, and to provide an improved basis for studying the impact of climate change on Honey Bee forage. With participation from USGS, USDA, Arizona State U., and the Mid-Atlantic Apicultural Research and Extension Consortium, the 3 year project will utilize the Decision Support System at the National Institute for Invasive Species Science at Ft. Collins, CO. The activity is based on a) success in applying 1 km satellite-derived fields to predict invasive plant species habitats, and b) a tight functional relationship between satellite observations and the dates of nectar flows (scale hive data) in central Maryland. The onset of the nectar flow is advancing at the rate indicated by the satellite vegetation index of spring green-up (both at 0.57 d/yr, since the 1970-1980 period) due primarily to climate change in MD. Scale hive sampling of nectar flows is nicely matched to the resolution of satellite and climate observations, and might be achieved through a volunteer beekeeper network. Dates of nectar flows require updating, and relationships between the local nectar flows and satellite phenology must be developed for regions. Some regions of the US show delays in green-up. The addition of bee forage phenology derived from satellites with historical and modern scale hive data is expected to significantly improve the skill in predicting the future distribution of AHB, over current models that use primarily temperature and humidity. Equally important, the database will be useful for assessing current and future impacts of climate on European Honey Bee health and management. NASA has established a web-based interface **http://honeybeenet.gsfc.nasa.gov** to serve as a focus and a repository of scale hive data for this activity. Participants from over 28 states are expected to contribute data in 2008. We encourage broad participation in the scale hive network in order to assess the regional differences between phenology and nectar flows that occur over this diverse continent.

10. Giovenazzo, P.[l], and P. Dubreuil[m] - INTEGRATED PEST MANAGEMENT AGAINST *VARROA DESTRUCTOR* : TREATMENT THRESHOLDS AND EFFICACY OF THE COMBINED USE OF FORMIC ACID, OXALIC ACID AND THYMOL - Our research goal was to test two different integrated pest management strategies that combine the use of organic pesticides (formic acid, oxalic acid and thymol) and mite economic level thresholds. In spring 2006, forty-eight colonies of equivalent strength were selected. These hives survived wintering without receiving a fall varroa treatment and therefore varroa infestation rates were high and variable (7 ± 11 mite fall/day). The genetic bee stock was a mixture of Primorsky and local Italian *Apis mellifera*. Hives were randomly distributed in experimental groups (Table). The oxalic acid solution (40g/litre in a 1:1 sugar solution) was dripped between each frame of bees (5ml/frame). Each Mitewipe® contained 35 ml of 65% formic acid and two pads were placed on top of the frames of the second brood chamber. Other treatments were applied by the label recommendations. Hives were followed for a year. Statistical data analysis was done using the ANOVA procedure with the SAS software.

Spring treatments in both IPM strategies are inefficient. Daily mite fall increased from mid May to end of July (8.1 ± 3.0 (mean ± standard error) to 17.8 ± 3.0 in IPM1 and from 5.7 ± 1.4 to 21.3 ± 6.6 in IPM2). Mid summer treatments with Mitewipes (2 pads/3x/3days interval) reduced mite fall from 17.4 ± 3.0 mites/day to 12.0 ± 1.8 mites/day. Oxalic acid was inefficient during the same time period: varroa mite fall went from 21.3 ± 6.6 mites/day to 25.6 ± 6.8 mites/day.

Autumn treatments are the most efficient in reducing mite populations and there was no significant difference between strategies from September to November. Analysis of honey (2 samples from total honey harvested in each experimental group) from IPM2 hives showed the presence of formic acid residues (July honey=330 ppm and 317 ppm; September honey=127 ppm and 113 ppm). From May to September the bee and brood numbers and the honey yield were similar between groups. In November 2006 hives were significantly stronger in the IPM1 group (frames of bees: IPM1=8.2 ± 0.2 > IPM2=5.0 ± 0.6; $\alpha<0.01$), a similar result was also measured in May 2007 (frames of bees: IPM1=7.1 ± 0.4 > IPM2=3.4 ± 0.5; $\alpha<0.01$). We suggest that the high varroa levels present in the IPM2 hives at the beginning of September (25.6 ± 27.3 mites/day) were mainly responsible for the low colony strengths measured before and after wintering. The Apiguard® treatment reduced mite populations with good efficacy (92%), but the high September mite load had already affected the physiology of the wintering bees. In conclusion, we suggest that a good varroa control strategy must aim an optimal fall treatment in order to avoid a spring treatment. A mid summer treatment with Mitewipes (2 pads/3x/3 days interval) will reduce mite populations, but care must be taken to avoid formic acid residues in honey.

	Threshold	IPM1	IPM2
Spring	1	MiteAwayII®	Oxalic acid (2x/7days interval)
Summer	11	Mitewipe (3x/3days interval)	Oxalic acid (2x/7days interval)
Autumn 1	11	MiteAwayII®	Apiguard®
Autumn 2	1	Oxalic acid (1x)	Oxalic acid (1x)

Table - Integrated pest management strategies tested.

11. Harris, J.W.[h] – VARROA SENSITIVE HYGIENE AND DRONE BROOD - Honey bees have been bred to express high levels of varroa sensitive hygiene (VSH), which is the removal of mite-infested pupae from capped worker brood. This hygienic behavior is a complex interaction of bees and brood in which brood cells sometimes are inspected, and then brood is either removed (especially if diseased or varroa infested) or recapped (especially if healthy). Previous work has shown that VSH bees uncap and remove significantly more varroa-infested worker pupae than do non-hygienic bees (Harris, *2007 J Apic Res 46: 134-139*), but nothing is known about the reactions of VSH bees towards mite-infested drone brood.

This study compared the reactions of VSH bees to mite-infested worker and drone brood in a field test. Brood combs of both types were chosen so that the majority of host pupae had untanned bodies and non-pigmented eyes. The infestation rate for each comb was estimated before and after placing it into the center of the broodnest of a VSH colony for 1 week. Each colony was given a comb of worker brood; followed by a comb of capped drone brood within 1-2 weeks of the first trial.

Results indicated that VSH bees inspected brood cells containing mite-infested drone pupae, but they did not remove significant numbers of pupae from cells (Figure). Some hygienic responses (e.g. uncapping of mite-infested pupae) were positively correlated to an increasing initial infestation rate in worker brood, but there were no correlations between initial infestation and hygienic responses to drone brood. These results suggest that mite populations in VSH colonies could increase more rapidly when drone brood is available.

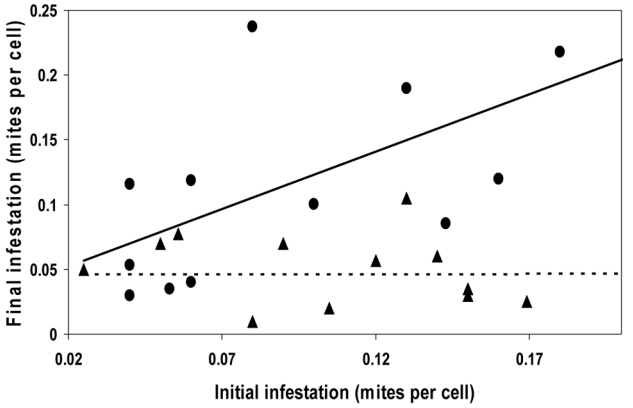

Figure – Comparison of varroa sensitive hygiene directed to mite-infested drone (circles) and worker (triangles) brood during a 1-week exposure of combs to VSH colonies. The final and initial infestation rates should be the same if no hygiene occurred, and the slope of a regression line should equal 1. The slope for drone brood (solid line) was significantly greater than the slope for worker brood (dashed line). Worker brood showed a significant reduction of the final infestation rate caused by removal of mite-infested pupae.

12. Hood, W.M.[n] – RISK OF FEEDING HONEY BEE COLONIES POLLEN SUBSTITUTE PATTIES IN WINTER WHEN SMALL HIVE BEETLES ARE PRESENT – Small hive beetles (SHB), *Aethina tumida* Murray, adults are capable of successfully overwintering in the colony cluster in temperate areas of the world (Hood, *2000 Bee World 81: 129-137*). Normally other life stages of SHB do not overwinter inside or outside the hive in winter. As temperatures increase in spring, the adult SHB break cluster with the bees and will begin the first reproductive cycle, depending on favorable conditions which are unknown at this time. However, some commercial beekeepers feed pollen substitute patties in winter to maintain or increase colony strength for package bee production in spring or early-year pollination purposes. Anecdotal reports by this author and others have indicated that beetle larvae appear in winter when colonies are fed pollen substitute patties, even in cold weather when SHB larvae were not expected. This investigation was conducted to determine the level of risk taken when beekeepers feed pollen substitute patties in winter when adult SHB are present.

Thirty-two honey bee colonies were equalized for bees and brood in fall, 2006, and four apiaries were established with eight colonies per yard in Pickens County, South Carolina. Four colonies in each apiary were randomly selected to receive pollen substitute patties (Global Patties, Bay 2 -8 Eastlake Way NE, Airdrie AB T4A 2J3) continuously from 10 December – 20 March 2007. One pollen substitute patty containing 4% bee pollen was placed across the top bars in the brood chamber of each colony. The remaining four colonies in each apiary received no pollen substitute patties. Capped brood was measured as a colony strength parameter in each colony by placement of a scribed 25cm² piece of Plexiglas over each brood frame on 10 December and during each follow up visit to the apiaries. SHB adults and larvae were surveyed in colonies on 3 January, 15 January, 27 February, and 20 March. SHB adults were surveyed by counting beetles under the inner cover and on the three interior sides and the bottom board of the brood chamber following removal of five brood frames. A total colony examination including frames was conducted to assess SHB larvae counts.

SHB larvae occurred throughout the investigation in colonies having pollen substitute patties and were mainly found concentrated in the pollen patty. SHB larvae were unable to develop past the larval stage and apparently were unable to survive once leaving the warmer area just above the cluster as many dead larvae were found on the bottom board. SHB larvae rarely occurred in control colonies having no pollen substitute patties during this investigation. There was a significant ($P \leq 0.05$) increase in capped brood found in colonies fed pollen substitute patties compared to control colonies that received no pollen patties.

The results of this investigation indicate low risk by beekeepers who feed their colonies pollen substitute patties in winter when conditions are unfavorable for SHB development. However, beekeepers should be conservative in feeding pollen substitute patties when SHB adults are present in late winter or early spring when mild temperatures may persist and result in beetle regeneration earlier than normal.

13. Maisonnasse, A.[o], G. Costagliola[p], F. Choteau[p] & Y. Le Conte[o] - IDENTIFICATION OF VOLATILE COMPOUNDS EMITTED BY EUROPEAN HONEY BEE LARVAE (*APIS MELLIFERA*) - Like many social insects, honey bees have evolved a complex social system in which the brood is completely dependent on nurse bees. To provide optimal care to the larvae, nurses must recognize the various brood instars, their age, sex and needs. Chemical communication seems to be a major factor in this interaction. In 1990, a blend of ten esters that function as a brood pheromone was identified by Le Conte et al. *(Naturwissenschaften 77: 334-336)*. Their experiments focused on the recognition of larvae by nurse bees. However, little is know about the recognition of larval needs. One compound of the blend, methyl linoleate, can increase the amount of royal jelly deposited in cells by workers (Le Conte et al., *1995 J Econ Entomol 88: 798-804*), but we do not know if that compound or others are produced by larvae as a hunger signal.

In this study we focus on volatile compounds that could be produced by larvae as a hunger signal, informing nurses about their needs. We used solid-phase microextraction to sample the volatile compounds emitted by starved larvae. Two candidate molecules (X and Y) were identified. The production of compound X increased during the first 6 h of larval starvation and stabilized at 24 h. Molecule Y production was constant and low during the first 6 h, and increased strongly at 24 h. We hypothesized that the 2 compounds could be hunger signals. Four testable hypotheses related to larval food needs were established (Figure) to determine the biological function of molecules X and Y. Preliminary results indicate that molecule Y can induce increased royal jelly deposition in queen cells during queen rearing. Molecule Y can also significantly delay the age at first foraging in a single cohort colony. Our results suggest that compound Y functions as a hunger signal in honey bee colonies. Additional experiments are ongoing to confirm the biological function of molecule Y and to determine the role of compound X.

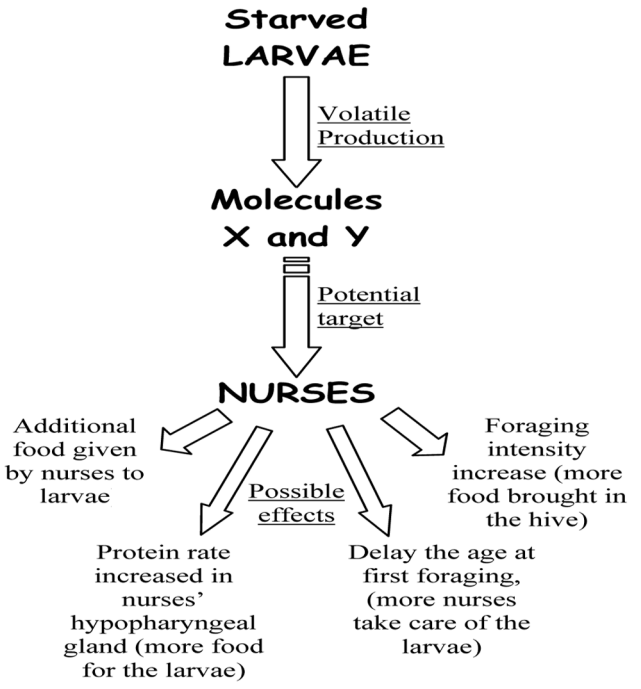

Figure - Four hypotheses we are investigating to determine the roles of 2 potential feeding signals.

14. Martín-Hernández, R.[q], E. Garrido-Bailón[q], A. Meana[r] & M. Higes[q] - NATIONAL SURVEY OF BEE HEALTH IN SPAIN: MONITORING BEE DEPOPULATION SYNDROME - Due to the increasing losses of honey bee colonies in Spain, a research project is being developed by the Apicultural Centre (CAR) of Marchamalo, the Veterinary Faculty of Madrid (UCM) and Chemistry Faculty of Valladolid in order to study the pathological and toxicological agents that could be related with bee depopulation.

Beekeeping associations and veterinary services were contacted to ask for their participation in the study. The epidemiological design for the survey (95% confidence) was made according to the number of hives (2,700,000) and beekeepers (45,000) in Spain and also with the prevalence of depopulation (estimated in 40% due to the absence of official data). All regions were included in the study and it was made throughout 2006 and 2007, sampling in spring and autumn both years.

The hives were randomly selected and all samplings (total of 4) were made on the same sentinel colony, properly identified. Each hive sample included a questionnaire, adult bees, brood, honey and pollen.

So far we have received 1916 samples from the most of the regions of Spain. The Table shows partial results for *Nosema* spp. and *Varroa* prevalence (other determinations on samples are still going on). The prevalence of *Varroa* was higher in autumn than in spring and up to now, all of Korean haplotype (Anderson & Fuchs, *1998 J Apic Res 37: 69-78*). The prevalence of positive samples to *Nosema* spp. was very high in all the studied periods and *Nosema ceranae* has been shown as the most prevalent bee microsporidiosis in Spain. Mixed infections (*N. apis* and *N. ceranae*) have been also observed, especially high in Spring 2007.

N. ceranae is a recently described new microsporidian pathogen of honey bee (Higes et al., *2006 J Invertebr Pathol 92: 81-83*). Our data indicate a very high presence of this parasite and due to its highly pathogenicity for this host (Higes et al., *2007 J Invertebr Pathol 94: 211-217*) it must be taken into account when studying bee losses. On the other hand, although one *Varroa* treatment per year is compulsory and subsidized by the Spanish Government, our data demonstrate that no good *Varroa* control has been reached.

Table - Prevalence of *Nosema* and *Varroa* in samples of honey bees surveyed in Spain.

	Spring 06	Autumn 06	Spring 07	Autumn 07
Varroa	32%	57%	42%	52%
Nosema spp.	52.8%	43.5%	45.7%	ND
Nosema ceranae	38.5%	35.0%	32.6%	ND
Nosema apis	8.4%	4.5%	2.6%	ND
N. apis + *N. ceranae*	5.9%	4.0%	10.5%	ND

ND = no data

15. Niño, E. L.[s], D.R. Tarpy[t] & C.M. Grozinger[u] - BEHAVIORAL, PHYSIOLOGICAL, AND MOLECULAR CHARACTERIZATION OF FACTORS AFFECTING REPRODUCTIVE QUALITY OF HONEY BEE QUEENS (*APIS MELLIFERA* L.) - Commercially raised honey bees used for crop pollination are of tremendous value to our agriculture. The increase in crop value directly attributable to honey bee pollination has been estimated to $14.6 billion in the U.S. alone (Morse & Calderone, *2000 Bee Culture 128: 1-15*). However, the populations of honey bees and native pollinators have declined dramatically over the last twenty years (Kremen et al., *2002 PNAS 99 (26): 16812-16816*). The recent scare by Colony Collapse Disorder (CCD) has brought much needed attention to the current state of our pollinators. Although they are very important for colony survival, honey bee queen health and quality are often overlooked. Since the queen is the only reproductive female in the colony, even minor reduction in her quality and fitness could have profound effects on colony productivity and health. After mating, a queen experiences multiple behavioral and physiological changes (Tanaka and Hartfelder, *2004 Arth Str Devel 33(4): 431-442*; Slessor *et al.*, *2005 J Chem Ecol 31(11): 2731-2745*) which could determine her fate in the hive and the productivity of her colony. In my research, I will focus on understanding the molecular and physiological mechanisms responsible for these dramatic post-mating changes and what factors may modulate these changes.

The first component of my research project will focus on the effects of insemination volume and semen quantity on behavior, physiology, pheromone production, and brain and ovary gene expression of instrumentally inseminated (II) queens. Next, I will consider the effects of carbon dioxide treatment at the behavioral, physiological, and molecular level, since CO_2 is commonly used as the anesthetic agent in II and it stimulates reproduction in virgin queens. Finally, I will consider the effects of sublethal doses of commonly used pesticides on queen rearing and reproductive quality, since pesticides have been implicated as one possible factor in CCD. My preliminary data shows a significant negative effect of oxalic acid and imidacloprid on honey bee queen rearing and quality. Queens were reared in the presence of different concentrations of these pesticides. Number of emerged queens, their total body weights, as well as their lipid weights was observed. We propose to further investigate the effect of pesticides on queen mating behavior (e.g., mating flights, egg-laying pattern), physiological changes (e.g., ovary development, pheromone production, lipid storage), and gene expression patterns in brain and ovaries. We will also extend these studies to determine the effects pesticide treatment may have on immune function and disease resistance in queens.

This research will lead to improved basic knowledge of insect reproduction. It will elucidate the factors affecting honey bee queen mating and begin to relate these to queen reproductive ability. It will also enable improvements in instrumental insemination techniques that are becoming increasingly important for selection of disease-resistant strains and for preventing hybridization with Africanized bees.

16. Nolan IV, M.P.[n] & W.M. Hood[n] - MARK, RELEASE, AND RECAPTURE TECHNIQUES FOR THE SMALL HIVE BEETLE - The small hive beetle (SHB), *Aethina tumida* Murray, has spread rapidly throughout the United States since it was first collected in Charleston, South Carolina in 1996 (Hood, *2000 Bee World 81: 129-137*). By the year 2003 the SHB had spread to 30 states (Hood, *2004 Bee World 85: 51-59*). This rapid migration across the country is primarily attributed to the movement of infested hives, packages of bees, and beekeeping equipment. Although long distance SHB movement has been reported, little is known about SHB movement within an apiary or between apiaries. In order to study SHB short range movement, a mark, release, and recapture technique is needed.

Five apiaries were established in the Clemson University Forest, Pickens County, South Carolina, and were located at least one mile apart. Each apiary contained five colonies started from 2-pound packaged bees and a laying queen purchased from Wilbanks Apiaries, Claxton, Georgia. The colonies were established on 31 March - 1 April 2007. The five colonies were arranged in a straight row all facing the same cardinal direction. On 3-4 May, 150 marked SHB adults were introduced into the first, third, and fifth colonies. Each of the three colonies had uniquely marked beetles.

The marking technique began with lab-raised SHB. Beetles were placed in a freezer briefly to immobilize them. They were then placed in a glass Petri dish on an ice bath to keep the beetles immobilized. Beetles were marked on the posterior end of the ventral side of the abdomen. The marker tested in this project was Testors® model car paint. Releasing was accomplished by opening the inner cover and quickly dumping the beetles in to minimize beetle escape. Beetles were captured using the Hood beetle trap (Brushy Mountain Bee Farm, Moravian Falls, North Carolina)

loaded with vinegar as the attractant and food grade mineral oil as the lethal agent. Two traps were placed in each colony, one in the top super and one in the brood chamber. Traps were placed in the first or tenth frame position. The traps were serviced one week after beetles were introduced, then every two weeks for the next three servicings, then every three weeks for the remainder of the study. Total capped brood for each colony was estimated by using a piece of Plexiglas scribed with 25cm^2 squares placed over the brood frames. Traps were taken out and replaced. Colony beetle numbers were surveyed by adding the number of beetles counted on the bottom side of the inner cover to the number of beetles counted on the three exposed walls and bottom board of the brood chamber with five frames removed.

Of the 2,250 beetles released 175 or 12.86% were recaptured. Of these 18 or 10.3% were beetles captured from colonies they were not introduced. Most of these beetles were captured within the first few weeks of trapping. More marked beetles were likely captured, but we suspect the markings were not holding up. We feel this recapture rate can be improved by use of a more lasting paint. A study using other marking materials will be conducted in 2008. Once an effective mark, release, and recapture technique has been perfected, studies can be performed on SHB movement between local apiaries and within colonies in the same apiary.

17. Ostiguy, N.[v] – FACTORS IMPACTING *VARROA* MONITORING AND POPULATION GROWTH - *Varroa* monitoring increases the time spent on colony maintenance. We explored using the mite drop per day measured in one colony to predict adjacent colony mite levels. While the low-density apiary did show a positive correlation (r=0.8) in adjacent colonies, this relationship did not occur until after day 175 (October) – too late to be practical (**Figure 1**).

In 2007 two apiaries were established with fifteen colonies each. The spacing between colonies in the low-density apiary was 12 feet while the spacing in the moderate-density apiary was 6 feet. Mites were continuously monitored with sticky boards. The moderate density apiary reached the provisional economic threshold (50 mites/day) 28 days earlier than the low-density apiary (p=0.0029). Increasing the distance between colonies delayed mite population growth such that the threshold was delayed until mid-September (**Figure 2**).

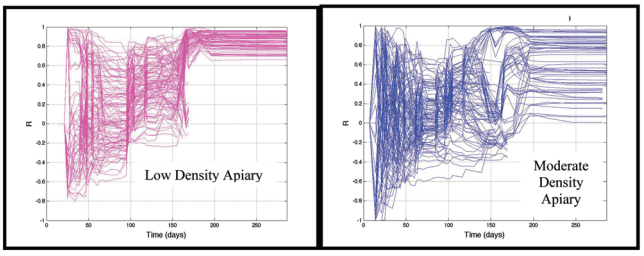

Figure 1 - Mite drop correlation between adjacent colonies through time. A positive correlation between adjacent colonies useful for reducing the number of colonies needing to be monitored does not occur in the moderate-density apiary, while in the low-density apiary a positive correlation is not observed until day 175 (October).

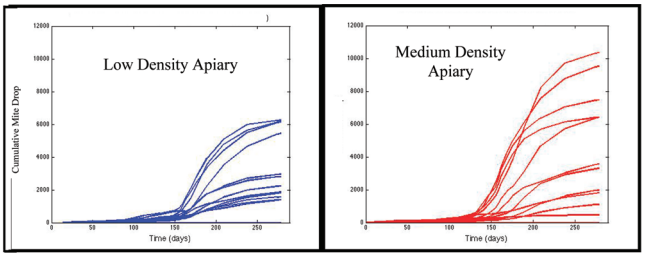

Figure 2 - Cumulate mite drop in low- and medium-density apiaries. Provisional threshold exceeded on day 159 (mid-August) in medium-density apiary, while threshold not exceeded until 187 (mid-September) low-density apiary.

18. Pernal, S.F.[w], J. Pettis[x] & A.P. Melathopoulos[w] - A PRELIMINARY EVALUATION OF CONTROL METHODS FOR *NOSEMA APIS* AND *NOSEMA CERANAE* – *Nosema ceranae* is a highly adapted fungus that is an emergent pathogen of *Apis mellifera*. The organism is now known to be distributed widely throughout Europe, the U.S. and Canada (Klee et al*., 2007 J Invertbr Pathol 96: 1-10*; Chen et al., *2007 J Invertbr Pathol 96: in press*). Though this parasite is similar to the more commonly known *Nosema apis*, increasing evidence has linked *N. ceranae* to the rapid depopulation and demise of colonies (Martín-Hernández et al., *2007AEM 73: 6331-6338*). In order to mitigate the impact *N. ceranae* in Canada, we undertook a preliminary study to survey for its presence, assess its pathology and determine therapeutic control options.

During the summer of 2007, trials were conducted in which 100 newly-emerged *A. mellifera* workers were placed in screened wooden cages. Cages were administered one ml of 1:1 (v/v) sucrose syrup mixed with one of four strains of *N. apis* or one strain of *N. ceranae* (3×10^6 spores/ml). One day after inoculation, bees were fed untreated sucrose syrup or syrup containing fumagillin (25 mg ai/L) *ad libitum* (*n*=5 cages per strain*treatment). Additional cages of bees inoculated with one strain of *N. apis* were also treated with thymol (60 mg ai/L) or lysozyme (5 g ai/L). *Nosema* spores were microscopically counted in dead and live bees after 8, 15 and 22 days. Results showed that both species of *Nosema* appeared to cause similar rates of worker mortality and that fumagillin at prescribed label doses suppressed infections for both *N. apis* and *N. ceranae.* Other treatments were ineffective.

A second series of assays, using three-hole queen shipping cages, were employed to further investigate alternative therapies for *N. apis.* In these trials, ten newly-emerged workers were placed in each cage and were allowed to feed on candy containing medication. These included fumagillin (25, 250 mg ai/L 2M sucrose) and thymol (6, 60, 600 mg ai/L 2 M sucrose); to form candy, medicated syrups were mixed with icing sugar. Albendazole was also tested, formulated as candy directly (60, 600, 6000 mg ai/kg). Four to seven replicates were used per treatment. After seven days, all concentrations of fumagillin were found effective at suppressing *N. apis* infections, though thymol and albendazole were not.

Large-scale colony trials were conducted in the Canada and the U.S. during the fall of 2007, in which medications were assessed for their ability to suppress field infections of both *Nosema* species. Treatments were applied at two intervals, two weeks apart. In Canada, the label rate of fumagillin (190 mg ai/colony) was effective at suppressing infections *N. apis* when examined 28 days after treatment. Field experiments in the U.S. showed reductions in levels of *N. ceranae* spores for treatments of fumagillin fed in syrup at 1, 2 and 4× label rates as well as 1× label rate applications formulated as a sucrose spray or in 20 g icing sugar dustings. Nevertheless, levels of spores in all colonies decreased substantially, including those untreated. These confounding seasonal effects prevented us from drawing firm conclusions about these and other alternative treatments. Additional research is necessary to screen alternative therapies against *N. ceranae* as well determining optimum seasonal strategies for control.

19. Sammataro, D.[y], B. LeBlanc[y], J. Finley[y] & M. Weiss[y] – RECENT FINDINGS IN HIGH FRUCTOSE CORN SYRUP FED TO BEES - High fructose corn syrup (HFCS) is incorporated into numerous processed foods consumed by humans. It is also used by beekeepers to supplement feeding bees during dearth times. Hydroxymethylfurfural (HMF) is a thermally formed, aromatic impurity that is associated with a darker syrup color and is believed to form mostly from the dehydration of fructose. HMF levels are associated with the quality and therefore the value of HFCS, honey, maple syrup and other liquid sweeteners. Samples of HFCS were obtained from domestic manufacturers and were characterized and subjected to isothermal temperatures. The HMF levels were studied at four temperatures, over time, by a variation of a spectrophotometric technique. The samples where analyzed for carbohydrate content by liquid chromatography pulsed amper-

ometric detection. Preliminary results show that starches or complex carbohydrates are present from about 1 to 11% by mass.

20. Simone, M.S.[z], J. Evans[x], & M. Spivak[aa] - COLONY-LEVEL IMMUNITY BENEFITS OF RESIN COLLECTION BY HONEY BEES (*APIS MELLIFERA*) - One surprising finding from the recent genome sequencing project of the honey bee, *Apis mellifera*, is that honey bees have a substantially decreased diversity of immune-related genes when compared to other insect genomes. Honey bees are highly social insects that live in large colonies, which can increase the rate of disease transmission. One way that individuals within a densely populated society, such as a honey bee colony, can compensate for their reduced physiological ability to recognize and resist pathogens is through the evolution of defenses that emerge at the colony-level due to the collective behaviors of individuals.

This study aimed to determine if the use of resins, complex plant secretions with diverse antimicrobial properties, is a colony-level defense against pathogens by honey bees. The harvesting of antimicrobial compounds from the environment and the incorporation of these compounds into social nest architecture as propolis is an exciting but relatively unexplored colony-level defense.

We hypothesized that the presence of propolis within the colony reduces the amount or diversity of pathogenic microbes within the nest and thus results in a lowered physiological investment in the production of antimicrobial peptides in bees throughout the colony. Colonies were experimentally enriched with a propolis extract from Brazil, a propolis extract from Minnesota, or were deprived of propolis. Bees of known age (1-day old, 1-week old) were collected from each colony for analysis. We measured gene transcript levels of 4 antimicrobial peptides (abaecin, apidaecin, defensin1, hymenoptaecin) and a gene involved in cellular immunity (AmEater) to determine if the presence of propolis in colonies has the effect of down-regulating or decreasing the inducible immune response of individual adult bees. In addition, we measured gene transcripts of the 16S rRNA loci as a generic indicator of the presence of eubacteria, and of vitellogenin as a general stress indicator.

Among the 1-week old bees, significant differences were found for only two gene transcript levels: hymenoptaecin and AmEater. In both cases, bees collected from the colonies treated with Brazilian propolis had reduced levels of the gene transcripts as compared to those from the propolis-deprived colonies. 1-week old bees from the MN-propolis treated colonies had significantly less hymenoptaecin than bees from the propolis-deprived colonies, but were intermediate for AmEater gene transcript levels. Results suggested that individual bees' immune systems in propolis-deprived colonies were up-regulated, as indicated by higher immune-related gene transcript levels. Our results, however, did not necessarily indicate that the down-regulation seen in bees from propolis treated colonies was due to a reduction in microbes found in the nest, as there were no differences in general eubacteria. Future studies will investigate whether propolis in the nest causes a reduction in specific or pathogen-related microbes in addition to general eubacteria.

It is important to note that the colonies used in this experiment were not challenged by pathogens and in general under no stress, and future research will investigate the effect of challenging bees in a propolis-rich environment. This research is the first report that we know of showing that the nest environment can affect immune expression in honey bees.

21. Strange, J.P.[f] & N.W. Calderone[e] – EVALUATION OF ECONOMIC TRAITS IN PACKAGE BEES - We examined 48 packages representing six lines of bees purchased in the spring of 2006. We estimated levels of the parasitic mite *Varroa destructor* Anderson and Trueman and the percentage of drone (male) honey bees received in packages. We surveyed for presence of the tracheal mite *Acarapis woodi* (Rennie) and a microsporidian parasite, *Nosema* spp., in the shipped bees. We found significant differences in both the mean varroa mite per bee ratios (range from 0.004 to 0.054) and the average percent drones (range from 0.04 % to 5.1 %) in packages from different producers. We found significant differences in the number of *Nosema* infected packages (range from 0.0% to 75.0 %) among the six lines. No packages contained detectable levels of *Acarapis woodi*. Considering the variability among purchased packages, beekeepers should be aware of the potential for pest and disease infestations and high drone levels in packages.

Table - Evaluation of package bees.

Producer	Line (n=)	Queen failure	*Varroa* (mites/bee)	*Varroa* SER*	Packages w/*Nosema*	Percent Drones	Tracheal mites
A	1	12.5 %	0.034 ± 0.008bc	5.71 ± 1.30	25.0 %	5.1 ± 1.3b	ND**
B	2	12.5 %	0.054 ± 0.017c	9.01 ± 2.81	37.5 %	4.5 ± 2.3b	ND
C	3	0.0 %	0.004 ± 0.002a	0.67 ± 0.30	0.0 %	0.6 ± 0.2a	ND
D	4	12.5 %	0.032 ± 0.006bc	5.42 ± 1.05	0.0 %	0.04 ± 0.04a	ND
D	5	12.5 %	0.027 ± 0.007ab	4.46 ± 1.13	75.0 %	0.2 ± 0.1a	ND
D	6	0.0 %	0.032 ± 0.005bc	5.35 ± 0.84	50.0 %	0.09 ± 0.06a	ND
	All (n = 48)	8.33 %	0.030 ± 0.004	5.10 ± 0.67	31.25 %	1.75 ± 0.53	

***SER = standardized 300-bee ether roll count **ND = none detected**

Swanson, J.[aa], S. Kells[aa], B. Torto[bb] & M. Spivak[aa] - ODE D' DISEASED BROOD: VOLATILES THAT ELICIT HONEY BEE HYGIENIC BEHAVIOR - Honey bee hygienic behavior is an economically important mechanism of resistance against brood disease. Colonies selected for hygienic behavior detect, uncap and remove diseased brood from the colony before the pathogen reaches an infectious stage, thus reducing disease transmission. Hygienic bees have greater olfactory sensitivity to the odor of diseased brood compared to non-hygienic bees, but the volatile compounds that elicit the behavior have not yet been determined. The goal of this study was to determine which compounds associated with diseased brood the bees detect and to assay whether the application of these compounds to healthy brood would elicit the motor tasks of uncapping and removal of the brood.

Volatile compounds were collected from healthy honey bee larvae and from larvae infected with chalkbrood *Ascosphaera apis*. The volatiles were analyzed by mass spectrometry and the chemical profiles compared to determine which compounds were unique to chalkbrood-infected larvae. Gas chromatography coupled with electroantennographic detection (GC-EAD) determined that adult honey bees detected three compounds unique to diseased larvae: benzyl alcohol, phenyl alcohol and phenethyl acetate.

Proboscis extension response studies demonstrated that bees from hygienic and non-hygienic colonies could be conditioned to respond equally well to all three compounds and a mixture of the three compounds at two concentrations. However, a field bioassay showed that only one compound, phenethyl acetate, and a mixture of the three compounds elicited hygienic behavior. In this assay a picospritzer was used to apply individual compounds onto healthy fifth instar larvae within a colony. Removal of treated larvae by bees from 12 colonies varying in their levels of hygienic behavior based on a freeze-killed brood assay was monitored at 4 and 24 hours. Phenethyl acetate and the mixture showed a significant relationship between the level of hygienic behavior of a colony and the proportion of treated larvae that were removed. In the future, beekeepers may use this bioassay to select their most hygienic colonies for breeding.

23. Taylor, M.A.[cc] & E. Guzmán-Novoa[dd] – NEW ANSWERS TO AN OLD QUESTION: IMPROVED METHODS FOR THE CRYOPRESERVATION OF HONEYBEE (*Apis mellifera* L.) SEMEN - The objective of this study was to test diluents, cryoprotectants, and semen dilutions using a new freezing protocol to improve post-thaw spermatozoa viability of cryopreserved honeybee (*Apis mellifera* L.) semen for instrumental insemination (II) purposes. Previous attempts have mixed semen with various extenders (diluents) and cryoprotectants (glycerol or DMSO), before storing samples in liquid nitrogen. However, the use of relatively simple freezing protocols coupled with inadequate diluents

and cryoprotectants, has resulted in poor post-thaw sperm viability. We assessed multiple diluents, cryoprotectants, and diluent:semen collection ratios, as a means of improving post-thaw viability. In addition, samples underwent cooling and freezing cycles in a computer-controlled rate freezer. Initially, semen was collected and pooled from mature drones for each diluent at a 1:1 ratio. Samples were analyzed for viability using the dual SYBR-14 and propidium iodide (PI) staining method, and visually assessed for motility. Based upon their elevated motility levels, diluent 2 and 4 were used in all subsequent experiments. Specific protocols were then developed to control the freezing and thawing rates of the samples. Semen samples collected at ratios of 1:1 and 3:1, were added to one of three cryoprotectants (DMSO, glycerol, DMA), and frozen. Diluent 4 provided significantly higher post-thaw viability than either diluent 2 or the control (Kiev diluent). Post-thaw viability was significantly higher for samples collected at a 3:1 ratio than a 1:1 ratio, while DMSO produced significantly higher post-thaw viability levels than glycerol or DMA. To determine optimal collection ratios, diluent 4 in combination with DMSO was added to samples collected at a 6:1, 9:1, and 12:1 diluent:semen ratio. Collection ratios significantly affected sperm post-thaw viability. Dilution ratios of 6:1 and higher produced significantly higher post-thaw viability levels than lower dilution ratios. There was no significant difference among 6:1, 9:1, and 12:1 ratio treatments. These new methods improve post-thaw viability of honeybee spermatozoa, with viability levels as high as 68.3% ±5.4, and thus provide potential for selecting specific genetic characteristics to directly influence colony fitness.

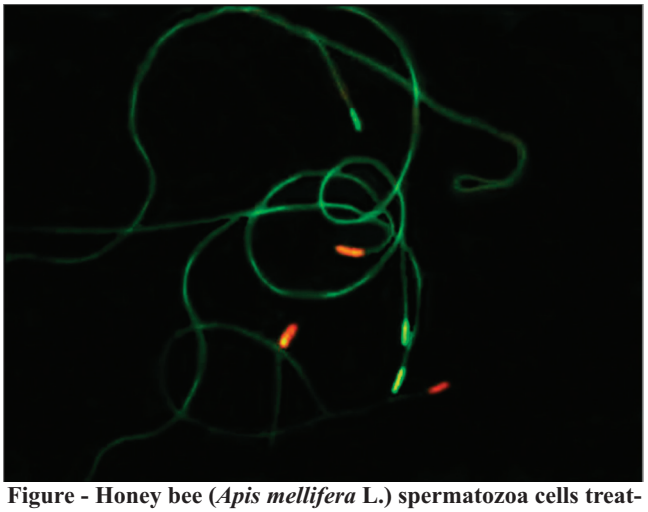

Figure - Honey bee (*Apis mellifera* L.) spermatozoa cells treated with the dual-fluorescent stain SYBR-14 and propidium iodide (PI).

24. Villa, J.D.[h] – EVALUATIONS OF RESISTANCE TO TRACHEAL MITES IN U. S. BEES – Honey bees in the United States have been exposed to tracheal mites for more than two decades, but problems still are found when certain factors coincide. To maximize our efficacy in finding infested colonies for research, we sample unselected colonies in the winter in Louisiana and neighboring states. It is common to find between a quarter and a third of colonies from unselected sources with infestations above the economic threshold of 20%, especially in colonies moved from northern states for the winter.

During the last ten years we have evaluated the genetic resistance of colonies from eleven sources using bioassays of newly emerged workers exposed to tracheal mites in infested colonies (Gary & Page, *1987 Exp Appl Acarol 3: 291-305*). In 1999, the resistance of colonies from eight commercial sources (n = 6-19 per source) was compared to colonies known to be resistant or susceptible (Danka & Villa, *2000 Am. Bee J. 140: 405-407*). Using these standard colonies, a resistance index can be used to place each colony on a common scale from 0 to 1 (similar to resistant or susceptible standards, respectively). Five out of eight commercial sources showed mean susceptibilities above 0.4 and a high amount of variability between colonies (Danka & Villa 2000, cited above, results summarized in Figure). In subsequent years, colonies from three of those sources (n = 9-63 per source per year), plus colonies from three additional sources (n = 5-88 per source per year) were evaluated with the same procedures. The levels of resistance in four of the six sources appear to have deteriorated or not improved through time, with their most recent mean resistance indices being higher than 0.5 (Figure). Beekeepers should be aware of the fact that uniformly high resistance to tracheal mites is not found in all commercial sources. Active selection for resistance by breeders and purchasing of resistant breeding material by queen producers is recommended to solve these persistent problems with tracheal mites.

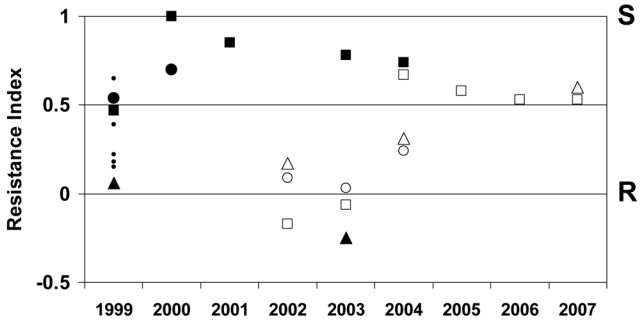

Figure – Mean tracheal mite resistance indices of colonies from 11 sources evaluated through time. Infestations of young workers exposed in highly infested colonies are scaled to infestations of similarly treated workers from standard resistant (R) and susceptible (S) colonies. Colonies from eight commercial sources were evaluated in 1999 and are indicated with solid symbols. Colonies from three of those sources (●, ■ or ▲) plus three additional ones (○, □ or △) were tested in subsequent years and mean indices obtained in different years are indicated.

25. Wagnitz, J.[ee] & M. Ellis[ee] - COMBINING AN ARTIFICIAL BREAK IN BROOD REARING WITH OXALIC ACID TREATMENT TO REDUCE VARROA MITE POPULATIONS - Sixty-two colonies were set up to test the hypothesis that varroa control with oxalic acid (OA) can be enhanced by combining treatment with late summer requeening. Each colony consisted of two full depth boxes and nine frames. All colonies were queen right at the beginning of experiment and had brood present. Moving sealed frames of brood three weeks prior to the study equalized mite infestation. Pre-treatment alcohol samples were taken to determine varroa mite populations. Approximately 300 adult bees were collected pre- and post-treatment to calculate mite per adult bee using the alcohol wash technique (Shimanuki & Knox, *Agricultural handbook No. AH-690*). This experiment consisted of four treatment groups, requeen plus OA, requeen only, OA only, and controls (untreated). Sister queens were grafted to be used in the requeening treatments. Queens were caged in the requeen treatment groups five days prior to placing a sealed queen cell in the colonies. This provided a period of 18-21 days without egg laying, which allowed all of the brood present to emerge. The absence of brood resulted in all of the varroa mites becoming phoretic.

A 50 ml application of a 3.0% OA sugar water solution (sugar:water) (1:1) (w:w:w) was applied to the requeen plus OA, and OA only treatment groups. The two-story colonies were separated and each half received approximately 25 ml of the OA solution. The OA solution was trickled from above the frames into the bee-ways with a 100 ml syringe and an effort was made to maximize adult bee contact with the solution. OA treatment occurred later in the afternoon when most of the colony's adult bee population was present. Post-treatment alcohol samples were taken four days after treatment. The data indicates that the mite populations

did change between pre- and post-treatment for all of the treatment groups (**Figure**). The two groups that were requeened were the only treatments that exhibited a significant decrease in mite population. Data indicates that combining late summer requeening with OA treatment significantly reduces mite populations compared to untreated colonies, colonies that are requeened but not treated, and colonies only treated with OA.

Even though we had a significant decrease in the treatment group that was requeened and treated with OA, we found evidence that the presence of brood is not the only factor affecting the efficacy of oxalic acid treatments.

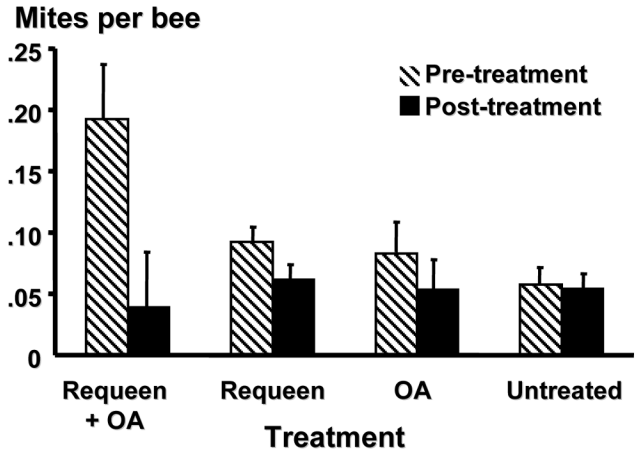

Figure. Pre-treatment and post-treatment mites per adult bee

26. Webster, T.C.[ff] & E.M. Thacker[ff] - THE EFFECTS OF SELECTED AGENTS ON *NOSEMA APIS* AND *NOSEMA CERANAE* SPORE VIABILITY – The effects of chemical and physical treatments on *Nosema apis* and *Nosema ceranae* spores were studied. One goal is to develop treatments for contaminated bee hive equipment that are safe, effective and inexpensive for beekeepers. We also wish to compare the hardiness of spores of the two *Nosema* species. Perhaps spore hardiness will help to explain the distribution and persistence of *N. apis* and *N. ceranae*, in the United States and other parts of the world.

Spores were obtained from infected honey bee colonies kept at Kentucky State University. Liquid chemical treatments were either methanol, 70% ethanol, isopropanol, or 3% hydrogen peroxide. For these treatments a purified preparation of spores was placed into the liquid for 20 minutes (alcohols) or 30 minutes (hydrogen peroxide). The spores were then cleaned of the treatment by rinsing with water and centrifuging five times. Spores were then stained in a test for membrane integrity (Green et al., *2000 J Clin Microbiol 38(10): 3811-3814*). In this method, the sytox green will enter spores with ruptured membranes and stain the DNA. Spores are then examined by fluorescent microscope to determine which contain sytox green, indicating that they have ruptured membranes and must be dead.

Volatile chemical treatments were either Apilife VAR or paradicholorobenzene (PDB). A preparation of spores was allowed to dry on a microscope slide and then contained with the treatment inside glass Petri dishes for 24 hours. Staining followed as above. Physical treatments were heat at 60^{O} C (for 30, 60 or 120 minutes) or short-wavelength ultraviolet (UV) light for 1, 5 or 10 minutes. Staining followed.

Methanol, ethanol and isopropanol killed 52%, 74% and 52% of the *N. apis* spores, respectively. These alcohols killed 69%, 50% and 86% of the *N ceranae* spores. Hydrogen peroxide killed 70% and 52% of *N. apis* and *N. ceranae* spores, respectively. In comparison, control preparations of *N. apis* and *N. ceranae* contained 26% and 14% non-viable spores, respectively.

ApiLifeVAR killed 64% and 82%, respectively. PDB killed 67% and 70%, respectively. Treatment with 60^{O} C for 30, 60 or 120 minutes killed 47%, 53% and 53% of N. *apis* spores, respectively. The corresponding values for *N. ceranae* spores killed were 42%, 78% and 88%. UV light for 1, 5 or 10 minutes killed 84%, 96% and 100% of *N. apis* spores. The corresponding values for *N. ceranae* spores were 59%, 49% and 65%.

These results encourage us to test the above agents for longer exposure periods, in hopes that control will approach 100%. The comparison of *N. apis* spore hardiness to that of *N. ceranae* is intriguing. However, it will be necessary to test spores from multiple locations for both species to make any compelling argument about the relative hardiness of these *Nosema* species. Also, this staining method may be used to evaluate the viability of spores that are used to inoculate bees in controlled experiments.

27. Williams[gg], G.R., A.B.A. Shafer[gg, hh], D. Shutler[gg], R.E.L. Rogers[ii] & D.T. Stewart[gg] – *NOSEMA* AND BEES HAVE INCONGRUENT PHYLOGENIES: HOW, WHY, AND WHAT MIGHT THIS PORTEND? - Cophylogenetic studies investigate the evolutionary history of interacting organisms to reveal macroevolutionary processes that shaped present-day interactions. Unfortunately, cophylogenies of many host-parasite associations remain unclear; for example, previous phylogenies of microsporidians of the genus *Nosema* have produced conflicting results (e.g., Fries et al., *2001 J Apic Res 40:91-96*; Slamovits et al., *2004 J Eukaryot Microbiol 51: 207-213*; Vossbrinck and Debrunner-Vossbrinck, *2005 Folia Parasitologica 52:131-142*). *Nosema* species associated with bees have considerable economic importance. Historically, *Nosema apis* and *N. ceranae* were known from Western (*Apis mellifera*) (Fries, *1993 Bee World 74: 5-19*) and Asian honey bees (*A. cerana*) (Fries et al., *1996 Europ J Protistol 32: 356-365*), respectively, and *N. bombi* from multiple bumble bee (*Bombus*) species (Tay et al., *2005 J Eukaryot Microbiol 52: 505-513*), but recent evidence suggests *N. ceranae* has made a host switch to *A. mellifera* within the last decade (Klee et al., *2007 J Invertebr Pathol 96: 1-10*). Symptoms of *Nosema* disease vary among host-parasite systems, but generally are associated with reduced colony productivity (Moeller, *1972 J Apic Res 11: 117-120*; Schmid-Hempel, *1998 Apidologie 29: 525-535*; Higes et al., *2006 J Invertebr Pathol 92: 93-95*).

Clarifying the coevolutionary history of *Nosema* infecting bees may help explain host specificity and virulence, and improve treatment against *Nosema*. Our first objective was to reanalyze evolutionary relationships among *Nosema* species infecting *A. mellifera, A. cerana,* and *Bombus* spp. (Hymenoptera: Apidae). Our second objective was to assess congruence between host and parasite phylogenies.

Using established protocols, large and small subunit ribosomal RNA sequences of *Nosema* species infecting bees were analyzed separately. In nearly all analyses, we observed a sister relationship between *N. ceranae* and *N. bombi*. When compared to the phylogeny constructed from cytochrome *b* data of their respective hosts, two plausible scenarios emerged, each consisting of a cospeciation, a sorting event, and a host- switch. The first scenario has a common ancestor of *N. bombi* jumping from a historical *Bombus* lineage to *A. cerana*. *N. bombi* infects a variety of different host tissues in multiple bumble bee species (Fries et al., *2001 J Apic Res 40: 91-96*); this low host specificity may have facilitated host-switching. The second scenario has an ancestral *N. ceranae* jumping to a species of *Bombus*. The recent jump by *N. ceranae* to the Western honey bee (Huang et al., *2007 Apidologie 38: 30-37*) suggests a common ancestor could possibly switch hosts when suitable conditions occur (e.g., overlapping of ranges).

Although *N. ceranae* is not likely to be the sole cause of recent colony deaths in North America, it may be a contributing factor (Oldroyd, *2007 PLoS Biology 5: 1195-1199*). Work is needed to test whether fumagillin, an antibiotic used to control *N. apis*, is efficacious against *N. ceranae*, because it was ineffective against *N. bombi* infecting *Bombus occidentalis* (Whittington and Winston, *2003 J Invertebr Pathol 84: 54-58*). In addition, our results showing *N. ceranae* closely related to *N. bombi*, as well as experimental infections showing susceptibility of bumble bees to the more distant *N. apis* (Fantham and Porter, *1913 Ann Trop Med Parasitol 7: 569-579*), suggest that this genus may be vulnerable

to *N. ceranae*, especially as other bumble bee species encounter this parasite around the world.

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