Evaluation of Selected Biopesticides for the Late Fall Control of Varroa Mites in a Northern Temperate Climate

Published in ABJ, June 2004, page 475. Volume 144. NO 6.

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ABSTRACT

ApiLife VAR®, the MiteGone™ liquid formic acid delivery system, an oxalic acid trickle technique, and multiple spray application of sucrose octanoate esters (Sucrocide™) were compared to Apistan® as late-fall treatments to control varroa mites (*Varroa destructor* Anderson & Trueman) in single-deep and double-deep honey bee (*Apis mellifera* L.) colonies under northeastern US conditions. Mite mortality in single-deep colonies was 97% for Apistan®, 96% for Sucrocide, 92% for oxalic acid, 91% for ApiLife VAR®, 79% for formic acid, and 23% in untreated control colonies. Mite mortality in double-deep colonies was 95% for Apistan®, 93% for oxalic acid, 69% for ApiLife VAR®, 66% for formic acid, and 15% in untreated control colonies. No significant relationships were detected between the amounts of ApiLife VAR® or formic acid that evaporated and corresponding mite mortality or fluctuations in apiary temperature. The advantages and disadvantages of each biopesticide are discussed.

Note: For those who would like to know a fast answer to formic acid treatment the following is an excerpt from the discussion on page 7. Please keep in mind that only one full length pad was used on purpose in 2 high colonies capable of a 40 day treatment. The test was discontinued at 30 days in mid November. A standard treatment requires 2 pads emitting 12 grams of acid per day and treatment is recommended at the end of August: visit MiteGone website www.mitegone.com for more details.

The levels of varroa mite control found here using formic acid in the MiteGone delivery pad system were similar to those reported for the gel-based formulation, Apicure® (ca. 70%) (Feldlaufer et al. 1997), and were slightly higher than the single-application MiteAway™ formic acid pads (51-56%) tested in New York (Calderone 1999, Calderone and Nasr 1999). The average formic acid evaporation rate *per one pad* found here (4-6 g/day) is generally considered insufficient for adequate (i.e., stand-alone) varroa control (Feldlaufer et al. 1997, Thomas 1997, Calderone 1999, Calderone and Nasr 1999, Imdorf et al. 1996); and our results concur.

Increasing the level of varroa control using MiteGone pads may be possible by implementing one or more of the following adjustments in treatment application: (1) extending the treatment period beyond the 30-day period evaluated here, (2) using more than one delivery pad to increase the amount of formic acid that is released per day, and/or (3) beginning treatments earlier in the fall season to take advantage of typically warmer daily temperatures, which should increase formic acid evaporation (Ruzicka 2004). Adjustments in the application of MiteGone formic acid delivery pads to increase evaporation rates and subsequent varroa control appears to be realistic, as only 52-60% of the initial volume of formic acid evaporated during our study.

INTRODUCTION

The parasitic mite, *Varroa destructor* Anderson & Trueman, is arguably the single greatest challenge to successful beekeeping in the United States. Without intervention, honey bee (*Apis mellifera* L.) colonies typically die within two years after initial varroa infestation (DeJong et al. 1982a). For over 15 years, effective varroa control strategies in the US have centered on the use of conventional pesticides. However, the consistent and exclusive use of these products to control varroa mites have led to additional problems for beekeepers; the most serious being the development of varroa populations that are resistant to fluvalinate (Apistan®, Wellmark, Bensenville, IL) and coumaphos (Checkmite+®, Bayer, Shawnee Mission, KS) (Baxter et al. 1998; Elzen et al. 1998; Elzen and Westervelt 2002), and the potential contamination of hive products with pesticide residues (Wallner 1999).

Alternative strategies for varroa control are numerous and exhibit a wide range of efficacy and practicality. Physical and cultural controls such as screen bottom boards and varroa trapping in drone comb are typically not stand-alone strategies. However, they do represent options that can be incorporated into an integrated pest management (IPM) program that utilizes multiple strategies for suppressing varroa populations in bee colonies. Selective breeding and bee stock importation programs (e.g., the USDA-ARS *Suppression of Mite Reproduction* (SMR) and Russian bee projects) show great potential (Harbo and Harris 2003; Tubbs et al. 2003), but more studies are needed on the adaptability of these bees to different regions of the US.

Other alternative approaches to varroa control are the use of biologically-derived compounds. These "biopesticides" may offer beekeepers practical, yet effective means of parasite control, and are therefore strong candidates for incorporation into varroa IPM and pesticide resistance management programs. A considerable amount of research on tracheal mite (*Acarapis woodi* (Rennie)) and varroa mite control with organic acids (formic, lactic, and oxalic acid) and essential oils (thymol, eucalyptol, etc.) has been conducted in Europe, the US, and elsewhere (Imdorf et al. 1996; Fries 1997; Thomas 1997; Nanetti et al. 2003).

In the late 1990s, a formic acid gel formulation (Apicure®) was developed for use in the US for the control of tracheal mites and suppression of varroa mites (Feldlaufer et al. 1997). Despite reasonable efficacy against both mites, Apicure® was removed from the market shortly after its introduction due to packaging problems. No other formulations of formic acid have yet been approved in the US. However, various formic acid pads and dispensers are available and used routinely by beekeepers in other countries (Nasr 1996; Calderone 1999; Fries 1997).

Several oxalic acid formulations have been tested in Europe, with some reaching >90% varroa control (Thomas 1997; Brødsgaard et al. 1999; Buchler 2000; Nanetti et al. 2003). One promising application technique is the "trickle method", whereby variable concentrations of oxalic acid (1.8-4.5%) are dissolved in sucrose solutions (0-60%) and trickled onto adult bees, the volume of solution depending on bee colony size and strength (30-50 ml/colony) (Nanetti et al. 2003). Currently, oxalic acid is not approved for use by beekeepers in the US.

Of the many essential oils tested for efficacy against varroa mites, thymol has possibly received the most attention (reviewed by Imdorf et al. 1999). In 2003, the Italian thymol-blend product, ApiLife VAR®, was granted a Section 18 Emergency Exemption in various US states. The degree of varroa control achieved with ApiLife VAR® has ranged from 66-99% in Europe (Imdorf et al. 1999) and 65-97% in the southeastern US (Ellis et al. 2001).

The latest biopesticide to be registered for use by beekeepers in the US is sucrose octanoate esters (EPA 2002; Sheppard et al. 2003). This compound was originally isolated from tobacco (*Nicotiana* spp.) leaves, and a synthesized formulation is approved for use against various soft-bodied insect and mite pests of food and ornamental crops, mushroom production systems, and for varroa mites (EPA 2002). Sheppard et al. (2003) developed an effective application technique (direct spraying of bees on combs with a 0.25% active ingredient solution), and achieved an average of 68% varroa mortality (range 38-87%) with single applications of this compound. The formulation is now available to beekeepers as "Sucrocide™" (Dadant & Sons, Inc., Hamilton, IL), and is hereafter referred to by this name.

Most of the studies on using formic and oxalic acid to control varroa mites have been done in Europe. This complicates the interpretation and applicability of these data to US conditions, as those studies often employ different beehive designs (e.g., Swiss hives) and have explored a wide array of delivery systems, dosage rates, and timings of applications. To be useful to US beekeepers and to provide information through which unregistered compounds could become approved, these organic acids and delivery systems need to be evaluated and adapted to environmental conditions and bee management practices in various regions of the US. Studies on ApiLife VAR® in the US are few and limited to specific regions, such as the Southeast (Ellis et al. 2001). Likewise, more studies on Sucrocide are needed to evaluate the efficacy of multiple-applications on varroa mite and honey bee populations.

In this study, we evaluated the efficacy of ApiLife VAR®, the MiteGone™ (Kelowna, Canada) formic acid delivery system, an oxalic acid trickling method, and multiple spray applications of Sucrocide as late fall treatments to control varroa mites in the northeastern US. Mite mortalities obtained with the four biopesticides were compared to those obtained with Apistan® (standard treatment) and untreated (control) colonies. We also evaluated the effects of temperature on the evaporation rates of ApiLife VAR®, and the effects of hive size (single versus double, full-depth hive bodies) on treatment efficacy.

MATERIALS AND METHODS

Colonies. Two test apiaries ("Van Waganer" and "Wilson") were established within a 10-mile radius of Cream Ridge, New Jersey, in the fall of 2003. The Wilson apiary contained 30 honey bee colonies of mixed commercial origin housed in "double-deep" hives (two-story, full-depth Langstroth hives); and the Van Waganer apiary contained 30 colonies housed in "single-deep" hives (single-story, full-depth, Langstroth hives). Each colony consisted of a queen and worker bees covering 6-10 (single-deep) or 8-12 (double-deep) frames, and all colonies had similar levels of open and sealed brood (1-2 frames total). The double-deep colonies contained honey stores sufficient for overwintering, while single-deep colonies were fed 3.8 liters (1 gallon) of sucrose solution (50:50 w/v; using division board feeders) before the application of treatments and again mid-way through the study. The placement of beehives was similar between apiaries with respect to sun exposure and windbreaks, and hives were positioned to reduce the drifting of foragers between colonies (Jay 1966).

Treatments. All colonies were identified as being infested with varroa mites by using the alcohol wash technique (DeJong et al. 1982b), and treatments were assigned randomly to colonies within apiaries having comparable varroa mite infestation levels (% infestation = mites/bee x 100). The 30 bee colonies in the Van Waganer apiary were divided into 6 groups of 5 colonies, where each group received treatment with ApiLife VAR®, formic acid, oxalic acid, Sucrocide, Apistan®, or was left untreated (control). Treatments in the single-deep hive study began on 14 October and ended on 15 November 2003. Similarly, the 30 bee colonies in the Wilson apiary were divided into 5 groups of 6 colonies, where each group received treatment with ApiLife VAR®, formic acid, oxalic acid, Apistan®, or was left untreated (control). Treatments in the double-deep hive study began on 21 October and ended on 21 November 2003.

Colonies in the Apistan® treatment group received two (single-deep) or four (double-deep) 10% Apistan® strips (2 strips per hive body). Strips were installed between frames such that adequate contact with bees was possible. Apistan® was used as the standard chemical control, as the colonies used in this study were not suspected of hosting fluvalinate-resistant varroa mites. The handling time to install Apistan® strips was 1-2 minutes/colony.

Colonies in the ApiLife VAR® treatment group received 3 applications of ApiLife VAR® (2 tablets/packet) at 10-day intervals. In each application, the tablets were broken in two, creating four pieces that were placed on top of the frames in the four corners of the upper or only hive body in each treated colony. ApiLife VAR® pieces were not enclosed in screen mesh. The handling time to install ApiLife VAR® tablets was 1-2 minutes/colony/treatment.

Colonies in the formic acid treatment group each received a single, plastic-covered MiteGone pad soaked with 250 ml (7.5 oz.) of a 65% formic acid solution. The solution was added by trimming one end of each pad to expose 0.9 x 9 cm (3/8 x 3 ½ in.) of evaporating surface. Pads were attached vertically to an outermost frame in the upper or only hive body, with the opening oriented downwards to allow the formic acid to evaporate. MiteGone pads measure 9 x 22 x 0.9 cm (3 1/2 x 8 1/2 x 3/8 in.), and are made of a lightweight, highly porous material. The handling time to install formic acid pads was 1-2 minutes/colony.

Colonies in the oxalic treatment group each received 50 ml (1.5 oz.) single applications of a 3.2% oxalic acid solution. Oxalic acid solutions were trickled directly onto bees between the frames using a large syringe at a rate of 5 ml (0.15 oz.) of solution per frame space. In double-deep colonies, the solution was trickled only between the frames of the top hive body. The 3.2% oxalic acid solution was prepared by adding 44.8 g (1.6 oz.) oxalic acid dihydrate (99% purity; 71.4% oxalic acid) to 1 liter (ca. 1 qt.) of a 50% sucrose solution (w/v). The handling time to apply oxalic acid was 1 minute/colony.

Colonies in the Sucrocide treatment group received 3 applications at 10-day intervals. The application technique developed by Sheppard et al. (2003) was used, whereby the Sucrocide solution was applied to all frames containing adult bees at rate of 50 ml (1.5 oz.) per frame (25 ml or 0.75 oz./side) using a handheld garden sprayer set on a fine mist pattern. Adult bees were wetted completely with solution. Diluted Sucrocide for application was prepared by adding 25 ml (0.75 oz.) of 40% sucrose octanoate esters (AVA Chemical Ventures, Portsmouth, NH) to 3.8 liters (1 gallon) of distilled water, giving a final concentration of 0.625% product (0.25% active ingredient). Five single-deep and no double-deep colonies were treated with Sucrocide. The handling time to apply Sucrocide was about 5 minutes/colony/treatment.

Mortality Data. Mite mortality was measured using laminated paper sticky boards (15 x 12 in. (l x w = 38.1 x 30.5 cm)) covered with hardware cloth (size 8 = 64 openings per in^2) to prevent bees from contacting the petroleum jelly used to capture falling mites. Sticky boards were placed on the bottom boards in each test colony at 10-day intervals for a period of 30 days (3 sticky boards readings from each colony during treatment). On day 30, Apistan® strips were placed in all test colonies to quantify the varroa mite population remaining after the experimental treatment regimes. Mite mortality from the evaluation Apistan® strips (2 in single-deep hives and 4 in double-deep hives) was determined for a 20-day period (16 Nov. to 5 Dec. 2003 for single-deep colonies, and 22 Nov. to 11 Dec. 2003 for double-deep colonies).

Due to our use of separate apiaries and starting dates, mite mortalities from single-deep and double-deep colonies were analyzed separately. Percent mortality (proportion of mites collected during treatment vs. total mites collect during treatment + Apistan® evaluation periods) were arcsine-squareroot transformed and analyzed using one-way analysis of variance, with mean separation tests performed using Tukey's tests (Minitab 2000).

Evaporation of Formic Acid and ApiLife VAR. MiteGone pads soaked with formic acid were weighed before placement in colonies and at the end of the 30-day treatment period. ApiLife VAR® tablets were weighed before placement in colonies and 10 days later when tablets were removed and/or replaced. Temperature records were obtained from weather monitoring stations near the Van Waganer apiary (Pemberton, NJ) and Wilson apiary (Freehold, NJ).

Correlation Analysis. Pearson (r) or Spearman (r_s, for non-normal data) correlation analyses were performed to detect possible relationships between: (1) the pre-treatment mite infestation level estimates using the alcohol wash technique and the total number of mites in each colony collected throughout the treatment + evaluation periods (r, square-root transformed to normalize data); (2) the total number of mites in a colony and corresponding mite mortality (r); (3) the amounts of formic acid or ApiLife VAR® that evaporated during treatment periods and corresponding mite mortality (r_s); and (4) the amount of ApiLife VAR® evaporating to the maximum, minimum, and average daily temperatures during the individual 10-day treatment periods (r_s).

RESULTS

Treatment Effects. Treatment effects on varroa mite mortality were significant in both single-deep and double-deep colonies ($P \leq 0.0001$). For single-deep colonies, there were no differences in total mite mortality for colonies treated with Apistan®, Sucrocide, oxalic acid, or Apilife VAR® (Table 1). Colonies treated with formic acid and the control group had significantly lower mite mortality and also differed from each other. Mite mortality data for the single-deep study are presented as the proportion of total mites collected during each treatment period and the subsequent treatment evaluation period using Apistan® (Fig. 1A).

For double-deep colonies, there was no difference in total mite mortality between Apistan® and oxalic acid (Table 1). Mite mortality was similar in colonies treated with ApiLife VAR® and formic acid. In double-deep control colonies, mite mortality was lower than all other treatment groups. Mite mortality data for the double-deep study are presented as the proportion of total mites collected during each treatment period and the subsequent treatment evaluation period using Apistan (Fig. 1B).

Pre-treatment mite infestation levels determined by the alcohol wash technique did not differ significantly between the 6 treatment groups in the single-deep colonies or between the 5 treatment groups of the double-deep colonies (Table 1). The average total number of mites collected per colony also did not differ between treatment groups in either the single-deep or the double-deep colonies (Table 1).

The numbers of bee colonies dying during treatment are presented in Table 1. In the single-deep study, one colony from each of the Apistan® (treatment period 1), ApiLife VAR® (treatment period 1), and control (treatment period 3) groups died during the study. In the double-deep study, one colony from each of the Apistan® (treatment period 1), ApiLife VAR® (treatment period 2), and formic acid (treatment period 1) groups, and two colonies from the oxalic acid (both in treatment period 1) group, died during the study. The cause of colony death in all cases was presumed to be exceptionally high varroa mite infestation levels (among the highest in both studies; alcohol wash estimates exceeded 35% infestation of worker bees).

Evaporation of ApiLife VAR and Formic Acid. The total amount of ApiLife VAR® that evaporated in each colony during the 30-day treatment period was 13.44 ± 0.86 g (ca. 20.3% of the cumulative weight of three packets) in single-deep colonies and 9.50 ± 1.24 g (ca. 14.4%) in double-deep colonies (Table 2). In the single-deep study, there was no difference between treatment periods 1, 2, or 3 for the amount of ApiLife VAR® that evaporated ($P = 0.4410$). In the double-deep study, there was no difference between treatment periods 1, 2, or 3 for the amount of ApiLife VAR® that evaporated ($P = 0.1335$). Very little or no portions of the ApiLife VAR® tablets appeared to have been chewed by bees. The total amount of formic acid that evaporated during the 30-day treatment period was 165.4 ± 7.23 g (60% of initial volume) in single-deep colonies and 144.8 ± 4.15 g (52.5% of initial volume) in double-deep colonies (Table 2). Average formic acid evaporation rates were 5.56 g per day for single-deep colonies, and 4.82 g per day for double-deep colonies.

Correlation Analyses. The alcohol wash estimates of pre-treatment mite infestation levels were significantly correlated with the cumulative number of mites collected on sticky boards during the treatment + evaluation periods ($r = 0.781$, $P < 0.001$). In the single-deep study, mite mortality was not correlated with the total number of mites in a colony in the ApiLife VAR® group ($r = 0.505$, $P = 0.386$), the formic acid group ($r = 0.365$, $P = 0.545$), the oxalic acid group ($r = 0.769$, $P = 0.231$), the Sucrocide group ($r = -0.262$, $P = 0.738$), or the Apistan® group ($r = 0.108$, $P = 0.863$). In the double-deep study, mite mortality was not correlated with the total number of mites in a colony in the ApiLife VAR® group ($r = -0.560$, $P = 0.621$), the formic acid group ($r = -0.096$, $P = 0.877$), the oxalic acid group ($r = -0.870$, $P = 0.328$), or the Apistan® group ($r = 0.151$, $P = 0.809$).

The amount of ApiLife VAR® that evaporated during a treatment period was not significantly correlated with the corresponding mite mortality in either single-deep colonies ($r_s = 0.975$, $P = 0.144$) or double-deep colonies ($r_s = 0.933$, $P = 0.235$). The amount of formic acid that evaporated in a colony was not significantly correlated with the corresponding, cumulative mite mortality of the 30-day treatment period in either the single-deep colonies ($r_s = -0.489$, $P = 0.403$) or double-deep colonies ($r_s = 0.746$, $P = 0.464$).

The amount of ApiLife VAR® that evaporated during a treatment period was not correlated with the average daily minimum temperature ($r_s = -0.0161$, P = 0.761), maximum temperature ($r_s = 0.020$, P = 0.970), or mean temperature ($r_s = -0.030$, P = 0.955) during the treatment period (data combined across apiaries). Minimum, maximum, and daily average temperatures during each treatment period are presented in Table 2.

DISCUSSION

All four of the biopesticides we evaluated (ApiLife VAR®, formic acid, oxalic acid, and Sucrocide) show promise as viable alternatives, alone or as part of a multi-tactic IPM approach, to conventional pesticides for the late-fall control of varroa mites. Alternating a conventional pesticide with a biopesticide treatment (i.e., spring and fall) would reduce conventional pesticide use by 50%, and could serve as a tool to combat fluvalinate- and coumaphos-resistance in varroa populations by decreasing the frequency of mite exposure to these pesticides. Percent varroa control resulting from these biopesticide treatments were not related to the overall numbers of varroa mites in the colonies, suggesting that these biopesticides should be equally effective against various levels of mite infestation (e.g., high or low infestations).

The sticky boards used here to capture falling mites during treatment, in effect, served the same function as screen bottom boards, as mites (if alive) trapped on the boards were prevented from reattaching to host bees. Therefore, mite mortality reported here should be considered the cumulative effect of chemical treatment and the culling of varroa from the brood nest through the physical barrier of a sticky board/screen bottom board. This aspect is particularly important for treatments such as ApiLife VAR® whose efficacy relies, in part, on increasing grooming behavior by acting as an irritant to bees, which leads to increased rates of varroa removal from adult bees through behavior modification.

Our use of different apiaries and treatment starting dates for the single- and double-deep hive studies does not allow us to directly compare treatment efficacies between these two hive size categories. However, due to similarities in average total mite infestation levels (Table 1) and average temperatures (Table 2) between apiaries during the studies, some general trends can be noted for informational purposes. Specifically, the efficacy of chemicals that act by contact (Apistan® as an axonic (nerve) poison) or consumption (oxalic acid, probably as a protoplasmic poison by acidifying the host bee's hemolymph) by varroa mites were unrelated to hive size; whereas chemicals whose efficacy relies on evaporation rates (ApiLife VAR® and formic acid) were more effective at killing varroa in single-deep hives than in double-deep hives. Sucrocide was only tested in single-deep colonies; however, the mode of action for sugar esters (presumably both suffocation and desiccation through the dissolving of the mite's cuticular wax layer) (Puterka et al. 2003) suggests that it would be equally effective in larger hives as long as the application of solution was sufficient to cover all adult bees in the colony.

The levels of varroa mite control found here using formic acid in the MiteGone delivery pad system were similar to those reported for the gel-based formulation, Apicure® (ca. 70%) (Feldlaufer et al. 1997), and were slightly higher than the single-application MiteAway™ formic acid pads (51-56%) tested in New York (Calderone 1999, Calderone and Nasr 1999). The average formic acid evaporation rate *per one pad* found here (4-6 g/day) is generally considered insufficient for adequate (i.e., stand-alone) varroa control (Feldlaufer et al. 1997, Thomas 1997, Calderone 1999, Calderone and Nasr 1999, Imdorf et al. 1996); and our results concur.

Increasing the level of varroa control using MiteGone pads may be possible by implementing one or more of the following adjustments in treatment application: (1) extending the treatment period beyond the 30-day period evaluated here, (2) using more than one delivery pad to increase the amount of formic acid that is released per day, and/or (3) beginning treatments earlier in the fall season to take advantage of typically warmer daily temperatures, which should increase formic acid evaporation (Ruzicka 2004). Adjustments in the application of MiteGone formic acid delivery pads to increase evaporation rates and subsequent varroa control appears to be realistic, as only 52-60% of the initial volume of formic acid evaporated during our study.

Varroa mortality obtained here using ApiLife VAR® was very similar to that reported in Europe and the southeastern US (Imdorf et al. 1999; Ellis et al. 2001). Interestingly, the evaporation rates of ApiLife VAR® and formic acid in our study were not correlated to temperature (min, max, or daily average), which is in contrast to data reported by Calderone (1999), who found significant correlations between evaporation rates and temperatures for formic acid and a thymol-blend of similar composition to ApiLife VAR®. Perhaps the low temperatures fluctuations during our study were insufficient to detect this relationship.

Mite mortality was also not correlated with the amount of ApiLife VAR® or formic acid that evaporated during our study, which was unexpected, because, as Calderone (1999) noted, the efficacy of both materials should be related to the amount of material to which mite populations are exposed. Calderone (1999) and Calderone and Nasr (1999) found this same discrepancy between evaporation rates and corresponding mite mortality using formic acid and the thymol-blend they evaluated. The higher mite mortality for ApiLife VAR® and formic acid in single- versus double-deep colonies found here was, presumably, due to the greater exposure (closer proximity) of bees to chemical fumes in smaller hives than in larger hives.

Each of these biopesticides has their own advantages and disadvantages. Sucrocide provided excellent control of varroa, can be used throughout the season, and is safe to use; but the application technique may be too labor intensive to be adopted by beekeepers with substantial numbers of colonies. In addition, more studies on Sucrocide are needed to determine what, if any, effects varying levels of brood have on treatment efficacy, and whether or not honey bee eggs and young larvae may be affected by direct spray applications. Oxalic acid also provided good control of varroa; however, it is typically reserved as a late-season treatment or for other times in which there is little or no brood production, as there are conflicting reports to its effects on bees, ranging from no deleterious effect to impaired queen performance and overwintering success (Hiiges et al. 1999; Nanetti et al. 2003). This aspect needs to be better resolved before commercialization of an oxalic acid product in the US can be attempted. Treatment with ApiLife VAR® or formic acid would be likely more efficacious if used in conjunction with other varroa-reducing strategies (e.g., mite-resistant bee stock). ApiLife VAR® use is also restricted to fall treatments, due to its negative effect on brood production (Ellis et al. 2001), making it counterproductive as a spring treatment. The potential of formic acid to be re-approved for use by US beekeepers is unclear.

The positive correlation between the pre-treatment mite infestation level in a colony determined using the alcohol wash technique (DeJong et al.1982b) and the corresponding cumulative number of mites collected on sticky boards during the treatment + evaluation periods suggests that the alcohol wash technique may be a useful indicator to estimate and monitor colony-level varroa infestation levels. Delaplane and Hood (1997) and Calderone and Nasr (1999) previously reported similar relationships using the ether roll technique.

In conclusion, ApiLife VAR®, the MiteGone formic acid delivery system, a 3.2% oxalic acid trickle application, and Sucrocide each have potential to be valuable additions to the beekeepers' arsenal of varroa control products. Increasing the diversity and availability of varroa control options are of critical importance to the US beekeeping industry, considering the widespread reports of mite resistance to conventional pesticide products.

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ACKNOWLEDGEMENTS

The use of trade names in this publication does not imply endorsement by the authors of the products named or criticism of similar ones not mentioned. We thank Tony Barrington of AVA Chemical Ventures and Bill Ruzicka of MiteGone Enterprises, Inc. for donating supplies; Walter Wilson for technical assistance and the use of his bee colonies; J. Van Waganer and C. Pettite for allowing us to establish test apiaries on their respective properties; Bob Hughes for managing the bee colonies prior to the start of the study; the Harvey family for supplying a portion of the test bee colonies; and Dr. James Barry for early manuscript reviews and statistical support.

Table 1. Pretreatment mite infestation level estimates (mean ± SE), total average number of mites in colonies (mean ± SE), and mite mortality (mean ± SE) resulting from 6 treatments in single-deep colonies and 5 treatments in double-deep colonies.

Hive Size Treatment	n [z]	Pre-treatment mite infestation [y]	Total number of mites/colony [x]	Mite mortality from treatment [w]	Bee colony death during treatment
Single-Deep Colonies	30				
Apistan	5	9.3 ± 1.90	2473 ± 529.2	0.97 ± 0.007 a	1
Sucrocide	5	12.4 ± 2.75	1825 ± 422.5	0.96 ± 0.008 a	0
Oxalic Acid	5	10.2 ± 4.35	1473 ± 382.1	0.92 ± 0.019 a	0
ApiLife VAR	5	24.0 ± 9.07	2077 ± 440.8	0.91 ± 0.035 a	1
Formic Acid	5	22.4 ± 9.41	2435 ± 633.6	0.79 ± 0.049 b	0
Control	5	10.7 ± 3.43	1363 ± 158.1	0.23 ± 0.004 c	1
Mean	-	14.8 + 2.69	1941 + 193.0	-	-
Double-Deep Colonies	30				
Apistan	6	25.9 ± 3.94	1818 ± 222.0	0.95 ± 0.007 a	1
Oxalic Acid	6	12.8 ± 1.02	2369 ± 538.3	0.93 ± 0.022 a	2
ApiLife VAR	6	12.2 ± 1.04	2355 ± 473.3	0.69 ± 0.012 b	1
Formic Acid	6	20.3 ± 1.84	1117 ± 243.0	0.66 ± 0.067 b	1
Control	6	15.6 ± 4.33	2091 ± 357.9	0.15 ± 0.012 c	0
Mean	-	17.4 + 2.57	1950 + 231	-	-

[z] Original sample size.

[y] Pre-treatment alcohol wash estimates of percent varroa infestation did not differ between treatment groups in either the single-deep ($P = 0.3696$) or the double-deep ($P = 0.1047$) colonies.

[x] The average total number of mites collected per colonies did not differ between treatment groups in either the single-deep ($P = 0.4051$) or the double-deep ($P = 0.6870$) colonies.

[w] Proportion of mites collected during treatment periods in relation to the total number of mites collected during the treatment + evaluation periods. Values in columns followed by the same letter were not significantly different from each other ($P = 0.05$). Mean separation data from single-deep colonies and double-deep colonies are independent from each other.

Table 2. Amount (mean + SE) of ApiLife VAR® and formic acid evaporating, and the corresponding minimum, maximum, and average temperatures during and across treatment periods.

Hive Size Treatment Period	ApiLife VAR Evaporated (g) [z]	Formic Acid Evaporated (g) [y]	Temperature ^{O}C (^{O}F) Min	 Max	 Mean
Single-Deep Colonies					
1 (14 Oct. - 24 Oct.)	5.63 ± 1.83	-	7.2 (44.9)	17.3 (63.2)	12.1 (53.7)
2 (25 Oct. – 5 Nov.)	3.56 ± 0.33	-	8.7 (47.7)	19.1 (66.4)	13.8 (56.9)
3 (6 Nov. – 15 Nov.)	4.25 ± 0.53	-	4.8 (40.6)	12.4 (54.4)	8.5 (47.3)
Total (14 Oct. – 15 Nov.)	13.44 ± 0.86	165.4 ± 7.231	6.8 (44.2)	17.1 (62.7)	12.1 (53.8)
Double-Deep Colonies					
1 (21 Oct. - 31 Oct.)	3.61 ± 1.42	-	7.2 (45.0)	15.6 (60.0)	11.2 (52.2)
2 (1 Nov. – 10 Nov.)	2.44 ± 0.92	-	7.7 (45.8)	16.7 (62.1)	11.9 (53.4)
3 (11 Nov. – 21 Nov.)	3.45 ± 0.62	-	5.0 (41.0)	13.1 (55.5)	8.7 (47.7)
Total (21 Oct. – 21 Nov.)	9.50 ± 1.24	144.8 ± 4.15	7.0 (44.6)	15.7 (60.2)	10.8 (51.4)

[z] Original total weight of ApiLife VAR tablets (2 per package) was 22.0 g.

[y] Original total weight of formic acid solution in delivery pads was 275.0 g.

Fig. 1. Proportion (mean + SE) of the total number of *V. destructor* mites in a colony collected during each of the treatment periods in single-deep colonies (A) and double-deep colonies (B).