Proceedings of the American Bee Research Conference

The 2006 American Bee Research Conference was held January 9-10 at the Embassy Suites Hotel in Baton Rouge, Louisiana. The twentieth American Bee Research Conference will be held in conjunction with the American Honey Producers' Association at the Sheraton Crescent Hotel in Phoenix, Arizona on January 8-13, 2007. The following are abstracts from the 2006 Conference.

1. Cox, R.L.[a], and F. A. Eischen[a] – TEST OF AN INTEGRATED VARROA MANAGEMENT PLAN WITH AFRICANIZED HONEY BEES IN SOUTH TEXAS – In order to slow development of resistance in varroa mites to miticides and avoid the resultant damage to honey bee colonies, integrated pest management strategies are needed. Genetically resistant bee stocks and equipment modifications are two non-chemical tools than can be utilized to reduce pest populations. Africanized honey bees and bees carrying the SMR trait have shown genetic resistance to varroa mites (Guzman-Novoa, 1996 *Apidologie* 27: 93-103; Harbo and Harris, 2003 *Am. Bee J.* 143: 213-216). Screened bottom inserts have also reduced varroa mite loads in honey bee colonies (Pettis & Shimanuki. 1999. *Am. Bee J.* 139; 471-473). The purpose of the present study was to determine how different bee stocks and screen bottom inserts affect the varroa mite and bee population growth, honey production and time until the economic threshold is reached for mite treatments in honey bee colonies located in a southern Texas thorn brush forest.

Colonies were established with 2.5 lbs. package bees and one of four types of queens: Italian, SMR, Africanized (AHB) and SMR mated to AHB drones (SMR × AHB) in April 2005. Although not significant, SMR × AHB colonies were heavier and had more frames of honey than either Italian or SMR colonies (see Table below). Italian colonies had significantly higher varroa populations compared to other breeds. Bottom type had no overall effect on the number of varroa, brood or adults, but colonies with screened bottoms had significantly fewer frames of honey and smaller colony weight than with solid bottoms. However, when populations were high in October, it did appear that varroa numbers were reduced when using screened bottoms ($P<0.05$). Overall, trends indicated that colonies with SMR × AHB or AHB queens were stronger, heavier and carried fewer varroa mites. These results suggest that these stocks could be included in an integrated honey bee colony management plan to control varroa in south Texas.

Table – Colony strength (mean number of frames of adult bees, brood area and colony weight), honey production (frames of stored honey) and varroa population (mites on sticky board inserts) of honey bee colonies with either an Africanized (AHB), Italian, SMR or SMR × AHB queen. Means are for monthly measurements for each colony for each type of queen from June through November, 2005.

Type of Queen	Number of colonies	Colony Weight (kg)	Frames of:			Varroa mite fall
			Honey	Adult Bees	Brood (cm^2)	
SMR × AHB	15	38.0a[2]	5.1a	12.4a	4618b	7.2b
AHB	15	36.3a	4.1a	12.9a	5650a	11.0b
SMR	15	35.6a	4.2a	10.0b	3754c	15.5ab
Italian	15	35.4a	4.0a	10.4b	4096c	24.3a
F-value		1.56	1.9	4.34	8.24	3.32
df		3, 54.7	3, 46.8	3, 48.5	3, 48.5	3, 51.3
p-value		0.209	0.134	0.009	<0.001	0.027

[2] **Means followed by the same letter in a column are not significantly different (α= 0.05) from one another according to Fisher's protected LSD (Proc Mixed using repeated Measures statement, SAS 2004)**

2. Cox, R.L.[a] & P.J. Elzen[b] - SUITABILITY OF RUSSIAN HONEY BEES TO SOUTH TEXAS DRY SUBTROPICAL CONDITIONS - Honey bees introduced from Far East Russia have shown genetic resistance to varroa mites (Rinderer *et al.,* 1999, *Am. Bee J.* 139: 287-290). This study was designed to compare the performance of Russian honey bee colonies to Italian honey bees in the dry subtropical climate of extreme southern Texas when managed according to the specific recommendations for Russian honey bees (Tubbs *et al.,* 2003, *Am. Bee J.* 143: 819-820).

An apiary was established near Weslaco, TX with 20 bee packages installed in single hive bodies on new comb foundation April 7, 2004. On April 15th ten colonies were requeened with marked Russian queens obtained from the USDA Baton Rouge Bee Lab, and the other ten colonies were requeened with marked Italian queens from a California queen breeder. Colonies were arranged in the apiary so that every other colony was Russian. Every month for 14 months (until June 2005) the colonies were evaluated for colony weight, the number of frames of brood, adult bees and honey and the condition of the brood and queen performance.

There were no significant differences in colony strength between colonies headed by Italian or Russian queens (Table). However, Russian colonies were numerically stronger and more productive than the Italians. Small sample size (10) may have limited our ability to detect statistically significant differences

Table – Comparison of colony strength of Russian and Italian honey bees in an apiary in deep south Texas near Weslaco from May 2004-June 2005. Means for each variable are for all monthly measurements for each colony for each breed combined. The mean varroa mite density is for samples collected in June 2005.

Bee Breed	No. Cols	Colony Weight (kg)[1]	Frames of:			Mite Density 6/13/05
			Honey	Adult Bees	Brood	
Italian	10	42.7a	6.50a	11.51a	3.19a	3.06a
Russian	10	45.4a	7.38a	12.18a	2.77a	1.49b

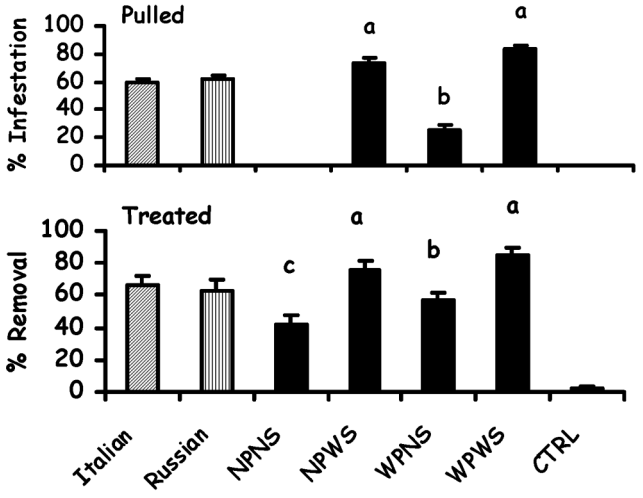
[1] **Means followed by the same letter in a column are not significantly different at 0.05 level by the GLM procedure (SAS Enterprise Guide 2004)**

between the two stocks. Additionally, the differences between the colonies were probably lessened later in the study because of queen supersedure and subsequent matings of replacement queens with the same local drone population. In August and December 2004 varroa mite populations were significantly smaller in the Russian colonies as measured by mite fall on sticky boards. At the end of the study (June 2005) the varroa mite density on adult bees in Russian colonies was significantly smaller as measured by an alcohol wash (Table). In this study Russian bees appeared to be as strong and productive as Italian bees and therefore, suitable for beekeeping in extreme southern Texas.

3. de Guzman, L. I.[c] & A. M. Frake[c] - PRELIMINARY RESULTS ON THE REMOVAL RESPONSE OF RUSSIAN HONEY AGAINST BROOD INFESTED WITH SMALL HIVE BEETLES - Removal response of Russian (n = 9) and Italian (n = 9) honey bees against brood infested with small hive beetles (SHB) was compared. SHB-infested brood was obtained as described by Ellis *et al.* (2003, *Naturwissenschaften* 90: 532-535) with modifications. For each colony, three sections of brood (58 cm^2 each) were caged as follows: control (no beetle), treated (20 beetles) and pulled (20 beetles). Adult beetles were caged for 15 h. Each brood section was examined under the microscope for the presence of perforations and then mapped using photo prints. All brood cells in the pulled section were opened and examined for the presence of eggs, which provided infestation data for the treated section. Brood removal was assessed 20 h after brood frames were returned to their colonies. For analyses, brood cells in each section were grouped as follows: a) NPNS = no perforation on capping and cell wall; b) NPWS = with cell wall perforation only; c) WPNS = with capping perforation only; and d) WPWS = with capping and cell wall perforations.

The figure shows that brood removal in the Italian and Russian honey bees were similar (P=0.715). However, the rate of brood removal was influenced by the presence of perforations on the cell wall (P< 0.0001). Brood removal was highest in the NPWS and WPWS groups, which corresponded well with their infestation levels. Our results were similar with those observed by Ellis *et al.* (2004, *Ann. Entomol. Soc. Am.* 97: 860-864) in SHB-resistant Cape bees (67%) and European bees (57%). However, when restricting the analysis to only the NPWS and WPWS data, a higher removal rate of about 80% was observed for both stocks. Thus, our data suggest that Russian and Italian honey bees detected and removed SHB-infested brood. This ability of both stocks may contribute to the regulation of beetles in their colonies.

Figure - Percentage of SHB infestation (pulled) and 20 h-brood removal (treated) by Italian and Russian honey bees and in the different groups of cells.



4. Delaplane, K.S.[d] & J.D. Ellis[d] - VARROA IPM: DOES IT WORK? DOES IT PAY? - An integrated pest management (IPM) approach to Varroa control has been a goal of labs around the world for decades. Specific practices, such as genetically resistant bees, screen hive floors, drone brood trapping, and dusts have been shown to eliminate mites from a colony or limit population growth without the use of acutely toxic miticides. In recent years the enterprise has matured to show that complete systems integrating multiple tactics are efficacious (Ellis *et al.* 2001, *Am. Bee J.* 141: 813-816; Rinderer *et al.* 2003, *Am. Bee J.* 143: 410-413; Rinderer *et al.* 2004, *Am. Bee J.* 144: 481-485; Rice *et al.* 2004, *Am. Bee J.* 144: 791-795; Sammataro *et al.* 2004, *Int. J. Acarol.* 30: 71-76; Delaplane *et al.* 2005, *J. Apic. Res.* 44: 117-122).

No matter how well IPM controls Varroa, beekeepers will not adopt it until it has been shown to be advantageous practically and economically. We here report one year's data from a two-year project comparing mite control and economic performance of three management schemes. Six collaborating beekeepers in Georgia are each providing 21-30 colonies. Within apiary, each test colony is assigned one of three treatments: (1) "coumaphos" in Feb and Aug, (2) "IPM," consisting of Russian queen + screen hive floor, or (3) "control" consisting of no coumaphos, solid floor, and non-selected queen. Queens of the two types were marked and replaced as needed over the course of the study. On five sampling episodes between Feb and Nov 2005, mite levels (24-hr sticky sheets) were numerically highest in controls and lower, but not different between, coumaphos and IPM. By December, 21 control colonies had reached the lower treatment threshold of ≥60 mites / 24-hr sticky sheet of Delaplane & Hood (1999, *Apidologie* 30: 383-395) compared to 6 coumaphos colonies and 8 IPM. Summed honey production was 2800 pounds in control colonies, 2950 in coumaphos, and 3200 in IPM. Summed beekeeper work hours was 18.3 hr in control colonies, 19.1 in coumaphos, and 16.8 in IPM; these numbers do not yet include time spent counting sticky sheets which was done by experimenters. The percentage of colonies requiring queen replacement over 7 months was 48.9% in control and coumaphos colonies compared to 44.7% in IPM. Number of colony deaths was 6 in the control colonies, 7 in coumaphos, and 2 in IPM. In summary, at the half-way point in this study it appears that IPM provides Varroa control at levels equal to coumaphos with no cost to honey production, beekeeper colony labor, queen survival, or colony survival.

5. Eischen, F.A.[a], R. H. Graham[a], & R. Rivera[a] - ADULT BEE PROTEIN LEVELS IN COLONIES POLLINATING ALMONDS IN CALIFORNIA - Colonies brought to California for almond pollination occasionally experience severe dwindling, sometimes to the point of colony collapse. This study was done to establish a baseline of nutritional status among pollinating colonies as a first step in determining if nutritional stress is involved in dwindle. We randomly sampled 916 colonies belonging to 46 beekeepers, whose bees were pollinating almonds in California during February, 2005. New adults and adults sampled from the broodnest were examined for soluble protein using the Bradford reagent technique. All data are averages of 20 bees/colony.

Overall, protein levels in new adults ranged from 5.3 – 36.0 mg per bee. Broodnest bees ranged from 8.3 – 49.5 mg/bee (protein levels in bees with or with their guts were not significantly different). Protein levels of newly emerged adults were, on average, positively correlated with their weight ($r = 0.52$, $P < 0.0001$, $n = 179$), and with the protein levels of the adults bees sampled from the broodnest of these colonies ($r = 0.231$, $P < 0.0001$, $n = 914$).

The protein levels of broodnest adults in the smallest colonies (1-3 frames) were on average 24.9 mg/ml, while levels in the strongest colonies (≥ 14 frames) averaged 29.6 mg/ml ($P < 0.05$). Similarly, new adults in the smallest colonies had 19.8mg of protein, while the largest colonies had 21.5mg of protein ($P < 0.05$). In both the new and older workers, a stepwise progression of increasing protein levels was found in colonies with increasingly larger

adult worker populations. In general this was not true of broodnest size, i.e., larger broodnests were not always associated with higher protein levels.

Individual beekeepers had colonies that uniformly showed striking asymmetries of protein levels in older bees and new adults, i.e., older bees with relatively high protein levels, while new adults were quite low. The reverse was occasionally true as well. We do not know the cause for this.

Previous studies have found that weight is associated with protein levels and both are correlated with longevity (de Groot, 1953, *Physiol. Comp. Oecologia* 3: 197-285; Eischen *et al.*, 1982, *J. Apic. Res.* 21: 19-25). Haydack (1934, *J. Agr. Res.* 49: 21-28) reported that emerging bees in Minnesota had about 13% of their fresh body weight composed of protein. Occasionally we saw protein levels this low or lower in new adults, but generally levels were higher. Kleinschmidt & Kondos (1976, *Austr. Beekeeper* 78: 36-39) on the other hand observed that wintering bees in Australia had protein levels of 45%. This exceeds many of those in this survey.

Colonies with long lived bees, in theory, should grow faster than colonies producing young bees with marginal levels of protein because bees with higher protein levels will tend to live longer. Colonies producing long lived adults are less likely to exhibit dwindle.

6. Frake, A. M.[c] & L. I. de Guzman[c] - COLONY INVASION OF SMALL HIVE BEETLES: THE EFFECTS OF HONEY BEE TYPE AND ENTRANCE REDUCERS - First detected in Florida in 1998, small hive beetles (SHB) are now found in at least 30 states. Although SHB can kill colonies (Elzen *et al.*, 1999, *Apidologie* 30: 361-366), survival of infested colonies may differ between different honey bee stocks. De Guzman *et al.* (*Am. Bee J.* in press) showed that SHB-infested Russian honey bee colonies live longer than SHB-infested Italian colonies. Several approaches have been tested to control SHB population in European honey bees, including the use of modified hive entrances such as polyvinyl chloride (PVC) pipes. However, SHB control was inconsistent, and this technique negatively affected brood production, water drainage and amount of floor debris when a regular wooden bottom board was used (Ellis *et al.*, 2003, *J. Econ. Entomol.* 96: 1647-1652; Hood, 2004, *Bee World* 85: 51-59).

In this study, we compared the effects of standard hive entrance reducers on SHB population using 18 Italian and 17 Russian honey bee colonies. Entrance reducers were installed in nine colonies for each stock, while the entrances to nine Italian and eight Russian colonies remained fully open and served as controls. Prior to the installation of entrance reducers, all colonies were rid of beetles by rigorously examining individual frames. Four observations were made. All beetles were counted and collected during each observation. The numbers of frames of adult bees and brood for each colony were also estimated.

Our results showed no significant (P = 0.1596) effect of entrance reducers on the number of SHB with an average (mean ± SE) of 12.7 ± 2.6 beetles in the control (without entrance reducers) colonies and 10.4 ± 2.3 beetles in colonies with entrance reducers. However, we found that honey bee stock significantly (P = 0. 018) influenced the number of SHB in the colonies. Russian colonies (8.5 ± 1.9) supported lower population of beetles than the Italian colonies (14.5 ± 2.9). In addition, the presence of entrance reducers did not affect number of frames of bees (14.6 ± 0.8 with reducer and 15.5 ± 0.8 without reducer, P = 0.385) or number of frames of brood (6.3 ± 0.4 with reducer and 6.58 ± 0.5 without reducer, P = 0.559), which did not agree with the findings of Ellis *et al.* (2003, *J. Econ. Entomol.* 96: 1647-1652). The two stocks had similar numbers of frames of adult bees (15.6 ± 0.9 and 14.5 ± 0.6, P = 0.271) and frames of brood (6.4 ± 0.4 and 6.3 ± 0.4, P = 0.926).

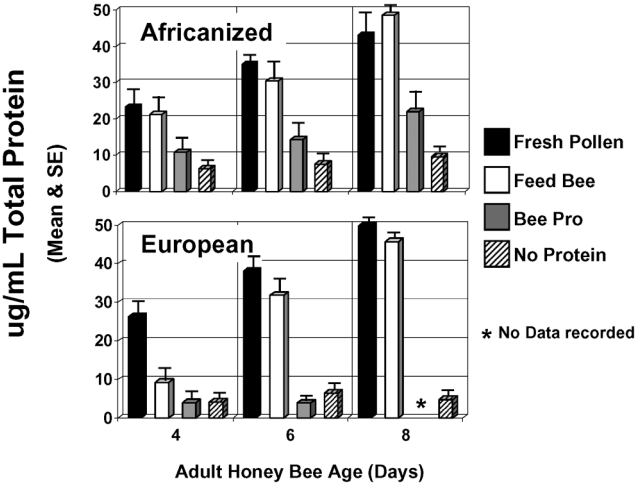
Our preliminary results suggest that Italian bees were more susceptible to SHB invasion than Russian colonies. However, further research is needed to fully establish and understand the importance of honey bee stock in the regulation of SHB population.

7. Gregory, P.G.[a] – PROTEIN DIETS AND THEIR EFFECTS ON WORKER WEIGHT, LONGEVITY, CONSUMPTION AND HEMOLYMPH PROTEIN LEVELS OF *APIS MELIFERA* – Physiological parameters of Africanized and European honey bees were explored when fed 4 different protein diets. The treatments were: (1) freshly frozen bee collected pollen, (2) dry powdered old pollen, and two artificial protein diets, (3) Bee Pro® and (4) Feed Bee®. Bee Pro® is a soy meal based diet and has been the industry's standard. Feed Bee® is a recently developed non-soy based diet. Results are from a laboratory caged experiment that started with 100 newly emerged bees and from a field cage experiment carried out in 5 frame nucs started with a queen and 500 grams of newly emerged bees.

The laboratory caged experiment demonstrated that honey bees consumed as much Feed Bee® as freshly collected pollen and bees weighed as much as bees fed fresh pollen. The field experiment showed that longevity varied among bees fed different diets (fresh pollen > Feed Bee® > Bee Pro® > old pollen. From the laboratory caged experiment the total hemolymph protein levels (via Bradford assays) were similar between bees fed a diet of fresh pollen and Feed Bee® (Figure). Bees fed Bee Pro® and old pollen had lower total hemolymph protein than those fed Feed Bee® or fresh pollen.

The sugar content of the diets was analyzed for two artificial protein diets. Sugars were extracted by homogenizing the samples in 80^{O}C ethanol and analyzed using HPLC with a refractive index detector. Feed Bee® contained 34.9 mg sucrose and 2.03 mg stachyose, whereas Bee Pro® contained 8.85 mg sucrose and 4.55 mg stachyose. Stachyose is toxic to honey bees; however, the toxic effects are reduced when diluted with 50% sucrose to 4% or less (Barker, 1977, *J. Nutrition* 107: 1859-1862). Future research will be conducted to determine which dietary components of artificial protein diets have negative physiological results on honey bees. From these data, different ingredients may be eliminated or substituted.

Figure- Total protein analysis of hemolymph from adult bees of various ages. Africanized and European honey bees that were fed different diets have differences in their protein levels with regard to age and diet.



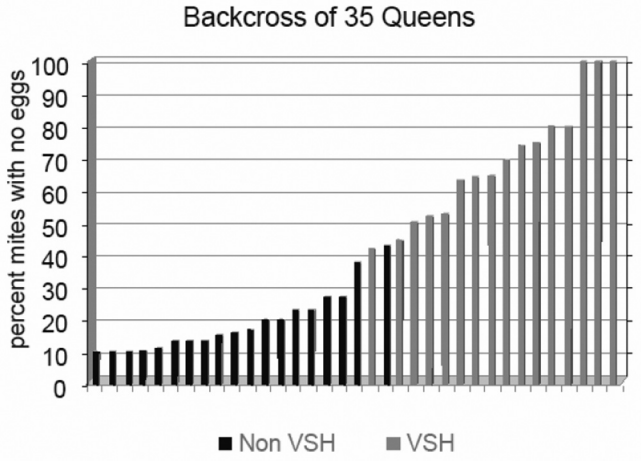
8. Harbo, J.R.[c] & J.W. Harris[c] – VARROA-INFESTED CELLS THAT ARE NOT REMOVED BY BEES WITH VARROA-SENSITIVE HYGIENE —The mite-resistance trait called suppression of mite reproduction (SMR) can be explained by a form of hygienic behavior that we call varroa sensitive hygiene (VSH). With VSH, adult honey bees remove worker-bee pupae from brood cells infested with *Varroa destructor*. Objectives were (1) to define which brood cells are removed by bees and which are not and (2) to describe the most effective way to measure VSH.

We produced 35 colonies that ranged from having 0 to 100% expression of VSH (see figure). The distribution in the figure sug-

gests that most or all of the genes for VSH are additive, differing from the recessive genes that control hygiene for resistance to American foulbrood (Rothenbuhler, 1964, *Am. Zool.* 4: 111-123). We measured both the removal of infested cells and the frequency of nonreproducing mites in all colonies. An increase in the rate of removal of infested cells was strongly related to a decrease in all categories of reproductive mites, even mites that produced eggs too late to mature. However, removal rates were not related to the number of mites that produced no progeny. This selective removal of egg-laying mites creates an increase in the proportion of mites that lay no eggs. Therefore, the simplest way to measure VSH is to measure the frequency of mites that lay no eggs.

For example, a population of mites typically has those that enter cells but do not lay eggs. The average frequency of these nonreproducing mites is about 12%. When examining worker cells that are >7 days postcapping, a colony that has 12% of the mites with no eggs has had little or no removal of infested cells and probably has none of the genes that express VSH. When 45, 70, or 100% of the infested cells have mites that lay no eggs, the colony has about 50, 75 or 100%, respectively, of the genes that express VSH (Figure).

Figure - 35 colonies ranked for their expression of varroa-sensitive hygiene. Each queen (19 with no alleles for VSH [black bars] and 16 with 100% of the VSH alleles [gray bars]) was mated to a single drone. The 35 drones were produced by a queen that was heterozygous for VSH, so they represented a random segregation of genes for VSH (0 – 100%). The Y axis is presented as both percentage and ratio (the second number of a 1:n ratio).



9. Harris, J.W.[c] & J.R. Harbo[c] – VSH BEES PERFORM HYGIENE ON VARROA-INFESTED HOSTS AGED 4-7 DAYS POSTCAPPING – Honey bees bred for high percentages of nonreproducing varroa mites hygienically remove mites with offspring from capped brood cells. This behavior is a form of varroa-sensitive hygiene (VSH). The objective of this experiment was to determine if VSH bees respond equally to varroa-infested pupae of different ages.

Varroa-infested worker brood was placed into the center of the broodnest for each of 12 colonies (6 VSH and 6 controls) for 40 hours. An infestation rate was determined before and after the test period by sampling 235-300 capped brood cells. The ratio of number of infested pupae to number of uninfested pupae was compared between the initial and final infestation rates to calculate the removal rate for varroa-infested pupae from each comb. Removal rates were also estimated for each of 3 mutually exclusive age cohorts of pupae (Table). Cohorts were defined by body color and eye pigmentation that are associated with different periods during the metamorphosis of worker bees (Jay, 1962, *Bee World* 43:119-122). The final infestation rate for each cohort was determined by sampling pupae with morphological characteristics expected after 40 hours of development from the starting age. All removal rates were compared between stocks of bees using an analysis of variance with type of bee as a fixed effect and source of infested combs, source of bees, and the interaction of these two factors as random effects.

Combs placed in control colonies had a 11 ± 24% (mean ± SD) reduction in number of varroa-infested pupae for all ages, while those placed in colonies of VSH bees had a 53 ± 21% reduction (P=0.0072). VSH bees removed significantly more varroa-infested pupae from cohorts I (P=0.041) and II (P=0.028) than controls (Table). The two types of bees did not differ in removal rates for cohort III (P=0.785). These results suggest that varroa-infested host pupae in cohorts I and II have a stimulus (or stimuli) which elicits hygienic removal of the host, while hosts in cohort III either do not possess the same stimulus, or they have other stimuli that prevent hygienic removal of infested hosts. The stimulus triggering varroa-sensitive hygiene may be related to the onset of oviposition by varroa mites or the appearance of mite offspring within the brood cell, which correspond to the pupal stages in cohorts I and II (Martin, 1994, *Exp. & Appl. Acarol*. 18: 87-100).

Table – Changes in number of varroa-infested pupae within different age cohorts after capped brood combs were placed into two different types of colonies for 40 hours. VSH bees caused a significantly greater reduction in the number of varroa-infested cells for cohorts I and II than did controls. There were no significant differences in the reduction of varroa-infested cells for cohort III between the two types of bees.

Type of Bee	Cohort	Initial Age (days postcapping)	Baseline		After 40 hours		% Decrease in Infested Cells
			% Infested	Total Cells	% Infested	Total Cells	
controls	I	2 - 4	21 ± 9	74 ± 42	17 ± 9	118 ± 60	6 ± 57
	II	5 – 7	18 ± 12	67 ± 17	15 ± 10	106 ± 32	22 ± 29
	III	8 – 9	19 ± 9	43 ± 26	16 ± 10	88 ± 36	15 ± 43
VSH bees	I	1 - 4	26 ± 6	71 ± 41	12 ± 5	119 ± 41	59 ± 25
	II	5 – 7	24 ± 9	72 ± 25	11 ± 4	116 ± 26	59 ± 23
	III	8 – 9	27 ± 16	54 ± 15	21 ± 8	97 ± 28	4 ± 49

10. Hood, W.M.[e] - A COMPARISON OF TWO SMALL HIVE BEETLE TRAPS - The small hive beetle (SHB) continues to spread to new regions in the United States and other parts of the world. When conditions are favorable for beetle reproduction, the pest can become a serious problem. Alternative control measures that are simple, economical, and efficient are needed to manage this pest.

Field trials were conducted in 2005 to compare the effectiveness of two SHB traps. A plastic box trap known commercially as the "Hood Small Hive Beetle Trap" is a one-way beetle trap that can be fastened by screws to a hive frame bottom bar and placed in the top or bottom of a hive depending on season and SHB activity. The trap lid is constructed in a manner which impedes beetle escape especially when the trap is partially filled with certain liquids such as cider vinegar or food grade mineral oil. This trap was compared to a "jar trap" which is fastened underneath the hive bottom. The jar trap consisted of a 1.15 kg (2.5 lb) honey jar with lid secured underneath the hive bottom which had a 38 mm (1.5 inch) hole drilled through the hive bottom and jar lid. A funnel screen (bee escape board cone, Better Bee, New York) was also fastened to the jar lid which protruded into the jar to impede SHB escape. A 10 x 10 cm (4 x 4 inch) piece of corrugated plastic was secured over the hole on the hive bottom to prevent bee entry and provide SHB harborage.

Twelve honey bee colonies were established on 29-30 March 2005 with 0.9 kg (2 lb) package bees free of SHB in each of two apiaries located in Colleton and Bamberg Counties, South Carolina where beetles had been problematic. Colonies were allowed to become naturally SHB infested from nearby infested colonies. On 30 June, four colonies in each apiary were randomly selected to receive one of three treatments: a Hood SHB trap, a jar/modified bottom trap, or control with no trap. Both treatment traps were one-third filled with cider vinegar and the Hood SHB traps were placed in hive body position number one or number ten. Colonies were serviced at 3-week intervals through 9 November

to count dead SHB in traps and to refill traps with cider vinegar. During each 3-week visit, colony strength was measured by counting number of 25cm² capped bee brood and colony SHB population was surveyed by adding the number of beetles counted under the colony inner cover to the number of beetles counted on the three exposed vertical hive body surfaces and hive bottom following removal of five frames. No attempt was made to count number of beetles on frames.

The numbers of dead SHB adults counted in the two trap types were not significantly different and the amount of capped bee brood did not vary by treatment during the trial period. However, there was a significant decrease in number of SHB surveyed in the Hood SHB trapped colonies when compared to the number SHB counted in control treatment colonies over the 5 months trapping period which suggests a higher control efficiency. All test colonies survived the trial period except one control treatment colony.

Table – Least square means ± SE (n) of dead small hive beetles (SHB) counted in two treatment traps, amount of capped bee brood by treatment, and colony SHB populations sampled. Data in columns followed by the same letter are not different at the $\alpha \leq 0.05$ level.

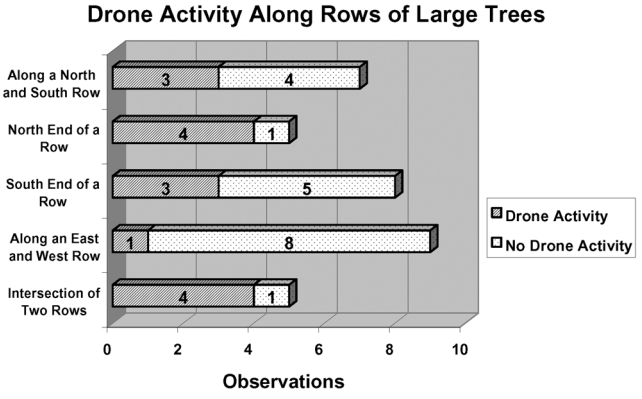
Treatment	Dead SHB	Capped Bee Brood ($25cm^2$)	Colony SHB Population Surveyed
Hood SHB Trap	8.75 ± 3.70 (8)a	98.85 ± 5.68 (8)a	.98 ± .86 (8)a
Jar/Hive Bottom SHB Trap	15.40 ± 3.70 (8)a	101.86 ± 5.73 (8)a	1.60 ± .86 (8)ab
Control (No Trap)	---	88.04 ± 6.40 (7)a	3.81 ± .98 (7)b

11. Mack, S.[f], J. Wagnitz[f] & M.D. Ellis[f] - WHERE DO DRONE CONGREGATION AREAS FORM IN MIDWESTERN AGRICULTURAL SETTINGS? - We investigated the formation of drone congregation areas (DCAs) in a Midwestern agricultural setting. This research was done at the University of Nebraska Agricultural Research and Development Center (ARDC) near Mead, Nebraska. The ARDC covers 9,500 acres including 5,000 acres used for row crops. We located flyways and DCAs and explored their relationship to landmarks.

By using a weather balloon and a lure containing queen pheromone, we were able to observe the amount of drone activity in various locations. Observations were made between 3:00pm and 6:00pm. The lure was flown at heights of 30 to 40 feet. Areas observed included rows of trees, open areas, a lake, low elevation areas, and prominent man-made structures. Drone activity appeared to be most closely associated with the rows of trees. Activity was most commonly found along rows running north and south and at the intersection of two rows. The north ends of the rows showed activity more frequently, but with fewer drones than the south ends (Figure). Observations in open areas resulted in no activity during three out of four observations. The open area where drones were found was bordered by rows of trees serving as flyways. It is possible that drones were attracted from the flyways. We were unable to find activity near a large reservoir or in areas of relatively low elevation. Observations near man-made features included roads, buildings, and a water tower. In the few cases where drones were attracted near a road, the activity appeared to be the result of an accompanying row of trees. Drones were never found near buildings or water towers.

Considerable activity was found in several different locations on certain afternoons, while no activity could be found on other afternoons with similar weather conditions. Similarly, areas that attracted many drones did not always produce the same results when revisited. Although these inconsistencies make interpretation difficult, drone activity was consistently associated with rows of trees.

Figure - Number of successful and unsuccessful observations in association with rows of large trees. Number of observations = 34.



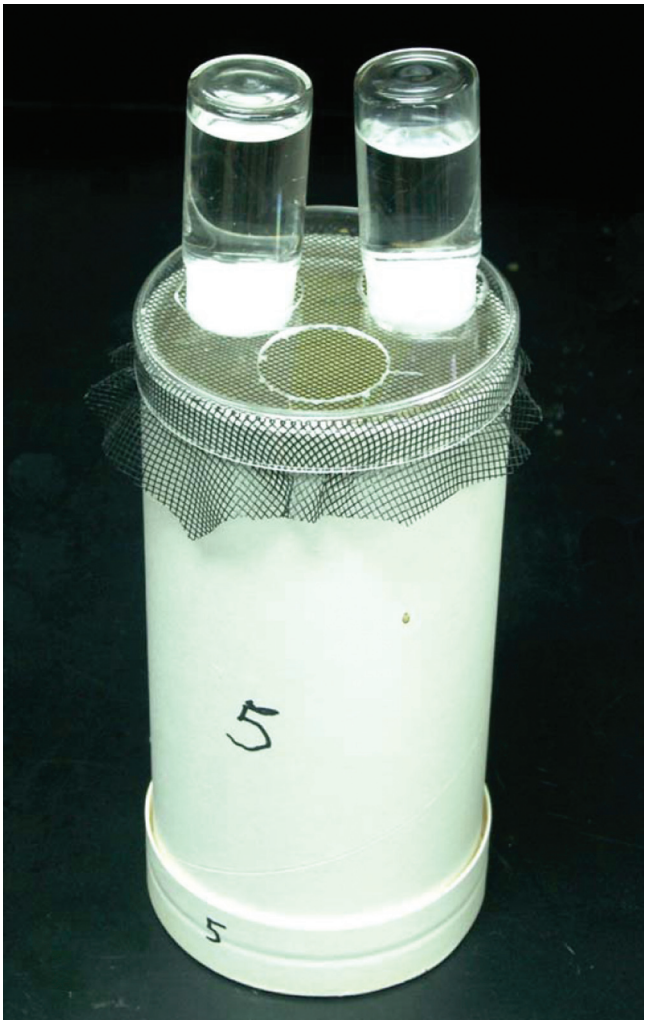
12. Rivera, R.[a], F.A. Eischen[a], and H.R. Graham[a] - MODIFICATION OF STANDARD TECHNIQUES FOR SCREENING POTENTIAL VARROACIDES IN THE LABORATORY - The Weslaco Honey Bee Research Laboratory's success in developing *Varroa destructor* control products is due to laboratory screening of many potential Varroacides. Laboratory screening saves time, money, and bees and mainly helps the beekeeping industry. One good example of this achievement is laboratory testing, field-testing and emergency registration of Coumaphos in 1998 for varroa control. This laboratory testing led to control of Small Hive Beetle in the hive using coumaphos.

We use a modified Plapp Vial Test (Plapp & Vinson, 1977, *Environ. Entomol.* 6: 381-384) for testing varroa resistance to registered acaricides. This vial testing is effective in challenging both susceptible and resistant mites to "new" and improved compounds. The lethal dose to varroa of experimental compounds is determined using this vial test. The modified Plapp test consists of 20 ml glass scintillation vials coated with potential Varroacides in a solvent, which is evaporated. Varroa from brood cells are placed in the vials, (Elzen, *et al.*,1999, *Am. Bee J.* 139: 362), kept in an incubator at 24°C, 58% RH and checked at 24 hours. We modified the procedure by adding a 100-µl H_2O moistened paper towel to the vial cap to keep mites hydrated. Mites were considered dead if they did not exhibit leg movement when touched by a probe (Hillesheim et al., 1996, *Exp. & Appl. Acarol.* 20: 283-296).

Laboratory wooden longevity cages are used for topical applications, feeding trials, synergy of hive treatments, and toxicity of compounds to honey bees. Toxicity and dosages are determined in laboratory setting instead of on a full size colony, as some compounds are toxic to varroa, but also toxic to honey bees. These cages have to be cleaned and autoclaved to ensure non- contamination of future tests.

We developed and modified quart cardboard ice cream containers **(**W.L. Enterprises, Newark, NJ) to cages for quick screening of potential Varroacides. The bottom of the cage is fitted with a wire mesh insert for monitoring Varroa drop. The bottom lid is waxed so the varroa stick to it. The top of the cage is a lid from a plastic Petri dish, with three 1" holes to hold feeder vials (50% sugar/water and water). The Petri dish fits over fiberglass window screen to contain the bees. The treatments can be presented as a strip, sprayed or in the feeder vials. About 200 bees are introduced into the cages with treatment, held in an incubator and monitored daily for up to 72 hours for mite drop and for bee mortality. The cages are disposed of to prevent cross-contamination. This data from the cages helps to determine effective dosages. These "Weslaco Bee Lab Screening Cages" facilitate identifying candidate compounds for varroa control. Presently we are testing several promising Varroacides.

Figure - Weslaco Bee Lab Screening Cage.

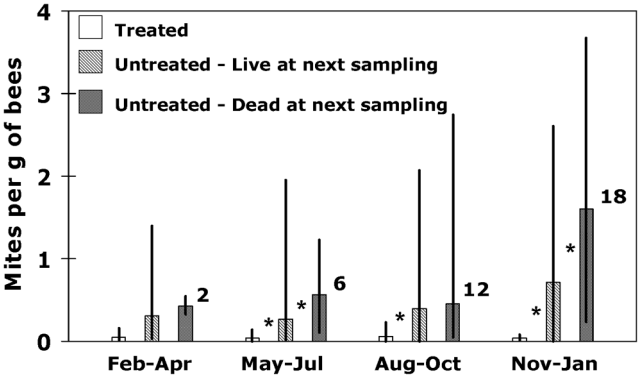


13. Villa, J.D.[c] - THE EFFECTS OF SEASONAL FLUCTUATIONS IN POPULATION DENSITIES OF VARROA MITES ON THE SURVIVAL OF UNTREATED COLONIES – Rapid increases of varroa mites in colonies, summer mortality of untreated colonies, and the disappearance of feral colonies were common after the detection of mites in 1992 in Baton Rouge, Louisiana. Recently, local observations and reports from various areas of the United States, suggest that some of these negative effects may have moderated (e.g. Seeley, 2003, *Bee Culture* 131: 24-27). I monitored the density of varroa mites in untreated colonies for six years (2000-2005) to observe seasonal trends in infestation, to relate infestation to colony mortality, and to use this information to develop economic thresholds.

Samples of adult workers (*ca.* 150 g) were taken four times per year from colonies not receiving miticide treatment. Fifty colonies produced a total of 277 samples (1-21 samples per colony). A total of 38 colony deaths were observed, and as colonies died they were replaced to maintain about 15 colonies available for sampling at a given time. Five colonies were kept under an annual treatment with either Apistan® or Check-mite®.

Half of the colony deaths occurred after the sampling period of Nov-Jan. For that period, the infestation of colonies that died was significantly higher than that of surviving colonies. Another significant period of mortality occurred after Aug-Oct, but for those samples, subsequently dead vs. live colonies did not differ significantly in infestation. Pooling the data from all seasons, only 9% of colonies with infestations below 0.25 mites per g of adults died, while 73% of those above 1.5 mites per gram died. Based on these data, and on observations of symptoms of infestation, 0.5 mites per gram of adult bees may be a useful economic threshold for southern Louisiana.

Figure - Density of varroa mites on adult bees in treated and untreated colonies (which survived or died). Maximum and minimum values are indicated by vertical lines, numbers indicate colonies that died in each period.



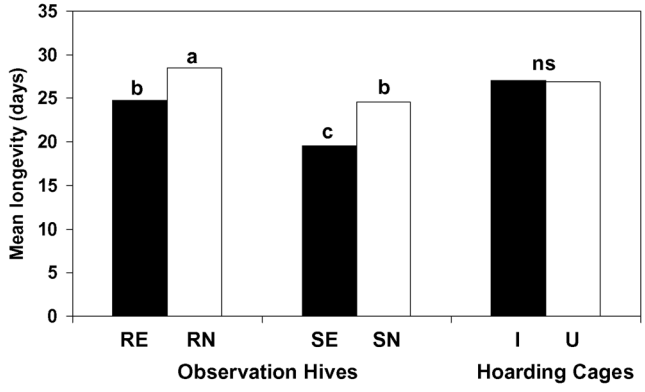
∗ - Significant differences (P<0.05) between adjacent means.

14. Villa, J.D.[c] - DO TRACHEAL MITES REDUCE THE LONGEVITY OF WORKERS? - *Acarapis woodi* reproduction can lead to apparent obstruction of the prothoracic tracheae and darkening of the tracheal walls. Despite these symptoms, reported negative effects on worker longevity have been small or nonexistent (e.g. Gary & Page, 1989*, J. Econ. Entomol*. 82: 734-739). The longevity of individual workers exposed to tracheal mites at different times of the year was evaluated in observation hives and in hoarding cages.

Young workers (< 24 h) from colonies known to be highly resistant (R) and highly susceptible (S) to infestation were painted or tagged with plastic-numbered discs. They were introduced into the most highly infested colonies available at the time or into uninfested colonies. Workers were then retrieved and placed either into a common observation hive or into hoarding cages. A subsample of marked workers were dissected to estimate the resulting level of infestation in bees exposed to infestation (E), and to verify the lack of infestation in bees not exposed (N). For workers treated as above but placed into hoarding cages, it was possible to determine actual infestation of each dead bee.

In observation hives, there was a significant reduction in the longevity of workers exposed to infestation (E vs. N in figure). However, results were not homogeneous between trials. Resulting infestation of E workers was highly variable. Also, seasonal, genetic and fostering colony effects confounded the effects of tracheal mite infestation. In experiments in hoarding cages, which

Figure - Mean longevity of workers in observation hives and in hoarding cages. In observation hives, R and S origin, as well as exposure (E vs. N) to tracheal mite infestation had significant effects; significant differences (P<0.05) between the four means are indicated by different letters. In hoarding cages, the infestation of each dead bee as I or U was possible; no significant differences were found.



permitted determining the infestation of each individual bee as infested (I) or uninfested (U), there was no reduction in longevity. As suggested by earlier literature, the effects of tracheal mites on worker longevity may be variable and even absent under some circumstances.

15. Wagnitz J.[f], N. Aliano[f], S. Mack[f] & M.D. Ellis[f] - CAN OXALIC ACID OR SUCROCIDE™ BE USED TO REDUCE VARROA POPULATIONS IN PACKAGE BEES? - In May 2005 we conducted an experiment to test the effectiveness of oxalic acid (OA) and Sucrocide™ for reducing varroa mite populations in package bees. We began the experiment by shaking 25 kg of bees into a bulk bee-box. We quantified initial mite infestation by collecting 8 alcohol samples of adult bees (totaling 2,550 bees) from the bulk bee-box. We subdivided the bees in the bulk bee-box into 30 packages each weighing approximately 0.84 ± 0.07 kg. Each package contained 1 caged queen and 1 liter of sugar water (1:1 solution). One package represented an experimental unit.

We recently conducted laboratory bioassays to estimate the acute contact toxicity of OA to varroa mites and their honey bee host. We used the information from the bioassays to estimate the optimum dosage of OA that would effectively control varroa mites and minimize adult bee mortality.

We randomly assigned three treatments to the 30 packages (OA, Sucrocide™, or untreated) and applied the treatments 8 hours prior to package installation. We applied 25 ml of a 2.0% OA solution directly on to the package bees through the screen with a 50 ml applicator. We followed the label for our Sucrocide™ dosage, and determined that the correct dosage would be 167 ml. We applied the Sucrocide in a similar manner to the OA.

The packages where installed in single story Langstroth hives just outside of Lincoln, NE. One week later, we collected ±300 adult bees in alcohol from each hive to quantify the post-treatment mite infestation. The percent reduction for each treatment was then calculated. The oxalic acid treatment significantly reduced varroa infestation by 62.79 ± 14.77% when it was compared to the untreated packages. Sucrocide™ did reduce varroa infestations by 32.35 ± 14.77%, but this reduction was not significant when compared to the untreated packages.

16. Webster, T. C.[g], K. Pomper[g], G. Hunt[h], E. M. Thacker[g] – *NOSEMA APIS* DETECTION BY POLYMERASE CHAIN REACTION (PCR) – The PCR technique was used to detect *Nosema apis* DNA in honey bee tissue. This is more sensitive than an examination of ventriculi and rectums for spores (Webster *et al*., 2004, *Apidologie* 35: 49-54).

We examined *Nosema*-inoculated queens and brood that later developed in the hives with those queens. This was to assess possible transovarial movement of the disease, as is common for other Microsporidia infecting other invertebrates. Sister queens were established in 20 small colonies at one apiary. After each had established a good worker brood pattern, they were each fed approximately 10^6 *N. apis* spores in sucrose solution (18 queens) or sucrose solution without spores (2 queens), and then returned to their respective colonies. Eggs (5 samples of 10 eggs) and larvae (5 samples of 2 larvae) were collected from each colony 10 days, 20-21 days, and 38-39 days after the queens were inoculated. Queens were removed and dissected 38 and 39 days after inoculation. Eggs, larvae, queen ventriculi and queen ovaries were frozen in separate, plastic microcentrifuge tubes and stored at -70^{o}C until analysis. Great care was taken in sterilizing dissection tools before each sample was collected, to avoid cross-contamination. No *N. apis* DNA was detected in any egg, larva or queen ovary sample, although most of the inoculated queens had infected ventriculi.

To evaluate the effects of mailing queens through the U. S. mail, we inoculated 90 caged queens that had been donated by a commercial beekeeper (Wilbanks Apiaries, GA). Twenty of the spore-inoculated queens were then mailed from Kentucky State University to Purdue University, and mailed back. The 70 remaining queens were kept in the dark, and at room temperature in the laboratory at KSU. After 5 days, the mailed queens had returned to KSU. On that day and the following day, all queens were dissected. The ovaries and ventriculi were frozen until DNA extraction could begin. All of the 14 surviving mailed queens had infected ventriculi by the PCR test, while 42 of the 49 surviving queens kept at KSU had infected ventriculi. This difference is not highly significant (P=0.33). None of these queens had *N. apis* DNA in their ovaries, according to the PCR test.

These results suggest that transovarial infection may not occur for *N. apis* in honey bee queens. However, the possibility of such a mode of infection cannot be completely eliminated.

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