Removal of Drone Brood From *Apis mellifera* (Hymenoptera: Apidae) Colonies to Control *Varroa destructor* (Acari: Varroidae) and Retain Adult Drones

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J. Econ. Entomol. 102(6): 2033–2040 (2009)

ABSTRACT The parasitic mite *Varroa destructor* Anderson & Trueman (Acari: Varroidae) has plagued European honey bees, *Apis mellifera* L. (Hymenoptera: Apidae), in the Americas since its introduction in the 1980s. For many years, these mites were sufficiently controlled using synthetic acaricides. Recently, however, beekeepers have experienced increased resistance by mites to chemical pesticides, which are also known to leave residues in hive products such as wax and honey. Thus there has been increased emphasis on nonchemical integrated pest management control tactics for *Varroa*. Because mites preferentially reproduce in drone brood (pupal males), we developed a treatment strategy focusing on salvaging parasitized drones while removing mites from them. We removed drone brood from colonies in which there was no acaricidal application and banked them in separate "drone-brood receiving" colonies treated with pesticides to kill mites emerging with drones. We tested 20 colonies divided into three groups: 1) negative control (no mite treatment), 2) positive control (treatment with acaricides), and 3) drone-brood removal and placement into drone-brood receiving colonies. We found that drone-brood trapping significantly lowered mite numbers during the early months of the season, eliminating the need for additional control measures in the spring. However, mite levels in the drone-brood removal group increased later in the summer, suggesting that this benefit does not persist throughout the entire season. Our results suggest that this method of drone-brood trapping can be used as an element of an integrated control strategy to control varroa mites, eliminating a large portion of the *Varroa* population with limited chemical treatments while retaining the benefits of maintaining adult drones in the population.

KEY WORDS honey bee, *Varroa destructor*, IPM, drone-brood trapping

Varroa destructor Anderson & Trueman (Acari: Varroidae) is an ectoparasite of the European honey bee, *Apis mellifera* L. (Hymenoptera: Apidae). It was introduced to the United States from mainland Asia in the 1980s and has since been the largest concern facing the U.S. apiculture industry. Its range is very widespread, and it is now found nearly worldwide. The mite's native host is the Asian honey bee, *Apis cerana* F., but it has shifted hosts to parasitize European honey bees as well (Oldroyd 1999, Anderson and Trueman 2000). The mite is fairly innocuous to its native host because it reproduces only within the cells of developing males (drone brood), leaving developing workers unparasitized and restricting the mite's population growth to those times of the season during which drone brood is present (Boot et al. 1995, 1999; Chandler et al. 2001). *A. cerana* is also adept at detecting and removing mites from brood cells and adult workers but *A. mellifera* does not possess these behavioral defenses against *V. destructor* (Boot et al. 1999); thus, the mite has proven to be far more injurious to *A. mellifera* than it is to *A. cerana.* Consequently, both managed and feral European colonies experience high rates of mortality and morbidity as a result of parasitism (Kraus and Page 1995, Sammataro et al. 2000).

Parasitism by *V. destructor* is directly damaging to late-instar larvae, pupae, and adult honey bees because vital nutrients are lost when the mite consumes the bees' hemolymph. Most of this injury is incurred during the bees' pupal development, during which time the invading female (foundress) mite and her developing offspring are actively feeding. This is a critical stage of development for the honey bee, and parasitism results in decreased adult body weight and longevity, as well as sperm count, mucous gland weight, and seminal vesicle weight in drones (DeJong et al. 1982, Rinderer et al. 1999, Janmaat and Winston 2000, Zoltowska et al. 2007). Indirectly, however, Varroa is even more detrimental due to its competency as a vector for numerous viral diseases, as well as its ability to activate otherwise latent infections. This condition has been termed parasitic mite syndrome or varroosis (Shimanuki et al. 1994).

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0022-0493/09/2033–2040$04.00/0 © 2009 Entomological Society of America

Synthetic acaricides have long been the most common method for beekeepers to control varroa mites. Among them are the pyrethroid fluvalinate (Apistan) and the organophosphate coumaphos (Checkmite+). Although once highly effective, varroa mites are developing increased resistance to these chemicals, in many cases making them insufficient for controlling infestations (Elzen et al. 1998, 2000; Spreafico et al. 2001; Elzen and Westervelt 2002; Sammataro et al. 2005). Resistance has been propagated by overuse and misuse of pesticides, as well as exposure of mites to sublethal residues left behind in honey and wax (Korta et al. 2001). Because of their lipophilic properties, fluvalinate and coumaphos accumulate in beeswax over time and persist even after the industrial processing that occurs before it is recycled and reused as wax foundation (Martel et al. 2007). Moreover, pesticide residues pose potential health hazards for human consumers of honey and beeswax products. Alternative pesticides are available (such as oxalic and formic acids), as well as plant-derived pesticides (such as the tobacco derivative sucrose octanoate and essential oils such as thymol). Although these acaricides may be appealing alternatives to synthetic substances, they are often more labor-intensive to apply and may not offer sufficient mite control when used alone (Imdorf et al. 1999, Gregorc and Planinc 2001, Rice et al. 2004, Stanghellini and Raybold 2004). Nonchemical control measures (such as application inert dusts, fungal pathogens, and use of screen bottom boards) are sometimes used, but these measures do not offer consistently high efficacy when used alone and may be highly disruptive to the colony (Pettis and Shimanuki 1999; Chandler et al. 2001; Fakhimzadeh 2001; Kanga et al. 2003; Aliano and Ellis 2005; Delaplane et al. 2005; Kanga et al. 2006; Coffey 2007; Meikle et al. 2007, 2008).

Another method of nonchemical control of varroa mites is "drone-brood trapping," which is designed to take advantage of the mite's natural preference for parasitizing developing males (Boot et al. 1995, Wilkinson and Smith 2002, Charriere et al. 2003, Calderone 2005). Reproducing in drone cells increases the reproductive potential of a female mite, because reproducing females within brood cells lay eggs singly and at 30-h intervals. The drone pupal stage is longer than workers, allowing the female mite to produce more mature offspring before the bee emerges (Calderone and Kuenen 2001). Traditionally, to perform the drone-brood trapping technique, frames of drone comb are placed into a hive, left there until there is capped drone brood in the cells, and then removed from the colony to be frozen. The drone brood acts as a sink for varroa mites, which are removed from the colony in a way that has minimal impact on worker brood. Drone-brood trapping has been shown to maintain low mite populations through the late summer when used without any other mite treatment, and it is unlikely that varroa mites will develop any behavioral resistance to this form of treatment (Boot et al. 1995, Wilkinson and Smith 2002, Charriere et al. 2003, Calderone 2005, Coffey 2007).

This application of drone-brood trapping and subsequent freezing effectively kills the mites trapped within it (Calderone 2005), but it also kills all of the developing drones. Genetic diversity is vital to the health of a colony, and this is accomplished when a queen honey bee mates with many drones. When the queen does not mate with a sufficient number of males, her colonies are often weaker and more susceptible to parasitism and disease (e.g., Seeley and Tarpy 2007). Current methods of drone-brood trapping result in a decrease in the drone population available for mating with local queens. Also, exertion of selection pressure from mites on drones may result in increased survival of individuals that have increased mite tolerance. Drones are haploid, and so any alleles responsible for such tolerance would be passed on to each offspring. Thus, it would seem beneficial if drones could be salvaged after their removal from colonies while killing the mites that are present with them, particularly because parasitism does not seem to unduly affect their ability to mate (Rinderer et al. 1999). A method of treatment that accomplished this would maintain the mating population and promote the natural selection of those individuals which are most fit under the conditions of parasitism.

In this study, we investigated a variation of drone-brood removal that permits the survival of the drone population, thus leaving them to bolster the mating population and potentially act as vehicles to disperse any mite-tolerant alleles they may possess. We hypothesize that this method will decrease the number of mites within a colony with very limited use of synthetic acaricides, which can be applied only to a small subset of colonies in the apiary.

Materials and Methods

We began the study in early March 2008 with 20 ten-frame colonies in standard Langstroth hives. The colonies were European honey bees of Italian stock. The hives were placed in a single row directly adjacent to a corn field on the Lake Wheeler Research Station in Raleigh, NC. Each hive included two frames of drawn drone comb placed in positions 2 and 9, approximately one-and-a-half frames of pollen, two frames of honey, and four frames of brood. To supplement the colonies' nutrition and encourage rapid colony growth and development, all colonies were fed supplemental sugar syrup and pollen patties consisting of 1 part irradiated bee-collected pollen (GloryBee, Eugene, OR) to 1 part 50% sucrose solution. As colony populations increased, second hive bodies were added on a colony-by-colony basis. These additional brood boxes each consisted of two foundation frames placed in the outmost positions and eight frames of drawn, empty comb. Colonies were thereafter managed using standard apicultural techniques (i.e., swarm management, adding and removing supernumerary hive boxes such as brood boxes and medium-sized honey supers, feeding sugar syrup to supplement nectar flow, and installation of robbing screens). Varroa mites, however, were managed according to treatment group

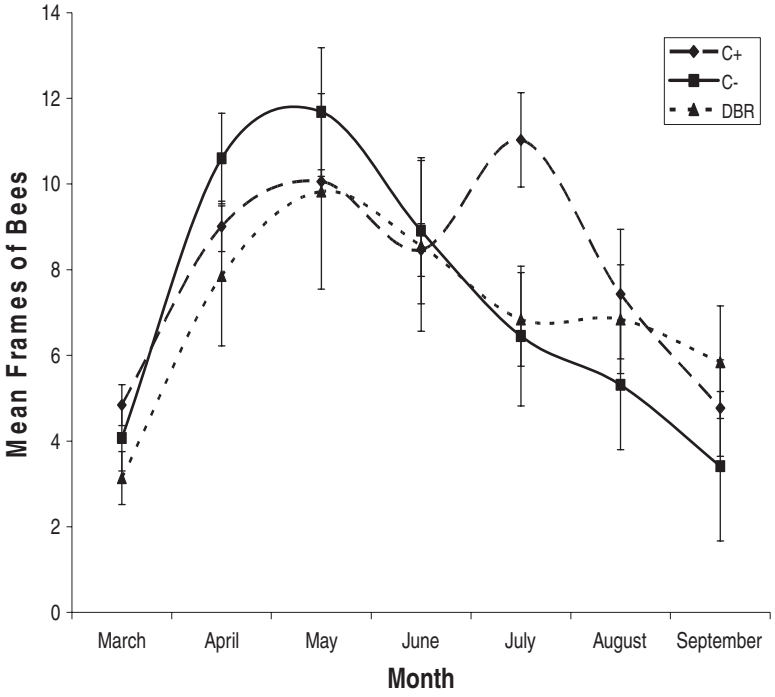


Fig. 1. Comparisons of adult bee population in positive control, negative control, and drone brood removal groups (C+, C−, and DBR, respectively) measured by visually estimating total frames covered by adult bees in each colony (mean ± SE). No groups differed significantly at the outset, during, or at the conclusion of the study. This indicates 1) that any effects seen during the experiment were not a result of unequal adult populations before treatment was applied, and 2) that no treatment applied was detrimental to the adult population of colonies.

assigned (see below). At outset of the study, all colonies were treated with the antibiotic Fumagillin to protect against *Nosema*, a microsporidium disease of adult honey bees.

Before we assigned treatment groups, we evaluated various aspects of colony strength. We measured adult worker population by estimating the proportion of each frame's surface area that was covered by adult bees. We then summed these proportions to estimate the total number of frames of adult bees per colony. Similarly, we estimated the percentage of each comb occupied by immature bees to find the number of frames of brood in each colony, which included all stages of development. Colony weights—including bees, brood, combs, and stored food—were measured in the field during the day using a digital scale. During the study, before any equipment was added or removed from a colony, it was weighed without bees to ensure accurate measurement of changes in hive equipment weight.

Initial measurements of mite populations in each colony were also made by taking 24-h mite drop counts using adhesive "sticky boards" (Delaplane and Hood 1999, Ostiguy and Sammataro 2000, Sammataro et al. 2002, Tarpy et al. 2007). Sticky boards (Mann Lake Ltd., Hackensack, MN) consisted of adhesive-coated cardboard, which were placed on the bottom boards of hives for 24 h. For a more accurate evaluation of proportion of bees infested with mites, these counts were also corrected for the total number of adult bees in each colony. This is because the total number of mites within a colony is a linear function of its population, thus larger colonies would be expected to have higher mite loads than smaller colonies, even when mite prevalence (percentage of infestation) is the same. Therefore, comparing the daily mite drop to the number of frames of adult bees (estimated as previously described) allowed mite drop counts to be more meaningfully compared among colonies of varying sizes.

We then assigned one of three treatment groups to each colony, controlling for position effects within the apiary using a semirandom block design. Group 1—Negative control ($n = 5$), with no preventative treatment for varroa mites. Group 2—Positive control ($n = 5$), with two Apistan strips applied per brood box, which were removed and replaced with new strips every 6 wk. To offset the potential impact of development of mite resistance to fluvalinate, colonies were also treated with Apilife VAR (thymol tablets) according to label instructions in late summer. Group 3—Drone-brood removal ($n = 8$), where drone frames were removed, shaken to remove adult bees, placed into one of two "drone-brood receiving" colonies (after the majority of drone brood present was capped), and replaced with spare drone frames. The two drone-brood receiving colonies were treated with Apistan and Apilife VAR in the same manner as those

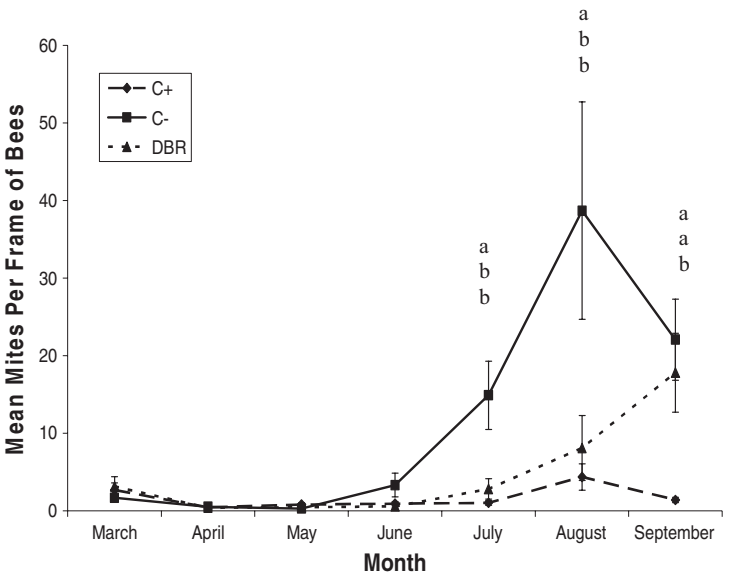


Fig. 2. Comparisons of sticky board mite counts measuring 24-h mite counts per frame of bees in each treatment group (positive control [C+], negative control [C−], and drone brood removal [DBR]) during each month of the study (mean ± SE). During July and August, mite counts in the C− group were significantly higher than were those of the C+ or DBR groups. In September, C− and DBR groups had significantly higher counts than did the C+ group.

in the positive control group. All of the banked drone frames were marked by date and left for 2 wk to ensure that all adult drones had emerged, after which they were removed from drone-brood receiving colonies, placed in a freezer at −20°C (to kill any new brood produced by drone-brood receiving colony queens), and redistributed as replacement frames in other drone-brood removal colonies when needed.

All treatments were initiated in mid- to late March, continued over the course of the spring and summer, and ended when colonies ceased drone production in early September. We recorded monthly mite counts from each colony during the study by using sticky boards (see above) and "sugar shakes" (Fakhimzadeh 2001). Sugar shakes were performed on ≈200 bees collected from brood frames in each colony. Monthly estimates of adult bee population were also taken and compared with 24-h sticky board mite counts in the manner described previously. Population estimates were performed over several days preceding sticky board installation. However, sticky boards were installed no <12 h after the end of physical manipulation of hives. This was an important consideration, because the increased bee activity resulting from human disturbance could result in artificially high mite counts.

Three colonies died during the study. Both drone-brood receiving colonies were gradually weakened throughout the study and ultimately died, as did one colony in the negative control group. Another colony in the drone-brood removal group was removed from the study after being diagnosed with European foulbrood. After the cessation of treatments, colony strength was assessed by estimating adult bee and brood populations as described previously. Also, final colony weights were measured and the change in weight was determined for each colony.

Statistical Analysis. Means of pretreatment measurements of strength were compared among treatment groups using analysis of variance (ANOVA) to ensure that there were no differences in starting conditions. These measurements included total frames of adult bees, frames of brood, and initial colony weight. Mean 24-h mite counts from sticky boards were divided by total number of frames covered by adult bees to control for differences in colony strength, and treatment groups were compared by ANOVA to determine whether the mite counts among treatment groups differed significantly from each other during each month of the study. The mean mite counts of treatment groups measured using this method and measured by sugar shakes were compared using repeated measures analysis to determine whether there were significant differences among groups during the course of the study. Tukey post hoc tests were used to compare sugar shake counts among treatment groups during each month of the study. Similarly, the mean number of frames covered by adult bees was compared using repeated measures ANOVA to determine whether groups differed significantly during the entire study and Tukey post hoc tests during any one month. Finally, means of posttreatment measurements of colony strength were compared among treatment groups using ANOVA, including total frames of adult bees, frames of brood, and change in colony weight. All means are reported as ± 1 SEM with $\alpha = 0.05$ (JMP version 7.0; SAS Institute, Cary, NC).

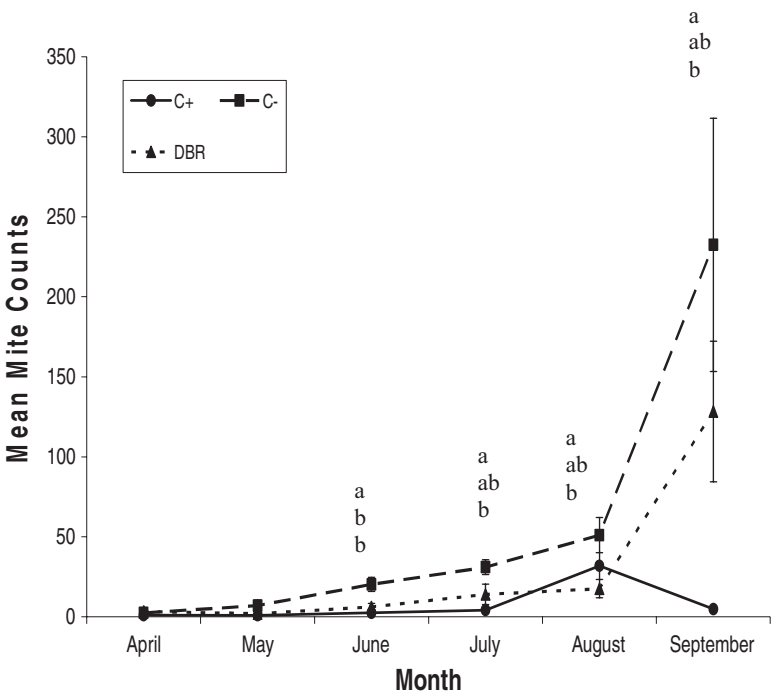


Fig. 3. Comparisons of sugar shake mite counts in positive control, negative control, and drone brood removal groups (C+, C−, and DBR, respectively) during each month of the experiment (mean ± SE). In June, the C− group had significantly higher mite counts than did the C+ or DBR group. In July, the DBR group was intermediate between the C− and C+ groups. In August, the C− group had significantly higher mite counts than the DBR group, and C+ group was intermediate between then two. In September, C− and DBR groups had significantly higher sugar shake counts than did the C+ group.

Results

There were no significant differences among any of the pretreatment groups at the outset of the study concerning measures of colony strength; they were statistically similar with regard to mean adult bee population ($F = 1.83$; df = 2, 15; $P = 0.19$) (Fig. 1), mean brood population ($F = 0.03$; df = 2, 15; $P = 0.97$), and mean colony weight ($F = 0.99$; df = 2, 15; $P = 0.40$). Likewise, they did not differ significantly with regard to mean 24-h mite drop on sticky boards per frame of adult bees ($F = 0.46$; df = 2, 15; $P = 0.64$) (Fig. 2).

Monthly 24-h sticky board mite drop counts were adjusted by the number of frames covered by adult bees for each colony to quantify the number of mites per frame of bees (Fig. 2). Over the course of the season, treatment groups differed significantly with respect to mean number of mites per frame of bees ($F = 1.44$; df = 2, 13; $P < 0.005$). During March, April, May, and June, there were no significant differences in the number of mites counted per frame of bees among the different treatment groups (all $P > 0.05$). In July and August, however, drone brood removal (2.82 ± 2.167 and 8.10 ± 6.940 for July and August, respectively) and positive control colonies (1.02 ± 2.564 and 4.36 ± 8.211 for July and August, respectively) had significantly fewer mites per frame of bees than did the negative control group to which no mite treatment was applied ([14.90 ± 2.564] $F = 8.98$; df = 2, 14; $P < 0.01$ for July; [38.69 ± 8.211] $F = 5.47$; df = 2, 14; $P < 0.05$ for August). September measurements indicated that the mean number of mites per frame of bees was significantly higher in both negative control (22.07 ± 5.224) and drone brood removal colonies (17.81 ± 3.949) compared with the positive control group ([1.41 ± 4.672] $F = 5.28$; df = 2, 13; $P < 0.05$).

Mite counts from monthly sugar shake measurements (Fig. 3) differed significantly among treatment groups throughout the study ($F = 1.04$; df = 2, 13; $P = 0.0096$), and specifically in June, July, August, and September. In June, the negative control group had significantly higher mean mite counts (20.20 ± 2.940) than did the drone brood removal (6.14 ± 2.484) and positive control groups ([2.40 ± 2.940] $F = 10.43$; df = 2, 14; $P < 0.005$). In July, mean mite counts from the negative control group (31.00 ± 5.620) were significantly higher than were those of the positive control group (4.20 ± 5.620), and the mean counts from the drone brood removal colonies were intermediate between the two ([14.00 ± 4.750] $F = 5.85$; df = 2, 14; $P < 0.05$). Mean sugar shake counts in August were significantly higher in the negative control colonies (51.00 ± 8.803) than in the drone brood removal colonies (17.57 ± 7.440), and mite counts in the positive control group were intermediate between the two ([31.80 ± 8.803] $F = 4.21$; df = 2, 14; $P < 0.05$). In September, the negative control group had a sig-

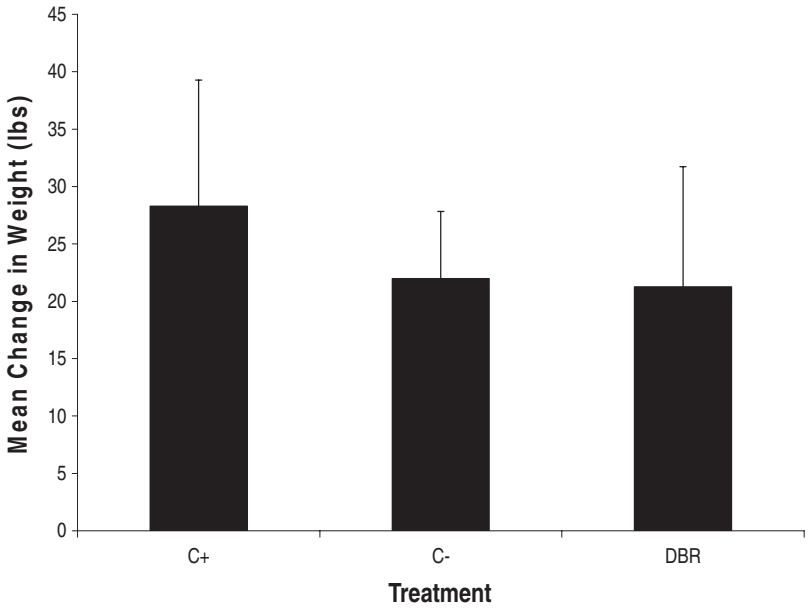


Fig. 4. Comparisons of change in colony weight over the course of the study in positive control, negative control, and drone brood removal groups (C+, C−, and DBR, respectively; mean ± SE). No significant differences were observed in changes in colony weight, indicating that treatments did not have harmful effects on colony productivity.

nificantly higher mean mite count (232.50 ± 54.830) than did the positive control group (4.80 ± 49.041), and mean drone brood removal sugar shake counts were intermediate between the other two groups ([128.29 ± 41.448] $F = 4.87$; df = 2, 13; $P < 0.05$).

Treatment groups did not differ significantly in measures of colony strength at the conclusion of the study in regard to mean frames of brood ($F = 0.29$; df = 2, 13; $P = 0.7520$) or mean change in colony weight ($F = 0.14$; df = 2, 13; $P = 0.8712$; Fig. 4), nor did they differ significantly at any point during the study in mean frames of adult bees ($F = 0.016$; df = 2, 13; $P = 0.9006$) (Fig. 1).

Discussion

Results of our study support the hypothesis that this method of drone-brood removal provides sufficient varroa mite control to forgo at least one seasonal application of acaricides. During much of the study, colonies undergoing drone brood removal had significantly fewer mites than those without mite treatment (negative control group). This method of drone brood removal maintained varroa populations low until mid-to-late summer without the application of synthetic acaricides in the spring. Furthermore, the drone population was preserved while in the absence of acaricides during larval development. Our findings agree with those of previous studies using drone-brood removal to control mites (e.g., Calderone 2005).

During the summer months, when varroa mite populations naturally increase (DeGrandi-Hoffman and Curry 2004), those undergoing drone brood removal (with no other mite control) maintained mite populations that were either equivalent to those of colonies treated with synthetic acaricides (positive control group) or were intermediate between the positive and negative control groups. In late summer and early fall, however, the rate of growth of mite populations increased in drone brood removal colonies, leading to mite numbers similar to those of the negative control group. This agrees with previous findings concerning drone-brood removal (Charriere et al. 2003). It is important, therefore, that when this technique is used by beekeepers, they may need to apply a late summer varroa treatment in addition to drone-brood trapping in the spring. It is very detrimental to the health of overwintering colonies to have high mite levels during the time that the overwintering worker population is developing (DeGrandi-Hoffman and Curry 2004). For this reason, we do not suggest drone-brood trapping as a stand-alone treatment, which agrees with the findings of other studies done on drone-brood trapping techniques (Charriere et al. 2003). Moreover, monitoring for drone brood can be incorporated into existing swarm management monitoring schedules (Calis et al. 1999), particularly for those beekeepers with a relatively small number of hives.

Results from this study suggest that drone brood removal does not interfere with colony development and has no adverse effect on colony strength. At the conclusion of the study, there were no significant differences between any of the treatment groups with regard to change in colony weight, adult bee population, or brood population (also see Calderone 2005, Coffey 2007).

A critical aspect of this drone-brood trapping strategy was that drone frames were removed from their

natal colonies when the majority of the brood present was capped. Colonies were monitored and the decision to remove drone frames was made on a colony-by-colony basis. This was done in such a way as to ensure that no drones emerged from the frames within their original colonies, which is important because if they were allowed to emerge, mite numbers would probably increase significantly in those colonies because there would be no pesticides to kill the large numbers of mites emerging with the drones.

The two colonies in this study designated as drone-brood receiving colonies, in which drone frames removed from other colonies were banked until all drones had emerged, died during the course of the study. Observations suggested that these colonies were weakened as a result of supporting such a large drone population; they had very low honey stores and relatively small worker populations. They seemed less able to defend against otherwise innocuous pests, such as small hive beetles, *Aethina tumida* Murray, which did significant damage to both colonies at various times. These observations are speculative, however, because we did not empirically measure the precise cause of collapse. Thus, if beekeepers choose to implement this method of drone-brood trapping, they should be prepared for the possibility that the colonies used to bank drones will not produce much, if any, honey and are more likely to die before the end of the season.

In conclusion, drone-brood trapping and banking has much potential for being an effective element of an IPM plan to control varroa mites, as the technique can be effective in maintaining low mite levels while preserving the drone population. It has been shown by Sylvester et al. (1999) that drones parasitized by mites do remain reproductively competitive, which indicates that this strategy could bolster mating populations, especially in comparison with drone-brood trapping techniques that destroy all drone brood. This method of mite control would allow beekeepers to manage varroa mite populations in a way that requires only very limited pesticide application, and which allows the potential natural selection of those individuals who are most fit after experiencing parasitism.

Acknowledgments

We thank Jennifer Keller and Matthew Mayer for assistance with data collection and processing as well as Joe Flowers for helpful advice. This research was funded by the North Carolina Agriculture Foundation, the North Carolina State Beekeepers Association, and a grant from the Southern IPM Center.

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Received 8 June 2009; accepted 23 August 2009.