THE EFFECTS OF LOW LEVELS OF THE PESTICIDE MONITOR ON H0NEY BEE BROOD REARING ACTIVITIES

Report to: California State Alfalfa Seed Growers Association
Date submitted: December 1, 1986

Principle investigator: Dr. C.Y.S. Peng
Co-investigators: Dr. T. C. Webster
Mr. J. Marston
Department of Entomology
University of California at Davis

I. INTRODUCTION

This project was designed as an investigation of the effects of Monitor (methamidophos, an organophosphate pesticide) on honey bees. Our particular interest was in the ways in which this chemical affects the brood-rearing abilities of bee colonies. After a pesticide application, dead brood inside a hive may be less conspicuous than the many adult worker bees which usually die and accumulate in large numbers outside the entrances to hives. However, the long term consequences of brood loss in a honey bee colony are a reduced adult bee population, and hence a limited ability to produce honey or to pollinate crops.

It is also important to understand the effects of an exposure to pesticide, in terms of subsequent reduction in colony viability. We list here four possible ways for pesticide damage to occur. Any combination of these effects is possible.

1. Worker bees foraging in the field may be poisoned and die there or shortly after returning to the hive. This results in the pile of dead bees frequently seen after a pesticide application. The effect on the colony is a reduction in the amount of pollen and nectar being brought in. The colony must send new, inexperienced forager bees into the field to replace those that died. It will probably need to consume some of its stored honey and pollen. The colony may starve if stored food is not sufficient.

Signs of this effect : a) Orientation flight behavior characteristic of new, inexperienced foragers. b) Reduced honey and pollen stores. c) "Gentle" colony disposition because the bees most prone to sting, the forager bees, have died.

2. Foraging workers may return with pesticide to the hive, where it is fed to larvae and/or to the queen. This could cause the loss of many larvae, the queen may lay fewer eggs, the eggs she lays may not hatch, or the queen may die.

Signs of this effect : a) Disappearance of larvae and eggs. b) Disappearance of the queen.

3. Foraging workers may return with pesticide, and then feed it to nursing workers in the hive. These nursing workers then become poisoned in such a way that the glands they use to feed the larvae may atrophy. Eventually, the nursing workers are not able to feed the larvae very well, and many larvae die. Since these nurse bees also feed the queen, the queen may receive less food also.

Signs of this effect : a) Nursing workers with atrophied hypopharyngeal glands. b) Disappearance of larvae. c) Fewer eggs laid by queen. d) Disapppearance of queen.

4. Foraging workers may avoid the crop which has been sprayed because of the strong odor of pesticide. They may fly to more distant flowers, either another crop or other available

flowers. However, flight to another food source (i.e. flowers) would probably be less profitable for the bees and they would be expected to consume some of their stored honey and pollen, If no other food sources are available, the forager bees may remain inside the hive. This effect is somewhat like #1 above, because the general outcome is colony starvation rather than poisoning, except that the forager bees are not killed.

Signs of this effect : a) Reduced honey and pollen stores. b) "Aggressive" colony disposition, because the forager bees are in the hive rather than out foraging.

II. TESTS PERFORMED IN THIS PROJECT

Our project included three sets of tests which were designed to show which of these effects are important when honey bees are exposed to the pesticide Monitor. The first experiment was carried out by making observations of colonies placed in a large alfalfa field, before and after an aerial application of the pesticide. Our intention for this part of the project was to use a newly developed device to record brood cell activities inside the colonies. Because of persistant problems with the electronic circuitry involved in recording the signals from this device, we were not able to get data pertaining to brood cell activities, as originally planned and proposed. Consequently, we made measurements and observations that were not part of the original proposal in order to make a meaningful study of the effects of the pesticide application. Of the three colonies brought to the alfalfa field, one became infected with a brood disease and was not used for our study. Therefore, we measured the effects of the pesticide on two colonies. Data from only two colonies allow us to make important observations, but our conclusions must be somewhat tentative. This experiment was conducted at the Boswell Ranch, at Corcoran, California. The tests are indicated as follows:

A. Field tests conducted at Boswell Ranch:

1. The amount of brood in the colonies was measured before the pesticide was applied, and at two times afterward.

2. The amount of honey stored in the colonies was measured before and after pesticide application.

3. Nursing worker bees were sampled from the colonies, and their hypopharyngeal glands were removed for protein determinations. Bees were sampled before and after pesticide application.

4. The numbers of bees foraging for water at a barrel with water exposed to pesticide and a barrel protected from the pesticide were counted. These observations were meant to show

whether water exposed to the pesticide had a repellant effect on the bees. A repellant effect at the water barrels probably pertains also to the alfalfa field, since the field was sprayed in the same way as the barrels.

5. General observations of colony disposition (i.e. "aggressiveness"), numbers of dead bees in front of the hives, and numbers of foraging bees were made before and after pesticide application.

B. Feeding experiments with small colonies at Davis, California:

In addition, we performed feeding experiments at Davis, California, in which small colonies were fed low concentrations of Monitor in sugar syrup. These are as follows:

1. Tests were performed to determine the lowest concentrations of Monitor which affect brood rearing. This part of the project also allowed us to discover the stages of brood most susceptible to this pesticide: the egg and young larval, or the older larval stage. In these experiments, bees in small "nuc" hives were established outdoors. In each of the small colonies there were three frames for brood and honey storage. When the colonies had established brood, each received Monitor mixed in sugar syrup, which the bees took from feeders inside their hives. Bees feeding on sugar syrup are receiving nourishment in much the same way as if they were drawing nectar from flowers. The brood on one side of the center frame was censused just before the introduction of the pesticide, and again three days later. The brood in individual cells was recorded as being either in the egg, larva or pupa (capped cell) stage, at each census.

2. The viability of eggs laid by queens after bees in the small colonies consumed pesticide in sugar syrup was determined.

3. These experiments also included observations of the effect of the pesticide treatments on the queen's egg-laying behavior, the survival of the queens, and the repellant effect of the pesticide.

C. Experiments with electronic device for measuring brood cell activities:

This device has been designed to measure brood cell activities that are expected to change when a colony is exposed to pesticide in some manner. Since the problems which we have had with this technique are nearly resolved, we plan to use it in early 1987. Our tests will be measurements of changes in brood cell activities that are associated with pesticide exposure.

III. RESULTS AND DISCUSSION

A. Field study at Boswell Ranch

The results of these experiments indicate that the aerial application of pesticide did not have a dramatic effect on brood rearing in the colonies which were exposed, during the 12 day period of our observations. We must emphasize, however, that this 12 day period is less the length of time for development from egg to adult bee. An effect over a longer time period is possible. An effect might have been seen also if more colonies had been exposed to the aerial application of pesticide.

The amount of uncapped (younger) brood in Colony A (Table 1) actually increased following pesticide application on the evening of Aug. 14. It is possible that some mortality occurred among older brood, since the amount of capped brood was seen to decrease. Data from Colony B cannot be directly compared to those from Colony A, since the queen in Colony Bwas removed and later replaced. However, the early decline in capped brood in Colony B (Aug. 14 - 19) might have been due to a small amount of pesticide-induced mortality in older brood.

The measurements of hypopharyngeal gland development (Table 2) also support the idea that the pesticide did not seriously damage brood rearing. Gland development increased following pesticide application, although it reached a low level at the final sample date, Aug. 26. Data for Colony B are provided for comparison, and show a decline in gland development that would be expected after the queen was removed.

Visitation to water barrels clearly shows that the pesticide had a repellant effect for bees that were foraging for water. Nearly all the bees foraging were on the barrel that had been protected by being covered with a plastic bag the night of the spray application. This remained true even after the two barrels were switched. After the switch, the bees foraged at the unprotected barrel in its new position. This indicated that a visual cue was not important for the bees, and that they preferred the protected barrel probably because it had less repellant odor.

Honey bee activity changed in some ways after the aerial spraying. While some foraging activity on alfalfa bloom was seen before the spray, virtually no bees were seen on alfalfa afterwards. Dead bees were observed in front of the colony entrances after the spray. The colonies were quite docile after the spray. Bees returning to hives after the spray exhibited a "zig-zag" approach characteristic of bees that learning to forage, and are replacing experienced foragers. These observatons point to a loss of foraging bees due to poisoning in the field.

The amount of honey stored in Colony A declined after the spray (Table 1). This supports the idea that the bees were obliged to consumed their stored food because forage from the alfalfa field was not available.

Overall, this study indicates that the primary effect of the pesticide application was to kill a large fraction of the adult workers foraging on alfalfa. This is the first of the four effects listed in the Introduction of this report. The pesticide also clearly had a repellant effect for the bees so the foraging bees which survived the pesticide application avoided the alfalfa crop. This is the fourth effect which we listed. There was no indication that pesticide was being brought into the hive by bees, although we cannot completely rule out that possibility. The overall effect of the pesticide application was a loss of incoming forage, and a need for the bees to draw upon their stored honey under food shortage conditions.

B. Small colony study at Davis

This controlled study showed that a pesticide concentration of approximately 1.0 ppm (parts per million) in sugar syrup was enough to affect brood mortality in the small colonies (Table 4). We can also see that the younger stages of brood were more vulnerable than the older stages. This was seen in Trial 3 in which the proportion of eggs later appearing as young larvae (the "egg-larva" column) was very low. Our statistical analysis of this data is forthcoming, and is expected to support these conclusions.

It is also apparent that the season or environmental conditions were important. The effect of a 1.0 ppm treatment in July was less than the same treatment in September. This is probably because of a greater availability of natural forage for the bees in July compared to September. It is likely that the bees brought in considerable nectar from flowers during July so that the pesticide they consumed was, in a sense, diluted by this uncontaminated food.

The viability of eggs laid by the queen was not affected at all by the consumption of 1.0 ppm of pesticide in syrup in the colonies (Table 5). The proportion of eggs hatching was close to 90%, for queens in both the treatment and control colonies. It is possible that the queen never receives any pesticide when workers in colonies consume it in low concentrations.

ACKNOWLEDGEMENTS

Our work at the Boswell Ranch was made possible by the extensive assistance and cooperation of Mary Wadsworth. Linda Franke assisted with many stages of the project at Davis, including pilot studies, construction of small disposable hives and frames, and censuses of brood in small colonies exposed to pesticide. This work was funded by a grant from the Alfalfa Seed Growers.

FIELD STUDY AT BOSWELL RANCH AT CORCORAN, CALIFORNIA

TIME TABLE FOR FIELD STUDY

Aug. 7, 1986	University bee hives established in field site, on Boswell ranch near Corcoran, CA.
Aug. 12 - 14	Data and sample collection
Aug. 14 (evening)	Aerial application of pesticide to field site
Aug. 16 - 19	Data and sample collection
Aug. 25	Sample collection. Bee hives returned to Davis (evening)
Aug. 26	Data and sample collection from hives returned to Davis

TABLE 1. AMOUNT OF BROOD AND HONEY IN COLONIES MEASURED BEFORE AND AFTER AERIAL APPLICATION OF PESTICIDE

	Capped brood (in. ^{2})	Uncapped brood (in. ^{2})	Honey (frames)
Colony A			
Aug. 14	350	250	9.5
Aug. 19	275	325	8.5
Aug. 26	150	450	7.0
Colony B			
Aug. 14	375	250	9.0
Aug. 19	250	0	10.0
Aug. 26	50	150	9.5

Amounts of brood and honey in colonies before and after aerial application of pesticide, on the evening of Aug. 14. Capped brood is pupae and prepupae. Uncapped brood is eggs and larvae. The amount of brood is expressed in terms of the total number of square inches of frames covered with brood in the colony. The queen in colony B was removed on Aug. 14, in order to allow bees in that colony to produce queen cells. She was replaced on Aug. 19. Changes in the amount of brood reflect this temporary absence of the queen.

TABLE 2. ASSESSMENT OF BROOD REARING POTENTIAL BY MEASURING PROTEIN CONTENT OF NURSE BEE HYPOPHARYNGEAL GLANDS

	Aug. 14	Aug. 16	Aug. 17	Aug. 18	Aug. 26
Colony A	161 (9)	278 (10)	347 (10)	291 (10)	126 (10)
Colony B	178 (11)	156 (10)	115 (10)	----	----

Mean protein content (in micrograms) of hypopharyngeal glands. The numbers of bees sampled on each day in in parentheses.

TABLE 3. VISITATION TO WATER BARRELS

	Protected barrel	Unprotected barrel
Aug. 17 pm	14 (7)	0 (7)
Aug. 18 am	10 (5)	0 (5)
Aug. 18 pm	21 (2)	0 (2)
Barrels switched [a]		
Aug. 18 pm	19 (1)	2 (1)
Aug. 19 pm	28 (3)	3 (3)

Mean number of bees visiting water barrels for water collection, on three days following aerial application of pesticide, which occurred on the evening of Aug. 14. The number of observation times are given in parentheses.

a) On Aug. 18, the positions of the protected and the unprotected barrels were switched.

SMALL COLONY STUDY AT DAVIS, CALIFORNIA

TABLE 4. BROOD SURVIVAL IN SMALL COLONIES

	Egg-egg	Egg-larva	Larva-larva	Larva-pupa	Larva-larva or pupa
Trial 1 :					
May - June, 1986					
control (6)	----	63%	----	----	90%
0.1 ppm (9)	----	53%	----	----	83%
Trial 2 :					
July, 1986					
control (9)	42%	49%	36%	47%	
0.2 ppm (9)	29%	53%	30%	42%	
0.5 ppm (10)	31%	43%	37%	43%	
1.0 ppm (9)	26%	53%	35%	51%	
Trial 3 :					
Sept., 1986					
control (9)	39%	41%	38%	31%	
1.0 ppm (9)	38%	14%	21%	24%	
5.0 ppm (9)	24%	4%	21%	25%	

The mean proportion of brood in surviving in small colonies, according to the stage they were in before pesticide introduction and the stage observed three days later. For instance, "egg-egg" refers to the proportion of the eggs that were observed as eggs three days later. "Egg-larvae" are the proportion of the eggs for which larvae were observed three days later. Colonies were fed pesticide mixed with sugar syrup at the concentrations indicated. Control colonies consumed syrup without pesticide. The numbers of colonies tested for each group are given in parentheses.

TABLE 5 : EGG VIABILITY

	Control	1.0 ppm
Replicate 1	90.5% (84)	92.5% (93)
Replicate 2	90.2% (82)	86.2% (87)
Replicate 3	89.5% (95)	90.3% (103)
Total	90.0% (261)	89.8% (283)

Viability of eggs from queens in small colonies of bees which had consumed Monitor mixed in sugar syrup. Eggs from queens in three colonies which had been fed sugar syrup only and from three colonies which had been fed 1.0 ppm Monitor in sugar syrup were observed for viability. The number in parentheses is the total number of eggs observed from each queen.