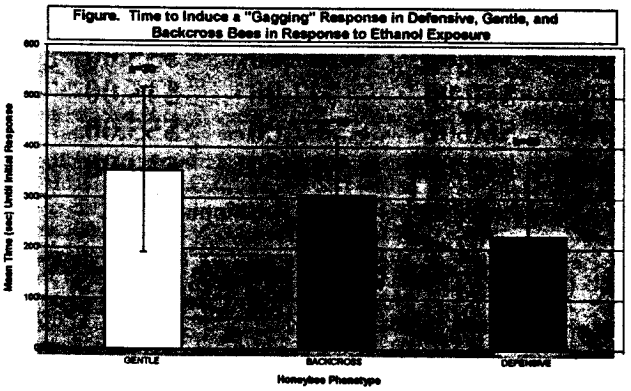
Proceedings of the American Bee Research Conference

The 2005 American Bee Research Conference was held in Reno, Nevada on January 13 - 14. The meeting was dedicated to the memory of a former member, Patti Elzen, who died on June 5, 2004. Dr. Elzen had a reputation for responding to crises in the beekeeping industry, such as the small hive beetle and varroa, and communicating her findings with beekeepers. She was a frequent contributor to this journal.

The next research conference will be held on January 9-10 at the Richmond Suites in Baton Rouge, Louisiana. The following are abstracts from the 2005 conference.

1. Ammons, A.D.[a] & G.J. Hunt[a] – IS THERE A CONNECTION BETWEEN DEFENSIVE BEHAVIOR, ALCOHOL SENSITIVITY, AND LEARNING IN HONEY BEES? - Gene regions influencing defensive behavior in honey bees have been previously identified from populations of Africanized "stingers" and European "guards" (Hunt *et al.*, 1998 Genetics 148:1203-1213; Arechavaleta-Velasco *et al.*, 2003 Behav. Genetics 33:357-364). Sequencing of the honey bee genome has allowed the detection of specific genes in these regions. A literature search suggested that a few of these genes have the potential for influencing other behaviors, in addition to colony defense, such as sensitivity to alcohol and learning ability. Experiments were conducted to develop assays designed to reveal specific differences in individual behavior between bees from a highly defensive European colony and bees from a very gentle European colony. Upon exposure to ethanol fumes, defensive bees displayed significantly higher ethanol fume sensitivity than gentle bees of the same age. Workers derived from crosses between the two original colonies showed intermediate sensitivity (See Figure). Alcohol consumption studies indicated the gentle bees (which were less sensitive to ethanol vapor) were also more willing than defensive bees to feed on higher concentrations of alcoholic sugar syrup, particularly under stressful conditions. Defensive bees also performed better than gentle bees in olfactory-based associative learning trials at certain times of the day. Molecular analyses will indicate if the same genes that influence defensive behavior also influence these other behaviors. These studies will add to our understanding of how alcohol affects the nervous system and what causes bees to sting.



2. Cobey, S.[b] - COMPARISON STUDIES OF INSTRUMENTALLY INSEMINATED AND NATURALLY MATED QUEENS AND FACTORS AFFECTING THEIR PERFORMANCE - Instrumental insemination is an essential tool to control honey bee mating. Additionally, it enables the creation of specific crosses that do not occur naturally, providing significant advantages to research and stock maintenance. It also provides a method of short term semen storage and shipment.

Widely used in research, a factor in the reluctance of the industry to utilize this technique is the perception of poor performance by instrumentally inseminated queens, IIQs. The varying, pre- and post-insemination treatments may explain these differences.

The majority of studies report similar colony performance in honey production, brood production and longevity: Roberts 1946 *ABJ.* 85:186, 211; Vesley 1984 *Bienenvater* :332-371; Konopacka 1987 *Proc. Apimondia 31st Congress*; Nelson & Laidlaw 1988 *ABJ.* 128:279-280; Cobey 1998. *Proc. Congreso Ibero-Latinamericano de Apiculture*; Skowronek et. al.2002. *JAR.* 46:85-95; and Pritsch & Bienefeld 2002 *Apidologie* 33:513.

Several studies show higher performance in colonies headed by IIQs: Szalai 1995 *Pszcelnicze Zeszyty. Nnaukowe* 39:61-69; Cermak 2004 *Vcelarstvi* 57:148-149; Wilde 1987. *Proc. Apimondia 31st Congress*; and Woyke & Ruttner 1976 Instr. Insem. of Queen Bees. Ruttner (*ed.*) 87-92. This is attributed to selection and uniform semen dosage. One comparison study reported lower queen performance, Harbo & Szabo 1984 *JAR* :31-36. The difference in this study is the treatment of queens.

In studies reporting equal or higher performance, queens were inseminated when 6 to 8 days old, given 8 to 12 µl of semen and introduced by direct release methods into nucleus colonies or packages. In the Harbo & Szabo study, queens 2 to 3 weeks old were inseminated twice with 2.7µl of semen, banked another 2-3 weeks and introduced into full size colonies.

Queens undergo physiological changes in preparation for egg laying. Many factors influence the rate of changes and affect; sperm storage, egg laying and performance. Queens mated after 14 days store less sperm. Banked queens also store less sperm and are subjects to injury from aggressive workers. Results are also influenced by semen dosage and handling techniques, and introduction methods; population size; temperature and nutrition.

IIQs may have an initial delay in development though catch up by the first cycle of brood. Onset of oviposition is delayed, averaging 6 to 10 days, compared to 2 to 3 days for NMQs. This may explain the lower queen acceptance rates among IIQs, though can be overcome with beekeeping management.

Influenced by treatment effects, differences between IIQs and NMQs can be eliminated with proper care. An understanding of the conditions necessary to enhance performance of IIQs will provide better results and confidence in the technique.

3. Cox, R.L.[c] F.A. Eischen,[c] & R.H. Graham [c] - NOSEMA DISEASE IN HONEY BEE COLONIES IN THE WESTERN UNITED STATES - A large gathering of honey bee colonies (1.4 million in 2003) from the Western U.S. is assembled each Spring in California for almond pollination. Many beekeepers have expe-

rienced unexpected weakening or losses of colonies when they were moved into the almond groves. Nosema disease causes spring dwindling of the colony, queen supersedure and subsequent loss of honey production. Treatment with fumagillin is recommended when Nosema infestation levels are greater than one million spores per bee. We conducted a survey of honey bee colonies in almond groves in the San Joaquin valley of California to determine the level of Nosema infection and any regional differences in the level of infection.

In February 2003 we sampled 560 colonies belonging to 95 beekeepers. These colonies originated from 16 western states, but California accounted for the greatest number of beekeepers (41) from any one state. Six randomly selected colonies were sampled from each beekeeper. About 100 bees from the brood nest were collected and stored frozen until examined. In order to count the number of Nosema spores we ground up the abdomens of 30 bees in 30 ml of water with a mortar and pestle. After allowing the resulting slurry to settle for 10 minutes, the clear liquid was extracted and spores counted in a hemocytometer under a phase-contrast microscope at 400x.

We found that 39% of the beekeepers averaged one million or more spores for their six samples. Approximately one-third of the colonies were either negative (30%), had < one million spores (35%), or had one million or more spores (35%) of Nosema. Therefore, about one-third of the colonies in the survey had spore levels that warrant treatment with fumagillin (i.e. one million or mores spores).

There were significant regional differences in the levels of Nosema spores. For example, the Pacific Northwest and the Upper Midwest had the highest levels of spores. In contrast, spore levels in the Rocky Mountains and Central regions were the lowest, while intermediate levels of spores characterized the Southwest and California.

According to this survey, Nosema infestation could be a significant factor in the weakening of colonies pollinating almonds in the Spring. It would also follow that the level of Nosema spores in other colonies in the same regions from which the colonies originated would also be high enough to warrant treatment.

4. Currie, R.W.[d], Tahmasbi, G.[e] - THE ABILITY OF HIGH AND LOW GROOMING LINES OF HONEY BEES TO REMOVE *VARROA DESTRUCTOR* IS AFFECTED BY ENVIRONMENTAL CONDITIONS - The objective of this study was to examine the effect of temperature and humidity on the ability of honey bee workers to groom varroa mites from their bodies.

One hundred worker bees from each of two lines of workers were placed in cages and were infested with 40 to 45 varroa mites per cage. Cages from each line of workers were randomly assigned to a combination of temperature and humidity treatments. Individual cages were held in incubators at 10, 25 or 34°C and enclosed in bags with regulated air flow of low, medium or high humidity. The entire experiment was replicated three times (18 cages per replicate). The proportion of mites falling into the base of the cage (grooming), or migrating within the closed system was monitored on day 2, 4 and 6 of the experiment. At the end of the experiment the proportion of mites on live bees, dead bees and in the equipment was also quantified.

The results showed significant differences in the ability of the two lines to groom mites off their bodies and that the relative effectiveness of the grooming in the two lines of bees was dependent upon the combination of temperature and humidity to which they were exposed. The difference in the ability of the two lines to groom mites off their bodies was highest at 25°C. At this temperature, the high grooming line removed 60 ± 4% of the mites through grooming by day six, but the low grooming line had removed only 36% ± 4% of mites. Overall mite mortality was greatest at 34, but at this temperature there were no differences in the amount of mite fall in cages containing high and low grooming lines of bees. Much of the mite mortality at higher temperatures was attributed to a higher incidence of migrating mites. The results show that programs that are attempting to breed for increased worker grooming behavior to enhance resistance to *Varroa destructor* will have to consider the environmental conditions under which experiments are carried out as environmental factors can mask differences between lines of bees.

5. Delaplane, K.S.[f], N.H. Mendizabal,[f] & J.A. Berry [f]- A MULTI-TRAIT SELECTION PROGRAM AT THE UNIVERSITY OF GEORGIA - The University of Georgia has begun a breeding program designed to select for disease and varroa resistance simultaneously with other characters of economic interest. A dedicated apiary of fifty nucleus colonies was established in 2002 to house queens, perform selections, and propagate naturally-mated daughters. Initial queens were purchased or donated from 10 producers from GA, CA, and TX. Some queens were used that had already been selected for suppressed mite reproduction or hygienic behavior. In spring of each year, every colony is equally reconstituted with *ca.* 2 pounds of worker bees, brood of all stages, stored honey, and a clipped and marked queen. In 2003, daughters were reared from every over-wintered 2002 mother and each instrumentally inseminated with a pool of semen representing drones from across the population; this homogenized genetic variation across colonies in the population. Beginning in 2004, all subsequent matings will occur naturally via drone saturation.

We are selecting for six characters: low Varroa mite levels (24-hr sticky sheet counts), high hygienic behavior (percentage freeze-killed brood removed by bees), high brood viability (number of occupied cells / brood square of 100), high brood production (cm^2), high honey production (colony kg gain) and low defense behavior (no. stings on leather patch / 120-sec exposure on top of combs). All traits are measured and their values transformed into z-scores in order to normalize units. Weighted z-scores are inserted into the following formula to derive a selection index: SI = (% brood solidness x 0.3) + (cm^2 brood x 0.1) - (Varroa x 0.2) + (% hygienic x 0.2) + (weight gain x 0.1) - (no. stings x 0.1). Colonies (queens) are ranked in order of SI score and the top 20% selected to propagate daughters for the next generation.

Changes in characters between two generations in a bee breeding program at the University of Georgia. Values are mean ± standard error. Numbers in parentheses = *n* (no. colonies) except for 2003 when all *n*=24. Asterisks indicate significant (α<0.05) difference between generations.

Breeding Character	2003	2004
Brood solidness (no. brood cells occupied/100)	88.4 ± 1.4	86.2 ± 1.2 (49)
Brood production (cm^2, all stages)	4361 ± 342	6587 ± 230 (50)*
Varroa mites (mites/24 hr sticky sheet)	0.5 ± 0.2	0.9 ± 0.1 (50)
Hygienic behavior (% freeze-killed brood removed)	56.3 ± 7.4	88.8 ± 1.8 (46)*
Honey production (kg, col weight gain during flow)	2.4 ± 0.4	4.2 ± 0.4 (50)*
Defense behavior (no. stings on leather patch/120 sec)	26.0 ± 5.7	18.5 ± 2.4 (50)

6. Eischen, F.A.[c], R.H. Graham,[c] & R. Cox[c] - THE IMPACT OF FEEDING FUNGICIDE PREPARATIONS OF CAPTAN TO HONEY BEE COLONIES [x] - Captan preparations are applied to control blossom fungal diseases of almonds. This crop requires more than one million honey bee colonies for pollination. Honey bees come in contact with Captan preparations directly through sprays, but more commonly by eating contaminated pollen and nectar. Nucleus colonies fed Captan-treated pollen produced significantly less brood and smaller adult progeny that lived much shorter lives. Caged nucleus colonies given diets containing Captan levels routinely encountered in almond orchards, had about 50% as many surviving nurse bees after four cycles of brood as did control colonies. Brood rearing was impaired with treated colonies rearing about 65% of eggs to the sealed stage, while controls reared about 80%. The greatest impact was on brood quality.

Emerging adult progeny in treated colonies weighed 76 – 85% of controls, but lived only 20 – 54% as long. Many of these adults lived less than 48 hours. These bees represent a net loss to their parent colonies.

The conditions of the test, i.e., extended feeding of Captan-treated pollen, is not ordinarily encountered during almond pollination. However, there are situations were Captan products are applied early in the day during peak bloom and is accompanied with intense foraging. If this situation is followed by several days of inclement weather forcing colonies to consume treated pollen for an extended period, it would result in damage to colonies. Pollinating honey bees receiving exposure to Captan in other crops should be examined for impact.

7. Ellis, J.D.,[f] K.S. Delaplane [f]& W.M. Hood [g]– PROGRESS TOWARD AN ECONOMIC THRESHOLD FOR THE SHB/VARROA COMPLEX – Hosting a pathogen/parasite can lower an individual's response threshold to an additional pathogen/parasite (Schmid-Hempel, 1998 *Parasites in Social Insects*). Herein we report preliminary data on determining an economic threshold for the SHB/varroa pest complex.

The experimental design was a split-plot ANOVA blocked on state (Georgia or South Carolina). Within each state, each whole plot consisted of one of 5 apiaries in which average colony SHB populations were manipulated to achieve 0, 150, 300, 600, or 1200 adults. Within each whole plot (apiary) each colony was randomly assigned one of three fluvalinate treatment dates (continuous, August, or October, two colonies per treatment) to experimentally vary varroa numbers (0, 3172, and 6662 respectively; Delaplane & Hood, 1997 *J. Apic. Res.* 36: 125-132). Colony strength parameters (cm^2 brood, adult bee population, bee weight, number of varroa on a 24-hour sticky screen, number of SHB in a 12-hour sample jar, level of tracheal mite infestation and colony weight) were sampled bi-monthly.

Results from the first season are reported in the table. There was no significant effects of SHB population on colony weight, number of adult bees, or cm^2 brood, nor was there an effect of varroa treatment time on colony weight or cm^2 brood. There was an effect of varroa treatment time on bee population ($F = 4$; df = 2, 26; $p = 0.04$).

We achieved a strong correlation between the number of SHB collected in a 12-hour sampling device and colony SHB population ($R^2 = 0.91$), but the sampling protocol proved unreliable during cooler temperatures. The work of Ellis *et al.* (2003, *Apidologie* 34: 399-408) suggests that SHB compromise bee populations and brood area. A second season of data should more conclusively illuminate how SHB and varroa interact to the detriment of colonies.

Table: The effects of varying SHB populations (0-1200) and varroa treatment date (month) on colony strength parameters in December. Data are mean ± std. error (*n*). Columnar data followed by the same letter are not different at the $\alpha \leq 0.05$ level.

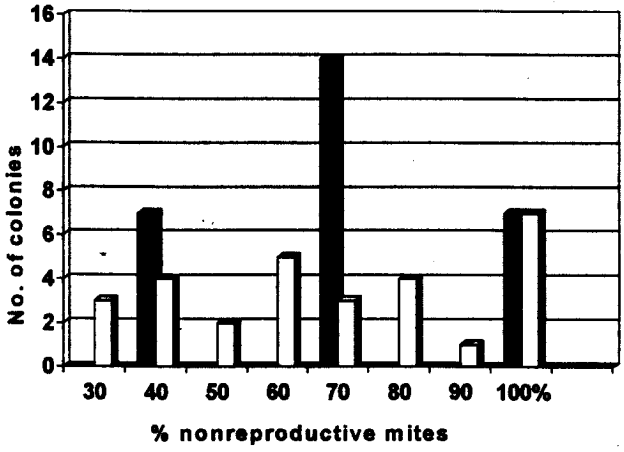
no. beetles	colony weight (kg)	no. adult beetles	cm^2 brood	varroa treatment time	colony weight (kg)	no. adult beetles	cm^2 brood
0	32 ± 1.5 (12) a, b	11829 ± 752 (12) a	48 ± 24 (12) b	continuous	33 ± 1 (17) a	12673 ± 550 (17) a	351 ± 142 (17) a
150	34 ± 1.5 (11) a	11954 ± 803 (11) a	606 ± 276 (11) a	August	31 ± 1 (20) a, b	11476 ± 830 (20) a, b	348 ± 139 (20) a
300	30 ± 1 (11) a, b	11314 ± 1162 (11) a	130 ± 82 (11) a, b	October	30 ± 1 (18) b	9499 ± 710 (18) b	155 ± 42 (18) a
600	32 ± 1 (12) a, b	11006 ± 1042 (12) a	267 ± 96 (12) a, b				
1200	29 ± 0.8 (9) b	9553 ± 1267 (9) a	426 ± 130 (9) a, b				

8. Harbo, J.R.[h] & J.W. Harris [h]– THE NUMBER OF GENES INVOLVED IN THE SMR TRAIT – The SMR (suppressed mite reproduction) trait is explained by the hygienic removal of reproductive mites, *Varroa destructor* (see abstracts #9 and #10). This report describes what a breeder may expect when outcrossing or backcrossing bees with the SMR trait.

To estimate the number of genes, we evaluated 28 gametes (drones) produced by a queen that was heterozygous for the trait. For example, if one gene is involved in the SMR trait, about half of the drones (14) from a heterozygous queen would have the SMR allele and 14 would not. If there were two genes involved, about 7 drones would have no SMR alleles, 14 would have one, and 7 would have two. As the number of genes increases, we expect fewer drones with none or all-possible alleles (the number of drones with none or all should be equal).

For the field test, we mated 28 drones (single drone inseminations) to queens that (in our judgment) had 100% of the alleles for the SMR trait. We established 28 colonies with these queens and measured mite reproduction after the queens had been laying for 8 weeks. We calculated the percentage of nonreproducing mites in each colony by evaluating 30 mite-infested worker cells 8-10 days postcapping.

Results suggest that there may be two genes involved in the SMR trait (see figure) and that the alleles may be additive. However, two is only a working hypothesis and estimate. The strongest case for 2 genes is the 1:2:1 distribution. About 25% of the colonies had 100% expression of the trait, about 25% were in a low grouping, and the rest were grouped in the middle.



Frequency of nonreproductive mites in 28 test colonies (white bars). Gray bars represent an expected distribution of 28 colonies if there are: 2 genes involved, no colony variation, and no measurement error. Except for ¼ of the expected data being at 100% nonreproduction, the other expected frequencies have no predicted percentages and were subjectively placed to provide the best fit.

9. Harris, J.W.[h] & J.R. Harbo[h]– THE SMR TRAIT EXPLAINED BY HYGIENIC BEHAVIOR OF ADULT BEES - We bred varroa-resistant honey bees by selecting colonies with low percentages of reproductive mites (Harbo & Harris, 2001, *J Econ Entomol* 94: 1319-1323). The trait causing this effect was termed "suppression of mite reproduction" (SMR) because we thought that the bees were increasing mite infertility (Harris & Harbo, 2000, *Apidologie* 31: 689-699). Ibrahim and Spivak (2004, *ABJ* 144: 406) found that SMR bees were hygienic and were able to remove varroa-infested pupae from capped brood cells. They suggested that SMR bees may selectively remove pupae with reproductive mites.

We tested this hypothesis by transferring combs with naturally infested and recently capped worker brood from 7 source colonies into control and SMR colonies. Combs from source colonies had an average infestation of 12 ± 8 mites per 100 capped worker cells and 71 ± 18 % reproductive mites (mean ± SD) a few weeks before the test. At least 1 comb from each source was transferred into each type of recipient. Immediately prior to this test, the control recipients had 80 ± 10 % reproductive mites, while the SMR recipients had 13 ± 6 % reproductive mites (mean ± SD).

The infestation rate and percentage of reproductive mites were

measured for 17 transferred combs after 7-9 days in recipient colonies. We defined three types of reproductive success for mites: (1) reproductive mites produced an adult daughter (including unmated daughters) before the host emerged, (2) mites with nonviable offspring may have produced daughters, but no daughter matured before the host emerged, and (3) nonreproductive mites did not lay eggs.

Fewer mites were found in combs given to SMR colonies than in combs given to controls (Table). This suggests that SMR bees hygienically removed infested pupae. Combs exposed to SMR bees had > 90% fewer pupae with reproductive mites. They also had 58% fewer pupae with mites that had nonviable offspring. The number of nonreproductive mites was equal in the two groups, which suggests that SMR bees did not remove mites that did not lay eggs.

Table – Comparison of varroa mites from combs that were transferred into control and SMR colonies. Newly capped cells of naturally infested brood were transferred, and mite reproductive success was evaluated 7-9 days later by examining cells containing pupae that were 3 days from emergence.

Variable[a]	Combs into control colonies (n=8)	Combs into SMR colonies (n=9)	Comparison of Least Squares Means t	df[b]	Pr > |t|
Percentage of reproductive mites	71 ± 5%	20 ± 5%	7.78	8.05	<0.0001
No. reproductive mites[c]	6.4 ± 0.7	0.4 ± 0.6	6.62	15	<0.0001
No. mites with nonviable offspring[c]	1.2 ± 0.2	0.5 ± 0.2	3.18	15	>0.007
No. nonproductive mites[c]	1.0 ± 0.2	1.0 ± 0.2	0.24	15	>0.8
No. dead mites [c,d]	0.4 ± 0.1	0.2 ± 0.1	1.60	15	>0.13
Total infested cells[c]	9.0 ± 0.9	2.2 ± 0.8	5.55	15	<0.0001

[a] **Least squares mean ± SE for each group of combs.**
[b] **Degrees of freedom were estimated using the Kenward-Roger method (Proc Mixed, SAS Institute). The *source* of brood combs was a random effect in the analysis of variance.**
[c] **Values reported as number of mites per 100 capped worker brood cells. We examined 315 ± 35 and 563 ± 180 (mean ± SD) capped worker cells in each comb that had been transferred into control and SMR colonies, respectively.**
[d] **All dead foundress mites had no progeny.**

10. Ibrahim, A.[i] & M. Spivak [i]– HONEY BEE RESISTANCE TO VARROA: HOW MUCH OF THE SMR TRAIR IS DUE TO HYGIENIC BEHAVIOR? - Suppression of Mite Reproduction (SMR) is an important, heritable mechanism of bee resistance to *Varroa destructor* (Harbo & Harris, 1999 *J Econ. Entomol.* 90:893-897). How bees suppress the reproductive success of the mite is not known. In 2002, we noted that colonies from the SMR line bred by J. Harbo (USDA Baton Rouge) also displayed hygienic behavior (HYG). Our preliminary experiments explored the relationship between these two traits (Ibrahim & Spivak, 2004 *Am. Bee J.* 144: 405-6). Here, using different methods, we addressed the same questions: (1) Do bees bred for Suppression of Mite Reproduction (SMR) detect and remove mite-infested pupae? (2) If so, do SMR bees preferentially remove pupae infested with reproductive mites leaving pupae with nonreproductive mites? (3) What is the reproductive success of mites from SMR colonies when bees are not allowed to remove mite-infested brood? For each question, we compared colonies from the SMR and HYG line.

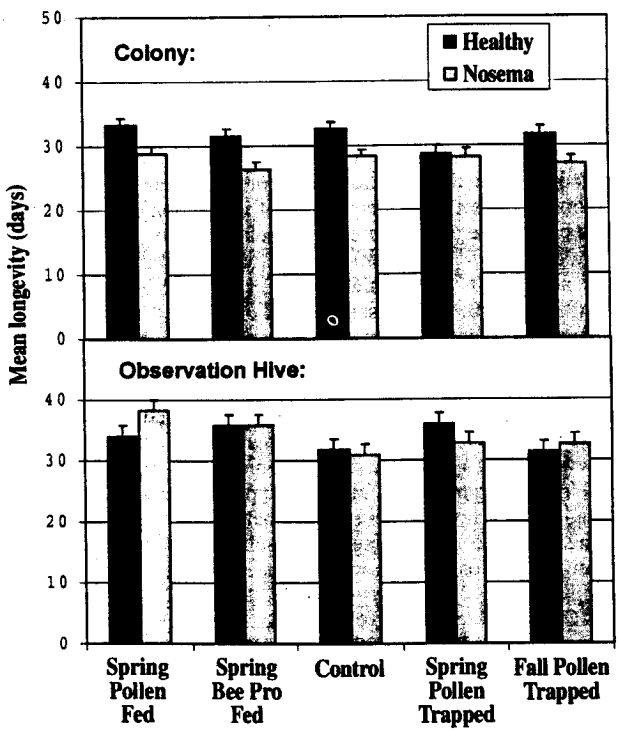
Mites were collected from an infested SMR and HYG colony, and were introduced into recently capped worker brood cells within 3 SMR and HYG mite-free colonies; each recipient colony received 40 mites from both source colonies. Mite source had no effect on the proportion of infested pupae removed by either line of bees. SMR colonies removed significantly more mite-infested pupae than HYG colonies (82.3% ± 10.8 vs 63.7% ± 8.5, respectively; $P < 0.001$; Survfit Procedure, R Statistical Software, 2004).

The reproductive success of the mites that were not removed by the bees was examined. Of the 241 mites introduced into the 3 SMR colonies, only 27 remained. Of these, 17 (63%) oviposited, but only 1 (3.7%) reproduced successfully, defined by the presence of an adult female offspring on a pupa with gray wing pads (Martin, 1994, *Exp. & Appl. Acarol.* 18:87-100). In contrast, of the 82 mites remaining in the HYG colonies, 70 (85%) were fertile and 22 (26.8%) produced adult female offspring (Fertility: bee type P = 0.014; mite type P = 0.293; Viable offspring: bee type P = 0.03, mite source $P = 0.25$ Log-likelihood ratios, SAS)

To determine if bee brood can affect mite reproductive success, we repeated the above experiment, but placed infested pupae in an incubator (34°C, 50% RH) until they reached the gray wing pad stage. Mites collected from both source colonies had significantly lower fertility on SMR brood (146 of 225, 65% oviposited) compared to HYG brood (178 or 230, 77%) (bee type P 0.003; mite type 0.126), and produced significantly fewer viable female offspring on SMR brood (5 of 225, 2.2%) than on HYG brood (26 of 230, 11.3%) (bee type: $P < 0.001$; mite type = 0.344)

In summary, bees bred for SMR do detect and remove mite-infested pupae, and tend to remove those pupae infested with reproductive mites, leaving pupae with mites that have low reproductive success. SMR colonies remove more infested pupae and are more selective about removing pupae with reproductive mites than are HYG colonies. In addition to this strong adult bee effect, there appears to be a physiological effect of SMR brood on mite reproduction because mites had significantly less reproductive success on SMR brood compared to HYG brood. Mites that develop for several generations on SMR brood come to have reduced reproductive potential due to the combination of the adult bees selective removal of reproductive mites and the brood effect which limits mite reproduction in an unknown way.

11. Mattila, H.R.[i] & G.W. Otis [i]– DOES SPRING POLLEN FEEDING PROVIDE RELIEF FROM THE SYMPTOMS OF *NOSEMA* INFECTION? - *Nosema* disease is widespread but often undetected in apiaries across North America. Rates of infection may be low throughout the year, but *Nosema* levels rise over winter and can reach damaging levels by spring. Infected workers experience reduced longevity, loss of nursing capacity and early onset of foraging, in part due to the effects of impaired nutrient absorption in the mid-gut. High levels of *Nosema* coincide with



The mean longevity of healthy and *Nosema*-infected workers in diet-treated colonies or introduced into an observation hive.

the time at which many beekeepers choose to supplement pollen diet in colonies. This increased availability of nutrients could potentially counteract some of the symptoms of *Nosema* infection.

We manipulated the spring availability of pollen/diet in colonies (fed pollen or Bee-Pro patties in the spring, pollen trapped in the fall or spring, or left alone [control]). From each treated colony, newly emerged workers were marked, half were inoculated with *Nosema*, and 100 workers were returned to the source colony and 20 were introduced into an observation hive. Worker longevity was followed in colonies and the observation hive and the latter was monitored to determine differences in nursing and foraging patterns.

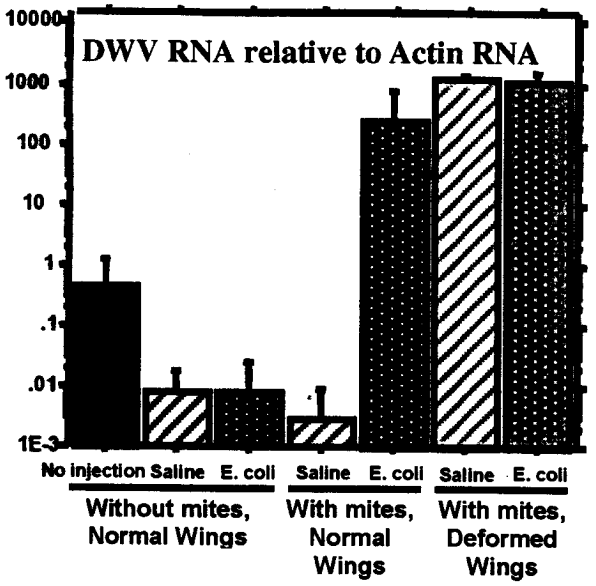
In colonies, *Nosema* infection reduced worker lifespan relative to healthy workers ($P<0.001$), and increases in pollen/diet availability did not offset this ($P = 0.10$, see figure). The opposite occurred in the observation hive, where pollen treatment had the greatest influence on worker longevity ($P=0.009$) and the effects of *Nosema* infection were lost ($P=0.79$, see figure). Workers from colonies with spring surpluses were more likely to be found in the brood area and were seen resting on the comb the least. Feeding pollen to colonies increased brood rearing, spreading nutritional resources over more workers rather than increasing nutritional investment per bee. This reduced the effects of feeding at the worker level and provided the opportunity for the symptoms of *Nosema* to be expressed. In the observation hive, workers experienced similar rearing demands and the pollen/diet conditions of the source colonies took precedence.

12. Nasr, M. [k] - EFFICACY OF SUBLIMATED OXALIC ACID IN CONTROLLING OR CONTROL OF VARROA MITES IN HONEY BEE COLONIES IN CANADA - Field trials were carried out in fall 2004 to evaluate the efficacy of sublimated oxalic acid against the varroa mite, *Varroa destructor* in Alberta, Canada. A homemade machine (C. De Wit, Leduc, AB) designed with 4 outlets, allowed for the application of sublimated oxalic acid to four hives at once. Each outlet was provided with a heating chamber to heat oxalic acid. Produced fumes were then forced by pressured air into the bee colony. In this test 8 unwrapped bee colonies and 8 bee colonies wrapped for wintering were used. For each colony with two brood chambers, 2 g of oxalic acid dehydrate were applied to the heating chamber and fumes were forced into the hives. Treatment date was October 7, 2005. To evaluate treatment efficacy, samples of 300-400 bees were taken out of each colony before treatment to determine the percentage of mite infestation per colony. 21 days after treatment, another sample of 300-400 bees/hive was collected to determine the percentage of mite infestation after treatment. The efficacy was then calculated as follows ((the percentage of mite infestation before treatment – the percentage of infestation after treatment)/(the percentage of mite infestation before)*100).

In unwrapped bee colonies the before and after treatment average number of varroa mites were 19.43±16.24 and 8.75±14.58 varroa mites/100 bees, respectively. For wrapped bee colonies, the before and after treatment average number of varroa mites were 19.08±9.00 and 1.37±1.42 varroa mites/100 bees, respectively. There was a significant difference between the percentage of varroa mites after treatment between the unwrapped and unwrapped bee colonies. The efficacies of sublimated oxalic acid treatment of unwrapped and wrapped bee colonies were 61.06±41.43 and 93.80±7.40, respectively. Time for applying treatment averaged 2 min/4 hives. These results suggest that sublimation of oxalic acid using this type of machine will result in effective and efficient control of varroa mites in wrapped bee colonies.

13. Ostiguy,N.[l], D. Cox-Foster,[l] X. Yang,[l] D. Caron [m] & M. Embrey [n]- BEES, MITES AND VIRUSES - Several tactics for reducing mite numbers are being explored in the Mid-Atlantic region. The first summer of a several year project testing summer requeening and dequeening show reduced mite numbers ($p\leq0.05$) if the interruption of the brood cycle is 15 days or more. Additionally, mite numbers were significantly reduced in colonies started from new packages and apiaries with small (5) colonies, $p = 0.04$, $p = 0.001$, respectively. In Pennsylvania we are also exploring the prevalence of three viruses - Sacbrood (SBV), Kashmir (KBV), and deformed wing (DWV) - along with the relationship between mites and viruses. Thus far we have observed a positive linear relationship between mite levels and KBV and SWV ($p = 0.029$ and $p = 0.046$, respectively). In addition we have observed colony death after miticide treatment of the colony for elevated mite levels in the absence of these three viruses. Both vertical and horizontal transmission of viruses is possible. KVB and SBV can be transmitted from workers to larvae through food; in samples of honey, pollen, royal jelly, and brood food SBV and KBV levels ranged from 33% to 100% of samples. Between 82% and 100% of adult workers and larvae sampled were positive for KBV and SBV. Co-infection/co-occurrence of KBV and SBV has been found in 33% to 90% of samples. Vertical transmission from the queen to the larvae is also possible. KBV and SBV were found in queens and in her eggs. Not all eggs from an infected queen will be positive for virus. An additional complication we have been exploring is the interaction between viruses and bacteria. In normal wing bees with mites injected with the bacteria *E. coli*, DWV virus levels were significantly greater than in bees not injected with *E. coli*. DVW levels in normal wing bees injected with saline did not differ from bees without mites with normal wings. Both saline only and *E. coli* plus saline injected bees with deformed wings were especially at risk for high levels of DWV.

Figure – Deformed-winged bees: A combination of mites and bacteria may interact to produce high viral level



14. Reyes- Carrillo, J.L.[o], P. Cano-Ríos, [p] & F. Eischen [c] - PLANT COMPETITION FOR HONEY BEE POLLINATORS DURING MUSKMELON BLOOM IN LA LAGUNA, MEXICO - This work was carried out in the Comarca Lagunera, which is located in the states of Coahuila and Durango in northern Mexico. During the spring and summer of 2002, we collected anthers of wild, cultivated and ornamental plant species. Our goal was to isolate and identify the pollen, using the acetolisis technique.

Plants were photographed, collected and desiccated to be preserved in the herbarium of the university. The pollen was processed and then observed with a microscope (Olympus model BH-2) that was connected to a TV screen. The pollen was measured with an objective micrometer at 100X immersion oil and photographed at 40X and 100X with a reflex Minolta SRT 101 camera, Rokkor lens PF 58 mm in a tripod. We used color slide film ASA 100, f9 at ½ second speed. We obtained at least 2 images of each pollen type at different angles and scanned in a 3500C HP scanner. We have 105 species of pollen in our collection –wild, cultivated and ornamentals - to serve as a reference

database for identifying plants visited by honey bees during cantaloupe pollination.

During the first blooming month, a six ha cantaloupe field was pollinated with 18 bee colonies, half of them had a pollen trap. Pollen was collected twice a week, weighed and frozen. An aliquot of the corbicular pollen was processed and mounted to identify the pollen and counted in the light microscope by fields at 40X. The results showed: cantaloupe pollen 8.72 %, 9.81%, 17.56 %, 9.29 %, 28.12% and 83.48%, respectively. The number of different plant species including muskmelon in the samples were: 7, 11, 13, 17, 21, and 15. The main plants competing with muskmelon during the first blooming month (see table) were mesquite, alfalfa, creosote bush, cucumber, London rocket, and sorghum, as shown in the table below.

Table - The main plants represented by pollen trapped in 9 colonies of honey bees located next to a field of cantaloupe. Data are in percentage of pollen sample.

	DATE					
Plant Species	**Apr 17**	**Apr 21**	**Apr 24**	**May 1**	**May 4**	**May 12**
Mesquite (*Prosopis juliflora*)	64.55	29.34	30.47			
Alfalfa (*Medicago sativa*)	25.45	26.46	45.16	3.17	0.04	0.26
Cantaloupe (*Cucumis melo*)	8.72	9.81	17.56	9.29	28.12	83.48
C. bush (*Larrea divaricata*)	0.85	8.28	1.58	82.79	23.23	0.62
Cucumber (*Cucumis sativus*)	0.10	20.25	0.09	0.37	0.32	0.18
L. rocket (*Sisymbrium irio*)	0.13	2.43	2.88	0.26	0.00	0.07
Sogham (*Sorghum vulgare*)					42.00	12.66

15. Rivera, R.[c], F.A. Eischen,[c] R.H. Graham,[c] & G.M. Acuña [c]– VARROA CONTROL TRIALS WITH THE THYMOL-BASED GEL PRODUCT [x]– *Varroa destructor*, a major pest of honey bees, is becoming resistant to the two chemical products currently registered by US EPA for use in honey bee colonies. The Weslaco Honey Bee Laboratory is currently screening compounds that have acaricidial properties to control *Varroa destructor*. Thymol is one compound that shows promise to control Varroa. Thymol is used in food products and is considered safe. Researchers have tested thymol in liquid and powdered form in Europe, (Imdorf, *et al.* 1999), Canada, (Otis 1999, Mattila *et al.* 2000), and the U.S. (Calderone 1999, Ellis *et al.* 2001). This compound is currently used in Europe with good beekeeper acceptance.

In the past we tested thymol in a liquid presentation and observed a rapid evaporation of active ingredient and a negative effect on honey bees. We tested Apiguard®, thymol in a food grade gel, that controls the release of the active ingredient to prolong the effect on varroa. We treated the colonies using 25 g three times at 7-day intervals and 50 g twice at 14-day intervals, applied above the brood chamber. We evaluated the frames of adult bees and frames of brood, monitored mite levels before the application and after 42 days. The trials were conducted in winter at Weslaco, TX, in summer at Ada, MN, summer in Weslaco, TX, and repeated in the summer in South Texas. We are collaborating with researchers at the Centro de Investigaciones Apicolas Tropicales in Costa Rica using the gel-based Apiguard® to control Varroa in a tropical setting.

Table - No. of Varroa at end of Test

Treatment	Texas	Minnesota	Texas	Texas
Control	724	523	292	483
Apiguard*50g 2x	119	35	16.5	47.2
Apiguard*25g 3x	224	24	13.7	
Check Mite™		8	12.5	407

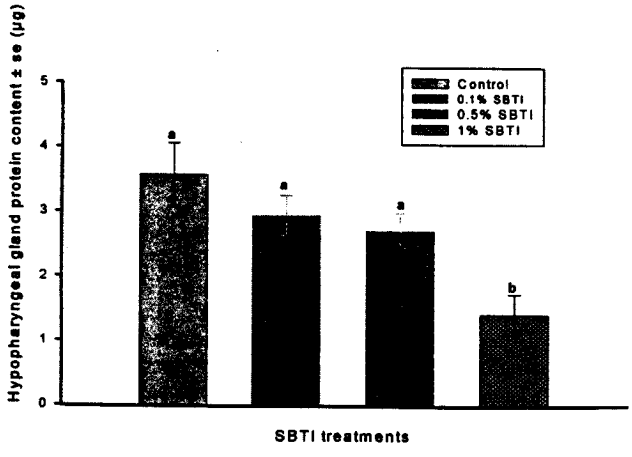
There were no apparent adverse effects on the bees, as we found no significant difference in brood area and adult bee numbers before treatment and after 42 days of treatment. We used Check Mite+™® as our positive control to compare effectiveness of the test chemical. We determine good control if we got a 10-fold difference between negative control and experimental treatments (Table 1.). This gel-based formulation of thymol controlled Varroa effectively in four trials in climatically different locations and different seasons of the year in the US.

16. Sagili, R.R.[q], T. Pankiw [q]& K. Zhu-Salzman [q]– EFFECTS OF SOYBEAN TRYPSIN INHIBITOR ON HYPOPHARYNGEAL GLAND PROTEIN CONTENT, TOTAL MIDGUT PROTEASE ACTIVITY AND SURVIVAL OF THE HONEY BEE (*APIS MELLIFERA* L.) - Insecticidal properties of protease inhibitors have been established in transgenic plants. In the wake of continuous research and rapid development of protease inhibitors it is important to assess possible effects on beneficial insects like the honey bee (*Apis mellifera* L.). We hypothesized that soybean trypsin inhibitor would have deleterious effects on honey bee protein digestion. In this study we evaluated effects of soybean trypsin inhibitor (SBTI) on hypopharyngeal gland protein content, total midgut proteolytic enzyme activity and survival of adult honey bees. Newly emerged caged bees were fed pollen diets containing three different concentrations (0.1%, 0.5% and 1% w: w) of soybean trypsin inhibitor (SBTI). Hypopharyngeal gland protein content, total midgut proteolytic enzyme activity and survival of these bees were measured.

Significant differences in hypopharyngeal gland protein content of bees were observed between 1% SBTI and remaining diets i.e. 0.1%, 0.5% and control ($F_{3,156}$=6.4, P<0.003, P<0.007, P<0.0001 respectively) (see figure). Midgut enzyme activities of bees fed with 1% SBTI were significantly different from control, 0.1% and 0.5% SBTI ($F_{3,156}$=237.5, P<0.0001, P<0.0001, P<0.0001, respectively). There were no significant differences between control, 0.1% and 0.5% SBTI. Bees fed with 1% SBTI diet had the lowest survival, followed by 0.5% and 0.1%, over a 30 day period.

This study has revealed that SBTI at 1% of pollen diet can negatively impact the hypopharyngeal gland development, midgut protease activity and survival of honey bees. In contrast it also showed that lower doses of SBTI were not deleterious to adult bees. As honey bee larvae are completely dependent on the hypopharyngeal gland secretions of nurse bees for their nutritional needs, the deleterious effects of SBTI on gland production quality and quantity could negatively impact colony growth and maintenance. However, the threshold response shown in this study suggests that pollen diets containing less than 1% SBTI are unlikely to affect colonies.

Figure - Mean hypopharyngeal gland protein quantities of bees (±SE) fed with different dosages of SBTI in pollen. Different letters indicate significant differences among the treatments (P < 0.0001).



17. Timm, P.C.[r] & M.D. Ellis [r]– A BEEKEEPING CURRICULUM FOR AGRICULTURE EDUCATION PROGRAMS – The role a mentor plays in training apprentices and passing on knowledge is a crucial one. This time-tested model of apprenticeship learning is used to train new doctors, mechanics, and graduate students in the sciences. Agriculture Education instructors are trained to seek opportunities to engage youth in apprenticeship learning. Projects that have low start-up cost, require only a small amount of land, and teach principles that apply to all agricultural enterprises are invaluable in agricultural education, and beekeeping represents a great tool for teaching youth about animal husbandry, plant science, environmental interactions, conservation, record keeping, marketing, value-added products, and many other skills that will serve them well in any agricultural enterprise.

Agriculture instructors use three unique, yet interwoven, components to prepare students: classroom instruction, supervised agriculture experiences (SAE for short), and the National FFA Organization. Classroom instruction provides the first exposure to agricultural science and practice. The SAE component provides real-life training through business internships, personal entrepreneurial ventures, conducting agriculture research, and part-time employment outside of the school. The National FFA Organization provides students with opportunities to develop leadership skills.

In agriculture education programs, beekeeping provides an excellent opportunity to engage students in a form of production agriculture that has all the characteristics of other agricultural operations that have higher start-up cost and space requirements. Students learn entrepreneurship by managing a small farming operation that includes all the steps from preparing a business plan to marketing. When several students participate they can share resources, and some beekeeping organizations have programs to support and encourage young beekeepers that offer opportunities for leadership development.

To gain widespread acceptance, a beekeeping curriculum must address the required agriculture education standards. In Nebraska, Agriculture Education has ten essential learning standards (Katt *et al.*, 2003 *Nebraska Department of Agriculture Education*). Seven of the ten standards can be addressed by studying bees. The seven standards include: agribusiness management, agricultural mechanics, agricultural sales and marketing, food science, horticulture, natural resources, and plant science.

We are developing a beekeeping curriculum for agriculture education programs that will provide educators with the tools necessary to incorporate beekeeping training and experiences into agriculture education classrooms, while meeting the required agriculture education standards. The curriculum will be distributed to agriculture instructors on CDs with lesson plans, supporting worksheets, quizzes and tests, visual aids and additional materials. In addition, workshops will be offered to provide apiculture training to agriculture education specialists who want to use the beekeeping curriculum. The project is funded by the Nebraska Beekeepers Association. The expected completion and dissemination date is April 2006.

18. Underwood, R.M.[d] & R.W. Currie [d]- INDOOR WINTER FUMIGATION OF HONEY BEES WITH FORMIC ACID - Formic acid is generally used as a fumigant in individual hives outdoors in spring or fall. This work tests the feasibility of fumigating indoors in winter using small experimental rooms housing 21 colonies each. Fumigating indoors in winter is advantageous because labor is reduced, the proportion of mites on adult bees is increased, and the applicator can control the ambient conditions.

Indoor winter fumigation can be applied as either a long-term low concentration or a short-term high concentration of formic acid. Each has its advantages and disadvantages. Long-term low concentration fumigation is effective as a varroa mite control technique, bringing the mean abundance under 2 mites per 100 bees for the start of the subsequent honey production season. In addition, long-term low concentration fumigation does not harm workers or queens. However, fumigation must be carried out over a long period of time (*i.e.* a month or two). Short-term high concentration fumigation is also effective as a varroa mite control technique. However, worker and queen bees can be killed if the proper precautions are not taken. The use of temperature-dependent step-wise ventilation during fumigation with a high concentration of formic acid may prevent queen loss. Low room temperature (*i.e.* < 4°C) also may be effective in queen loss prevention. Overall, whether a low or high concentration is used, the amount of formic acid per hive is approximately the same (*i.e.* 200 ml/hive) and is equivalent to the amount used outdoors.

Before this technique can be used by commercial beekeepers, a few things need to be studied further. The concentration of formic acid in the hive air is substantially less than that in the room air and varies between hives. Therefore, monitoring the total amount of acid to which the bees are exposed requires measurements of acid concentrations inside the hives. If the variability among hives can be reduced, possibly by standardizing equipment and/or using temperature-dependent step-wise ventilation, the efficacy of this method will be improved.

19. Wenner, A.M.[u], R.W. Thorp,[v] & J.F. Barthell [w]- BEE COLONY MORTALITY DUE TO VARROA IN A CLOSED ECOSYSTEM - In 1988 we began a program to remove feral European honey bee colonies from the Channel Islands National Park. We did so to help restore an ecosystem (including enhancing pollination of native plants by native bees) and to forestall a takeover of island colonies by Africanized honey bees.

A rapid fennel invasion and early 1993 damaging rains led us to investigate the use of varroa mites as a biological control. That mite met all the requisite characteristics for introduction into a nature preserve, including target host specificity.

Those mites had additional promise in three ways: (1) should mites fail to eliminate all colonies, we could have a mite resistant strain of bees, (2) if all bee colonies perished, we would have met our original goals (above), and (3) we would gain valuable information about progressive mortality due to mites in a closed ecosystem.

We introduced a small number of individual varroa mites only in selected spots and only on the eastern half of the island in midwinter of 1993-94. None of 117 monitored colonies perished during the first two years, but all those colonies had succumbed before January 1998 (Wenner & Thorp, 2002 *Proceedings of the 2nd International Conference on Africanized Honey Bees and Bee Mites*. 159-166; Wenner, *et al.* 2002, *Proceedings of the Fifth California Islands Symposium*. 256-260).

Nevertheless, we could occasionally find a few foragers at 19 sites of "magnet" plant species, apparently due to a long term persistence of 2-3 colonies. By contrast, the past five seasons (2000-2004) experienced a steady decline in the number of sites at which bees could be found foraging and in the total number of times we saw bees at those sites (see figure).

The steady demise of remaining island colonies becomes more evident in the fact that we caught no swarms after 1996. That lack of swarm capture occurred despite our placing fresh lures each year in 2-3 dozen swarm hives positioned near previous foraging sites.