Varroa in the Mating Yard: II. The Effects of *Varroa* and Fluvalinate on Drone Mating Competitiveness[a]

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Revised manuscript received for publication Dec. 14, 1998

ABSTRACT

An experiment was conducted to determine whether drones that are produced in *Varroa*-infested or Apistan®-treated honey bee, *Apis mellifera*, colonies are able to mate and produce worker offspring in competition with drones from uninfested and untreated colonies. The experimental treatments were identified in workers and drones using a DNA restriction fragment length polymorphism (RFLP). Virgin queens naturally mated to the three types of drones at an isolated mating area. It was found that the ratio of workers descended from each type of drones was the same as the ratio of parental drones available for mating. This indicates that the drones that survive to mating age from *Varroa*-infested, Apistan®-treated and control colonies are equally able to mate and produce offspring.

INTRODUCTION

In the last few years there have been reports that new queens were not surviving introduction into colonies or were being superseded within the following two to three months (Webster 1998, Williams 1998). There is a question as to whether the reported problem of queen performance is related to varroa mites. Varroa could affect queen establishment through interfering with drone production, survival, or mating. Therefore, in 1997 we designed experiments to determine whether varroa infestations or chemical treatments to control varroa were having an effect on drone production, survival, and competitive mating ability.

Other articles (Rinderer *et al.* 1998, de Guzman *et al.* 1998) report findings on the effects of varroa, Apistan®, and formic acid on the production, survival and semen production of drones. However, there are still other aspects of mating that could be affected by varroa or Apistan®.

It is critical to determine if drones from Apistan®-treated or varroa-infested colonies that survive to mating age are able to mate successfully. We report here the results of an experiment to measure the competitive mating ability of drones from varroa-infested, Apistan®-treated and untreated (control) colonies. Specifically, we asked, do mature drones from each of the treatment groups have equal probability of mating and producing offspring? Molecular markers were used to identify the parental drone types and distinguish the offspring of each type of drone.

MATERIALS AND METHODS

To minimize variation among treatment groups, a group of colonies headed by queens purchased from one queen producer was used for the test colonies. These colonies were screened for their DNA patterns for DNA RFLP probe #27 (lambda phage clone available from HAS) and 3 distinguishable patterns were identified using the methods detailed in Oldroyd *et al.* (1994). A group of colonies was chosen with each pattern and assigned to one of the treatments. All colonies were started from packages that had been treated with Apistan® to suppress mite populations.

Mites were encouraged to develop in the varroa treatment group by adding frames of emerging mite-infested drone brood into the colonies. By June 9, when the sealed drone brood combs were moved to nurse colonies, 9% of the sampled worker brood and 42% of the sampled drone pupae were infested. The Apistan® treatment group was treated with Apistan® strips (10% fluvalinate, according to the manufacturer's directions), while the experimental drones were being reared. The control group was not inoculated with mites or treated with Apistan@. All of the experimental drones were reared in drone combs that had been recently drawn under conditions to minimize the possibility of Apistan® residues.

After the treatments began, drones were raised from each group, transferred to nurse colonies for emergence, marked, counted, raised to sexual maturity, counted again, and then taken to a completely isolated coastal mating area on Marsh Island, Louisiana. There was a high mortality of drones in both treatment groups (data not reported) but all surviving treatment drones were used. To avoid biasing the mating results, the number of surviving control drones released was arbitrarily reduced to somewhat less than the total of the two treatment groups. The high early mortality of the treatment drones was not anticipated and we then expect-

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[a]Mention of a proprietary product does not constitute an endorsement by U. S.D.A.

ed there might be further mortality. To avoid providing too few drones and thereby producing inadequately mated queens, we decided to provide approximately equal numbers of drones from the control group and from both treated groups together. We felt this was preferable to further reducing the number of control drones to approximately the same as each of the treated groups, which had been our original intention. Sister virgin queens from a single queen mother were allowed to mate and produce offspring. The virgins and drones were taken to Marsh Island for mating on June 23 and brought back to Baton Rouge on July 3. Samples of brood were collected from these 21 queens and stored at -70° C. DNA was extracted from 50 workers from each queen and processed to produce RFLP banding patterns using the methods detailed in Oldroyd *et al.* (1994).

RESULTS AND DISCUSSION

In this experiment, those drones that were able to survive to mating age were equally competitive, regardless of varroa mite infestation or Apistan® treatment.

The overall percentage of daughter workers of each mating type is almost the same as the percentage of parental drones of that type that were available to mate (Table 1). Therefore, no statistical analysis was conducted. Several colonies had very different ratios of the three types of workers than the overall ratio. The DNA test we used could only distinguish which type of drones mated with a queen, but could not determine the number of drones that mated with a queen. Some queens may have only mated with a few drones or only one type of drones. In any case, the individual queen differences average out over the 21 queens.

Early drone mortality has been demonstrated to result from exposure to varroa and Apistan® (Rinderer *et al.* 1998). Since we were not able to identify individual drones which had been parasitized by varroa, we do not know that the surviving drones from the varroa treatment colonies were themselves actually parasitized by varroa. Therefore, the drones, from the varroa treatment colonies, that survived and mated may have been only unparasitized drones. However, it may be assumed that all of the drones in the Apistan® treatment colonies were equally exposed to Apistan®.

Choi and Woo (1974) reported that *Varroa* caused a reduction of pupal weight in workers and drones. In drone pupae, from 1 to 8 mites per pupa caused nearly a 10% loss in pupal weight while 10 to 13 mites caused about a 20% loss. Schneider (1986) found a correlation between the degree of infestation of drone brood and a reduction in emergence weight, size of seminal vesicles and mucus glands. The number of spermatozoa decreased by 50% when a drone pupa was infested with more than 3 mites. The impact on drone survival to maturity is probably more important than the effect on the number of spermatazoa. Since a queen will normally mate with a large number of drones and not all of the semen is transferred to and stored in the spermatheca (Woyke 1962), a varroa-damaged drone that survives to mate, even with a reduced number of spermatozoa, may be nearly as fit as a normal drone. However, a drone that does not survive to mate has zero fitness. This experiment indicates that drones from varroa-infested, Apistan® treated and control colonies produce worker offspring in the same proportion as the drone availability during mating, when about 90 drones per queen are available for mating. However, this might not be the case if insufficient drones are available for mating. It is possible that the deficiencies in the drones reported by Rinderer *et al.* (1998) could reduce the sperm contribution in the spermatheca of the queen if the queen mates with insufficient drones. Under such conditions, a higher percentage of the drones would mate and a higher percentage of each drone's sperm may enter the spermatheca. Less fit drones from *varroa*-infested and Apistan®-treated colonies would be more likely to mate with queens. Their reduced sperm production could then further reduce the amount of sperm actually entering the spermatheca compared to normal drones.

It is important to understand that the presence of many drones in a colony does not necessarily mean that there are sexually mature drones. There may be an abundance of immature drones, many of which will not survive to sexual maturity if the colonies are infested with *varroa* or treated with Apistan® during the drone production period. The production of properly mated queens depends on the availability of adequate numbers of sexually mature drones for mating. It is now clear that effective and safe methods of controlling varroa will make it much easier for queen producers to provide adequate numbers of sexually mature drones.

Table 1. Number of daughter workers of each parental drone type in each test colony.

	Treatment			
Colony No.	Varroa	Control	Apistan®	Total
1	1	27	21	49
2	7	19	23	49
3	3	29	16	48
4	0	9	39	48
5	21	24	2	47
6	6	29	9	44
7	4	8	38	50
8	31	18	0	49
9	0	37	6	43
10	17	13	8	38
11	1	22	27	50
12	48	2	0	50
13	7	15	28	50
14	3	36	11	50
15	0	31	11	42
16	0	29	20	49
17	15	30	5	50
18	10	16	20	46
19	50	0	0	50
20	4	28	9	41
21	8	35	4	47
Total daughter workers	236	457	297	990
percent	24	46	30	100
Total father drones	426	873	584	1883
percent	23	46	31	100

ACKNOWLEDGEMENTS

This research was conducted in cooperation with Louisiana Agricultural Experiment Station.

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* Reprinted from Volume 139, No. 3, March, 1999 American Bee Journal