Special Feature

Proceedings of the American Bee Research Conference

The 1989 American Bee Research Conference was held at the Hoblitzelle Center of the Texas A & M Agricultural Experiment Station in Weslaco, Texas on October 3 and 4, 1989. A Tracheal Mite Symposium was held at the same place on October 1 and 2, and a few of the participants in that symposium chose to publish their abstracts with those of the research conference. Abstracts from the mite symposium are noted as such. Complete articles from the Tracheal Mite Symposium will be published by OneBetter Publishing (watch for details in bee journals).

The fifth American Bee Research Conference will be held in Tucson on October 1 and 2, 1990. The following are abstracts from the 1989 conference.

1. Clark, K. J.[a] D. L. Nelson,[b] and D. McKenna[c] – EFFECT OF MENTHOL ON QUEEN REARING – The effect of three dosages of menthol was investigated during the rearing of queens in northern Alberta in June and July, 1989. Twenty-five mating nucs in each of four treatments received 0, 10, 20 and 40 grams of menthol pellets in 15 cm. square, 2 mm. nylon mesh packets placed on their top bars. Queen cells within 2 days of emergence were installed in the center of each nuc at the same time that menthol was applied. The menthol packets were weighed 3 times over a treatment period of 17 days. During the first week, the weight of menthol which evaporated in each treatment was approximately 2.7, 3.8 and 5.3 grams, respectively. Following the treatment, the queens were tagged, caged and introduced into small colonies which were allowed to build up for the rest of the summer.

The table shows the number of queens surviving each treatment as recorded on day 7 (emergence in mating nuc), day 16 (egg laying in mating nuc), day 26 (acceptance in small colony) and day 33 (egg laying in small colony). In the group which received no menthol, queens emerged from 80% of the cells, and the number of queens accepted and laying after transfer to the small colonies was 76% of the number of installed cells. In the three menthol treatment groups, live queens emerged and were present at day 7 in only 48 to 56% of the nucs, while the number of queens accepted and laying in the small colonies was between 48 and 28% of the number of installed cells (see Table).

Most of the reduction in success occurred in the first week after installation of the cells, and appeared to result from lack of emergence from the cell or destruction of the cell by worker bees. Bees involved in the study had no tracheal mites. (Presented at the mite symposium)

Table – Number of queens surviving from 25 cells installed per treatment.

Dose (gm. Menthol)	Day 7	Day 16	Day 26	Day 33
0	20	19	19	19
10	13	10	8	7
20	14	13	12	12
40	12	7	7	7

2. Coelho, J. R.[d] – THE EFFECT OF THORAX TEMPERATURE AND BODY SIZE ON FLIGHT SPEED IN HONEY BEE DRONES – The temperature of the thorax (T_{th}) of a flying insect is believed to be an important determinant of its flight performance because increases in T_{th} improve flight muscle function (up to a limit). However, few studies have actually demonstrated the effect of T_{th} on any aspect of flight performance itself. Honey bee drones consistently have a T_{th} ~3°C higher than workers; therefore, it might be concluded that this results in faster, more agile flight, which would presumably result in greater success during aerial mating attempts. Drones are approximately twice the size of workers, which should also result in more rapid flight for drones. This study examined the effects of T_{th} and size on flight velocity (V) in an attempt to test these ideas.

Individual drones were captured while returning to the hive, taken ~30 m from the hive, marked with paint and

released. One investigator measured V with the use of K-ban Doppler radar, while another captured the drone as it again returned to the hive, measured T_{th} with a digital thermocouple thermometer, and stored the drone for later morphometric analyses. The percentage of drones which returned to the hive was improved from 23% to 80% when a single drone was captured, quickly taken away from the hive, marked and released, as opposed to capturing many drones, taking them away and releasing them one at a time. Rapid recovery of drones was improved when the first investigator held the drone aloft, rotated it slowly in a complete circle, then released it in the direction of the hive. Data were taken only for drones that returned within ~15 s in order to minimize potential changes in T_{th} which may have occurred since V was measured.

V (meters per second) was related to T_{th} (°C) according to the following equation: $V = 0.287(T_{th}) - 5.86$, $r^2 = 0.21$. V was also related to thorax mass (M_{th}), an index of body size: $V = 61.7\ (M_{th}) + 0.367$, $r^2 = 0.15$. However, M_{th} and T_{th} were correlated ($T_{th} = 101\ (M_{th}) + 31.4$, $r^2 = 0.16$). Multiple regression analysis including all three variables showed that T_{th} was significantly related to V ($P < .05$), but M_{th} was not ($P > .05$): $V = 38.8\ (M_{th}) + 0.226\ (T_{th}) - 6.7$, $r^2 = 0.26$. V was not related to wing loading ($r^2 = 0.05$). Mean V for drones (5.37 ± 0.93 m/s, n = 184) was significantly different from that of workers (5.90 ± 0.57 m/s, n = 130, $p < 0.0001$). Mean V of nectar-loaded workers was 5.58 ± 1.22 m/s (n = 48), while that for unloaded workers was significantly higher, 6.08 ± 0.95 m/s ($n = 82$, $p < 0.0001$).

The rate of physiological processes generally increases two- to three-fold with a 10 degree Celsius increase in temperature (Q_{10} = 2 to 3). Since metabolic rate in drones increases with T_{th} with a Q_{10} of 2.1, one might expect a similar effect on V. The Q_{10} of V in drones in this study was 1.69. The increased aerodynamic drag associated with higher V may result in a lower Q_{10}. In free-flying sheep blowflies, V increased linearly with T_{th}, and Q_{10} ranges from 1.23 to 1.33 (Yurkiewicz & Smyth, *J. Insect Physiol.* 12:189-194), perhaps indicating that V in endothermic insects (such as bees) is more temperature sensitive than in ectothermic insects (such as flies).

T_{th} has a positive effect on V of drones, at least within the range of T_{th} observed. Furthermore, T_{th} is more important than size in determining V. These results underscore the significance of thermoregulatory strategy in establishing flight performance. Greater size only augments V because it results in higher T_{th}. Thus, because drones have greater size and T_{th}, one would predict that they should be faster flyers than workers. The fact that this is not the case indicates that drones have not evolved large body size and high T_{th} as a means of improving V. It is possible that T_{th} is still important for competition among drones during mating attempts. A hotter drone may reach the queen faster than a cooler drone by virtue of more rapid flight and, consequently, succeed in copulation.

3. Collins, A. M.[e] — CONSIDERATIONS ON BREEDING HONEY BEES RESISTANT TO MITES — Chemical treatments for tracheal mites are being developed and used with some success (Cox *et al.*, *Amer. Bee J.* 129:129-131; Burgett and Stringer; *Gleanings* 117:522-524). However, the likelihood is that mites will be able to develop resistance to these materials. In the long term, the most effective solution for mite control is to breed honey bees that are resistant to tracheal mites.

For any success with a breeding program, it is first necessary to have variation, phenotypic as well as genotypic, and to be able to measure it in a biologically significant way. Gary and Page (*Exper. & Applied Acarol.* 3:291-305) reported that phenotypic variation in the level of infestation of tracheae does exist. They went on to select for resistance in their highly variable population and quickly (in few generations) achieved some success (personal communication), not an unusual occurrence in honey bee selection. Certainly the reports from beekeepers of the variable effects in individual apiaries, where some colonies will be devastated and others only mildly infested by mites, show that sufficient stock differences do occur in the U.S. To look at the results of this past year's heavy losses in a positive light, we can say that surviving colonies represent a pool of naturally selected raw material readily available to us for further controlled selection.

Additionally, scientists are in the process of importing resistant stocks from abroad to take advantage of selection that has already been done. These include stocks from Great Britain where long exposure to the parasites may have selected phenotypes that are resistant (Morse, personal communication). Rinderer and Kulincevic have done several generations of controlled selection for *Varroa* resistance and are now transferring stocks from Yugoslavia (Kulincevic, *et al.*, *Apidologie* in press).

As with any honey bee selection program, care must be taken to begin with populations of sufficient variation that inbreeding depression does not counteract any selection success. The closed population breeding system of Page *et al.* (*Amer. Bee J.* 122:350-355; see also Lawrence and Coby, *Amer. Bee J.* 25:687-688, Severson *et al.*, *Amer. Bee J.* 126:93-94) is a good model for any prospective breeders. Also line breeding as used in the past (Mackenson and Nye, *J. Apic. Res.*, 5:79-86; Nye and Mackenson, *J. Apic. Res.* 9:61:64) will continue to prove effective, but requires more care and skill. The emerging field of biotechnology deserves some mention also. It has the potential for allowing us to tap the resistance mechanisms present in the original host species of honey bees and transfer them to *Apis mellifera*.

The ease with which any such selection program can be done is also dependent on the ways in which the level of resistance is measured. At the current time, we are still limited to dissection and visual counting of dead or living mites for clear indications of infestations. This is extremely labor intensive and limits our efforts. I am pleased to see that some of the papers to be presented in this symposium are addressing potential assay techniques to speed our characterization of colony status. (Presented at the mite symposium)

4. Collins, A. M.,[e] H. V. Daly,[f] T. E. Rinderer,[g] and J. R. Harbo[g] — CORRELATIONS BETWEEN IDENTIFICATION AND DEFENSIVE BEHAVIOR TRAITS — Because of difficulties in visually identifying Africanized honey bees in the field, the suggestion has been made that defensive behavior might be a suitable character for preliminary identification (Spivak *et al.*, in "Africanized Honey Bees and Bee Mites," Needham *et al.*, eds., pp. 313-324). Currently the only widely accepted identification of Africanized bees is the body measurement (morphometric) system of Daly and Balling, *J. Kansas Entomol. Soc.* 51:857-869. If significant correlations exist between these measures and defensive behavior, the vigor with which a colony defends itself would be an appropriate way to choose possible Africanized colonies.

In 1979 two populations of honey bees, 150 colonies in Louisiana, USA, and 148 colonies in Monagas, Venezuela, were measured for defensive behavior (Collins and Kubasek, *Ann. Entomol. Soc. Amer.* 75:383-387), alarm pheromones (Collins *et al.*, *J. Chem. Ecol.* 15:1747-1756), and body size (morphometric identification). Using a refined morphometrics database (Buco, unpublished), the Louisiana bees were identified as all European and the Venezuelan bees were identified as European, Africanized, hybrid and questionable. Using the pooled values from both populations, correlations were determined for the 25 morphometric measures

with the 7 defense measures and the 12 pheromones. Values in the table indicate that some defensive behavior traits had significant correlations with the morphometric measurements and could usefully be employed for quick identification purposes.

Table – Correlation Coefficients Between Various Traits[1]

	Morphometric Measures				
	Forewing Length	Forewing Width	Hindwing Length	Femur Length	Angle 34
Defense measures					
No. stings	-0.393	-0.548	-0.186	-0.308	-0.318
Time to react to:					
pheromone	0.376	0.448	0.188	0.387	0.219
target	0.318	0.492	0.128*	0.324	0.230
No. of bees at 60 s	-0.201	-0.310	-0.106ns	-0.200	-0.179
Pheromone production level					
hexyl acetate	-0.647	-0.619	-0.485	-0.595	-0.177
heptanol	-0.553	-0.506	-0.421	-0.538	-0.072ns
nonanol	-0.573	-0.552	-0.442	-0.532	-0.127*
isopentyl acetate	-0.024ns	-0.010ns	0.018ns	-0.014ns	0.078ns
2 heptanone	-0.001ns	0.104ns	-0.058ns	0.043ns	-0.017ns

1 – All traits except isopentyl acetate and 2 heptanone production were significantly different for the two populations.

All values are significant at $P < .01$ except * = $P < .05$ and ns = not significant.

5. Danka, R. G.,[g] J. L. Williams,[g] and T. E. Rinderer[g] – ACEPHATE BAITING TECHNOLOGY: REFINEMENTS AND PRELIMINARY FIELD TESTS[ff] – Initial development of a baiting system designed to eradicate undesirable honey bee colonies was reported previously (Williams *et al.*, *Apidologie* 20: 175-179). In initial tests colonies were destroyed after foragers collected sucrose-honey solution containing 250 ppm acephate from feeding stations located 10 m away. More recent tests examined the effect of treatment distance, levels of insecticide residues in treated colonies, and practical aspects of eradicating populations of feral colonies.

Each of 12 colonies treated from 500 m with 500 ppm acephate were successfully destroyed (see Table). In addition, treatment distances of up to ca. 700 m did not limit successful treatments during field tests (see Table).

Residues of acephate and its metabolite methamidophos were quantified by gas chromatogaphy after extraction from dead bees and food-storage comb (*i.e.*, a mixture of wax and honey, syrup or nectar). Samples were collected from 5 combs in each of 5 colonies treated with 500 ppm acephate at 500 m. In dead bees, acephate peaked at ca. 10 ppm 1 day after treatment and dropped to 2 ppm after 10 weeks. Methamidophos levels were at 2-3 ppm throughout the sampling period. In comb samples, acephate peaked at ca. 1.2 ppm and methamidophos at ca. 0.1 ppm; ca. 50% of these levels were present after 10 weeks. The low residue levels probably represent a minimal environmental hazard, especially since an average of only 31 mg of acephate was collected during all treatments (see Table.)

Four sequential replications of a simulated eradication program were conducted on an isolated barrier island (Grand Terre) in southeast Louisiana. Simulated feral colonies were moved into randomized positions on the island; densities ranged from ca. 1-7 colonies/sq km. Baits typically were placed at 500-m-grid intersections, and treatments with 500 ppm acephate ranged from 88 to 691 m. During the first three replications, 12 of the 13 colonies that were treated (and 12 of 14 overall) were eradicated (see Table). In the fourth replication, colony density was high, leading at times to multiple colonies per feeder; there was also an increasing nectar flow. At least 7 colonies were known to be treated, and 9 died (9 of 15 overall). The system thus was almost always successful when treatments were made to individual colonies that were foraging actively. Difficulties arose, however, when foraging activity at bait stations was inadequate because of a nectar flow, poor flight conditions, or multiple colonies per feeder.

Table – Responses of honey bee colonies treated with 500 ppm acephate. Colonies were baited to feeding stations that delivered sucrose-honey syrup. When foraging activity was acceptably high (>100 bees per feeder), plain syrup was replaced with acephate-treated syrup. Tests at Baton Rouge, Louisiana, evaluated baiting performance at a longer distance than was previously attempted. Tests on Grand Terre Island, Louisiana, simulated an eradication program.

Site	Rep.	Treatment distance, m (x̄, range)	Total No.	No. treated	No. died	Max. no. foragers on feeder during treatment	ml treated syrup collected	mg acephate collected
Baton Rouge		500	12	12	12	205 ± 67	67 ± 23	34 ± 12
Grand Terre	I	154 (88-198)	6	5	5	152 ± 90	37 ± 24	19 ± 12
	II	132 (88-198)	5	5	5	178 ± 84	54 ± 10	27 ± 5
	III	477 (265-691)	3	3	2	165 ± 44	63 ± 17	32 ± 9
	IV	376 (182-566)	15	>7[1]	9	173 ± 56[2]	56 ± 16[2]	28 ± 8

1. Multiple colonies were known to be treated from one feeder.
2. n = 3

6. Davidson, F.,[h] T. Udagawa,[i] N. Lakey[h] and G. B. Kitto[h] – HONEY BEE IDENTIFICATION: CHARACTERIZATION OF THREE PROTEINS SPECIFIC TO AFRICANIZED BEES – The arrival of africanized honeybees (AHB) in the U. S. will necessitate rapid, economical methods for their detection. Antibodies, directed against AHB-specific proteins, can be used in ELISA and dipstick-type immunological assays and provide a rapid, highly specific and economical methodology. We describe here the characterization of three proteins which appear to be unique to africanized honeybees.

Using pH 5-7 non-denaturing isoelectric focusing in polyacrylamide gels, we have identified three proteins (A1, A2 and B1) which are restricted to AHB. Proteins A1 and A2 have isoelectric points (pI) of 5.51 and 5.49, respectively. Protein B1 has a pI = 5.11 while protein B2 (found in all european honeybees and in ca. 50% of all AHB) has a pI (5.09) very similar to but distinct from that of B1. We have screened 16 different sources of AHB from Mexico, Honduras, Costa Rica, Venezuela and Brazil; A1 is found in 60% of the populations, A2 in 24% and B1 in 45%. Seven per cent of the AHB populations have neither A1 nor A2 nor B1. We have not found AHB-specific proteins in any of eight european bee samples we have tested. Using antibodies directed against the three AHB-specific proteins, we anticipate we will be able to detect africanized honey bees in approximately 93% of bonafide AHB samples.

The AHB specific proteins are found in the thoraces of africanized honey bees but not in the abdomens or heads. To further characterize the subcellular distribution of AHB-specific proteins, 50 AHB thoraces were pooled and the mitochondria isolated by differential centrifugation: the AHB-specific proteins were found only in the cytoplasmic and not the mitochondrial fraction. Since several proteins can have the same isoelectric point, IEF gels of AHB samples were stained with Coomassie Blue and the A1, A2 and B1 bands cut out. The excised bands were then electrophoresed on slab 10% SDS-PAGE gels. A single major band corresponding to each of the AHB-specific proteins was found on

the SDS-PAGE gel. The molecular weights of A1 and A2 appear to be identical (ca. 43,000 daltons). The molecular weights of B1 and B2 are also identical and are also approximately 43,000 daltons. We are currently doing amino acid composition and sequence studies to further elucidate structural relationships among the four proteins. We are also raising antibodies against each of the four proteins for use in ELISA immunoassays.

7. Dawicke, B. L.,[j] G. W. Otis,[j] and C. D. Scott-Dupree[j] — PREDICTING TRACHEAL MITE INFESTATIONS AND EFFECTS ON COLONIES — Economic damage from the honey bee tracheal mite in the form of decreased honey yields (Eischen, F. *et al., Apidology* 20:1-8) and increased winter mortality (Otis, G. W. *et al.*, 1986, Proceedings of the Honey Bee Tracheal Mite Scientific Symposium, St. Paul, MN) has forced beekeepers to seek out methods of control against the mite. The expense of these treatments necessitates knowledge of optimum treatment times which could be based on trends of mite prevalence within colonies.

Using data from four different years (1985-1989) and four different locations we examined correlations of mite prevalence from one month to the mite prevalence of another month within that same year. We also correlated the mite prevalence of a given colony for a given month to the brood area of that colony found in the spring (May).

Beginning with the month to month correlations from the four years of data, we noted general trends. These trends are represented by the data of 1988-1989. The August 1988 mite prevalence was highly correlated with that of September, ($r = .90$, $p < .001$) and November 1988, ($r = .78$, $p < .001$). However, the correlations with the subsequent months tended to decline and became negligible by May 1989. The month of January appeared to be a crucial one in that it correlated with all of the months, including the previous summer and the following spring. The May 1989 correlations re-emphasized a lack of relationship between summer or late summer mite prevalence levels with mite prevalence levels of the following spring.

The correlations indicate that the mite prevalence of months which closely follow each other are highly significant but the mite prevalence correlations decline as the span between months increases. This decline prevents the use of summer mite prevalence values as predictors of spring trends.

The trends for the correlations of mite prevalence to spring brood area were not consistent from year to year. In 1985-1986 we found that no relationship of mite prevalence with brood area existed until into the winter months; January, February, March and April. For 1986-1987 the pattern of correlations was different. The significant relations began earlier and continued into the winter; October, December and March. The data for 1987-1988 was similar to the previous year with October, November and March mite prevalence values being significant with the spring brood area.

The mite prevalence values when correlated to spring brood area indicate that a relationship exists between mite prevalence of colonies in the fall and winter with spring brood area. However, the correlation trends for this method are not consistent from year to year and the low correlation values suggest other variables are more important for influencing spring brood.

In conclusion, the magnitude of changes of mite infestation which occur during the fall and winter make summer and early fall measurements not useful as predictors for treatment. (Presented at the mite symposium)

8. Delfinado-Baker, M.[k] — INTRODUCING OTHER TRACHEAL MITES: *LOCUSTACARUS BUCHNERI, L. TRACHEALIS* AND *L. MASONI* (PODAPOLIPIDAE: ACARI) — This is a review of parasitic mites related to Tarsonemidae *(Acarapis)* that infest the tracheal system of insects other than honey bees *(Apis)*. Within the superfamily Tarsonemoidea are two families, Tarsonemidae and Podapolipidae, that contain true parasites of insects having a great diversity in living habits and of structure of the body, legs and mouthparts. Included in this group are tracheal parasites of the genera *Acarapis* (Tarsonemidae) and *Locustacarus* (Podapolipidae). They live and multiply in the tracheal system, injuring their hosts by piercing the walls of tracheae to feed on haemolymph and hindering respiration by blocking the tracheae. The importance of these mites is related to agriculture. Among the tarsonemids, only the honey bee tracheal mite, *Acarapis woodi*, is considered of importance economically. This species infests adult honey bees, *Apis cerana* and *A. dorsata* in Asia, and *A. mellifera* worldwide. The possible importance of podapolipids is largely unknown. All podapolipids are internal or external parasites of several insect groups. They occur in the vaginal membranes and oviduct sacs of beetles, in the tracheal air sacs of bees and orthopterans, under the elytra of beetles, and on the wings and bodies of locusts, grasshoppers and cockroaches. The tracheal parasites *Locustacarus buchneri* infest bees of the genera *Bombus* and *Psithyrus* in Europe and North America, while *L. trachealis* and *L. masoni* infest acridiids (locusts and grasshoppers) in the U.S., Africa, New Zealand and Australia. They are considered to be more harmful to the host than are external parasites. Mites may be recovered in the tracheal air sacs in the abdomen of the host by making an incision between two sternites (IIS-IIIS) and pulling them apart to expose the sacs. Infested air sacs appear dark brown. Frequently mites are found in the posterior section of the air sacs. (Presented at the mite symposium)

9. Diaz-y-de-la-Garza, C.,[l] W. L. Rubink,[e] and W. T. Wilson[e] — FOLKLORE AND MAGIC IN RUSTIC APICULTURE OF TAMAULIPAS, MEXICO — The majority of the rustic beekeepers are primarily uneducated senior citizens in rural areas where the per capital income is low. Their knowledge of the biological processes of honey bees is small to nonexistent. Family tradition has preserved extensive and varied folklore and superstition regarding honey bees and their husbandry. Also, honey is used in combination with herbs for traditional magic healing medicines sold in "Yerberias" (herb apothecary) in Texas along the Rio Grande Border and throughout most of Mexico. These medicines are used by "Curanderos" and "Shamans" (good witch doctors). A good example of medicinal herbs is "Ajo" (garlic) mixed with honey. It is used for dandruff and applied to scorpion bites.

Some of the rustic beekeepers believe that bees are endowed with senses much keener than man and also with human intelligence. Because bees were felt to be in sympathy with a family, it is a custom for them to tell the bees about marriages, births, and deaths that were taking place in the home.

Since none of the beekeepers surveyed owned veils or tools, a widely believed superstition, and one that will be rather dangerous with the incoming Africanized bees, is that bees will not sting a person if he holds his breath or clenches his fist (the fact that a bee may not sting at times under such a circumstance is probably because a person is apt to be more quiet and less disturbing to the bee). Most other superstitions compiled along that line can be easily explained the same way (de Lys, *Philosophical Library*, 45-50).

The following beliefs are stranger and not so easily explained:

- A girl that is a virgin can go through a swarm of bees without being stung.

- Bees flying into a house means a visitor is coming.
- When bees swarm on a dead tree there will be a death in the family.

Since capturing swarms represents the principal means of establishing new colonies, myths also surround that practice:

- To make a flying swarm settle down, make loud noise by beating pans or ringing cow bells.
- Urinating in a rustic bee hive (box or hollowed out tree trunk) will attract bees.

Most of the superstitions and myths regarding beekeeping in Tamaulipas are not from literature or native to Mexico. They are of European origin, part of the legacy that Spaniards brought to the new world when they also introduced the European honey bee *(Apis mellifera)* in the 16th century.

Native Mexican indians had extensive folklore associated with stingless bees (*Melipona beecheii* and *M. favosa*), the Toltecs and Aztecs, mainly in the central plateau of Mexico (Byer, *University of Texas Press*, 280-283), and the Mayas in the Yucatan peninsula and Central America (Sepulveda, *Editorial Everest*, 26-28).

10. Dietz, A.,[m] J. F. Leitner,[n] C. Vergara,[m] and M. Mejia[m] – EFFECT OF PROLONGED CONFINEMENT IN A REFRIGERATION CHAMBER ON THE SURVIVAL OF AFRICANIZED AND EUROPEAN HONEY BEE COLONIES – A comparative study was conducted from June 9 to Sept. 9, 1985 in Nogoyá, Entre Rios, Argentina to determine the survival of 6 Africanized and 6 European honey bee colonies under constant low-temperature conditions. The method employed was similar to the one used in our 1984 survival study (Dietz *et al.* pp. 237-42, In: Needham *et al.* eds, 1988, AHB and Bee Mites). The only modification was the replacement of the glass inner cover with a masonite board. This modification prevented condensation inside of the colonies. The board was replaced temporarily with a piece of glass during the regular inspection periods.

The results showed that there was no significant difference between the rates of survival of confined Africanized and European honey bee colonies. A total of four colonies, two Africanized and two European, died during the 91 day study period. One colony each of the Africanized and the European honey bees was found dead on day 56. The other two, one Africanized and one European colony, died on days 70 and 77, respectively. The remaining four Africanized and four European colonies survived the entire 91-day period.

Although the colonies of Africanized honey bees died sooner than colonies of European bees in our 1984 study (cited above), this was not the case in the present study. Not only was there an identical number of Africanized and European colonies surviving, but additionally both groups of honey bees were confined about 2 weeks longer than those colonies tested in our 1984 refrigeration chamber study. A possible explanation for the increase in the colony survival rate has been a concerted effort to reduce colony disturbances as much as possible during our routine inspection periods.

Based on the present results, and our previous studies on overwintering in Cordoba (Krell *et al. Apidologie* 16:109-118, 1985), San Juan (Dietz *et al. Proc. AHB Symp., Atlanta* 87-91, 1986), and the discovery of Africanized honey bees near the 39°S in the province of Rio Negro, Argentina (Dietz *et al. Apidologie* 16:99-108, 1985), it is clear that the ability to survive low temperatures for extended periods of time is not the main factor in limiting the distribution of Africanized honey bees in Argentina, or in most areas of the United States where the Italian honey bee, *Apis mellifera ligustica* is kept throughout the year. Food sources and nesting sites, which ultimately are influenced by weather conditions, *i.e.*, temperature and rain, appear much more limiting than long periods of confinement and low temperature conditions.

The evidence from this study, combined with other cold survival studies (Krell *et al. Apidologie* 16:109-118, 1985; Dietz *et al. Proc. AHB Symp., Atlanta* 87-91, 1986; Dietz *et al.* pp. 237-42, In: Needham *et al.* eds, *AHB and Bee Mites*, 1988; Spivak, *Am. Bee J.* 126:834, 1986; Villa, *Am. Bee J.* 128:835, 1986) demonstrates that the distribution and the survival of Africanized honey bees in Argentina, and probably most areas of the U.S., is not limited mainly by temperature conditions. These findings also do not support the contention of Taylor (*Bull. Ent. Soc.*, 31:14-24) that 60°F is the overwintering limit temperature for Africanized honey colonies in the U.S. Our conclusions are consistent with data that reveal the past existence in Europe of a group of bees now confined to the tropics and subtropics (Culliney, *Bee Wld.* 64:29-38).

A recent comparative overwintering experiment in Germany again demonstrated that there were essentially no differences in survival between Africanized and Italian honey bees (Rinderer, personal communication). Since numerous German beekeepers and beekeeping organizations were opposed to the presence of Africanized honey bees in Germany, the experiment was unfortunately terminated in February, 1989.

11. Harbo, J. R.[g] – EFFECTS OF PLASTIC COMBS ON HONEY BEE POPULATIONS[gg] – Plastic combs produced in Germany (ANP combs) may affect *Varroa* mites. The explanation was that workers produced in plastic combs had a shorter development time and consequently provided less time for *Varroa* mites to reproduce in capped brood (Posern, *Am. Bee J.* 128:698-702). Although I had no *Varroa* in Louisiana to test this hypothesis, I did measure how these plastic combs affected the development time of workers as well as other characteristics (see table).

The development time of brood was measured with known aged larvae compared side by side in plastic or wax cells in the same colony. No significant differences were found (see table).

Population growth and honey production were evaluated in 7 colonies with plastic combs and in 10 colonies with wax combs. Colonies were established on April 21 with 5141 ± 139 (mean ± SD) worker bees, sister queens, and 5 Zander frames (8¾ X 16½ inches) in each colony. A queen excluder and a super of 5 wax combs were added on May 24. Colonies were evaluated June 14. Methods for establishing and evaluating colonies were described by Harbo, *J. Apic. Res.* 25:22-29. These colonies provided all the data in the table except for worker development time.

Table – Characteristics of bees reared in wax or plastic cells.

	Wax combs	Plastic combs	Probability that means are equal
Worker development time:[1]			
Uncapped larva	4d & 19h	4d & 23h	ns[2]
Capped cell	11d & 19h	11d & 18h	ns
Total dev.[3]	19d & 13h	19d & 16h	ns
Wt of emerging adult worker	107 ± 8mg (40)[4]	131 ± 11mg (36)	<0.01
No. of ovarioles per worker	9.4 ± 4.4 (48)	8.9 ± 3.0 (37)	ns
Estimated adult longevity	26.5 ± 3.7 d	30.6 ± 2.3 d	<0.05
Colony evaluations:			
Adult population	13554 ± 2149	10206 ± 1880	<0.01
Cells of brood	13927 ± 1970	10007 ± 1683	<0.01
Grams of honey	4004 ± 1413	3261 ± 496	ns

1. Mean times for 3 different stocks (38 bees)
2. ns = not significantly different at the 0.05 level
3. Assuming 71 hours for egg development
4. These and following data are in mean ± SD (number measured)

There was no indication that plastic combs shortened worker development time in Louisiana. In colonies starting with 5000 bees, plastic combs retarded the first 8 weeks of population growth. However, workers produced in plastic cells were larger and may have lived longer as adults. I emphasize that I do not know if plastic combs can reduce populations of *Varroa* mites, but if they do, it is probably not because of a shorter development time of worker bees.

12. Harris, J. W.[o] and J. R. Harbo[g] — OVARY DEVELOPMENT OF WORKER BEES WHEN CAGED WITH WORKERS FROM DIFFERENT STOCKS — Three stocks that differed in time required for workers to lay eggs were evaluated. Stocks A and B became laying workers rapidly (*ca.* 9 days), and stock C was slow (*ca.* 25 days). To test effects that mixing different stocks might have on the ovary development of worker bees, 5 "select" workers from stock A were marked and placed into each of 40 incubator cages. Each cage was given an additional 25 workers (attendants) from stock A (10 cages), from stock B (15 cages) or from stock C (15 cages). All bees were less than 24 hours old at the start of the experiment. Each cage received a section of drone comb and honey, water and fresh pollen *ad libitum*. All cages were maintained through 10 days in an incubator (34°C; 50% RH). On the 10th day all select workers were removed from their cages, and the attendants were maintained for another 24 hours to check for egg production from them. Ovary development in the select workers was evaluated using a ranking system described by Velthuis (*Ent. Exp. & Appl.* 13:377-394; Class I — resting or inactive; Class II — early stages of development when eggs appear round to bean shaped; and Class III — fully mature ovaries having sausage-shaped eggs). Means reported for number of ovarioles per ovary were weighted by the number of select workers examined for each cage.

Select workers in cages containing stock C attendants required two extra days to begin laying when compared to the control group (stock A attendants). Since none of the cages containing stock C attendants produced eggs after removal of the select workers, egg production in that group was probably from select workers only. The percentage of select workers with developed ovaries was highest when attended by their supersisters and lowest when attended by stock B workers.

Table — Ovary development of "select" workers and overall egg production in cages. Groups of 5 select workers from stock A were caged with 25 attendants from their own or from other stocks.

	Attendant Stock (mean ± SD)[1]			Probability of no differences
	Stock C (n = 15)	Stock B (n = 15)	Stock A (n = 10)	
Oviposition onset in days	10.3 ± 0.5, b	8.0 ± 0.4, a	8.2 ± 0.4, a	<0.01
No. of eggs after 10 days	6 ± 6, b	28 ± 17, a	32 ± 24, a	<0.01
No. of eggs after removal of select workers	0 ± 0, b	11 ± 12, a	12 ± 8, a	<0.01
Ovaries/ovary for select workers	4.0 ± 1.2	3.9 ± 0.8	3.7 ± 0.9	n.s.
Percentage of select workers with developed ovaries[2]	47 ± 22, a,b	34 ± 19, b	56 ± 15, a	<0.03

1. Means in the same row having the same letter do not differ using the LSD multiple comparison.
2. Class II or Class III ovaries (see text).

13. Ibay, L. A.[o] and D. M. Burgett — BIOLOGY OF THE TWO EXTERNAL ACARAPIS SPECIES OF HONEY BEES: ACARAPIS DORSALIS MORGENTHALER AND ACARAPIS EXTERNUS MORGENTHALER — The tracheal mite, *Acarapis woodi* Rennie, is the only *Acarapis* species for which detailed biological studies have been carried out. Although the two external *Acarapis*, *A. dorsalis* and *A. externus*, are known to be hemophagic parasites (Orosi-Pal, *Bee World* 15: 93-94), they have been largely ignored since they are considered to be harmless to honey bees. No studies have been done to determine the impact of these *Acarapis* species on the colony health. For this reason, biological studies were undertaken in order to better understand the interaction of these two external *Acarapis* species and their honey bee hosts.

Both external *Acarapis* species were observed to have *ca.* 8-9 days total developmental period. *A. dorsalis* required four days for embryogenesis and 4-5 days more before the emergence of new adults. For *A. externus*, the egg incubation period took only three days, but the immature stages lasted for 5-6 days. In both species, males emerged earlier than females.

Variations in mite load and percent infestation of both *Acarapis* mites were monitored on marked bees as the bees became older. Decreases in mite load and infestation rate of *A. dorsalis* were observed as the bees aged. However, *A. externus* seemed to maintain its population on older bees.

The seasonal population fluctuations of both species were also monitored. *A. dorsalis* had the highest infestation levels recorded in spring months (March to June) when suitable hosts were emerging, and during mid-late summer (August and September) when brood rearing started to decline. For *A. externus*, infestation was highest in the fall (October and November), which was coincidental with the decreasing brood rearing activities inside the colonies. During this period there was a higher proportion of older bees, which may indicate that the age of bee hosts has little effect on *A. externus* population.

The lowest infestation rates of *A. dorsalis* were recorded in January when no suitable hosts for the mites were available and in July, which coincided with the peak bee emergence inside the colonies. This drop in July could indicate the dilution of mite populations due to more new, uninfested bees emerging during this month. Percent infestation by *A. externus* was also lowest in July.

Both species were observed to reproduce year round as shown by the constant presence of immatures throughout the sampling period. However, fecundity decreased during the winter months (December and January). The average female: male ratios were established at 1.9:1 for *A. dorsalis* and 2.07:1 for *A. externus*. (Presented at the mite symposium)

14. Kitto, G. B.,[h] E. Broussard,[h] J. Lemburg,[h] L. Davidson,[h] F. Davidson,[h] W. Rubink[e] and O. Taylor[q] — MALATE DEHYDROGENASE (MDH) PROFILES OF MEXICAN TRAPLINE HONEY BEES PRIOR TO AFRICANIZATION — The establishment of honey bee traplines in Mexico and South Texas and the collection of samples from these areas prior to Africanization, by the USDA/ARS personnel at Weslaco, Texas, provides an exceptional opportunity to examine in detail what happens to local European bee populations as the African bees move through these locations. In addition to providing essential and specific information about the temporal changes that occur as ingression by the Africanized bees proceeds, studies of trapline samples can also prove useful, in a more general sense, by providing data for the development of models for the dynamic interaction of two competing insect populations.

Previous studies have established that malate dehydrogenase (MDH) is one of the few polymorphic enzymes found in honey bees, with three allelic forms occurring in most European strains. Samples of *A.m. scutellata* from Africa are essentially monomorphic for one of these allelic forms (Nunamaker *et al.*, *J. Kans. Entomol. Soc.* 57(4):622-631). We have determined MDH allele frequencies for both

Mexican and South Texas trapline samples by starch gel electrophoresis at pH 8. The information obtained, to date, is presented in the Table. While the Mexican trapline samples show both general constancy from site to site and a relatively low frequency of the MDH-5 allele, this is in marked contrast to the South Texas trapline samples where a significantly higher frequency of the MDH-5 allele was observed.

Such site specific variation in MDH allele frequencies have also been observed in previous studies (Nunamaker *op cit.* and Gartside, *Experientia* 36:649-650) and these findings highlight the need for long-term evaluation at specific trapline sites prior to and throughout the time of the Africanized bee ingression.

Determination of MDH allele frequencies provides just one measure with which to evaluate the changes which will occur as the Africanized bees pass through the trapline areas. We anticipate that, through collaborative interactions, this picture will be enhanced by additional information such as that for morphological variation, mitochondrial DNA analyses and cell size assessment.

These studies were supported in part by a grant from the Texas Advanced Technology Program.

Table – MDH Allozyme Frequencies for Mexican and South Texas Honey Bee Trapline Samples.

LOCATION	# BEES	MDH ALLELE FREQUENCIES 1	3	5
Mexico				
Bait hives	257	34%	54%	12%
Rustic Hives or Feral Colonies	134	30%	58%	12%
Modern Managed Colonies	10	25%	65%	10%
South Texas Trapline				
Bait Hive Transect	102	40%	29%	33%

15. Labougle, J. M.,[r] M. Mancera,[r] and O. R. Taylor[q] – A MORPHOMETRIC AND ELECTROPHORETIC STUDY OF THE AFRICAN HONEY BEE IN SOUTHERN MEXICO – African honey bees (AHB) entered México in September 1986. The feral African bee population spread rapidly along both coasts, advancing approximately 240 miles per year (if measured from Tapachula, Chiapas to González, Tamaulipas). In this study we attempted to determine whether the feral population entering Mexico differed from other feral neotropical African bee populations and from European bees used by beekeepers. Because of numerous speculations that African bees would be modified through hybridization with large European bee populations in Mexico, we utilized morphometrics and allozymes to determine whether such modifications have occurred. Samples were obtained from feral swarms collected in Guatemala (n = 31) during 1986, from managed hives along the coastal area of Chiapas state in Mexico (n = 471) during 1986 and from 80 feral swarms as AHB moved into the region in 1987. In addition, 18 feral European swarms were collected in Oaxaca in 1986. These were compared with 44 feral swarms collected in this region in 1987 and 165 collected in 1988. Another 68 feral swarms were obtained from Las Choapas in Veracruz.

The electrophoretic data are based on collections made during 1987 and 1988 at Tapachula, Chiapas, and in January 1989 at Tapanatepec, Oaxaca, and Las Choapas, Veracruz. Two allozymes, malate dehydrogenase (MDH) and hexokinase (HK), which differ markedly in frequencies between African and European bees, were used to characterize the samples from feral and managed colonies.

Our data indicate that migratory swarms are African bees which do not differ substantially in phenotypic or genotypic characteristics from African bee populations in Central and South America. Non-parametric tests and discriminant analyses of morphometric data clearly distinguish European and African honey bees. The frequency data for MDH and HK alleles support the morphometric data. The morphometrics and allelic frequencies of the feral population during the first year of migration suggest that modifications due to hybridization with European bees were minimal. Frequencies for the MDH fast allele were 0.81 at Tapanatepec and 0.86 at Las Choapas. These values are higher and more "African-like" than obtained at Tapachula, Chiapas indicating that the feral African population is not becoming "Europeanized " as it advances further into Mexico.

Both the morphometric and allozyme data indicate that the migrating feral bees found along the Gulf Coast are more similar to the AHB type than those found on the Pacific coastal region, however, the differences are small. At this time there is no clear explanation for these differences. The morphometric data are particularly difficult to interpret because of possible environmental interactions and, as has been reported by Boreham and Roubik (*Bull. Ent. Soc. Am.* 1986) in Panama, there is a trend toward smaller size the longer the feral African bees are resident in a given area.

16. Labougle, J. M.,[r] E. Yarce,[r] and O. R. Taylor[q] – SWARM CHARACTERISTICS AND BAIT HIVE SELECTION BY AFRICAN HONEY BEES IN SOUTHERN MEXICO – Migratory swarms of African honey bees (AHB) entered Mexico in September of 1986. They reached the southern portion of the Isthmus of Tehuantepec in May and the northern part in September of 1987. Our study on the swarming biology of AHB was conducted at Tapanatepec, Oaxaca, and Las Choapas, Veracruz. In July of 1988 we established a bait hive line 20 kms long at each area. Our objectives were to test two types of bait hives: 1) a pressboard bait hive, and 2) a cardboard bait hive. We also tested the efficiency of a synthetic Nasanov pheromone lure. The null hypotheses tested in this study were that characteristics of swarms did not differ 1) between areas, 2) at different seasons within these areas, 3) throughout the transect and 4) did not differ among bait hive types.

The swarming biology and the characteristics of swarms were very different between areas. In a six month period we captured 178 swarms at Tapanatepec and 70 at Las Choapas. Pressboard bait hives were preferred over cardboard ones at both study sites. Most swarms were captured in bait hives containing Nasanov lures. The average swarm weight for Tapanatepec was 616.2 gr., for Las Choapas 2087.35 gr. Another important difference between the two areas was the number and distribution of drones; there was a significant number of swarms (n = 66) with drones at Tapanatepec and almost none in Las Choapas (n = 4). At Tapanatepec there was a distinct drone season. Few drones were found in swarms from July-Sept. However, a large proportion of the swarms collected from October to January contained drones. At Las Choapas we found that most swarms were captured from bait stations 46 to 70 (from a total of 80). This relatively small portion of the transect seemed to represent a migratory pathway. Swarms in and out of the pathway were statistically different.

In each case the null hypotheses were rejected. The characteristics of swarms differed between areas, seasons, locations on the transect and other factors such as type of bait hive and the presence or absence of the Nasanov lure. The pressboard bait hive is strongly recommended over the cardboard bait hive. Not only does it capture more swarms but larger ones. These larger swarms are more likely to survive and reproduce than the smaller swarms attracted to cardboard bait hives. The pressboard bait hive has the additional advantages of minimal preparation time and great durability. Depending on the environment and the number of colonizing swarms, the half-life of pressboard

bait hives is usually more than two years. Cardboard bait hives seldom last more than a few months.

17. Loper, G. M.[s] and R. K. Smith[t] – CUTICULAR OLEFIN ASSAY OF AFRICAN HONEY BEES VISITING COTTON FLOWERS – The African honey bee *(Apis mellifera scutellata)* has variously been described in the popular literature (especially newspaper articles) as being either less-efficient than managed honey bees or just as good, if not better. Most of the confusion comes from a lack of definitive research studies, but a lot also comes from the bias of the particular reporter.

In one study, I (GML) used individual colonies inside screen cages (3m x 6m x 2m) and observed their behavior including visitation to flowers of cotton. The study was conducted near Tapachula, Chiapas, Mexico. I had 3 cages of AHB and 3 cages of EHB in each of 2 years, 1987 and 1988. The AHB colonies were obtained from the La Norteña Apiary maintained by the Mexican Agricultural Agency (SARH) under the direction of Ing. Meliton Fierro ('87) or Lic. Francisco Choy ('88). The colonies originated from AHB swarms caught by SARH personnel in the local area. The EHB colonies were headed by queens purchased in California as "Ultra-lite" yellow Italians. All colonies were fed sugar water and water, and they all had pollen stores at the beginning of the experiment. The colonies were placed in the cages when at least 20% of the cotton plants had flowers.

In general, the AHB colonies attempted to abscond; most of the foragers went to the upper corners of the cages and formed large groups hanging on the screen. Most of them returned to the hive in late afternoon. Additionally, they exhibited considerable aggressive behavior for at least the first several days. Also, the AHB generally ignored both the cotton flowers and the extra floral nectaries. The EHB colonies also exhibited some of the "escape" behavior, hanging in much smaller numbers on the screen, but very quickly many foragers began visiting the flowers and nectaries, collecting both pollen and nectar.

In all the cages, we caught individual foragers at the cotton flowers and from the groups of bees hanging on the screen. These samples were air-dried (at room temperature) and olefin assay performed (by RKS) for determination of AHB or EHB. This technique is able to discriminate subgroups within both population types (Smith and Lavine, *Proc. Am. Bee Res. Conf.* 1987).

The olefin profiles of all EHB samples from this study were identical, but the AHB foragers that did visit the cotton flowers – in both years – had unique hydrocarbon characteristics placing them in a separate sub-group from the rest of the workers in the same cage (total of 4 reps, 4 floral visitors/rep).

We are not sure how to interpret these results. The AHB workers that did visit the flowers may represent a separate patri-line within the colony – one more genetically inclined to forage under these confined conditions than were their half-sisters which were distinguished by a detectably unique olefin profile.

18. Lozano de Haces, L.,[u] W. L. Rubink,[e] W. T. Wilson,[e] and M. Guillen-M.[u] – NOSEMA AND HONEY BEE TRACHEAL MITE INTERACTION IN SWARMS FROM NORTHEASTERN MEXICO – Recent studies have suggested that the role of *Acarapis woodi* in causing decreases in colony production may be related to mite-associated diseases, and not the tracheal mite itself (Gary and Page, *J. Econ Entomol.* 82:734-9). *Nosema apis* is a disease which has been shown to have a significantly greater effect on honey bee longevity when tracheal mites are present (Bailey, L. *Parasitology* 48:493-506). We evaluated the relationship between levels of *Acarapis woodi* infestations and Nosema in 181 honey bee swarms captured over a one year period in central Tamaulipas state, Mexico. This study is part of an ongoing characterization (Rubink *et al.*, *Amer. Bee J.* 128:807-8, Rubink *et al.*, *J. Kans. Entomol. Soc.*, in press) of honey bees of the region prior to their Africanization. The captured swarms probably had primarily feral and rustic-colony origins.

Standard methods were used for the *Nosema* detection and spore counts (Cantwell, *Amer. Bee J.* 110:222-3). Tracheal mite infestation levels, measured in terms of the number of adult mites per worker bee, were evaluated by simple dissection. Ten bees per swarm were examined.

Nosema levels were generally light, ranging from 2.5 x 10^5 to 4.8 x 10^6 spores per bee. Of 181 swarms (1810 bees) examined, a total of 21 bees from 13 swarms were found to be infected. Swarms were infested at rates from 10% to 30%. *A woodi* was present in 300 bees from 76 of the swarms; infestation rates ranged from 10 to 100% per swarm. Comparisons of mite-infested/non-infested and *Nosema*-infected/non-infected bees and swarms in 2x2 contingency tables showed no statistically significant association between the presence of the two diseases. The presence of tracheal mites is not associated with increased susceptibility to *Nosema* in swarms of probable feral origin.

Nosema levels were higher in early (spring) season, and lower in late season samples. Tracheal mite levels followed a similar trend. Interestingly, *Nosema* levels showed remarkable geographic variation. Swarms captured in the Coastal plain region on the Gulf of Mexico coast were more frequently infected than those from farther inland. The drier inland climatic conditions may play a role here, or the observed results may be a result of the introductions of more susceptible bees in the last two decades of increased modern apicultural practices in the coastal region.

19. Mohamed, M. A.,[g] H. A. Sylvester,[g] B. P. Oldroyd,[g] and J. A. Stelzer[g] – AN EFFICIENT METHOD FOR THE ISOLATION OF MITOCHONDRIAL AND NUCLEAR DNA FROM SINGLE BEES[gg] – Our protocol capitalizes on the direct invagination of the tracheoles into insect cells. Flash freezing the entire organism followed by lyophilization dehydrates the bee and allows controlled access to organelles, mitigating premature cellular disruption prior to homogenization. Additionally, a substantial proportion of hemolymph contents such as lipids and proteins are removed prior to cellular disruption.

The method is as follows: single bees are flash frozen in liquid nitrogen, transferred to 1.5 ml Eppendorf tubes, and lyophilized overnight in a vacuum desiccator over a bed of Drierite. Each dried sample is cut longitudinally. The tissue is washed by gentle vortexing in 0.5 ml of ice-cold buffer 1 (composed of 50 mM Tris, pH 7.4, 250 mM sucrose, 1 mM EDTA, 2 mM $CaCl_2$ and 5 mM $MgCl_2$). The buffer is discarded and the tissue is resuspended in buffer 1, homogenized with a Tissuemizer, and then centrifuged at 1000g at 4°C for 10 minutes. The supernatant is carefully removed and discarded. The pellet is resuspended in 0.5 ml buffer 1 supplemented with Triton X – 100 (0.01% or 0.025% for the larvae or adults, respectively) and then dounced with a loose fitting pestle. Prior to this douncing, for the efficient recovery of mt DNA, the adult homogenate should be filtered to remove chitinous debris (a glasswool pad in a perforated lower half of an Eppendorf tube is used to filter the homogenate into an intact tube by a 1000g/2 min. spin in a centrifuge). The sample is then centrifuged at 1000 g at 4°C for 10 minutes to pellet nuclei and intact cells. The pellet can be processed once again to enrich the cytosolic organelle fraction. The supernatant is pooled and centrifuged at 15,000 g at 4°C for 30 minutes to recover the mitochondria. The nuclear and mitochondrial pellets are lysed in 0.3 ml of a second buffer (10 mM Tris, pH 8.0, 0.5 mM sodium acetate, 10 mM EDTA, 5 mM $MgCl_2$, 10 µl Proteinase K (10 mg/ml), 5 µl RNAase A (DNAase free, 10 mg/ml), and 0.5% N-lauryl sarcosine), at 42°C for 30 minutes.

Restriction enzyme digests of single bee mt and genomic DNA (fig. 1a) show a clean separation of these two classes of nucleic acids. The quality and quantity of extractable DNA is evident from the homogenously stained lanes of genomic adult DNA down to the level of 0.25 equivalents of a single bee extract (fig. 1b).

The initial treatment of the entire bee to facilitate tissue dehydration allows a controlled access to cellular components free of grossly contaminating entraneous derbis, hemolymph lipids and proteins. Cellular disruption in the presence of a mild detergent is thus rendered efficient for the fractionation of nuclei and cytosolic components. This method, coupled with pre-existing DNA extraction protocols (Gross-Bellard, 1972; Eur. J. Biochem. 36:32) render the bee mitochondrial (mt) and nuclear genomes accessible in a manner that is unambiguous, efficient and reproducible for projects that entail rapid screening of large samples.

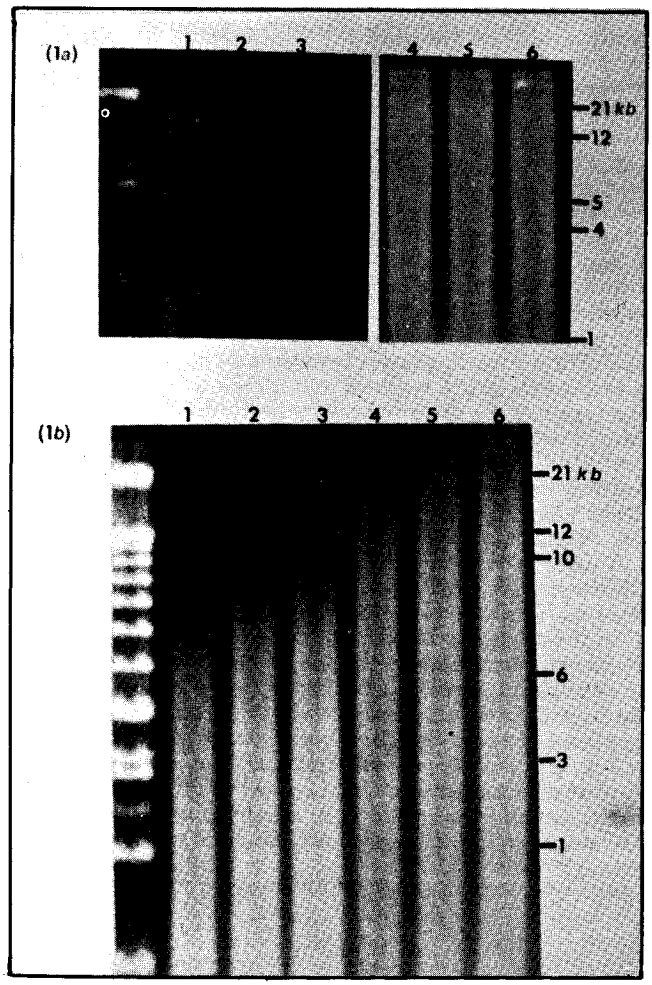


Fig. 1. (a) Mitochondrial (lanes 1, 2, 3) and genomic DNA (lanes 4, 5, 6) from single bee larvae. The DNA extracted from each bee was digested with Eco R1 for 45 minutes. (b) Genomic digest of European (1-3) and Africanized (4-6) adult honey bees at varying concentrations — 0.25, 0.5 and 1.0 (lanes 1-3 and 4-6) equivalent of DNA extracted from a single bee. DNA is extracted with SS Phenol/$CHCl_3$, and precipitated with 1/10 volume sodium acetate and 2-3X volumes of cold EtOH. The DNA is resuspended in TE buffer, digested with a restriction enzyme (Eco R1, 37° C/1.5 hrs), and electrophoresed in a 0.8% Agarose gel in TAE buffer.

20. Mora-R., P.,[v] F. Lavín-O.,[w] and W. L. Rubink[e] — BEEKEEPING IN CENTRAL AND SOUTHERN TAMAULIPAS, MEXICO AND PREPARATIONS FOR THE ARRIVAL OF THE AFRICANIZED HONEY BEE — Tamaulipas is the Mexican state bordering Texas to the south through which the Africanized honey bee is expected to first enter the United States. It is unique in the northward dispersal of the Africanized bee because it may be the first area where substantial numbers of feral European-origin bees exist (Rubink *et al., J. Kans. Entomol. Soc.* in press). As such, and because the central and northern parts of the state are ecologically similar to southern Texas, the response of the Africanized bee in Tamaulipas will provide important insights into the preparations necessary in Texas. The first Africanized bee swarms in Tamaulipas were reported only weeks ago, but preparations for its arrival have been anticipated for more than a year.

The southern/central region of Tamaulipas has some of the most intensive apiculture in the State. Most of the state's 45,000 modern and rustic bee colonies are located there. Annual honey production is estimated at approximately 1500 tons of honey and 30 tons of wax, ranking 14th among the 31 Mexican states. The southern and central zones have better floral sources for longer periods than are available in the drier north. The most productive regions are Mante, Xicotencatl, Ocampo, Gomez Farías Llera, Victoria, Guemez, Padilla, Hidalgo. Modern apiculture now and in the past has been complemented with large numbers of rustic colonies, which by census, number at least 5000 in the southern half of the state; as many as 10,000 more uncensused rustic colonies have been estimated to exist in this and other areas of the state (Resendez *et al., Amer. Bee J.* 128:806-7). These rusticly-maintained colonies present a unique problem to local livelihoods with the advent of Africanization.

In accord with the Mexican Department of Agriculture (SARH) National Program for the Control of the African Honey Bee, objectives now being carried out in southern Mexico, the Tamaulipas African Bee Action Plan has been divided into 4 phases: preparation, containment, control, and management/improvement. In the first and presently active phase, public information service, through the Secretariat of Public Education (SEP), the University of Tamaulipas (UAT), the Tamaulipas State Department of Agriculture (SFA), and the Department of Welfare (DIF), have presented talks, courses, and prepared pamphlets, radio and TV interviews, and videocassettes to inform both the general public and apiculturists of the possible social repercussions which may present themselves.

Initial preparations for honey bee Africanization have been in the following areas: (1) relocating apiaries, (2) monitoring honey bee populations through swarm capture and sample identification (cooperative effort in conjunction with the Mexican Program, SARH/USDA/ARS, and the SARH/USDA/APHIS Cooperative Program), (3) training apiculturists (cooperative effort between Tamaulipas State Beekeepers Association and the State Department of Agriculture), (4) queen replacement, marking, and establishment of a queen rearing facility at Jaumave, and (5) the conversion of rustic apiaries to modern equipment and retraining rustic apiculturists (21% of 4807 censused rustic colonies had been modernized at the time of writing), and (6) providing first aid information and training for Africanized honey bee stinging incidents.

As further movements of Africanized honey bees are detected in the state, other phases of the action plan will be put into operation.

21. Oldroyd, B. P.,[g] H. Sylvester[g] and T. Rinderer[g] — ATTEMPT TO GENETICALLY TRANSFORM HONEY BEES WITH FOREIGN DNA USING SPERM AS THE VECTOR — The ability to transfer genes between species promises to be one of the most important tools for animal and plant genetic improvement. Currently, gene transfer between animal species is extremely costly, and in most species (including honey bees) it has not been achieved at all. Recently however, Lavitrano *et al.* (*Cell* 57:717-23) mixed mouse sperm with plasmid DNA, fertilized mouse eggs *in vitro*, and then transferred the embryos to the

oviducts of foster mothers. The bacterial gene was detected in about 30% of the resulting progeny. Furthermore, there is evidence that the method works in other species, and may be a universally effective technique.

We have attempted similar experiments with honey bees. The bacterial gene which codes for the enzyme β-galactosidase, was used to attempt transformation. A construct containing this reporter gene was kindly provided by Dr. Kousoulas, LSU.

The plasmid DNA was mixed in various proportions with honey bee semen and semen diluent. After incubation at 37° C. for 30 minutes, the semen was reconcentrated by centrifugation. Virgin queen bees were then instrumentally inseminated with 4 ml of the treated semen mixture.

To assess whether or not our honey bees were transformed, larval progeny were extracted for hemolymph and cystolic proteins, and genomic DNA. Homogenates from putatively transformed and control larvae were tested for β-galactosidase activity (30 min./37° C) using the substrate pNPG (paranitrophenolgalactoside) in a buffer consisting of 100 mM Tris, pH 7.4; 10 mM KCl; 1 mM DTT and 5 mM PMSF. Hydrolysis of this substrate by this enzyme produces a yellow color. Both putatively transformed larvae and controls gave the same positive result. Since honey bees possess a lactase (Peng *J.Apic.Res.* 19:105-11), with a pH optimum of 4-5, we inferred that the color development observed in both our controls and putatively transformed larvae was the result of honey bee lactase activity. Further experiments at pH 9.5 gave the same results. In addition, southern blots of putatively transformed larvae followed by hybridization with the β-galactosidase gene also gave negative results.

We conclude that this simple procedure did not successfully transform honey bees, as far as we can determine. Bacterial β-galactosidase is an inappropriate "reporter" gene for honey bees, as honey bees have an enzyme of similar function.

Thanks to Wilbanks Apiaries and Weaver Apiaries for providing virgin queens at very short notice.

22. Otis, G. W.,[j] and C. D. Scott-Dupree[j] – TRACHEAL MITE INFESTATIONS AND COLONY PERFORMANCE IN NEW YORK – Despite the presence of honey bee tracheal mites (HBTM) in North America for at least five years, we still have very little information on the effects of mites on honey bee colonies. Our studies to obtain such information began in 1985 and are ongoing.

Mite infestations have been monitored during four winters, each year utilizing different apiaries belonging to three different beekeepers. We used the thoracic disc method of examining for mites. In every year, mite prevalence values were significantly greater than reported from England (Bailey, *Bee World* 42:96-100). Mite prevalence values >20% were recorded in 30-44% of the colonies in New York, as compared to only 11% over a five year period in England.

Colonies with higher mite prevalence experienced greater mortality. Data are presented in the table. Colony mortality at given levels of mite infestation in New York were remarkably similar to those reported from England (Bailey, *Bee World* 42:96-100), with very high mortality of highly infested colonies. However, because heavily infested colonies were more common in New York, the total colony mortality was substantially greater. It is common for beekeepers who typically experienced 3-8% winter mortality in the past to have mortality increase to 30-50% after their bees become infested with mites. Such correlations do not prove that mites cause colony mortality, but are highly suggestive of that given the magnitude of change in mortality.

There are significant correlations between winter mite infestations and spring brood areas in every year. Again these correlations fail to prove that mite infestation causes reduced brood areas, but clearly indicate that there is a consistent relationship between the two variables. Correlations of spring brood areas with either the simple measure of mite prevalence and with the more involved parasite load score (based on estimated numbers of mites) yielded r-values of approximately the same magnitude.

In conclusion, our data establish clear relationships of winter HBTM infestations with increased mortality and reduced spring brood areas. (Presented at the mite symposium)

Table – Colony Mortality During Winter

	Mite Prevalence in October		
	0-20%	21-60%	61-100%
1987-88	9.1% (n = 46)	23.1% (n = 12)	75.0% (n = 8)
1988-89	8.3% (n = 60)	48.5% (n = 33)	85.7% (n = 14)

23. Pettis, J. S.,[e] and W. T. Wilson[e] – REPRODUCTION OF ACARAPIS WOODI AS RELATED TO SEASONAL HOST LONGEVITY – The honey bee tracheal mite *Acarapis woodi* (Rennie) lives and reproduces within the prothoracic tracheal tubes of honey bees, *Apis mellifera* L. Longevity of the host bees varies depending on the season in which they are reared. This study examined the reproduction of *A. woodi* within bees from four seasons. Large cohorts of bees (ca. 2,000/hive) were marked upon emergence from incubated brood combs and then placed in a hive infested with tracheal mites. Four hives were utilized per season. Live dissections were performed on 30 bees per hive at four-day intervals over the life span of the host bees, and all life stages recorded (see figure). Mite incidence in marked bees ranged from 20 to 40% over the four seasons. Mean number of foundress females recorded on day 8 were 0.8, 1.5, 0.9, and 3.0 for the winter, spring, summer, and fall periods, respectively. In the spring sample, bees were dissected every two days to yield life stage interval estimates. All time intervals are in days from emergence of host bee. Foundress females began egg laying on day three and the number of individuals continued to increase in all seasons until approximately day 24. The egg stage was estimated to be four days. Newly emerged adult males and females first appeared on days 8 and 10, respectively. Peak numbers of mites occurred between days 24 and 30 and then began to decline reflecting the number of daughter mites beginning to disperse to new host bees. Limited egg laying by foundress's daughters could not be ruled out, however there was no evi-

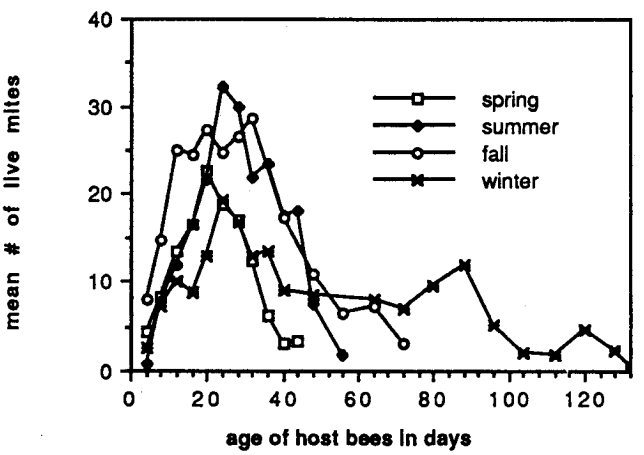


Figure — Mean number of live honey bee tracheal mites (all life stages) dissected from bees (30/colony) during four seasons of sampling. Four colonies were used per season.

dent second generation. One generation of mites was reared per host bee in all seasons with the majority of offspring apparently migrating before the host bee died. The fall and winter generation time was extended in concert with the longevity of the host. (Presented at the mite symposium)

24. Ramirez, B. W.,[x] and R. Marin-R.[x] – THE USE OF DISTILLED WATER, ROYAL JELLY AND COLA AS A SUBSTRATA FOR TRANSFERRING BEE LARVAE TO PRODUCE QUEENS – Three substances were used as substrata to transfer honey bee larvae (*Apis mellifera* L.) to produce queens. The experiment was designed to find a substance for priming queen cell cups which does not require refrigeration, does not decompose, and is easy to obtain.

The Doolittle method for queen production and artificial wax cups was used. Larvae came from the same mother and were less than 24 hours old. A little drop of each substrata was placed in each cell cup before the bee larvae were transferred. The hive used to raise the queens was a queenright colony. Four variables were evaluated: (1) time from transfer of larvae to emergence of adult queens, (2) weight of the recently emerged queen, (3) percentage of emergence, and (4) the length and width of the anterior and posterior wings. Once the queen cells were capped, they were introduced into an incubator at 35°C and 70% RH. The newly emerged queens were weighed as soon they eclosed from the cells. No food was available for them.

The results of the experiment are detailed in the table. There was no statistical difference between the values obtained; thus, the three substrata produced the same effects in development period and weight and size of the queens produced. There was, however, a high statistical difference between the three substrata in percentage of queens produced. A high positive correlation was found between the following variables: weight and development time, weight and size of the wings, time and size of the wings, and between the size of the anterior and the posterior wings.

It is concluded that the three substrata used do not have any apparent effect on the developmental period, weight and size of the queens produced.

Table – Effects of different substrata in the queen cup on the resulting queens.

	Royal Jelly	Distilled water	Cola Beverage	Average
Average weight	226.6 mg	213.8 mg	225.2 mg	221.9 mg
Larval period*	278.4 h	272.9 h	276.8 h	276.0 h
Percentage of emergence	56.8%	46.4%	26.2%	42.5%
Length of Wings (Anterior)	9.94mm	10.00mm	9.99mm	9.98mm
Width of Wings (Anterior)	3.36mm	3.42mm	3.46mm	3.41mm
Length of Wings (Posterior)	7.20mm	7.23mm	7.29mm	7.24mm
Width of Wings (Posterior)	2.24mm	2.26mm	2.30mm	2.27mm

* This is the number of hours from the time the cups with the larvae were put into the hive until emergence of the young queens.

25. Rivera, R.[e] and W. T. Wilson[e] – PRESENCE AND REMOVAL OF MENTHOL IN HONEY AND BEESWAX – Naturally occurring menthol is found in wild and cultivated mint plants. About 135 species of mint plants are found in the United States (L. Goltz, *Am. Bee Jour.*, Oct. 1987). Plants in the mint family are often grown as garden herbs or food seasoning such as, peppermint, spearmint, sage, thyme, and rosemary. Also, horsemint, wild bergamot, and bee balm are considered important honey plants. Since honey bees *(Apis mellifera)* gather nectar from mint plants, honey and beeswax have been found to have "background" or "naturally" occurring menthol.

A comprehensive survey was started in October 1987 to determine levels of naturally occurring menthol in honey and beeswax. The samples were collected before menthol was acceptable as a legal treatment for tracheal mite *(Acarapis woodi)* in honey bees. The beekeepers who provided the samples for testing had not used menthol as a therapeutic agent to treat the colonies. Honey and wax samples were collected from various parts of the United States, Mexico, Central and South America.

Menthol residue analyses were done by gas chromatography. One hundred honey samples were purchased from or sent in by beekeepers, apiary inspectors, researchers, and commercial firms. The floral sources for the honey were identified on the label. Eighty wax samples were received and analyzed.

The range of menthol in the 100 untreated honey samples was from 0 to 1.03 (0.11 avg.) parts per million (ppm) and in the 80 untreated wax samples was from 0 to 8.4 (1.07 avg.) ppm. In the 49 honey and 24 wax samples that contained menthol, the average residue was 0.25 and 3.62 ppm, respectively.

Removal of menthol from honey and beeswax was achieved by heating. Honey and wax were spiked with known concentrations of menthol. The quantities added were 0.1 ppm, 0.5 ppm, 1.0 ppm, 2.0 ppm, and 5.0 ppm. The spiked honey and wax were heated and held at 65°C in a water bath. Samples were analyzed daily for menthol residues. The menthol in honey was completely dissipated in two days, but there were trace amounts (<0.1 ppm) of menthol in the wax. However, menthol was not detectable in beeswax after three days of heating.

26. Royce, L. A.,[p] P. A. Rossignol,[p] and D. M. Burgett[p] – A SUGGESTED ROLE OF SWARMING IN TRACHEAL MITE PARASITISM – The parasitic tracheal mite, *Acarapis woodi* (Rennie), inhabits the thoracic tracheae of the European honeybee, *Apis mellifera* L. Understanding regulation of this host-parasite relationship presents particular problems because the mite requires two host populations for transmission, namely, a population of colonies, which displays Malthusian growth parameters, and, within a colony, a population of worker bees, which grows linearly. We propose a model in which swarming and colony distribution act as mechanisms to provide oscillatory stability to the parasite population. This model implicates modern techniques of hive management in the sudden historical appearance of the mite on the Isle of Wight. (Presented at the mite symposium)

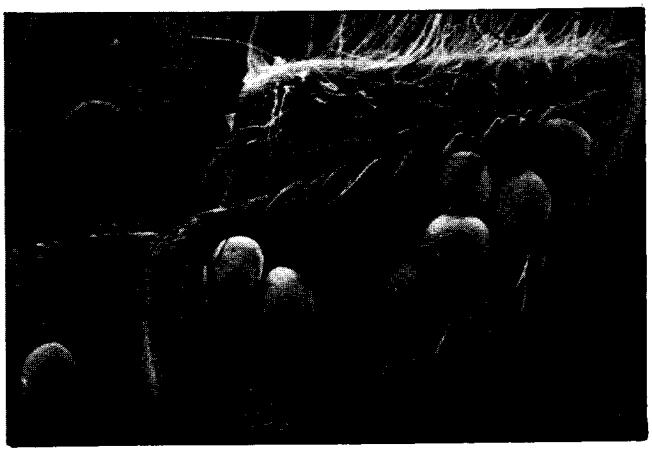
27. Rubink, W. L.,[e] Delfinado-Baker[k] W. T. Wilson,[e] T. Sanford,[y] M. L. Gonzales-G.[v] and A. M. Collins[e] – PHORETIC BEHAVIOR OF A UROPODID MITE FROM HONEY BEE SWARMS IN NORTHEASTERN MEXICO. In a survey of honey bee swarms of Tamaulipas, Mexico and Texas, USA, honey bees from two alcohol-preserved Mexican swarm samples had phoretic uropodid mites attached to the legs. This is the first association of this family with *Apis mellifera.*

The mites (see Figure) are ovoid and have a length and width of ca. 0.48 X 0.37 mm. They are sclerotized, pale brown, sub-adult deutonymphs of the family Uropodidae (Acari: Mesostigmata); the adult forms are unknown. The mites attach to their carriers, honey bees, by means of an anal pedicel. Other species of phoretic uropodid deutonymphs are known, but from termites or ants. They are not parasitic, but on ants some feed from the debris on the carrier's surface.

The phoretic association reported here is unusual in that the mites were found attached almost exclusively to the outside surfaces of the basitarsi and in the pollen baskets. In one swarm 100% of the bees in a 50 bee sample had at least one mite attached; another had only 5 bees with a few attached mites in a 32 bee subsample.

The infested swarms came from two wooded sites in Central Tamaulipas. They were collected in late October and in early December and probably represent secondary swarms. Numerous tree holes and other natural cavities exist

which could provide overlapping habitats for mites and bees. Apicultural activity is low in the region, so we surmise that the captured swarms emanated from natural cavities occupied by *Apis mellifera*. The mite-bee relationship reported here is most likely accidental; both species are migrants exploiting the same or overlapping natural habitats.



Hind leg of honey bee with attached uropodid mites.

28. Rubink, W. L.,[e] W. T. Wilson,[e] A. M. Collins,[e] and J. Vargas-C.[z] – ACARAPIS WOODI INFESTATION LEVELS IN HONEY BEE SWARMS IN SOUTHERN TEXAS AND NORTHEASTERN MEXICO – The tracheal mite dispersed rapidly through North America after its initial introduction. The means by which this might have taken place is still not entirely understood. Human-assisted migration undoubtedly played a significant role initially. In the case of the Africanized bee, swarms are considered a major factor in tracheal mite proliferation in Brazil (Flechtmann *et al.*, *Ann. Ent. Soc. Brasil* 130-31), although migratory Africanized swarms in Panama were found mite-free (Roubik and Reyes, *Amer. Bee J.* 125:665-7). Since swarms must play a major role in dispersal, especially in feral populations, and only a few studies have dealt with tracheal mite populations in swarms of European or African-origin bees, we initiated a study of tracheal mite levels in swarms captured in bait-hive transects (Rubink *et al.*, *J. Kans. Entomol. Soc.*, in press) in northeastern Mexico and southern Texas. The objective of this study was to elucidate variations in tracheal mite infestation levels in subtropical populations of honey bees from southern Texas and central Tamaulipas. Initial findings of this study are presented here, along with suggestions for consistent reporting of tracheal mite infestation levels.

Honey bee tracheal mite infestation data from widespread populations demonstrated a need to redefine the terminology associated with infestation rates. I suggest and use the following hierarchical terminology, which is determined by the sampling unit involved: First is the lowest practical sampling unit, the individual tracheal tube. Mite populations need be measured in terms of volume, or number of individuals per trachea to provide a Tracheal Infestation Level (TIL). Second in the hierarchy is the infestation level per bee, the Individual Infestation Level (IIL), measured either as the mite population per bee, or coded as bilateral, unilateral, or non-infested. Third is the Colony Infestation Level (CIL), measured as the proportion of the bees in a colony that are infested. At the fourth and highest level the fraction of colonies infested in a given population provides a Population Infestation Level (PIL). Using this system it is then possible to succinctly express data derived from various possible sampling situations.

Overall PILs in Mexican swarms differed greatly when estimated from worker (46% of swarms infested), drone (24%) or queen bees (5%). PIL analyses of Texas swarms gave 59% (workers), 10% (drones), and 0% (queens). Drone and worker infestation levels were not strongly correlated, and the presence of tracheal mites in drones almost always signaled their presence in workers. High worker infestation levels were not indicative of drone infestations. In all castes monthly PILs were generally highest in March to July and lowest in August to November. IILs, based on the fraction of the tracheal tube filled with mites, also showed a general decrease from March to late in the year, ranging from 78% in March workers and 68% in March drones to 60% in November workers and 45% in July drones. Initial analyses from Texas origin samples showed similar trends.

Analyses of CILs over wide geographic areas of the Mexican and Texas transects showed no readily apparent variations with regard to altitude or distance inland from the Gulf of Mexico, and appear similar for central Tamaulipas and southern Texas. (Presented at the mite symposium)

29. Smith, A. W.,[aa] P. L. Phelan,[bb] G. R. Needham,[aa] and R. E. Page, Jr.[cc] – CHEMICAL ECOLOGY OF THE HONEY BEE TRACHEAL MITE – It is well documented that adult bees greater than four days old are highly immune to infestation by migrating female tracheal mites, and that adult bees less than four days old are quite susceptible (Morgenthaler, *Bee. Wld.* 11:49-50; Bailey, *Parasitology* 48:493-506; Lee, *J. Ins. Pathol.* 5:11-15; Gary and Page, *Exp. Appl. Acarol.* 3:291-305). In this study, chemical analyses, behavioral bioassays, and field work were conducted to demonstrate that female tracheal mites use contact chemoreception for discerning between young and old bees.

Gas chromatography revealed that the surface cuticular hydrocarbons are qualitatively and quantitatively different in young and old bees.

A laboratory bioassay was developed to test the tracheal mite's ability to discriminate between young and old bee cuticle extracts. Females had a significant preference for young adult bee extracts in three out of four colony sources tested (50 female mites/test). However, they were not repelled by the old bee extracts and strongly preferred them over hexane controls. Other experiments indicated that the mites are responding to a complex blend of saturated and unsaturated hydrocarbons on the integument of the bees.

Cage studies demonstrated that the use of vegetable oil can completely obstruct the successful infestation of bees less than 24 hrs. old. Laboratory and field tests were also conducted to demonstrate that the use of vegetable oil can completely disrupt the successful migration of tracheal mites to young adult bees. We speculate that vegetable oil masked the true cuticular hydrocarbon composition of the workers, confounding the female tracheal mites' ability to locate a suitable young bee host. The potential use of vegetable oil for tracheal mite control will be discussed. (Presented at the mite symposium)

30. Smith, A. W.,[aa] D. L. Wrensch,[aa] and G. R. Needham[aa] – SOURCES OF HETEROGENIETY IN TRACHEAL MITE INFESTATIONS REVEALED BY POPULATION DYNAMIC STUDIES – Four mite-infested honey bee colonies were monitored from June, 1987 until July, 1989 in Delaware County, Ohio northeast of Columbus. A sample of 100 bees was collected biweekly from each colony, except in late fall and winter when monthly samples were taken to reduce colony stress. The thoracic tracheal trunks from each bee were excised (Smith *et al.*, *Am. Bee J.* 127:433-444) and examined for the presence of all developmental stages (eggs, larvae, male and female adults), and the number of infested bees was scored for each colony.

Infestation rates and bee load (number of mites/infested bee) varied significantly among colonies, demonstrating the potential heterogeneity of hives in their susceptibility to the

mite. However, seasonal trends in sex ratio and developmental stages in the four colonies were similar. Sex ratio was generally depressed (fewer females) and fewer immature stages were present in the winter.

Due to our sampling procedure which allowed us to visualize whether mites were dead or alive, we noticed numerous dead mites inside tracheae during the final winter (1989) of sampling. There was significant variation in the relative proportions of live and dead mites among the colonies and this kind of information may lend clues about the host-parasite interaction. In one colony, so many mites perished that the infestation level dropped from about 82% in the fall to less than 2% the following spring. Another colony died in February with over 80% of the bees infested harboring live mites. Possible reasons for these trends will be discussed. (Presented at the mite symposium)

31. Smith, D. R.,[dd] O. R. Taylor,[q] G. Lopers and W. Rubink[e] – INFERRING THE MATRILINEAL DESCENT OF FERAL AMERICAN BEES USING MITOCHONDRIAL DNA AND ALLOZYMES – Until recently, feral honey bee populations in the United States were believed to consist largely of recently escaped swarms from domestic *A.m. ligustica* and *A. m. carnica* populations. Recent studies (Sheppard, *Ann. Entomol. Soc. Amer.* 81:886-889) show that genetic evidence of early introductions of *A. m. mellifera* and other subspecies is detectable in feral American populations.

We have used mitochondrial DNA (mtDNA) genomes to infer the matrilineal origins of several feral American honey bee populations. Animal mtDNA is maternally inherited without recombination. Thus all the offspring of a queen carry her mtDNA, and hybrids carry only the queen's mtDNA, not a mixtue of the two parental types.

We used DNA restriction enzymes to survey and characterize mitochondrial genomes of Old World populations of *A. m. mellifera iberica, carnica, lamarkii, capensis* and *scutellata* (Smith, pp. 303-312 in Needham *et al., Africanized honey bees and bee mites*, Ellis Horwood Ltd. NY; Smith and Brown, *Experientia* 44:257-260; Smith et al., *Nature* 339:213-215; Smith and Brown, *Ann. Entomol. Soc. Amer.* in press). We found three large classes of mtDNA: *A. m. mellifera* and *iberica* from northern Spain; *ligustica, carnica* and *lamarkii;* and *scutellata, capensis* and *iberica* from southern Spain. The restriction patterns generated by the enzymes, XbaI, EcoRI, BglII, and NdeI, give distinct patterns for each of the three classes. (Other analyses distinguish subspecies within each class).

We prepared mtDNA from 12 feral hives from Emporia, Kansas (12 km from the nearest commercial apiary), 12 feral hives from Tucson, Arizona (3 km from the nearest commercial apiary), and 5 feral hives from Texas/northern Mexico, and surveyed the mitochondrial genomes by digestion with the enzymes XbaI and EcoRI.

All 12 Kansas hives had mtDNA typical of *carnica* or *ligustica*. Two Arizona hives had mtDNA typical of *ligustica* or *carnica*, and the remaining 10 had mtDNA typical of *mellifera* or northern *iberica*. Of the Texas hives, four had mtDNA typical of *ligustica* or *carnica*, and one had mtDNA typical of *mellifera* or northern *iberica*. Allozyme data from the Kansas population showed that among workers the frequency of the $Mdh1^{80}$ allele (which occurs in very high frequencies in *A. m. mellifera* and very low frequencies in *ligustica* and *carnica)* is 0.36.

The isolated feral populations in Arizona are matrilineal descendants of *A. m. mellifera* or *A. m. iberica*, and may be remnants of very old introductions. The evidence of the mtDNA genomes shows that there has been little gene flow into this population in the form of swarms from managed *ligustica* and *carnica* populations. In the Kansas population, there is evidence of *A. m. mellifera* ancestry, as evidenced by the allozyme data, but these bees are not direct matrilineal descendants of *A. m. mellifera*.

This preliminary study indicates that the interactions between feral and domestic populations in the United States are more complicated than might have been expected. Management of incoming populations of African bees should consider the interactions between managed European bees, feral African bees, and feral European bees.

32. Smith, R. K.[t] – A RAPID ACARACIDE DETECTION METHOD FOR HONEY ANALYSIS – Honey (50 gm) was diluted with distilled water to 700 ml in a beaker. Pesticide spike in acetone solution (0.300 ml) was added, followed by addition of sodium phosphate dibasic (5.0 gm). The solution was filtered through a #4 Whatman paper filter (pre-washed with distilled water). The SPE cartridge (C-18, 6 ml size, Baker #7020-07) was attached to a 1 L. vacuum flask and pre-washed with 2 column volumes of hexane, two column volumes of acetone, and two column volumes of distilled water, taking care to not allow the absorbent bed to go dry. The filtered honey solution was passed through the SPE cartridge with house vacuum assistance at a rate of 30 to 50 ml per minute. The cartridge was washed with distilled water (25 ml) then air dried under vacuum suction for 15 to 20 minutes. The cartridge was eluted with 2 column volumes of acetone, 1 column volume of acetone:hexane (1:1) and 2 column volumes of hexane. The combined eluents were concentrated under nitrogen stream of 0.5 ml, then reconstituted with acetone to 2.00 ml. Internal standard (20 µl of 1.658 mg/ml dicyclohexyl phthalate in 2,2,4-trimenthylpentane) was added and the solution assayed by GC-MS (Finnigan OWA 1020, upgraded to 1050 Autoquan software; SGE aluminum clad 25 m x 0.33 mm i.d. HT-5, 0.1 um film, capillary column). Conditions: injector 310 degrees, separator 310 degrees, manifold 80 degrees, Helium carrier, splitless injector, splitless time 30 seconds. Temperature program: 50 degrees for one minute, 8 degrees/minute to 300, hold 5 minutes. MS program: 70 eV electron impact, 40 to 450 amu quadrupole scan every 0.55 sec.

Dr. Al Dietz, Department of Entomology, University of Georgia provided the sample of Apitol. This report made possible by a grant from the Georgia Beekeepers Association. (Presented at the mite symposium)

Table – Recoveries of tested acaracides and other pesticides with SPE.

Compound	Spike Level	Recovery
Amitraz	0.51 ppm	49 %
Baythroid	0.64 ppm	53 %
Carbaryl (Sevin)	0.37 ppm	90 + %
Carbofuran	0.40 ppm	90 + %
Chlordimeform	0.38 ppm	90 + %
Chlorobenzilate	0.49 ppm	90 + %
Chlorpyrifos (Dursban)	0.33 ppm	45 %
Coumaphos (Perizin)	0.35 ppm	90 + %
Cymiazole (Apitol)	0.40 ppm	90 + %
Diazinon	0.42 ppm	90 + %
Fluvalinate (Apistan)	0.51 ppm	55 %
Malathion	0.44 ppm	90 + %
Menthol	1.01 ppm	0
Pentac	2.20 ppm	0
Propoxur (Baygon)	0.55 ppm	90 + %
Tetradifon (Tedion)	1.44 ppm	50 %

33. Smith, R.-K.[t] – EUROPEAN HONEY BEE STOCK CERTIFICATION VIA OLEFIN ASSAY – In an earlier publication (Smith, *et al., ABJ* 128:676-678) the possibility of using olefin assay as a means of stock certification for European queens was advanced. At that time a problem was present in the analysis due to apparent segregation of queens

from the same stock depending on whether the queens were roaming free in the broodnest or had been stored for prolonged periods in queen banks. Segregation was not seen between free virgin and active egg-laying queens. At the last ABRC, Smith and Taylor presented evidence indicating that the total separation of Africanized and European queens required ignoring the queen specific compounds in the olefin assay (*ABJ* 128:808-809). Application of this procedure to the stock certification problem removes the storage history dependant segregation in queens from the same apiary, without effecting the separation between stocks. This suggests that the queen specific olefins, which are localized in the area of the tergal glands on the abdomen, are possibly linked to a reproductive or hive cohesiveness function of the European queen.

Comparison of the separation seen between Africanized and the general European queen database with that obtained from an apiary specific queen model (Weaver and Sons) demonstrates that an at least 10-fold increase in sensitivity in the olefin assay is obtained. As the individual mean values for the olefins are almost identical for the general European model and the apiary specific model, the increase in sensitivity is probably due to the narrowing of the standard deviations (used in the auto-scaling step) in the apiary model. It is important to note that although the sensitivity of the Africanized/European separation is vastly increased, the separation between European stocks (in this case Rossman and Weaver queens) is affected to a much lesser degree.

The idea has been advanced (GA Bee. Assoc. Meeting, July, 1988) of using mating nuclei with virgin queens and olefin assay of offspring workers to monitor feral gene pools around mating apiaries. Using the offspring workers of Kona queens mated in Hawaii and Kona queens allowed to free mate in Venezuela (provided by R. Hellmich) it is possible to separate the two groups of workers to a large degree. The experiment is muddied by the presence of both European and Africanized drones in the feral Venezuela population at the time of the matings, however overall the results indicate that the methodology is useful. The utility can be increased by deliberately mating virgin European queens with Africanized drones and using the worker offspring to establish a database for future identification of local backcross populations.

As pointed out earlier, successful queen stock certification depends on establishing apiary specific models prior to any local Africanization. To date certification files have been established for the following apiaries: Weaver and Sons, Navasota, TX; Calvert Apiaries, Calvert, AL; Rossman Apiaries, Moultrie, GA; Swords Apiaries, Moultrie, GA; Wilbanks Apiaries, Claxton, GA; York Bee Company, Jesup, GA; High Shoals Apiaries, High Shoals, GA; University of Georgia Apiary, Athens, GA; and University of Kansas Apiary, Lawrence, KS.

This report is made possible through a grant from the Georgia Beekeepers Association.

34. Smith, R. K.[t] and O. R. Taylor[q] — OLEFIN ASSAY FOR DETECTION OF AFRICANIZATION IN COMB WAX — As a first approximation the olefin analysis of comb wax should be a straight forward extension of the chemical analysis of worker bee hydrocarbon extracts, as the workers are the biosynthetic source of the wax. Examination of the olefins (unsaturated straight-chain hydrocarbons; *i.e.*, alkenes and alkadienes) from Africanized and European combs gave the initial impression that the alkene profile is identical for workers and wax, however the alkadiene presence is severely diminished in the wax.

To test the observation, several swarming workers with well developed wax scales were frozen, then the wax scales removed. Olefin assay of the individual worker and her wax scales showed that indeed the alkadienes were present in only trace amounts in the wax, as compared to the rest of the bee. This is in agreement with previous work (Tulloch *Bee World* 61:47-62) which did not list alkadienes as present in comb wax. Differential wax component production has been demonstrated between wax glands and epidermal cells (Blomquist *et al.*, *Insect Biochem.* 10:313-321). Now alkadiene synthesis can be added to the list of differences.

Aside from this very interesting observation, the analysis of the olefin profile for Africanization in comb wax is an identical problem to that presented by the workers and queens and is handled in the same fashion. A blind test of wax samples (63) gave 100% accurate Africanization identification using the worker identification criteria established by Smith for olefin assay.

Measurements of the span of ten worker cells in honey comb has been used as a quick field determination of Africanization in bees. Samples measuring an average of less than 4.99 cm are considered Africanized, above 5.25 cm European and the intermediate values (5.0 to 5.2 cm) are for Africanized bees backcrossed to the European side (Spivak's values from Costa Rica). The method has not been very reliable in the field in Mexico (Dietz, pers. comm.; Taylor, pers. obs.) possibly due to the large range of cell diameters which bees will build for various purposes such as rearing drone brood and worker brood, honey and pollen storage, and possible contributions from nutritional factors. The genetic inheritence of cell size in Africanized hybrid bees has not been delineated.

Comparison of the results from olefin assay and cell measurements indicates that the extremes of the measurements (less than 4.99 for Africanization, and greater than 5.25 cm for European) are probably reliable for identification. However values between 5.0 and 5.2 cm could be anything. A confirmatory method, such as olefin assay, is absolutely necessary for reliable identifications.

This report is made possible through a grant from the Georgia Beekeepers Association.

35. Sugden, E. A.,[e] A. M. Collins,[e] and W. T. Wilson[e] — PRELIMINARY SURVEY OF HONEY BEE SCOUT DENSITY IN THREE SOUTH TEXAS HABITATS — Honey bee forager "scout traps" were placed in five sites composed of riparian (two sites), brushland (two sites), and suburban (one site) habitats in the Rio Grande Valley of South Texas. Data from 47, 48, and 30 traps, respectively, were recovered after 33 days of sampling during nectar flow conditions in March and April, 1989. These yielded 0.72, 1.17, and 5.6 bees per trap respectively. Significant differences were found between the catches from different habitats, but not between sites within riparian and brushland habitats.

Equal numbers of traps at each of the above sites during the test were baited with synthetic Nasanov pheromone (1:1 citral-geraniol) (P), honey (H), and honey plus pheromone (HP). The means obtained were 0.36, 2.21, and 3.66 bees per trap, respectively. Comparisons were made between catches of the three bait types. Significant differences were found between P vs. H and P vs. HP, but not between H vs. HP, although HP baited traps caught notably more bees. The latter observation indicates a possible synergism between pheromone and honey baits.

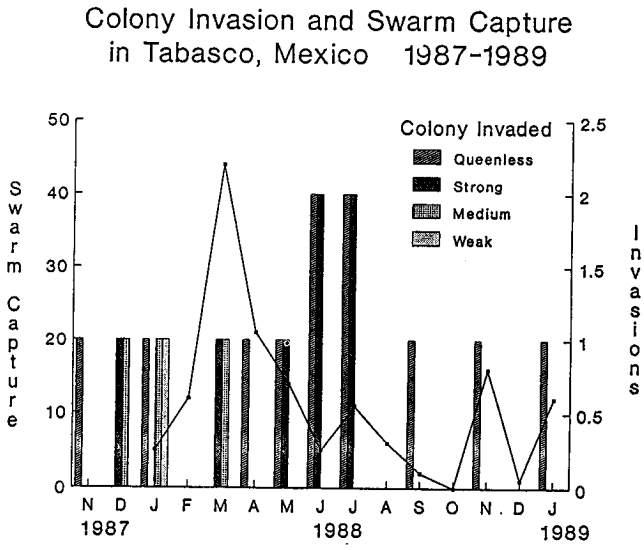
Major habitat types in South Texas thus displayed detectable and significant differences in honey bee forager scout density as measured by this technique. Honey baited scout traps can provide valuable, habitat-sensitive, low-input honey bee population census data over relatively short sampling periods. Synthetic Nasanov pheromone may increase trap yield.

36. Vergara, C.,[m] A. Dietz[m] and A. Perez[m] — USURPATION OF MANAGED HONEY BEE COLONIES BY MI-

GRATORY SWARMS IN TABASCO, MEXICO – The usurpation, or take-over, of established European honey bee colonies by migratory swarms in areas being invaded by Africanized honey bees is a phenomenon frequently observed by beekeepers (Michener, *Annu. Rev. Entomol.*, 20:399-416; Dietz in Hermann ed., 1982, Social Insects, III:323-360). Queen usurpation has been observed by us in San Juan, Argentina (Dietz *et al. Proc. Int. Apic.* Congr. 32: in press). Using DNA markers, Hall and Muralidharan *(Amer. Bee J.* 128:803) also found evidence of usurpation in honey bees. The frequency of this phenomenon may vary in different regions, but it plays an important role in the process of Africanization.

The present study has been in progress at 2 monitoring sites in Southeast Mexico since November 1987. Six apiaries, with a total of 240 colonies, were regularly examined at monitoring Line 1. After the first year, the colony number was reduced to 120. Line 1 is located south of the Tehuantepec Isthmus, near Villahermosa, Tabasco. The apiaries in Line 1 have 4 colony groups: queenless, weak, medium, and strong. The populations of bees in these groups are maintained by removing or adding brood and bees. All queens are marked and only European queens, obtained from Africanized honey bee-free areas of Mexico, are used to requeen. Line 2, is situated north of the Isthnus, near Nautla, Veracruz. Along this line, we are monitoring 120 colonies which are located in six apiaries. Additionally, we are monitoring 120 bait hives in Line 1 and 60 in Line 2.

With the exception of a single recent invasion at monitoring Line 2, all colony take-overs have occurred in colonies located at monitoring Line 1. So far, a total of 21 confirmed invasions have been recorded. These take-overs occurred in 1 weak, 2 medium, 8 strong and 11 queenless bee colonies. There were statistically significant differences in usurpation between strong and queenless colonies. Usurpation does not seem to be tied to distinct seasonal periods, or the increase in the number of swarms captured in the bait hives located near the study sites. The observed temporal distribution of the phenomenon suggests that invading swarms are most prevalent during the early stages of the Africanization process.



37. Waller, G. D.[s] and D. N. Byrne[ee] – HONEY BEE MORTALITY COMPARING DIURNAL WITH NOCTURNAL APPLICATION OF DIMETHOATE[ff] – The use of dimethoate to control citrus thrips *Scirtothrips citri* Moulton, on lemons, *Citrus limon* L., oranges, *Citrus sinensis* L. and other citrus cultivars kills forager honey bees, *Apis mellifera* L., because dimethoate is a broad-spectrum contact-systemic insecticide (Waller *et al., J. Econ. Entomol.* 77:70-74). Arizona citrus growers have been restricted to night applications for the use of dimethoate on blooming citrus. However, the loss of honey bees located near citrus is a continuing concern to beekeepers. In 1988 and 1989 a moratorium on the use of dimethoate has been in effect during peak bloom in part of Yuma County, Arizona.

Because of these concerns about honey bee losses and the inconvenience of applying insecticides in the dark, we have compared the impact of daytime versus nighttime treatments of dimethoate to citrus in bloom.

Three semi-isolated orchards of mixed plantings of lemons, oranges, and mineolas (*C. reticulata* Blanco x *C. paradisi* Macs.), were used – one for daytime treatment (8.1 ha), one for nighttime treatment (8.1 ha), and one as a control (30 ha). Two weeks prior to treatment we placed into each orchard six honey bee colonies having populations of 35,000 to 45,000 bees. Each hive was fitted with a dead bee trap and half of the colonies (3) also had a pollen trap. Populations of worker bees were estimated prior to the move to the orchard and after removing the colonies from the orchard. A baseline for bee mortality and incoming pollen was established for one week prior to treatment. The daytime treatment was made March 29, 1989 ca. 9:30 a.m. and the nighttime treatment on March 30, 1989 ca. 12:15 a.m. Both treatments were applied by airplane using 40% emulsifiable concentrate at 1.4 kg active ingredient per hectare. Data from dead bee traps and pollen traps were collected daily for 9 days following treatment.

Daytime treatment killed a mean of 14,211 bees per colony compared with a mean of 8,398 bees killed by nighttime treatment, as determined by dead-bee-trap data for a nine-day period following treatment. Colony population losses averaged 9.2 frames of bees and 4.2 frames, for day and night treatments, respectively. Using 1,500 honey bees per frame (GDW unpublished data) the estimated per colony population losses were 13,750 and 6,250 for day and night treatments, respectively. That these latter figures provide a lower estimate than do the dead-bee-trap data seems appropriate since emerging brood resulted in some recovery of bee losses. The highest bee kill occurred the first 24 hours following the daytime treatment when the mean per colony mortality was 4,962 bees. Dead bee traps on control colonies collected a mean of 2,095 dead workers during the nine-day post-treatment period while showing an estimated population increase of 4,750 bees per colony.

Pollen traps accumulated a mean of 146.4 g/colony/day prior to treatment. For one week following treatment, colonies in the day-treated orchard essentially ceased pollen gathering. Colonies getting the night treatment also reached a zero level of collection the fourth day after treatment. The presence of the pollen traps interfered with the removal of dead bees and the ingress and egress of foragers. Pollen traps should not be used when bee losses of this magnitude need to be removed from the hive by the "undertaker" bees.

Our results showed that daytime treatment caused nearly double the bee losses that resulted from nighttime treatment. Although bee losses resulting from even the latter may be unacceptable by the beekeeping industry, certainly it is reasonable that Arizona's regulation forbidding daytime treatment of blooming citrus with dimethoate remain in effect.

38. Wilson, W. T.[e] and A. M. Collins[e] – VAPORIZING MENTHOL TO REPEL ADULT WORKER BEES FROM HONEY SUPERS – During harvesting, many beekeepers utilize chemicals to repel adult bees *(Apis mellifera)* from storage supers containing combs filled with honey. Carbolic acid was used for many years, but currently beekeepers

apply either benzaldehyde or n-butyric anhydride to fume boards to drive adult bees out of supers. Butyric anhydride is effective but it has a strong, unpleasant odor. Recently, Wilson *et al.* (unpublished data, 1989) noted a strong bee repellency when treating colonies with menthol for *Acarapis woodi* control. Additionally, Collins and Hellmich (*Am. Bee J.* 128:801) sprayed spearmint oil and benzaldehyde in oil into the air near an apiary to repel bees and disrupt defensive behavior.

In recent studies, three formulations, each containing menthol, were applied to black fume boards and placed on top of open colonies to drive bees out of honey supers. The formulations tested were: 1) Menthol crystals (400 grams) dissolved in ethyl alcohol (500 ml), 2) Menthol crystals (500 g) dissolved in solid vegetable shortening (lipid) (500 g) by liquefying at 66°C, 3) Menthol crystals (500 g) dissolved in liquid vegetable oil (500 g) at 66°C. The menthol-containing mixtures were stored in a freezer.

In field tests, direct sunlight heated the black fume board and vaporized menthol from the alcohol solution quickly. In more than 100 colonies in south Texas, 75% of the adult worker bees left the honey super in about one minute and 95% or more were out within 3 min. (ambient temperatures 30°C & above with intense sunlight). Comparisons were made between menthol and butyric anhydride. Fumes from the two chemicals were equally effective in repelling bees from honey supers. The only observable difference was immobility in a few of the adult bees caused by intense and prolonged exposure to butyric anhydride.

One application of 40 to 60 ml of menthol-alcohol (ethyl) solution to absorbent cloth was effective in repelling bees from four different colonies over a one-hour period. The two menthol-lipid formulations were spread over cloth pads at ca. 50 g of mix per fume board. The lipid was quickly absorbed into the cloth and gave off menthol fumes for more than one hour. They were as effective as the menthol-alcohol solution in repelling bees, but less convenient during formulation and application.

A commercial beekeeper in Wyoming reported excellent bee repellency from the menthol-alcohol (isopropyl) solution provided that the black fume board received direct exposure to the sun. However, an Iowa beekeeper reported poor repellency during cool weather when a black fume board was not used. Without solar heating of the fume board, the menthol will not vaporize.

39. Wilson, W. T.,[e] J. S. Pettis[e] and A. M. Collins[e] — EFFICACY OF DIFFERENT ISOMERS OF MENTHOL AGAINST THE HONEY BEE TRACHEAL MITE — Four forms of menthol were tested under laboratory conditions for efficacy against honey bee tracheal mites, *Acarapis woodi* (Rennie). The four forms of menthol tested were: 1) Natural plant extract crystals, 2) Synthetic crystals, 3) L optic isomer crystals and, 4) D optic isomer crystals. Twenty-five grams of each type were liquified in petri dishes at 65°C and allowed to resolidify at room temperature, thus forming a solid cake. Fumigation chambers consisted of a deep hive body (24x41x50 cm) with a bottom board and telescoping cover. The entrance to the bottom board was not closed during the three-day fumigation period. Approximately 200 adult worker bees in a wood and screen laboratory cage (8 x 15 x 18 cm.) were placed in each hive body with a 25 g cake of menthol or no menthol for the controls. All worker bees came from a single mite-infested colony. During fumigation, temperatures ranged form 27 to 38°C. After three days of fumigation, 30 live bees from each cage were dissected and counts made of live and dead adult mites in the prothoracic tracheal tubes. Dead adult mites were shrunken and most were yellow in color. Three or four replications of each treatment were performed. There was a significant increase in mite mortality in all four treatments when compared with controls. However, between the four menthol treatments the D isomer was significantly less effective than the other three forms. No significant difference in efficacy was observed between natural, synthetic, and the L form of menthol. Menthol fumes were highly effective in killing adult mites, but many immatures appeared to survive. From these limited tests the D isomer of menthol alone does not offer adequate control of tracheal mites. The commercially available synthetic pellets and naturally extracted crystals appear to be of equal efficacy and both are readily available to the beekeeping industry.

Mortality of honey bee tracheal mites *Acarapis woodi* after 72 hrs. of fumigation (27-38°C) with various forms of menthol.

menthol treatment	# of bees infested/n	adult mite alive	adult mite dead	percent mortality
untreated	44/119	275	20	6.7a[1]
D-form	39/90	136	80	37.0b
L-form	22/60	5	195	97.5c
synthetic	30/90	5	142	96.5c
natural	55/120	2	176	98.8c

[1]Duncan's new multiple range test.

ADDRESSES OF AUTHORS

a. B.C. Ministry of Agriculture and Fisheries, Dawson Creek, B.C., Canada
b. Agriculture Canada, Beaverlodge, Alta., Canada
c. Fairview College, Fairview, Alta., Canada
d. Dept. of EPO Biology, Univ. of Colorado, Boulder, CO 80309-0334
e. Honey Bee Research, USDA-ARS, 509 West 4th St., Weslaco, TX 78596
f. Dept. of Entomological Sci., Univ. of California, Berkeley, CA 94720
g. Honey Bee Breeding, Genetics, and Physiology Lab., 1157 Ben Hur Rd., Baton Rouge, LA 70820
h. Chemistry Dept., Univ. of Texas, Austin, TX 78719
i. Graduate School of Biomedical Sci., M. D. Anderson Health Science Center, Houston, TX 77030
j. Dept. of Environmental Biol., Univ. of Guelph, Guelph, Ont., Canada
k. Beneficial Insects Lab., USDA-ARS, Bldg. 476. BARC-East, Beltsville, MD 20705
l. Univ. of Texas-Pan American, Edinburg, TX 78539
m. Dept. of Entomology, Univ. of Georgia, Athens, GA 30602
n. INTA, 3150 Nogoya, Entre Rios, Argentina
o. Dept. of Entomology, Louisiana State Univ., Baton Rouge, LA 70803
p. Dept. of Entomology, Oregon State Univ., Corvalis, OR
q. Dept. of Entomology, Univ. of Kansas, Lawrence, KS 66045
r. Centro de Ecologia, UNAM, Box 70-275, Coyoacan 04510, D.F. Mexico
s. Carl Hayden Bee Research Center, USDA-ARS, 2000 East Allen Rd., Tucson, AZ 85719
t. Apichemical Consultants, 5419 South Lake Dr., Douglasville, GA 30135
u. Dept. de Parasitologia Agricola, Facultad de Agronomia-Victoria, Cd. Victoria, Mexico
v. SARH, Calzada Luis Caballero 925, Cd. Victoria, Tamaulipas, Mexico
w. Secretario de Fomento Agropecuario, 19 Gutierrez de Lara, Cd. Victoria, Tamaulipas, Mexico
x. Escuela de Fitotecnica, Faculdad de Agronomia, Univ. de Costa Rica, Cd., Universitaria, Costa Rica
y. Dept. of Vet. Sci., Univ. of Wyoming, Laramie, WY 82070
z. SARH-INIFAP, Apartado Postal 172, 88900 Rio Bravo, Tamaulipas, Mexico
aa. Acarology Lab., Dept. of Entomology, The Ohio State Univ., 484 W. 12th Ave., Columbus, OH 43201
bb. OARDC, Dept. of Entomology, Wooster, OH 44691
cc. Dept. of Entomology, Univ. of California, Davis, CA 95616
dd. Museum of Zoology, Insect Div. and Lab. for Molecular Systematics, Univ. of Michigan, Ann Arbor, MI 48109
ee. Dept. of Entomology, Univ. of Arizona, Tucson, AZ 85721

ff. This report gives the results of research only. Mention of a pesticide does not constitute recommendation by the U.S. Department of Agriculture for use, nor does it imply registration under FIFRA as amended.
gg. Mention of a commercial or proprietary product does not constitute an endorsement by the USDA.

Reprinted from December, 1989, American Bee Journal
Vol. 129 (12): 811 - 826