Varroa in the Mating Yard: I. The Effects of *Varroa jacobsoni* and Apistan® on Drone Honey Bees[a]

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ABSTRACT

Although drones are more likely to be parasitized than worker bees by *Varroa jacobsoni*, the effects of *Varroa* infestation on drones have not been extensively researched. Likewise, the effects of Apistan® on drone honey bees are not known. We considered whether or not *Varroa* or Apistan® had an effect on the quality or quantity of drones that were produced in drone source colonies that were placed near mating apiaries by beekeepers and conducted these experiments to answer these questions. Colonies were established that exposed developing drones to *Varroa*, Apistan®, or neither as a control. The numbers of drones produced by the three types of colonies were quite similar. However, the survival of the drones differed strongly. At the end of a 1 day emergence period, the drones that emerged in control colonies were mostly (97.5%) alive. In contrast, significantly fewer (86.1 %) of the drones that emerged in colonies treated with Apistan® were alive. Only 59.7% of the drones emerging in *Varroa*-infested colonies survived their first day of adult life. These trends continued as the drones developed to sexual maturity. Results indicated that both *Varroa* infestation and Apistan® also had minor negative effects on drone weights, mucus gland and seminal vesicle weights, and numbers of spermatozoa.

Several recommendations are offered to queen producers to overcome the negative mating yard consequences of *Varroa* infestation and Apistan® use. 1) Drone source colonies should be specifically supplied in sufficient numbers. Based on current knowledge, we estimate that about 60 drones should be supplied for each queen. Twenty drones that successfully mate are essential. In order to achieve this rate of success, additional drones must be supplied, since drones are often lost before mating. In addition, it is important to estimate the number of drones in relation to both the number of queens to be mated and the age of the drones in the drone supply colonies. Each cycle of queens into the mating nucleus colonies must be supplied with an adequate number of adult drones that are at least 12 days old to assure that they are sexually mature. 2) Drone source colonies should be treated for *Varroa* just before drone rearing begins or during drone rearing. Apistan® treatment causes substantial early drone mortality. However, this mortality is not as great as the drone mortality caused by *Varroa*. Where it is possible, queen breeders can reduce the premature death of drones produced by both causes by treating colonies to reduce *Varroa* numbers prior to rearing drones in the colonies. 3) Monitoring infestation levels in drone brood is a better indicator of final effective drone abundance than the presence of drone brood or adults. Colonies with heavy *Varroa* 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 they are infested with *Varroa*, most of these drones will die before sexual maturity.

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. 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 (Dejong *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, 1992). 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.

The loss to *Varroa* of colonies without chemical protection is so dramatic that other effects of parasitism have received little attention. Although drones are more likely to be parasitized than worker

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bees (Fuchs, 1990; Schulz, 1984), the effects of *Varroa* infestation on drones have not been extensively researched. One report suggests that drones are also adversely affected, but provides few details (Ritter, 1988). A second report indicates that *Varroa* parasitism reduces the level of glycoprotein expression of honey bee sperm (Del Cacho, 1996). A third report suggests that parasitized drone pupae may have reduced weight (Choi and Woo, 1974). In response to concerns about the quality of at least some commercially produced queens, we wondered if *Varroa* could be an underlying cause. Certainly the loss of feral colonies would reduce the number of drones available in the environs of commercial mating yards. We considered whether or not Varroa or Apistan® had an effect on the quality or quantity of drones that were produced in drone source colonies that were placed near mating apiaries by beekeepers. Mating with an insufficient number of drones may result in an early supersedure of queens (Camargo and Goncalves, 1971).

MATERIALS AND METHODS

Honey Bee Manipulations

Experiment 1.

Three types of colonies (five colonies per type) were established: those that had no or very few *Varroa*, those that had no or very few mites and were treated with Apistan®, and those that had a large number of *Varroa*. On February 28, 1997 fifteen 1.14 kg packages of bees were taken from a large pool of bees using the methods of Harbo (1986). Queens of the same commercial stock were placed into queen cages and then into the packages. Each package was given an Apistan® strip for package bees which hung into the cluster of worker bees. The packages were stored in a darkened cool room for 5 days and then installed into Langstroth hives comprised of materials that were free of *Varroa* and had never been exposed to any treatment for the control of mites. The treatment groups were spaced 50 M apart along a road . Within each group, 5 m separated colonies and entrances were systematically oriented to further discourage the drifting of bees between colonies. Colonies were reared using stimulative feeding of 50% sugar syrup and frames of pollen.

On March 10, 1997 each colony was given a frame containing drone comb. Within the next three days, all queens had laid eggs in half or more of the drone cells. At this time, two Apistan® strips per colony were placed into five of the hives, one strip on either side of the drone comb. One caged frame of drone brood which was heavily infested with *Varroa* was placed into each of the five colonies intended to have *Varroa* infestations in order to provide an inoculation of *Varroa* into the colonies. Mites from this emerging brood were allowed to enter the colonies and the cages with the emerged drones were removed from the colonies. The development of drones in the test colonies was monitored daily. The drone brood from the five colonies with *Varroa* was transferred to highly infested colonies one day prior to the cells being sealed in order to assure high infestation levels in the drone brood. After the drone brood was sealed, the frames were returned to their original colonies. The remaining five colonies contained drone combs that were not exposed to mites or Apistan® and produced drones which served as controls.

Prior to the emergence of drones, 100 cells of worker brood and 25 cells of drone brood were opened and examined for the presence of infesting *Varroa* mites. The remainder of the drone brood was placed in a hive chamber above a queen excluder so that emerging drones could be collected and paint marked. Two worker brood frames and a frame of honey were also placed in the hive chamber to assure an abundance of worker bees on the drone combs and abundance of food.

On each day of the three-day period that the drones emerged, the hive chambers containing the newly emerged drones were examined. Dead drones were counted and discarded. 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 and placed below the queen excluders, allowing them free flight as they became sexually mature. The colonies were inspected on May 13 and May 19 and surviving drones were counted to provide information on survival through time. An additional group of drones was produced from the combs, paint-marked and weighed on their emergence date of May 12.

When the drones were between 1 and 14 days old, the flight times of marked drones were recorded. Both incoming and outgoing drones were recorded. One colony in each of the three groups was observed simultaneously for two minutes. In the first minute, the incoming drones were recorded, in the second minute, outgoing drones were recorded. Each colony in each group was observed in rotation. Observations started before drones flew and ended after the flight had stopped.

When marked drones were at least 12 days old, they were considered sexually mature 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 was estimated using a haemocytometer and light microscope (Rinderer *et al.* 1985).

Experiment 2.

Unexpected drone mortality in experiment 1 may have diluted the real treatment effects of treatments and made them undetectable. Hence, a second experiment was conducted in which individual drones with known exposure to *Varroa* and Apistan® were followed to sexual maturity and measured for several characteristics related to reproduction.

For this experiment, nine colonies were treated with two Apistan® strips placed next to the drone comb. Ten colonies received no Apistan® treatment. In each colony, an empty drone comb was introduced for egg laying. On the 8th day, before the cells were capped, all drone frames were introduced into *Varroa*-infested colonies for infestation. On the 19th day, drone frames were examined under a dissecting microscope and cells were opened as drones started to chew their way out of the cells. This technique enabled us to know with certainty which drones were infested and to count the total number of *Varroa* inside the cells. In both treatments, newly emerged drones from each colony were grouped as uninfested (0 mites), or having from 1 to 5 infesting mites. Too few drones had more than 5 infesting mites to provide sufficient numbers to analyze. A maximum of 30 newly emerged drones from each group per colony were individually weighed. Drones from each colony were then paint marked to denote their colony number and their level of *Varroa* infestation. Marked drones were introduced in equal proportions into 3 host colonies not having Apistan® and remained there until sexual maturity. On the 12th day, drones were collected and their reproductive organs were dissected. Mucus glands and seminal vesicles were weighed and spermatozoa were counted. All marked drones were searched for in the storage colonies and the numbers surviving were recorded.

Statistical Analysis

Experiment 1.

All the data from experiment 1 were analyzed as a completely randomized design with a repeated measure treatment structure. Experiment 1 had five dependent variables: drone survival, weight of ten drones, seminal vesicle weight, mucus gland weight, and sperm count. For the drone survival data a generalized linear model for binary response data was used (Chamber and Hastie, 1992). The effects used to model the drone survival data were treatment (control, Apistan®, and infested), date of emergence, and date of inspection. A linear model was used to describe the weight data for drones, seminal vesicles, and mucus glands. For the sperm count data, a generalized linear model for Poisson response data was used (Chamber and Hastie, 1992). The effects used to model the weight and count data were treatment (control, Apistan®, and infested) and emergence dates.

Experiment 2.

All the data from experiment 2 were analyzed as a completely

randomized design with a one-way treatment structure. Experiment 2 had five dependent variables: drone survival, weight of ten drones, seminal vesicle weight, mucus gland weight, and sperm count. For the drone survival data, a generalized linear model for binary response data was used. The effects used to model the drone survival data were treatment (control and Apistan® and infestation level (0 *Varroa* and 1-5 *Varroa*). A linear model was used describe the weight date for drones, seminal vesicles, and mucus glands. For the sperm count data, a generalized linear model for Poisson response data was used. The effect used to model the seminal vesicle weight, mucus gland weight, and sperm count data was treatment (control and Apistan®). To account for colony heterogeneity, weighted least squares estimates were calculated, where the weights were the inverse of the variance of the dependent variable for a colony. The effects used to model the drone weight data were treatment (control and Apistan®) and infestation level (0 *Varroa* and 1-5 *Varroa*).

RESULTS

An inspection of infestation rates in the experimental colonies (Experiment 1) one day prior to the emergence of the drones confirmed that colony manipulations produced the expected levels of *Varroa* parasitism. Colonies intended as control (no or few *Varroa* and no Apistan® had only 1.0% infestation in drone brood, colonies treated with Apistan® had *Varroa* infestations below detection levels, and colonies that were intended to have *Varroa* had an average of 58.6% of their drones infested (Table 1).

Drone survival

The numbers of drones produced by the three types of colonies in Experiment 1 were quite similar (Table 2). However, the survival of the drones differed strongly. At the end of a 1 day emergence period, the drones that emerged in control colonies were mostly (97.5%) alive. In contrast, significantly fewer (86.1 %) of the drones that emerged in colonies treated with Apistan® were alive. Only 59.7% of the drones emerging in *Varroa*-infested colonies survived their first day of adult life. The percentage of drones surviving after one day in each group differed significantly from the survival percentages of drones in the other groups (P = 0.05).

All colonies lose drones through time. When the drones were between 5 and 11 days old, about half (53.6 %) of the marked drones in control colonies remained, 43.0% of the drones in the colonies treated with Apistan® remained, and about a third (33.1%) of the drones in colonies infested with *Varroa* remained. Again, the percentage of drones remaining in each group differed significantly from the percentages in the other groups. (P = 0.05).

By the time the drones were from 12 to 18 days old, the numbers of drones in each group was again reduced. About a third of the drones (37.5% and 33.1%) in control colonies and Apistan® treated colonies remained. The percentage of drones in the *Varroa* treated colonies was reduced to about 20%. This percentage is significantly less than the percentages for the other two groups.

The survival of drones known to be infested or not to be infested in experiment 2 followed similar trends (Table 3). Although survival was low in all groups, perhaps as a result of extracting the drones from the rearing cells, *Varroa* infestation caused significantly increased drone mortality in contrast to the mortality of drones not infested with *Varroa*. Once again, Apistan® treated drones that were not infested with *Varroa* had a significantly greater mortality than drones that were not treated with Apistan® and not infested with *Varroa*.

Weights of drones

In Experiment 1, (Table 4) a significant (P> 0.05) treatment by date interaction was found for the weight of drones. Drones which emerged on May 5 that had been reared while exposed to Apistan® weighed significantly less (P> 0.05) than the drones not exposed to Apistan®. Also, drones which emerged from combs containing drones that were infested with *Varroa* weighed significantly less (P> 0.05) than the drones not infested with *Varroa*.

These suggestions of weight reductions in drones caused by Varroa and Apistan® were confirmed by the results of Experiment 2 (Table 5). In the comparison of drones known to be infested or not infested with *Varroa*, those drones infested with *Varroa* weighed about 7% less than drones not infested with *Varroa* (P < 0.00001). Also, drones exposed to Apistan® weighed about 5% less than drones not exposed to Apistan® (P < 0.05).

Seminal vesicle weights, mucus gland weights, and sperm counts

In Experiment 1, (Table 6) seminal vesicle weights and sperm counts were not differentially affected by *Varroa* infestation or

Table 1. Experiment 1. *Varroa* infestation in drone brood studied and worker brood in experimental colonies one day prior to the emergence of experimental drones.

Colony treatment	Percentage of brood infestation	
	Drone	Worker
Control	1.0	0.6
Apistan®	0	0
Varroa	58.6	16.2

Table 2. Experiment 1. The number ($\bar{X}$±SEM) and survival of drones produced in colonies having no *Varroa* and not treated with Apistan® (control), colonies treated with Apistan® and colonies treated with *Varroa* mites

Treatment	Number colonies	Total number of drones produced*	Percentage Survival to time		
			1 day	5 to 11 days	12 to 18 days
control	5	1128	97.49±0.01 a	53.64±0.07 a	37.53±0.07 a
Apistan®	5	1105	86.13±0.02 b	43.02±0.08 b	33.06±0.03 a
Varroa	5	1359	59.68±0.05 c	33.06±0.07 c	19.99±0.05 b

Analysis of variance

Factor	df.	mean square	F	P
Treatment (T)	2	4.46	0.24	0.75
Residuals (A)	12	18.42		
Drone Emergence (E)	2	62.63	10.06	0.0004
E*T	4	6.76	1.08	0.46
Residuals (B)	24	6.22		
Days (D)	2	948.27	299.41	0.0001
D*T	4	38.54	12.15	0.0001
D*E	4	7.39	2.33	0.063
D*E*T	8	1.79	0.56	0.80
Residuals (C)	72	3.17		

*All treatment groups produced similar numbers of drones (ANOVA P=0.67).

Table 3. Experiment 2. The survival of drones known to be infested with between 1 to 5 *Varroa* mite females and drones known not be be infested from ten colonies not treated with Apistan® and 9 colonies treated with Apistan®.

Infestation level	Colony Treatment	
	Varroa present	Apistan®
	11 day survival (proportion alive)	11 day survival (proportion alive)
Not infested	0.16122	0.11139
Infested with from 1 to 5 mites	0.02672	0.04412
Chi-Square for equality for proportions	1df 31.14 P<0.0001	1df 5.04 P=0.0001

Table 4. Experiment 1. The weights in grams ($\bar{X}$± SEM) of 10 living drones (two samples for each colony produced in colonies having no *Varroa* and not treated with Apistan® (control), colonies treated with Apistan® and colonies treated with *Varroa* mites (5 colonies per treatment) within three days of emergence for four emergence dates.

Emergence date	Treatment		
	Control	Apistan®	*Varroa*
May 2	2.694 ± 0.016 a	2.630 ± 0.005 a	2.574 ± 0.017 a
May 5	2.406 ± 0.097a	1.852 ± 0.084 b	2.432 ± 0.020 a
May 8	2.270 ± 0.671a	2.256 ± 0.696 a	2.556 ± 0.037 a
May 12	2.616 ± 0.001a	2.562 ± 0.029 a	1.604 ± 0.098 b

Analysis of variance				
Factor	d.f.	mean square	F	P
Treatment (T)	2	2.50	0.87	0.56
Residuals (A)	12	2.86		
Date (D)	3	0.49	10.06	0.18
D *T	6	0.71	1.08	0.04
Residuals (B)	35	0.29		

Table 5. Experiment 2. The weights in grams ($\bar{X}$± SEM) of individual drones infested with from 1 to 5 female *Varroa* produced in eleven colonies having no *Varroa* and not treated with Apistan® (control) or seven colonies treated with Apistan®.

Drone weights	Colony Treatment /infestation rate			
	Control		Apistan®	
	0 infesting mites	1-5 infesting mites	0 infesting mites	1-5 infesting mites
By treatment and infestation level	0.266 ± 0.020	0.249 ± 0.022	0.252 ± 0.020	0.235 ± 0.027
By infestation level	0 infesting mites		1-5 infesting mites	
	0.260 ± 0.021		0.244 ± 0.025	
By treatment	Control		Apistan®	
	0.259 ± 0.022		0.246 ± 0.024	

Analysis of variance				
	d.f.	Mean Square	F	P
Apistan® Treatment	1	0.037275	5.349	0.035
Treatment X Level	1	0.004375	0.627	0.440
Residual A	15	0.006967		
Mite Level	1	0.057770	167.397	0.000
Treatment X Level	1	0.000286	0.829	0.362
Residual B	829	0.000345		

Apistan® treatments. However, both seminal vesicle weights and sperm counts were numerically smaller in the *Varroa* and Apistan® treatment groups. Mucus gland weights were significantly less ($P < 0.05$) for drones reared with Apistan® when compared to the weights of mucus glands of drones in the control group.

In Experiment 2, (Table 7) the weights of mucus glands were again the most noticeably affected characteristic. In this case, *Varroa* infestation resulted in reduced mucus gland weights ($P < 0.05$). In colonies not treated with Apistan®, there was again a numerical tendency for the weights of mucus glands, weights of seminal vesicles and spermatozoa counts to be reduced by *Varroa* infestation. In colonies treated with Apistan® there was a tendency for the weights of seminal vesicles and numbers of spermatozoa to be increased for drones that were infested with *Varroa*. These two tendencies led to significant treatment by infestation rates for seminal vesicle weights ($P < 0.05$), mucus gland weights ($P < 0.05$) and a fairly strong interaction for number of spermatozoa ($P < 0.018$).

Flight times

Drones from all treatment groups followed similar patterns for both outgoing and incoming flights. The time at which flights began, the time at which flights ended, the peak times of flight and the number of flights all were similar for all groups.

DISCUSSION

The early death of drones resulting from infestation by *Varroa* or exposure to Apistan® is the most important information from this study. This is especially important in light of several other important circumstances. First, the majority of the feral honey bee colonies in the United States have died because of *Varroa* (Kraus and Page, 1995; Loper, 1997). Surveys of the occurrence of feral colonies prior to *Varroa* have shown that feral honey bee colonies once were very 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 *Varroa* mite infestations. Also, recent studies using precise DNA methods indicate that *Apis mellifera* queens mate with 15 to 20 drones (Estoup *et al.*, 1994) in comparison to the seven to ten that was once thought to be the number of drones that mated with an *A. mellifera* queen prior to the availability of DNA technology (Page and Metcalf, 1982). Estimates of the number of matings by *Apis mellifera* queens stand in contrast to estimates of the number of matings by other cavity nesting honey bees. *Apis cerana* queens mate with up to 27 drones (Oldroyd *et al.* 1998) and *A. koschevnikovi* queens mate with up to 40 drones (Rinderer et al., 1998). The estimates of mating numbers for *A. mellifera* were made in places having *Varroa*, *Varroa* control chemicals, and few or no feral honey bee nests. It may be that these estimates have been restricted by these circumstances and the more natural condition for *A. mellifera* queens is to mate with more than 15 to 20 drones.

In any event, it is reasonable to speculate that in some years in some commercial queen mating yards, *Varroa*, through its effects on drone survival and feral colony survival, may reduce the functional numbers of drones to insufficient numbers to assure adequate matings. Assuring an adequate number of matings is an essential component of producing quality commercial queens. Queens that mate with too few drones are quickly superseded when placed in commercial colonies (Camargo and Goncalves, 1971).

Consequently, we offer several recommendations to queen producers: 1) Drone source colonies should be specifically supplied in sufficient numbers. Based on current knowledge, we estimate that about 60 drones should be supplied for each queen. Twenty drones that successfully mate are essential. In order to achieve this rate of success, additional drones must be supplied, since drones are known to be eaten by predators and lost in other ways (Ambrose, 1978; Coleman, 1986; Grant, 1945). Also, the propagation of drones is relatively inexpensive and some additional number of drones can easily be supplied as a numerical buffer against various adverse circumstances such as sudden storms or a migration of predators that eat drones into the area of the queen mating yard. Some queen breeders may consider the supply of 60 drones per queen to be few in their specific circumstances. In addition to these considerations, it is important to estimate the number of drones in relation to both the number of queens to be mated and the age of the drones in the drone supply colonies. Each cycle of queens into the mating nucleus colonies must be supplied with an adequate number of adult drones that are at least 12 days old to assure that they are sexually mature (Kurennoi, 1953).

2) Drone source colonies should be treated for *Varroa* just before drone rearing begins or during drone rearing. Apistan® treatment causes substantial early drone mortality. However, this mortality is not as great as the drone mortality caused by *Varroa*. Where it is possible, queen breeders can reduce the premature death of drones produced by both causes by treating colonies to reduce *Varroa* numbers prior to rearing drones in the colonies. It is not always possible to treat colonies before drone production begins, since the industry

Table 6. Experiment 1. The weights in grams (X̄±SEM) of seminal vesicles, mucus glands and sperm counts from one seminal vesicle of drones produced in five colonies having no *Varroa* and not treated with Apistan® (control), five colonies treated with Apistan® and five colonies treated with *Varroa* mites.

Variable	Treatment		
	Control	Apistan®	*Varroa*
Seminal vesicle weights	0.003358 ± 0.000517	0.003199 ± 0.000491	0.002994 ± 0.000534
Mucus gland weights	0.01383 ± 0.00041	0.01320 ± 0.00050*	0.01353 ± 0.00113
Sperm counts	4.254×10^6 ± 4.236×10^5	3.593×10^6 ± 7.267×10^5	3.672×10^6 ± 4.707×10^5

Analysis of variance				
Analysis /Factor	d.f.	Mean Square	F	P
Seminal vesicle weights				
Treatment	2	2.8×10^{-13}	1.43	0.28
Control vs. Apistan®	1	5.6×10^{-15}	2.83	0.12
Control vs. Infested	1	7.8×10^{-13}	0.03	0.85
Residuals	11	2.0×10^{-13}		
Mucus gland weights				
Treatment	2	3.8×10^{-12}	2.78	0.11
Control vs. Apistan®	1	7.6×10^{-12}	5.39	0.04
Control vs. Infested	1	2.3×10^{-13}	0.17	0.69
Residuals	11	1.4×10^{-12}		
Sperm counts				
Treatment	2	1.1×10^{24}	2.24	0.15
Control vs. Apistan®	1	3.9×10^{17}	2.88	0.11
Control vs. Infested	1	2.0×10^{17}	1.48	0.26
Residuals	11	1.4×10^{17}		

* significantly different from control, P< 0.05.

Table 7. Experiment 2. The weights (X̄mg±SEM) of seminal vesicles of drones infested with from 1 to 5 female *Varroa* produced in eleven colonies having no *Varroa* and not treated with Apistan® (control) or seven colonies treated with Apistan®.

Variable	Colony Treatment /infestation rate			
	Control		Apistan®	
	0 infesting mites	1-5 infesting mites	0 infesting mites	1-5 infesting mites
Seminal vesicle weights	0.00346 ± 0.0003542	0.002797 ± 0.0004273	0.003045 ± 0.0002525	0.003167 ± .0.0004671
Mucus gland weights	0.01485 ± 0.000603	0.01257 ± 0.002198	0.01316 ± 0.0009152	0.01313 ± 0.0006103
Sperm counts	2.009×10^6 ± 1.019×10^6	1.236×10^6 ± 9.145×10^5	1.817×10^6 ± 6.916×10^5	2.017×10^6 ± 1.03×10^6

Analysis of variance				
Analysis /Factor	d.f.	Mean Square	F	P
Seminal vesicle weights				
Treatment	1	7.6484×10^{-9}	0.0516	0.822
Infestation rate	1	3.7200×10^{-7}	2.5123	0.127
Treatment x Infestation rate	1	7.2566×10^{-7}	4.9007	0.038
Residuals	22	1.4807×10^{-7}		
Mucus gland weights				
Treatment Residuals	1	2.0766×10^{-6}	1.2274	0.279
Infestation rate	1	1.0092×10^{-5}	5.9221	0.023
Treatment x Infestation rate	1	8.1779×10^{-6}	4.8337	0.039
Residuals	22	1.6918×10^{-6}		
Sperm counts				
Treatment Residuals	1	222923	0.4415	0.513
Infestation rate	1	362068	0.7171	0.406
Treatment x Infestation rate	1	964957	0.9111	0.181
Residuals	22			

places a premium on early queens. When it is not possible to treat colonies prior to the production of drones, it is preferable to treat the colonies rather than suffer the even greater loss of drones caused by *Varroa*.

3) Monitoring infestation levels in drone brood is a better indicator of final effective drone abundance than the presence of drone brood or adults. Colonies with heavy *Varroa* 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 they are infested with *Varroa*, most of these drones will die before sexual maturity. Only by painting drones according to age were we able to determine that the drones were lost before sexual maturity.

In addition to the death of drones caused by *Varroa* and Apistan®, surviving drones had several defects which may have adversely affected their reproductive success. Both *Varroa* and Apistan® caused drones to have about a 5% reduction in body weight. These effects appear to be additive since drones that were both infested with *Varroa* and exposed to Apistan® had about a 10% reduction in body weight. There was a tendency for both *Varroa* and Apistan® to reduce the weights of mucus glands, weights of seminal vesicles and spermatozoa counts. Apistan® significantly reduced mucus gland weights. However, drones that were both infested with *Varroa* and were exposed to Apistan® during development had numerically increased spermatozoa counts and seminal vesicle weights when compared to drones only exposed to Apistan®. Perhaps feeding mites differentially take materials from developing drones that cause reduced spermatozoa counts and seminal vesicle weights in Apistan®-treated drones. Collectively, this information suggests that, providing drones survive the initial mortality caused by *Varroa* infestation or exposure to Apistan®, they are left with some deficiencies that may restrict their potential to be fully successful as mates. Differences may not have been detectable for flight times because of the sampling procedure used to monitor drone flight or because the more adversely affected living drones in the treatment groups may not have flown at all. Also, flight may not be a good indicator of mating competitiveness.

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REFERENCES

AMBROSE, J T. (1978) Birds. *In*: Honey Bee Pests, Predators and Diseases. *Eds*. Morse, R A; Nowogrodzki, R. Cornell University Press 1978; pp 243-260.

BALL, B V. (1985) Acute paralysis virus isolates from honey bee colonies infested with *Varroa jacobsoni*. *Journal of Apicultural Research* 24: 115-119.

BUHLMANN, G; GERIG, L; VOM HOVEL, R; MULLER, B; PERSCHIL, F; RITTER, W; WILLE, H. (1984) Populationsdynamische untersuchungen an *Varroa*-befallenen fretfliegenden bienenvolkern in Baden-Wuerttemberg. 1. Teil: Welche parameter lassen sich aus den populationsmessungen der beinenvolker ableiten? *Apidologie* 15: 252-254.

CAMARGO, J M F; GONCALVES, L S. (1971) Manipulation procedures in the technique of instrumental insemination of the queen honey bee *Apis mellifera* L. (Hymenoptera: Apidae). *Apidologie* 2: 239-246.

CHAMBERS, J M; HASTIE, T. (1992) Statistical Models in S. Wadsworth & Brooks/Cole Advanced Books & Software, Pacific

BALL, B V. (1985) Acute paralysis virus isolates from honey bee colonies infested with *Varroa jacobsoni. Journal of Apicultural Research* 24: 115-119.

BUHLMANN, G; GERIG, L; VOM HOVEL, R; MULLER, B; PERSCHIL, F; RITTER, W; WILLE, H. (1984) Populationsdynamische untersuchungen an *Varroa*-befallenen fretfliegenden bienenvolkern in Baden-Wuerttemberg. 1. Teil: Welche parameter lassen sich aus den populationsmessungen der beinenvolker ableiten? *Apidologie* 15: 252-254.

CAMARGO, J M F; GONCALVES, L S. (1971) Manipulation procedures in the technique of instrumental insemination of the queen honey bee *Apis mellifera* L. (Hymenoptera: Apidae). *Apidologie* 2: 239-246.

CHAMBERS, J M; HASTIE, T. (1992) Statistical Models in S. Wadsworth & Brooks/Cole Advanced Books & Software, Pacific Grove, California.

CHOI, S Y; WOO, K S. (1974) Studies on the bionomics of bee mite, *Varroa jacobsoni* Oudemans, and its chemical control (II). (In Korean with English summary). *Research Review ORD* 16(L): 69-76.

COLEMAN, R. (1986) Birds take the buzz out of queen breeder profits. *American Bee Journal* 126: 208-209.

DEL CACHO, E; MARTI, J I; JOSA, A; QUILEZ, J; SANCHEZ-ACEDO, C. (1996) Effect of *Varroa jacobsoni* parasitization in the glycoprotein expression on *Apis mellifera* spermatozoa. *Apidologie* 27: 87-92.

DE JONG, D; MORSE, R A; EICKWORT, GC. (1982) Mite pests of honey bees. *Annual Review of Entomology* 27: 229-252.

ENGELS, W; SCHATTON, K. (1986) Changes in haemolymph and weight loss in worker bees due to *Varroa* parasitization. *Varroa* Workshop Feldafing, 5.

ESTOUP, A; SOLIGNAC, M; CORNUET, J-M. (1994) Precise assessment of the number of patriline and of genetic relatedness in honey bee colonies. *Proceedings of the Royal Society of London, Series B* 258: 1-8.

FUCHS, S. (1990) Preference for drone brood cells by *Varroa jacohsoni* Oud in colonies of *Apis mellifera carnica. Apidologie* 21: 193-199.

GLINSKI, Z F; JAROSZ, J. (1984) Alterations in haemolymph proteins of drone honey bee larvae parasitized by *Varroa jacobsoni. Apidologie* 15: 329-338.

GRANT, C. (1945) Drone bees selected by birds. *Condor* 47: 261-263.

HARBO, JR. (1986) Effect of population size on breed production, worker survival and honey gain in colonies of honey bees. *J. Apic. Res.* 21(1):22-29.

KURENNOI, N M. (1953) When are drones sexually mature? *Pchelovodstvo* 30: 28-30. (In Russian)

KRAUS, B; PAGE, R. (1995) Effect of *Varroa jacobsoni* (Mesotigmata, Varroidae) on feral *Apis mellifera* (Hymenoptera, Apidae) in California. *Environmental Entomology* 24: 1473-1480.

KOSH, W; PITTER, W. (1987) Laborversuche zum zusammenhang zwischen dem *Varroa* befall von adulten bienen und der bakterienkonzentration der hamolymphe. *Apidologie* 18: 382-383.

LOPER, G M. (1997) Over-winter losses of feral honey bee colonies in southern Arizona, 19921997. *American Bee Journal* 137: 446.

OLDROYD, B P; CLIFTON, M J; PARKER, K; WONGSIRI, S; RINDERER, T E; CROZIER, R. H. (1998) Evolution of mating behaviour in the genus *Apis* and an estimate of mating frequency in *Apis cerana* (Hymenoptera:Apidae). *Annals of the Entomological Society of America.*

PAGE, R E; METCALF, RA (1982) Multiple mating, sperm utilization, and social evolution. *American Naturalist* 119: 263-281.

RINDERER, T E; COLLINS, A M; PESANTE D G. (1985) A comparison of Africanized and European drones: Weights, mucus gland and seminal vesicle weight, and counts of spermatozoa. *Apidologie* 16(4):407-412.

RINDERER, T.E; STELZER, J A; OLDROYD, B P; TINGEK, S. (1998) Levels of polyandry and intracolonial genetic relationships in *Apis koschevnikovi. Journal of Apicultural Research.* In press.

RITTER, W. (1988) *Varroa jacobsoni* in Europe, the tropics, and subtropics. NEEDHAM, G R; PAGE R E JR; DELFINADO-BAKER, M; BOWMAN C E *Eds.* Africanized Honey Bees and Bee Mites. Chichester: Ellis Horwood Ltd.; 1988; pp 349-359.

RITTER, W; LECLERCQ, E; KOCH, W. (1984) Observations des populations d'abeilles et de varroa dans les colonies a differents neveaux d'infestation. *Apidologie* 15: 389-400.

SEELEY, T D. (1978) Life history strategy of the honey bee, *Apis mellifera. Oecologia* (Berlin) 32: 109-118.

SHIMANUKI, H; CALDERONE, N W; KNOX, D A. (1994) Parasitic mite syndrome: The symptoms. *American Bee Journal* 134:827-828.

SCHULZ, A E. (1984) Reproduction and population dynamics of the parasitic mite *Varroa jacobsoni* Oud. and its dependence on the brood cycle of its host *Apis mellifera L. Apidologie* 15:401-420.

SAMMATARO, D. (1997) Report on parasitic honey bee mites and disease associations. *American Bee Journal* 137: 301-302.

WEINBERG, K P; MADEL, G. (1985) The influence of the mite *Varroa jacobsoni* Oud. on the protein concentration and the haemolymph of the brood of worker bees and drones of the honey bee. *Apidologie* 16 421-436.

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