

## Evaluation of Drone Brood Removal for Management of *Varroa destructor* (Acari: Varroidae) in Colonies of *Apis mellifera* (Hymenoptera: Apidae) in the Northeastern United States

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**ABSTRACT** The efficacy of drone brood removal for the management of *Varroa destructor* Anderson & Trueman in colonies of the honey bee, *A. mellifera* L., was evaluated. Colonies were treated with CheckMite+ in the fall of 2002. The following spring, quantities of bees and brood were equalized, but colonies were not retreated. The brood nest of each colony consisted of 18 full-depth worker combs and two full-depth drone combs. Each worker comb had <math><12.9\text{ cm}^2</math> of drone cells. Standard management practices were used throughout the season. Colonies were randomly assigned to one of two groups. In the control group, drone combs remained in place throughout the season. In the treatment group, drone combs were removed on 16 June, 16 July, 16 August, and 16 September and replaced with empty drone combs (16 June) or with drone combs removed on the previous replacement date. In the early fall, the average mite-to-bee ratio in the control group was significantly greater than the corresponding ratio in the treatment group. Drone brood removal did not adversely affect colony health as measured by the size of the worker population or by honey production. Fall worker populations were similar in the two groups. Honey production in treatment colonies was greater than or similar to production in control colonies. These data demonstrate that drone brood removal can serve as a valuable component in an integrated pest management program for *V. destructor* and may reduce the need for other treatments on a colony-by-colony basis.

**KEY WORDS** *Varroa destructor*, *Apis mellifera*, drone removal, drone trap, IPM

THE PARASITIC MITE *Varroa destructor* Anderson & Trueman remains a major threat to honey bees and to those sectors of the agricultural community requiring honey bees for pollination services wherever beekeeping is based on the honey bee, *A. mellifera* L. Mite-resistant stocks of bees are available (Harbo and Harris 1999, 2001, 2003; Rinderer et al. 2001a, b), but their performance is variable, and it will require many years to incorporate them into the honey bee population. Consequently, colonies will require supplemental control measures for the foreseeable future.

Several chemical options are available in the United States. Apistan (fluvalinate) and CheckMite+ (coumaphos) are highly effective, whereas Sucrocide (sucrose octanoate) and Api-Life VAR (thymol) are somewhat less effective. However, pesticide resistance is a growing problem (Baxter et al. 1998; Elzen et al. 1998, 1999; Elzen and Westervelt 2002), and reinfestation also adds to colony mite loads (Greatti et al. 1992). Consequently, beekeepers continue to experience significant losses, especially during the late summer and fall when mite levels rapidly increase and end-stage symptoms known as parasitic mite syndrome occur (Shimanuki et al. 1994). A colony exhibiting early stages of this syndrome can usually be saved by the application of an effective miticide; how-

ever, in the northeastern United States, these symptoms typically occur during or just before the fall nectar flow when chemical treatments are proscribed by label restrictions. Delaying treatment until the end of the flow results in either the death of the colony or serious damage to the colony (Ritter 1981, DeJong 1990, Amdam et al. 2004). Therefore, methods that maintain low mite levels during the summer and early fall are needed to protect colonies until the end of the flow and the beginning of a legal treatment window.

The reproductive behavior of *V. destructor* suggests a nonchemical method for suppressing mite populations. Mites reproduce on their host's immature stage. Those that reproduce on drone brood average 2.2–2.6 female offspring per host, whereas those reproducing on worker brood average 1.3–1.4 female offspring per host (Schulz 1984, Fuchs and Langenbach 1989). Mites do not reproduce on queen brood (Romaniuk et al. 1988, Rehm and Ritter 1989, Harizanis 1991, Santillan-Galicia et al. 2002). Differences in fecundity are correlated with the duration of the capped stage of each host type, which is greatest in drones, intermediate in workers, and shortest in queens (Jay 1963). Host choice by female mites mirrors the reproductive opportunities afforded by the different host types. Mites are found more often on drone brood than on

worker brood, with average differences between five- and 12-fold (Grobov 1977; Sulimanovic et al. 1982; Issa and Goncalves 1984; Schulz 1984; Fuchs 1990, 1992; Boot et al. 1991; reviewed in Fries et al. 1994; Calderone and Kuenen 2001). Mites are rarely found on queen brood (Harizanis 1991, Calderone et al. 2002, Santillan-Galicia et al. 2002). Therefore, by removing capped drone brood from an infected colony, a disproportionately large number of mites is removed without adversely affecting the size of the worker population, and also mites with the greatest fecundity are removed.

In Europe, where drone brood removal has been used for many years, the practice typically involves the construction of special combs, the destruction of drone brood with the requirement that colonies build replacement drone comb, and short replacement intervals (Santas and Lazarakis 1984; Rosenkranz and Engels 1985; Marletto et al. 1990a, b; Marletto et al. 1991; Charriere et al. 2003). Others studies combine drone brood removal with additional, labor-intensive techniques such as a heat treatment (Brodsgaard and Hansen 1994), swarm control measures (Schmidt-Bailey et al. 1996), or, most commonly, a short broodless period created by temporarily caging the queen (Fries and Hansen 1993, Calis et al. 1999). Difficulties involved in implementing these methods have prevented their widespread adoption by beekeepers in the United States.

The goal of this study is to determine whether a simple application of the drone brood removal method using commonly available equipment can maintain mite populations at levels consistent with good colony health until the end of the fall flow and the beginning of a legal treatment window. In addition, because host-parasite population dynamics are highly sensitive to environmental conditions (Fries et al. 1994, Lodesani et al. 2002, Harris et al. 2003), I evaluated this method in the northeastern United States.

### Materials and Methods

Experimental colonies were kept in apiaries within 10 km of Ithaca, NY. Colonies were treated with CheckMite+ during the fall of 2002 according to label instructions. The following spring, 41 colonies were each reduced to a single full-depth hive body (50.48 by 41.28 by 24.5 cm) with 10, full-depth worker combs. Combs were covered with worker bees and contained the equivalent of eight combs of brood. Colonies were not retreated. A second full-depth hive body containing eight empty worker combs and two empty drone combs was added to each hive to complete the brood nest. Each of the 18 worker combs in the brood nest had <12.9 cm<sup>2</sup> of drone cells. The two drone combs were maintained in the upper brood chamber in the second and ninth positions. Standard management practices were used throughout the season, including the addition of honey supers above a queen excluder.

Each colony was randomly assigned to one of three apiaries, and colonies in each apiary were randomly assigned to one of two groups. In the control group,

drone combs were left in place throughout the season. In the treatment group, drone combs were removed on 16 June, 16 July, 16 August, and 16 September 2003 and replaced with empty drone combs (16 June) or with drone combs removed on the previous replacement date. When not in a colony, drone combs were kept in a freezer at -20°C. Bees were required to clean out the dead brood in the drone combs provided on the last three dates. Several variables were assayed between June and November 2003.

**Mite-to-Bee Ratios.** The ratio of the number of adult mites per adult bee in each colony was estimated from a sample of worker bees collected from brood combs on 7 October 2003 according to the method of Calderone and Turcotte (1998). Samples from each colony were collected after reducing colonies to two full-depth hive bodies, but before a fall application of CheckMite+.

Mite-to-bee ratios for each sample were converted to standardized 300-bee ether roll (ER) counts using the formula  $ER = (R \cdot B) / 1.783$ , where R is the mite-to-bee ratio, and B is the number of bees in a sample. The conversion factor (1.783) is from Calderone and Turcotte (1998). This conversion factor (reflecting 56.1% recovery of mites) is similar to the 59% recovery rate reported by Ellis and Baxendale (1994). Standardized 300-bee ether roll counts were used for comparisons with published economic thresholds.

**Fall Worker Population.** The fall worker populations, measured as the number of combs of adult bees at an ambient temperature of -2 to 0°C, were estimated on 14 November 2003 using the method of Nasr et al. (1990).

**Weight Gain.** Colony weight gain, primarily a measure of honey production (McLellan 1977), was determined by weighing colonies ( $\pm 0.23$  kg) on 11 June, 18 August, and 25 September 2003 and calculating weight gains or losses after adjusting for the weights of supers added and removed. Weight gains or losses were calculated for the periods 11 June-18 August (period 1), 18 August-25 September (period 2), and 11 June-25 September (seasonal gain).

**Analysis.** Data for the above-mentioned variables were analyzed with PROC MIXED (SAS Institute 1996) using a complete factorial, fixed effects model with treatment and apiary as main effects. Significant interactions between main effects were further analyzed with the Tukey-Kramer test (SAS Institute 1988). Proportion data (p) for mite-to-bee ratios were transformed using the arcsine $\sqrt{p}$  function to equalize variances. Colony weight gain data were transformed with the square root function to equalize variances.

**Number of Cells of Capped Drone Brood Removed from Treatment Colonies.** Drone combs were photographed after being removed from treatment colonies on each replacement date, and the number of cells of capped drone brood removed from each colony was determined by counting. Data are reported for informational purposes.

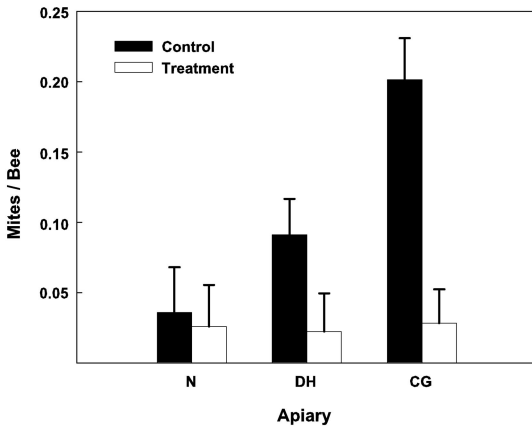


Fig. 1. Average mite-to-bee ratios in treatment and control groups in each of the three apiaries on 7 October 2003 (N, Nelson; DH, Durfee Hill; CG, Cole Grove). Average number of bees and mites per sample (AVG. and SE) and sample sizes (*n*) are given in Table 1. *P* values for comparisons of means within apiaries were determined by Tukey–Kramer tests: *P* < 0.70 for N, *P* < 0.01 for DH, and *P* < 0.0001 for CG.

**Results**

**Mite-to-Bee Ratios.** Mite-to-bee ratios in the two groups were significantly different. Colonies in the control group had an average ratio of 0.109 ± 0.017 (LSMean ± SE), compared with a ratio of 0.025 ± 0.016 in the treatment group (*F* = 21.27; *df* = 1, 35; *P* ≤ 0.0001). The lowest and highest ratios were 0.012 and 0.441, respectively, in the control group, and 0.000 and 0.070, respectively, in the treatment group. Apiary effects were significant. The average ratio was 0.110 ± 0.019 in the Cole Grove apiary, 0.067 ± 0.019 in the Durfee Hill apiary, and 0.031 ± 0.022 in the Nelson apiary (*F* = 6.23; *df* = 2, 35; *P* ≤ 0.0049). The interaction between treatment and apiary was significant (*F* = 6.02; *df* = 2, 35; *P* ≤ 0.0121). Tukey–Kramer tests revealed significant treatment effects in the Cole Grove and Durfee Hill apiaries (Fig. 1). The average numbers of mites and bees, mite-to-bee ratios, and standardized 300-bee ether roll counts are given in Table 1.

**Fall Worker Population.** The average fall worker populations in the two groups were not significantly different. The average number of combs of bees in the control group was 6.24 ± 0.68 (LSMean ± SE), compared with 6.71 ± 0.63 in the treatment group (*F* = 0.25; *df* = 1, 35; *P* ≤ 0.6204). Average worker populations in the three apiaries were not significantly different. The average number of combs of bees was 7.83 ± 0.77 in the Cole Grove apiary, 5.46 ± 0.75 in the Durfee Hill apiary, and 6.13 ± 0.88 in the Nelson apiary (*F* = 2.55; *df* = 2, 35; *P* ≤ 0.0925). The interaction between treatment and apiary was not significant (*F* = 0.94; *df* = 2, 35; *P* ≤ 0.4009).

**Weight Gain in Period 1.** Average weight gains in the control and treatment groups were significantly different. The average gain in the control group was 20.87 ± 3.12 kg (LSMean ± SE), compared with

Table 1. Numbers of mites and bees, mite-to-bee ratios and calculated ether roll counts (mean ± SE) for samples of brood nest bees collected on 7 October

Apiary	Group	Variable	<i>n</i>	Mean	±SE
Cole Grove	Control	Mites	6	49.67	10.74
		Bees	6	254.83	10.83
		Ratio	6	0.20	0.051
	Treatment	ER	6	33.89	8.65
		Mites	9	6.11	1.18
		Bees	9	221.89	16.20
		Ratio	9	0.03	0.01
		ER	9	4.75	0.90
		ER	9	4.75	0.90
Durfee Hill	Control	Mites	8	19.00	7.49
		Bees	8	227.50	9.81
		Ratio	8	0.09	0.04
		ER	8	15.32	6.89
	Treatment	Mites	7	4.71	1.69
		Bees	7	218.86	14.11
		Ratio	7	0.02	0.01
		ER	7	3.75	1.49
		ER	7	3.75	1.49
Nelson	Control	Mites	5	8.60	2.25
		Bees	5	242.80	16.59
		Ratio	5	0.04	0.01
		ER	5	6.02	1.61
	Treatment	Mites	6	6.67	1.52
		Bees	6	253.33	7.26
		Ratio	6	0.03	0.01
		ER	6	4.35	0.91
		ER	6	4.35	0.91

*n*, sample size; mites, number of mites in sample; bees, number of bees in sample; ratio, mites/bees; ER, standardized 300-bee ether roll count calculated using the formula ER = ((R\*B)/1.783)/(B/300), where R is ratio, B is bees, and the conversion factor (1.783) from Calderone and Turcotte (1998) and Ellis and Baxendale (1994).

30.76 ± 2.89 kg in the treatment group (*F* = 6.07; *df* = 1, 35; *P* ≤ 0.0188). Average gains in the three apiaries were also significantly different. The average gain was 12.59 ± 0.90 kg in the Cole Grove apiary, 8.45 ± 0.92 kg in the Durfee Hill apiary, and 7.61 ± 1.03 kg in the Nelson apiary (*F* = 8.85; *df* = 2, 35; *P* ≤ 0.0008). The interaction between treatment and apiary was not significant (*F* = 0.84; *df* = 2, 35; *P* ≤ 0.440).

**Weight Gain in Period 2.** Average weight gains in the control and treatment groups were not significantly different. The average gain in the control group was 36.02 ± 2.72 kg (LSMean ± SE), compared with 38.09 ± 2.52 kg in the treatment group (*F*<sub>1, 35</sub> = 0.44; *df* = 1, 35; *P* ≤ 0.5129). Average gains in the three apiaries were not significantly different. The gain was 38.68 ± 3.07 kg in the Cole Grove apiary, 40.00 ± 3.01 kg in the Durfee Hill apiary, and 32.48 ± 3.52 kg in the Nelson apiary (*F* = 1.19; *df* = 2, 35; *P* ≤ 0.3154). The interaction between treatment and apiary was not significant (*F* = 0.05; *df* = 2, 35; *P* ≤ 0.9543).

**Seasonal Weight Gain.** The average seasonal gain in the control group was 56.89 ± 4.83 kg (LSMean ± SE), compared with 68.84 ± 4.47 kg in the treatment group (*F*<sub>1, 35</sub> = 3.47; *df* = 1, 35; *P* ≤ 0.0709). Average seasonal gains in the three apiaries were significantly different. The gain was 77.21 ± 5.45 kg in the Cole Grove apiary, 58.14 ± 5.35 kg in the Durfee Hill apiary, and 53.26 ± 6.26 kg in the Nelson apiary (*F* = 4.18; *df* = 2, 35; *P* ≤ 0.0236). The interaction between treatment and apiary was not significant (*F* = 0.17; *df* = 2, 35; *P* ≤ 0.8421).

**Table 2.** Number of cells of capped drone brood (mean  $\pm$  SE) removed from colonies in the treatment group in each apiary on each replacement date

Date	Cole Grove	Durfee Hill	Nelson
16 June	2,860.56 $\pm$ 192.74	1,711.75 $\pm$ 344.75	1,587.50 $\pm$ 563.39
16 July	1,599.50 $\pm$ 270.87	2,247.29 $\pm$ 193.70	1,497.83 $\pm$ 261.11
16 Aug.	1,863.00 $\pm$ 503.34	1,965.14 $\pm$ 442.21	2,678.17 $\pm$ 270.70
16 Sept.	1,272.89 $\pm$ 250.22	1,501.14 $\pm$ 398.34	1,350.50 $\pm$ 276.99
Total <sup>a</sup>	7,354.50 $\pm$ 1,014.03	7,506.14 $\pm$ 762.40	7,114.00 $\pm$ 961.89

*n* = 9 for Cole Grove, *n* = 7 for Durfee Hill, and *n* = 6 for Nelson.

<sup>a</sup> Average number of cells of capped drone brood removed from each colony during the entire experimental period.

### Number of Capped Cells of Drone Brood Removed.

The average number of capped cells of drone brood removed from treatment colonies in each apiary on each replacement date is given in Table 2.

## Discussion

The drone brood removal method suppressed mite levels throughout the summer and early fall. Mite-to-bee ratios in colonies that had drone combs removed four times during the spring and summer were relatively low on 7 October (average ratio  $\leq 0.03$  in each apiary) compared with ratios in colonies that did not have drone combs removed (average ratio of 0.10 for the three apiaries; Fig. 1). The lowest and highest ratios were 0.012 and 0.441, respectively, in the control group, and 0.000 and 0.070, respectively, in the treatment group. The average mite-to-bee ratios in the treatment group in the three apiaries remained  $\leq 0.03$ , regardless of the mite levels in the corresponding control group. This suggests that the amount of drone brood removed was more than sufficient to trap the available mites.

Drone brood removal did not adversely affect colony health as measured by the size of worker populations or by honey production. Fall worker populations were similar in the two groups. Average honey production in the treatment group was significantly greater than production in the control group during period 1, similar to production in the control group during period 2, and perhaps greater overall when measured over the season ( $P < 0.07$ ). This suggests some added benefit from drone brood removal in addition to the maintenance of lower mite levels. Increased honey production could be a direct result of lower mite levels, or it could be due to colonies in the treatment group not needing to support as many adult drones. Seeley (2002) suggested that this might partially explain his finding that colonies that rear and care for drones gain less weight than colonies that do not rear and care for drones. Although both groups in this experiment reared drones, only the control group cared for them as adults.

Charriere et al. (2003) also examined the effects of drone brood removal on colony health. Like the findings in this study, treatment and control colonies in their experiment had similar worker populations in the fall. However, whereas data presented here suggest

greater honey production in the treatment group, production in the control and treatment groups in their study was similar. This could be a consequence of their removing drone comb by cutting it out of the frames, thereby requiring treatment colonies to invest in expensive new drone comb construction (reviewed in Seeley 1985, Winston 1987), which reduced honey production.

The results from this study have implications for the frequency of miticide applications. Colonies in this experiment were not treated in the spring, the last miticide application being made the previous fall. The low mite-to-bee ratio in the treatment colonies (LS-Mean = 0.025  $\pm$  0.016) after a full year without chemical treatment suggests that drone brood removal may eliminate the need for a spring treatment; and, on a colony-by-colony basis, it also may permit one to skip an occasional fall treatment.

The decision-making process for determining whether to treat with a miticide is based on economic thresholds. Delaplane and Hood (1999) proposed an ether roll count between 15 and 38 as an economic threshold in the fall in the southeastern United States. A ratio of 0.025 in a sample of 229.5 bees (average ratio and sample size for samples collected from treatment colonies in this study) translates into a standardized 300-bee ether roll count (per Delaplane and Hood 1999) of four or five mites (conversion of ratios to counts based on data in Calderone and Turcotte 1998 and Ellis and Baxendale 1994), well below this recommendation. However, Strange and Sheppard (2001) recommended a 300-bee ether roll count of only three mites in the fall for the state of Washington. Conditions in the area where that study was conducted are similar to those in upstate New York and are likely to be more relevant for the northeastern United States. Therefore, on average, the colonies in the treatment group in this study would still require a fall application of a miticide. However, considered individually, several of the colonies would not require treatment. Five of the 22 treatment colonies (23%) had ratios below the recommended 3-count threshold and do not need to be treated. Had additional drone comb replacements been used, beyond the four in this study, the proportion of colonies with mite levels below the recommended 3-count threshold would likely increase. Clearly, it is necessary to estimate the mite-to-bee ratio on a colony-by-colony basis in the fall to make the appropriate decision.

An added benefit of drone brood removal was the maintenance of relatively low mite levels in those colonies in the treatment group that exceeded the recommended 3-count threshold. The average standardized 300-bee ether roll counts for colonies in the control and treatment groups that exceeded this recommendation were 21.80  $\pm$  5.12 (*n* = 16) and 5.34  $\pm$  0.61 (*n* = 17), respectively. The low mean and standard error in the treatment group indicate that all of the colonies in that group with counts exceeding the recommended 3-count threshold actually had mite levels very near the threshold, even if they still required a fall application of a miticide. However, most

of the colonies in the control group with counts exceeding the recommended threshold exceeded it by a wide margin.

Other issues need to be investigated to understand the limits and long-term value of this method. Paramount is a determination of the degree to which colonies will add drone cells to the worker combs in the brood nest. This could reduce the efficacy of the method by allowing drone production in nontrap combs, or it could require that worker combs be culled at an economically unacceptable rate. Seeley (2002) found that colonies provided with 20% drone combs (including both brood chambers and honey supers) added additional drone cells to significantly fewer worker combs than did colonies without any drone combs. In the present experiment, colonies were provided with only two drone combs in the brood nest (10%), and no drone combs were added to the honey supers. Therefore, the propensity of colonies to add drone cells to worker combs under these conditions needs to be determined.

These issues notwithstanding, this study suggests that drone brood removal holds significant promise as a major component in an IPM program for *V. destructor* in colonies of *A. mellifera*. The fact that it provides suppression of mite populations during the summer and early fall is significant in itself. The maintenance of low mite levels during this period will reduce the incidence of late summer and fall collapse, which is common in mite-infested colonies. This, in turn, will ensure that colonies going into the winter have healthy workers. It also may eliminate the need for a spring miticide treatment, and, on a colony-by-colony basis, reduce the frequency of annual fall treatments.

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