

# Proceedings of the American Bee Research Conference

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*The 1998 American Bee Research Conference was held on January 16 and 17 at the Doubletree Hotel/World Arena in Colorado Springs, CO. The following are abstracts from the 1998 conference.*

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1. Baxter, J.<sup>a</sup>, F. Eischen<sup>a</sup>, J. Pettis<sup>b</sup>, W.T. Wilson<sup>a</sup> & H. Shimanuki<sup>b</sup>—DETECTION OF FLUVALINATE-RESISTANT VARROA MITES IN U.S. HONEY BEES—*Varroa jacobsoni* was discovered in honey bee colonies in the United States in 1987. Apistan<sup>®</sup> strips (EPA registered) and other fluvalinate-application methods soon became widely utilized in varroa control. The fluvalinate-based treatments were highly effective, and soon it was difficult to find varroa populations in most commercial beekeeping operations during the mid 1990s. Unfortunately, varroa populations with resistance to fluvalinate were reported from Italy in 1992 (Lodesani et al. *Apidologie* 26:67-72).

In August 1997, bee inspectors in South Dakota (R. Reiners) and Florida (D. Westervelt & L. Cutts) using the ether-roll method for varroa detection (Ellis et al. *Am. Bee J.* 128:262-64) reported about 30 to 50 mites on 300 adult bees in colonies containing up to 3 Apistan strips. They reported their findings to scientists at the USDA-ARS Honey Bee Research group, Weslaco, Texas (TX). In early September 1997, in a continuing study of new varroacides, 7 different treatments with 20 colonies per treatment was established in South Dakota (SD). Surprisingly, applying 2 Apistan strips (10% fluvalinate) per colony was not statistically different ( $P = .0001$ ) from untreated control colonies, with no varroa reduction over a 4-week period. However, colonies treated with plastic strips impregnated with 7.5% amitraz (not EPA registered), or an experimental miticide, reduced the varroa population by more than 95%. The SD colonies were traceable back to Florida.

In a similar study in mid-September in Florida (FL), 9 separate treatments (6 fluvalinate, 2 experimental and 1 control) ( $n=135$ ) demonstrated that 2, 4 or 8 Apistan strips or 2 cardboard strips soaked in 1:1 water/Mavrik (fluvalinate) solution per colony were ineffective and the mite kill was not statistically different from untreated control colonies. Excellent varroa control was achieved with amitraz or an experimental miticide in plastic. The same Apistan strips that were ineffective in FL were retested in two susceptible varroa populations in TX and found to be highly effective. This evidence demonstrates that some varroa populations in the U.S. are becoming resistant to fluvalinate.

The only conflicting information came from the use of old Apistan (known as section 18 strips) containing DIOP plasticizer,

and stored in sealed containers for about 10 years. In FL, these strips resulted in excellent varroa knockdown that was equal to amitraz strips. In a follow up study in November 1997 in FL, the section 18 Apistan strips were wiped with tissue paper to remove oily fluvalinate that had migrated to the surface over the 10-year period and then compared with unwiped strips. The data suggest that the high level of efficacy may be based on an unusually high concentration of surface fluvalinate and a deterioration of the plasticizer.

2. Calderone, N.W.<sup>c</sup>—SUB-SAMPLING ALGORITHMS FOR ESTIMATING VARROA COUNTS ON STICKY-BOARDS—A method to estimate the number of varroa mites on a sticky board was developed based on stratified random sampling. The sticky board consists of 12 columns (i) and 17 rows (j) and has a collection area measuring 30.48 cm x 43.18 cm (12 in x 17 in) divided into a grid of 204 equal-sized squares. The collection area was divided into 96 strata, each consisting of 2 adjacent squares in the same column [(i, j) and (i, j+1)]. The first stratum included the two squares designated [(i=3D1, j=3D1) and (i=3D1, j=3D2)], the last stratum included the two squares [(12, 15) and (12, 16)], and no square was included in more than one stratum. Mites were counted in one randomly selected square in each stratum and in all 12 squares in the 17th row. A total of 108 of 204 squares were counted, and the number of mites in those squares was determined. The total number of mites on the board was estimated by multiplying the number of mites counted by 1.8889. The bias was calculated as the difference between the estimate and the actual number of mites on the board which was known. The |bias| as a percentage of the actual number of mites on the board was also determined. One thousand estimates were made for each of 126 boards and the average bias and % |bias| were determined. Two additional evaluations of the method were made in which the population of boards was restricted to those with > 500 mites or to those with > 1,000 mites. The average bias was 0.70 (all boards), 0.58 (boards w/> 500 mites), and -0.18 (boards w/> 1,000 mites). These values confirm that the method is unbiased. The corresponding values for the average % |bias| were 9.12, 1.94 and 1.69. Additionally, for these three populations of boards, there was a 95% probability that estimates would be within 38.46%, 5.93% and 5.03%, respectively, of the actual value.

removed after 56 days, and strips inserted in the continuously-treated colonies were replaced with new ones every 56-69 days.

By September-October, colony bee populations were highest in continuously-treated colonies, followed by colonies treated in February and August, August, and February. Number of brood cells was highest in continuously-treated colonies and in colonies treated in February and August. Brood with visibly abnormal symptoms occurred only in February-, August-, and non-treated colonies, but there were no significant treatment effects (see Table). The following January all Georgia colonies were assessed with a subjective "survivability score" to estimate the likelihood that the colony could survive under optimum management. Each of four observers independently ranked each colony from 0 (dead) to 3 (best condition and highest likelihood for survival).

In actual beekeeping practice, it is not advisable to treat colonies continuously as this is one of the surest ways to promote chemical resistance in mites. Continuous treatment was included in our study to provide information on colony conditions under a hypothetical best-control scenario. We argue that the February + August treatment regimen provided a satisfactory compromise between mite control and excessive chemical use. Colonies treated in February + August had comparatively large bee populations and brood cell number in September and October and good likelihood of survival the following January (see Table). This satisfactory level of mite control was achieved in colonies that, in February, had 300-bee ether roll mite levels of 2, overnight adhesive bottom board insert mite levels of 4.3, and colony mite populations of  $70 \pm 42$ , and that, in August, had ether roll levels of 14, bottom board insert levels of 187, and colony mite populations of  $4261 \pm 1585$ .

**Effects of different acaricide treatment schedules on colonies infested with *Varroa jacobsoni*.** Each colony received one of five treatments: (1) Apistan treatment in February, (2) in August, (3) in February and again in August, (4) continuously treated, or (5) never treated. Values presented are average  $\pm$  standard error. Column averages followed by the same letter are not different at the  $\alpha=0.05$  level. For the number of brood cells there were treatment effects for South Carolina only. Treatments did not significantly affect bee weight and percentage brood with visibly abnormal symptoms.

month treated	variables measured in September-October					measured following January
	bee population	mite population	bee weight (mg)	no. brood cells (SC only)	% abnormal brood	"survivability" score (0=dead, 3=best) (GA only)
Feb.	15506 $\pm$ 3384bc	5758 $\pm$ 1430a	128 $\pm$ 6	3146 $\pm$ 723b	4.4 $\pm$ 2.2	1.4 $\pm$ 1.2
Aug.	16515 $\pm$ 3202bc	37 $\pm$ 17b	131 $\pm$ 7	3327 $\pm$ 1206b	0.32 $\pm$ 0.3	2.3 $\pm$ 0.5
Feb+Aug	21258 $\pm$ 2776b	33 $\pm$ 33b	134 $\pm$ 7	7759 $\pm$ 1623a	0	2.3 $\pm$ 0.2
continuous	29442 $\pm$ 2220a	39 $\pm$ 24b	139 $\pm$ 3	9907 $\pm$ 1244a	0	2.8 $\pm$ 0.2
not treated	11488 $\pm$ 2178c	5847 $\pm$ 1215a	132 $\pm$ 5	1665 $\pm$ 1365b	5.6 $\pm$ 3.7	0.4 $\pm$ 0.4

**6. Eischen, F.A.<sup>a</sup>—THE EFFECT OF SUPPLEMENTAL POLLEN FEEDING ON POLLEN COLLECTION BY HONEY BEES**—Pollen is handled by two distinct groups of adult worker bees in a normal colony. Nurse bees and pollen foragers are distinguished by differing behavioral repertoires as well as differing physiological and chronological ages. However, the roles they play in the colony's nutrition are interrelated.

Twelve colonies of about equal strengths were randomly assigned to four treatment groups, i.e., control, wheat, mixed pollens, and a single specific pollen, viz. corn pollen. Modified Ontario Agricultural College Pollen Traps were used to monitor pollen collection during a 14 day period. Pollen was removed from the traps each day at 5.00pm. This pollen was weighed and sorted according to pollen loads. On days 6, 7, 8, and 9, thirty grams of either corn pollen, a mixture of non-corn pollens, or wheat was combined with 10 grams of sucrose and water into a

soft paste and placed just above the brood nest.

The Table shows that colonies fed one of the three supplementary feedings collected slightly more pollen than did the controls. The ratio of corn to non-corn pollens collected by the control, wheat, and non-corn pollen colonies was about 2:1. The corn-pollen group, however, showed a 1: 2.7 ratio of corn to non-corn pollen, i.e. almost the reverse of the other groups ( $P < 0.02$ , 1-way ANOVA).

**Table. Collection of corn and non-corn pollen by colonies during early August in Columbus, Ohio (N=3).**

Treatment	Average Pollen Collection/24 hrs. (grams)	% corn pollen	% non-corn pollen
Control	190	67.7	32.3
Wheat	249	69.5	30.5
Non-corn pollen	307	64.5	35.5
Corn Pollen	239	27.0	73.0

These results indicate that honey bee foragers can rapidly shift their collection efforts among available pollen types. This is not surprising since Free (1967) *J. Apic. Res.* 15: 134-144 found that pollen foragers rapidly changed to nectar foragers (and vice versa), depending upon the needs of the colony. Free also found that supplemental feeding inhibited pollen collection. The data supports this in part. In colonies that I fed corn pollen, the collection of that pollen was inhibited. However, total pollen collection was not. These results suggest that pollen foragers are either able to assess pollen needs independently or that nurse bees are able to communicate their pollen needs/preferences to pollen foragers.

**7. Eischen, F.A.<sup>a</sup> & W.T. Wilson<sup>a</sup>—THE EFFECT OF NATURAL PRODUCTS SMOKE ON *VARROA JACOBSONI*: AN UPDATE**—We have continued to evaluate natural products smoke for activity against *Varroa jacobsoni* (Eischen & Wilson 1997 *Am. Bee J.* 137: 122-123). Secondly, we have monitored the effect of plant smoke on honey bees. Materials were selected for testing based either on use by beekeepers or for properties previously reported. Plant materials screened for activity included alfalfa pellets (Purina rabbit chow), American pennyroyal (*Hedeoma pulegiodes*), camphor tree (*Cinnamomum camphora*), eucalyptus (*Eucalyptus* sp.), European/English pennyroyal (*Mentha pulegium*), incense cedar (pet bedding), juniper bark (*Juniperus* sp.), mountain mint (*Pycnanthemum muticum*), passion flower (*Passiflora foetida*), staghorn sumac (*Rhus typhina*), sorrel (*Oxalis rubra*), sweet Annie (*Artemisia annua*), tansy (*Tanacetum vulgare*), and twine (unweathered, partially weathered and weathered). Groups of infested bees (ca. 250) were exposed to the cool smoke of these materials for 60 sec. Mites initially knocked-down were counted and the bees placed on white cardboard ringed with a sticky material and placed in an incubator for 24 hrs. Knocked-down mites were again counted and the bees shaken in alcohol to determine the number of living mites.

The knockdown by the smoke reached levels of 70-90% in sorrel and sumac. Previous work with cedar, citrus, creosote bush, melaleuca, neem and tansy showed similar levels of activity. While this level of control is only marginally effective, it suggests strongly that these plants contain materials, that when burned, release products that impact this parasite. A laboratory test of honey bee longevity with the smoke of alfalfa pellets, sumac, and twine found no adverse effects. American pennyroyal and sorrel smoke caused anesthesia. Even though some of these plant products show varying degrees of activity against varroa, we do not recommend their use in a control program.

**8. Ellis, M. D.<sup>h</sup>, M. Spivak<sup>i</sup> & M. Reed<sup>h</sup>—AN EVALUATION OF COUMAPHOS IMPREGNATED PLASTIC STRIPS FOR *VARROA* CONTROL IN THE MIDWEST—**

Coumaphos impregnated plastic strips were assayed for varroa mite control in Nebraska and Minnesota. Tests were conducted in September and October of 1997, and all colonies were rearing brood at the start of the trial. Eighty two-story colonies were selected for the study. Varroa infestation was confirmed in all colonies by ether-roll or alcohol-wash testing. Twenty colonies served as untreated controls. The remaining colonies were divided into three treatment groups (20 colonies per treatment). Treatment groups were divided equally between the two states. Each group received two coumaphos impregnated plastic strips per colony. Three concentrations of coumaphos were assayed: 2.5%, 5.0%, and 10%. Strips were positioned for maximum contact with the cluster of bees. Mite drop during the initial 24 hours of treatment was determined by using DeWill® sticky boards. After six weeks of treatment, all coumaphos strips were removed and new Apistan® strips were placed in all colonies. Mite drop was again recorded for a 24 hour period following Apistan application by using DeWill sticky boards. Mite recovery during the initial and final recovery periods is shown in the accompanying table. Raw data were transformed for regression analyses using the log 10 transformation due to variability in the level of colony infestation. The regression for mite recovery during the first 24 hours of coumaphos treatment and strip concentration was significant ( $P = 0.0001$ ). Regression parameters were slope: 0.103 ( $\pm 0.023$ ), intercept 1.286 ( $\pm 0.128$ ). The regression for mites recovered during the first 24 hours of Apistan treatment and coumaphos strip concentration was also significant ( $P = 0.0001$ ). Regression parameters were slope: -0.204 ( $\pm 0.026$ ), intercept 2.474 ( $\pm 0.151$ ). The final mite recoveries were examined by least square means comparisons. Colonies treated with 2.5% strips did not have fewer mites than untreated colonies ( $P = 0.2802$ ), colonies treated with 5.0% strips had fewer mites than colonies treated with 2.5% strips ( $P = 0.0001$ ), and there was no difference in colonies treated with 5.0% and 10% strips ( $P = 0.3883$ ). Also, there was no difference in the results obtained in Nebraska and Minnesota trials ( $P = 0.7713$ ).

**Table. Comparison of the number of mites recovered from colonies treated with three concentrations of coumaphos impregnated plastic strips. Means followed by the same letter are not significantly different (lsmeans, alpha = .05).**

#### Initial recovery period

Concentration	Mean ( $\pm$ SEM)	Minimum	Maximum
10%	346.63 ( $\pm 84.41$ ) a	9	1399
5.0%	352.75 ( $\pm 108.04$ ) a	2	1845
2.5%	50.74 ( $\pm 12.23$ ) b	1	182
Control	49.15 ( $\pm 14.20$ ) b	0	223

Concentration	Mean ( $\pm$ SEM)	Minimum	Maximum
10%	13.39 ( $\pm 1.49$ ) a	0	76
5.0%	19.22 ( $\pm 1.47$ ) a	1	69
2.5%	831.29 ( $\pm 206.22$ ) b	15	2449
Control	771.21 ( $\pm 145.56$ ) b	29	1992

#### Final recovery period

**9. Fisher, J.J., K. Cramp, J. Finley & S. Camazine—EFFECTS OF TRACHEAL MITES AND NOSEMA ON COMMERCIAL U.S. QUEENS**—Endoparasitic tracheal mites (*Acarapis woodi* Rennie) and ectoparasitic varroa mites (*Varroa jacobsoni* Oudemans) have been implicated in the death of many honey bee colonies (Hung et al. 1995 *Am. Bee J.* 135: 702-704; Sammartaro 1997 *Am. Bee J.* 137: 301-302). Some states experienced losses as high as 80% in 1995-96 which is considerably higher than the normal 10% (Finley et al. 1996 *Am Bee J.* 136: 805-808).

In recent years, increased queen supersedures have been

reported in mite-infested colonies (Tew 1996 *Bee Culture* 124: 466-469). Increases in queen supersedure rates may negatively impact the beekeeping industry. The overall health of the queen can effect the productivity of the entire colony. Consequently, we examined the health of the queens produced by various commercial sources across the U.S.

We purchased commercially-available Italian queens from 13 breeders in 10 different states. We attempted to purchase 2 sets of 15 queens each from all the breeders; the first (spring) set was shipped during May or early June, 1997; and the second (summer) set was shipped during July, 1997. Breeders reported that the queens ranged from 12 to 30 days old at shipment.

Assays were performed to examine the total live weight, ovary weight (wet), developed egg count, developed egg length, sperm count, tracheal mite count, and Nosema spore count. Data were analyzed using analysis of variance, where  $P \leq 0.05$  was considered significant.

We received a total of 325 queens. Of the queens examined for tracheal mites, 20% were infested. Infestations of even a single mite in the trachea were detected. Nosema spores were found in 7% of the queens examined for Nosema. This low incidence of Nosema is most likely due to the fact that many breeders (50% in our study) routinely treat mating nuclei with Fumidil-B for Nosema prevention.

The presence or absence of tracheal mites caused significant differences in total live queen weight. The average weight of tracheal mite-infested queens was significantly lower than that of uninfested queens. This weight reduction may be caused by mite feeding on the host hemolymph and consequently reducing the total volume of hemolymph, which has been observed in varroa mite-infested bees (Weinberg & Madel 1985 *Apidologie* 16: 421-436). It is also possible that queens in mite-infested colonies receive poor care from infested attendants and that lower queen weight reflects malnutrition.

The presence or absence of tracheal mites also caused significant differences in sperm count. Infested queens contained fewer sperm than uninfested queens. The lower number of sperm in tracheal mite-infested queens could be explained by abnormal matings causing reduced mating success, or by malnutrition of the sperm during storage.

There was a significant correlation between Nosema infections and ovary weight. The average ovary weight (both ovaries wet) was lower in infected versus non-infected queens. The differences in weight may coincide with the early stages of ovary degeneration caused by a metabolic disturbance in Nosema-infected queens (Fyg 1964 *Ann. Rev. Entomol.* 9: 207-224; Gerson et al. 1988 In: *Africanized Honey Bees & Bee Mites* p. 420-424).

This study shows: 1) a significant incidence (20%) of tracheal mites and a moderate incidence (7%) of Nosema in 325 commercially-produced U.S. queens. 2) Tracheal mite infested queens weighed significantly less and had fewer sperm in their spermathecae than uninfested queens. 3) Nosema-infected queens had lower ovary weights than uninfested queens.

**10. Finley, J.J., J. Kuszniir, M. Frazier & S. Camazine—CAN WE IGNORE TRACHEAL MITES? BEE LOSSES IN PENNSYLVANIA 1995 TO 1997**—We began an annual survey of Pennsylvania beekeepers to assess colony losses after the regional losses epidemic in 1995-96. For 1995-96, we collected data from 227 PA beekeepers (4,622 colonies) (Finley et al. 1996 *Am. Bee J.* 136: 805-808). Approximately 51% of these colonies died. For 1996-97, 251 beekeepers responded (3,474 colonies), with a 26% loss rate. Approximately 2/3 of losses in both years occurred in late winter or early spring.

Untreated colonies had high rates of mortality: 82% in 1995-96 and 45% in 1996-97. Applications of Apistan and Fumidil-B each significantly reduced colony losses by 15% or more in both years. More than 75% of beekeepers routinely apply Apistan, but only 20% apply Fumidil-B. Previously, Nosema was linked to 15% of winter losses in Pennsylvania (1988-89), while tracheal

mites accounted for 36% (Frazier et al. 1994 *Bee Science* 3: 94-100).

Surveyed beekeepers reported that most winter losses corresponded with typical tracheal mite symptoms — lots of honey and very small clusters. Between 30 and 40% applied tracheal mite treatments (menthol or “grease” patties or both). However, tracheal mite treatments were not effective in reducing overall losses, perhaps because 1/3 were applied at the wrong time of year.

Menthol works as a fumigant and is highly-dependent on temperature. Unsuccessful treatments are common in Pennsylvania due to cool autumn temperatures. One of the authors has developed application timing recommendations for menthol based on temperatures inside the hive (Finley et al. *Bee Science* in prep.). Menthol is effective if applied at the top of the broodnest when average daily temperatures (max + min/2) are  $\geq 15^{\circ}\text{C}$  ( $60^{\circ}\text{F}$ ).

“Grease” patty treatments were also commonly mistimed. Since they do not kill tracheal mites immediately, they must be applied during summer to reduce mite infestations by winter (Sammataro et al. 1994 *J. Econ. Entomol.* 87: 910-916).

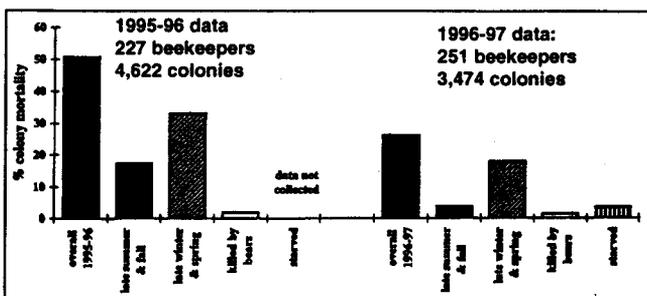


Figure. Colony losses in Pennsylvania in 1995-96 (left) and 1996-97 (right).

**11. Haarmann, T. K.<sup>k</sup>—HONEY BEES AS INDICATORS OF RADIONUCLIDE CONTAMINATION: INVESTIGATING CONTAMINANT REDISTRIBUTION PATHWAYS**—Honey bees have been referred to as mobile samplers that efficiently cover a sample area during their foraging flights, then return to a central location (Bromenshenk 1990 *Ecological Indicators*, Vol 1, Elsevier Applied Science, New York, NY). During their foraging flights, bees inadvertently contact and accumulate a wide array of environmental pollutants that are often returned to the colony (Bromenshenk et al. 1985 *Science* 227: 632-634). As part of ongoing research at Los Alamos National Laboratory concerning the use of honey bees as indicators of bioavailable radionuclide contamination, I conducted experiments to investigate the redistribution pathway of radionuclide contaminants and the factors that ultimately determine levels of radionuclides in the bees.

One aspect of my study investigated two factors that might influence the levels of contaminants found in a standard sample of honey bees. All experiments were conducted within a study site containing radionuclide contamination. The first experiment examined the contaminant level differences in forager bees and nurse bees. Might the proportion of forager bees to nurse bees in a particular sample influence the contaminant levels found in that sample? Bees were collected from colonies and analyzed for concentrations of radionuclides. Results indicated that there was no significant difference between the contaminant levels in forager and nurse bees. The second experiment compared the levels of radionuclides in three floral species frequently used by foragers: salt cedar (*Tamarix ramosissima*), white sweet clover (*Melilotus albus*), and rabbit brush (*Chrysothamnus nauseosus*). Results indicated that there was no significant difference in the amounts of radionuclides found in the flowers of these three plants.

Another aspect of my study investigated the redistribution of contaminants within a study site as the contaminants moved from the source, in this case, a radioactive waste lagoon, to the honey bees. The area adjacent to the lagoon contained many flowering

plants that were used by honey bees as forage. Samples were collected from beehives, flowering plants, and the lagoon in order to assess the dynamics of the contaminant pathway. My experiments were designed to investigate several questions: (1) From what source did the bees take up the majority of contaminants—the lagoon or nearby flowers? (2) Were the levels of contaminants in the bees, flowers, and water correlated and/or did they demonstrate similar trends? and (3) Was there an observable bioaccumulation of contaminants within the bees or flowers?

Samples of water, flowers, and honey bees were collected from the study site for two consecutive years. The samples were analyzed for concentrations of radionuclides, and the results were compared using rank sum, correlation, and trend analysis. Results were then used to assess the redistribution pathway of radionuclides within the study site. Results indicated that honey bees received the majority of their contamination directly from the source, a radioactive waste lagoon. The amount of contamination the honey bees received from flowers during nectar collection appeared to be insignificant compared to the amount received during water collection. Results did not demonstrate significant patterns of correlation or trend between the lagoon, bees, or flowers. Sample results showed a significant bioaccumulation of cobalt-60 and sodium-22 within the honey bees, but no significant bioaccumulation within the flowers.

**12. Harbo, J. R.<sup>c</sup> & J. W. Harris<sup>c</sup>—SELECTING HONEY BEES FOR SUPPRESSION OF THE REPRODUCTION OF VARROA JACOBSONI**—Nonreproduction of mites in brood cells was related to the growth of mite populations in field colonies (Harbo & Hoopingarner 1997 *J. Econ. Entomol.* 90: 893-898). This was a heritable characteristic of honey bees ( $h^2 = 0.44$ ), so we called it suppression of mite reproduction and proceeded to increase its expression through selective breeding.

Twenty-five uniform colonies of bees and mites were established on 21 June in Baton Rouge, LA. Bees and mites were collected from a population of mite-infested bees that had been collected into a large cage. Each colony began with 753 grams of bees, 433 mites, no brood, and a test queen (see Table caption). Mite reproduction was evaluated in each colony at 3 different times (3, 9, and 15 weeks after queen release) by counting 30 infested cells per colony.

The average mite population decreased by 37% during the 15-week test. The 4 best colonies had an 86% decrease in their mite populations.

When all colonies were equal except for the genotype of the brood (as during the first brood cycle), reproduction of mites was at the level of nonresistant bees and thus not suppressed (see Table). Therefore, brood did not suppress mite reproduction after a mite entered a cell. Suppression was caused either by previous experience in brood cells (as suggested by Martin et al., *Exp. & Appl. Acarol.* 21: 539-549) or by adult bees.

A high level of nonreproducing mites was first expressed

Table — Colonies were divided into 6 groups based on 6 drone source colonies. The 25 queens were supersisters whose mother had been mated to one drone and whose colony had suppressed reproduction in 72% of the mites. Inseminations from drone sources a and b were each with a single drone; c-f were with mixed spermatozoa. Data are means.

Insemination source	No. of cols.	% cells with nonreproductive mites			Final mite population
		week 3	week 4	week 15	
a	5	24	100	88	204
b	3	26	85	70	441
c	6	15	89	88	95
d	3	32	81	55	409
e	4	22	72	33	423
f	4	21	42	50	295
total	25	22	79	67	273

sometime between weeks 3 and 9 (see table), so this characteristic cannot be detected until this time. Thus mite populations continue to grow in mite-resistant colonies for at least one month, and an overall decrease in the mite population may not exist until the third month.

**13. Harris, J. W.<sup>c</sup> & J. R. Harbo<sup>c</sup>—LOW SPERM COUNTS AND DELAYED OVIPOSITION OF MITES IN COLONIES OF HONEY BEES THAT ARE RESISTANT TO *VARROA JACOBSONI*.**—Mites were categorized as reproductive or non-reproductive in colonies selected for their potential resistance to varroa (see Harbo & Harris). Non-reproductive mites were further subdivided into 3 groups: dead mites that had not laid eggs (usually <2% of the mite population in non-resistant colonies), mites that did not lay eggs, and mites that began laying eggs later than normal. The percentages of mites in the 4 categories significantly changed through time (Figure). In some colonies the proportion of dead mites was > 60%. High mortality resulted from mites becoming trapped beneath the cocoon of the bee larvae. During the 9th week 50% of all mites had not laid eggs, and 20% began laying eggs too late to permit maturation of the female progeny. In an earlier experiment, mites that had not laid eggs stored significantly ( $P < 0.001$ ) fewer spermatozoa in their seminal receptacle than normally reproductive mites [ $4 \pm 3$

( $n=33$ ) and  $27 \pm 3$  ( $n=45$ ) spermatozoa, respectively].

These results suggest that resistant bees suppress mite reproduction in several ways. In some cases, the bees cause the production of non-functional male mites that do not fertilize their sisters. Mortality of male mites was low and cannot explain non-mating. According to Martin *et al.* (*Exp. & Appl. Acarology* 21: 539-549), unmated female mites will not lay eggs during subsequent brood cycles. In other cases, the bees may cause mites to delay egg-laying. The fact that oviposition is delayed rather than totally inhibited suggests that activation of oviposition is not strictly an on/off mechanism.

**14. Hood, W.M.<sup>s</sup>, P.M. Horton<sup>s</sup> & J.W. McCreadie<sup>s</sup>—EVALUATION OF THE IMPORTED FIRE ANT FOR CONTROL OF WAX MOTHS IN STORED COMB**—The objective of this project was to assess the efficacy of the imported fire ant (IFA), *Solenopsis invicta*, in controlling wax moths, *Galleria sp.*, in stored comb. Field studies were conducted from 1995 to 1997 in 4 South Carolina counties that varied in IFA population density.

Supers of nine (dry) frames of undamaged drawn comb were stored by various arrangements on sites which ranged from 43 - 159 fire ant mounds per acre. All frames were placed in a freezer and held at 10°F (-12.2°C) for 12 hours to kill all life stages of wax moth prior to test. Each super was seeded with 3 wax moth larvae at field placement to ensure initial pest presence.

Tests plots were established each year during the first week in August with wax moth damage measurements made at 30-day intervals for 15-16 weeks. Wax moth damage was measured by placing a frame-size plexiglass grid (cm<sup>2</sup>) over each side of a test frame and counting all squares that contained damage. The total amount of damage per super of nine frames was the test variable. IFA activity density (active ant mounds per acre) was recorded at the beginning of each test by multiplying the number of mounds within a 60 foot radius of a plot center by a factor of 4.3.

In 1996, wax moth damage was significantly greater ( $P < .01$ ) in counties with lower IFA densities (Figure). In 1997 tests, significantly less ( $P < .01$ ) wax moth damage occurred when five supers were stacked crisscrossed on a wooden pallet placed on the ground in areas containing 120 IFA mounds per acre. Comparisons were made with equipment stacked similarly with IFA excluded on the same site. IFA exclusion was made possible by stacking supers on a table that had legs sitting in no. 10 vegetable cans one-half filled with used motor oil. Other sites having less IFA density (43 and 86 mounds per acre) had greater ( $P > .05$ ) wax moth damage.

The results of these tests indicate that beekeepers may use this method of biological control of wax moths only in areas having very high level IFA activity. The IFA did not damage the stored

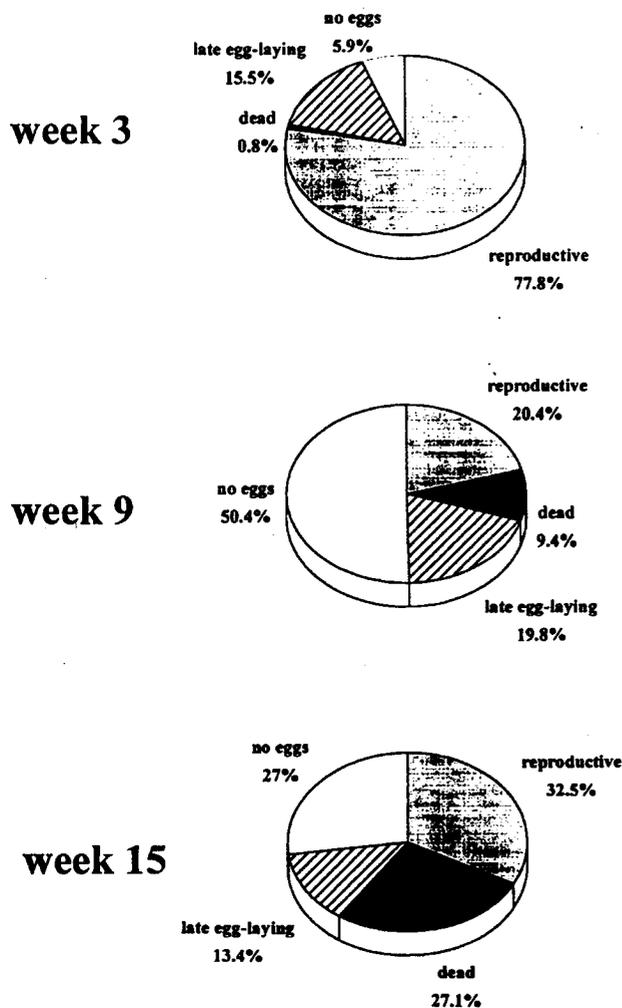


Figure - Reproductive status of mites found in 25 colonies of bees at three time periods. Frequencies of mites in all 4 groups changed significantly through time ( $\alpha = 0.05$ , MANOVA). The best colony had 43 mites, and the worst had 710 mites at the end of 15 weeks (each colony began with 433 mites).

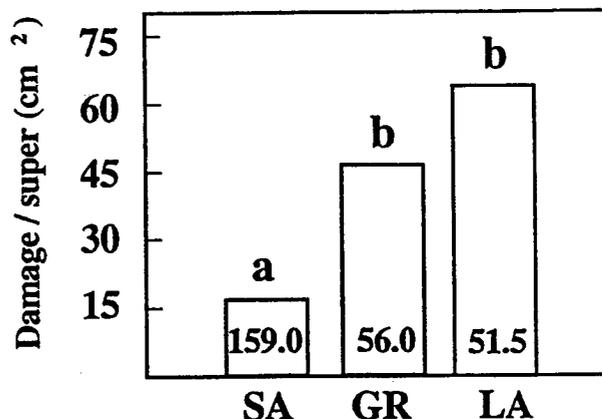


Figure. Comparison of wax moth damage (cm<sup>2</sup>) / super of nine frames. Numbers in bars represent the number of imported fire ant mounds per acre located on test sites in the 3 counties (Saluda, Greenville and Laurens).

comb or wooden ware during these studies.

**15. Huang, Z.-H.<sup>1</sup> & G.E. Robinson<sup>1</sup>—REGULATION OF ONSET OF FORAGING IN HONEY BEES: A WORKER PRIMER PHEROMONE IN THE MANDIBULAR GLANDS?**—Honey bee workers can respond to different colony conditions by changing their age of first foraging. It is not known what signal(s) workers perceive to modulate their rate of behavioral development. We proposed previously that a socially transmitted "inhibitor" regulates the behavioral development in workers, by modulating the juvenile hormone (JH) levels in the blood (Huang & Robinson, 1992 *Proc. Natl. Acad. Sci.* 89: 11726-29). In this study we examined the modality by which worker inhibition occurs and one possible source of the hypothesized inhibitor. In the first experiment, 1-day-old adult worker honey bees were reared for 7 days in a typical colony in one of three ways: individually in cages with double-screens that prevented physical contact with colony members, individually in cages with single-screens that allowed only antennation and food exchange with colony members, or with unrestricted access to colony members (control bees). Workers were then sampled to measure their rates of JH biosynthesis, JH titers, or were put into a colony to observe their tendency to become foragers. Bees reared in double-screen cages had significantly higher rates of JH biosynthesis and JH titers than control bees. Behavioral observations showed that bees reared in double-screen cages were significantly more likely to become precocious foragers than control bees. Because bees reared in double-screened cages experienced the same environment of volatile odors as other bees, but could not engage in direct social interactions with others, these results suggest that physical contact is required for inhibition. Bees reared in single-screen cages were only partially inhibited; their JH biosynthesis rates and titers, and their rate of behavioral development were intermediate between that of the control bees and bees reared in double-screen cages. These results indicate that bees with limited physical contact with colony members were only partially inhibited, again suggesting that physical contact is required for inhibition. The inhibitor therefore is unlikely to be a highly volatile chemical, but rather either a chemical that is passed in the food or during antennation, or a behavior that can be transmitted through a single screen.

Previous work here and elsewhere has shown that queen mandibular pheromone can inhibit rates of JH biosynthesis (Kaatz et al., 1992 *J. Comp. Physiol. B* 162: 588-592) and also delay the onset of foraging in workers (Pankiw et al., in press, *J. Insect Physiol.*). There is also structural similarity between 9-ODA (a major component of queen mandibular pheromone) and 10-HDA (a major product of the worker mandibular glands). Our second experiment therefore tested the hypothesis that worker mandibular glands contain an inhibitor of behavioral development. Older bees with their mandibular glands removed were significantly less able to inhibit the behavioral development of young bees than were sham-operated and control bees (unoperated). These results suggest that an inhibitor of behavioral development may be produced by the worker mandibular glands.

In summary, our results suggest the existence of a worker-produced primer pheromone that affects the behavioral development of individual workers and thus the organization of the entire honey bee colony. This pheromone may be produced in the mandibular glands, but further study is needed to reveal its chemical identity.

**16. Hunt, G. J.<sup>m</sup>, E. Guzmán-Novoa<sup>n</sup> & M. Ioannides<sup>m</sup>—CONFIRMING THE EFFECTS OF GENETIC LOCI THAT CONTRIBUTE TO THE STINGING BEHAVIOR OF AFRICANIZED BEES**—Africanized honey bees have had a serious impact on beekeeping in Mexico due to their stinging behavior. They now threaten important queen-rearing operations in the US. European and Africanized bees can mate with each

other to produce hybrid offspring and some hybrids are just as likely to sting as highly Africanized bees. If we had DNA markers for the genes that influence stinging behavior, we might be able to use them to determine which bees carry the African versions of the "stinging genes." These DNA markers may be useful tools for breeding gentle bees and for understanding how genes affect stinging behavior in our bees. We have been working to find diagnostic markers. We use markers that are generated from bees in the polymerase chain reaction (PCR). The markers appear as bands of DNA on gels. We can often distinguish the African version of the marker from the European, because the bands look different on the gel. We use the African versions of the DNA markers to follow the inheritance of African versions of genes.

Last year, we identified specific genetic markers from one honey bee chromosome that are linked to a gene that has an effect on stinging behavior. We are 95% sure of these results. In that study, we made a single cross between an African drone and a European queen to produce a hybrid queen. The gene was mapped by comparing the markers of the drones of the hybrid queen with the stinging behavior of the colony that each drone fathered. We used 172 colonies to do this. Drones that fathered colonies that stung excessively tended to have African versions of the DNA markers near the potential "stinging gene". Since then, four other possible gene locations have been mapped that influence the number of stings in our whole-colony assay.

Now we are trying to confirm the effects of these genes on stinging behavior by looking at the behavior of individual workers from new crosses. To test a gene's effect, we need to see if workers that inherited the marker from the African parent are more likely to sting. We made new hybrid queens from crosses between highly-defensive Africanized bees and gentle European bees in Mexico. Hybrid queens were backcrossed to either a European or African drone. Hundreds of workers from these crosses were tested to see if they were more likely to become guard bees. Many other bees were tested to see if they were likely to be the first individuals to sting a target. We also used a more general stimulus on about 600 workers. Individual bees were placed on an electrified grid attached to a constant-current stimulator (Isostim A320). Current was increased until the worker stung the leather patch. If those individuals with the African DNA marker are more likely to sting with less stimulus, we can confirm the gene's effect on stinging behavior. We are now testing for the association of African versions of the markers with all these aspects of stinging behavior.

**17. Masterman, R.<sup>i</sup>, K. A. Mescè<sup>i</sup>, B. H. Smith<sup>d</sup> & M. Spivak<sup>i</sup>—ODOR DISCRIMINATION BY HYGIENIC HONEY BEES USING PROBOSCIS EXTENSION CONDITIONING**—Hygienic behavior involves the detection and removal of infected pupae from the brood nest, and affords the bees a behavioral mechanism of disease resistance. We hypothesize that hygienic bees, as compared to non-hygienic bees, have a lowered stimulus response threshold for chemical cues that are perceived to be abnormal within a brood cell. We tested this hypothesis using proboscis extension reflex conditioning to evaluate the ability of hygienic and non-hygienic bees to discriminate between odors. In odor discrimination experiments, one odor (conditioned stimulus, CS+), is paired with an appetitive (+) unconditioned stimulus (sucrose) while the other odor (CS-) is paired with punishment, (-, salt solution). If bees are able to discriminate between odors, they will extend their proboscises upon presentation of the CS+ and withhold their proboscises in response to the CS-. In the first experiment, we used the floral odors of anise and geraniol to determine if there were overall differences in the abilities of the lines to discriminate odors. In the second experiment, we used the normal and abnormal brood odors of healthy and diseased pupae at low, intermediate and high stimulus levels to establish the stimulus response thresholds of each line. The results were analyzed using 2-way ANOVA (Table). Bees from both hygienic and non-hygienic lines were able to discriminate between floral odors. At low

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**15. Huang, Z.-H.<sup>1</sup> & G.E. Robinson<sup>1</sup>—REGULATION OF ONSET OF FORAGING IN HONEY BEES: A WORKER PRIMER PHEROMONE IN THE MANDIBULAR GLANDS?**

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**17. Masterman, R.<sup>i</sup>, K. A. Mescé<sup>i</sup>, B. H. Smith<sup>d</sup> & M. Spivak<sup>i</sup>—ODOR DISCRIMINATION BY HYGIENIC HONEY BEES USING PROBOSCIS EXTENSION CONDITIONING**

Hygienic behavior involves the detection and removal of infected pupae from the brood nest, and affords the bees a behavioral mechanism of disease resistance. We hypothesize that hygienic bees, as compared to non-hygienic bees, have a lowered stimulus response threshold for chemical cues that are perceived to be abnormal within a brood cell. We tested this hypothesis using proboscis extension reflex conditioning to evaluate the ability of hygienic and non-hygienic bees to discriminate between odors. In odor discrimination experiments, one odor (conditioned stimulus, CS+), is paired with an appetitive (+) unconditioned stimulus (sucrose) while the other odor (CS-) is paired with punishment, (-, salt solution). If bees are able to discriminate between odors, they will extend their proboscises upon presentation of the CS+ and withhold their proboscises in response to the CS-. In the first experiment, we used the floral odors of anise and geraniol to determine if there were overall differences in the abilities of the lines to discriminate odors. In the second experiment, we used the normal and abnormal brood odors of healthy and diseased pupae at low, intermediate and high stimulus levels to establish the stimulus response thresholds of each line. The results were analyzed using 2-way ANOVA (Table). Bees from both hygienic and non-hygienic lines were able to discriminate between floral odors. At low

stimulus levels, neither line was able to discriminate between healthy and diseased brood odors. At high stimulus levels, they discriminated between odors of healthy and diseased pupae equally well. There were differences in the hygienic and non-hygienic responses to brood odors at the intermediate stimulus levels when the CS+ consisted of 2 chalkbrood mummies. The ability of hygienic bees to discriminate between odors of healthy and diseased brood emerged between the low and intermediate stimulus levels. Hygienic bees discriminated between the CS+ of 2 and 4-chalkbrood stimuli equally well. The non-hygienic bees extended their proboscises less frequently ( $P < .01$ ) to the CS+ of 2-chalkbrood than to the CS+ of 4-chalkbrood, suggesting that their threshold level was between the intermediate and high stimulus levels, and therefore higher than that of the hygienic bees.

**Table. A summary of the results of the 2-way ANOVA for the main effects of odor discrimination and line of bee (hygienic and non-hygienic) for each PER experiment. ns = not statistically significant at  $\alpha \geq 0.05$ .**

Odors	CS+	Odor Discrimination	Line Differences
Floral	anise	$P < 0.01$	ns
	geraniol	$P < 0.01$	ns
<b>Brood</b>			
Low	1 pupa	ns	$P < 0.02$
	1 chalkbrood	ns	$P < 0.04$
Intermediate	2 pupae	$P < 0.01$	ns
	2 chalkbrood	$P < 0.01$	$P < 0.01$
High	4 pupae	$P < 0.01$	ns
	4 chalkbrood	$P < 0.01$	ns

**18. Nabors, R. A.<sup>o</sup> & R. E. Linhardt<sup>o</sup>—APICULTURE EXTENSION EDUCATION NEEDS IN THE U.S.**—The purpose of this study was to determine what extension programs should be made available for beekeepers to ensure apiculture remains viable. Pollination problems have been reported by producers of entomophilus plants. Feral honey bees have been reported as absent from large areas. Mite parasites of honey bees are considered a main cause of these problems. The numbers of beekeepers and honey bee colonies are perceived as decreasing. Economic factors have limited profits for beekeepers. Mites and Africanized bees have reduced demand for package bees and queen bees.

A Delphi study asked the opinion of experts (Extension Apiculture Specialists) from 25 states. The Delphi method was chosen to inventory the condition of Apiculture Extension Education and beekeeping industry problems in the United States. The Delphi instrument was initiated with questions suggested and validated by apiculture professionals. The first round of the Delphi collected demographic data and general opinions about questions regarding apiculture. Two additional rounds of questioning were used to gain consensus or expose disagreement. Responses were analyzed using measures of central tendency (mean, median and mode) along with the range and standard deviation.

Findings indicate too few apiculture educational opportunities are available. Experts agree that more and better educated beekeepers are needed. There is also a need for education about apiculture for growers of entomophilous plants. Public education about apiculture and the value of honey bees was deemed important and necessary. Resources to meet these needs were limited. Current apiculture specialists are 50 years old and devote less than 1 day per week to apiculture. These specialists have competing job responsibilities that take 80% of their time. Assistants are

rarely provided for apiculture specialists. Conclusions indicate public financial support would be needed for apiculture extension, education and research to continue. Private financial support from growers and the beekeeping industry should supplement and encourage public support of apiculture extension, education and research. There is a need for extension, education and research programs to address problems within the apiculture industry.

**19. Nasr, M.E.<sup>p</sup> & D. McRory<sup>q</sup>—INTEGRATED PARASITIC MITE MANAGEMENT IN HONEY BEES: FROM LABORATORY RESULTS TO FIELD IMPLEMENTATION**—Ontario beekeepers have created a tech-transfer program to adapt the developed technology of mite control to current beekeeping management practices. This program has focused on using multiple approaches; genetics, management, and chemical control in an integrated parasitic mite management (IPMM) strategy. Bee breeders have been using the bioassay for tracheal mite resistance (Gary & Page 1987 *Exp. Appl. Acarol.* 3:291-305; Nasr et al. *J. Econ. Ent.* in press) to evaluate their breeder queens annually (Table). The average mite abundance in the mite resistant bees decreased significantly from 13 mites/bee in 1992 to 1.5 mites/bee in 1997.

Liquid nitrogen has been used to kill the capped brood to test and evaluate bee stocks for the hygienic trait. Selected tracheal mite resistant bee stocks that also are hygienic have been propagated and utilized in commercial bee colonies.

Mite-away<sup>®</sup> as a single application of 65% formic acid has reduced the incidences of tracheal mites by 95% and increased the natural drop of varroa mites per frame of bees per 24 h by 95% in honey bee colonies in comparison to the control colonies in the spring. Bee colonies treated with Mite-away<sup>™</sup> in the spring have not required fall treatment for tracheal mites. The use of Apistan<sup>®</sup> to control varroa mites is recommended for use in the fall. Thus, Mite-away<sup>™</sup> can be used alternatively with Apistan<sup>®</sup> in bee colonies to reduce the risk for developing Apistan<sup>®</sup> resistant varroa, contamination of wax and cost.

Preliminary field trials have shown that providing wintering protection to bee colonies has helped to reduce the impact of mites on bees.

Current developed IPMM practices - requeening with mite resistant queens in the spring or summer, spring application of Mite-Away<sup>®</sup>, fall application of Apistan<sup>®</sup>, and providing winter protection to bee colonies - are compatible. The key to achieve successful control of mites has been monitoring populations of mites and applying the proper combination of control methods when mite populations approach the economic threshold. The economic threshold for tracheal mites is  $\geq 10\%$  mite prevalence in 150 bees which are sampled in equal numbers of bees from each colony in the bee yard in the spring. Currently, the presence of varroa in bee colonies in the spring and the fall is used to trigger the application of Mite-Away<sup>®</sup> and Apistan<sup>®</sup>, respectively. The economic threshold for varroa is being evaluated to provide a better estimate. Beekeepers who have adopted the outlined IPMM strategy have reduced their winter colony mortality to 10%.

Criteria	1992	1993	1994	1995	1996	1997
Number of beekeepers	5	10	22	22	22	28
Number of tested bee colonies	28	154	305	413	168	162
Number of tagged bees	1,350	9,990	22,058	32,220	12,460	12,600
Total number of tagged bees*	90,678					

\* The average percentage of retrieved tagged bees after exposure to tracheal mites in infested bee colonies was 76.5%.

**20. Ramírez B. W.<sup>r</sup>—A DRAWER FEEDER WITHOUT SCREENS THAT WORKS AS A QUEEN EXCLUDER FOR AFRICANIZED BEES**—The object of this work is to present a hive feeder that also works as a queen excluder. The African or Africanized queens are such good layers that it is common to find brood combs in the supers used to store honey. It has been report-

ed that the traditional queen excluder also acts as a honey excluder. Due to the high tendency of the Africanized bees to rob other hives during artificial feeding in the wet season, the author designed a drawer feeder with the standard lateral dimensions of the Langstroth hive but 4cm (about 2 inches) deep. The frame of the drawer is made of wood and has a tin pan inside which leaves a 4 cm passage in front for the feeding bees. The feeder is placed under a flat top cover, and all the hives are kept with two standard brood chambers year around. Since the Africanized bees also rob honey and become very defensive when the empty supers with extracted combs are returned to the hives, the supers have to be returned in the very late evening, almost when it is dark. One day, due to the lack of time and shortage of light, I had to put the empty extracted supers between the feeder and the cover; two weeks later I visited the apiary to rearrange the supers and found that most of them were almost full of honey already and did not have queens or brood of any age. Since that time I have used the feeder as queen excluder. The drawer feeder has other functions: 1) it works as an insulating air space during hot spells; 2) it collects dripping water during the wet season; 3) it allows for feeding with dry sugar or syrup; 4) it does not expose all the combs and bees during feeding or the extraction of supers, thus, less smoke has to be applied; 5) it allows easy feeding inside the hives; 6) when the boxes with extracted combs are placed over the feeder after the last honey extraction, the bees quickly move all the surplus honey down to the brood chamber leaving the combs dry, without wax moths, and clean for storage; 7) when burr combs with honey or brood are put in to the feeder, the bees remove the honey and the bees take care of the brood up to emergence; 8) it facilitates the introduction of the board with the bee escape and the removal of the bees with repellents; 9) it can be used as a space to apply fumigants against *Varroa jacobsoni*. The author has been using the feeder for three years as a queen excluder in his 100 africanized hives with great success and less expenditure of time and effort.

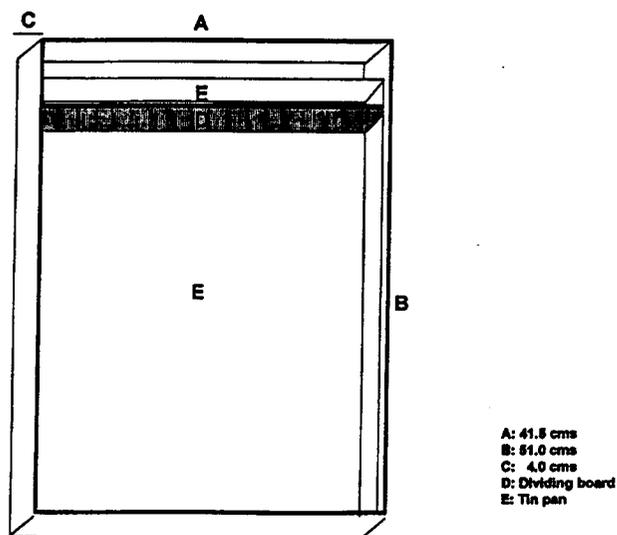


Figure. Diagram of the drawer feeder.

21. Spivak, M.<sup>1</sup>, Reuter, G. S.<sup>1</sup>—HYGIENIC HONEY BEES AND RESISTANCE TO VARROA AND BROOD DISEASES—Hygienic honey bees detect and remove diseased brood from the nest before the pathogen becomes infectious, and remove varroa-infested brood, interrupting the reproductive cycle of the mite. Colonies may be screened for hygienic behavior using a freeze-killed brood assay (Spivak & Downey, 1998 *J. Econ. Ent.* 91: 64-70).

If hygienic queens are to be utilized by the beekeeping industry, it is important to determine whether colonies with naturally mated queens from hygienic stock produce as much honey, have

less disease, and have lower levels of varroa mites than colonies bred from unselected, commercial stock. Our experiments in 1995-96 indicated that queens raised from a hygienic line had significantly less chalkbrood, no American foulbrood (AFB), and produced significantly more honey than colonies derived from a commercial line of bees (Spivak & Reuter, *Apidologie* In press). In three of four apiaries, the hygienic colonies had significantly fewer varroa mites (Figure); however, the mite levels were relatively low in all apiaries.

We repeated the experiment in 1997 for two reasons: 1) to compare the colonies from the hygienic line to Starline colonies, a line renowned for high honey production; and 2) to compare the levels of varroa mites between the two lines when the colonies had been left untreated for a longer period of time.

In March 1997, groups of sister hygienic and sister Starline queens mated naturally in an apiary of a commercial beekeeper in Mississippi. The colonies were transported to Minnesota in May and were distributed among four apiaries. Comparisons of the colonies during the summer and fall indicated that the hygienic colonies had significantly less chalkbrood, had no AFB, and produced as much honey as the Starline colonies. The hygienic colonies had significantly lower levels of varroa mites across all apiaries by mid-October (Figure). The mite levels in the untreated colonies will be evaluated again in the spring of 1998.

These results demonstrate that the effects of AFB, chalkbrood, and varroa mites can be alleviated if queen producers select for hygienic behavior from their own lines of bees.

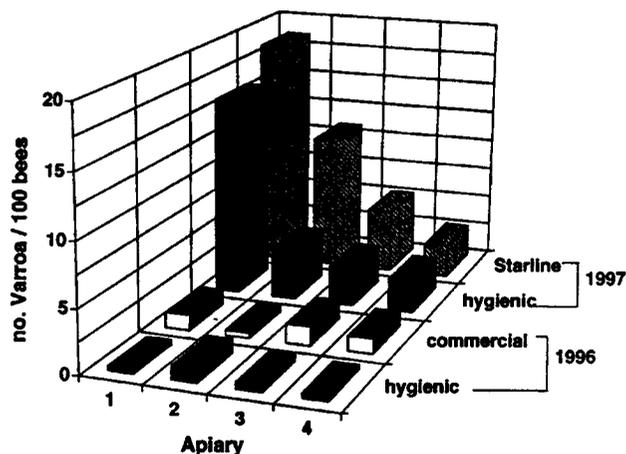


Figure. Comparison of the mite counts on adult bees in the 1996 and 1997 experiments. The colonies in each experiment had not been treated for one year. Mite counts were derived by sampling adult bees, straining the mites off the bees, and calculating the number of mites per 100 bees. Comparison of the two bee lines across 4 apiaries (2-way ANOVA) indicated that in 1996, the hygienic colonies ( $n = 49$ ) had significantly fewer mites than the commercial colonies ( $n = 46$ ) in three of four apiaries, although the mite levels were low in all colonies (line effect:  $F = 5.78$ ;  $df = 1, 87$ ;  $P = 0.013$ ). In 1997, the hygienic colonies ( $n = 55$ ) had significantly fewer mites than the Starline colonies ( $n=47$ ), even in highly infested apiaries (line effect:  $F = 4.56$ ;  $df = 1,94$ ;  $P = 0.035$ ).

22. Skinner, J. A.<sup>5</sup>, J. P. Parkman<sup>5</sup> & M. D. Studer<sup>5</sup>—COMPARING EFFICACY OF FORMIC ACID (GEL, BOARD) APILIFE VAR( AND 100% THYMOL CRYSTALS FOR MANAGEMENT OF VARROA AND TRACHEAL MITES IN TENNESSEE—Honey bees in North America have been devastated in the past decades by two parasitic mites, the varroa mite, *Varroa jacobsoni*, and the tracheal mite *Acarapis woodi*. However, only two treatments are registered in the USA

for mite control. This study evaluates alternative mite treatments which are needed to expand management options for beekeepers.

Two apiaries consisting of 25 colonies each were established at the Plant Sciences Unit experiment farm, Alcoa Hwy., Knoxville in April 1997. Beginning in August, four treatments for varroa and tracheal mites were evaluated: 1) formic-acid gel, a pre-formulated gel containing 65% formic acid enclosed in a plastic bag; 2) formic-acid board, 250 ml of 65% formic acid absorbed into a 10" x 10" Homosote board held in a Ziploc® vegetable bag; 3) Apilife VAR®, a European product consisting of essential oils absorbed into foam blocks; and 4) thymol (the major constituent of Apilife) crystals applied in petri dishes. Treatments were placed singly above the brood cluster of honey bee colonies; one treatment was applied per colony. Treatments were applied in mid-August and were removed 38 days after application. Then Apistan® was applied to kill and collect surviving varroa. Ten colonies were used per treatment. varroa drop was monitored continuously during the study with sticky-board traps placed on the bottom board of each hive. Thirty bees were collected from each colony 4 times to examine for tracheal mite infestation. Intensive colony counts of number of adult bees, amount of brood and food stores in each colony were made 3 times.

Formic-acid gel gave the greatest varroa mite drop at 3 days post-application; formic-acid board and Apilife gave similar drops at 3 days. These three treatments gave similar results for 6-38 days post-application. Thymol provided no control. Low mite drop for the Apilife-treated colonies after Apistan® application suggested Apilife may have given the best control. However, intensive counts revealed Apilife-treated colonies contained significantly less brood after treatment. The strong essential oil vapors of Apilife probably disrupted egg laying and brood production. Fewer varroa in these colonies was more a result of colony weakness (thus, fewer mite hosts) than of product efficacy. Varroa populations in the formic-acid treated colonies were rebounding by the time of Apistan application. Based on the examination of the first three tracheal mite samples, the formic-board treatment provided the best control (no mites found 4 weeks after treatment versus 10% mean colony infestation level before treatment). Apilife and formic gel reduced tracheal mite infestations from 8.7 to 1.8% and from 8.0 to 3.3%, respectively. Infestation levels rose in the thymol-treated (from 5.0 to 10.7%) and control (from 11.7 to 13.7%) colonies.

Failure of the formic acid treatments to provide better varroa control indicates exposure time to vapors must be increased. Delivery must be adjusted so that vapors are present in the hive for at least 42 days (= 2 honey bee brood cycles) so that two generations of varroa, which develop in capped brood cells, come in contact with vapors. In most colonies, the gel had completely desiccated by 21 days after application. Spring 1998 studies will focus on application methods and timing for formic acid.

**23. Webster, T. C.<sup>1</sup> & E. C. Cho<sup>2</sup>—EFFECTS OF DRIFTING BEES ON VARROA DISTRIBUTION WITHIN AN APIARY**—In order to develop a strategy for sampling a limited number of varroa-infested hives within a large apiary, a mathematical model was constructed for comparison with actual infestations. The model simulates the multiplication of mites and their dispersal via drifting bees. It was designed to determine the most successful strategy when overall infestations are very low. The model assumes an initial infestation in one hive, that mite dispersal is primarily through drifting host bees, and that mite-infested bees exhibit drifting behavior no different from uninfested bees.

Given these assumptions, we determined the probability of discovering the apiary's infestation when sampling only two of the hives in an apiary. Detection of an infestation in a linear apiary of 10 hives is most likely when hives at positions 3 and 8 are chosen. Samples taken from the end hives 1 and 10, or adjacent hives such as 4 and 5, are much less likely to discover the presence of the apiary's infestation.

By comparing the results of the model to actual apiary infes-

tations, the validity of the model's assumptions will be examined, and possibly the assumptions will be altered. Similar models descriptive of the multiplication and dispersal of tracheal mites and Nosema disease will have value in the generation of sampling strategies for large apiaries.

**24. Wilson, W.T.<sup>1</sup>, J. Baxter<sup>2</sup>, J. Ibarra & R. Rivera—PARASITES AND DISEASES OF HONEY BEES IN GUATEMALA**—In a 1980 examination of honey bee colonies in Mexico, Wilson et al. (*Am. Bee J.* 124:51-53) reported that overall the bees were healthy and no *Varroa jacobsoni* was present. Similarly in 1985, honey bee samples (in alcohol) were collected across the southern part of Guatemala. There were no *V. jacobsoni*, *Acarapis woodi* or *Nosema apis* seen in the colonies nor in the adult bee samples (n=200) that were analyzed at the USDA-ARS Honey Bee Unit, Laramie, Wyoming. No bacterial foulbrood was observed in the colonies. Honey bees in tropical climates have traditionally been healthier than those in temperate zones.

In 1996 and 1997, cooperative research projects between MOSCAMED (Guatemala), USDA-APHIS & USDA-ARS resulted in the broodnests of ca. 400 honey bee colonies being examined and more than 200 samples of adult bees in alcohol analyzed at the ARS Bee Lab, Weslaco, Texas. *A. woodi*, *V. jacobsoni* and *N. apis* were all present in the adult bees. However, amoeba (*Malpighamoeba mellificae*) cysts were not present in the Malpighian tubules. In the colonies, we saw no American or European foulbrood, no sacbrood (virus) and no chalkbrood (*Ascospaera apis*), although they may exist at low frequencies.

Varroa mites were spreading rapidly when we first observed them in southwestern Guatemala in the spring of 1996. Many beekeepers were already reporting weakened bee populations and the loss of colonies. Some beekeepers recognized that the damage was caused by varroa, while others attributed the bee loss to a wide variety of causes, and especially to insecticide applications. Due to the wide distribution of varroa early in 1996, we suspect that varroa mites entered Guatemala in 1995 via a natural migration of bee swarms from Mexico or from other countries through the importation of infested queens. Bee Parasitic Mite Syndrome was present, but rare.

We examined colonies (n=70) in a remote bee yard near Chicacao, Guatemala with a history of strong, healthy colonies and excellent honey production. However, early in 1996 the colonies suffered from a lack of adult bees (5 to 10 frames covered by workers) and a small amount of brood (ca. 2 frames) per colony. We were not able to identify a causative agent. Adult bee samples were collected in October, November and December 1996 from three bee yards near Chicacao with the same problem, and sent to the Weslaco ARS Bee Lab for analysis. Samples from the 3 months contained varroa, tracheal mites and nosema spores. Samples from November were the most heavily infested with colony prevalence of 55% varroa, 33% tracheal mite and 25% nosema. At these levels of parasitism, the bee populations were under stress and dwindling or dying bees would not be unexpected. The only remedy would be chemical intervention with the appropriate treatments. The level of Africanization during the 3 months was 79% (by FABIS). Varroa was present in 1996-97 bee samples from Honduras and Costa Rica.

**25. Wilson, W.T.<sup>1</sup>, J. Baxter<sup>2</sup>, J. Ibarra & R. Reiners<sup>3</sup>—MITICIDES IMPREGNATED IN PLASTIC FOR CONTROL OF VARROA JACOBSONI**—Adding agricultural pesticides to a plastic matrix is not a new concept. The cattle industry has been using ear tags for external parasite control since the 1970s (J. George, pers. comm. 1997). United States beekeepers started using fluralinate-impregnated plastic strips (Apistan®) in 1987 (Coindreau, pers. comm. 1998). The plastic meters an effective dose of chemical over a long period of time and in a manner that is safe for both the applicator and the animal being treated.

Currently, beekeepers in the U.S. have only one miticide (Apistan®) registered with EPA for control of *Varroa jacobsoni*. This leaves the beekeeping industry in a vulnerable position since varroa has become resistant to fluralinate in Europe, Argentina and the U.S. Consequently, testing of additional miticides impregnated into plastic has been carried out by the USDA-ARS Honey Bee group, Weslaco, TX and by universities.

In the spring of 1997, 60 one-story colonies were established in an apiary near Coatepeque, Guatemala. Five treatment groups of 12 colonies each were given compound A (amitraz) (see Table). A sticky board with screen was placed under each colony for 24-hrs. and removed to count varroa. After 45-days all treatments were removed; after a 48 hr. delay, 1 Apistan strip was placed in the broodnest plus a bottom sticky board for a final 24 hr. varroa count. With these 2 sets of data, the percent varroa reduction (miticide efficacy) was determined. All four chemical treatments were highly effective for varroa control, and all four were statistically different ( $P=0.001$ ) from the control.

In the fall of 1997, a similar study was done with compound C in Guatemala using 46 one-story colonies divided between 2 apiaries. There were 4 groups of 10 colonies each receiving chemical treatment and the remaining 6 colonies were untreated controls. Baseline data were collected in the first 24-hrs. and then treatments removed after 45 days with the final data collected on day 47. As seen in the table, the 10% strips were highly effective against varroa, but the 1% strip was ineffective and did not decrease the varroa population.

Table - Miticide efficacy tests, Coatepeque, Guatemala

Compound A (Spring 1997)		Compound C (Fall 1997)	
Treatment	% Varroa Change*	Treatment	% Varroa Change*
5%, 2 strips	-97 a	10%, 1 strip	-91 a
7.5%, 2 strips	-98 a	10%, 2 strips	-97 a
10%, 2 strips	-98 a	10%, 2 strips**	-91 a
12.5%, 2 strips	-99 a	1.0%, 2 strips	+48 b
Control	+76 b	Control	+71 b

\*Percent varroa with same letter not significantly different (ANOVA)  
\*\*Strips on bottom board

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