Proceedings of the American Bee Research Conference

The 1997 American Bee Research Conference was held on January 10 and 11 at the Memphis Marriott in Memphis, Tennessee. The American Bee Research Conference was restructured and will hereafter function as a committee of the American Association of Professional Apiculturists. The 1998 Research Conference will be held in conjunction with the American Beekeeping Federation in Colorado Springs, Colorado on January 17-19, 1998. The following are abstracts from the 1997 conference.

1. Al Ghamdi,[a] A. & R. Hoopingarner[a]—REPRODUCTIVE BIOLOGY OF *VARROA JACOBSONI* IN WORKER AND DRONE BROOD OF THE HONEY BEE *APIS MELLIFERA* UNDER MIDWEST CONDITIONS—The reproductive biology of the mite *Varroa jacobsoni* Oudemans was studied from June 30 to October 15, 1995 under Midwestern conditions in *Apis mellifera* colonies that were highly infested from the previous year. A total of 353 worker cells containing 697 mother mites and 192 drone cells containing 498 mother mites were found in 959 worker cells and 344 drone cells that were examined. Number of offspring were calculated two different ways, one included infestations that did not produce offspring, or produced male only offspring, and included dead offspring. The second method that is presented in some of the literature excluded these infestations. In an effort to compare this study with studies available in the literature, both methods are presented.

It was found that the mean number of female offspring reaching maturity before the bee emerged in worker and drone cells containing a single mother mite are 1.41 and 2.47 offspring respectively, when non-reproduction and male only reproduction were included in the average. When these components were excluded the number increased to 1.82 for workers and 2.69 for drones. In multiple infested cells, the average number of offspring was 1.09 for workers and 1.87 for drones, when non-reproduction and male only reproduction were included. These increased to 1.26 and 2.03, respectively, when non-reproduction and male only reproduction were excluded. This study found that 86.75 and 93% of the mites were fertile in worker and drone cells, respectively, when including those mother mites that produced male only offspring. When excluding these offspring, the fertility rate decreased to 82 and 90%, respectively, in worker and drone cells. The percentage of female mites that did not produce eggs are 11 and 7% in worker and drone cells, respectively. The percentage that produced only males are 5.1 and 3% in worker and drone cells, respectively. In addition, mortality of mother mites accounted for 2.29 and 2.7% of the total infested mites in worker and drone cells, respectively.

2. Danka, R. G.[b], J. D. Villa[b], J. R. Harbo[b] & T. E. Rinderer[b]—INITIAL EVALUATION OF INDUSTRY-CONTRIBUTED HONEY BEES FOR RESISTANCE TO *VARROA JACOBSONI*—Queens from honey bee colonies that had survived without acaricides for at least one year were provided by beekeepers from five states. Twenty-five colonies were established in Baton Rouge, Louisiana, with these queens on 21 May 1996 for a short test to measure the population growth of *Varroa jacobsoni* (final/initial adult mite population) and resultant worker brood infestation under standardized conditions (Harbo, *BeeScience* 4:100-105). Also measured in each colony were factors that potentially influenced this population growth and infestation: the proportion of all adult mites infesting sealed brood (which is directly related to the duration of the mite reproductive cycle), hygienic activity (effectiveness in removing freeze-killed brood from 54-95 capped worker cells within 24 hours), grooming activity (the percentage of adult mites, collected from the bottom board of the hive, that were missing legs or had a damaged idiosoma), non-reproduction of mites (the percentage of infested cells containing no viable female offspring), and the average duration of the postcapping period of worker bees.

Mite infestations were highly variable 11 weeks after establishing queens in colonies with uniform bee and mite populations. The best colony had only a slight increase in the adult mite population (and relatively low mite infestation in the brood), while the worst colony had a ten-fold increase in mite population (Table). The pro-

Table - Results of an 11-week evaluation of potential resistance to *V. jacobsoni* begun in May 1996 with 25 colonies. Each colony was established with approximately 915 g of adult bees and 363 *V. jacobsoni*. "Best" and "worst" refer to the responses of individual colonies within each measure. See text for explanations of methods.

	Mite infestation		Factors that potentially influence mite infestation				
Colony	Population increase of adult mites	Final Worker brood infestation	Proportion of mites in brood	Hygienic activity	Grooming activity	Mite non-reproduction	Mean post-capping period
Best	1.36	5%	0.49	99%	19%	32%	282 h
Average	4.96	14%	0.67	52%	13%	15%	286 h
Worst	10.23	29%	0.79	13%	5%	5%	292 h

portion of mites in brood, hygienic activity and postcapping duration were quite variable, but the first two of these traits explained a significant part of the variation in growth of the mite population (r^2=0.50 and r^2=0.12, respectively). Removal of adult mites by grooming and non-reproductive female mites occurred at relatively low levels and probably did not appreciably influence mite population growth. The potential influence of a host bee colony on the duration of the reproductive cycle of *V. jacobsoni* (by extending the duration of the phoretic phase through some unknown behavioral or physiological mechanism) was important in this test and should be considered for measurement in future experiments.

After seven months with no treatment (December 1996), approximately one-third of the test colonies had died or dwindled to inviable populations. The remaining colonies, especially the least infested, may be useful in a program to select and breed resistant bees. We plan to further test the best of these together with additional survivor queens provided by beekeepers. We encourage beekeepers with colonies possibly resistant to *V. jacobsoni* to contact us to arrange for testing.

3. Delaplane, K. S[c] & W. M. Hood[d] — EFFECTS OF DELAYED ACARICIDE TREATMENT IN COLONIES INFESTED WITH *VARROA JACOBSONI* IN THE SOUTHEASTERN U.S. PIEDMONT[y,z]—In April 1995, we set up 72 colonies of honey bees in six apiaries of six colonies each in the Piedmont of Georgia and in six apiaries of six colonies each in the Piedmont of South Carolina. Colonies were individually housed in single-chamber Langstroth hives + one honey super, started with standard mail-order 0.9 kg (2 lb) packages of bees containing small incipient populations of the parasitic mite *Varroa jacobsoni* Oudemans, and managed optimally as for honey production. Within each state, each apiary was randomly assigned one of the following treatments: (1) treatment with Apistan® acaricide at month 2 (June), (2) treatment at month 4 (August), (3) treatment at month 6 (October), and (4) no treatment by month 8 (December) as an non-treated check. At each treatment episode, some or all colonies were sampled to determine colony bee population, colony mite population, number of brood cells, and mite levels with an ether roll test and an adhesive bottom board insert. Apistan strips remained in hives for 56-62 days.

By December, colony bee populations in August-treated apiaries were 3.5 times higher than in non-treated apiaries, 1.8 times higher than in October-treated apiaries, and not different from those of June-treated apiaries. Month of treatment did not affect bee body weight (see table).

In December, there were state x treatment interactions for colony mite populations, number of cells of brood, and percentage brood with disease-like symptoms. In both states, the trend was for more mites in the June and December treatments. In Georgia, number of brood cells was highest in October and December treatments, and number of abnormal brood cells was highest in December (see table for overall means).

Table - Effects of delaying acaricide treatment in colonies infested with *Varroa jacobsoni*. Colonies were treated in June, August, or October, or never treated by December as a non-treated check. Colonies were dismantled in December and the following variables measured. Values presented are average ± standard error. For colony bee populations, column averages followed by the same letter are not different at the =0.05 level. State x treatment interactions obscured treatment effects for colony mite populations, number of cells of brood, and percentage abnormal brood with disease-like symptoms. Treatments did not affect bee weight.

	variables measured in December					
month treated	% colony survival	bee population	mite population	bee weight (mg)	no. cells brood	% abnormal brood
Jun	83.3±16.7	12155±754ab	1111±178	130±3.9	200±98	0
Aug	91.7± 8.3	16130±1352a	62±25	142±2.5	204±100	0
Oct	83.3±16.7	8928±933bc	5.8±2.7	133±3.5	362±138	6.2±5.3
Dec	58.3±20.1	6350±682c	2391±306	123±2.8	316±70	7.5±3.6

Our data suggest that late-season acaricide treatments in the southeastern U.S. Piedmont are justified at 300-bee ether roll mite levels of 15 and overnight adhesive bottom board insert mite levels of 117 in colonies with ~24,808 ± 2245 bees and ~1,825 ± 327 cm^2 brood; these conditions occurred in mid-August.

The relationship of ether roll levels with colony mite populations was explained by a model with linear, quadratic, and cubic terms, but the same relationship with adhesive bottom board inserts was explained by a simple positive linear model; thus adhesive bottom board inserts are more practical predictors of real mite populations.

4. Eischen, F. A.[e] & W. L. Rubink[e] —USING DRONE SURVEYS TO ESTIMATE AFRICANIZATION LEVELS[z]— Earlier drone flooding experiments suggested that surveys of flying drones could predict area Africanization levels. This study was designed to test this hypothesis. Flying drones were surveyed along 100 km of the Rio Grande River in South Texas. Drones were collected at eight trapping locations (10-20 km apart) using aerial traps baited with an alcoholic extract of queen honey bees. The trapping area paralleled that of a swarm trapline used by one of us (WLR) for several years. Drone trapping was done during the summer of 1996 after most of the commercial pollinating colonies were removed. Areas containing managed colonies were avoided. A second study area was surveyed at Welder Wildlife Refuge about 60 km north of Corpus Christi, Texas where the location of many of the resident wild colonies is known and sampled regularly (n = 70). Only five managed European colonies are present on this 3500 hectare refuge. Drones were collected in the refuge from four locations, all within 1 km of each other. Drones were sampled from traps about every 30 minutes between 1530 and 1800 hrs. for two days at each trapping location. Worker bee and drone samples were collected during the same time.

The presence of hexokinase 1 & 2 and malate dehydrogenase 1, 2 & 3 alleles in drones were assessed using gel electrophoresis (PhastSystem). Workers from swarm traps along the Rio Grande or natural colonies at the Welder site were examined similarly. The worker genotypes were used as a baseline for allele frequency comparisons. The probable queen genotype was deduced from worker progeny and her contribution to the worker genotype removed. Chi-square analysis was used to detect significant differences between worker and drone allele frequencies.

Hexokinase-2 frequency for drones trapped along the Rio Grande was 45.0% and was not significantly different than the 42.5% found in workers. A similar result was observed at the Welder Wildlife Refuge, where 20.7% of the drones exhibited the hexokinase-2 allele and was not significantly different than the 21.4% found in workers. Though our analysis of the malate dehydrogenase is not complete, these data indicate that surveys of flying drones can be an accurate estimator of Africanization.

Drone surveys are disadvantageous only in that they must be done when drones are active. Although we have not conducted a cost analysis, we suspect that they are far cheaper in both materials and man hours than swarm trapping. They can be used in areas where swarm traps are considered inappropriate or subject to vandalism. Drone surveys may also be useful to queen breeders wishing to evaluate possible mating locations.

5. Eischen, F. A.[e] & W. T. Wilson[e] — THE EFFECT OF NATURAL PRODUCTS SMOKE ON *VARROA JACOBSONI*[y,z]—The primary objective of this work was to assess the efficacy of natural products smoke for the control of *Varroa jacobsoni*. A secondary objective involved monitoring the effect of natural products smoke on honey bees. Plant materials screened for activity were black walnut (*Juglans niger*), burlap, catnip (*Nepeta*

cataria), cedar (*Juniperus virginiana*), cenzio (*Leucophyllum frutescens*), coffee (*Coffee arabica*), corrugated cardboard, creosote bush (*Larrea tridentata*), eucalyptus (*Eucalyptus* sp.), feverfew (*Chrysantheum parthenium*), grapefruit (*Citrus* sp.), hickory nut hulls (*Carya* sp.), lantana (*Lantana sp.*), magnolia (*Magnolia* sp.), marigold (*Tagetes* sp.), melaleuca (*Melaleuca quinquenervia*), mescal bean (= Texas mountain laurel, *Sophora secundiflora*), mistletoe (*Phoradendron tomentosum*), neem (*Azadirachta indica*), oleander (*Nerium oleander*), orange (*Citrus* sp.), osage orange (*Maclura pomifera*), pecan leaves, nuts, and hulls (*Carya illinoiensis*), pokeweed (*Phytolacca americana*), pot marigold (*Calendula* sp.), red pepper (*Capsicum frutescens*), pokeweed (*Phytolacca americana*), pot marigold (*Calendula* sp.), red pepper (*Capsicum frutescens*), sassafras (*Sassafras albidum*), tansy (*Tanacetum vulgare*) and tobacco (*Nicotiana* sp.). Groups of infested bees (ca. 250) were exposed to the cool smoke of these materials for 60 sec. Mites initially knocked down were counted. The bees were placed over 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 knock down by the smoke from tansy, creosote bush, citrus leaves, neem, melaleuca and cedar reached levels of 70-90%. 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. This provides a starting point for further investigations. Most of the plant materials tested did not appear to shorten the longevity of bees, however smoke from cardboard and oleander proved to be quite toxic in some instances. More tests are needed for a complete assessment. Bees responded to some other plant smoke by vomiting and anesthesia, most seemed to recover without ill effects. Even though some of these plant products show varying degrees of activity against Varroa, we do not recommend their use in a control program.

6. Ellis, M. D.[f], B. Siegfried[f], & B. Spawn[f]—THE EFFECT OF APISTAN® ON HONEY BEE (*APIS MELLIFERA* L.) RESPONSES TO METHYL PARATHION, CARBARYL, AND BIFENTHRIN EXPOSURE[y,z]—Honey bees treated with Apistan® exhibited greater susceptibility to bifenthrin in laboratory bioassays. Bifenthrin was 1.9 times more toxic to bees that were caged with Apistan® Queen Tabs than to bees held in cages without Apistan®. Carbaryl and methyl parathion were 1.4 and 1.1 times more toxic, respectively, however, the differences in relative toxicity were not significant. The possibility that honey bees being treated with Apistan® may be more vulnerable to injury by bifenthrin is supported by data obtained in this study. Data reported in this study do not support the hypothesis that Apistan® treatment affects the vulnerability of colonies to carbaryl or methyl parathion injury.

Table - Responses of bees treated with Apistan® and untreated bees to three commonly used insecticides.

Compound	n	Slope	LD_{50} (CL)[a]	Relative Potency
Bifenthrin	360	3.907±.332	.034 (.023-.058)	
Bifenthrin + Apistan	360	5.434±.564	.018 (.016-.020)	1.9*
Carbaryl	360	2.828±.293	.232 (.190-.279)	
Carbaryl + Apistan	360	4.384±.453	.175 (.137-.209)	1.4
Methyl Parathion	360	10.415±1.187	.041 (.037-.044)	
Methyl Parathion + Apistan	360	11.449±1.695	.039 (.033-.044)	1.1

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7. Haarmann, T. K.[g]—HONEY BEES AS INDICATORS OF RADIONUCLIDE CONTAMINATION; EXPLORING SAMPLE CONSISTENCY AND TEMPORAL CONTAMINANT ACCUMULATION—Inherent in the many processes involved in the research and development of nuclear-related materials is the production of excess radionuclide isotopes. A consequence of this research is that some of these radionuclide waste products have found their way into surrounding ecosystems. Recent interest in analyzing the influences of contaminants on living systems has generated new questions on how best to incorporate sampling data into ecotoxicological analysis. The primary concerns involve determining which methods are best to monitor these contaminants, as well as how to analyze the influences these contaminants have on biological systems. One innovative sampling method incorporates insects, honey bees (*Apis mellifera*), as monitors of environmental contamination.

Honey bees can be thought of as mobile samplers that efficiently cover a large sample area and then return to a central location (Bromenshenk, 1990, *Site-Specific and Regional Monitoring with Honey Bees: Case Study Comparisons*, Ecological Indicators, Volume 1, Elsevier Applied Science, New York, NY). Contaminants often become incorporated into the bee tissue, the wax, the honey, or the hive itself. The purpose of my research was to investigate in greater detail the use of honey bees as monitors of radionuclide contaminants and explore various aspects of sampling these bees. Beehives have been shown to contain radionuclide contaminants when the contaminants are environmentally available (Fresquez *et al.*, 1994, Los Alamos National Laboratory report LA-12872-MS). However, there are many unanswered questions, notably those dealing with sampling protocol issues and the interpretation of data.

I conducted two separate experiments. The first tested the variance found in tissue samples taken from hives in identical locations. Do tissue samples taken from similar beehives under similar conditions yield the same results? The second experiment tested the hypothesis that there is no difference in contaminant levels found in hives of varying ages located in the same area. In other words, is there a temporal accumulation of contaminants within hives that might mislead the interpretation of sample results?

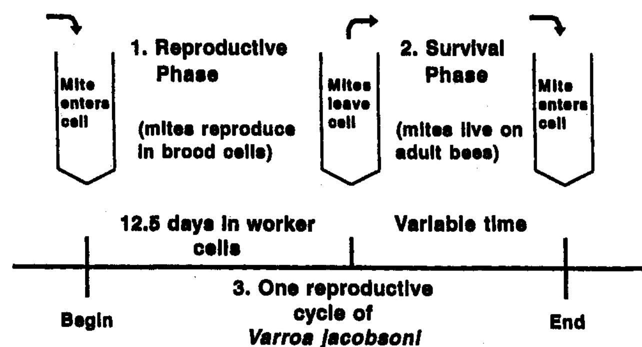
I placed bee hives in two separate study locations, one of which was next to a lagoon containing radioactive water. Results indicate that sample consistency is variable. Levels of tritium and sodium-22 in bee tissue samples were inconsistent, while levels of cobalt-57, cobalt-60, and manganese-54 were consistent. The second experiment, investigating the interhive accumulation of contaminants over time by comparing old and new hives, demonstrated that there is indeed an accumulation of radionuclides within hives.

8. Harbo, J. R.[b], R. A. Hoopingarner[a] & J. W. Harris[b]—EVALUATING HONEY BEES FOR RESISTANCE TO VARROA MITES: PROCEDURES AND RESULTS—We use a short period (9 to 16 weeks) to evaluate characteristics of a colony that may affect the growth of a mite population. Advantages of a short period of evaluation are (1) less time for beekeeping variables such as supersedure or swarming to enter the experiment, (2) colonies remain small and manageable, (3) seasons with low or no brood production can be avoided, and (4) a short testing period enables the use of test queens inseminated with one drone, thereby reducing genetic variability in a colony. A model for growth of the mite population (see figure) defines three components within the reproductive cycle of the mite. These components represent three general ways in which a colony of bees can affect the growth of a mite population.

A typical test begins with 25 colonies containing a test queen and 0.8 kg of bees taken from a single mixture of bees that has an infestation rate of 200 mites per kg of bees (measured when dispensing the bees). About 6 weeks into the test, we measured characteristics of adult bees such as hygienic and grooming behavior. At the end of the test, we measured each colony for non-reproduction of mites, populations of mites and bees, and amount of brood.

Analyses consisted of finding the variables that best describe the changes in the mite populations. A variable can be one of the three components in the figure or a characteristic within a conponent (*e.g.* non-reproduction and hygienic behavior are both within component 1; grooming behavior is within component 2). In 1995, only non-reproduction was significantly related to changes in the mite populations. In 1996, two variables, non-reproduction and the

duration of the mite's reproductive cycle, showed strong, negative relationships with changes in the mite populations (P = 0.005 and 0.0001 respectively, n = 24). These two variables fit components 1 and 3 (see figure).



Figure—Three distinct components within a reproductive cycle of *Varroa jacobsoni* that affect the growth of a mite population. Any resistance mechanism of the honey bee that controls the growth of the mite population must exist in one or more of these components, and resistance in more than one component should have an additive effect. Component 1 (reproduction) is the number of adult female mites that leave a brood cell per mite (always an adult female) that enters. Component 2 (survival) is the probability (ranging from 0 - 1) that a female mite will survive to enter another cell. Component 3 is the duration of the reproductive cycle. See Harbo, *BeeScience* 4: 100-105, for a mathematical description of this model and examples of its use.

9. Hood, W. M.[d]—FIELD TEST OF THE VARROA TREATMENT DEVICE (TM)[y,z]—The Varroa Treatment Device (VTD) filled with 85% formic acid (FA) was field tested for bee parasitic mite control in the Piedmont region of South Carolina from February - October, 1996. The base of the plastic reservoir box (152x80x25mm) was attached to the bottom bar of a brood chamber frame and placed next to the first broodless frame. The VTD was filled with sawdust to a fill line to allow absorption and controlled evaporation of the FA through the vented removable lid.

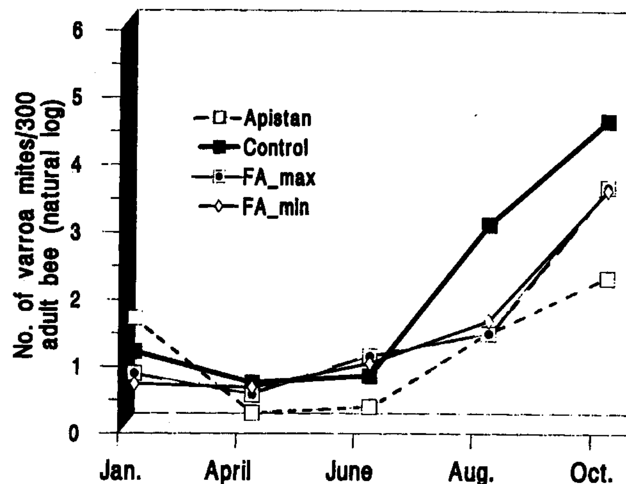
Three apiaries with twenty-eight (twelve in yard 1 and eight in yard 2 and 3) honey bee colonies were used in this test. Each colony was housed and managed in one, 10 frame Langstroth hive body and one 10-frame Illinois super. The colonies were managed for honey production with extra supers added only during the April and May nectar flows.

Two VTD/FA treatments, one Apistan (R) treatment and one control were replicated seven times for comparison of varroa and tracheal mite levels. Treatments were (1) two 60 day treatments with the VTD/FA, (2) continuous VTD/FA treatment except during the 2 month nectar flow period, (3) two 42 day treatments with Apistan, (4) VTD with sawdust but no FA as a control. Initial treatments were placed in colonies on February 19, and the second treatments of VTD/FA and Apistan were administered on August 6. The VTD/FA treated colonies were serviced at ca. 2 week intervals during the treatment periods.

Samples of ca. 300 adult bees were collected for mite diagnosis (alcohol wash method) on January 23, April 11, June 10, August 6, and October 15. One-hundred pupae from each colony were extracted and checked for varroa on the same dates beginning April 11. Thirty-three adult bees from each sample were diagnosed for tracheal mites by the thoracic disc method.

Varroa mite counts on adult bees collected from all treatments were significantly less ($P<.01$) than the control for the August and October samples (Figure). Although varroa mite counts on extracted bee pupae from all treatments were significantly less ($P<.01$) than the control for the August sample, the Apistan treatment was the only treatment that maintained significant ($P<.01$) varroa control in the brood for the October sample. One possible reason for the decrease in VTD/FA varroa control in October was the accumulation of propolis on the vents which began in June.

Although the results of this test indicate that the VTD/FA is less effective than Apistan in controlling varroa mites, the VTD/FA provides a viable alternative varroa mite control in combination with other mite control measures. Tracheal mite levels remained extremely low throughout the test, therefore treatment effects on tracheal mites were inconclusive.



10. Hood, W. M.[d] & W. Miller[h]—BIOCONTROL OF BOTRYTIS FRUIT ROT ON STRAWBERRIES USING HONEY BEES TO VECTOR AN AGENT[y,z]—Peng *et al (Can. J. Plant Pathol.* 14: 117-129) developed a naturally occurring biological control agent (*Gliocladium roseum*) that was effective for control of gray mold of strawberry. The fungus controlled gray mold fruit rot as effectively or more effectively than the fungicide Captan. They established the European honey bee as an effective delivery means for applying *G. roseum* to strawberry flowers (Redcoat cv.) in small field plots located near Guelph, Canada.

The objective of our research was to manipulate the same naturally occurring biological agent in an integrated approach in South Carolina to control botrytis in commercial fields of Chandler strawberries without putting fungicides on the berries. The efforts reported here represent two field studies, one conducted in 1993 and another conducted in 1995.

Infected leaves were collected from 15 different strawberry fields in 1992 and 12 different fields in 1994 and incubated in moist chambers for 6 to 8 weeks. The tissues were observed after extensive aging and necrosis for the presence and subsequent isolation of *G. roseum.*

Six sites were selected in 1992 and 12 sites selected in 1994 for field evaluations. The sites were selected as matched pairs with each pair representing a replication. Four treatments of fungicides were customized to each site in the fall of 1992 and 1994 immediately after planting. All growers planted the strawberry variety Chandler and used the annual hill production system sometimes known as plastic culture. The treatments were no fungicide, fall only fungicides, fall and spring fungicides and spring only fungicides. The variation in treatments was based on the grower's sprayer, the prevailing wind direction, and minimizing the size of the unsprayed plots and fall only sprayed plots to approximately 0.1 acre each. One member of each pair received *G. roseum* vectored by honey bees to the flowers in a talc mixture and the other received talc only.

Hive dispensers used in 1993 were modified, from ones made by Peng (ref. above) for nuc hives, to fit standard 10 frame Langstroth hives. Dispensers used in 1995 were designed by Gross

et al. (*Env. Entomol.* 23: 492-501). In 1993 and 1995, 1 and 1.5 hives per acre, respectively, were placed near the strawberry fields. In 1995, observations on bee flight activity were made approximately weekly.

The talc, with or without *G. roseum*, was placed in hive dispensers daily prior to bee flight activity beginning in late March and ending first week in May. Berry samples were taken at approximately weekly intervals starting 4 weeks after initiating dispensing talc.

No significant trends in botrytis control were observed in 1993. The spring of 1993 was dry with most areas receiving less than 3 inches of rain from late April to early June which contributed likely to the low occurrence of botrytis.

The disease pressure was higher in 1995 and the amount of disease increased significantly between successive fruit harvests, but no control of *botrytis* by the biocontrol agent was observed. Low bee counts suggested that bee flower visitations may have been inadequate to transfer successfully the biocontrol agent to the flowers. Bees from test colonies appeared to be attracted to other competing nectar-bearing plants during the 1995 study.

11. Hunt, G. J.[i], E. Guzman-Novoa[j], I. M. Ioannides[i] & R. E. Page[k] — DEVELOPING DNA MARKERS AS DIAGNOSTIC TOOLS TO DETECT THE STINGING GENES OF AFRICANIZED BEES — Researchers have developed diagnostic genetic markers that allow us to distinguish the mitochondrial DNA of Africanized bees from European bees. Since the markers are based on the polymerase chain reaction (PCR), the test is fairly rapid. The California Department of Food and Agriculture favors the PCR-based technique because the test is rapid and also because it gives unambiguous answers about the DNA of the bees. A PCR-based test that told us something about important behavioral genes would be useful for queen breeding and stock certification.

The problem with distinguishing European bees from Africanized bees is that African and European races of honey bees do mate with each other to produce hybrid offspring. These hybrids could be just as likely to sting as the pure African race, if the tendency to sting is dominant. If we had DNA markers for the genes that influence stinging behavior, we could use them to determine which bees carry the "stinging genes."

We are involved in mapping the genes that affect the stinging behavior of Africanized bees. We believe that a gene has been identified that has a major influence on the number of stings in our defensive-behavior assays. Our technique for mapping genes is to make a single hybrid queen (from an Africanized X European cross) and obtain many drones from her. Each of these drones from the hybrid queen is used in a single-drone insemination of a European queen (the queens are sisters). Each of the drones is the father of a different colony. We tested 162 of these colonies for their tendency to sting a leather patch in a defensive-behavior assay. Then, we analyzed the drone father's DNA for many different PCR-based markers. A drone can have either the African or European version for any marker. A drone will also have either the African or European version for any gene that affects stinging behavior. The next step was to determine which marker is associated with the number of stings in our assay. We found one location on a honey bee chromosome that had two adjacent markers that correlated with stinging. Workers that inherited the African version of either of these two markers were much more likely to sting the patch. Statistical tests tell us that we can be 95% sure that there is a gene at this point (between the two markers) that influences stinging behavior.

The next step in developing diagnostic markers will be to see if these two markers could be used to predict whether bees would tend to sting. Samples of bees from the study area were collected and we compared bees that had European mitochondria to those with African mitochondria. There was a 3 to 1 difference between these groups for the two versions of the marker that had the biggest correlation with stinging. Next, we plan to perform defensive-behavior assays of these colonies to see if the type of marker that the bees have can be used to predict their tendency to sting. If we are able to use these markers to predict tendency to sting, they will be useful for selecting stocks for breeding.

12. Kondapavuluru, V.[e] & F. A. Eischen[e]—IMMUNE RESPONSE TO HONEY BEE (*APIS MELLIFERA*) VENOM IN WHITE MICE (*MUS MUSCULUS*)—Honey bee venom stimulates mammalian adrenal glands to produce cortisol (Zurier *et al., Ann. Rheumat. Dis.* 32:466-70). Cortisol has two major functions, one is to help digest fatty acids in the blood, and the other is to act as an anti-flammatory hormone. The amount of cortisol normally secreted does not significantly affect inflammation, but large amounts block all stages of inflammatory response, including white blood cell (WBC) production. Because cortisol triggers an anti-inflammatory response, it can be inferred that cortisol is also an immuno-suppressant. The primary way to measure the activity of the immune system is to measure the concentration of white blood cells.

In this experiment, we sought to establish whether honey bee venom significantly affects WBC counts. Twenty young male white mice were randomly assigned to either an experimental group or a control group. The mice in the experimental group were stung on 6 consecutive days at 11:00 A.M. and their WBC counts were taken 4 hours later, while control mice were pricked with a small needle to simulate a bee sting at the same time as their counterparts, and had their WBC counts also taken.

A base line WBC count was established on day 1. The mean WBC count for the experimental group was 4370 WBC/µl of blood and the control group was 4260 WBC/µl of blood. These WBC counts are within the normal range for white mice and not significantly different.

The final mean WBC count for the control mice was 5118.8 WBC/µl of blood. These WBC counts are well within the normal range for white mice. The final mean WBC count for the experimental mice was 2792.9 WBC/µl of blood. These WBC counts are well *below* the normal range for white mice. A 1-way ANOVA revealed that over time, differences in WBC counts between the control and experimental groups were highly significant ($F = 135$, $P < 0.0001$). These data indicate that honey bee venom caused the drop in WBC count.

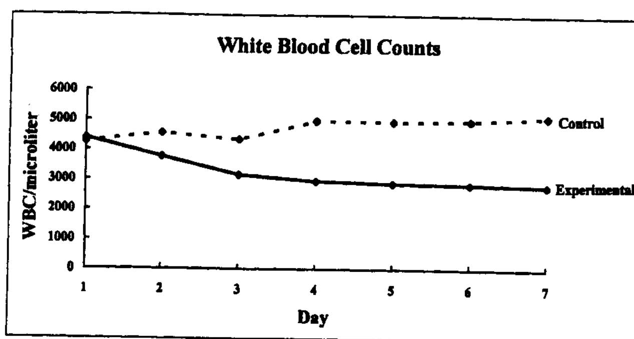


Figure - This graph shows differences in average white blood cell (WBC) counts between control and experimental groups; experimental mice were stung once a day starting on day 2.

13. Loper, G. M.[n] & O. R.Taylor, Jr.[o]—INFLUENCE OF AFRICANIZED DRONES ON MATINGS WITH EUROPEAN QUEENS IN SOUTHERN ARIZONA, APRIL 1996—Swarms of the Africanized honey bee (AHB) (*Apis mellifera scutellata*) have been arriving in S. Arizona since the Spring of 1993. Previous studies have documented the change in morphological, allozyme, and behavioral characteristics of some of these swarms. This study documents the degree of Africanization due to AHB drones mating with European (EHB) queens during the beginning of the spring (April) period of swarming (EHB) in and around Tucson, AZ. Virgin EHB queens were established in five

frame mating nucs; ten nucs were distributed within the Tucson city limits and ten about 60 miles N. of Tucson in my (GML) feral colony study area, where the first AHB swarms were documented in the spring of 1995. Just before EHB queen mating flights, any and all drones in the mating nucs were removed. Progeny from each successfully mated queen (eight in Tucson, ten in feral area) were removed as they were about to emerge as adults and stored at -74ºC in a laboratory freezer. Twenty-four progeny from each queen were analyzed electrophoretically for Malate Dehydrogenase (MDH) and Hexokinase (HK) allozymes.

The influence of AHB drones was estimated from the proportion of the progeny that exhibited the HK-2 allozyme and by factoring in the proportion of HK-2 in AHB populations (either 50% as per Del Lama et al (*Apidologie* 21: 271-280) for Central America AHB populations or 40% as per Taylor (unpublished 1996) for NE Mexico populations). Assuming no assortative mating, EHB queens in Tucson mated with 19.5% AHB drones. In the feral area, two sub-sets of five nucs each were located either (1) adjacent to a known highly AHB colony, yielding 27.1% AHB drone influence or (2) near an unknown number of EHB and AHB feral colonies, yielding 15.0% AHB drone influence. It is suggested that these levels of AHB are very significant in their potential impact on beekeeping in the area, especially to hobby beekeepers as highly defensive behavior typical of AHB can be imparted to colonies via AHB drones. Future repetitions of this study during various years and seasons are needed to determine the progress of Africanization in S. Arizona.

14. Mangum, W. A.[p] — MODELING THE POPULATION BIOLOGY AND THE POPULATION GENETIC DYNAMICS OF THE HONEY BEE, *APIS MELLIFERA* L., WHEN PARASITIZED BY THE MITE, *VARROA JACOBSONI* OUDEMANS—The varroa mite has caused extensive damage to temperate climate honey bee populations. One way to study varroa mite parasitism is through the use of mathematical models consisting of nonlinear difference equations. For the present study, varroa mite parasitism is described by a population biology model interacting with a haploid-diploid selection model. The population biology model describes the numerical changes in the honey bee colony and their associated varroa mite population. Some of the worker bees, referred to as remover bees, have the ability to remove their varroa mites by autogrooming. This remover trait is assumed to be under the control of one biallelic locus with the A_1 allele conferring the remover ability. The population genetics model describes the changes in the frequency of A_1 and the changes in the weighted average colony fitness that could occur in the corresponding population of mother queen bees.

Using only the population biology model, a theoretical scenario is described for detecting remover bees by a reduction in their varroa mite population from a constant value. Colonies with higher frequencies of more efficient remover bees would cause a greater reduction in their varroa mite populations, and these colonies should be easier to detect. The simulations demonstrate that controlling varroa mite population growth depends on the frequency and efficiency of the remover bees. More efficient remover bees control varroa mite population growth at lower remover bee frequencies. Although a colony may have remover bees, their frequency may be insufficient to protect the colony from varroa mite population growth.

Using both models with parameters that originally lead to a simulated parasite-induced extermination of the population of honey bee colonies, a selection experiment is described where the virulence of the varroa mite is reduced by an elevation in their death rate. When the varroa mite death rates are relatively small, the parasites are effectively more virulent, and the population of honey bee colonies is exterminated. If the elevated varroa mite death rates are large, as with a simulated long-term miticide treatment, the varroa mite's virulence is greatly reduced, and the population of colonies survives. Because the miticide treatment artificially removes the varroa mite's virulence, the increase in the frequency of A_1 is very small, *i.e.* the bee population does not develop genetic resistance. However, at intermediate varroa mite death rates, larger increases in the frequency of A_1 occur that would promote genetic resistance, and the extermination of the population of honey bee colonies does not occur. These simulations suggest that a moderate increase in the varroa mite death rate could help the bee population become genetically resistant to varroa mite parasitism.

15. Pettis, J. S.[q] & H. Higo[r]—QUEEN REARING— FACTORS AFFECTING QUEEN WEIGHT AND NUMBER OF QUEEN CELLS REARED—Honey bee colonies rear varying numbers of queen cells during emergency queen rearing situations. A single queen will eventually emerge to head the colony from the numerous new queens reared (range 11-49 cells, Fell & Morse, *Insectes Sociaux* 31:221-237), but is she the best queen? In these experiments we examined colony population and source (previously demonstrated, Hoopingarner & Farrar, *J. Econ. Ent.* 52:547-548), along with order of rearing as possible factors which could affect the number and weight of newly reared queens. Four-frame nucleus colonies were established from each of 12 parent colonies in Burnaby, BC, Canada in July 1994. Each colony contained two frames with pollen and honey and two frames with brood of mixed ages, all frames had adhering adult bees. Colonies were examined on day 4 to estimate adult bee population, and on day ten each queen cell was carefully opened and the pupal queen removed, weighed, and assigned to one of five development classes based on body and eye color. Regression analysis revealed a significant relationship between colony population and both queen weight (R^2=0.62, p=0.002) and number of cells reared (R^2=0.38, p=0.006). Additionally, analysis of variance showed that both colony source and development stage significantly influenced queen weight (p<0.0001). A means separation test revealed that, as a group, the second oldest queens (light tan, see figure) were significantly heavier than all other queens reared.

It was not surprising that small colonies reared smaller and fewer queens. However, it was surprising that there was a strong influence on queen weight based on the order that the queens were reared. In our protocol we did not control for age of larvae selected for cell rearing and thus our age groupings may not reflect the actual order of rearing. However, Fell and Morse found that 65% of queen cells were started over larvae <2 days old, thus our groupings should be representative of the true rearing order of cells. Apparently colonies undergo a lag time of 1-2 days before reaching full queen rearing potential and therefore the second group of queens are larger and thus more productive (Nelson & Gary, *J. Apic. Res.* 22:209-213). This lag time may reflect the time needed

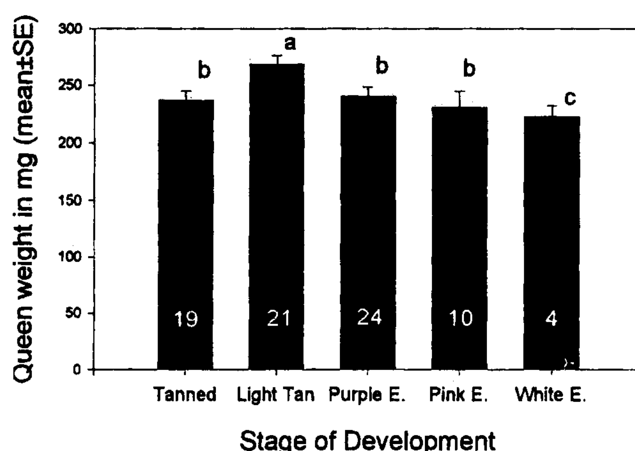


Figure - Average queen weight by pupal development class from 12 colonies of honey bees after 10 days of queenlessness. Numbers within bars represent the number of pupae weighed and bars with different letters are significantly different (ANOVA, P<0.0001, REGWF test, SAS 1988).

by nurse bees to reach optimal royal jelly production. Queen breeders should be able to produce larger queens using; stock selection, strong colonies, and timing the starter/finisher colonies to best utilize the peak queen rearing which occurs approximately two days into queenlessness.

16. Ragheb, M.,[s] — ENGINEERED POLYCULTURES WITH FRESH WATER AUGMENTATION AND BEE FORAGING — We consider the concept of Engineered Polycultures with fresh water augmentation and bee foraging as a sustainable approach for agriculture (Klinkenborg, *National Geographic* 188: 60-89) in arid regions of the world for the short term, and for space colonization in the long term. Arid areas need fresh water augmentation, through solar, fossil or nuclear processes, and judicious use of the available water resources. Fresh water augmentation would use brines and brackish supplies in interior regions, and sea water along sea shores (Ragheb *et. al.*, *Proc. of the International Symposium on Water Resources in the Middle East: Policy and Institutional Aspects*, 173-180, Urbana, Illinois, 1993). In space colonies, on the Moon or Mars, the initial water supply would be recycled. The resulting ecologies would primarily contain a symbiotic structure of mammals (humans), invertebrates (bees) and flowering plant species.

We first compare the two concepts of polyculture and monoculture and discuss their relative advantages and disadvantages (*Ayers, Amer. Bee J.*, 136: 197:201). Examples of polyculture are: pollinator trees interspersed in an orchard, insect-repelling plants providing a pest-free environment for other plants, legume plants fixing nitrogen and providing it upon their death to other plants, deep-rooted plants making deeper soil strata available to other species. The concept of overyielding in the polyculture approach is discussed within the context of niche filling (Vandermeer, *BioScience* 31: 361-364).

We mathematically analyze, in terms of a defined overyielding ratio, a prototype for implementation in arid areas of an overyielding polyculture consisting of deep rooted flowering trees, flowering climbing vines, and shade-tolerant herbaceous flowering ground covers. The main product would be honey as a carbohydrate harvested through bee foraging. Secondary products would be protein as pollen and bee brood, fatty substances as beeswax, wood for construction, furniture and fuel, fruit from vines and nitrogen from the ground covers. Interesting characteristics of such a system is that the main carbohydrate commodity (honey) is harvested by the foraging bees without destruction of the flowering plants from which it came.

It appears that the theoretical upper limit for the overyielding ratio is equal to n, where n is the number of flowering species in the polyculture. In our prototype case, this corresponds to a value of 3. However, because of incomplete niche filling a value around 1.16 should be practically attainable.

The engineered succession in polycultures is analyzed using the concepts of growth and decay constants. Niche filling and attainment of equilibrium appear to be crucial considerations. The overyielding ratio becomes in this case a function of time, implying that judicious adjustment of the growth and decay constants in view of achieving equilibrium among the flowering species is a necessary condition for the successful implementation of polycultures.

17. Scott-Dupree, C.[t], G.W. Otis[t], O. Welsh[t] & J. McCarthy[t]—INTRASPECIFIC VARIATION IN RESISTANCE TO *NOSEMA APIS* ZANDER IN HONEY BEES, *APIS MELLIFERA* L.—The objective of this study was to determine whether there are intraspecific differences in the susceptibility of adult honey bees to *Nosema apis*.

Sealed brood frames from 10 genetically different stocks of honey bees were placed in individual screened cages in an incubator at the University of Guelph on 29 May, 1996. The 10 honey bee stocks tested were: Treatment (TRT)1- Canadian; TRT 2- Carniolan/Australian; TRT 3 - Canadian; TRT 4 - Buckfast; TRT 5 - Buckfast; TRT 6 - Canadian; TRT 7 - Buckfast; TRT 8 - Canadian; TRT 9 - Buckfast; TRT 10 - Buckfast.

As worker bees emerged from their cells they were collected before contacting the comb to prevent contamination with Nosema spores. Ninety worker bees from each of the 10 bee stocks (n=900) were marked with a colored thoracic disc or a paint dot on their thorax identifying them as progeny of one of the 10 bee stocks being tested. Individually marked bees were placed in separate queen mailing cages and fed 10 µl of 1:1 honey-water solution plus inoculum (Dosage =700 Nosema spores/bee) with a microcapillary tube for a 24 hour period. Following inoculation bees were placed in either (1) a holding frame in a Nosema-free colony (Holding Frame Bioassay); or (2) clusters of 150 bees in a screened cage (Cage Bioassay). After 6, 9 and 12 days, bees were removed and analyzed individually for numbers of Nosema spores.

The results of the holding frame bioassay indicate significant colony effects (P=0.035). Spore levels in colonies increased significantly between sample days (P=0.0037). Three of the top four bee stocks in this Nosema bioassay were Buckfast in origin (Table 1); however, statistical analysis did not show a significant difference between Buckfast and non-Buckfast stocks (P=0.82).

The results for the cage bioassay proved to be much more difficult to interpret. For example, there were significant differences in the susceptibility to Nosema of bees of the same genetic bee stock maintained in the different bioassay cages (P=0.009). This effect was unexplained and partially obscured any colony effects. In addition, the CO_2 treatments appeared to have an overall negative impact on the behavior of the bees remaining in the cluster and resulted in defecation on cage-mates which would have affected Nosema infections on subsequent sample dates.

We have documented phenotypic variation in susceptibility to Nosema disease in honey bees. It is likely that this variation has a genetic basis. Our results suggest that breeding bees resistant to Nosema disease is possible.

Table - Mean number of Nosema spores (10^5) per worker bee for each genetic line of honey bees (TRT) averaged over 3 sample dates.

TRT.	STOCK	OVERALL AVERAGES		
		Mean	Lower CI*	Upper CI*
10	BUCKFAST	22.99	14.18	37.36
8	CANADIAN	28.37	18.46	43.64
9	BUCKFAST	32.78	21.32	50.47
7	BUCKFAST	37.03	23.65	58.03
1	CANADIAN	41.69	27.09	64.21
2	CARNIOLAN	43.21	28.75	64.99
4	BUCKFAST	43.34	20.25	93.05
6	CANADIAN	45.95	25.49	83.00
3	CANADIAN	46.60	25.96	83.78
5	BUCKFAST	70.37	47.47	104.34

*Upper and lower confidence intervals. Means and confidence intervals are back-transformed from a log transformation.

18. Skinner, J. A.[u] J. P. Parkman[u], & M. D. Studer[u] — HONEY BEE MITE STUDIES IN TENNESSEE: OILY, MINTY AND SMELLY[y,z]—Because only one compound, Apistan™ , is available in the USA for control of *Varroa jacobsoni,* a study was initiated in Tennessee to evaluate the efficacy of alternative control materials. Three apiaries, consisting of approximately 30 colonies each and managed by commercial beekeepers, were used. One each was located in western Tennessee near Dyersburg, central, near Chattanooga, and eastern, near Greeneville.

Colony strength was determined for each hive by counting frames with bees and capped and uncapped brood; treatments were assigned so that a variety of colony strengths appeared within each treatment. Eleven treatments were evaluated using a total of 92

colonies at the three sites: Formic acid veggie (200 ml of 65% formic acid absorbed into 3 layers of absorbent bed padding, placed in a 1-gallon Ziploc™ vegetable bag placed above the brood cluster); formic acid holes (200 ml of 65% formic acid absorbed into 3, 1 cm x 6.5 cm x 10 cm O-cello™ sponges, placed in a 1-quart Ziploc™ bag with a grid of holes punched in 1 side and placed above the brood cluster); formic acid slits (200 ml of 65% formic acid absorbed into 3, 1 cm x 6.5 cm x 10 cm O-cello™ sponges, placed in a 1-quart Ziploc™ bag with 3, 5-inch slits cut in 1 side and placed above the brood cluster); thymol (20 ml of a 76% thymol, 16% eucalyptus, 4% camphor and 4% menthol mixture absorbed into a 1 cm x 5 cm x 6.5 cm O-cello™ sponge placed above the brood cluster); spearmint syrup (1 ml of spearmint oil mixed with 1 pound of sugar in 1 quart of water and fed to the colonies); peppermint syrup (1 ml of peppermint oil mixed with 1 pound of sugar in 1 quart of water and fed to the colonies); shortening pattie (8 oz. shortening pattie, consisting of 2 parts sugar and 1 part shortening, placed above the brood cluster); peppermint pattie 1 (4.8 ml of peppermint oil mixed into an 8 oz. shortening pattie, consisting of 2 parts sugar and 1 part shortening, placed above the brood cluster); peppermint pattie 2 (0.8 ml of peppermint oil mixed into an 8 oz. shortening pattie, consisting of 2 parts sugar and 1 part shortening, placed above the brood cluster); Apistan (2 Apistan™ strips placed in the brood cluster); and a control. Because a wide variety of treatments and application methods were used, all the treatments were not evaluated at each site.

Varroa samples were taken 0, 30 and 60 days after treatment. A 'sticky board', surface area = 976.25cm², was placed on the bottom board of each hive and two Apistan™ strips were placed in the brood cluster for 24 hours. After 24 hours the strips and 'sticky boards' were removed; the 'sticky boards' were returned to the lab and the mites collected on the boards were counted.

Based on mean mite drop counts, percentage change in mite populations for the treatments (all sites combined) at 30 d varied significantly ($F=8.40$; $df=12, 70$; $P<0.0001$). Mean separation by Tukey's HSD after ANOVA failed to find a significant difference between the treatment giving the best control, Apistan (90% reduction in mites dropped), and the second best treatment, formic acid holes (12% reduction). Changes in mite drops for the other treatments ranged from a 47% increase for the formic veggie treatment to a 258% increase for the spearmint syrup treatment. Mite drop increased 100% for the control treatment. Due to colony decline and death, results were less clear at 60 d posttreatment.

Extreme variation in initial mite populations occurred among treatment groups. Mean 24-h drop counts at 0 d varied from 323 for the spearmint syrup group to 2436 for the formic veggie group. Results may have been different if initial mite populations had been lower and less variable. (Mite population levels will be standardized in future studies of some of the treatments.) Final sample and strength counts to be made in late winter 1997, plus analysis of tracheal samples taken from each colony at each sample date, should provide a better estimate of treatment efficacy.

19. Spivak, M.[v] & G. S. Reuter[v]—PERFORMANCE OF HYGIENIC COLONIES IN A COMMERCIAL APIARY— Hygienic behavior is one mode of resistance to both American foulbrood and chalkbrood and is a defense against Varroa mites. Hygienic bees detect and remove diseased brood from the nest before the pathogen becomes infectious, and remove mite-infested pupae interrupting the reproductive cycle of the mite. Colonies are selected for hygienic behavior using a freeze-killed brood assay in which the time taken to remove a 5cm x 5cm section of frozen pupae is recorded. Hygienic queens are reared from colonies that remove all of the freeze-killed brood within 48 hours over two trials. The queens are instrumentally inseminated with semen from drones of different hygienic colonies.

Inseminated queens are used as breeder stock but commercial beekeepers prefer naturally mated queens in production colonies. Genetic studies on hygienic behavior revealed that the hygienic trait is recessive (Rothenbuhler, *Am. Zool.* 4: 111-123). Our experiments in 1995 indicated that queens raised from inseminated stock retained the hygienic trait when they were outcrossed with unselected males (Spivak et al., *Am. Bee J.* 135: 830-831).

If hygienic queens are to be utilized by the beekeeping industry, it is important to determine whether colonies with open mated queens from hygienic stock produce as much honey, have lower incidences of chalkbrood, and have lower levels of *Varroa* mites than colonies bred from commercial stock.

In March 1996, hygienic queens were reared and open mated in an apiary of a commercial beekeeper in Texas. For comparison, unselected "commercial" queens were reared and mated in the same location. The colonies were transported to Wisconsin in May, and those with marked hygienic queens (n = 49) and marked commercial queens (n = 46) were distributed in four apiaries. In June, the colonies were evaluated for population size, incidence of diseases, and temperament. In September, the colonies were evaluated for honey production, and mite loads. The results indicated that the hygienic colonies had significantly lower levels of chalkbrood, and produced significantly more honey. Importantly, the hygienic colonies had significantly lower levels of mites in three of the four apiaries. All other measures were the same between the hygienic and commercial colonies.

Table - Comparison of hygienic (n=49) and commercial (n=46) colonies headed by open mated queens. Values shown for hygienic and commercial colonies are means ± std. dev. Evaluations of frames of bees, frames of brood, temperament, and chalkbrood were made in June 1996. Remaining measures were made in September 1996. All colonies were scored independently by 2 people and scores were averaged. Last column indicates whether values are statistically different: ns = not significant, P > 0.05. * = significant, P≤0.05 (2-way ANOVA comparing bee line and apiary site.)

Criteria	Measurement	Hygienic	Commercial	P
Frames Bees	range 1-20 frames	17.4 ± 1.38	17.3 ± 1.74	ns
Frames Brood	range 1-20 frames	10.1 ± 1.85	10.0 ± 1.52	ns
Temperament	# stings received: 0 = none; 1= one or more	0.14 ± 0.32	0.02 ± 0.15	ns
Chalkbrood	# mummies on 2 frames: 0= none; 1=<5; 2= 5-20; 3=>20	0.67 ± 0.85	1.78 ± 1.07	*
Honey Production	pounds harvested	90.0 ± 36.56	66.8 ± 32.20	*
Varroa mite load	# mites / 100 bees	0.6 ± 0.86	1.04 ± 1.09	*

20. Wilson, W. T.[e], J. Ibarra[w], R. Rivera[e], D. L. Maki[x] & J. Baxter[e] — HONEY BEE COLONY DEVELOPMENT FOLLOWING EXPOSURE TO SUREDYE BAIT IN GUATEMALA[y,z] — Control of the Mediterranean fruit fly (*Ceratitis capitata*) in the Western Hemisphere has often been accomplished by aerial spraying of malathion bait. In Guatemala, the bait spray has been applied to thousands of hectares of coffee plants under the direction of three agencies: USDA-APHIS, MOSCAMED-Guatemala and USDA-ARS. Although beekeepers were warned by MOSCAMED personnel to confine their bees before each spray, there were reports of honey bee (*Apis mellifera*) colony losses. The three agencies in early 1996 decided to test a new insecticide that would hopefully be less toxic to honey bees and less damaging to the environment. The new insecticide, called Suredye bait, contains: phloxine B (0.688%), uranine (0.312%), Mazoferm E802(R) (40%), fructose (20%) and water (39%).

Four apiaries were established in southwestern Guatemala with 15 colonies per apiary. Two apiaries were located within the spray area and two outside (unexposed controls). On April 1, each colony contained about 5 frames of brood, 7 to 9 frames covered with adult worker honey bees, and a laying queen housed in one deep Langstroth hive body. Five weekly applications of Suredye

bait were made starting on April 3, 1996. Aerial applications of Suredye were made directly over the colonies in the spray zone, and onto the surrounding coffee plants that were in bloom. The bees were not confined. An apron trap (*Amer. Bee J.* 118:671-672) was placed in front of each colony entrance to catch dead bees. On a weekly basis, dead bees were counted, number of frames of adult bees determined and square inches of capped brood measured for 7 weeks.

When the data were averaged on a per colony basis, no meaningful differences were observed between the development of colonies inside or outside the spray area. Adult bee mortality was very low (<10 bees/trap/week) for the entire 7 weeks. There were two exceptions when the count was 56 and 12 in an apiary where a migrating swarm attacked three colonies. No ants were seen removing dead bees. The overall lack of adult mortality was further substantiated by an increase of 2 to 3 frames covered by bees in each colony regardless of Suredye exposure. During the first 5 weeks, all four apiaries showed an increase in the amount (avg. sq. inches) of capped brood per colony, and all colonies expanded into a second deep hive body. Under field conditions, Suredye bait was not harmful to adult bees, immatures or queens. Egg laying continued throughout the study. Brood production did decrease in all of the colonies after the fifth week because the rainy season started. Rain interfered with worker-bee foraging, and may have washed the nectar from the coffee blossoms. The most productive apiary was located within the spray area.

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