

Am. Bee J. 122: 29-33) showed that chalkbrood may be reduced in colonies housed on new comb. Laboratory evidence indicates that chalkbrood symptoms may increase under conditions of elevated relative humidity (Flores *et al.* 1996 *Apidologie* 27: 185-192).

We were interested in testing the interactive effects of hygienic queens, comb age, and colony microclimate on the expression of chalkbrood symptoms. Forty-eight colonies were set up; 24 were assigned high interior relative humidity (60.5±1.7% RH) and 24 were assigned low relative humidity (58.1±0.6%). These differences were achieved by inserting a sheet of plastic between the inner and outer covers of each high humidity colony. Each colony within humidity class was assigned one of the following treatments: (1) new comb/non-hygienic queen, (2) new comb/hygienic, (3) old comb/non-hygienic, and (4) old comb/hygienic. Each colony was inoculated with the disease. For each of days 14, 21, 28, and 49, we determined for each colony the number of sealed brood cells and sum of chalkbrood cadavers.

Cells of sealed brood and number of chalkbrood cadavers in colonies treated as described in text Values are mean ± standard error, and number in parentheses = n.						
Day	Queen line		Comb		Humidity	
	Hygienic	Non-Hyg.	Old	New	High	Low
	Number of brood cells					
14	875±129 (17)	913±138 (24)	982±132 (20)	816±140 (21)	818±129 (21)	980±143 (20)
21	1840±242 (14)	1613±222 (22)	1743±237 (19)	1652±234 (18)	1522±150 (20)	1907±310 (17)
28	2623±240 (13)	2320±265 (19)	2458±294 (17)	2427±220 (15)	2213±191 (17)	2705±323 (15)
49	2602±247 (12)	2096±319 (16)	2478±305 (14)	2148±301 (14)	2044±321 (15)	2624±257 (13)
	Number of chalkbrood cadavers					
14	52±12 (17)	35±7.3 (24)	46±7.5 (20)	38±11 (21)	32±6.5 (21)	52±11 (20)
21	43±15 (14)	55±12 (23)	49±11 (19)	52±15 (18)	54±13 (20)	46±13 (17)
28	11±4 (13)	31±15 (19)	20±8 (17)	27±17 (15)	16±8 (17)	31±17 (15)
49	140.5 (12)	8.9±4.1 (16)	4.4±2.1 (14)	6.7±4.5 (14)	7.3±4.2 (15)	3.5±2.0 (13)

High variances and missing values resulting from superseded queens prevented any significant differences. On average, brood production was consistently highest in colonies with low humidity, old combs, and (except for day 14) hygienic queens. The number of chalkbrood cadavers did not vary meaningfully according to humidity. Except for day 14, the number of chalkbrood cadavers was consistently lowest in colonies with old combs and hygienic queens.

9. Eischen, F.A.^h & R.H. Graham^h - THE EFFECT OF COMBINED DOSES OF FUNGICIDES AND ACARICIDES ON ADULT HONEY BEES - Commercial pollinators of almonds in California have reported problems with honey bee health during the time that colonies are in the orchards. Several of them have suggested Captan®, a fungicide frequently applied to almonds, as its likely cause. We have begun a toxicological examination of Captan® and other fungicides in combination with mite control compounds. Specifically we measured the mortality of young adult honey bees when exposed to Apistan® (flouvalinate) and Captan® fed at varying levels in pollen.

The table shows that pollen consumption by bees varied dependent on the concentration of Captan® (in treated pollen) and the presence of Apistan®. Bees given untreated pollen ate an aver-

Measurement	Captan®	Captan® + Apistan®
Pollen Consumption:		
Control	48.1 mg/bee	38.3 mg/bee
454.5ppm Captan®	41.0 "	31.0 "
909.0ppm "	35.0 "	23.3 "
1818.0ppm "	21.0 "	11.7 "
3636.0ppm "	15.5 "	9.6 "
7272.0ppm "	9.3 "	8.7 "
LD ₅₀ (range)	2844ppm (2229-3666)	6973ppm (3459-22629)

Table. Consumption of pollen treated with Captan® and mortality caused by it alone or in the presence of the varroicide Apistan®.

age of 48 mg/bee. Consumption equaled 41, 35, 21, 15, 9 mg/bee for bees fed pollen treated with 454, 909, 1018, 3636, and 7272 ppm Captan®, respectively. Consumption of Captan® treated pollen was further reduced when an Apistan® strip (measuring 25 X 32mm) was presented in combination with the Captan treated diets.

The Table shows the LD₅₀ values (calculated with probits, Polo PC) for Captan® fed to young bees (0-24 hrs, 50 bees/longevity test cage) in pollen averaged 2844 ppm. Mortality observed for young bees fed Captan® and simultaneously exposed to flouvalinate was lower (LD₅₀ = 6973ppm). We suspect strongly that the lowered bee mortality in the presence of Apistan was caused by the reduced consumption of Captan®-treated pollen. We conclude, (1) that young honey bees consuming Captan®-treated pollen showed elevated mortality at our lowest level of exposure. Higher levels killed significant numbers of bees; and (2) that the simultaneous exposure of Captan® and Apistan® caused reduced Captan®-treated pollen consumption and consequently reduced mortality.

10. Harbo, J.R.¹ & J.W. Harris¹ - USING FREE-MATED QUEENS TO INTRODUCE GENES FOR VARROA RESISTANCE INTO A POPULATION OF HONEY BEES - We have selected honey bees to suppress reproduction of *Varroa jacobsoni* (Harbo & Harris, *Apidol.* 30: 183-196). Our objective is to introduce mite-resistant genes (in this case suppression of mite reproduction, SMR) into a bee population so that the population gains mite-resistant genes while retaining most of its former beekeeping characteristics. The plan is for commercial queen producers to raise daughter queens from mite-resistant queens and allow those queens to mate freely with their local drones. Our experiment is designed to see if those outcrossed queens retain measurable levels of resistance to varroa. If successful, beekeepers who receive those queens would obtain increased resistance to varroa, and the influx of genes for SMR would hasten the creation of a mite-resistant population of bees at their location.

Time	Resistant (n = 12)	Res x control (n = 28)	Control (n = 26)
18 days	64 ± 7% a	45 ± 5% b	33 ± 5% b
45 days	100 ± 0% a	58 ± 4% b	40 ± 5% c

Table. Data (means ± SE) are the percentage of foundress varroa mites that produced no viable daughters. Means within rows followed by different letters are different at P ≤ 0.05 level (Isd mean separation). About 15 mite-infested cells were examined in each colony to produce an estimate of SMR in each colony in the 2000 test. Worker pupae were examined when 15-18 d old, 18 and 45 days after the queens were released in their test colonies.

We began in early 1999 by sending SMR queens to 4 commercial queen producers. They produced free mated daughters (resistant x control) from those queens. Each queen producer sent us 5 resistant x control queens plus 5 free-mated queens from their commercial stock (control). Queens were tested in Baton Rouge in colonies that began with no brood and about 1kg of mite-infested bees (subdivided from a large population that had been collected on the previous day). After 108 days (Sept. 9) we evaluated each colony for mite reproduction and populations of mites and bees.

This year, we increased colony numbers to 66, added a third location, and added another treatment, resistant (mite resistant queens artificially inseminated with semen from mite resistant drones). It is a complete randomized block design with a resistant group added.

Results in 1999 showed that mite populations were 44% lower in resistant x control colonies than in control colonies (n = 16 and 17 respectively, P = 0.004). SMR averaged 46 and 34% in the two

groups, respectively ($P = 0.11$).

Data from 2000 thus far have shown (1) immediate SMR (heretofore SMR had not been detectable until the 6th week of the test), and (2) significant differences between resistant x control and control in suppression of mite reproduction. See table.

The results suggest that free-mated SMR queens can provide some immediate benefit to beekeepers. Any long-range benefits are beyond the scope of this test but provide an added incentive for using mite-resistant queens.

H. Hood, W.M.J. & S. Taber^k - SEARCH FOR EUROPEAN HONEY BEES THAT SHOW HIVE CLEANSING HABITS FOR REMOVAL OF THE SMALL HIVE BEETLE, *AETHINA TUMIDA* MURRAY, IN THE USA - Small hive beetles (SHB) were first identified in the USA in June, 1998 from beetles collected in St. Lucie, Florida. Although SHB have now been identified from colonies in 12 states in the USA (Pettis & Shimanuki, 2000 *Am. Bee J.* 140: 152-155), the new pest has been a problem in only four southeastern states, Florida, Georgia, North Carolina and South Carolina.

In 1999, we began investigations to identify European honey bee colonies that show small hive beetle cleansing habits. Lundie (1940, *S. Africa Dept. Agriculture & Forestry Science Bull.* 220: 30) reported that honey bees in South Africa have little difficulty in removing SHB larvae from comb. This trait has not been reported in the USA.

We began our investigations in April 1999 by establishing 41 small honey bee colonies (nucs) near Elgin, South Carolina which is located in the sandhills region of the state. Elgin is in Kershaw County where SHB were first identified in winter, 1998. All nucs were established using SHB-free bees shaken from Clemson University research colonies and were headed by queens raised from mothers selected for hygienic behavior (HYG). Each 5-frame nuc was placed on a wooden platform on a post 40 inches (100 cm) above ground level and separated by 10 feet (3 m) along two rows with nuc entrances facing variable directions.

All nucs were examined every ten days to determine queen status and to remove brood in excess of two frames and remove excess honey. Since all nucs were stocked with about 1 pound (.4 kg) of bees, the colonies built up rapidly during the nectar flows from April-June. From July-August, nectar flows subsided and many nucs were given frames of honey or fed sugar water. Nucs were tested for HYG behavior from 1-6 September by placement of a piece of dead sealed brood in the center of each brood nest to determine if it was removed in 48 hours or less. If all brood was removed in 48 hours, it was termed HYG. If any of the dead brood was not removed in 48 hours, it was termed non-HYG. Fifteen nucs were determined to be HYG and 19 were determined to be non-HYG. By 1 September, seven nucs were dead resulting from queen failure or other reasons apparently unrelated to SHB effects.

Ten SHB adults were added to each nuc on 6 May and 9 June for a total of 20 beetles per nuc. During the routine 10-day examinations, few beetle adults and larvae were seen in the nucs. All nucs were surveyed for SHB on 6 August by removal of all five frames of bees and counting all beetle adults and larvae under the nuc lid, all four inner sides and bottom. The mean numbers of SHB adults and larvae per nuc were 2.5 and 0, respectively. The SHB survey was repeated on 9 September and mean numbers of beetle adults and larvae were 2.8 and 0.6, respectively. There was no significant difference ($P > .05$) in SHB counts between HYG and non-HYG nucs. We did not observe any SHB damage to comb during surveys.

A failure of SHB buildup in all colonies was a surprise given that nucs are considered to be under stress and susceptible to SHB problems. Five possible reasons for SHB failure to buildup are: (1) possible SHB predation by the red imported fire ant, *Solenopsis invicta*, as beetle pupae enter the soil, (2) other site variables which may have contributed to failure of SHB buildup, (3) nuc construction with a 3/4 inch (19 mm) auger hole entrance drilled 3 inches above nuc floor may have restricted mature SHB larvae from exiting the colonies to pupate in the soil, (4) SHB introduced to test

colonies were reared in captivity which may have affected their reproductive potential, and (5) beekeeper manipulations on a 10-day schedule may have disrupted the SHB buildup.

12. Hung, A.C.F.¹ & J.D. Evans¹ - GENETIC EVIDENCE FOR COINFECTION OF HONEY BEES BY ACUTE BEE PARALYSIS AND KASHMIR BEE VIRUSES - Nucleotide sequence analyses were used to identify acute bee paralysis virus (ABPV) and Kashmir bee virus (KBV) isolated from a single honey bee colony. Most of the bees in this colony carried KBV. Surprisingly, some individual bees also carried ABPV, a coexistence not yet seen between these two viruses. Implications of coinfection on viral disease expression are discussed, along with a new diagnostic tool that can be used to discriminate between these two viruses.

13. Hunt, G.J.^m, Guzmán-Novoa, E.ⁿ - INTERACTIONS BETWEEN EUROPEAN AND AFRICANIZED BEES IN DEFENDING A COMMON NEST - In Africanized areas, European queens often mate with Africanized drones. The resulting colony has genetic diversity for defensive behavior, with unknown consequences.

We designed an experiment to determine how the two genotypes of bees would interact within a colony. We observed the guarding, pursuing and stinging behavior of European and Africanized honey bees that were reared together in roughly equal numbers in a small colony. The colony was established from newly emerged bees that were marked by genotype and age. In a second experiment, the duration of guarding behavior was also observed for various genotypes from controlled matings in their own colonies. In the mixed-genotype environment, Africanized bees performed the majority of guarding bouts (87%). The Africanized bees also guarded for much longer periods, up to 13 days, whereas European bees were not seen guarding for more than one day. Twenty percent of individually tagged Africanized bees guarded, but only 7% of European bees were seen guarding. However, there was no difference in tendency to pursue or to sting for co-fostered Africanized and European bees. In the second experiment, using instrumentally inseminated stocks, Africanized honey bees were also much more persistent in guarding than European bees when kept in separate hives. We marked about 40 guards in each of 18 colonies. After 6 days, Africanized bees were 25 times as likely as European bees to persist at guarding. Ten percent of the Africanized guards (12) were still guarding after 6 days, compared to 0.4% (1) of European bees. But colonies with hybrid or back-cross workers had intermediate proportions of workers that persisted in guarding.

We propose the following model to explain how response thresholds for guarding, pursuing and stinging are differentially influenced by interactions between individuals of different genotypes within the colony. (1) Some of the genes that influence guarding behavior also influence pursuing and stinging behavior, but an individual's guarding behavior is regulated by the colony environment in a different way than are the actions that lead to stinging. (2) For pursuing and stinging behavior, response thresholds of co-fostered individuals with different defensive-behavior genotypes converge, an effect that may be caused by the exchange of pheromones. (3) For guarding behavior, social interactions in mixed-genotype colonies exaggerate the differences in behavioral phenotypes between individuals of high- and low-defensive genotypes such that defensive-genotype bees become more likely to guard and gentle-genotype bees become less likely to guard than they would if they were in an unmixed colony.

14. Kochansky J.¹, D.A. Knox¹, M. Feldlaufer¹, & J.S. Pettis¹ - SCREENING ANTIBIOTICS AGAINST RESISTANT AND SUSCEPTIBLE AMERICAN FOULBROOD^x - Since resistance of the causative organism of American foulbrood disease to oxytetracycline (OTC) is becoming widespread in the United States, we began a search for effective alternative antibiotics. We investigated 27 antibiotics, primarily ones already registered with