Varroa in the Mating Yard: III. The Effects of Formic Acid Gel Formulation on Drone Production[a]

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ABSTRACT

The production of ample drones is essential to the commercial production of honey bee queens. Several factors such as the loss of feral colonies and new knowledge regarding the numbers of drones required for mating have made drone production more critical for commercial queen producers. Also, drone production is adversely effected by *Varroa jacobsoni* and Apistan®. Hence, this experiment was designed to determine the effects of treatment with formic acid gel on the production of drones. Formic acid treated colonies removed drone eggs from combs and delayed much of their drone production. Additionally, the formic acid reduced drone brood production: The treated colonies produced less than half as many drones as untreated colonies. Formic acid treatment reduced adult drone survival. Of drones ten days old, a much lower proportion survived in formic acid treated colonies (24%) when compared to the proportion of drones that survived in untreated colonies (49%). Additional recommendations are offered to queen producers to overcome the negative mating-yard consequences of *V. jacobsoni* infestation, Apistan® and formic acid use, the loss of drones through the loss of feral colonies, and the general loss of drones prior to sexual maturity.

When the goal of treating a colony to reduce *V. jacobsoni* numbers is to produce the largest possible number of healthy drones, formic acid should not be used, during active drone production. Also, in light of our overall observation that many drones do not survive to sexual maturity, even in colonies where stress factors are reduced as much as possible by management practices, one additional recommendation seems reasonable. Knowledge of early drone death should cause queen breeders to reconsider the number of drones that they must attempt to raise for their queen mating yards.

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INTRODUCTION

Since the discovery of *Varroa jacobsoni* in the United States in 1987, the parasite has become a major problem to beekeeping. Colonies throughout the country have acquired the mite and must be treated with miticide by beekeepers to prevent their death. Feral populations of colonies of honey bees are greatly reduced. Currently, most feral colonies are probably derived from recent swarms from colonies protected from the mites by beekeepers. *V. jacobsoni* feeds on pupal honey bees as they develop to adulthood. This feeding causes infested worker bees to have reduced body weight as adults (De Jong *et al.*, 1982, Engels and Schatton, 1986), sometimes to have deformed wings and abdomens (De Jong *et al.*, 1982) and to have a reduced life span (Ritter *et al.*, 1984; Buhlmann *et al.*, 1984). The mites also feed on adults between reproductive periods in brood. This feeding leads to a loss of proteins (Weinberg and Madel, 1985), and the possible spread of virus (Ball, 1985; Sammataro, 1997) and bacteria (Kosh and Ritter, 1987, Glinski and Jarosz, 1984). These various effects of feeding lead generally to a complex of symptoms called parasitic mite syndrome (Shimanuki *et al.*, 1994) which culminates in a sudden loss in numbers of worker bees in a colony and subsequent death of the colony.

Drones are about eight times more likely to be parasitized than worker bees (Fuchs, 1990; Schulz, 1984) and the effects of parasitism on drones can be devastating. In a study of the effects of varroa on drones, Rinderer *et al.* (1998) found that only 59.7% of drones emerging in *V. jacobsoni*-infested colonies survived their first day of adult life. In contrast, drones that emerged in control colonies were mostly (97.5%) alive after one day. These trends continued as the drones developed to sexual maturity. Varroa infestation also tended to have negative effects on drone weights, mucus gland and seminal vesicle weights, and numbers of spermatozoa. Since quality mating of commercial queens depends upon an ample supply of drones, they must be produced in colonies treated to reduce the numbers of *V. jacobsoni.*

In the same experiment, Rinderer *et al.* (1998) also found that Apistan treatment® produced similar although smaller negative effects on drones. Significantly fewer (86.1 %) of the drones that emerged in colonies treated with Apistan® were alive after one day. Apistan® treatments also had small negative effects on the

weights of drones and on the weights of their mucus glands.

Clearly, it is important for queen producers to protect colonies intended to produce drones from *V. jacobsoni* infestation. Because of the negative effects of Apistan® on drones, it would be ideal for treatments to end prior to the development of drones in the colonies. However, seasonal constraints do not always allow early treatment. Also, where colonies are required to produce drones for several months, mite populations may develop to harmful levels toward the end of the queen production season. Formic acid in gel formulation may offer a control of *V. jacobsoni* in drone producing colonies throughout the queen breeding season. Hence, this experiment was designed to determine the effects of formic acid treatment on the development and production of drones.

MATERIALS AND METHODS

In late June, 1997, ten colonies were chosen that each had an established laying queen, about 35,000 cells with brood, and 40,000 to 50,000 worker bees. All colonies had some drone brood, indicating a tendency to rear drones. The colonies had been treated with Apistan® earlier in the year. Colonies were chosen in which *V. jacobsoni* infestations were at low levels, since *V. jacobsoni* is known to adversely affect drones.

On June 26, 1997, colonies were each given a comb (42.5 cm X 15 cm) that had drone-sized cells. These combs were drawn from drone foundation and darkened by the production of many generations of brood. By June 27, 1997, queens in all colonies laid eggs in 70% or more of the cells in the drone comb. Five of the colonies, selected at random, were given formic acid gel packs as described by Feldlaufer *et al.* (1997) throughout the course of the experiment. The other five colonies were not treated with any material and served as controls.

The colonies were inspected daily when adult drones were emerging. Drones were reared above queen excluders which allowed the collection of all emerged drones. Dead drones were counted and discarded. All living drones were counted and three groups of 10 drones were weighed. Then, all living drones were paint marked, according to their day of emergence and their treatment group. The painted drones were returned to their colonies below the queen excluder, allowing them free flight as they became sexually mature. The colonies were inspected on appropriate days to determine both one day and ten day survival rates. Inspections were done in the morning prior to any drone flight.

When marked drones were at least 12 days old, they were considered sexually mature (Kurennoi, 1953) and about 30 were collected from each colony. The drones were dissected, the seminal vesicles and mucus glands were weighed and spermatozoa were counted. For the spermatozoa count, a single seminal vesicle from each drone was macerated in 10 ml 0.5% saline solution. Total spermatozoa from the one seminal vesicle was estimated using a hemocytometer and light microscope(Rinderer *et al.*, 1985).

Statistical Analyses

Five separate statistical analyses were performed. The dependent variables were drone survival, weight of ten drones, seminal vesicle weight, mucus gland weight, and spermatozoa count. In each analysis the independent variable consisted of the two treatment groups, formic acid and no treatment, or control. The drone survival data were analyzed as a completely randomized design with a one-way repeated measure treatment structure using a generalized linear model for binary data (Chambers and Hastie, 1992). For the spermatozoa count data, a generalized linear model for Poisson response data was used (Chambers and Haste, 1992). The dependent variables weight of ten drones, seminal vesicle weight, and mucus gland weight were analyzed using a two sample *t*-test. The number of drones produced in the two treatment groups over time was not analyzed using inferential statistics due to no production in two of the three time periods for the control group (Table 1). Despite this, it is obvious that a practical significance exists between the numbers of drones produced in the formic acid treated colonies and in the control colonies.

RESULTS

Drone production

Drone production was substantially hindered by the formic acid treatment. We observed that many eggs were removed from drone combs during the first one to five days of treatment by the treated colonies. All colonies had drone combs with eggs in at least 70% of the cells at the beginning of treatment. In the first week of treatment, treated colonies reduced the number of drone eggs to about 10% of the cells in drone combs having them. After the treatment had been on the colonies for about a week, much of the formic acid had evaporated. At that time, eggs were laid in the drone comb. This second round of egg laying accounts for the adult emergence timing reported in Table 1. However, the numbers of drones were also affected. Formic acid treated colonies produced less than half the number drones that were produced by the control colonies.

Drone survival

Not only were there strong differences in the production of drones, the survival of the drones also differed strongly (Table 2). At the end of a 1 day emergence period, most of the drones that emerged in control colonies were alive (94%) as were the drones that emerged in those colonies treated with formic acid (97%). However, by day 10, a much lower proportion of drones survived in formic acid treated colonies (24%) when compared to the proportion of drones that survived in untreated colonies (49%).

Weights of drones

The weights of recently emerged drones in the two groups were not significantly different. Groups of ten drones from formic acid treated colonies weighed 2.567 ± 0.1137 grams ($\bar{x}$ ± S.D.), while groups of ten drones from control colonies weighed 2.562 ± 0.1543.

Seminal vesicle weights, mucus gland weights, and spermatozoa numbers

Seminal vesicles and mucus glands of drones from colonies treated with formic acid and from the untreated control colonies

Table 1. Drone Production for three periods and total drone production in colonies treated with formic acid and untreated colonies as controls.

Treatment Group	Days of Drone Emergence, Numbers of Drones and Percentages of Total Production*			
	Days 1 through 6	Days 7 through 12	Days 13 through 19	Total Drones
Formic Acid	705 (46%)	670 (43%)	174(11%)	1549
Control	3800 (100%)	(0%)	(0%)	3800

*** Day one is the first day adult drones emerged. The numbers of drones are totals for each treatment group.**

Table 2. Average one and ten day survival of drones following emergence in five colonies treated with formic acid and 5 colonies not treated as a control.

Treatment Group	Average Number (Percentage) of Surviving Drones		
	Total Emerging Drones	Day 1 Survival*	Day 10 Survival
Formic Acid	344 (100%)	333 (97%) NS	94 (24%) S
Control	760 (100%)	720 (94%) NS	358 (49%) S

*** Day one is the first day adult drones emerged. The numbers of drones are totals for each treatment group. NS = not significantly different (P>0.05), S = significantly different (P<0.05).**

Table 3. Average ($\bar{x}$ ± S.D.) seminal vesicle weights (mg), mucus gland weights (mg) and spermatozoa numbers in one seminal vesicle of sexually mature drones produced in five colonies treated with formic acid and 5 colonies not treated as a control.

Treatment Group	Characteristic		
	Seminal Vesicle Weights	Mucus Gland Weights	Spermatozoa Numbers
Formic Acid	0.0036 ± 0.003 NS	0.01418 ± 0.0006 NS	$4.670 \times 10^6 \pm 9.4 \times 10^5$ S
Control	0.0035 ± 0.00009 NS	0.01418 ± 0.0012 NS	$3.264 \times 10^6 \pm 9.4 \times 10^5$ S

NS = not significantly different (P>0.05), S = significantly different (P<0.05).

were very similar (Table 3). Drones from colonies treated with formic acid averaged about 40% (P = 0.05) more spermatozoa than the average drone from control colonies.

DISCUSSION

Formic acid treatment caused several negative effects on drone production and drones, themselves. The formic acid treated colonies produced less than half the number of drones compared to controls and their drone production was delayed by several days. Adult drones that were produced had a much higher tendency to disappear from their colonies before sexual maturity. Most likely this disappearance was associated with death rather than drifting, since they were paint marked and marked drones were not observed in nearby colonies when they were inspected.

Formic acid treatment did not adversely affect drone weight, or the weights of mucus glands or seminal vesicles. Indeed, formic acid treatment resulted in surviving drones having a higher average number of spermatozoa. This, however, may be a result of so many of the drones from formic acid treated colonies having died before sexual maturity when spermatozoa counts were made. We speculate that perhaps only the most vigorous drones having higher spermatozoa counts survived.

We previously offered several recommendations to queen producers related to experiments determining the effects of *V. jacobsoni* and Apistan® (Rinderer *et al.*, 1998). We suggested that: 1) Drone source colonies should be supplied in sufficient numbers, 2) Drone source colonies should be treated for *V. jacobsoni* just before drone rearing begins or, if that is not possible, during drone rearing. 3) Monitoring *V. jacobsoni* infestation levels in drone brood is a better indicator of final effective drone abundance than the presence of drone brood or adults. This is recommended since colonies with heavy *V. jacobsoni* infestations in drone brood may appear to have ample drone brood and may have many drones walking on combs and taking orientation and cleansing flights. However, if their colonies are infested with *V. jacobsoni*, most of these drones will die before sexual maturity. The results of this experiment result in a fourth recommendation: When the goal of treating a colony to reduce *V. jacobsoni* numbers is to produce the largest possible number of healthy drones, formic acid should not be used, during active drone production.

In all of our experiments that have monitored drones through early adult life, we have noted that about half of the drones in control colonies, which have not been exposed to *V. jacobsoni*, Apistan®, or formic acid, have died before sexual maturity. This suggests that drone survival is low in the best of circumstances. The cause may reside in the genetic haploid nature of drones. However, whatever the cause, drones are clearly negatively affected by the three stresses of *V. jacobsoni*, Apistan®, and formic acid. Indeed, other stressful circumstances such as nutritional deficiencies, agricultural chemicals, etc. may also lead to early death of drones.

In light of our overall observation that many drones do not survive to sexual maturity even in colonies where stress factors are reduced as much as possible by management practices, one additional recommendation seems reasonable. Knowledge of early drone death should cause queen breeders to reconsider the number of drones that they must attempt to raise for their queen mating yards.

Several other factors have already caused concern about the numbers of drones available for mating in queen mating yards. First, the majority of the feral honey bee colonies in the United States have died because of *V. jacobsoni* (Kraus and Page, 1995; Loper, 1997). Surveys of the occurrence of feral colonies prior to the arrival of *V. jacobsoni* have shown that feral honey bee colonies once were abundant (Seeley, 1978). Probably, the typical queen mating apiary was once surrounded by a multitude of feral colonies which contributed hundreds of thousands of drones which mated with commercially produced honey bee queens.

These feral honey bee colonies and the drones they produced are now mostly gone because of *V. jacobsoni* infestations. Also, recent studies using precise DNA methods indicate that *Apis mellifera* queens mate with about 20 drones (Estoup *et al.*, 1994, Neumann *et al.*, 1998). This is more than twice the number of drones that were thought to mate with an *A. mellifera* queen prior to the availability of DNA analysis (Page end Metcalf, 1982). Queens that mate with too few drones are quickly superseded when placed in commercial colonies (Camargo and Goncalves, 1971). Recent evidence suggests that in many circumstances, commercially produced queens in the United States appear to have mated with an insufficient number of drones (Camazine *et al.*, 1998). Queen producers should be able to remedy this problem by increasing the numbers of drones that they raise for mating with queens.

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