FINAL REPORT

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Honeybee Breeding for Tracheal Mite Resistance in the Northeast United States

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OBJECTIVES:

To explore the development of resistant stock as an alternative to chemical pesticides for control of honey bee tracheal mites.

- **1.1** Demonstrate the presence of resistance to tracheal mites in three different lines of promising honey bee stock.
- **1.2** Test for the presence of resistance to tracheal mites in open-mated daughters of commercially-developed resistant breeding stock.
- **1.3** Determine the stability of resistance in stock produced by this project.
- **1.4.** Develop a new method of testing bees for tracheal mite resistance.

PROJECT RESULTS:

1.1 Many honey bee breeders have noted variation in response among their honey bee colonies in the face of challenge from tracheal mites. A few commercial queen producers have attempted to capitalize on this variation by selective breeding from lines showing relatively high survival rates. The end result is multiple claims regarding honey bee resistance to tracheal mites, but no controlled study actually assessing the level of resistance. Since beekeepers requeen regularly as a normal practice, identification of queen lines with true genetically controlled tracheal mite resistance would be of great practical value. Given that these bee strains also had other desirable attributes (e.g., manageability, disease resistance, good foraging capabilities), mite resistance would be a clear advantage.

We evaluated six different strains of honey bees for resistance to tracheal mites (Table 1). Three commercially available lines were purchased for inclusion in the project. The Weaver's Buckfast queen is advertised as resistant to tracheal mites and is quickly becoming a preferred queen in the

Northeast. Taber queens are open mated daughters of stock developed with instrumental insemination at Honeybee Genetics of Vacaville, California. This line is also advertised as resistant to tracheal mites. The third line, commercially available Italian queens reared by Holder Homan of Shannon, Mississippi, was included as a control. These bees are representative of queens used by many Northeast beekeepers, and Homan makes no claim of tracheal mite resistance for this line.

Three additional lines were produced by the project from special breeder queens. One breeder queen, Kirk, was chosen from the breeder's own operation which has suffered extensive losses due to tracheal mites. A second queen, Wagner, was selected from another heavily infested apiary in Vermont. Both of these queens were selected as breeders on the basis of their ability to thrive and produce high honey yields in the presence of tracheal mites in previous years. The third breeder was an instrumentally inseminated queen obtained from Mel Greenleaf of Hybri-Bees Inc. (LaBelle, Florida). This queen was reared from British honey bee stock imported by Roger Morse of Cornell University; this stock was imported as a potential mite resistance germ plasm source. Daughter queens were reared from each of the three breeder queens. The daughters were mated in an isolated mating yard in the Green Mountain National Forest, Vermont. Colonies headed by daughters of the Webster line, raised in the summer of 1989, as well as promising colonies of other Vermont stocks (exposed but not yet showing signs of infestation) were used as a drone source.

Parallel experiments were conducted in Vermont and Pennsylvania. Twenty queens of each of the six lines were represented at each location. In Vermont, the queens were overwintered and evaluated in nucleus hives, while, in Pennsylvania, queens were established in packages and overwintered as full strength colonies.

PENNSYLVANIA

In order to control initial mite densities, we purchased mite-free packages. However, these packages arrived already infested. Initial mite load was established by mite counts prior to introduction of queens. Four yards were designated as low mite levels, while the fifth yard (Manbeck) had a high mite level.

All experimental queens were received during the second week of June and introduced into randomly assigned colonies on 18 - 20 June. If a queen was not accepted within one month of introduction, or if a queen was superseded or became a drone-layer during the course of the summer, the colony was eliminated from the experiment. Also, if a colony was lost during the winter due to reasons other than tracheal mites, that colony was eliminated from the experiment. Table 1 shows the remaining colonies included in the experiment. All analyses were conducted on these colonies only.

Viable colonies of the six different lines were evaluated using the following criteria: brood production, tracheal mite infestation levels, and winter survival. Brood counts were made for all experimental colonies in September, when the majority of adult bees in each colony were the progeny of the experimental queen, and again in the spring. Tracheal mite samples (50 bees) were collected from each colony during September, February, March and May. Colony mortality was recorded throughout the winter.

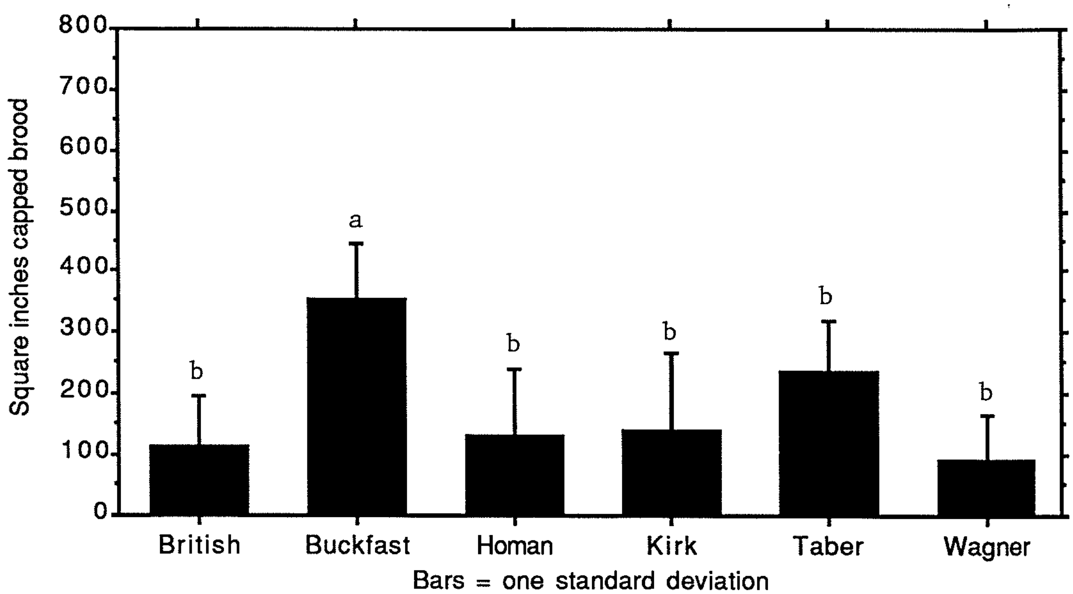
Table 1. Honey bee colonies available for analysis by yard and queen line.

Queen Line	Yard					Total by Queen
	Manbeck	Bayshore	Camp	Keeney	Kline	
British	2	2	0	1	4	9
Buckfast	2	4	3	4	3	16
Homan	2	3	3	3	3	14
Kirk	3	3	3	4	4	17
Taber	1	1	1	1	1	5
Wagner	3	4	4	3	4	18
Total by Yard	13	17	14	16	19	79

-Brood production

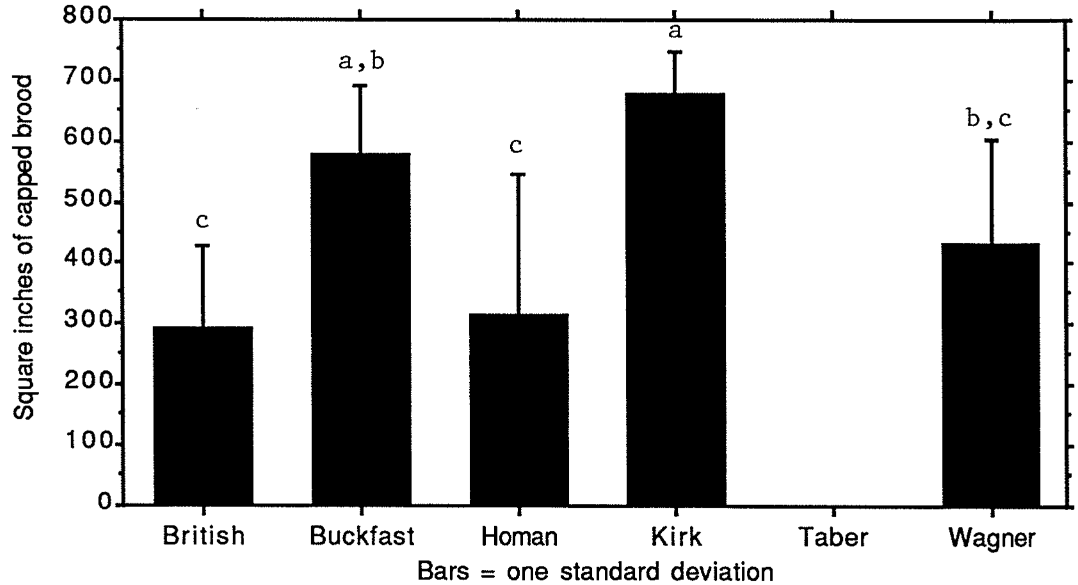
Average brood production was calculated for each queen line in September (Figure 1), and lines were compared analysis of variance (ANOVA). Fall brood production by the Buckfast line was significantly higher than that of all other lines ($p< 0.001$). There were no other significant differences in brood production between other lines.

Figure 1. Average brood production in Pennsylvania colonies headed by queens from each experimental queen line, Fall 1990.



The amount of brood in all surviving colonies was quantified again in May. Due to the large amount of brood in the colonies, brood counts were made over a three week period (May 3-4, 7-8, & 14-15). The brood counts conducted on May 3-4 were combined and analyzed as Date 1. Likewise, counts on May 7-8 and on May 14-15 were combined and analyzed as Dates 2 and 3, respectively. The average amount of brood produced by date was 381, 475 and 602 in^2 respectively. While there was an increase in brood production over time there was no date or yard interaction in brood production of the different queen lines. There was, however, a significant difference in brood production among queen lines (ANOVA; $F=3.49$, $df=48$, $p >0.001$). Average brood production by each line in spring, 1990, is shown in Figure 2. The Kirk and Buckfast lines were not significantly different in brood production (678 vs. 577 in^2). The British and Homan lines (371 and 314 in^2) were not different from each other, but were significantly lower in brood production than the Kirk and Buckfast lines. The Wagner line (432 in^2) was intermediate.

Figure 2. Average brood production in Pennsylvania colonies headed by queens from each experimental line, Spring 1991.



-Winter Survival

The number of colonies of each line lost over the winter in comparison to the total number of colonies in each yard included in the experiment appears in Table 2. All colonies headed by British queens survived the winter while no colonies headed by Taber queens survived. However, analysis of average survival values across all queen lines showed no significant differences between any of the lines. This was most likely due to the small number of colonies used for making the comparison. Comparing only the British to the average survival of all other lines demonstrated a significantly higher survival of British colonies ($\chi^2 = 3.44$, $p = 0.03$).

Table 2. Number of colonies lost due to tracheal mites during winter, 1990-91.

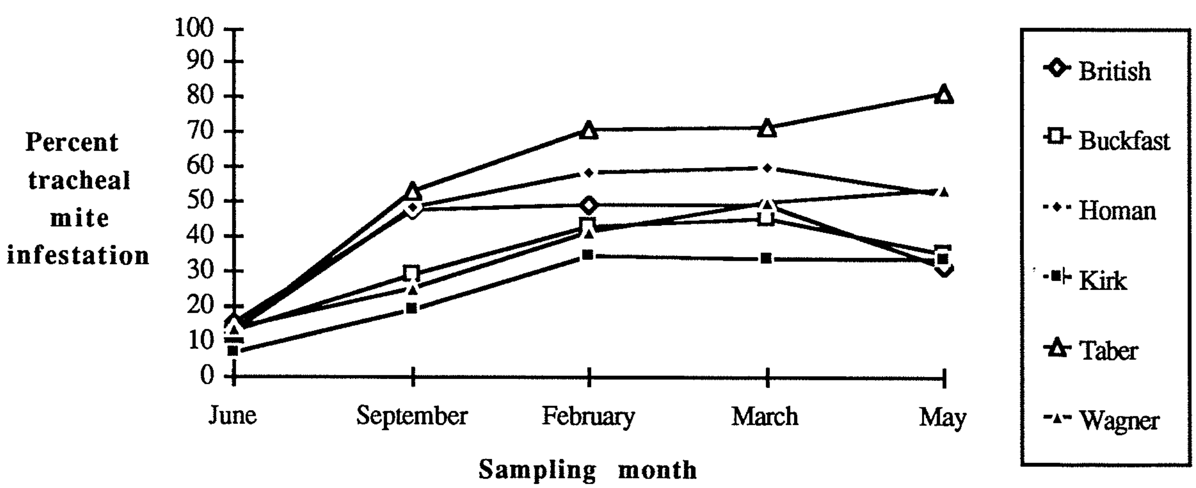
Queen Line	Yard					Total
	Manbeck	Bayshore	Camp	Keeney	Kline	by Queen
British	0/2	0/2	-	0/1	0/4	0/9
Buckfast	0/2	1/4	2/3	0/4	0/3	3/16
Homan	1/2	2/3	0/3	1/3	0/3	4/14
Kirk	0/3	2/3	0/3	1/4	0/4	3/17
Taber	1/1	1/1	1/1	1/1	1/1	5/5
Wagner	2/3	2/4	1/4	0/3	0/4	5/18
Total by Yard	4/13	8/17	4/14	3/16	1/19	20/79

–Tracheal mite infestation

Pre-treatment samples (June; before queen introduction) showed no significant difference in tracheal mite infestation levels, the proportion of bees infested with tracheal mites out of a sample of 50 bees, among the four low yards. However, the average infestation level of the high yard (64%) was significantly greater than the overall average of the four low yards (11%) ($p < 0.0001$). All percentages were arcsine-transformed prior to analysis. Further samples were collected from all colonies in September, February, March, and May. For the four low yards, average infestation was significantly lower in September than during the latter three sampling months. In order to include colonies that had died due to tracheal mite infestations over the winter, the average infestation level of these colonies at the last sampling date before colony death was calculated (82% infestation). This figure was used to represent dead colonies throughout the remainder of the analysis. During February, March, May, average tracheal mite infestation level was about the same across the four yards.

In June there were no significant differences in infestation levels either among the queen lines in the high yard or among the queen lines in the four low yards. Average infestation levels of the six different lines in the four low yards over the five sampling dates are shown in Figure 3. In September, the Kirk, Buckfast, and Wagner lines were not significantly different in infestation levels. The Taber line was significantly higher than the Kirk line, with no statistical differences from the remaining lines. This was due to the small number of colonies remaining in the Taber line and the large variability in infestation levels between colonies in this line.

Figure 3. Average tracheal mite infestation levels of six experimental queen lines in PA.



While the infestation levels of all of the lines increased by February, the Kirk line again had the lowest average infestation level (35%) of the six lines. This figure was significantly lower than the Homan and Taber lines. However there was no significant difference in the average infestation levels of the Kirk, British, Buckfast and Wagner lines. The Taber line again had the highest average level of infestation (71%), significantly higher than the Kirk, Wagner, and Buckfast lines.

Overall, infestation levels did not change in March. Only the Wagner line increased more than 3%. The Kirk line continued to have the lowest infestation level (34%) of the six lines, again, significantly lower than Homan and Taber. There was no significant difference among the Kirk, Buckfast, British and Wagner lines. The Taber line still had the highest level of infestation (72%), significantly higher than the Buckfast and Kirk lines.

In May, tracheal mite infestation levels decreased in the British, Buckfast and Homan lines, remained the same in the Kirk line, and increased in the Taber line. The British line had the lowest average infestation level (31%); however, it was not significantly lower than the Buckfast, Kirk, Homan or Wagner lines. The Taber line had a significantly higher infestation level than all other lines. However, at this time all colonies in the Taber line were dead (as described above, 82% represents the average infestation level of all colonies that died due to a tracheal mite infestation).

VERMONT

Results from the Vermont tests are described under objective 4, since the results are directly related to the development of a new method of assaying tracheal mite resistance.

1.2. An isolated mating yard was developed in the Green Mountain National Forest, Vermont, to permit us to raise a large number of daughter queens from a queen showing desirable attributes (used to rear daughters from breeder queens for objective 1). Undesirable lines from year 1 of the experiment were to be eliminated and replaced by open-mated daughters of commercially-developed resistant breeding stock. However, the project was terminated prior to implementation of this objective.

1.3. Stability of resistance requires a multi-year perspective. As the project was terminated at the end of the first year, it was not possible to achieve this objective.

1.4. VERMONT

Queens were introduced by July 15 into nucleus colonies (nucs). These nucs were made up of two frames of brood and bees, one frame of honey and one frame of empty comb. Twenty queens from each of the six lines were introduced, one per nuc, at this time. On July 21, all colonies were examined. Frames (0 to 2) of brood and bees were removed from each nuc as necessary to maintain the nucs (keep them from swarming). This information was recorded as an indication of how rapidly the different lines build up. Estimates were made of sealed brood and adult populations on August 30. Samples of 50 bees were taken from each nuc in late June and again in early September and sent to Penn State for tracheal mite examination. In mid-September, all nucs were evaluated for winter stores, and nucs with insufficient winter stores were fed sugar syrup. Nucs were later moved to regular commercial bee yards where they spent the winter on top of full size colonies. In November, all nucs were wrapped with cardboard cartons as added protection against winter weather. This system of wintering nucleus colonies has been used successfully in Vermont for the past four years.

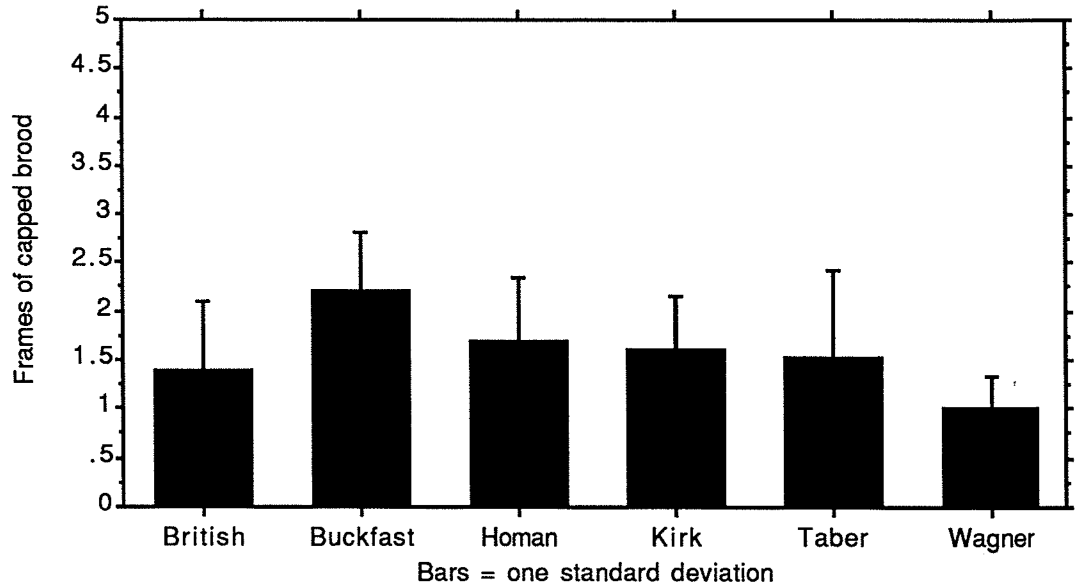
In some cases it was necessary to combine two nucs of the same line. This was done in cases where a queen was lost, a queen became a drone layer, or a particular colony did not exhibit adequate strength for wintering. All information concerning the uniting of nucs and reasons for uniting these units was recorded. The colonies remaining in the Vermont experiment as of August 30 are shown in Table 3.

Table 3. Colonies remaining in the Vermont experiment.

Queen Line	June 15	August 30
British	20	14
Buckfast	20	15
Homan	20	18
Kirk	20	18
Taber	20	14
Wagner	20	20

In the Vermont nucleus colonies, average brood production of the Buckfast line was significantly higher by August than that of the British line and the Wagner line ($p < 0.05$). It was not significantly different from that of the other lines, nor were the other lines significantly different from each other. However, it should be noted that brood counts of the Vermont colonies were made on a frame basis rather than on the basis of square inches. This is a much quicker method of calculating capped brood but is less accurate.

Figure 4. Average brood production in Vermont nucleus colonies headed by queens from each experimental queen line, Fall 1990.



Neither the nuc rearing system as a method of improved selection nor differences among queen lines could be evaluated from the Vermont section of the experiment because other data, such as spring brood production, tracheal mite infestation throughout the winter, and colony survival, were not collected and analyzed.

Discussion

Considering the limited time frame of this project it is difficult to draw concrete conclusions on the performance of the six different lines. However, some general observations can be made. It is clear from these data that the Taber line was the least desirable line overall under our experimental conditions. The queens were initially difficult to introduce, and many of those that were accepted were superseded or became drone-layers and had to be eliminated from the experiment. All five colonies that remained in the experiment in late fall were lost over the winter due to tracheal mites. In addition, the average tracheal mite infestation level of this line was higher than that of any other line throughout the period of the experiment. While the average brood production in the fall was greater than all other lines except Buckfast, this difference was not significant. In contrast, the Buckfast and Kirk lines performed very well. In terms of management the queens were easy to introduce and the majority were readily accepted. Few colonies superseded or became drone layers. Buckfast produced significantly more brood in the fall than any other line, and, in the spring, the Buckfast and Kirk lines produced more brood than any of the other queen lines. Tracheal mite infestation of the Kirk line was lower than any other line throughout the winter and spring (not different from the British line in May). The Buckfast line had a relatively low level of infestation when compared to the other lines (Figure 3). The Buckfast and Kirk lines lost 19% and 18% of their colonies over winter due to tracheal mites. However, this was significantly lower than only the British line.

There was less difference among the British, Wagner, and Homan lines. However, some observations are worth noting. Of particular interest is the British line, since they were imported specifically for their resistance to tracheal mites. This line displayed a number of undesirable characteristics such as increased susceptibility to disease (chalkbrood and EFB), reduced queen acceptance, tendency to run on the combs, and aggressive behavior. They showed no significant increase in brood production in either spring or fall. However, they did appear to overwinter better than any other line in spite of a substantial tracheal mite infestation. Also, the average tracheal mite infestation level of this line did drop in the spring. This may be an indication that resistance does not correlate directly to absolute levels of tracheal mite infestation. Resistant colonies may have some mechanism of "tolerating" moderate to high levels of tracheal mites.

The development of a simple, inexpensive test for evaluating honey bee stock for mite resistance (and other traits) is critical if this work is to be useful to other commercial beekeepers. The system explored in Vermont, testing the performance of lines using nucleus colonies, looks promising and has many other potentially valuable applications for northern beekeepers (see Economic Analysis). Even if winter survival proves to be the only reliable means of evaluating bees for tracheal mite resistance, this method will allow beekeepers to evaluate the largest number of colonies at the least cost.

C. Economic Analysis

High numbers of honey bee colonies (estimates run as high as 50%, R. Morse, personal communication) succumbed to tracheal mite during the winter of 1989-90. This situation has had serious effects on the supply of bees available for pollination in the Northeast and could be disastrous for the growers of bee-pollinated crops as well as beekeepers if such losses continue. Ensuring the survival of a strong beekeeping industry is paramount to agriculture in the Northeast.

Menthol is the only chemical approved for the treatment of tracheal mites. However, this treatment is difficult to time, could lead to resistance in the tracheal mite population and threatens the honey crop with direct exposure to chemicals. Honey is marketed on the basis that it is a pure, natural food. One or two isolated incidents of chemical contamination could potentially devastate the honey market. Most commercial and many sideline beekeepers derive their income from a combination of honey sales and pollination contracts. If it were no longer profitable to sell honey, it probably would not be profitable to keep bees even if pollination rentals increased. In addition, use of menthol to control mites has not been uniformly effective in the Northeast, perhaps due to problems associated with menthol application.

Chemical control of tracheal mites with menthol costs beekeepers approximately $2.00 per colony, plus labor costs for application. Other synthetic chemicals are not currently labeled for use on tracheal mites, may not be registered for some time, and are quite expensive ($10-12 per colony) to apply. While the cost of requeening is also expensive ($8-12), requeening on an annual or biennial basis is a management strategy already practiced by most beekeepers. If the cultural practice of requeening with resistant queens could be used in place of menthol or other chemical treatments to control tracheal mites, it would lead to a considerable savings in time, labor, and expense for beekeepers. By avoiding application of costly and potentially harmful chemicals, it would also maintain the availability of residue-free honey and an adequate supply of colonies for pollination at a reasonable price.

The methods being developed in this project to test stock for tracheal mite resistance can easily be adapted to test bees for other characteristics, including resistance to Varroa mite (considered a potentially far more serious pest than tracheal mite). The use of nucs represents a considerable savings in time, equipment and labor compared to the use of full sized colonies. This system can be efficiently used by both commercial beekeepers and researchers. By raising and maintaining these nucs through the winter, northern beekeepers can replace packages, normally purchased from the South ($20 - $25), take full advantage of adapted local stock and be in a position to supply other parts of the country with healthy, disease and mite resistant bees when African bees become a problem in the package-producing areas of the South.

D. Dissemination of Findings

An article describing this project was published in the *American Bee Journal* (Webster, K. 1992. Honey bee breeding in the Northeast–Starting again. Amer. Bee J. 131: 20-24). Webster and Tomasko have each presented information on this project at county and state beekeepers' association meetings. In addition, the results of this project are expected to be published in a refereed scientific journal.

E. Producer Involvement

Two beekeepers/queen breeders were intimately involved in the design and execution of this project. One of these individuals (Webster) was responsible for the initiation of the project proposal. The subject of this study is of acute interest to the beekeeping industry and groups on the periphery of the industry (fruit and vegetable grower). There is little disagreement among producer or research oriented apiculturists that the development of strains of bees resistant to parasites is the only viable long -term alternative for mite control.

15. AREAS NEEDING ADDITIONAL STUDY

Clearly, there is a great need for additional work in this arena. It is necessary to continue to search for resistant varieties of honey bees, to determine quick and accurate methods of selecting and breeding for resistance, to determine the extent to which resistance can be carried on from generation to generation and to develop a way to maintain resistant stock. Once this has been accomplished, making resistant stock commercially available to beekeepers at a reasonable cost will be the challenge. Also important is the identification of the mechanisms of resistance. Understanding how honey bees are resistant to tracheal mites would most likely make it much easier to select and breed for these characteristics.