Proceedings of the American Bee Research Conference

The 1999 American Bee Research Conference was held on January 4 and 5 at the Hilton Hotel in Baton Rouge, LA. The following are abstracts from the 1999 conference.

1. Baxter J.[1], P. J. Elzen[2] & W. T. Wilson[2]—CONTROL OF THE SMALL HIVE BEETLE *AETHINA TUMIDA*—With the recent discovery of the small hive beetle in Florida, and now in the coastal regions of Georgia, South Carolina and North Carolina, it is necessary to develop control measures for use both inside and outside of a colony. This new pest has the potential to be a serious threat to U. S. beekeeping.

One problem in controlling the small hive beetle inside a colony is finding a treatment that is effective but causes no damage to bees. A preliminary test was set up to screen 8 different insecticides in a plastic strip matrix (provided by Y-Tex Corp., Cody, WY and Bayer Corp., Shawnee Mission, KS). These strips were cut into 2cm x 5cm pieces and placed inside 20-ml scintillation vials. Either 3 adult beetles or 3 pre-pupae were placed in each vial. Each treatment was replicated 10 times for a total of 30 adults or larvae exposed to each insecticide. Untreated vials served as controls. Vials were held about 27° C for 24 hrs and then checked for mortality.

Zeta-cypermethrin, bifenthrin and chlorpyrifos have the potential to harm bees (Baxter, unpublished data, 1997) and therefore require caution. Ethion, amitraz and ivermectin provided inadequate control. Coumaphos was chosen for the in-hive treatment because it is safe for bees and has been used to control *V. jacobsoni* in Europe. It is now being developed for mite control in the U. S. Permethrin without PBO was selected as a ground drench. Permethrin can harm bees if it is applied directly on them.

Table. Mortality of *A. tumida* exposed to eight insecticides.

Treatment	Mean Percent Mortality	
	Adults	Larvae
Zeta-cypermethrin	100 a	100 a
Permethrin	100 a	100 a
Bifenthrin	100 a	100 a
Chlorpyrifos + PBO	100 a	100 a
Coumaphos	100 a	100 a
Ethion	13.3 b	26.7 c
Ivermectin + PBO	70.0 c	20.0 bc
Amitraz	13.3 b	3.3 bd
Control	4.9 b	7.0 d

Means within a column followed by different letters are significantly different (p<0.05,LSD).

Adult beetles hide under cardboard attached to the bottom board (D. Westervelt, Pers. Comm., 1998). Coumaphos strips attached to 15cm x 15cm pieces of cardboard with one side removed to expose the corrugations were stapled to the bottom board. This proved to be highly effective in controlling the beetles with 99.9% mortality after 72 hours. As an added bonus, larvae were also attracted to the cardboard and many of them were killed.

Permethrin (40%) was mixed at the fire ant rate of 5-ml per 4 liters of water and applied using a hand-held pump sprayer. Four liters were adequate to drench the soil in front of approximately 6 hives. This treatment caused high mortality (>90%) in both emerging adults and pre-pupae.

Although these are preliminary results, coumaphos as an in-hive treatment and permethrin as a ground drench show great promise against this newly discovered pest.

2. Bell, Jr. D. A.[1], S. Gloor[1], & S. M. Camazine[1]—BIOCHEMICAL MECHANISMS OF FLUVALINATE RESISTANCE IN *VARROA JACOBSONI* MITES—In recent years there have been reports of sporadic instances of fluvalinate (Apistan) resistance in *Varroa jacobsoni* mites. These cases of resistance have been scattered across the country and are generally associated with migratory beekeepers, especially those originating in Florida. Recently, Pennsylvania State University Honeybee Lab has been able to acquire hives with presumptively resistant mites. These hives were from a hobby scale beekeeper and were not in proximity to any migratory or commercial apiaries.

Resistance in these hives was tested using the USDA/Pettis protocol (Pettis *et al.*, July 1998 *Am. Bee J.* 538-541), which involves stapling a section of Apistan strip to an index card and placing the card against the wall of a Mason jar with a screen ventilation cover. A sample of mite infested bees are placed in the jar and allowed to walk over the Apistan strip for twenty-four hours.

After this time total mite mortality levels for both 2.5% and 10.0% Apistan dosages are determined by washing the surviving mites from the bees with a weak hot water/detergent solution. The accepted levels of mite kill for fluvalinate are 33% and 85% for 2.5% and 10.0% Apistan after twenty-four hours. Fluvalinate resistance in these mites was confirmed, as the mortality level of both dosages after twenty-four hours fell significantly below accepted kill percentages for fluvalinate, as determined by G-test.

To determine the biochemical pathway being utilized by the mites, various synergists were used to block detoxification pathways in the mites using a modified version of the USDA/Pettis protocol. In the modified protocol filter paper disks soaked in an acetone/liquid fluvalinate solution were used in place of Apistan strips. The efficacy of these disks was tested on mites known to be non-resistant to fluvalinate, and mite downfall levels were not significantly different between the disks and the strips. In the synergist testing experiments, 1:10 molar ratio of fluvalinate:synergist in acetone was used to prepare the filter paper disks. If a synergist blocks the pathway that is conferring resistance to the mites, then the mortality level after twenty-four hours should be restored to the acceptable kill levels.

Four different synergists blocking two different pathways were tested. Piperonyl Butoxide (PB) and Chlordimeform (CDF) were tested as blockers of the cytochrome p450 (Mixed Function Oxidase [MFO]) detoxification pathway. N-Ethylmaleimide (NEM) and Maleic Acid Diethyl Ester (DEF) were tested as blockers of the Glutathione S-Transferase (GST) detoxification pathway. In the cases of PB, NEM and DEF the mortality levels between the synergist:fluvalinate treated mites and the fluvalinate only treated mites were not significantly different, indicating that those biochemical pathways are not being utilized by the mites to breakdown fluvalinate. CDF did produce a mite downfall, however, but comparison of the mite downfall of CDF:fluvalinate and CDF alone treated mites indicated that the mite downfall was due to acaricidal effects of CDF alone rather than to a synergistic interaction by CDF. Therefore, the cytochrome p450 (MFO) and Glutathione S-Transferase detoxification pathways can be eliminated as being responsible for conferring fluvalinate (Apistan) resistance to *V. jacobsoni*. Future experiments will involve testing additional synergists that block the hydrolytic esterase and the hydrolase detoxification pathways.

3. Eischen, F. A.[a], J. Baxter[a], P. J. Elzen[a] & W. T. Wilson[a]-*VARROA JACOBSONI* INFESTATION LEVELS IN COLONIES OF HONEY BEES POLLINATING ALMONDS IN CALIFORNIA-During late February and early March of 1998 we examined honey bee colonies pollinating almonds in the Central Valley of California (Bakersfield to Modesto) for *V. jacobsoni*. Beekeepers representing the widest geographical area of the US were selected. About 20 colonies each from the apiaries of selected beekeepers were given a hasty inspection. If no particular sign of any disease was found, four of these colonies were randomly chosen for an ether roll. Bees from the brood nest (200-250) were killed with ether and the number of mites counted. If during our hasty inspection, colonies showing signs of distress were found, then those were included in our ether roll survey. In total, 112 beekeepers representing 18 states west of the Mississippi River (Nevada & New Mexico absent) plus Wisconsin were represented in the survey. Colonies originating from California predominated in our survey (35.8%), followed by Montana (12.8%), South Dakota (8.3%), North Dakota (6.4%), and Arizona (5.5%). Other states individually represented less than 5% of the total.

About 1400 colonies were hastily inspected and 456 examined by ether roll. Twenty-eight of the 112 beekeepers had infested colonies (25%). For those beekeepers with infested colonies Prevalence-c equaled 44.9 ± 26.9%. This number is probably somewhat inflated because we biased the sample by looking for distressed colonies. The average number of mites per ether roll equaled 4.6 ± 12.1. The figure shows the distribution of mite infested colonies.

About 50% of beekeepers had fluvalinate (Apistan) treatments on their colonies. In general, we found colonies to be in good condition and relatively few mites in most operations.

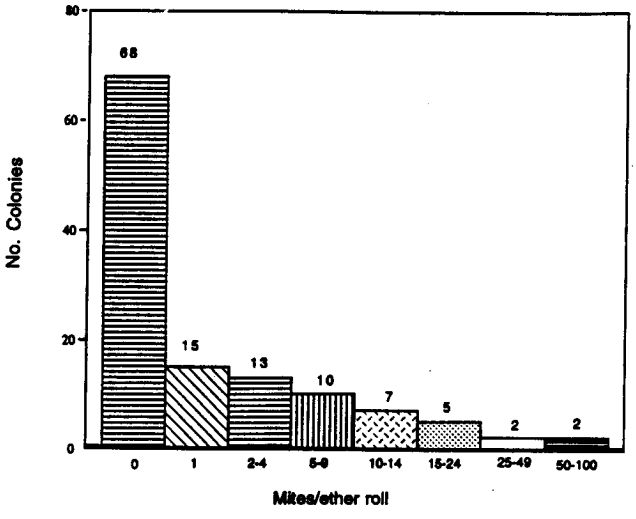

Figure. Distribution of *V. jacobsoni* per ether roll in infested colonies in California during almond pollination, spring 1998.

4. Eischen, F.A.[a], D. Westervelt[c], & C. Randall[d]—DOES THE SMALL HIVE BEETLE HAVE ALTERNATE FOOD SOURCES?—*Aethina tumida*, the small hive beetle, has recently become a serious pest of honey bee colonies in Southeastern United States. We have initiated a study of this sap beetle's diet breadth and preferences. Free flying beetles that were naturally infesting two apiaries in South Florida were presented with a range of fruit in 8-liter plastic pails. The St. Lucie apiary consisted of four living colonies, but had a few weeks previous held 90+ colonies, most of which had been killed or greatly weakened by small hive beetles. The soil in the vicinity of these hive sites contained many beetle larvae, pupae and emerging adults. The Volusia County apiary was a staging area for beekeeping operations, and involved hundreds of colonies, many of which were infested. Our hypothesis was that a portion of the adult beetles emerging from the soil in the St. Lucie apiary would find suboptimal feeding conditions. These beetles if offered a choice might select foods other than those found in a bee hive. On the other hand, adult beetles emerging in the Volusia County apiary would find a great many available honey bee colonies and would chose them over alternate foods.

Weekly observations starting 25 November 1998 showed that adult beetles in the St. Lucie apiary were highly attracted (100+/fruit) to a number of fruit, including cantaloupe and pineapple; moderately attracted (20+/fruit) to grapes, mango, and honeydew melons; and weakly attracted (2+/fruit) to avocado, banana, and starfruit (carambola). Beetles were not attracted to tomatoes. In the Volusia County apiary, beetles were weakly attracted to bananas only. Beetles were positively identified by M.C. Thomas, USDA, Gainesville, FL.

Feeding trials under captive conditions showed that mating, egg laying, larval development, and adult eclosion occur normally on a diet of cantaloupe. Additional tests are planned. We tentatively draw the following three conclusions: 1) *Aethina tumida* prefer honey bee colonies; 2) when the preferred diet is not readily available, these beetles will chose to eat and oviposit on selected fruit; and 3) beetle reproduction can occur on some of these fruit.

5. Ellis, M.D.[a] & M. E. Scharf[a]—EVALUATION OF THE VARROACIDAL PROPERTIES OF CYHEXATIN— Cyhexatin is an organotin compound that has been used extensively as a miticide in greenhouse, field and orchard crops. It is virtually non toxic to honey bees (LD_{50} adult bees and larvae = 36.7 and 25.08 (µg per bee, respectively) (Atkins, 1993, *Asian Apiculture* p. 639-643). We investigated the varroacidal properties of cyhexatin due to its low bee toxicity and its mode of action (that differs from existing varroacidal products). We bioassayed technical grade cyhexatin by coating 20 ml scintillation vials with serial dilutions of cyhexatin dissolved in 100% ethanol. One half ml of each concentration was added to each of six vials. The vials were then rotated on a vial roller until the solvent evaporated. This procedure provided a uniform residue of the test concentration on the walls of the vials. We then uncapped brood combs from a single mite-infested colony and transferred ten adult *V. jacobsoni* females to each vial using a camel hair brush. One mature pupa was added to each vial as a food source. All vials were transferred to a dark incubator at 26°C and held for 24 hours. After 24 hours of exposure, mortality was determined by touching mites with a probe and observing movement. The entire bioassay was repeated two times. In the first bioassay, we determined the range of activity. For the second bioassay, we selected a concentration that killed all the mites, a concentration that killed no mites, and four concentrations between the two extremes. A total of 720 mites were used to conduct the assays. Using probit analysis, we estimated a LC_{50} of 8.16 (5.01 - 11.53) (µg /vial and a slope of 1.92 ± .28. The LC_{90} was 37.82 µg/vial. Compared to existing varroacides, cyhexatin exhibited low mite toxicity (the LC90 for fluvalinate, a commonly used varroacide, is 2.4 (µg/vial).

A field test of 10% cyhexatin-impregnated plastic strips (Y-Tex Corporation) was also conducted from October 24 to December 5, 1999. We placed two strips per colony in the brood nests of each of six mite-infested colonies. Mite fall during the first 48 hours of treatment was determined using sticky boards. After five weeks of treatment, the cyhexatin strips were removed. Two days after cyhexatin removal, two new Apistan strips and sticky boards were placed in each colony and mite fall during the first 48 hours of Apistan treatment was determined. Two of the six colonies examined died during the experiment (both were heavily infested), and control was ineffective in the remaining colonies. From the remaining four colonies, we recovered a total of 7,442 mites. Ten percent (677) of the mites were recovered during the first 48 hours of cyhexatin treatment. The remaining 90% were recovered when cyhexatin strips were removed and replaced with Apistan strips. We conclude that while cyhexatin is virtually non-toxic to bees, it does not merit further investigation as a varroacide.

6. Elzen, P.[a], J. Baxter[a], F. Eischen[a], & W. T. Wilson[a]—RESISTANCE OF *VARROA JACOBSONI* TO FLUVALINATE IN THE U.S.—Since its discovery in the U.S. in 1987, *Varroa jacobsoni* has become a serious parasitic pest in U.S. bee hives. Only one chemical is legally registered in the U.S. for mite control—fluvalinate. Recently, fluvalinate resistance in *V. jacobsoni* has been reported in Italy (Lodesani *et al.*, 1995, *Apidologie* 26: 67-72) and in the U.S. (Baxter *et al.*, 1998, *Am. Bee J.* 138).

In the spring of 1998, a survey of fluvalinate resistance in U.S. *V. jacobsoni* mites was initiated using a standard laboratory method (Plapp & Vinson, 1977, *Environ. Entomol.* 6: 381-384). Technical grade fluvalinate, dissolved in acetone, was pipetted into 20 ml glass scintillation vials. The vials were rolled to evaporate the acetone, leaving a film of fluvalinate on the inner surface of the vials. Preliminary experiments determined that a dose of 2.4(µg of fluvalinate per vial caused approximately 80-90% mortality of susceptible mites. If mites from a location tested showed statistically less than 80% mortality after 24 hours, we considered these mites resistant. Three mites were placed in each vial, and each vial was replicated 33 times. *V. jacobsoni* from three main locations were tested: Texas, Florida, and migratory bee operations in California.

Mites tested in Texas were found to be susceptible to fluvalinate; the two bee operations tested resulted in an average of ca. 73% mortality. Mites tested in Florida were highly resistant, with an average of ca. 24% mortality. Mortality of mites tested in California also was significantly less than 80% with an average of ca. 65% mortality.

From these results it can be seen that fluvalinate resistance in *V. jacobsoni* is present in certain regions of the U.S. The need for additional chemicals to control the mites is underscored. There is a good likelihood that at least one additional chemical will be approved for use in honey bee hives for mite control. This compound will greatly aid the industry in its efforts to deal with these mites in the future.

7. Elzen, P.[a], J. Baxter[a], F. Eischen[a], & W. T. Wilson[a]—BIOLOGY OF THE SMALL HIVE BEETLE—The small hive beetle, *Aethina tumida* Murray, was first officially reported in North America in November 1996. Since that time it has been officially verified in Georgia and South Carolina. In U.S. areas where it occurs, this beetle has been found to be devastating to honey bee operations, resulting in loss of previously healthy hives.

Adult beetles are attracted to a combination of odors of hive products plus bees. It is also presumed that additional beetles are attracted to an aggregation pheromone released by adult beetles. Lundie (1940, S. Africa Forestry Sci. Bull. 220) has reported on the life cycle of the small hive beetle in South Africa: Once in the hive, eggs are laid in groups; the egg stage lasts 2-3 days. Small white larvae then hatch which feed on hive products for ca. 10-14 days. Thousands of larvae can be seen within the hive, all of apparently similar age. Larvae then enter a wandering stage, during which they cease feeding and migrate to soil outside the hive to pupate. The pupal stage lasts ca. one month, after which adults emerge and fly to hives. Adults can live up to six months. Five complete generations were completed in one year in South Africa.

Lundie reports that beetles primarily attack previously weakened hives; our observations in Florida, however, have shown that beetles attack and overtake previously healthy hives under good management. Lundie also reports that adults and larvae damage hives by feeding on honey and pollen only, but we have experimental data showing adults feeding on eggs and observations showing adults and larvae feeding on bee larvae and pupae, as well as honey and possibly pollen. Honey seeps from the frames, becoming fermented and fouled, resulting in a definite "rotten orange" smell. Honey is thus rendered unusable.

Because of its status as a new pest in the U.S., much is not known of its biology in this new geographic location. Key to such knowledge is why the small hive beetle is so devastating in the U.S., whereas it is considered a minor pest in South Africa. Such a difference implies that there are natural controls in South Africa which are not present in the U.S. Additionally, all infestations in the U.S. have been of European bees, which do not show hygienic behavior toward beetle infestations; perhaps African honey bees are more defensive against beetle infestations.

Much is left to learn about this new species attacking honey bees in the U.S. There is currently a combined effort of USDA laboratories, universities, and state agencies involved in studying the biology and potential control of the small hive beetle in the U.S.

8. Elzen, P.J.[a], J. R. Baxter[a], & W. T. Wilson[a]—SUSPECTED RESISTANCE TO AMITRAZ IN *VARROA JACOBSONI*— Within the past year, several reports have been published on resistance of *V. jacobsoni* to fluvalinate in the U.S. (Baxter *et al.*, *Am. Bee J.* 138: 291; Elzen *et al.*, *Am. Bee J.* 138: 674). Recently, the USDA Weslaco bee laboratory has conducted research on alternate chemicals to control fluvalinate-resistance mites. One such study was a comprehensive field and laboratory experiment using amitraz, conducted on fluvalinate-resistant mites in the Upper Midwestern U.S.

The study involved using the glass vial technique used in previous resistance testing in *V. jacobsoni* (Elzen *et al.*, *Am. Bee J.* 138: 674). A single discriminating concentration of technical grade amitraz was used: if less than 90% mortality resulted in mites exposed to this amount of amitraz, these mites were considered resistant. A total of 1·14 adult mites were tested, 3 mites per vial, from four different hives within the candidate bee yard.

The field test of the mites, conducted in the same bee yard from which the mites were taken to do the laboratory study, consisted of 51 hives treated with amitraz incorporated into plastic strips. Two strips were hung in the brood chamber of each hive and initial counts of mite drop were measured 24 hours later by use of standard-sized sticky boards inserted onto the bottom of each hive. Five weeks later, total mite numbers were measured by inserting coumaphos strips, shown to be effective on resistant mites, into each hive for 24 hours and then numbers of mites dropped were counted. At the time of treatment, colonies had less than 1 frame of brood.

Results of the laboratory test indicated some problems with amitraz in killing *V. jacobosni*. Less than 33% mortality was found, which is significantly less than the expected 90% mortality. The field test corroborated the laboratory test, with only approximately 72-81% mortality of mites treated with amitraz strips; expected mortality, based on previous experience, was greater than 95% mortality (Baxter et al., *Southwest. Entomol.*, in press).

From these results it can be seen that one population of *V. jacobsoni* is showing some signs of tolerance to amitraz. It is our opinion that this represents a case of multiple resistance of amitraz and fluvalinate, because in other locations, amitraz is providing excellent control of fluvalinate-resistant mites. Such generally good control by amitraz would not suggest cross resistance between fluvalinate and amitraz. These results create a difficult situation for the North American beekeeping industry and points out the need for a third, unrelated miticide.

9. Finley, J.[b], D. Sammataro[b], & S. Camazine[b]—QUEENS IN TRANSIT: SPYING ON THE POST OFFICE—Prior to this study, little was known of the shipping conditions that queens endure, except that they are highly variable depending on the weather and the knowledge and care of postal workers. We sent temperature and humidity monitoring devices (Stowaway Dataloggers, Onset Compter, MA) inside a total of 20 queen shipments from producers in California, Texas, Hawaii, Georgia and Tennessee. The shipping methods were via Priority Mail and Express Mail (US Postal Service), and UPS Next Day Air and UPS 2nd Day Air (United Parcel Service). All of these shipping methods delivered queens to us within 96 hours.

Queen shipments commonly experienced temperatures of 50°F to 95°F (10°C to 35°C) and relative humidity of 30 to 80%. A few shipments suffered damage, such as shattered wooden queen cages, broken data monitors and torn packaging, indicating improper handling.

Cool temperatures can be lethal to honey bees. Free & Spencer-Booth (1959 *Bee World* 40:173-177) found that groups of 10 bees died after 5 hours at 50°F (10°C). Bee muscles do not function below 50°F (10°C) (Esch 1988 *J. Exper. Biol.* 135: 109-117) and without internal heating from their flight muscles, bees cannot rewarm themselves; they go into "chill coma" and quickly die. During an airplane flight, one Express Mail shipment from California (battery cage) experienced temperatures below chill coma 50°F (10°C) for 4.5 hours and survived 20°F (-6°C) for 2.5 hours, see figure. We believe this package was placed in an unpressurized, unheated cargo compartment.

If exposed to hot temperatures, overheated bees which have access to water can cool themselves by evaporating liquids. However, bees in queen cages are not typically supplied with any liquid. Bees without water overheat and die at approximately 115°F (46°C). One queen shipment reached 109°F (43°C) for approximately 2 hours.

What affect does this have on the quality of the queen received by the beekeeper? It may affect queen health and may interfere with sperm storage. In future research, we plan to subject Penn State-raised queens to these types of temperature conditions and observe queen acceptance and performance.

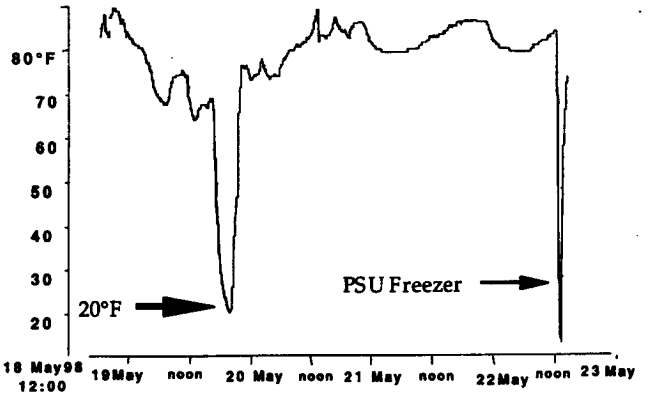

Figure. Queen Shipment from California

10. Harbo, J. R.[f] & J. W. Harris[f]—COMPARING COLONIES WITH QUEENS INSEMINATED WITH SEMEN FROM ONE OR SIX DRONES—When measuring characteristics of bees at the colony level, colonies with queens inseminated with one drone (identical spermatozoa) provide different conditions for selection of bees than do colonies with queens mated to many drones. We found that insemination with one drone may be needed in the early stages of selection, whereas multiple mating seems to be advantageous later.

The character that we measured was nonreproduction of the parasitic mite, *Varroa jacobsoni*, after a female mite had entered a brood cell. Nonreproduction of mites is a heritable characteristic of honey bees (Harbo & Harris, *J. Econ. Entomol.*, in press), and includes mites that die in the cell, those that produce only males, those that produce progeny too late to mature, as well as those that lay no eggs. However, nonreproduction in this report includes only mites that laid no eggs.

We compared nonreproduction of mites in colonies with queens mated to 1 drone to colonies with queens mated to 6 drones. The experiment was conducted twice; we used 24 colonies in 1994 and 25 colonies in 1998. The experimental designs were the same except 1994 queens were daughters of unselected stock and 1998 queens were daughters of a queen selected for suppressing mite reproduction. In both years, drones were collected from the entrances of >40 unselected colonies at three different apiaries near Baton Rouge, LA. The drones were mixed and recollected into cages so that each drone taken for insemination was a random representative of the population that had been collected. Queens were randomly assigned a treatment (one or six drones). Colonies for each experiment were established from a uniform mixture of bees and mites.

In 1994, colonies with queens inseminated with one drone had significantly larger means and variances than colonies with queens inseminated with 6 drones: 22 ± 16% nonreproducing mites (mean ± SD, n = 13) in colonies with queens mated to one drone and 6 ± 7% (n = 11) in colonies with queens mated to six drones (Harbo, Apiculture for the 21st Century, Connor and Hoopingarner [eds.], Wicwas, in press). The means were statistically different at the 0.01 level. In 1998, the means and variances were not different (36 ± 25% [n = 13] and 35 ± 26% [n = 12], respectively).

Therefore, the results from the two years were different. Single drone inseminations did not improve detection of nonreproduction in 1998 as it had in 1994, perhaps because the genes for nonreproduction were present at a higher frequency in 1998 than in 1994. With no difference in detection, advantage may shift to multiple-drone inseminations during the mid and latter stages

of selective breeding because (1) queens survive longer when inseminated with multiple drones (2) more creative selection schemes can be used (such as mating a group of queens with the same mixture of semen), and (3) daughter queens from a multiply-mated queen are more variable and would therefore reduce the rate of inbreeding at a time when the specific characteristic of selection is well-established.

11. Harris, J. W.[f], J. R. Harbo[r] & J. P. Woodring[f]—A COMPARISON OF HEMOLYMPH PROTEINS IN HONEY BEE LARVAE THAT ARE RESISTANT AND SUSCEPTIBLE TO *VARROA JACOBSONI*—Total protein and specific fractions of protein from the hemolymph of bee larvae were not significantly different (α=0.05) between bees that were resistant to *V. jacobsoni* mites and those that were susceptible to them. Resistant bees were selected for their ability to suppress mite reproduction (Harris & Harbo, *J. Econ. Entomol.*, in press). This heritable trait of bees includes mites that die in a cell, those that produce only males, those that produce progeny too late to mature, and those that do not lay eggs. Susceptible bees were unselected and known to not suppress mite reproduction.

We examined protein content of the hemolymph because (1) developing eggs in mites incorporate host-derived proteins and (2) stimulation of reproduction in mites occurs during a discrete period soon after the brood cell has been capped. Mites that feed before the host spins a cocoon will lay eggs, while those that begin feeding after spinning will not reproduce. One of the changes in bee larvae that correspond to this period is the release of larval storage proteins to the hemolymph from the fat body.

We compared the total protein content of the hemolymph using a standard spectrophotometric assay from Bio-Rad™. Samples of hemolymph were collected from last instar larvae in resistant and susceptible colonies (8 colonies per type). The average (mean±SE) percent of non-reproducing mites was significantly different between resistant (66.5 ± 9.1%) and susceptible (30.0 ± 2.1%) bees. Three classes of larvae were sampled from each colony: (1) uncapped larvae, (2) capped larvae that were feeding on brood food, and (3) capped larvae that were spinning cocoons. The two types of bees did not differ significantly in concentration of total protein. Resistant larvae averaged 48.2 ± 0.83 µg/µl, and susceptible larvae had 46.8 ± 0.85 µg/µl. In both types of bees, spinning larvae (58.7 ± 1.25 µg/µl had significantly higher protein concentrations than either uncapped larvae (42.5 ± 0.82 µg/µl) or capped larvae that were still feeding (41.3±0.96 µg/µl).

In a second experiment we compared profiles of specific protein bands between the two types of bees. High performance liquid chromatography with a gel filtration column (Bio-Silect™ SEC-250) and a UV detector was used to partition whole hemolymph samples into 5 bands that differed in molecular weight: Peak A (Mwt. > 300,000) at 6.8 min, Peak B (Mwt. ≅ 73,000) at 8.2 min, Peak C (Mwt. ≅ 34,000) at 9.0 min, Peak D (Mwt. ≅ 17,000) at 9.6 min and Peak E (Mwt. < 3,000) at 12.3 min. Peak A contained at least 3 co-eluents, and peak E contained at least 2 co-eluents.

Samples of hemolymph were taken from the two types of bees (5 colonies per type) at two different dates (mid-October and mid-November). The resistant bees had 90.9 ± 4.1% non-reproducing mites, and the susceptible bees had 29.6 ± 3.0 % non-reproducing mites in November. The same 3 classes of larvae that were used for total protein were sampled from each colony. The peak areas were not different between chromatograms for resistant and susceptible larvae. Some peaks changed significantly between the two dates, and some peaks were significantly different between the 3 classes of larvae.

12. Hart, T.[s] & R. Nabors[s]—POLLEN TRAPS AS A METHOD OF *VARROA JACOBSONI* CONTROL— A method of *V. jacobsoni* control using pollen traps was tested in honey bee colonies. Based on preliminary studies by J. Pettis and H. Shimanuki (personal communication), we tested whether pollen traps fitted with screens that keep bees off the bottom board while allowing mites to fall through would result in the death of mites and a reduction of mite populations.

Fifteen packages of bees were installed on April 24, 1998. On August 6, 1998 the bees were separated into 3 groups of 5. Each group received one of 3 treatments that were randomly assigned. One group was treated for mites with Apistan as the standard. The second group was left untreated. The third group was given a standard Glory Bee pollen trap. All treatments were removed at 42 days on September 20, 1998. Mites were counted on a sticky board after 24 hours using Apistan to induce drop.

The results indicated that the colonies treated with pollen traps had significantly (at alpha = 0.10) fewer mites than the untreated colonies. Apistan controlled mites significantly better than the pollen trap treated hives. We are 90% sure that a pollen trap under the brood nest will reduce the mite population by approximately 50%.

Although pollen traps did reduce the number of mites, the amount of reduction was not enough to control the mites. However, the reduction in mite build up could reduce mite populations enough to allow one treatment with a miticide rather than two.

Hive #	# Mites		Treatment
1	8		Apistan
6	14		Apistan
9	8		Apistan
14	27		Apistan
15	12		Apistan
4	108		Untreated check
7	747		Untreated check
10	686		Untreated check
11	952		Untreated check
12	1,332		Untreated check
2	183		Pollen trap treated
3	662		Pollen trap treated
5	31		Pollen trap treated
8	766		Pollen trap treated
13	559		Pollen trap treated

Confidence level Treatment	95% # mites / colony	90% # mites / colony
Apistan	13.80 b	13.80 c
Pollen trap treatment	440.20 a	440.20 b
UTC	765.00 a	765.00 a

Means followed by the same letter do not significantly differ (P = .05, LSD) or (P = 0.1, LSD)

13. Kaftanoglu[h], O. & K. Abak[f]—THE EFFECTIVENESS OF BUMBLEBEES (*BOMBUS TERRESTRIS*) FOR THE POLLINATION OF CROPS GROWN IN UNHEATED GREENHOUSES—There are > 18,000 hectares of greenhouses in Turkey. Tomatoes are grown in 50% of these, peppers in 15%, eggplants in 9%, and other crops in the remaining 26%. Most growers use hormones or growth regulators to achieve fruit set, but this practice reduces fruit quality. We have started a project to use domesticated bumblebees (*Bombus terrestris*) for pollinating greenhouse grown crops. This project is supported by the NATO SfS Program.

Bumblebees are bigger and hairier than honey bees. Their long tongues allow them to visit and pollinate flowers such as tomatoes which have long tubular corollas. Their ability to forage at low temperatures and low light intensities make them important pollinators in the greenhouses.

Bumblebee colonies were raised in the laboratory and used for

the pollination of tomatoes, peppers, eggplants, and melons in unheated greenhouses during the winter seasons of 1996 and 1997. Queens emerging from hibernation were fed with fresh pollen and 50% sucrose solution, and kept in dark rooms at 27°C and 65% relative humidity. New colonies were transferred to greenhouses when they had 20-30 worker bees.

Bumblebee pollination increased the early and total tomato yields by 32.9% and 40.2%, respectively, when compared with hand pollination. It also increased the fruit weight, height, diameter, acidity, and the number of seeds per fruit by 16.7%, 8.2%, 6.2%, 9.8%, and 43.1%, respectively.

Similarly, bumblebee pollination increased the early and total yield of peppers by 29.6% and 22.4%, respectively. The average fruit weight, diameter, volume, flesh thickness, and the number of seeds were 18.7%, 9.8%, 16.4%, 7.9%, and 52.3% greater, respectively, in bumblebee pollinated plants.

The effectiveness of bumblebees and honey bees for the pollination of melons was compared. Honey bee pollination increased the early yield and total yield by 31.3% and 10.9%, respectively. There were more fruits per plant in honey bee pollinated plants; however, fruit weight, height, and diameters were 17.7%, 13.2% and 21.4% greater, respectively, in bumblebee pollinated plants.

The average pollen production, pollen viability, and pollen germination rates were studied for the eggplant varieties 'Munica,' 'Mileda,' and 'Phaselis.' The production, viability, and germination rate of pollen increased throughout the season. The average pollen production in April, May, and June was 1.13 million, 2.01 million, and 2.77 million grains per flower, respectively. Pollen production and germination rates were higher in 'Munica' than in the other varieties. The average pollen viability was 42.6% in April, 90.0% in may, and 93.1% in June. The pollination effectiveness of bumblebees was also compared with hand pollination and hormone applications. Bumblebee pollination increased the total yield by 33.2% and 48.3% compared to hand pollination and hormone applications, respectively. Moreover, bumblebee pollination significantly increased the fruit weight, length, diameter, and volume.

In sum, the use of bumblebees facilitates the production of better quality and healthier crops which is of benefit to consumers.

14. Nasr, M. E.[1], A. J. Skinner[a]& P. G. Kevan[b]—OXYGEN CONSUMPTION, THERMOREGULATION AND WINTERING OF HONEY BEES INFECTED WITH TRACHEAL MITES (*ACARAPIS WOODI*)—Effects of the tracheal mite infestation on Oxygen (O_2) consumption, thermoregulation and wintering of honey bees were studied. The O_2 consumption of clusters of bees (100-250 bees) which were placed in a ventilation-temperature controlled chamber was monitored using S3A Oxygen analyzer. Clusters of bees infested with tracheal mites had lower O_2 consumption rates in comparison to uninfested bees when incubated at -20, 5, and 25°C. When the temperature was lowered from 5 to -20°C the O_2 consumption rate of the unifested bees increased by 2-4 times, whereas the O_2 consumption rate varied with the level of mite infestation (Figure). The O_2 consumption of individual bees was significantly lower in infested bees when bees were incubated at 15°C for 50 min in comparison to uninfested bees and bees with wax blocked first spiracle.

The cluster temperature for clusters of bees was constant (mean ± sd: 26.8 ± 1.4°C) when bees were held at 5°C regardless the level of mite infestation. When temperature was lowered to -20°C the cluster temperature was reduced significantly in the mite infested bees and varied with the mite infestation levels. Variable portions of bees in the infested clusters entered a chill-coma and died when incubated at -20°C.

A field evaluation in Ontario of three wintering systems (Davies system, cardboard wintering box, and wintering facility) showed that the Davies system and the wintering facility gave bee colonies a relatively consistent temperature through the winter at 5°C and 7°C, respectively. The temperature inside the cardboard wintering box fluctuated with the ambient temperature. The winter mortality rates of the uninfested colonies ranged from 0-25% in all three tested systems. However, the mortality rates of the infested colonies (≥50% mite prevalence) were significantly higher when the cardboard wintering box was used in comparison to the other two systems. Mite infested colonies with no winter wrap had a mortality rate of 50-75%. These results demonstrate that tracheal mites affect the O_2 consumption, thermoregulation and winterability of bees. Providing wintering protection to bee colonies can alleviate these effects of mites in northern climates.

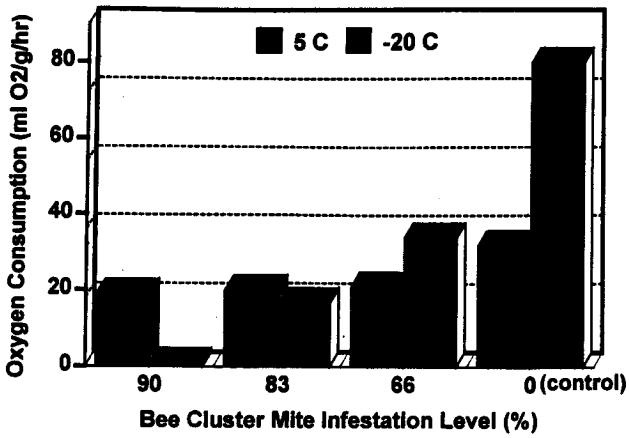

Figure. Oxygen consumption in clusters of 250 bees which had variable tracheal mite infestation levels incubated at 5 and -20°C. Values were taken after onset of exposure resulting from organized clustering of the group.

15. Ostiguy, N.[b], D. Sammataro[b], & S. Camazine[b]—HOW TO COUNT *VARROA JACOBSONI* WITHOUT GOING BLIND: A SANE APPROACH—Counting each *V. jacobosni* mite on a sticky-board is a formidable and not necessarily accurate method of determining the number of mites on a board. A sampling technique was developed to take advantage of the pattern of mites on sticky-boards.

If mites were evenly distributed on sticky-boards, the counting of the number of mites in a simple random sample of cells would be an efficient and accurate method for determining mite number. Two characteristics of bees and beehives exist to create a non-random distribution of mites. The mites are not even distributed on the sticky-board because of the physical nature of the beehive. The frames create stripes of mites. The behavior of bees also influences the pattern of mites. Bees, and therefore mites, cluster within the hive. The result of these two attributes of bees and beehives is that mites tend to occur within non-evenly distributed parallel bands.

Sticky-boards are divided into cells of one square inch or less. The sampling technique requires cells to be grouped into blocks of 6-9 cells. Each cell within a block is assigned a number between one and nine. Cells are selected randomly, without replacement, from each block. All mites within each of the selected cells are counted. The number of mites is determined by multiplying the mean number of mites per cell by the total number of sticky-board cells.

Six sampling regimes were tested. Two, three or four cells were randomly selected for each block on the sticky-board. Then, either the same randomly selected cells were sampled or new randomly selected cells were sampled for each sticky-board. The total mite number was determined by counting the all the mites on the sticky-board.

For each sampling technique the total number of mites was compared to the estimated number of mites. The adjusted R^2 and the error rates differed only slightly between techniques. When the estimates were compared to the total count, irrespective of the

number of mites per board, the adjusted R^2 was greater than 0.99 and the mean percent error was 8.6% (SD=7.2%) when 48 cells (22%) were counted. If 33% of the cells were counted (72 cells) the R^2 was unchanged and the mean percent error was 6.9% (SD=6.8%). If the number of mites per board was greater than 1000 and 22% of the cells were counted, the adjusted R^2 was 0.98 and the mean percent error was 6.8% (SD=4.5%). The adjusted R^2 increased to 0.99 if 33% of the cells were counted and the mean percent error was 5.1% (SD=4.3%). If the number of mites per board was less than 500 and 22% of the cells were counted, the adjusted R^2 was 0.94 and the mean percent error was 9.4% (SD=8.8%). The adjusted R^2 increased to 0.97 if 33% of the cells were counted and the mean percent error was 8.3% (SD=8.1%). If 44% of the cells were counted the adjusted R^2 increased to 0.98 and the mean percent error was 7.5% (SD=6.8%).

To obtain an adjusted R^2 of at least 0.97 and a mean percent error smaller than 8.3% (SD=8.1%), only one-third of each sticky-board needs to be counted. Selecting new random numbers for each sticky-board does not increase the accuracy of the technique.

16. Parkman, J. P.[1], J. A. Skinner[1] & M. D. Studer[1]—COMPARATIVE EFFICACY OF ALTERNATIVE HONEY BEE MITE TREATMENTS WITH AN EMPHASIS ON FORMIC ACID GEL ACTIVITY OVER TIME—Extension Entomology & Plant Pathology, University of Tennessee, Knoxville, TN 37901-1071 USA -Beginning in late May 1998, a study was begun to evaluate 1) 65% formic acid gel enclosed in a plastic bag; 2) formic acid board: 250 ml of 65% formic acid absorbed into a 9.5" x 8" Homosote board held in a Ziploc vegetable bag. Treatments were placed singly above the brood cluster, one treatment was applied per colony. Treatments were removed 21 d later. Then Apistan was applied to kill and collect surviving mites. Ten colonies were used per treatment. Mite drop was monitored with sticky-board traps placed on hive bottom boards. Thirty bees were collected from each colony before and after treatment to determine tracheal mite infestation. HOBO recorders monitored temperatures in the treatment and brood areas of four hives within each treatment group and within two control hives. Formic acid vapors were measured three times per week in six of the hives within each treatment group with a drager gas detector pump.

In early September, a second study was begun to study formic acid gel and three other treatments: 1) Apilife VAR, a European product consisting of essential oils absorbed into foam blocks; 2) plant extract oil strips, cardboard strips impregnated with vegetable oil and plant extracts; 3) Apistan . Treatments were kept on colonies for 42 d. Formic gel and Apilife were replaced at 21 d, oil strips were replaced every 7 d. Ten colonies were used per treatment. Mite drop, tracheal mite infestation, within-hive and ambient temperatures, and formic acid vapors were measured/monitored as before. Bee and brood abundance were estimated. Apistan was not placed on colonies after treatment.

In the spring, low mite numbers hampered efforts to determine mite control. Formic acid vapors in gel-treated colonies dropped from a mean of 56 ppm at 1 d post-application to 17 ppm at 6 d. Mean vapor concentration dropped to < 5 ppm by 13 d. Mean vapor concentrations in formic board-treated colonies were 36, 23 and 15 ppm at 1, 6 and 13 d post-application, respectively. Results suggested formic acid treatments may need to be replaced during a 42-d treatment period. Temperatures fluctuated greatly in hive treatment areas, often exceeding 35°C. Formic board reduced tracheal mite infestation by 94%; gel-treated colonies did not contain tracheal mites before or after treatment.

In September/October, formic gel and Apistan provided the best mite control, Apilife gave intermediate control, oil strips provided no control. Mite drop in the post-treatment period remained significantly lower in formic gel- and Apistan-treated colonies. There was a significant association between formic acid vapor concentration and mite drop. Because of cooler ambient temperatures, vapors did not dissipate as rapidly as in the previous study. There were significantly fewer bees in oil strip-treated colonies after treatment. The formic acid gel pack treatment showed the greatest promise of the alternative treatments tested; but its delivery method should be improved to provide uniform release of vapors. Continued monitoring of *V. jacobsoni* populations in study colonies will determine long-term control of these mites. Tracheal mite samples are still being examined; at least 90% control is expected for formic acid gel.

17. Pettis, J.[m] & H. Shimanuki[m]—DISTRIBUTION OF THE SMALL HIVE BEETLE (*Aethina tumida*) IN SOIL SURROUNDING HONEY BEE COLONIES—The small hive beetle, *Aethina tumida* Murray, was recently identified in the United States and was previously known only from sub-saharan Africa. The distribution and severity of this pest in bee hives in North America is largely unknown at this time. To begin to understand the biology of the beetle we examined the distribution of beetle life stages in the soil and determined the longevity of beetles held without food. Larvae, pupae and newly eclosed adult beetles were found from 1-20 cm deep in sandy soil in south central Florida, nearly 80 percent in the first 10cm of soil. Eighty-three percent of all beetle life stages were collected within 30cm of the entrance of hives, 17% at 90cm and no beetles were found at 180cm. Some adult beetles survived for five days without food or water and thus can survive long enough without food to be easily transported to new areas. Our data on beetle distributions in the soil indicate that, in sandy soil, larvae do not crawl far from the hive to pupate. However, the distance and depth of beetles reported here will surely change with changing soil type. These observations provide a start for future studies on the biology and control measures for the small hive beetle in North America.

18. Rubink, W. L.[s], Orley R. Taylor[h] & William T. Wilson[s]—COMPARATIVE RESPONSE OF AFRICANIZED AND EUROPEAN HONEY BEES TO *VARROA JACOBSONI* MITE INFESTATION: PRELIMINARY RESULTS—There is accumulating evidence that Africanized bees (AHB) are more tolerant of *V. jacobsoni* than are European bees (EHB). This is supported by our personal observations in Texas, as well as observations made by DeJong in Brazil and Guzmán in Mexico. Now that Africanized bees have become a permanent part of Texas' Lower Rio Grande Valley habitat we have been afforded the possibility of studying this difference in susceptibility to the mites, and elucidating the factors which make Africanized bees less susceptible.

This study was carried out at the USDA/APHIS/PPQ Moore Air Base facility, Mission, Texas, where we maintain experimental Africanized honey bee apiaries. Twelve, specially-designed, observation colonies were used to monitor comparative honey bee response to artificial infestations of mites. Each observation colony has a drawer ("refuse" trap) located immediately beneath the combs to collect falling debris, mites, etc, and also has sampling ports to collect samples and introduce mites. In addition, the outside of each colony is equipped with a dead bee trap as part of a mechanism which creates a separate hive entrance and exit, and forces one-way traffic into and out of the observation hive.

Six EHB and six AHB colonies, identified using behavioral, morphometric and isozyme characteristics, were treated with Apistan and/or Amitraz over a 6 week period to eliminate existing mite populations, and then were transferred to observation colonies for 4-6 weeks for acclimation and observation. On June 19-21, 1998 each of the observation colonies was artificially-infested with 50 female Varroa mites obtained from infested bee colonies. Observation colonies were monitored twice weekly for: 1) bee populations and activity levels. 2) live, dead or damaged mites in the refuse traps, 3) dead bees and mites in the dead bee traps. The experiment was terminated on October 2, 1998.

Although bee population levels at the time of introduction to the observation hives were similar, at about midway through the experiment EHB colonies were generally less populous than the AHB colonies, and they fared less well under the extreme drought

conditions present throughout the experiment. EHB population levels were lower, ranging from 1600 to 8000, while AHB populations ranged from 7000-12000 bees/colony. EHB depleted stores and generally had more empty comb and less stored honey or pollen than the AHB.

Results, cumulative mite totals for all AHB and EHB colonies, showed that only a small proportion of partial, damaged mites was present in refuse traps. A slightly higher total cumulative mitefall was found for AHB vs. EHB (~1900 vs. ~1400 mites). Mitefall in refuse traps consisted of 87% whole dead mites, 4% whole live mites and 9% partial mites for AHB, and 79%, 7% and 15%, respectively, for EHB.

Mites found in the dead bee traps, on the other hand, showed interesting differences. Cumulative totals of ~100 mites were found in the AHB colony dead bee traps, but only ~40 in EHB colony dead bee traps. In addition, AHB mite totals were a result of continual removal of mites from the colony, but EHB mite removal became negligible within 2 weeks after the initial infestation. This suggests that there may be a greater degree of mite removal from the interior of the colony by AHB. We plan to evaluate this and other factors more thoroughly in further experiments now underway.

19. Wheeler, S.[a] & M.D. Ellis[a]—THE EFFECT OF CARBON DIOXIDE NARCOSIS OF YOUNG BEES ON HOST PREFERENCE BY THE TRACHEAL MITE, *ACARAPIS WOODI* (RENNIE)—Carbon dioxide narcosis of honey bees results in profound changes in their behavior and biology. Artificially inseminated queens and virgin queens begin egg laying sooner (Mackensen, 1947, *J. Econ. Ent.* 40: 344-349), nurse bees become foragers at an earlier age (Ribbands, 1950, *J. Exp. Biol.* 27: 344-349), and the pharyngeal glands of nurse bees retrogress at an accelerated rate (Simpson, 1954, *Bee World* 35(8): 149-154). These findings suggest that CO_2 narcosis may initiate hormonal changes in bees. We sought to further examine the effect of CO_2 on worker bees by determining if narcosis affected the attractiveness of newly emerged bees to tracheal mites.

A single colony served as a source of tracheal mite-free bees (no mites detected in a 250 bee sample). Likewise, highly infested bees were obtained from a single colony (percent infestation = .88 ± .03). Frames of sealed brood were taken from the mite-free colony, and all adult bees were removed with a bee brush. Brood frames were then placed in an incubator for 24 hours and newly emerged bees were harvested. Newly emerged bees were then divided into two groups. One group received sixty seconds of CO_2 narcosis. The second group was not narcotized. A third group of returning foragers was collected at the hive entrance. Bees from each of the three groups were marked with a drop of enamel paint on the thorax, and cohorts of 100 marked bees were introduced into each of two cages containing approximately 400 heavily infested bees. After three days, the bees in both cages were killed by misting them with 70% ethanol. Bees were then sorted by color and examined for mites by cutting disk from the prothorax, dissolving the muscle in 8% KOH and examining the tracheae at 30X magnification.

There were no significant differences in the three treatment groups (see table), but all three groups exhibited significantly fewer infested bees than the known infested stock ($p = .0005$). If CO_2 narcosis affects host selection by tracheal mites, a longer delay between narcosis and exposure to infested bees may be needed to demonstrate the effect. Since the overall mite movement to young bees was less than expected, other factors may have also affected the results. Our source colony may have been resistant to mites, mites in the source colony may have been dead or past the phoretic stage, or the marking technique may have reduced host attractiveness.

Treatment Group	Mean % of Bees Infested ± SEM	Min.	Max.
Foragers	.04 ± .04 b	0.0	.08
Young Bees	.10 ± .07 b	.03	.17
Young Bees + CO2	.20 ± .004 b	.20	.21
Infested Stock	.88 ± .025 a	.85	.90

Tracheal mite infestation of bees introduced to cages of infested stock. Treatments followed by the same letter were not significantly different ($\alpha = .05$).

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