FINAL REPORT

An Integrated Pest Management Program for Mite Pests of Honey Bees
Project No. 95-MS2

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Summary of Accomplishments

1. Honey bee colonies bred for hygienic behavior display 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. Tests conducted at the University of Minnesota over three summers (1994-1996) using a line of bees derived from the Italian race (*A. mellifera ligustica*) indicated that the hygienic colonies detected, uncapped, and removed significantly more infested pupae than non-hygienic colonies. The results from 1994 and 1996 were comparable: the hygienic colonies detected and removed 70% of the experimentally infested pupae within 10 days, whereas the non-hygienic colonies removed under 23%. In 1995, the hygienic colonies removed 25% of the infested pupae when one mite per cell was introduced, and 50% when two mites were introduced. Environmental conditions (higher mite loads, foraging or weather conditions) influenced the variation in removal response between the years because some of the same colonies (headed by the same queens) that removed low numbers of mites in 1995 removed significantly more mites when retested in 1996. 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 commercial stocks, other lines of bees from bee breeders in California were tested for hygienic behavior in 1995 and 1996. 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 for hygienic behavior, and hygienic breeder queens have been propagated from this stock. In the summer of 1997, these hygienic colonies will be tested for removal of Varroa mites at the University of Minnesota.

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 the hygienic colonies had significantly lower levels of chalkbrood (a fungal disease of immature bees), and produced significantly more honey. Importantly, the hygienic colonies had significantly lower levels of mites in three of the four apiaries. All other measures were the same between the hygienic and commercial colonies. These promising results indicate that colonies headed by open mated hygienic queens are healthier and more profitable than unselected colonies.

4. Another critical aspect of this project is to help the beekeeping industry maintain the inseminated breeder lines of hygienic 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. Future research of this project, funded by the Almond Board and California State Beekeepers Association will investigate 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.

In addition, we will continue to offer short courses and instructional materials on beekeeping, queen rearing, and instrumental insemination to foster technology transfer of techniques used in breeding programs.

Detailed Summary

Introduction

This project is a continuation of 95-MS1. This year, there was widespread media attention on the plight of honey bees, which heightened the public's awareness of the importance of honey bees in the pollination of economically important crops. This research project was highlighted in many news reports as one of the few in the United States to develop alternative solutions to the problem of the parasitic mites plaguing honey bees.

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 by:

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

Honey bees which are bred for hygienic behavior demonstrate one mechanism of defense against *Varroa* mites. Hygienic bees are able to detect and remove a portion of mite-infested pupae from the nest which interrupts the reproductive cycle of the mite. Colonies are selected for hygienic behavior using a freeze-killed brood assay in which the time taken to uncap and remove frozen pupae is recorded. To establish hygienic lines (breeder stock), daughter queens are reared from colonies that consistently remove all of the freeze-killed brood within 48 hours. The daughter queens are instrumentally inseminated with male bees (drones) from different hygienic colonies to control the genetics of the breeder stock. The hygienic colonies are then tested for their ability to remove mite-infested pupae using a more precise behavioral assay. This assay utilizes a commercially available apparatus called a Jenter Box®. Individual *Varroa* mites are introduced into specific cells containing pupae by removing a plug from the bottom of the cell within the box. Nine days after the mites are introduced (or one day before the pupa is due to emerge from the cell as an adult) the cells are inspected to determine if the infested pupa has been removed or not.

Tests conducted at the University of Minnesota over three summers (1994-1996) using a line of bees derived from the Italian race (*A. mellifera ligustica*) indicated that the hygienic colonies detected, uncapped, and removed significantly more infested pupae than non-hygienic colonies (Figure 1). The results from 1994 and 1996 were comparable: the hygienic

colonies detected and removed close to 70% of the experimentally infested pupae within 10 days, whereas the non-hygienic colonies removed under 23%. In 1995, the hygienic colonies removed only 25% of the infested pupae when one mite per cell was introduced, and 50% when two mites were introduced. It is still unclear why the removal response in 1995 was lower. However, it appears that environmental conditions (higher mite loads, foraging or weather conditions) influenced the response because some of the same colonies (headed by the same queens) that removed low numbers of mites in 1995 removed significantly more mites when tested in 1996.

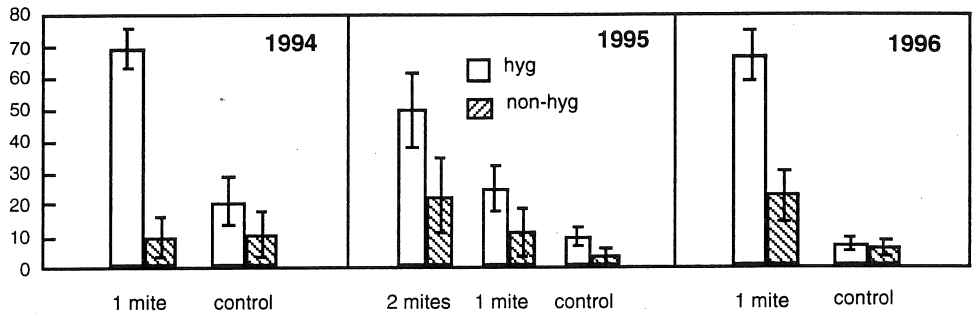

Figure 1. The percent removal of mite-infested pupae by hygienic and non-hygienic colonies 10 days after introduction of the mites through cell bases of Jenter Box. In 1994 and 1996, the hygienic colonies (n = 4 and 10, respectively) removed significantly more pupae infested with one mite per cell than the non-hygienic colonies (n = 3 and 6) ($P < 0.01$; split-plot 2-way ANOVA for each year). There also was a significant difference between the removal of infested pupae and controls ($P < 0.05$) in both years. Tests in 1995 (n = 7 hyg, 4 non-hyg) revealed a significant difference only when two mites per cell were introduced (treatment effect: $P < 0.01$). Results from 1994 and 1995 in Spivak (1996), and from 1996, unpublished.

To augment the selection of commercial stocks, other lines of bees from bee breeders in California were tested for hygienic behavior in 1995 and 1996. 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. Hygienic breeder queens have been propagated from this stock. In the summer of 1997, these hygienic colonies will be tested for removal of Varroa mites at the University of Minnesota, using the methods described above. This stock has also been selected for reduced incidence of tracheal mite infestations and is showing good winter survivability without treatment. Mite infestations during the critical period of spring buildup in 1991 averaged 48% and has steadily decreased to under 5% infestation in 1996.

To enhance the ability of the beekeeping industry to utilize the genetic lines of mite tolerant bees, we examined the 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. The results from our experiments in 1995 indicated that daughter queens raised from inseminated breeder stock retained the hygienic trait when they

were outcrossed with unselected males. Another study was conducted to determine if the open mated queens are commercially profitable.

In March 1996, hygienic queens were introduced into the apiary of commercial beekeeper in Texas. The queens were allowed to mate naturally, and were introduced into 50 colonies. For comparison, 50 unselected "commercial" queens were raised and allowed to mate in the same location. The colonies were transported to Wisconsin in May, and equal numbers of hygienic and commercial colonies were placed in four apiary locations. In June, the colonies were evaluated for population size, incidence of diseases, and temperament. In September, the colonies were again evaluated for diseases, honey production, and mite loads. The results (Table 1) indicated that the hygienic colonies had significantly lower levels of chalkbrood (a fungal disease of immature bees), and produced significantly more honey. Importantly, the hygienic colonies had significantly lower levels of mites in three of the four apiaries. All other measures were the same between the hygienic and commercial colonies. These promising results indicate that colonies headed by open mated hygienic queens are healthier and more profitable than unselected colonies.

Another critical aspect of this project is to help the beekeeping industry maintain the inseminated breeder lines of hygienic 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

Criteria	Measurement	Hygienic	Commercial	*P*
Frames Bees	range 1-20 frames	17.4 ± 1.38	17.3 ± 1.74	ns
Frames Brood	range 1-20 frames	10.1 ± 1.85	10.0 ± 1.52	ns

Temperament	# stings received:
	0 = none, 1 = one or more
		0.14 ± 0.32	0.02 ± 0.15	ns

Chalkbrood	# mummies on 2 frames:
	0= none; 1=<5; 2= 5-20;
	3=>20
		0.67 ± 0.85	1.78 ± 1.07	*

Hygienic assay	% freeze-killed brood removed within 48 hrs.	94.2% ± 12.16	82.3% ± 22.91	*
Honey Production	pounds harvested	90.0 ± 36.56	66.8 ± 32.20	*
Varroa mite load	# mites / 100 bees	0.6 ± 0.86	1.04 ± 1.09	*

Table 1. Comparison of hygienic (n=49) and commercial (n=46) colonies headed by open mated queens. Values shown for hygienic and commercial colonies are means ± std. dev. Evaluations of frames of bees, frames of brood, temperament, and chalkbrood were made in June 1996. Remaining measures were made in September 1996. All colonies in June were scored independently by 2 people and scores were averaged. Last column indicates whether values are statistically different: ns = not significant, $P > 0.05$. * = significant, $P \geq 0.05$ (2-way ANOVA comparing bee line and apiary site.)

at large. For example, methodological questions still arise regarding the introduction of queens into colonies. Also, factors related to colony performance, such as, queen longevity and egg laying rate, are reputed to differ between II and naturally mated (NM) queens. This summer we began to compare queen performance by measuring brood areas of inseminated and open mated queens. The queens were from the Carniolan stock maintained in a cooperative effort between the California Bee Breeders Association and Ohio State University. Preliminary data indicate that there was no significant difference in number of cells of brood within the two sets of colonies. An average frame of brood contains 3000 cells of brood; therefore, on average the inseminated queens produced 10.2 frames of brood, and the open mated queens produced 9.3 frames ($P > 0.05$). Comparisons of queen longevity and introduction will be conducted in 1997.

Short courses and instructional materials are effective means to foster technology transfer of techniques used in breeding programs. Currently, an introductory short course on beekeeping, and an intensive queen rearing course are offered at the Univ. of Minnesota every summer. A manual and video have been developed to accompany both courses. An intensive course on instrumental insemination is offered at the OSU which covers techniques used to maintain breeder stock. A manual and video currently are being developed to compliment the course on instrumental insemination.

Publications

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

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1997 25th Annual Apiary Inspectors of America Workshop "The Performance of Hygienic Honey Bees in a Commercial Apiary" Beltsville, MD March 4-5.

1997 Canadian Association for Professional Apiculturists and Manitoba Beekeeping Association. "The Performance of Hygienic Honey Bees in a Commercial Apiary" and "The Use of Botanical Oils to Treat Mites" January 24-25.

1997 American Honey Producers Assoc. "Are hygienic bees commercially profitable?" January 9-10.

1996 Almond Board of California. "Research Progress" Modesto, California. December 3.

1996 University of California - Davis, Dept. Entomology Seminar. "Hygienic Behavior and Defense against Varroa mites" December 4.

1996 X Congress Programa Nacional para el Control de la Abeja Africana, Veracruz, Mexico. "Breeding Programs for Honey Bees" August 1-4.

1996 University of Wisconsin, Madison. Dept. Entomology seminar. "Hygienic Behavior of Honey Bees." March 29.

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

Research Presentations: S. Cobey 1996-1997

1997 Ohio Apiary Inspectors Meeting Columbus, OH. "Selection For Mite Resistance" April.

1997 Instrumental Insemination Class. Coatepec, Verarcuz, Mexico. Beekeeprs Assoc. of Coatepec. March.

1997 Central Ohio Beekeepers Association, Columbus, Ohio. "Update On Bee Research At OSU"

1997 American Honey Producers Assoc. /AAPA Memphis "The Stumbling Blocks of Bee Breeding" and "Maintaining A Bee Breeding Program" January.

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