# FINAL REPORT

## An Integrated Pest Management Program for Mite Pests of Honey Bees
## Project No. 95-MS1

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## Summary of Accomplishments

1. Honey bee colonies bred for hygienic behavior displayed a natural mechanism of defense against the parasitic mite, *Varroa jacobsoni.* The damage caused by this mite has reduced the health and number of bee colonies available for almond pollination. The hygienic colonies, bred from an Italian "Starline" stock using instrumental insemination, detected and removed significantly more pupae experimentally infested with *Varroa* mites in 1994 than non-hygienic colonies. In 1995, the removal response was statistically significant only when pupae were infested with two mites per cell. The reasons for this difference should be evident with continued testing. The results appear very promising as they suggest that this behavior can be incorporated into beekeeper's selection programs to help bee colonies combat the mite.

2. To augment the selection of hygienic colonies, other lines of bees from bee breeders in California were tested in 1995. Hygienic colonies were found in a Carniolan stock, maintained in a cooperative effort between the California Bee Breeders Association and the Ohio State University, and in another Italian stock, maintained by a reputable breeder in northern California. In 1996, these colonies will be tested for removal of *Varroa* mites.

3. Large scale field tests were conducted in collaboration with a commercial beekeeper in Wisconsin to evaluate the viability and performance of naturally mated hygienic queens. Inseminated queens are used in research to control the genetics of the stock; however, beekeepers use naturally mated in their colonies used for pollination. The results indicated that hygienic trait was retained in naturally mated queens, again providing support for the incorporation of the trait into production bee stocks. Continued evaluations will be made of the naturally mated hygienic colonies for mite levels, pollen hoarding, honey production, disease resistance and temperament.

4. Future research of this project, funded by the Almond Board and California Apiary Board will investage the relative performance of naturally mated and inseminated queens to establish the level of confidence that beekeepers can have in the insemination technique for maintenance of genetic lines of bees.

## Publications on Research Results 1995-1996:

Spivak, M., Reuter, G. S. 1996. Video: Beekeeping in Northern Regions. Minnesota and Wisconsin Extension Services. To be released in April, 1996.

Cobey, S. 1995. Instrumental Insemination Equipment: Sophistication andSimplification in Designs. ABJ. Vol 135. No. 10 pp.697-701.

Calderone, N.W., Spivak, M. 1995. Plant extracts for control of the parasitic mite, *Varroa jacobsoni* (Acari: Varroidae), in colonies of the Western honey bee, *Apis mellifera* (Hymenoptera: Apidae). *J. Economic Entomology* 88(5): 1211-1215.

Spivak, M., G. S. Reuter, M. Lamb. 1995. Frequency of hygienic behavior in naturally mated daughters of a hygienic breeder queen. *Am. Bee J.* 135: 830-831.

Cobey, S. 1995. A Course on the technique of Instrumental Insemination of Honey Bee Queens. ABJ. Vol. 135. No. 3 pp. 189-192

## Manuscripts submitted for publication

Boecking, O., Spivak, M. Drescher, W. In search of tolerance mechanisms of the honey bee *Apis mellifera* to the mite *Varroa jacobsoni.* Review book chapter submitted to: *Mites of the Honey Bee.* WicWas Press, 1996.

Spivak, M. Honey bee hygienic behavior as a defense against *Varroa jacobsoni.* Submitted to *Apidologie.*

## News Articles

Great Lakes Vegetable Growers News. November, 1995. "Minnesota Researcher Looking for Bees that Clean House."

Bee Times California Beekeeping Association Newsletter. 1996. "IPM Program for Management of Mite Pests of Honey bees"

## Research Presentations: M. Spivak

1996 American Beekeeping Federation, Portland, Oregon. with Sue Cobey: "Practical Selection Techniques" January.

1995 Wisconsin Beekeepers Association, Sheboygan, MI. "Research Update, University of Minnesota." November

1995 Michigan Beekeepers Association, Lansing, MI. "Hygienic Behavior", October.

1995 Missouri Beekeepers Association, Lake of the Ozarks. Three talks: "Breeding Bees for Resistance to Mites," "Alternative Controls for Mites," and "Queen Rearing." October

1995 Eastern Apicultural Society. Wooster, Ohio. "Field Assay for Disease Resistance" August.

1995 Minnesota Honey Producers, Willmar, MN "Research and Extension Update" July 14.

1995 Federal Council of Australian Apiculturists Association, Victoria, Australia. "Hygienic Behavior of Honey Bees" June 6.

1995 Manitoba & Saskatchewan Beekeepers Assoc. Canada "Research at the University of Minnesota" February.

## Research Presentations: S. Cobey

1996 Michigan State University Agricultural and Natural Resources Week Program. East Lansing, MI "Instrumental Insemination, From Watson To The Present" March.

1996 Nova Scotia Beekeepers Association, Kentville, NS. "Living With Mites" February.

1995 French National Queen Breeders Association. Valance, France. "Instrumental Insemination" December.

1995 Almond Industry Conference. Modesto, Ca. "An Integrated Pest Management Program For Mite Pests of Honey Bees" December.

1995 Eastern Apicultural Society, Wooster,OH, "Races Of Bees" August.

1995 Queen Breeders Association Of Mexico. Cuernavaca, Mexico "Queen Rearing Methods" "Instrumental Insemination" January.

# Detailed Summary

## Introduction

The long-term goal of our research is to develop an integrated pest management program for the treatment of two economically important parasitic mite pests of honey bees; the Varroa mite (*Varroa jacobsoni*), and the tracheal mite (*Acarapis woodi*). Since their introduction into the U.S. in the 1980's, these mites have reduced the quality and quantity of colonies available for almond pollination. Alternative non-pesticide controls of both mites have been tested with success in our laboratories (Sammataro et al. 1994; Calderone and Spivak 1995). Our current objectives are to supplement these control measures in the following ways:

1. Continue our breeding program to select among various commercial bee stocks that demonstrate mechanisms of defense against the most destructive pest, the *Varroa* mite;
2. Investigate methods that enhance the ability of the beekeeping industry to utilize the genetic lines of mite tolerant bees;
3. Ensure technology transfer of techniques used in breeding programs through short courses and instructional materials.

## Tests for Hygienic Defense against Varroa Mites

Honey bees which are bred for hygienic behavior demonstrate one mechanism of defense against the ectoparasitic mite, *Varroa jacobsoni* Oudemans. This mite is the most destructive pest of honey bees in the U.S. and Europe. Because of the risks and disadvantages of using chemical treatments in mite-infested colonies (Lodesani et al., 1992, 1995), it is important to determine if honey bees have any heritable defense mechanisms against the mite which may be readily incorporated into breeding programs.

One defense may be hygienic behavior, in which the bees are able to detect and remove a portion of mite-infested pupae from the nest. This behavior interrupts the reproductive cycle of the mite inside sealed brood cells in two ways: 1) the immature mites are killed which decreases the average number of offspring per mother mite; and 2) the mother mite may be damaged which increases the mortality of fertile mites (Rath and Drescher, 1990; Fuchs et al., 1994).

Hygienic behavior is considered the primary mechanism of resistance to at least two disease of larval and pupal honey bees, American foulbrood caused by the bacterium *Bacillus larvae* (Rothenbuhler, 1964) and chalkbrood caused by the fungus, *Ascosphaera apis* (Gilliam et al., 1983; 1988). Hygienic bees have the ability to remove diseased brood from the nest before the causative organisms reaches the sporulating stage (Woodrow and Holst, 1942). Rapid hygienic behavior occurs at a relatively low frequency in most honey bee populations thus far studied (Spivak and Gilliam, 1993).

A two-way selection program for hygienic behavior was initiated at the University of Minnesota in 1992. Lines of hygienic and non-hygienic colonies were bred and tested for their ability to remove pupae infested with *Varroa* mites.

The hygienic and non-hygienic lines used in the experiment were bred from "Starline" stock, derived from Italian *A. mellifera ligustica*. The degree of hygienic behavior in the colonies was determined by a freeze-killed brood assay in which the amount of time was recorded for bees to detect, uncap, and remove a comb section containing freeze-killed pupae (frozen at -20 C. for 24 hours). Colonies that removed the freeze-killed brood within 48 hours were considered hygienic; colonies that took longer than one week to remove the dead brood were considered non-hygienic (Taber and Gilliam 1987). To establish and maintain the lines, queen bees were raised from colonies that displayed the most rapid and least rapid removal rates. The daughter queens were inseminated with 4-6 µl of semen from drones of different hygienic or non-hygienic colonies. All colonies were wintered outdoors and were tested again the following spring using the freeze-killed assay. Only the most hygienic and least hygienic colonies based on the second freeze-killed brood assays were used in the experiments to test whether the colonies would remove brood infested with *Varroa*.

In 1994, the experiments included four hygienic and three non-hygienic colonies, and in 1995 they included seven hygienic and four non-hygienic colonies. All colonies were treated with fluvalinate (two Apistan strips per colony) the previous fall, and were sampled for *Varroa* in the spring. No mites were detected in any of the hygienic or non-hygienic colonies in the spring of 1994 or 1995 before the experiments began. All colonies were maintained in standard Langstroth equipment, and had approximately 8-12 frames of brood when they were tested for removal of *Varroa* mites.

A commercially available apparatus called a Jenter Box® was used to test whether the selected hygienic and non-hygienic colonies of bees would remove pupae experimentally infested with *Varroa* mites (following methods of Boecking and Drescher, 1991, 1992). The box contains approximately 300 plastic worker cells and fits into a standard brood frame. Ninety of the cells within the box have false bottoms fitted with removable plugs which allows access to individual larvae or pupae within the box through the base of the cell.

The inseminated queens in each experimental colony were confined within the box until they had laid eggs in most of the cells (6-24 hours). Eight or nine days later, *Varroa* mites were introduced through the plugs in the cells containing fifth instar larvae. Care was taken to introduce mites only into cells which had been sealed with wax within the last 6-8 hours, or before the fifth instar larvae had spun a cocoon and begun pupation. All mites were collected off adult workers and drones from one highly infested colony located in an apiary over 5 km away. Care was taken to introduce mites that were fully

pigmented, however, the reproductive status of the mites at the time of collection and introduction was not known. The mites were introduced into the cells using a fine, camel-hair paint brush following the methods of Boecking (1992). In 1994, one *Varroa* mite per cell was introduced into 10 - 20 cells containing fifth instar larvae. Another group of cells served as controls, whereby the plugs were removed and replaced without introducing a mite. The infested and control cells were marked on a transparent sheet of plastic (following Infantidis, 1983), and were inspected on days 1, 2, 4, 7, and 10 after infestation to determine if the bees had detected and removed the infested brood. In 1995, two mites per cell were introduced onto other larvae within the box in addition to larvae infested with one mite and the controls. In both years, on the tenth day of the experiment, or one day before the pupae were due to eclose as adults, all cells containing infested pupae that were not removed by the bees were opened to determine if the remaining mites reproduced within the cells.

The differences in the results of the freeze-killed brood assays between the hygienic and non-hygienic colonies was analyzed using a student's t-test for each year (SYSTAT, Version 5.2.1). The mean percentages of mite-infested and control pupae removed from the Jenter Box on day 10 of the experiment was analyzed on arcsine transformed data using a split-plot two-way ANOVA for each year. The error term for bee type-was colony (bee-type), and for the treatment effect was the residual error (SAS Version 5.2.1).

**Results**

The results of the freeze-killed brood assays conducted before the mites were introduced into the colonies in 1994 and 1995 are presented in Figure 1. In both years, the hygienic colonies removed significantly more dead brood than the non-hygienic colonies within 48 hours ( 1994: $t = 6.53$; $df = 5$, $P = 0.001$; and 1995: $t = 6.65$, $df = 6$, $P = 0.001$). In 1995, there was no difference between the rate of removal by colonies containing queens inseminated with the sperm of one or of many drones, therefore, the results from all hygienic colonies were pooled together for the remainder of the analyses.

The results of the assay for the ability of the hygienic colonies to detect, uncap, and remove mite-infested pupae from the cells within the Jenter Box are given in Table 1 and Figures 2a and b. In 1994, the four hygienic colonies removed significantly more pupae infested with one mite per cell by day 10 than the three non-hygienic colonies and the controls. The same assay in 1995 yielded different results. The seven hygienic colonies did not remove significantly more infested pupae than the non-hygienic colonies or the controls when one mite was introduced per cell. However, significantly more pupae were removed that were infested with two mites per cell than the controls (Tukey HSD: $P < 0.05$). Continued testing will determine if the variation between years was due to genetic or environmental causes.

## Breeding Program and the Beekeeping Industry

To augment the selection of commercial stocks, other lines of bees from bee breeders in California were tested for hygienic behavior in 1995. Breeder colonies from a Carniolan stock (derived from *A. m. carnica*), maintained in a cooperative effort between the California Bee Breeders Association and Ohio State University, were screened using the freeze-killed brood test. Of 24 breeder colonies tested during the summer of 1995, three colonies displayed an average of above 82% removal of freeze-killed brood in repeated trails. Daughter queens and drones reared from these colonies were crossed using instrumental insemination and established in colonies. Next spring these breeder colonies will be tested for removal of *Varroa* mites. Additional colonies from another breeder in California who sells commercially proven Italian queens were also screened and will be tested to increase the gene pool of the hygienic lines.

To enhance the ability of the beekeeping industry to utilize the genetic lines of mite tolerant bees, we have begun to examine the viability and performance of naturally mated hygienic lines of bees. Queens which are instrumentally inseminated are used as breeder stock; however commercial beekeepers use naturally mated queens in the colonies used for almond pollination. Early genetic studies on hygienic behavior revealed that the alleles conferring the trait are recessive. It is critical, therefore, to determine what percentage of daughter queens raised from inseminated breeder stock retain the hygienic trait when they are outcrossed with unselected males, and to determine the commercial utility of the stock.

In June 1995, Starline hygienic queens were introduced into the apiary of commercial beekeeper in Wisconsin to examine the viability and performance of naturally mated hygienic lines of bees. Queens which are inseminated are used as breeder stock; however commercial beekeepers use naturally mated queens in their colonies used for production and pollination. The tests were initiated with Starline hygienic lines in 1995-1996, and will continue with both Starline and Carniolan hygienic lines in 1996-1997. The queens were allowed to mate naturally, and the frequency of hygienic behavior of these queens was measured in late August and early September, 1995. The results indicate that the average percent freeze-killed brood removed from the hygienic colonies over three trials (82.9% ± 10.5 n = 36) was significantly higher ($P = 0.00$) than the percent removed from the commercial, unselected bee colonies (59% ± 22, n = 56) (Figure 3). These results indicate that the hygienic trait is retained in naturally mated production colonies. In 1996 and 1997, these and other Carniolan hygienic colonies will be evaluated for hygienic behavior, mite levels, pollen hoarding, disease resistance, and temperament.

**Table 1.** Percent removal (mean ± std. dev.) of pupae from the Jenter Box on day 10 after treatment. One or two mites were introduced per pupae through the plug in the treatment groups. Controls refer to cells in which the plug was removed and replaced without introducing a mite. Last row shows results of split-plot 2-way ANOVA on arcsine transformed data, in which the error term for bee type = colony(bee type).

| Plastic Comb (Jenter Box) | 1994 | 1995 |
|---|---|---|
| **Hygienic** | n = 4 | n = 7 |
| 2 mites | -- | 49.8 ± 30.49 |
| 1 mite | 69.2 ± 16.41 | 24.7 ± 20.06 |
| control | 21.1 ± 19.92 | 9.9 ± 7.51 |
| **Non- Hygienic** | n = 3 | n = 4 |
| 2 mites | -- | 22.5 ± 3.54 |
| 1 mite | 10.0 ± 10.00 | 11.3 ± 6.29 |
| control | 10.4 ± 10.02 | 3.1 ± 6.25 |
| | bee type: F =45.87; df = 1,5; *P* = 0.001<br>treatment: F = 6.35; df = 1,5; *P* = 0.05<br>bee type*treatment: F = 4.86; df =1,5; *P* = 0.08 | bee type: F = 3.96; df = 1,9; *P* = 0.10<br>treatment: F = 9.03; df = 2,16; *P* = 0.002<br>bee type*treatment: F = 0.00; df= 2,16; *P* = 1.00 |

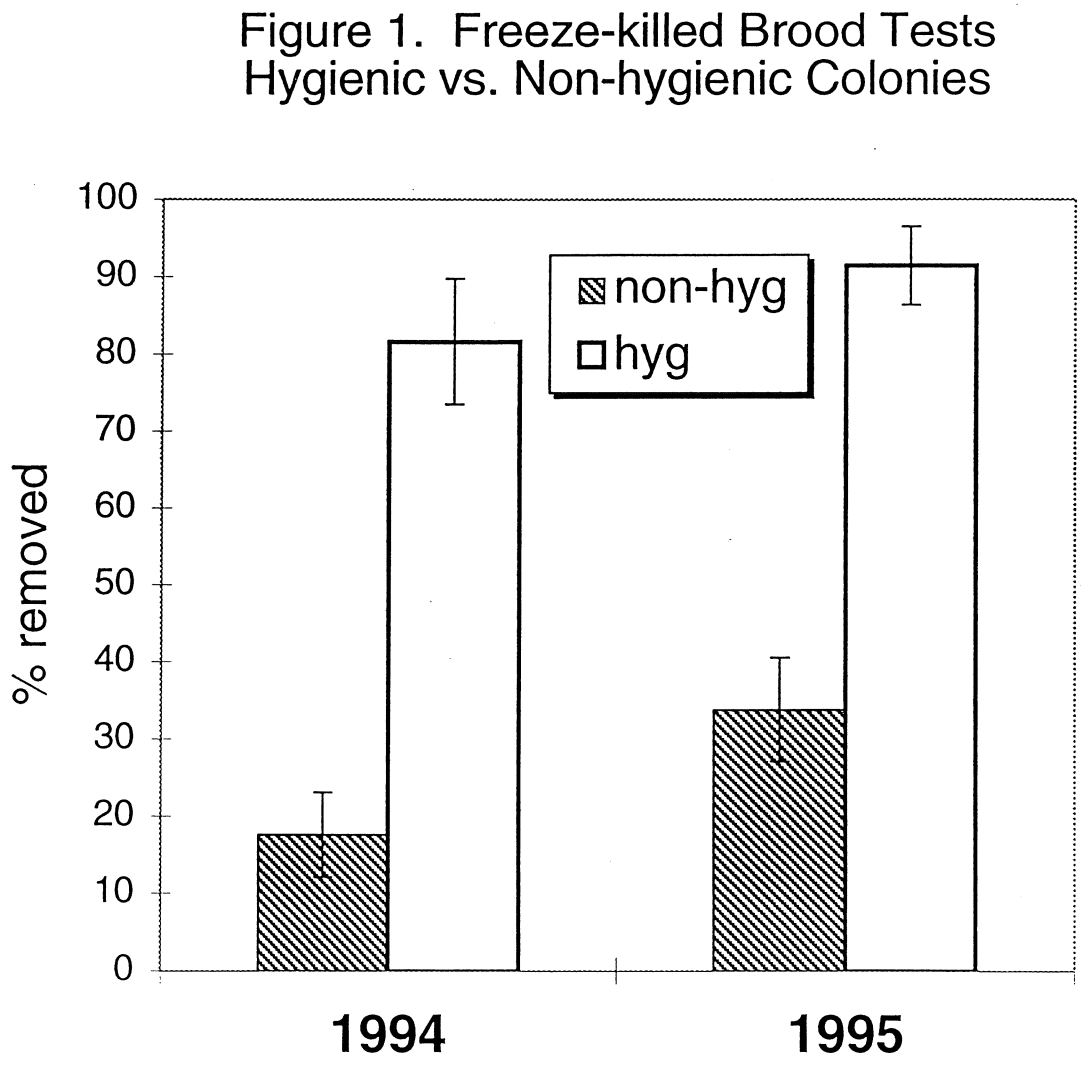
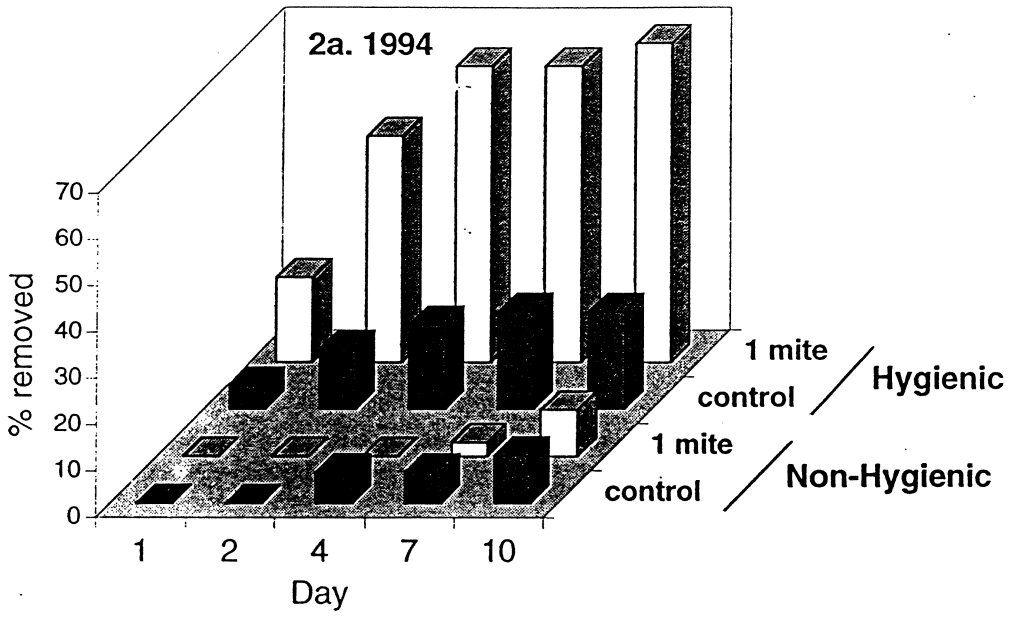
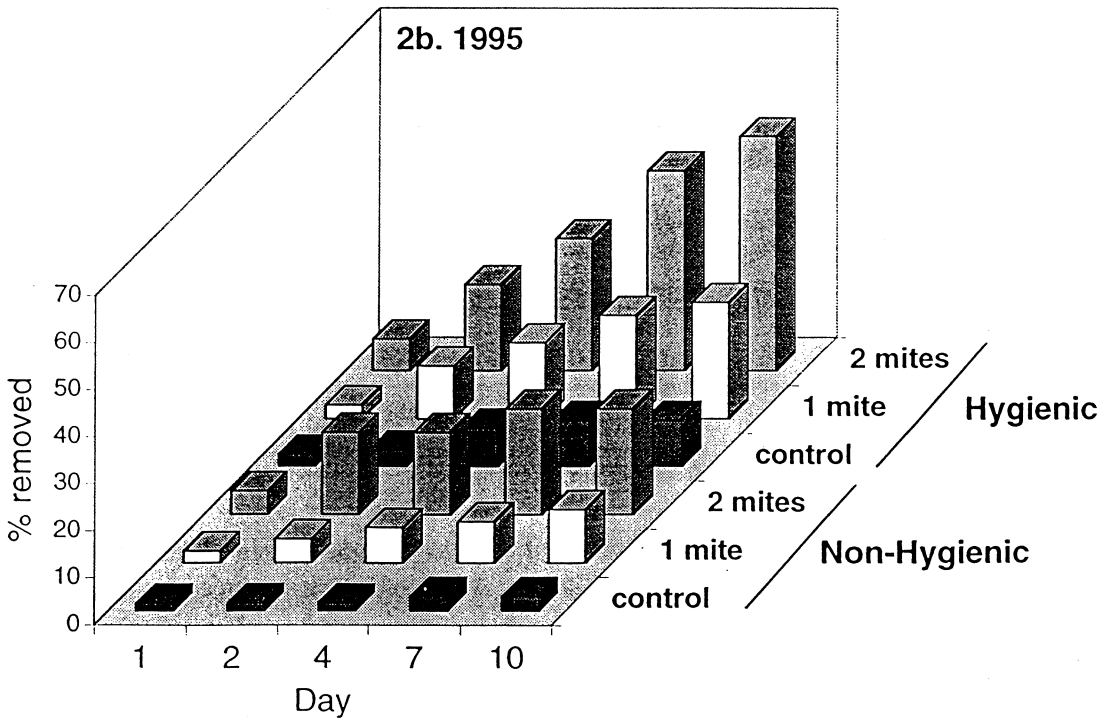



**Figure 1.** The mean (± std. error) percent freeze-killed brood removed from the cells within 48 hours by 4 hygienic and 3 non-hygienic colonies in 1994, and by 7 hygienic and 4 non-hygienic colonies in 1995. Student's t-test, 1994: t = 6.53, df = 5, $P = 0.001$; 1995: t = 6.65, df = 6; $P = 0.001$.

# Figures 2a and b. Removal of Mite-Infested Brood from Jenter Box




**Figures 2a and b.** The mean percent removal of mite-infested pupae from the cells of the Jenter Box by the hygienic colonies and non-hygienic in 1994 (a) and in 1995 (b) on days 1, 2, 4, 7, and 10 after the mites were introduced. One mite per cell was introduced into 10-20 cells in each colony through the plug at the base of the cell. The controls represent cells containing 5th instar larvae in which the plug was removed and replaced without introducing a mite.

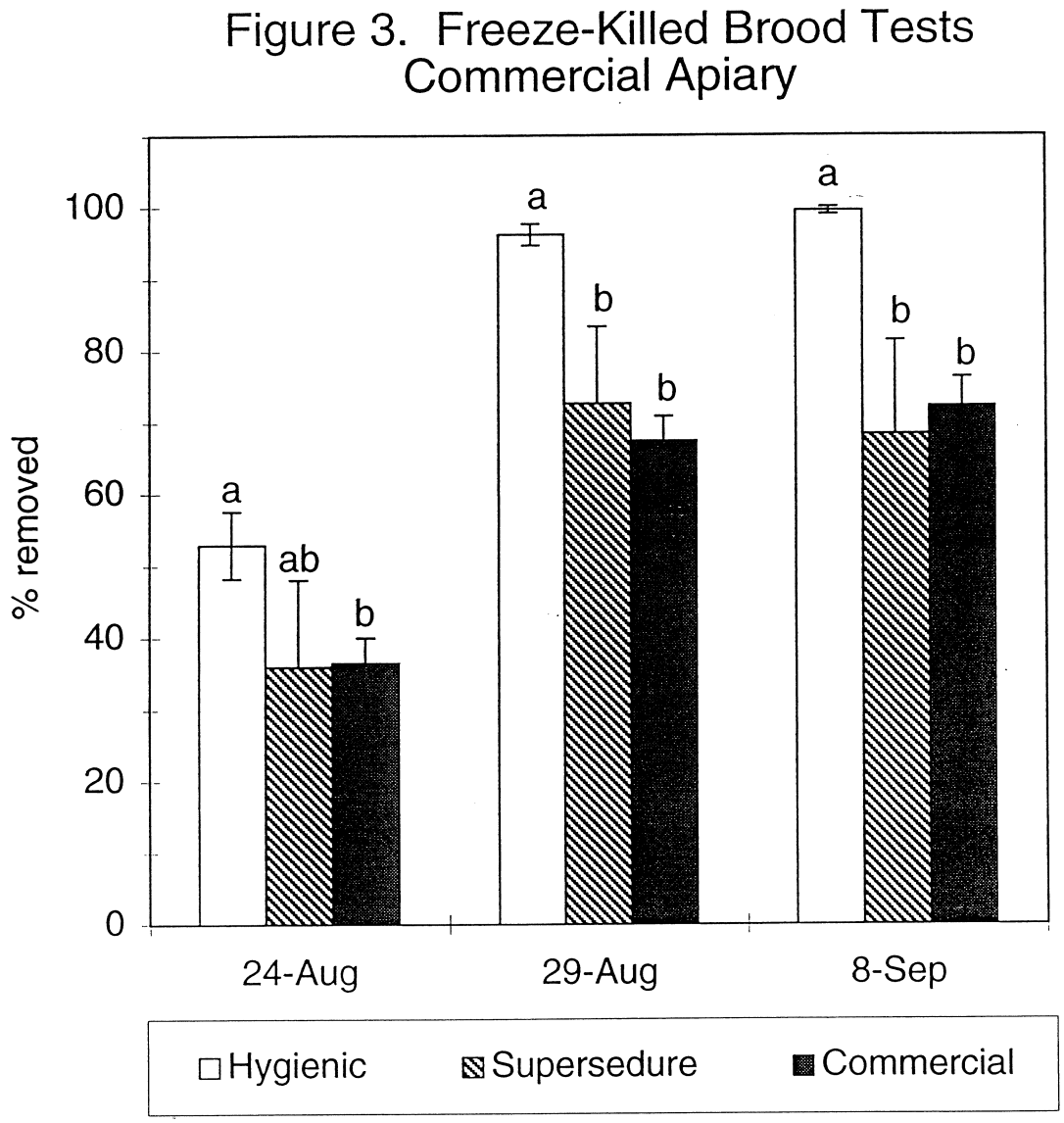



**Figure 3.** Mean (± standard error) percent freeze-killed brood removed by 36 hygienic, 7 hygienic-supersedure (hygienic queens that were replaced naturally by the bees with a new queen), and 56 commercial colonies over three trial dates. All measurements were collected 48 hours after introducing the dead pupae. ANOVA: $F = 18.45$; $df = 2,96$; $P = 0.00$. Means with different letters above bars indicate significant differences within a particular trial date (Tukey's HDS test).

## Methods to Enhance Utilization of Genetic Lines

Another critical area of this research is to help the beekeeping industry maintain the inseminated breeder lines of mite resistant bees. Problems associated with the technique of instrumental insemination (II) must be addressed in order to make it practical and usable to the beekeeping industry at large. For example, many beekeepers assume that II queens perform poorly in the field and are superseded (replaced by the bees) prematurely, which may be unfounded and may inhibit the use of this technique. Factors related to colony performance, such as, queen longevity and egg laying rate, are reputed to differ between II and naturally mated (NM) queens. Variation in the performance of II queens may be related to differences in techniques that researchers and beekeepers employ. Thus, II and NM queens must be more intensively studied so as to improve the overall usefulness of the II technique. A comparison study is being planned at OSU this spring (1996).

## Technology Transfer

An important and often neglected component in an effective program is to ensure technology transfer of information and techniques to the beekeeping industry. The University of Minnesota and the Ohio State University each offer intensive short courses which are highly complementary and have been well received by the beekeeping industry. A two-day short course in Queen Rearing is offered yearly at the University of Minnesota. The goal of the course is to teach experienced beekeepers methods to raise their own queen bees to gain control of the genetics of their stock. Also, sound and effective methods of stock selection are taught, including selection for hygienic behavior and methods of avoiding inbreeding. The course is supplemented by a manual and video (Spivak & Reuter, 1994).

S. Cobey at the Ohio State University annually offers a short course on Instrumental Insemination and bee breeding. The class is designed for commercial beekeepers who plan to establish or are involved in a breeding program. A practical hands-on approach to instruction is provided. Various breeding systems and practical methods of selection are presented. Participants receive a booklet of reprints reviewing methods of bee breeding and the technique of instrumental insemination. These materials are being developed currently into a training manual and video.

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