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Evaluation of Mite-Away-II™ for fall control of *Varroa destructor* (Acari: Varroidae) in colonies of the honey bee *Apis mellifera* (Hymenoptera: Apidae) in the northeastern USA

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Abstract Mite-Away II™, a recently-registered product with a proprietary formulation of formic acid, was evaluated under field conditions in commercial apiaries in upstate New York (USA) for the fall control of *Varroa destructor* Anderson & Trueman in colonies of the honey bee, *Apis mellifera* L. Ambient temperatures during the treatment period were in the lower half of the range recommended on the label, but were typical for early fall in upstate New York. Average mite mortality was 60.2 ± 2.2% in the Mite-Away II group and 23.3 ± 2.6% in the untreated control group. These means were significantly different from each other, but the level of control was only moderate. These results demonstrate that Mite-Away II may not always provide an adequate level of control even when the temperature at the time of application falls within the recommended range stated on the product's label. To make the best use of temperature-sensitive products, I suggest that the current, single-value, economic treatment threshold be replaced with an economic treatment range. The limits for this range are specified by two pest density values. The lower limit is the usual pest density that triggers a treatment. The upper limit is the maximum pest density that one can expect to reduce to a level below the lower limit given the temperatures expected during the treatment period. When the actual pest density exceeds the upper limit, the product should not be recommended; or, a warning should be included indicating that acceptable control may not be achieved.

Keywords *Varroa destructor* · *Apis mellifera* · Honey bee · Formic acid · Economic thresholds

Introduction

Varroa destructor Anderson & Trueman is the most serious pest of the honey bee, *Apis mellifera* L., throughout the world (De Jong et al. 1982; De Jong 1997); and it has caused

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significant damage to the US beekeeping industry since it was first reported there in 1987 (Anon 1987). The use of synthetic pesticides can cause damage to bees (Haarmann et al. 2002; Pettis et al. 2004) and leads to the contamination of hive products, especially honey and wax (Slabezki et al. 1991; Liu et al. 1993; Wallner 1995; Kochansky et al. 2001; Floris et al. 2004). Recent studies have found fluvalinate and coumaphos to be common contaminants in pollen and wax from colonies in the US (Frazier et al. 2008). These factors provide a strong incentive to seek alternative control strategies based on natural products and Integrated Pest Management (IPM) principles.

Numerous alternative methods for managing *V. destructor* have been explored (reviewed in Imdorf et al. 1995; Fries 1997; Thomas 1997; Calderone 2005). Formic acid has received considerable attention because of its activity against *V. destructor* (Maul et al. 1980; Hoppe et al. 1989; Eguaras et al. 1996, 2003; Nasr et al. 1996; Hood and McCreadie 2001; Feldlaufer et al. 1997; Calderone 2000; Currie and Gatien 2006) as well as *Acarapis woodi*, and *Tropilaelaps clareae* (Hoppe et al. 1989).

Formic acid has several highly desirable qualities that make it an ideal replacement for Apistan and CheckMite+. There are no reported cases of resistance to formic acid, it has been granted an exemption from tolerance by the US-EPA, and it is acceptable to the organic community because it is considered to be a natural product with negligible human toxicity. As a result, both hobbyist and commercial beekeepers in the northeast and elsewhere have a strong interest in formic acid.

The goal of this study is to determine the level of control (defined as the % mite mortality occurring during the product's recommended 21 day treatment period) that can be obtained with Mite-Away II under conditions typically encountered during the fall in upstate New York. Mite-Away II is a recently-registered pesticide that has not previously been evaluated. This formic-acid based product has several unique characteristics that distinguish it from previous formic acid products. Therefore, it is essential to learn as much as possible about this product in order that it can be used most effectively. I also examine the current use of economic thresholds and propose a new management tool—the economic treatment range—that incorporates the actual level of control obtained with a miticide into the beekeeper's decision-making process.

Materials and methods

Colonies

Ten colonies of the honey bee, *A. mellifera*, were selected for use in each of five apiaries located within 20 km of Venice Center, NY. At the end of the fall nectar flow in late September 2005, and prior to initiating treatments, honey supers were removed from hives, leaving each colony in two, full-depth (~24.5 cm), 10-frame hive bodies. Each colony was comprised of approximately 20, full-depth combs of worker bees (at 10–16°C) and a queen; and each had sufficient stores to successfully survive the winter. Treatments were initiated in early October because the fall nectar flow in this region usually extends through the third or fourth week of September. Honey supers must be removed prior to treatment according to the product's label, and that takes a substantial amount of time in a mid- to large-scale operation, making early October the first realistic treatment window for this group of beekeepers.

Treatments

Treated colonies received a single-application Mite-Away II pad (NOD Apiaries, Canada). Mite-Away II employs a novel packaging and delivery system and a new formulation of formic acid, all of which may affect the release of the active ingredient. The product contains 189 grams of formic acid in a proprietary formulation (part of it as an azeotrope, part of it as water soluble esters of formic acid). The total weight of the formic acid solution (which is between 60 and 65% formic acid) is 292 g.

Colonies in each apiary were randomly assigned to the treatment group or to an untreated control group. In each of the five apiaries, six colonies were treated with Mite-Away II, and four served as controls. A single Mite-Away II pad was left in place for the duration of the treatment period. Due to cool weather during the last week of the recommended 3 week treatment period, pads were left in place for an additional 10 days. This resulted in the Mite-Away II pads being on the colonies for 31 days rather than the recommended 21 days.

Treatment and follow-up periods

The treatment period ran from 9 October to 8 November. Mites were collected on sticky-board collection devices (Calderone and Spivak 1995). For purposes of mite collection, the treatment period was divided into four collection intervals. The first sticky-board was in place from 9 to 16 October, the second from 16 to 23 October, the third from 23 October to 1 November, and the fourth from 1 to 8 November (total of 31 days). One colony in the Mite-Away II group suffocated during the first interval because the sticky board frame reduced the bees' ability to leave the hive resulting in the entrance being blocked.

At the end of the treatment period, the Mite-Away II pads were removed; and follow-up miticides were applied to all colonies to kill remaining mites. To avoid possible problems with pesticide resistance, each colony received four strips of Apistan and four strips of CheckMite+ (two of each type per hive body; Elzen et al. 2000). Two sticky boards were used during this follow-up period, one from 8 to 22 November and the other from 22 November to 26 December (total of 49 days).

Level of control

For each colony, the level of control, defined as the percent mite mortality that occurred during the treatment period, was calculated by dividing the number of mites collected during the treatment period (9 October–8 November) by the number of mites collected during both the treatment (9 October–8 November) and follow-up periods (8 November–26 December) (Feldlaufer et al. 1997; Calderone and Nasr 1999; Sheppard et al. 2003) and converting the resulting proportion to a percent. Treatment effects were evaluated with PROC Mixed (SAS Institute Inc 1996; Littell et al. 1996) with treatment, apiary and their interaction term modeled as fixed effects. Analysis was performed on log transformed data to satisfy the assumption of homogeneity of variances.

Changes in weights of pads

Mite-Away II pads were weighed before being placed on colonies and after each collection interval. The weight change during each 7–10 days interval, expressed as a quantity (g)

and as a percentage of the initial weight of the formic acid solution in each pad (292 g for each pad), was analyzed with a repeated measures model using PROC Mixed in SAS (SAS Institute Inc 1996; Littell et al. 1996) with colony as the subject for repeated measures. Interval, apiary and their interaction were modeled as fixed effects. Tukey–Kramer tests were used to resolve significant main effects. The total weight change during the 31 day treatment period was also calculated.

Temperature

Temperature data were obtained from the nearest weather station and average values (mean ± SE) for the daily minimum, maximum and overall daily temperatures (24 h basis) were calculated.

Results

Level of control

The average level of control (lsmean ± SE) was 60.2 ± 2.2% in the Mite-Away II group and 23.3 ± 2.7% in the control group (Fig. 1). Treatment effects were significant ($F_{1,39} = 125.18$; $P < 0.0001$); but apiary effects ($F_{4,39} = 0.87$; $P = 0.49$) and the interaction between apiary and treatment ($F_{4,39} = 0.40$; $P = 0.81$) were not significant. The number of mites collected during the treatment and follow-up periods in each apiary are given in Table 1.

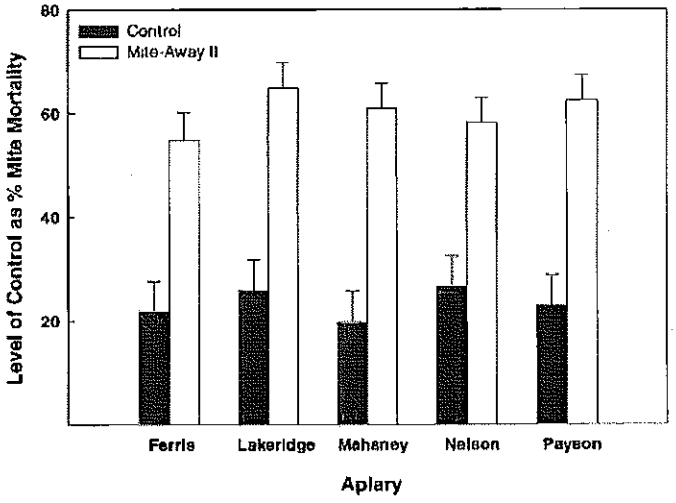

Fig. 1 Average level of control (% mite mortality) in the treatment and control groups during the 31 day treatment period from 9 October to 8 November 2005. Means within each apiary were significantly different from each other ($P < 0.05$), but apiary effects and the interaction between apiary and treatment were not significant

Table 1 The number of mites (mean ± SE) collected during the treatment (T) and follow-up (F) periods in each apiary in the fall ($n = 4$ for controls and $n = 6$ for Mite-Away II in each apiary)

Apiary	Period	Control colonies	Mite-Away II colonies
Ferris	T	357.8 ± 165.4	542.4 ± 185.5
	F	1,292.0 ± 556.7	433.8 ± 116.4
Lakeridge	T	530.3 ± 42.0	2,405.8 ± 683.0
	F	1,579.8 ± 246.3	1,211.8 ± 234.4
Mahaney	T	731.3 ± 142.3	1,614.5 ± 159.9
	F	3,046.3 ± 660.8	1,151.0 ± 254.5
Nelson	T	1,076.3 ± 343.4	2,350.0 ± 392.5
	F	2,586.5 ± 570.7	1,643.3 ± 228.4
Payson	T	740.5 ± 137.3	1,295.0 ± 111.1
	F	2,532.8 ± 360.5	867.0 ± 237.3

Mite-Away II pads were in treated colonies during period T. Follow-up treatments were in all colonies during period F

Change in weight of products

The average gross starting weight of the Mite-Away II pads (including the fiber pad and the inner plastic enclosure) was 408.3 ± 0.5 g ($n = 30$) with a difference of 13.6 g between the heaviest and the lightest pads. The average change in the weights of the pads during each of the four collection intervals is presented in Table 2. The effect of interval on weight change was significant ($F_{3,\ 72} = 36.26$; $P < 0.0001$); but apiary effects ($F_{4,\ 25} = 0.20$; $P = 0.94$) and the interaction between apiary and interval ($F_{12,\ 72} = 0.64$; $P = 0.80$) were not. Over the 31 day treatment period, pad weight decreased by an average of 153.2 ± 6.1 g or 52.5 ± 2.1% of the initial 292 g of formic acid solution. Statistics for percentage active ingredient are the same as for weight.

Temperature

The average daily temperature and the average daily maximum and daily minimum temperatures during each of the four collection intervals during the 31 day treatment period are reported in Table 3.

Table 2 Average change (lsmean ± SE) in the weight of Mite-Away II pads ($n = 29$ each interval) during each interval; and total change in pad weight over the 4 week treatment period

Weight change	Period 1 (9–16 Oct)	Period 2 (16–23 Oct)	Period 3 (23 Oct–1 Nov)	Period 4 (1–8 Nov)	Total (9 Oct–8 Nov)
g/colony	76.4 ± 4.4a	33.8 ± 4.5bc	13.9 ± 4.5d	28.9 ± 4.5c	153.2 ± 6.1
As % initial quantity formic acid solution[a]	26.2 ± 1.5a	11. 6 ± 1.5bc	4.8 ± 1.6d	9.9 ± 1.6c	52.5 ± 2.1

Data are given as quantities (g) and as a percentage of the initial quantity of formic acid solution. Means are compared across rows (Periods 1–4), and means with different letters are significantly different (Tukey–Kramer, $P < 0.05$)

[a] 292 g formic acid solution (total of 189 g formic acid available in a Mite-Away II pad)

Table 3 Average daily temperature high, average daily temperature low and average daily temperature (mean ± SE; all in °C) during each collection period and over the entire 31 day treatment period

Average	Period 1 (9–16 Oct)	Period 2 (16–23 Oct)	Period 3 (23 Oct–1 Nov)	Period 4 (1–8 Nov)	Total (9 Oct–8 Nov)
High	12.5 ± 1.0	14.8 ± 1.0	7.9 ± 1.2	18.2 ± 1.5	13.1 ± 0.9
Low	8.9 ± 0.7	3.4 ± 1.5	−0.1 ± 0.8	5.7 ± 1.5	4.2 ± 0.8
Daily	10.7 ± 0.8	9.2 ± 0.8	4.1 ± 0.6	12.1 ± 1.3	8.8 ± 0.7

Discussion

This study provides the first evaluation of Mite-Away II, a recently registered product for the control of *V. destructor* in honey bee colonies. The average level of control during the 31 day treatment period in the Mite-Away II group (60.2 ± 2.2%) was significantly greater than in the control group (23.3 ± 2.6%). This moderate level of control is likely due to the fact that the level of mortality obtained with fumigants is partially dependent on temperature (Ostermann and Currie 2004; Bahreini et al. 2004). The Mite-Away II label states that the "Outside daytime temperature highs should be between 50 and 79°F at the time of application." The average outside daytime temperature high during this study was 12.46 ± 0.98°C during the first interval, exceeding the recommended 10°C (50°F; see Table 2) at the time of application specified on the product's label. The performance of Mite-Away II obtained here, while significant, was lower than one might expect, given that the ambient temperatures during the study were within the recommended range.

Although not listed as requirements, several other measures of temperature also exceeded the recommended 10°C. The average outside daytime temperature highs exceeded 10°C during all but the third interval (7.9 ± 1.2°C) (to achieve the product's 3 week treatment period, treatments were extended for a fourth interval during which time the average outside daytime temperature high was 18.2 ± 1.5°C). Overall, the 31 day average outside daytime temperature high was 13.1 ± 0.9°C, and the outside daytime temperature high exceeded 10°C on 22 day of the 31 day treatment period. The average outside daytime high during those 22 day was 15.3 ± 0.9°C.

The level of control obtained with the new Mite-Away II pad with its novel packaging and delivery system and proprietary formulation of formic acid was similar to that seen with formic acid in previous studies (Feldlaufer et al. 1997; Calderone 1999; Calderone and Nasr 1999; Satta et al. 2005; Espinosa-Montano and Guzman-Novoa 2007), but less than that seen in others (Calderone 2000; Satta et al. 2005). Calderone (2000) reported mortality of 94.1 ± 4.1% under similar temperature conditions in the same beekeeper's operation as in the current study. The average changes in pad weights in the three apiaries in that study were 293.3 ± 5.8 g (85.5% of an initial 342.9 g of a 65% formic acid solution), 302.1 ± 53.8 g (88.1%) and 304.9 ± 5.76 g (88.9%), considerably greater than the 153.2 ± 6.1 g (52.5 ± 2.1%) change seen in this study (Table 2). The pad in the earlier study uses 300 ml of a 65% formic acid solution, about 20% more than the Mite-Away II pad. Additionally, the surface area of that pad is about 29% greater than that of the Mite-Away II pad; and the area of the openings in the vented plastic enclosure is 2.03 times greater. The combined effect of these differences likely accounts for the higher rate of evaporation and greater % mite mortality reported in the previous study and suggests ways to increase the level of control that can be obtained with the current Mite-Away II product.

Levels of control obtained with fumigants, especially under cooler temperatures, raise questions about the use of published economic treatment thresholds. Thresholds provide beekeepers with a tool to help them decide if a specific pest density warrants treatment. It is assumed that the treatment will reduce the pest density below the threshold so that the colony will remain healthy until the next treatment window. However, because of the temperature dependent nature of fumigants, one may not always achieve that goal with all combinations of pest density and temperature. Consequently, beekeepers need to know how to use these products effectively; and that requires a method for incorporating temperature-related variation in the level of control into the decision-making process.

The concept of a flexible economic treatment threshold has been used in other cropping systems (Archer and Bynum 1990), and the concept can be adapted for use by beekeepers. To ensure that temperature-sensitive products achieve the goal of maintaining colony health, I propose that the traditional economic treatment threshold be replaced by a flexible economic treatment range (ETR). This range is defined by two pest densities: a lower limit and a product-specific, temperature-dependent upper limit. The lower limit is simply the current economic threshold that triggers a treatment. The upper limit is the maximum pest density that one can expect to reduce to a level below the lower limit. This new upper limit is based on two variables. One is the lower limit. The other is the actual level of control one will obtain with a specific product at a specific temperature. The upper limit is calculated as:

$$\mathrm{UL} = \mathrm{LL}/(1 - \mathrm{MMpt}),$$

where UL is the upper limit, LL is the lower limit and MM_{pt} is the level of control (i.e. proportion of mites expected to be killed) obtained with a specific product and temperature. Adoption of an ETR will help beekeepers use these products more effectively by enabling them to target colonies with mite levels appropriate for the expected level of control.

An ETR for Mite-Away II under the current temperature conditions can be calculated using data from the current study and several published economic thresholds. Three economic treatment thresholds are available for northern regions. Strange and Sheppard (2001) recommend a fall 300-bee ether roll count $\geq$3. Currie and Gatien (2006) recommend a mite-to-bee ratio $\geq$0.04 (equivalent to a 300-bee ether roll count $\geq$7; conversion based on data from Calderone and Turcotte 1998). Ellis and Baxendale (1996) recommend treatment when the fall ether roll count is $\geq$6 mites. The thresholds reported in these studies vary because they apply to different times of the season. The upper limit (standardized to a 300-bee ether roll count) with Mite-Away II and a 60.2% level of control (this study) would be 7 (based on Strange and Sheppard 2001), 17 (Currie and Gatien 2006) or 14 (Ellis and Baxendale 1996). The resulting ETRs are: $3 \leq x \leq 7$, $7 \leq x \leq 17$ and $6 \leq x \leq 14$, respectively. To the degree that mite levels exceed the UL, treatments will become increasingly ineffective in maintaining colony health. However, as the temperature (and, therefore, the level of control) increases, the upper limit increases. Improvements to the product that increase the average level of control should be sought.

Variability in the level of control presents a second issue that affects the determination of the optimal ETR. Not only does the average level of control depend on the ambient temperature; in addition, there is considerable variability in control from colony-to-colony at any given temperature. Using the average level of control to determine the UL only ensures that 50% of the colonies will be adequately treated (assuming that the level of control is normally distributed). The other 50% will not be adequately treated because the level of control in those colonies will, by definition, be below average. If the variation is

small or the mite load only slightly above the LL, then the consequences will be minimal; however, as variation in control increases and the actual mite levels exceed the LL by larger amounts, colonies are at increased risk. With fumigants, variation in the level of control is greater than that seen with previous synthetics. The coefficient of variation (CV) is a measure of a treatment's performance from colony-to-colony. Calderone (1999) reported a CV of 13.43 for a thymol treatment, 23.53 for a formic acid treatment, and 0.20 for an Apistan treatment. The ratios of the CVs of the thymol and formic acid treatments to that of the Apistan treatment were 67.15 and 117.65, respectively. The CV for the Mite-Away II treatment in the present study was 23.05, similar to that reported by Calderone (1999) using a similar dose under similar conditions.

One solution to inadequate treatment is to factor the colony-to-colony variability into the decision-making process. In the present case, the average level of control and standard deviation for Mite-Away II was 60.4 ± 13.9%. To determine a value for UL that will ensure that the product reduces the mite population below the LL 95% of the time, the proper value of MM_{pt} to use in the current example would be 32.6% (the average level of control minus two SD). Based on that value, the ETRs calculated above would be: $3 \leq x \leq 4$, $7 \leq x \leq 10$ and $6 \leq x \leq 8$, respectively. This more conservative standard reduces the risk of inadequate treatment, but severely limits the situations in which these products can be used with a high degree of confidence. Nonetheless, these are the risks beekeepers need to understand when using these products.

Economic treatment thresholds are central to IPM programs. The results from this study offer several important findings related to their use with fumigants. Beekeepers should not assume that these products will effectively control mite populations simply because the temperature at the time of application falls within the recommended range. The actual level of control is a function of the ambient temperature during the treatment period, and that can vary widely. Therefore, the decision to treat must take into account the actual level of control obtained at a given temperature, the recommended treatment threshold and the actual pest density in the colony. The ETR, with its lower and upper limits, provides the beekeeper with one tool with which to make this decision. When the actual pest density falls above the UL, the product should not be recommended; or, beekeepers should be advised that their bees are at increased risk. Beekeepers should also be made aware of the fact that that even when using an ETR, all colonies may not be adequately treated because a product will not produce an average level of control in all colonies.

A beekeeper's choice of miticide is but one component in their IPM program. Colonies must remain healthy between treatment windows. This is especially critical when the approaching the fall treatment window because there is no time for a colony to recover if a serious mite problem has already compromised its health. Beekeepers must incorporate methods into their IPM programs that maintain mite populations at levels that do not compromise colony health between treatments. Even a miticide that provides a 100% level of control is of little use if the beekeeper has allowed mite levels to rise to a point where the colony's health has been seriously compromised. Therefore, recommendations for the use of fumigants should be conservative; and beekeepers should be advised to monitor mite populations throughout the season and to incorporate other methods into their IPM programs, if needed, to keep mite populations at levels consistent with good colony health.

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Comparison Between Two Thymol Formulations in the Control of *Varroa destructor*: Effectiveness, Persistence, and Residues

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ABSTRACT An apiary trial on the use of two acaricide formulations (gel-Apiguard and vermiculite and Api Life VAR) in the control of *Varroa destructor* (Anderson & Trueman) was conducted in summer 2001 in Sardinia (Italy). The main goals were 1) to determine their effectiveness against *V. destructor*, taking into account natural mite mortality in control hives; and simultaneously 2) to determine the persistence of both formulations and residues in honey and wax, by using a new extraction method. Both thymol formulations, after the treatments, reduced significantly the levels of mite infestations of adult bees and sealed brood, but their efficacy, expressed as percentage of mortality, was lower for both products (Api Life VAR 74.8 ± 13.1 and 81.3 ± 15.5, Apiguard 90.4 ± 8.3 and 95.5 ± 8.7 for sealed brood and adult bees, respectively) than the efficacy previously obtained with the same products in other experimental conditions. Moreover, a considerable colony-to-colony variability was recorded, and a significant negative effect of the thymol treatments on colony development was observed. During 2 wk of treatment, the bees removed nearly 95% of all the applied product (gel or vermiculite). Residues found in honey collected from the nest varied from 0.12 to 4.03 mg/kg for Api Life VAR and from 0.40 to 8.80 mg/kg for Apiguard. The residues were relatively higher in wax (Api Life VAR = 21.6 ± 13.0; Apiguard = 147.7 ± 188.9) than in honey, because thymol is a fat-soluble ingredient.

KEY WORDS *Varroa destructor*, thymol, effectiveness, persistence, residues

CONTROL IN THE APIARY of the mite *Varroa destructor* (Anderson & Trueman) is carried out with acaricides that often are synthetic compounds and, therefore, give rise to problems of resistance (Milani 1999) and residues in the hive products (Wallner 1999). Over the past few years, the worldwide trend has been toward the use of natural substances, particularly some organic acids and thymol (Calderone 1999, Imdorf et al. 1999, Whittington et al. 2000).

To date, thymol has been tested in powered form with different quantities and application intervals (Imdorf et al. 1999), impregnated in porous ceramic carrier (Api Life VAR, Chemicals LAIF, Vigonza (PD) Italy) (Imdorf et al. 1995), or included in a gel (Apiguard, Vita Europe, Basingstoke, United Kingdom) (Colombo and Spreafico 1999, Arculeo 2002). However, vermiculite and gel thymol-based treatments have never been compared with respect to effectiveness, using a method of evaluation that takes into account natural mite mortality, residues, and persistence of thymol.

In many countries, according to the national regulations, no official limits of thymol residues (maximum residue level) have been established for honey and wax (Wallner 1999). However, for the European food legislation, foreign odors or tastes are not allowed in honey. To date, studies on residues have been carried out only for Api Life VAR, and the sensory perception threshold was detected at the concentration level of 1.1 mg/kg (Bogdanov et al. 1998). No data on Apiguard residues are reported in the literature. In addition, the extraction methods used for thymol (Bogdanov et al. 1998, Martel and Zeggane 2002, Nozal et al. 2002) have some limits because of the presence of interference peaks in the chromatogram that do not allow an accurate quantification of the acaricide.

The present work aimed at 1) comparing the efficacy of the two thymol formulations Api Life VAR and Apiguard in the control of varroosis; 2) determining the persistence of the two commercial formulations; and 3) assessing thymol residues in the honey and wax, by using a new extraction method.

Materials and Methods

Apiary Trial. The trial was carried out in an apiary of 15 colonies of bees of local strain, derived from *Apis mellifera ligustica* Spinola, placed in Dandant-Blatt hives, during June and July 2001 in Oristano (southern Sardinia, Italy). Before the trial, both the mite infestation level and the size of all colonies were

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monitored to obtain three homogeneous experimental groups (two treated and one control) of five hives each. The mite infestation levels of sealed worker brood and adult bees were estimated in all hives by inspecting a total of 300 cells, in a crosswise manner, from three combs per hive (Floris 1992a) and by brushing ≈300 bees per hive from at least three combs (Ritter and Ruttner 1980). To establish the size of the colonies, we estimated the surface of sealed brood by using one-sixth of a Dadant-Blatt frame (188 cm^2) as a unit of measure (Marchetti 1985).

Two thymol-based formulations were used, separately, as treatments: Api Life VAR and Apiguard. According to the label, the first formulation consists of wafers of ≈12 g of vermiculite containing ≈74% thymol (equal to 9 g of thymol per tablet), 3.7% menthol, 3.7% camphor, and 16% eucalyptol. The second formulation contains 25% thymol in a gelatin, manufactured as 50-g gel portions (12.5 g of thymol). One group of hives was treated with Api Life VAR, one with Apiguard, and the third was used as control. Both formulation treatments were applied on 21 June 2001 by using two half wafers of Api Life VAR or one portion of thymol in a gel, respectively, per hive. A second treatment was carried out on 6 July 2001.

During the trial, fallen mites and dead adult bees were counted in the control and treated hives twice a week by using a white petroleum jelly-covered plastic sheet inserted at the bottom of each hive to count mites, and Gary traps to count dead bees (Gary 1960).

Before, during, and after each treatment, honey and wax samples were collected from each treated and control hive, to estimate the presence of thymol residues. Honey samples were collected by sucking them with a 50-ml syringe from ≈100 unsealed cells from three combs having honey in the brood nest. Wax samples were obtained from each hive by cutting three frames (≈3 cm^2) from different nest combs.

During the trial, to evaluate the persistence of thymol in the formulation, samples of gel and vermiculite from treated hives were also collected after 1 and 2 wk from the start of the treatment. All samples were stored at -20°C until analysis.

The efficacy of the treatments was evaluated as percentage of mite mortality (Henderson and Tilton 1955, Floris et al. 2001).

Chemicals. Analytical standards, thymol (98%), cineole (99%), menthol (96%), and camphor (96%) were purchased from Aldrich, Janssen (Geel, Belgium) and Carlo Erba (Milan, Italy), respectively. A standard stock solution (1000 mg/kg) was prepared in acetone. Working standard solutions were obtained daily by dilution with diethyl ether extracts from untreated (control) honey and wax. Diethyl ether was used as the solvent for analysis.

Extraction Procedure from Formulations. One gram of each formulation was weighed in a 50-ml screw-capped tube, 20 ml of methanol (Chromanorm for high-performance liquid chromatography, Prolabo) were added, and the tube was agitated (15 min) in a rotary shaker and then sonified for 5 min in an ultrasonic bath (Transsonic T460). An aliquot of methanol (100 μl) was diluted to 5 ml with acetone and injected into a gas chromatograph (GC).

Extraction Procedure from Honey. One gram of honey was weighed in a 15-ml screw-capped tube and dissolved in 2 g of water. Two milliliters of diethyl ether was added, and the tube was agitated (10 min) in a rotary shaker. The phases were allowed to separate, and an aliquot of the extract was injected into a GC.

Extraction Procedure from Wax. One-half gram of wax was weighed in a 25-ml screw-capped tube. 5 ml of methanol/water (1:1) was added, and the tube was plunged in hot water at 70°C until the wax dissolved, and then agitated for 1 min in vortex. After cooling at room temperature, 5 ml of diethyl ether was added, and the tube was agitated for 15 min in a rotary shaker. The phases were allowed to separate, the ether extract was centrifuged and an aliquot was injected into a GC.

Recovery Assay. Untreated samples of honey and wax were fortified with 0.1, 0.5, 2.0, and 5.0 mg/kg thymol, menthol, cineole, and camphor, respectively, and processed according to the procedure described above. At each fortification level, four replicates were analyzed.

Apparatus and Chromatography. Samples were analyzed using an HRGC 5160 Mega series gas chromatograph fitted with a flame ionization detector (FID) 80, an AS 800 autosampler, and a split-splitless injector (Carlo Erba, Milan, Italy), connected to a HP 3396 A reporting integrator (Hewlett Packard, Avondale, PA). The column was a fused silica capillary DB-5 MS (5% phenyl-methyl-polysiloxane, 30 m by 0.25 mm id and film 0.1 μm) (J&W Scientific, Folsom, CA). The injector and the detector were at 100 and 200°C, respectively. The samples (2 μl) were injected in the split mode (1:10) for the formulations and in the splitless mode (30 s) for the other samples. The oven temperature was programmed as follows: 60°C hold 5 min, raised to 130°C (2°C/min) hold 4 min, raised to 180°C (10°C/min). Helium was the carrier and the makeup gas at 1.8 and 30 ml/min, respectively. The air and hydrogen flows for the FID flame were at 250 and 30 ml/min, respectively. The calibration curves were calculated between peak height and concentration using the external standard method.

Statistical Analysis. The data were analyzed by analysis of variance (ANOVA) after arcsine transformation (arcsine $\sqrt{y/100}$), in the case of percentages, to reduce the heterogeneity of the variance. The tables and figures show the nontransformed values. When the F-tests were significant, means were separated applying the least significant difference (LSD) test ($P < 0.05$) (Statigraphics Plus 1998).

Results

Efficacy of Treatments. Table 1 shows that before treatment no statistically significant differences existed between the three experimental groups regarding the size of sealed brood area ($F = 0.00$, df = 2, $P = 0.9953$), and the infestation level of both sealed worker brood ($F = 0.00$; df = 2; $P = 1$) and adult bees ($F =$

Table 1. Mite infestation level and sealed brood area (mean ± SD) in the experimental hives groups before and after treatment

Treatment	Worker brood infestation (%)		Adult bee infestation (%)		Sealed brood area (cm^2)	
	Before treatment	After treatment	Before treatment	After treatment	Before treatment	After treatment
Apiguard	2.6 ± 1.2a	0.7 ± 0.5a	3.1 ± 1.7a	0.4 ± 0.5a	2387.6 ± 1071.6a	902.4 ± 319.6a
Api Life VAR	2.6 ± 1.0a	1.7 ± 0.6a	2.8 ± 1.0a	1.5 ± 1.0a	2425.2 ± 1128.0a	1090.4 ± 263.2a
Control	2.8 ± 2.2a	8.4 ± 4.8b	2.1 ± 0.9a	8.0 ± 6.1b	2368.8 ± 977.6a	1598.0 ± 282.0b

Means in each column followed by different letters are significantly different at the $\alpha = 0.01$ level (ANOVA followed by LSD test)

Table 2. Efficacy of thymol treatments against *V. destructor* evaluated by the following equation *M%* (percentage of mortality) = 100[1 − (*Bc*·*At*/*Bt*·*Ac*)], where *Bt* and *At* are the percentage of mite infestation levels in treated colonies before and after treatment, respectively, and *Bc* and *Ac* are the same parameters in untreated colonies

Hives	Sealed brood		Adult bees	
	Apiguard	Api Life VAR	Apiguard	Api Life VAR
1	92.9	94.9	97.6	98.5
2	88.9	61.1	100.0	58.9
3	80.0	66.7	80.0	92.8
4	100.0	79.2	100.0	80.7
5	[a]	72.2	100.0	75.6
Mean ± SD	90.4 ± 8.3	74.8 ± 13.1	95.5 ± 8.7	81.3 ± 15.5

Means are not significantly different (ANOVA, $P > 0.05$).
[a] Unavailable due to absence of sealed brood in the hive.

0.84, df = 2, $P = 0.4541$). At the end of the treatment, infestation levels fell considerably in the treated hives and were significantly different from the control one ($F = 14.14$, df = 2, $P = 0.0017$ for sealed brood; $F = 11.86$, df = 2, $P = 0.0014$ for adult bees) (Table 1).

In the Apiguard-treated group, the efficacy of the treatments, expressed as percentage of mortality, was 90.4 ± 8.3 and 95.5 ± 8.7% for the sealed brood and the adults, respectively (Table 2). In the group treated with Api Life VAR, mortality values were 74.8 ± 13.1 and 81.3 ± 15.5%, respectively, for sealed brood and adult bees. Differences between both formulations were not statistically significant ($F = 3.82$, df = 1, $P = 0.0915$ for the sealed brood; $F = 4.63$, df = 1, $P = 0.0635$ for the adults) (Table 2).

The pattern of fallen mites during the trial is shown in Fig. 1. The highest mortality was found in the Apiguard-treated group in the first week of treatment, and, after replacement of the doses, in the third week. However, differences between both formulations were not statistically significant. Differences between treated groups and the control were statistically significant only at the end of the first week ($F = 4.54$, df = 2, $P = 0.0341$).

The sealed brood area was significantly lower in the treated groups than in the control ($F = 5.42$, df = 2, $P = 0.0285$) (Table 1). Neither adult bee mortality nor robbery was observed.

Thymol Persistence and Residues. The titers of thymol in Api Life VAR and Apiguard used in the trial were 56 and 18%, respectively, whereas the titers described on their labels were 74 and 25%, respectively. Based on these titers the thymol initial contents of the formulations were 6.7 g against 8.9 g for Apilife Var, and 9.0 g against 12.5 g for Apiguard. During the trial, 1 wk after the treatment, the thymol content fell to 3.4 ± 0.6 g and to 1.4 ± 0.5 g for Api Life VAR and Apiguard, respectively. After 2 wk, the residual formulation looked "propolized" and scattered by the bees. The mean released amount of thymol from the formulations was ≈50% for Api Life Var and ≈85% for Apiguard in the first week.

Thymol residues in the honey collected before treatment, from the control and treated hives, when present, were very low (0.11 ± 0.21 mg/kg). Concerning the hives treated with Api Life VAR, the mean thymol residues in the honey were 1.97 ± 1.54 and

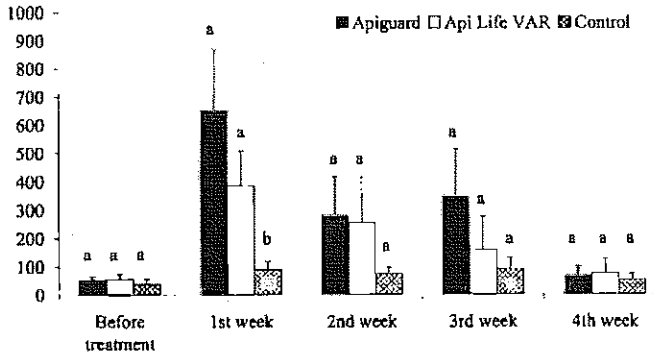

Fig. 1. Weekly average number ± SEM of fallen mites during treatment period. Within each date, different letters above bars indicate significant differences at the $\alpha = 0.01$ level (ANOVA followed by LSD test).

Table 3. Honey thymol residues (means ± SD) in treated colonies after 7 and 14 d from the start of the treatment. The two consecutive treatments were statistically analyzed separately

Acaricide	Treatment 1		Treatment 2	
	Samples collected after 7 days (mg/kg)	Samples collected after 14 days (mg/kg)	Samples collected after 7 days (mg/kg)	Samples collected after 14 days (mg/kg)
Api Life VAR	1.97 ± 1.54a	0.75 ± 0.44b	1.05 ± 1.01a	0.62 ± 0.57a
Apiguard	3.07 ± 1.80a	0.69 ± 0.70b	2.55 ± 3.50a	0.96 ± 0.61a

Means within each treatment followed by different letters are significantly different at the $\alpha = 0.01$ level (ANOVA followed by LSD test)

0.75 ± 0.44 mg/kg at the end of the first and the second week of treatment, respectively. After 2 wk, the wafers were replaced and the residues increased considerably in the third week (1.05 ± 1.01 mg/kg) to fall again in the next 7 d to the value of 0.62 ± 0.57 mg/kg (Table 3).

In the Apiguard-treated hives, the variability in the concentration of thymol residues and the residue fall, observed between the first and the second week and between the third and the fourth week of treatment, were similar to those found in the Api Life VAR-treated hives (Table 3). The two-factor ANOVA considering as factors the type of acaricide and the time of sample collection (7 and 14 d from the start of the treatment) showed no statistically significant differences between acaricides ($F = 1.04$, df = 1, $P = 0.3226$ for the first treatment; $F = 1.83$, df = 1, $P = 0.1941$ for the second treatment), whereas the decline in thymol residues observed for both acaricides between the first and second week of administration was statistically significant for the first treatment ($F = 8.70$, df = 1, $P = 0.0090$) but not for the second one ($F = 1.04$, df = 1, $P = 0.3226$).

Thymol residues in the wax collected before treatment and at the end of the fourth week of treatment are shown in Table 4. At the end of the treatments, Apiguard-treated hives showed the highest wax thymol residue, Api Life VAR-treated hives were intermediate, and the control showed the lowest residue ($F = 22.97$, df = 2, $P = 0.0001$).

Discussion and Conclusion

In this study, both formulations had a significant negative effect on colony development, showing a lower efficacy compared with previous trials carried out with the same acaricides in different experimental conditions or in the same environment (Imdorf et al. 1995, Colombo and Spreafico 1999, Arculeo 2002). A high colony-to-colony variability of the thymol effectiveness against *V. destructor* found in our apiary was similar to the one described in the above-cited articles. Approximately 95% of the wafer and gel were removed by the bees during the first 10 d after the treatment. After 2 wk, residues in the honey collected from the nest were below the perception sensory threshold established by Bogdanov et al. (1998).

Table 4. Wax thymol residues (means ± SD) in treated and control hives before and at the end of the two treatments

Acaricide	Before treatment (mg/kg)	At the end of the two treatments (mg/kg)
Api Life VAR	nd	21.0 ± 13.0a
Apiguard	1.0 ± 0.7	147.7 ± 188.9b
Control	2.9 ± 4.6	1.5 ± 0.8c

nd, not detectable.

Means detected at the end of the two treatments followed by different letters are significantly different at the $\alpha = 0.01$ level (ANOVA followed by LSD test)

The negative treatment effect on colony development, recorded as a reduction in brood area, was probably due to a partial brood removal by the bees during the treatment period, as observed previously by Imdorf et al. (1995) for Api Life VAR. In our trial, this effect was quantified and significant in treated hives, for either product, compared with the untreated ones. However, the observed reduction in brood area did not affect colony productivity, because the treatment was performed in summer, when a natural decrease in colony size usually occurs in our environment (Floris 1992b). Therefore, thymol-based treatments should not be performed during the period of natural increase in colony size.

The high variability in the efficacy of the two thymol-based treatments suggest that the action of this volatile compound was influenced by biological and/or climatic factors. The outside mean daily temperature during the whole treatment period was on the average 24.4°C and daily values always remained above 11.6°C. The sealed brood was present in the hive during the treatment period as previously observed in a study on population dynamic of bees conducted in the same environment (Floris 1992b).

An important difference observed between the two formulations regarded the amount of thymol released in the first week of the treatment, which was a lot higher from the gel of Apiguard. Based on these results, we think that the use of Apiguard could be improved using a shorter application interval (10 d) than the one recommended in the label (15 d).

Concerning residues, the method devised in the present work for the extraction of the thymol was extremely rapid and required no clean up, unlike the methods described in previous reports (Bogdanov et al. 1998, Martel and Zeggane 2002, Nozal et al. 2002), because the extracts showed no interference peaks. The average recovery for honey was 97% (range 84–111%) with a maximum coefficient of variation (CV) of 5.6%, whereas for wax it was 89% (range 82–98%)

with a maximum CV of 8.5%. A good linearity was achieved in the 0.1–5.0 mg/kg range with a correlation coefficient of 0.9994. The limit of determination (Their and Zeumer 1987) was 0.1 mg/kg.

Residues in honey were extremely variable, as reported by Bogdanov et al. (1998). The more abundant release of thymol in the first week of application (wafer or gel) is partly retained by the honey; however, some of it evaporates from the honey during the second week. Considering that thymol is highly volatile, variability could be due to different microclimatic conditions in the hives. The higher presence of thymol found in the wax than in the honey was likely due to apolar nature of both thymol and wax.

Finally, a better use of the thymol formulations tested, such as shorter application interval, particularly for the gel one, could represent an alternative to synthetic acaricides in integrated control strategies of *V. destructor* under the same environmental conditions. A very important advantage in using thymol is that no maximum limit for its residues in honey is imposed, and no mite resistance has been found yet.

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Comparing Oxalic Acid and Sucrocide Treatments for *Varroa destructor* (Acari: Varroidae) Control Under Desert Conditions

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ABSTRACT The effectiveness of oxalic acid (OA) and Sucrocide (S) (AVA Chemical Ventures, L.L.C., Portsmouth, NH) in reducing populations of the varroa mite *Varroa destructor* Anderson & Trueman (Acari: Varroidae) in honey bee, *Apis mellifera* L. (Hymenoptera: Apidae) colonies was measured under the desert conditions of Arizona, USA. OA and S were applied three times 7 d apart. A 3.2% solution of OA was applied in sugar syrup via a large volume syringe, trickling 5 ml per space between frames in the colony. S was applied at a concentration of 0.625% (mixed with water), according to the label directions, using a compressed air Chapin sprayer at 20 psi to apply 59 ml per frame space. Varroa mites, collected on a sticky board before, during, and after the treatments, were counted to assess the effectiveness of the treatments. This study showed that a desert climate zone did not confer any positive or negative results on the acaricidal properties of OA. Even with brood present in colonies, significant varroa mite mortality occurred in the OA colonies. In contrast, we found that Sucrocide was not effective as a mite control technique. Despite its ability to increase mite mortality in the short-term, varroa mite populations measured posttreatment were not affected any more by Sucrocide than by no treatment at all.

KEY WORDS *Apis mellifera*, *Varroa destructor*, oxalic acid, Sucrocide, desert conditions

The parasitic honey bee mite *Varroa destructor* (Anderson & Trueman 2000) (Acari: Varroidae) is a serious pest to honey bee (*Apis mellifera*) colonies, and it has caused large bee losses for >10 yr (Krause and Page 1995; Finley et al. 1996). These mites have been responsible for colony deaths in part because of their role in transmitting bee viruses (Allen and Ball 1996; Ball 1993; Kevan et al. 2006). More recently, mites and virus have been considered at least partially responsible for the colony losses associated with colony collapse disorder reported in 2007 (VanEngelsdorp et al. 2007). Varroa mites are becoming resistant to the registered chemical treatments fluvalinate and coumaphos, both in the United States (Eichen 1995; Elzen et al. 1998, 1999; Elzen and Westervelt 2002) and in Europe (Milani 1994, 1995; Lodesani et al. 1995; Lodesani 1996, Thompson et al. 2002). This is driving investigations into alternative treatment regimes, including botanical oils (Imdorf et al. 1999), selection of mite resistance in bee lines (Spivak and Reuter 1998; Rinderer et al. 2000, 2001) and exploration of fungal pathogens (Kanga et al. 2003). Recently, organic acids, such as oxalic acid, and sucrose esters, have emerged as tools to control resistant mites.

Oxalic acid (OA) has been used successfully in Europe and Canada (Gregorc and Planinc 2001, 2002; Charrière and Imdorf 2002; Nanetti et al. 2003; Gregorc and Poklukar 2004) by trickling a sugar (sucrose) syrup and OA solution on bees, or by heating OA crystals in hives, creating a vapor (Rademacher and Harz 2006a,b). In the vapor stage, OA can be effective, but it has low volatility (Aliano et al. 2006); therefore, it must be heated, making it difficult to control the dosage. In addition to being imprecise, vapor phase application could be hazardous to the operator, because OA is harmful if inhaled and can cause severe irritation and burns (MSDS 06044). The trickling method, using 3 to 3.5% solutions has been reported to have an efficacy of 90–95% (Rademacher and Harz 2006b). Although the mode of action against varroa mites is not clearly understood, it seems that direct contact with the low pH of OA has a deleterious effect on the mite (Nanetti et al. 2003).

Another compound, Sucrocide (S), is a product registered for varroa mite control in the United States (AVA Chemical Ventures, Portsmouth, NH). The active ingredient (sucrose octanoate esters), was reported to have rapid toxic effects on soft-bodied arthropods, and it was shown to be effective at controlling varroa mites (Sheppard et al. 2003; Stanghellini and Raybold 2004, Stanghellini et al. 2005). The mode of action has been somewhat controversial and could be a result of rapid suffocation by soap particles, damage to the mites' cuticular surface causing them to desiccate, or toxic fatty acids (Puterka et al. 2003). Sucrocide is applied as a liquid so the material comes

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in direct contact with the phoretic mites on bees. Because both OA and Sucrocide work upon direct contact with the mite, neither will be effective against immature varroa mites that are in sealed bee brood. Thus, these treatments are reported to work best when the brood levels are low, such as in the fall, when more mites are phoretic and thus vulnerable to treatments.

Although both of these treatments are reported to be effective as a varroa mite control, their efficacy in dryer climates has not been investigated. In addition, brood production rarely stops in Arizona, so varroa mite populations can remain high. The purpose of this study was to test the efficacy of liquid treatments of OA and Sucrocide in reducing varroa mite populations in the desert southwest (Arizona) even when brood is present.

Materials and Methods

Colonies of bees were owned by a local commercial beekeeper who rarely used chemical controls. They were located at the Willow Springs Ranch, Oracle, Pinal Co., AZ. Before application, colonies were screened for mite loads by using sticky boards (Ostiguy and Sammataro 2000). Mites that dropped onto the boards (mite drop) were considered killed by the treatment. For each treatment, we selected 10 colonies that were in two deep Langstroth hive bodies and contained an average of 17 frames of bees. Treatments began in October, when the mite population reached >50 mites on the pretreatment sticky boards. Cluster sizes were assessed pre- and posttreatment by estimating the number of frames of bees in each colony to determine whether the treatments had a detrimental effect on the bee populations. Brood was present in all colonies, with an average of five frames of capped brood, but brood area was not officially counted due to constraints of time and colony temperament. Colonies were randomly assigned to three treatments: 1) untreated control, 2) OA treated, and 3) S treated.

Oxalic Acid. OA was applied in sugar syrup. To obtain a 3.2% OA solution, 1 kg of sucrose was added to 1 liter of warm water and stirred until the sugar was dissolved. Then, 75 g of oxalic acid dihydrate was added to the syrup and the resulting solution (3.2% OA; 50% sugar, wt:vol) was enough to treat ≈25 hives (10 frames per hive). A large-volume syringe (60 ml) was used to deliver 5 ml per interspace between two frames end to end. Treatments were only delivered to frame spaces that contained bees; any empty frames were not treated. Average dose per colony was 50 ml of OA. Colonies were given three treatments 7 d apart.

Sucrocide. S was applied at a concentration of 0.625% (mixed with water), according to the label directions. The solution was applied using a compressed air Chapin sprayer at 20 psi through a Chapin 0.2 gallons per minute flat fan nozzle. The nozzle design allowed the tip to be guided between the frame spaces during application, eliminating the need to remove each frame. Spray was administered to each interspace between frames at a rate of 59 ml per frame space (8 s calibrated on the sprayer); an average of 590 ml was applied to each colony. Treatments were only delivered to frame spaces that contained bees; any empty frames were not treated. The bees were treated three times every 7 d.

Estimating Mite Mortality. Sticky boards (Great Lakes IPM, Vestaburg, MI) were covered with 8-mesh hardware screens stapled to a preglued paper (Ostiguy and Sammataro 2000), and they were in place on the bottom board in all colonies during each sampling period. The pretreatment period was for 7 d before treatment. There were a total of three treatment applications and corresponding "treatment week" sticky boards. New sticky boards were inserted within 30 min of each treatment application and were removed 7 d later. Posttreatment samples began 7 d after the last treatment and lasted for 7 d. Posttreatment mite drop was enhanced by the insertion of acaricide strips (following label directions for Apistan (Zoëcon, Schaumburg, IL). Because this beekeeper reported that he rarely used chemical miticides, we were confident that mites in these colonies were not significantly resistant to fluvalinate; this was confirmed by the high postmite drop counts.

Statistical Analysis. Mite drop onto sticky boards (and cluster size) was analyzed using a repeated measures analysis of variance (ANOVA) (PROC MIXED, SAS Institute 1999). When a significant treatment × time interaction was found ($P < 0.05$), mite drop at each time period was compared with drop during the pretreatment period. Contrasts were used to determine differences between treatments in each time period using Bonferroni-corrected α values.

Results

Varroa mite drop onto sticky boards was affected by treatment over time ($F = 9.16$; df = 4, 108; $P < 0.0001$). Mite drop in each treatment increased significantly from pretreatment samples to those collected during each of three during-treatment sampling periods compared with no increase in control colonies (df = 2, 27; $P < 0.05$) (Fig. 1). Comparison of mite drop between pre- and posttreatment samples showed that only the oxalic acid-treated colonies had a significantly smaller increase than the control colonies (OA: $F = 9.05$; df = 1, 27; $P = 0.0168$; S: $F = 1.34$; df = 1, 27; $P = 0.7692$).

Discussion

OA in syrup and Sucrocide in water were used in this experiment because of the similarities of application techniques (trickling and spraying) and the fact that they needed to be reapplied over time. Additionally, little research has been done comparing the performance of these products in dry climates and whether the drier climates would influence the efficacy of liquid applications of acaricides.

OA was effective as a varroacide in a desert environment and assuming all of the remaining mites were killed during the 7-d posttreatment sample with Apistan in colonies, 70% of mites were killed by the OA treatment. This compares with published reports of

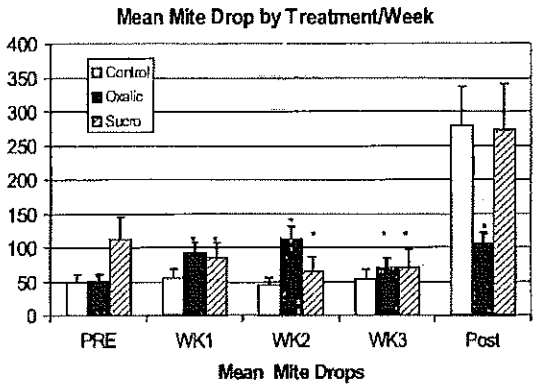

Fig. 1. Mean ± SE varroa mite drop onto sticky boards in control colonies and colonies treated with oxalic acid or Sucrocide before treatment (PRE), in each of 3 wk during treatment (WK1-3), and after treatment (Post) when Apistan was applied. An asterisk indicates a significant difference in the change in mite drop over time in the control versus treatment colonies for each time period compared with the pretreatment period ($P < 0.05$).

efficacy rates of 39 to >90% depending on the time of year OA was applied and the concentration of OA in the syrup (3–7%). The presence of brood seems to be the deciding factor in lower efficacy in the summer (Rademacher and Harz 2006a), especially in temperate climates. Higher OA concentration does not necessarily mean better control of mites and in some cases was detrimental to bees (Rademacher and Harz 2006b). The low pH (Nanetti and Stradi 1997, Nanetti 1999) of the OA solution may be a factor in the mode of action against varroa, because direct contact of the mites with the solution is needed to kill mites. By mixing OA in sugar water, the solution is attractive and distributed by bees and thus would come in contact with bees and mites through the colony.

Sucrocide was not effective in this study, a finding that is contrary to published reports by Sheppard et al. 2003, Stanghellini and Raybold (2004), and Stanghellini et al. (2005). Although mite drop significantly increased in our S-treated colonies compared with the untreated controls during the three weeks of treatment, the change in mite drop from the pre- to posttreatment period was no different from that in control colonies that were left untreated. In addition, wet bees from Sucrocide-treated colonies were observed crawling at the hive entrance, probably a result of the large amount of liquid that was administered to each colony. In some instances, such disoriented bees could attract robbing foragers and may be problematic given the right conditions. Crawling bees were observed on several occasions, although the bees did recover eventually and no adverse effects were observed ($F = 2.34$; df = 2, 227; $P = 0.1160$) when posttreatment cluster size was measured (data not shown).

In general, we found that the method we used to apply OA was effective. One potential hurdle to overcome with using a liquid-applied treatment is the need to develop an economically feasible application method for commercial beekeepers that reproduces the results of our "trickle" method. Sprayers can be used to apply OA, but if the mist is too fine, there is a potential danger that the operator could inhale the caustic vapor. Other coarse sprayers could be used as long as the correct dose was applied and inhalable mist was not created. Also, because of the caustic nature of OA, corrosion of metal parts is possible, so noncorrosive equipment is essential. As indicated in our Sucrocide treatment, delivery concentrations may need to be adjusted to minimize the amount of liquid delivered to each colony; there is a limit to how much liquid bees can tolerate. Under our hot desert conditions, bees dried very fast, but in more temperate climates, wet bees may be chilled or unable to fly back to their colony and could die.

This study showed that a desert climate did not confer any positive or negative results on the acaricidal properties of OA. Even with brood present in colonies, significant varroa mite mortality occurred. In conclusion, OA is an effective control method for varroa mites if applied as directed. By treating several times a year in the desert climate where brood production does not stop, OA treatments should be enough to offset the buildup of mite populations and keep them from reaching critical levels. The timing of miticides in relation to mite population is essential in the survivorship of bee colonies infested with varroa mites (DeGrandi-Hoffman and Curry 2004). OA is an attractive alternative mite control technique in that there are no residues in beeswax and propolis as there are with other chemical acaricides, due to the hydrophilic properties of OA (Rademacher and Harz 2006b). Using the recommended dosage, it is unlikely that OA will be detected in honey, because of the small volume applied during treatment. Also, natural concentrations of OA have been recorded from 3.3 to 761.4 mg/kg (Bogdanov et al. 2002, Rademacher and Harz 2006b) depending on the concentrations in the nectar from various botanical origins. As long as OA is carefully applied, its toxicity to the operator by inhalation or skin contact will be avoided. In contrast, we found that Sucrocide was not effective as a mite control technique. Despite its ability to increase mite drop in the short-term, varroa mite populations measured posttreatment were not affected any more by Sucrocide than by no treatment at all.

Milani (2001) has suggested that glycerol added to sugar solutions may act as a synergist, causing OA (and possibly sucrose esters) to become more hygroscopic. Experiments are currently underway in our lab to investigate this property and to test the effects on mite and bee mortality by mixing glycerol in both Sucrocide and OA solutions.

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THE RESISTANCE OF VARROA MITES (ACARI: VARROIDAE) TO ACARICIDES AND THE PRESENCE OF ESTERASE

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ABSTRACT - Varroa mites (*Varroa destructor* Anderson and Trueman, 2000) are becoming resistant to acaricide treatments via metabolic and/or target site desensitivity. Results of a survey of mites from the Carl Hayden AZ lab and from cooperators in five locations (Arizona, California, Florida, Maine, North Dakota) showed that some mites were susceptible to all three acaricides (Amitraz, Coumaphos, Fluvalinate) in the spring of 2003, but by fall most mites were resistant. Mites were resistant to all chemicals, even from beekeepers that do not treat colonies with acaricides. We used esterase native activity gels to test for the presence of specific esterases which might be involved in pesticide resistance in varroa. All mites tested had positive bands for esterase, even those exhibiting susceptibility to some acaricides. Based on the differences between the esterase activity gel profile of the susceptible and cross-resistant *V. destructor*, it is possible that an esterase-mediated resistance mechanism is operative in the population of the mites we analyzed. However, a combination of other resistance mechanisms may be present which make the esterase activity gel method unreliable for use in identifying varroa mites with multiple resistance.

Keywords - Varroidae, varroa mites, *Varroa destructor*, acaricide resistance, honey bees, esterase

INTRODUCTION

During the past decade, chemicals, such as the pyrethroid fluvalinate, and coumaphos, an organophosphate (OP), have been used by beekeepers to control varroa mites (*Varroa jacobsoni=V. destructor* Anderson and Trueman) in honey bee colonies. Mite resistance to both fluvalinate and coumaphos has been observed in Europe (Milani, 1995, 1999; Trouiller, 1998; Vedova *et al.*, 1997) and now is being found in the United states (Elzen *et al.*, 1998, 1999a, b, 2000; Pettis *et al.*, 1998 a, b). Fluvalinate-resistant mites first were reported in the U.S. in 1997 (Baxter *et al.*,1998) and more recently, Varroa mites resistant to coumaphos have been found (Elzen *et al.*, 2000, 2001; Elzen and Westerveldt, 2002). Amitraz, a formamidine, has been used for mite control sporadically since 1992 and is no longer registered for bee mites; nevertheless, resistance to this material has also been found (Elzen *et al.*, 1999c, Mathieu and Faucon 2000).

Reports of resistance to fluvalinate, coumaphos and amitraz throughout the U.S. indicate that resistance is spreading and that cross-resistance might be evident in some locations (D. Westerfelt and A. M. Jadzack, pers. comm.). Bees are transported across the U.S. for pollination and in the sale of queens and packaged bees. Since many beekeepers have relied upon single-chemical control regimes for about 10 years, resistant mites could exist in every beekeeping operation in the U.S. Currently, the trend is to use multi-chemical rotations so that mites are exposed to widely varying treatment regimes. However, mites could develop cross-resistance.

Organisms develop resistance via behavioral changes (e.g. avoiding the pesticide), reduced penetration (e.g. cuticle thickening), detoxification of the pesticide by enzymes (i.e. metabolic) or target site desensitivity (modifications of action site, e.g. sodium channel mutation) (Scott, 1990; Baars and Driessen, 1984; Hillesheim *et al.*, 1996; Watkins, 1996; Wang *et al.*, 2002). Carrière (2003) suggested that haplodiploid arthropods could develop resistance differently than diploid species. Since varroa are haplodiploid, as well as highly inbred, they may be able to develop pesticide resistance quickly. Resistance mechanisms also could change over time.

In Europe and Israel, the mechanisms of varroa resistance to fluvalinate are reportedly due to high levels of metabolic esterases (Hillesheim *et al.*, 1996; Gerson *et al.*, 1991; Mozes-Koch *et al.*, 2000). Strains of the tick, *Boophilus microplus*, which exhibit cross resistance to both pyrethroids and organophosphates (OPs), possess high levels of metabolic esterase activity (Jamroz *et al.*, 2000). Esterases oxidize and detoxify synthetic pyrethroids and significantly reduce their effect on mites. A second mechanism, target site desensitivity, has been described in pyrethroid resistant mites in the U.S. (Wang *et al.*, 2002) and involves mutations in the sodium channel gene sequence. The sodium channel is the target site of pyrethroid binding.

The purpose of this study was to determine the prevalence of resistant mites in beekeeping operations in the U.S. We tested for resistance to fluvalinate, coumaphos and amitraz. We also investigated whether the esterase native activity gel technique used to determine the presence of esterase in cattle ticks and horn flies resistant to pyrethroids and OPs (Guerrero *et al.*, 1997, 1998, 1999, 2001; Pruett *et al.*, 2002; Miller *et al.*, 2001) would be useful for testing resistance in varroa mites.

MATERIALS AND METHODS

Sources of mites - In 2002, mites were collected from untreated colonies at our Laboratory apiary (Carl Hayden Bee Research Center [CHBRC] Tucson, AZ) and from treated colonies in Maine. The mites from Maine were determined to be resistant because they had survived colony treatments with fluvalinate and coumaphos (A. M. Jadczak, pers. comm.). Mites were collected by shaking 200-300 live bees in a quart jar covered with a wire mesh lid. The jar was shaken to dislodge attached mites. The mites were collected into glass vials and stored in a -70°C freezer until ready for esterase activity analysis.

In 2003, mites were collected from colonies located in North Dakota, Florida, Arizona, California, and Maine and from colonies moved between Maine and Florida, as well as colonies from the CHBRC that had annual fluvalinate treatments and one coumaphos treatment. To obtain mites, frames of drone foundation were sent to each cooperator to be placed in their colonies. After the frames were drawn and filled with capped drone brood (and varroa), the frames were returned to CHBRC via overnight mail. Upon arrival, the drone brood frames were stored in an incubator (30°C, 50% RH) until mites could be analyzed for resistance using the vial bioassay.

Vial bioassay - Varroa were collected and tested for miticide resistance according to the protocol for varroa described in Elzen *et al.* (1998). Drone cells were uncapped and the brood removed with forceps. Attached live adult female varroa were collected with slender probes and five mites were placed into 20 mL glass scintillation vials for each treatment. The vials were treated with either 0.5 mL acetone (control), 123 µg amitraz, 53 µg coumaphos, or 2.4 µg tau-fluvalinate (Elzen *et al.*, 1998). Acaricide amounts were set to produce approximately 90% mortality in susceptible mite populations (Elzen *et al.*, 1999a, 2000). To ensure mite survival in the low humidity of Arizona (typical ambient humidity in the collection room was 18-25%), the protocol of Elzen (1998) was modified by wetting a 7 mm diameter disc (punched out by a paper punch) of No. 5 Whatman filter paper with 3 to 5 µL of distilled water. One disc was added to each vial during mite collection. A minimum of three replicate vials (5 mites/vial) of each acaricide vs. control was tested for each colony. Depending on the number of mites found in the frame, a minimum of 15 and a maximum of 65 mites were tested. Vials with mites were incubated for 24 hours (Little Giant Still Air Incubator, Miller Mfg., St. Paul, MN) at approximately 30-32°C and approximately 80% RH.

After 24 hours, the vials were examined under a dissecting microscope. Mites were gently prodded with a probe to encourage movement. Non-moving mites were scored as dead and the mortality rates for each vial were recorded. For each colony tested, total mortality rates from all vials of each type: control, amitraz, coumaphos and fluvalinate were tallied. Vial sets with more than 10% mortality in the control vials were discarded. Samples of susceptible and resistant mites from the vial bioassay were tested for esterase activity.

Esterase activity analysis - Live and dead mites from the pesticide treated vials were separated and tested for esterase activity. All mites in the control vials also were tested. Mites were frozen at -70°C in 1.5 ml microcentrifuge tubes and shipped on dry ice for analysis. Ten to 40 mites were used for each esterase activity test.

The mites were pulverized using a disposable pellet pestle (Kontes, Vineland, NJ) and extracted in buffer containing 0.01 M sodium phosphate (pH 6.5), 20% sucrose, 0.001 M EDTA, and 0.5% Triton X-100. Extracts were centrifuged at 4°C, 15,000 RPM for 10 min, and then stored at -80°C. The equivalent of a single mite was loaded onto a lane of a Novex pre-cast 4-12% gradient polyacrylamide Tris-glycine gel (Invitrogen, Carlsbad, CA) and electro- phoresed under native conditions at 4°C. Esterase activity was detected in the gel using the method of Hughes and Raftos (1985) with some modifications, by incubation of the gel in 0.1 M phosphate buffer (pH 6.5) containing 3.2 mM α- or β-naphthyl acetate and 2.4 mM Fast blue BB salt for 60 min, in the dark, at 37°C. The naphthyl acetate stock solutions were prepared in 1 ml acetone to aid their solubility in the phosphate buffer. Replicate gels were pre-incubated for 15 min in the dark in phosphate buffer with $1X10^{-3}$, $1X10^{-4}$, and $1X10^{-5}$ M eserine sulfate or triphenyl phosphate, which are specific inhibitors of acetylcholinesterases (AchE) and carboxyl-

esterases, respectively, prior to detection of esterase activity with the naphthyl acetate method. To ensure AChE inhibition throughout the 60 min esterase detection step, eserine sulfate was added to the naphthyl acetate-Fast blue BB buffer system. Since this electrophoretic analysis of proteins with esterase hydrolytic activity is performed under native conditions, molecular weights of visualized proteins cannot be determined.

Statistics - Mortality from each treatment in the vial bioassay was tested separately using Chi-Square Test for Independence. Survival of mites from each vial per treatment chemical was compared to the expected survival from the control vials. The null hypothesis was that treatments did not differ from control and if the χ^2 value was greater than the critical values ($\alpha = 0.05$), the hypothesis was rejected. Rejected values were recorded as *miticide-susceptible* (S); samples below the critical values were *miticide-resistant* (R).

RESULTS

Vial bioassay - In April 2003, the three colonies tested from the CHBRC apiary (AZ Lab-1, 2 3) were susceptible to amitraz (Table 1). AZ Lab-1 and 3 also were susceptible to fluvalinate and coumaphos but AZ Lab-2 was resistant. Subsequent testing of AZ Lab-3 was conducted twice in the spring and again in the summer. While the mites were still susceptible to amitraz in the spring, we found resistance to coumaphos and fluvalinate. By the summer, the colony's mites were resistant to all three acaricides. AZ Lab-4 and 5 were tested in the fall and were resistant to all three chemicals.

Mites from the apiary of the Arizona cooperator who reported using no chemical treatments had only one sample of fluvalinate-susceptible mites in May (Table 2). All other samples were resistant to all three acaricides. Samples from cooperators in other states also were resistant to all chemicals, with the exception of mites from Florida; they were all susceptible. The mite samples from a cooperator in Maine (ME) and one that moves colonies between Maine to Florida (Migratory ME/FL) all tested resistant. Migratory ME/FL provided mites from untreated and treated colonies, but all were resistant to the three acaricides. Mites obtained in early July samples from North Dakota were susceptible to amitraz and fluvalinate, but were resistant to coumaphos. In late July, mites from the same cooperator were resistant to all three chemicals.

Esterase activity analysis - There was an intense band of esterase activity present in the OP-resistant mites

Fig. 1. Native Esterase Activity Gel. Test run of esterase gel of varroa vs. *B. microplus* larvae (from TX lab) with various toxicological profiles. These were assayed for general esterase activity by extraction in phosphate buffer containing Triton X-100, fractionation by native polyacrylamide gel electrophoresis, and incubation with α-naphthyl acetate and Fast blue BB. Lanes represent one mite equivalent. Lane 1: *B. microplus* Gonzalez strain susceptible to both Pyrethroid and OP; Lane 2: OP-resistant Tuxpan strain of *B. microplus*; Lane 3: Coatzoacoalcos pyrethroid-resistant strain of *B. microplus* ; Lane 4 and 5: Verbal report of Pyrethroid- and OP- resistant varroa from Maine/Florida mites, 2002; Lane 6 and 7: varroa susceptible to both pyrethroid and OP (Lab mites, Tucson AZ 2002). The pesticide resistant ticks possess both qualitative and quantitative differences in esterase activity.

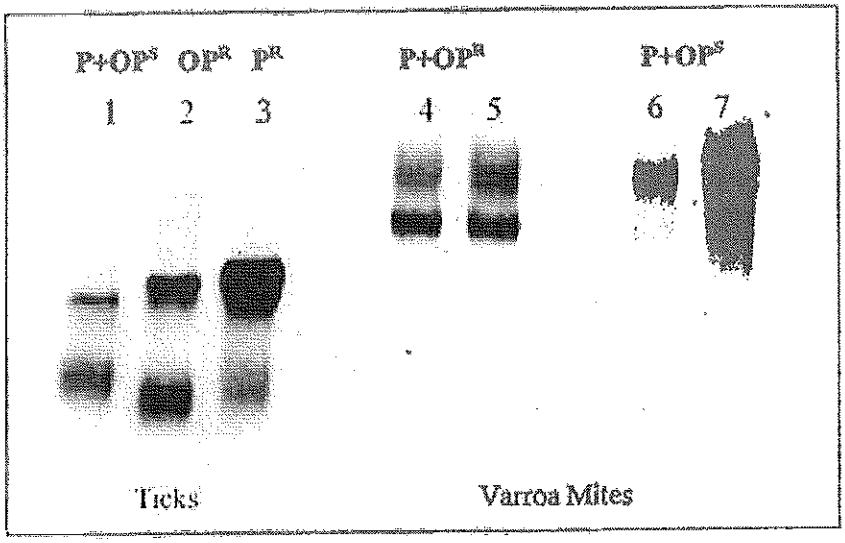

Table 1. Vial bioassay results from varroa mites from Carl Hayden Honey Bee Research Center (CHRC), Tucson AZ. The null hypothesis was that treatments did not differ from control and if the χ^2 value was greater than the critical values (α= .05), the hypothesis was rejected. Rejected values were recorded as *miticide-susceptible* (S); samples below the critical values were *miticide-resistant* (R). The *Time of sampling* included Early Spring (April/May), Spring (June) and Summer/Fall (July-Sept). The number of samples taken at each time is represented by the letters in each column. For example, in Lab-3 colony, two samples were taken in the Spring and the Summer. The numbers under the Chi Square column correspond to the sampling times, and indicate degrees of freedom and Chi Square value at α=.05. Mite samples from Lab 2 and 3 colonies were used in the esterase activity gel in Figure 2.

CHRC Bee Lab			*Time of sampling*		Esterase	*df, Chi Square* α= *.05*		
Source	Colony	Treatments	E.Spring	Spring	Summer/Fall	E.Spring	Spring	Summer/Fall
AZ lab	Lab-1	Amitraz	S			3, 10.25		
		Coumaphos	S			3. 8 0		
		Fluvalinate	S			3, 7.25		
	Lab-2	Amitraz	S			1, 5.56		
		Coumaphos	R			1, 2.93		
		Fluvalinate	R			1, 0.04		
	Lab-3	Amitraz	S	S S	R R	2, 5.89	2, 9.98; 4, 19.72	2, 1.2; 4, 3.4
		Coumaphos	S	R S	R R	2, 4.11	2, 4.96; 4, 13.18	2, 1.0; 4, 0.2
		Fluvalinate	S	R S	R R	2, 5.44	2, 3.54; 4, 10.92	2, 1.0; 4, 0.2
	Lab-4	Amitraz			R	2, 0.84		
		Coumaphos			R	2. 0.25		
		Fluvalinate			R	2, 0.25		
	Lab-5	Amitraz			S			2, 5.99
		Coumaphos			R			2, 1.46
		Fluvalinate			R			2, 1.46

Fig. 2. Native gel profiles of varroa from 2003 multi-state vial assay survey. Lane 1: AZ lab-1 susceptible; Lane 2: FL mites (susceptible); Lane 3: AZ lab-2 (mixed resistance); Lane 4: AZ cooperator reported no treatments (mites mostly resistant); Lane 5: Migratory ME/FL (treated), mites all resistant; Lane 6: AZ lab-3(mixed resistance); Lane 7: ND #1 (mixed resistance); Lane 8: *Boophilus microplus* Coatzoacoalcos. Table II represents the colonies that were tested in this gel run. The mites in Lane 2 (FL) tested out susceptible in the vial assay; however they came up positive for esterase activity in the gel profile.

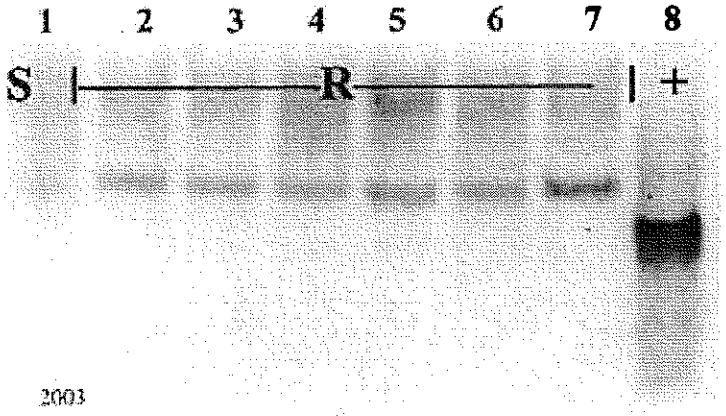

Table 2. Vial Bioassay results of varroa mites from the cooperators. Miticide-susceptible (S); and miticide-resistant (R) varroa are indicated in the columns. The *Time of sampling* included Spring (May), Summer (June/July) and Fall (Aug-Sept). The number of samples taken at each time is represented by the letters in each column; for example in AZ, there were three spring samples and 2 summer samples taken. The *Esterase* column (shaded) indicates whether the mite sample was used for the activity gel test (Y= Yes). The numbers under the Chi Square column correspond to the sampling times, and indicate degrees of freedom and the Chi square critical value, at α=.05.

COOPERATORS		Time of sampling			Esterase	df, $\chi 2$; α=0.05		
	Treatments	Spring	Summer	Fall	Y=tested	Spring	Summer	Fall
AZ	Amitraz	R R R	R R			2, 4.25; 1, 0.66; 2, 1.0	4, 0.76; 3, 0.22	
not treated	Coumaphos	R R R	R R		Y	2, 0.6; 1, 0.66; 2, 2.3	4, 2.26; 3, 0.22	
	Fluvalinate	S R R	R R			2, 1.53; 1, 1.93; 2, 0.77	4, 0.14; 3, 0.22	
FL	Amitraz	S				1, 2.43		
	Coumaphos	S			Y	1, 0.41		
	Fluvalinate	S				1, 1.29		
CA	Amitraz		R				4, 13.9	
	Coumaphos		R		*		4, 11.o	
	Fluvalinate		R				4, 13.2	
ME	Amitraz		R				7, 12.18	
	Coumaphos		R		*		7, 2.33	
	Fluvalinate		R				7, 6.88	
ND #1	Amitraz		S S				4, 20.1; 4, 11.0	
early July	Coumaphos		R R		Y		4, 7.3; 4, 3.6	
	Fluvalinate		S R				4, 12.4; 4, 9.23	
ND #2	Amitraz		R R				4, 1.31; 4,2.55	
Late July	Coumaphos		R R		*		4, 0.4; 4, 1.7	
	Fluvalinate		R R				4, 0.83; 4, 1.7	
Migratory ME/FL	Amitraz			R				3, 3.86
not treated	Coumaphos			R	*			3, 0.266
	Fluvalinate			R				3, 0.157
Migratory ME/FL	Amitraz			R				2, 1.32
treated	Coumaphos			R	Y			2, 0.021
	Fluvalinate			R				2, 0.062

that was missing in the susceptible varroa from the 2002 samples (Fig. 1). The pesticide-resistant tick strains (Lanes 2 and 3) possess both qualitative and quantitative differences in esterase activity compared with the pesticide-susceptible strain (Lane 1). The esterase profile for both the susceptible and resistant mites was not affected by eserine sulfate or triphenyl phosphate (data not shown), indicating the esterases were probably not acetylcholinesterases or carboxylesterases.

The column labeled *Esterase* in Table II identifies those samples that were tested by the gel method, shown in Fig. 2. The "Y" in the column identifies those mites in the sample that were tested for esterase activity. Lane 1 was the original susceptible colony in our research yard from 2002 which subsequently died. This colony had no esterase band. The mites from the Florida cooperator (Lane 2 in Fig. 2) had a strong esterase band although in the bioassay they were susceptible to all chemicals. All other mites run in the gel had esterase activity, regardless of their resistance history; the results in the vial bioassay showed strong resistance to all acaricides.

DISCUSSION

Mites from our lab generally were susceptible to the acaricides used for controlling varroa. However, almost all the mites we received from our cooperators, regardless of the treatment regimes used, had resistance to all acaricides. The only exception was the mites from Florida which were susceptible despite being in a region that has been heavily treated with acaricides. In colonies where we were able to test mites more than one time, we found mites susceptible in the spring but by late summer the mites became resistant to all three acaricides, despite not being treated with amitraz. We were unable to test the susceptible Florida mites again in the fall to determine if this trend continued. Esterase activity was not a reliable indicator of resistance in our samples. Mites resistant to some acaricides had an esterase band, as did the susceptible mites.

The presence of resistant mites in most colonies, especially those sampled in the fall, may be explained by the over-use or misuse of registered varroa acaricides. Surprisingly, mites also showed resistance to amitraz, which is not a registered acaricide. These results suggest either cross-resistance between amitraz and other registered chemicals, or mite exposure to amitraz. Resistance could increase quickly in colonies because mites not killed by acaricides reproduce, thus reinforcing resistant genes. It was surprising to find resistant mites from cooperators who did not treat with acaricides (e.g. the Arizona and Maine cooperators). The presence of resistant mites in their operations may be due to: 1) bees robbing honey from a weak or dying hive (with resistant mites) within the flight range of the apiary and in the process acquiring those mites, 2) introduction of package bees and queens from other states that have resistant mites, or 3) drifting bees, a common phenomenon in large apiaries where phoretic mites can be swiftly distributed throughout the whole apiary in a short time.

Our finings indicated that as the summer progresses, the population of bees and (resistant) mites increased. Perhaps the resistant mites were able to out-compete the susceptible mites. This may explain the switch from susceptible to resistant mites from spring to fall. Why the resistant mites appear to overwhelm colonies may be expressed in genetic terms. Resistance is rarely totally dominant (Carrière, 2003) but could be expressed at some level in heterozygotes especially if the resistance confers a gain of function, e.g. detoxification of chemicals by enzymes, reduced penetration and enhanced elimination of toxins. Females that have two copies of a resistant allele (RR) would produce offspring that also are homozygotes. Heterozygote foundress mites would produce 0.5 heterozygote and 0.5 homozygote susceptible offspring if the male parent had the susceptible allele, and 0.5 homozygous resistant and 0.5 heterozygous offspring if their male parent carried the resistant allele. Therefore, each heterozygote has a 0.5 probability of producing all resistant individuals and a 0.5 probability that half of their offspring will be resistant depending on the genotype of the male parent. The homozygous resistant state would not change due to brother-sister mating and its frequency would increase with each generation. Under these conditions, it is not surprising to find increased frequency of resistant individuals over time, especially if pressure from acaricides is removing homozygous susceptible individuals from the population. Varroa resistance could also be sex-linked, but since varroa males do not come in direct contact with the acaricides (other than through the accumulation in the wax) and their resistance has never been tested, this is only a speculation. Of course, unless we are able to rear varroa off host and in an artificial environment, we can only hypothesize such events.

Based on the esterase activity gel profile of the susceptible and resistant *V. destructor* from 2002, it is possible that an esterase-mediated resistance mechanism is operative in the population of mites we analyzed. However, the reliability of this method was not apparent in the 2003 tests, as all mites had the esterase band. Since other resistance mechanisms are operating on varroa mites, including esterase detoxification (Gerson *et al.*, 1991; Thompson *et al.*, 2002), monooxygenases in the P450 system (Hillesheim *et al.*, 1996), and sodium channel mutations (Wang *et al.*, 2000), testing varroa for any one method may not be the most reliable way to determine mite resistance.

Once the operating systems of varroa resistance are determined, it may be possible to develop a successful management program to counteract resistant varroa. According to Milani and Vedova (2002), resistant mites left untreated for 4-6 years will lose their resistance to

fluvalinate. Why this is so and what mechanisms are being used for such a switch, need to be determined. Identifying resistance mechanisms in varroa will be challenging though, because it will require rearing esterase-free and susceptible mites in an isolated area and subjecting them to known chemical regimes. This will require strains of mites (and colonies) that are not contaminated from outside sources or mites of known resistance reared in the laboratory.

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ORIGINAL RESEARCH ARTICLE

A national survey of managed honey bee 2010-11 winter colony losses in the USA: results from the Bee Informed Partnership

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Summary

This study records the fifth consecutive year that winter losses of managed honey bee (*Apis mellifera*) colonies in the USA have been around 30%. In April 2011, a total of 5,441 US beekeepers (an estimated 11% of total US beekeepers) responded to a survey conducted by the Bee Informed Partnership. Survey respondents reported that they had lost an average of 38.4% of their colonies, for a total US colony loss of 29.9% over the winter of 2010-11. One-third of respondents (all classified as backyard beekeepers, i.e. keeping fewer than 50 colonies) reported no winter loss. There was considerable variation in both the average and total loss by state. On average, beekeepers consider acceptable losses to be 13.2%, but 68% of all responding beekeepers suffered actual losses in excess of what they considered acceptable. Of beekeepers who reported losing at least one colony, manageable conditions, such as starvation and a weak condition in the fall, were the leading self-identified causes of mortality. Respondents who indicated that varroa mites (*Varroa destructor*), small hive beetles (*Aethina tumida*), poor wintering conditions, and / or Colony Collapse Disorder (CCD) conditions were a leading cause of mortality in their operations suffered a higher average loss than beekeepers who did not list any of these as potential causes. In a separate question, beekeepers who reported the symptom "no dead bees in hive or apiary" had significantly higher losses than those who did not report this symptom. In addition, commercial beekeepers were significantly more likely to indicate that colonies died with this symptom than either backyard or sideliner beekeepers.

Una encuesta nacional sobre las pérdidas invernales de colonias manejadas de abejas melíferas 2010-11 en los Estados Unidos: resultados de la Bee Informed Partnership

Resumen

Este estudio registra por quinto año consecutivo que las pérdidas invernales de abejas manejadas (*Apis mellifera*) en Estados Unidos están en torno al 30%. En abril del 2011, un total de 5,441 apicultores de los EE.UU. (se estima que el 11% del total de apicultores de EE.UU.) respondieron a una encuesta realizada por la Bee Informed Partnership. Los encuestados indicaron que habían perdido un promedio de 38.4% de sus colonias, con una pérdida total de colonias en EE.UU. del 29.9% durante el invierno de 2010-11. Un tercio de los encuestados (todos ellos clasificados como apicultores aficionados, es decir, con menos de 50 colonias) indicaron que no tuvieron pérdidas de invierno. Hubo una variación considerable tanto en la media como en el total de pérdidas por Estado. Por término medio, los apicultores consideran aceptables pérdidas del 13.2%, sin embargo, el 68% de todos los apicultores encuestados sufrieron pérdidas reales superiores a lo que consideran aceptable. Entre los apicultores que informaron de la pérdida de al menos una colonia, las principales causas de mortalidad identificadas por ellos fueron condiciones de manejo, tales como el hambre o una condición débil de las abejas en el otoño. Los encuestados que indicaron como principales causas de mortalidad de sus colmenas a los ácaros de Varroa (*Varroa destructor*), los escarabajos de las colmenas (*Aethina tumida*), las malas condiciones de invernada y / o condiciones del Síndrome de Colapso de las Colmenas (SCC), sufrieron una pérdida media mayor que aquellos apicultores que no incluyeron ninguna de estas causas potenciales. En una cuestión aparte, los apicultores que indicaron el síntoma "sin abejas muertas en la colmena o apiario" tenían pérdidas muy superiores a aquellos que no registraron ese síntoma. Además, los apicultores comerciales fueron significativamente más propensos a indicar que las colonias morían con este síntoma que los apicultores aficionados o los apicultores semi-profesionales.

Keywords: Honey bee, overwinter, mortality, USA, 2010-11

Introduction

Over the last few years, high rates of overwintering mortality have been reported in honey bee (*Apis mellifera*) colonies in many European and North American countries (vanEngelsdorp *et al.*, 2008, 2010, 2011a; Currie *et al.*, 2010; Neumann and Carreck, 2010; Nguyen *et al.*, 2010; Potts, 2010). In the US specifically, high overwintering losses of 32%, 36%, 29% and 34% for the winters of 2006-7, 2007-8, 2008-9, and 2009-10, respectively, have been reported (vanEngelsdorp *et al.*, 2007, 2008, 2010, 2011a).

It is clear that these losses, verging on 30% or more annually have not resulted in a pronounced decrease in the total number of honey-producing colonies managed by US beekeepers in the subsequent summers (USDA-NASS, 2009). The USDA-NASS Bee and Honey Inquiry is a survey that estimates the total number of US honey producing colonies on an annual basis, for operations with more than five colonies. From 2008-10, an increase in total colonies has been recorded in the USDA-NASS *Honey* report starting from 2.34 million colonies (rounded) in 2008; to 2.50 million in 2009; and to 2.68 million in 2010 (USDA-NASS, 2009, 2010, 2011). This apparent discrepancy may be explained by beekeepers who, fearing heavy losses, overwinter excess colonies to ensure they will have enough colonies to meet spring's pollination demands (vanEngelsdorp and Meixner, 2010). Beekeepers can increase the number of colonies they manage by either purchasing package bees or splitting existing hives. In addition, development and management of nucleus colonies has become more widespread as a hedge against heavy losses. A recent survey of Pacific Northwest beekeepers revealed that in both 2008 and 2009, beekeepers replaced more colonies than they lost in the preceding winter (Caron *et al.*, 2010). Another possible explanation for this discrepancy may be differences in survey methods and the respondent population.

Heavy losses cannot always be replaced by dividing colonies and buying packages, however. A decrease of 142,000 colonies from 2007 -8 is reflected in the 2009 USDA-NASS *Honey* report. This is the only decrease from the previous year recorded by USDA-NASS during the period between 2006 and 2010. This loss could have occurred during the winter of 2007-8 where the "winter loss survey" recorded colony losses for the same time period of around 36%; the highest loss in four years of surveys (vanEngelsdorp *et al.*, 2008).

The reason for the high level of losses is not completely understood. While annual overwintering loss surveys are not designed to identify factors responsible for losses, each survey has asked beekeepers to self-identify the reasons they believe high losses

occurred. Among the most mentioned factors have been queen failure, starvation, and varroa (*Varroa destructor*) mites (vanEngelsdorp *et al.*, 2007, 2008, 2010,2011a). Whilst not conclusive, these self-identified causes of mortality do suggest that a multitude of factors are contributing to colony mortality, and so suggest that efforts aimed to reduce losses will need to be as diverse as the causes.

In keeping with previous years' efforts, this survey's objective was to quantify the mortality of colonies in the USA over the winter of 2010-11. Here we report average and total colony losses for the country and by state, we compare the rate of loss by operation size, activity, and by the symptom of "no dead bees in the hive or apiary", and we quantify the prevalence of suspected reasons for loss as self-reported by survey respondents.

Materials and methods

An email soliciting responses to an online survey posted at InstantSurvey.com was sent to state apiarists (n = 42), presidents of national and state beekeeping organizations (n = 110), industry leaders (n = 125), honey bee brokers (n = 17; for almond pollination in CA), online beekeeper list servers, and posted on web-forums. A total of 2,877 individual emails were sent to participants in previous years' surveys who had indicated a desire to be contacted in future years. In addition, 621 individual emails were sent to persons who had "signed up to participate" at the beeinformed.org web site. These emails encouraged beekeepers to forward the request to other beekeepers. As in previous years, a number of large commercial beekeepers were contacted by telephone, with a total of 25 being successfully interviewed. The convenience and snowball sampling for this survey's solicitation effort precludes an ability to calculate survey response rate, because the exact number of beekeepers contacted is not known. Based on subscription rates of electronic listservers such as BEE-L and Catch the Buzz, however, we estimate that over 20,000 beekeepers were contacted (Flottum, 2010). The questions asked are shown in Box 1.

Box 1.

1. In what state(s) did you keep your colonies in 2010?

2. How many living colonies did you have on 1 October 2010?

3. How many living colonies did you have on 1 April 2011?

4. Did you make splits, increases or buy / sell colonies between 1 October 2010 and 1 April 2011?

5. How many splits, increases, and / or colonies did you make / buy between 1 October 2010 and 1 April 2011?

6. How many splits, increases, and / or colonies did you sell between 1 October 2010 and 1 April 2011?

7. What percentage of the colonies that died between 1 October and 1 April were lost without dead bees in the hive or apiary?

8. What percentage of loss, over this time period, would you consider acceptable?

9. In your opinion, which factor(s) was the main cause(s) of colony death in your operation between 1 October 2010 and 1 April 2011?

10. What percentage of your hives did you send to California for almond pollination?

11. How many times, on average, did you move your colonies last year?

12. Would you be willing to be contacted by our survey team in order to participate in other honey bee related surveys and/or to validate this survey and to receive a summary of survey results?

For question one, which asked in which state(s) the respondent kept bees, a list of all US States, the District of Columbia, and an "other" category was provided. Respondents could check more than one option. Those checking "other" were asked to specify the location of their colonies. For the question 9, pertaining to the perceived cause of losses, respondents could choose from a list of common responses from previous survey efforts (vanEngelsdorp *et al.*, 2011a). These included: queen failure; starvation; varroa mites; *Nosema* disease; small hive beetles; poor wintering conditions; pesticides; weak in the fall; Colony Collapse Disorder (CCD); don't know; did not suffer losses; and other. Those responding "other" were asked to specify their perceived cause of loss. For all other questions, possible answers were not provided and beekeepers were expected to type out answers in the fields provided.

This survey design and distribution was approved by the University of Nebraska-Lincoln Institutional Review Board (UNLIRB #200608523 EP) to ensure compliance with US Federal Law regarding research with human subjects. As in previous years, to help ensure loss estimates could be compared internationally, core survey questions were in keeping with efforts of Working Group 1 of COLOSS, an international network of honey bee researchers dedicated to the prevention of honey bee COlony LOSSes (Nguyen *et al.*, 2011; van der Zee, 2012).

Survey responses were solicited and collected between 1 and 18 April 2011. Once complete, the data were edited to permit processing (i.e. changing text to numbers (e.g. 2 instead of two) where appropriate). Filters were also developed to exclude from the analysis responses such as surveys with incomplete answers or those that were obviously duplicate answers. As in previous efforts, beekeepers were assigned to operational size groups by the following criteria; beekeepers managing 50 or fewer colonies were classified as "backyard beekeepers"; those managing between 51 and 500 colonies were classified as "sideline beekeepers"; and those managing 501 or more colonies were classified as "commercial beekeepers".

Calculations and statistical analysis

Total and average colony losses were calculated in keeping with the approach and standard outlined by vanEngelsdorp *et al.* (2011b). Confidence Interval (CI) calculations for total losses were conducted using R (R Development Core Team, 2009; code provided by Y Brostaux and B K Nguyen). The mean percentage of individual operation colony loss was calculated to determine the average loss among all respondents and subgroups. Average loss 95 % Confidence Intervals (95% CI) were calculated using the statistical program SAS JMP (SAS, 2007) as outlined in vanEngelsdorp *et al.* (2011b).

Unlike in previous years, total loss values were only calculated and reported for the entire nation and individual states with sufficient response rates to permit reporting. Whilst total loss values are the most accurate representation of losses suffered within a region, they are biased by overly representing the losses of larger operations because they manage more colonies. Total loss calculations were not therefore calculated for sub-classifications other than those based on region. Instead, potential differences between sub-groups of the responding beekeepers were explored by calculating and comparing average operational losses using the Kruskal-Wallis rank sum test.

When calculating losses in individual states, colonies belonging to operations which managed colonies in more than one state were counted multiple times; once in each listed state. This same practice is used by the National Agricultural Statistics Service when calculating the number of honey-producing colonies in each state (USDA-NASS, 2009). Responses for groups containing fewer than nine respondents are not reported, to protect the privacy of respondents. The total number of colonies lost with the symptom of "no dead bees in the hive or apiary" was calculated for individual operations by multiplying the number of colonies lost in an operation by the reported percentage lost without dead bees. The ratios of beekeepers grouped by operation size who suffered losses with the symptom of "no dead bees in the hive or apiary" were compared using the Chi square test.

Results

Average and total losses

National losses

The survey recorded 5,770 responses, of which 36 were duplicates and 51 did not reside in the US so were removed. An additional 242 respondents did not provide all the information needed to quantify overwintering losses. The remaining 5,441 respondents managed a total of 309,200 living colonies on 1 October 2010, representing 11.5% of the estimated 2.68 million honey-producing colonies being managed in the US in 2010 (USDA-NASS, 2010). These same 5,411 beekeepers reported 267,089 living colonies on 1 April 2011. When colonies that were made, bought (n = 80,707) or sold (n = 8,670) are factored into the calculation, the 5,411 respondent beekeepers lost an average of 38.4% (95% CI: 37.4 - 39.4%) of their colonies, while the total loss suffered by this group was 29.9% (95% CI: 29.2 - 30.4%). One-third of responding beekeepers, all of whom were backyard beekeepers, reported no winter losses.

Losses by state

There was considerable variation in both the average (Table 1; Fig. 1) and total (Table 1; Fig. 2) losses suffered by beekeepers operating in different states. The percentage of colonies and operations in any given state which operated exclusively in that state is summarized (Table 1). As outlined above, operations managing bees in more than one state had their responses reported in all states in which they operated. Some caution is therefore needed when comparing state colony losses where a large proportion of the colonies are managed by beekeepers with bees in several states.

Losses by operation classification

Average losses suffered by commercial beekeepers tended to be lower than those suffered by sideline and backyard beekeepers, but this difference was not significant ($P = 0.25$, Table 2).

Two percent of survey respondents reported maintaining colonies in more than one state. Although numerically lower, there was no statistical difference ($P = 0.58$) in the average loss experienced by those beekeepers who maintained colonies in more than one state (30.4%; 95% CI: 23.6 – 37.3%; n = 114) when compared to those who maintained colonies exclusively in one state (38.5%; 95% CI: 37.6 – 39.6%; n = 5,327).

Only 1.6% of respondents indicated that they utilized at least some of their operation for almond pollination during the survey period. On average, beekeepers pollinating almonds moved 83.6 ± 2.8% of their colonies into the almond orchards. The average loss experienced by beekeepers who moved colonies into almond orchards

Table 1. The number of operations and colonies contributing to the percentage of average and total losses by state (also summarized in Fig. 1 and Fig. 2) and the percentage of operations and colonies in each state that operated exclusively in that state. Operations reporting managing colonies in more than one state have had all of their colonies counted in all states in which they reported managing colonies. Results for states with fewer than nine respondents are not presented.

State	No. Operations	Operations exclusively in state (%)	Total No. Colonies	Colonies exclusively in state (%)	Average Loss mean (95 % CI)	Total Loss mean (95 % CI)
Alabama	35	97.1	514	98.1	15.2 (7.2-23.2)	11.3 (6.84-18)
Alaska	3					
Arizona	7					
Arkansas	43	95.3	305	96.1	22.4 (13.4-31.3)	30.2 (22.8-38.8)
California	328	82.6	310650	14.1	39 (35.1-42.9)	27.2 (25-29.4)
Colorado	137	97.1	1156	82.5	37.7 (31.9-43.5)	53.4 (48.5-58.3)
Connecticut	102	95.1	906	57.8	51.0 (43.8-58.2)	46.6 (41.2-52.1)
Delaware	15	93.3	97	91.8	32.2 (11.5-52.9)	58.8 (37.7-77.1)
Florida	133	93.2	38242	6.8	25.6 (20.5-30.7)	40.32 (37-43.8)
Georgia	143	93.7	8270	19.4	28.1 (22.9-33.3)	63.93 (58.6-69)
Hawaii	42	100.0	5520	100.0	44.6 (33.0-56.2)	7.7 (3.8-15.3)
Idaho	27	81.5	10033	1.0	30.4 (16.9-43.9)	5.8 (3.9-8.4)
Illinois	136	97.8	1102	95.7	54.9 (48.5-61.3)	45 (39.8-50.3)
Indiana	151	100.0	1228	100.0	41.9 (35.9-47.9)	37.5 (34-41.1)
Iowa	28	96.4	765	99.7	45.1 (31.7-58.5)	66 (57.4-73.7)
Kansas	21	95.2	401	98.0	22.1 (8.9-35.3)	14.5 (9.6-21.1)
Kentucky	55	98.2	991	97.0	28.3 (18.9-37.7)	30.6 (24.5-37.4)
Louisiana	18	94.4	3515	19.8	17.1 (4.7-29.5)	25.1 (22.4-28)
Maine	105	95.2	22764	2.9	48.8 (41.2-56.4)	45.9 (41.8-50.2)
Maryland	172	97.7	1622	95.1	37.2 (32.1-42.3)	49.5 (44.5-54.4)
Massachusetts	219	95.4	19931	11.9	46.3 (41.3-51.2)	34.7 (33.2-36.2)
Michigan	278	98.2	22631	21.0	62.7 (58.3-67.0)	34.8 (31.4-38.4)
Minnesota	55	83.6	128099	11.4	51.7 (41.9-61.4)	32.1 (28.5-35.7)
Mississippi	16	68.8	118909	0.1	12.8 (0-25.6)	26.5 (22.4-31)
Missouri	161	100.0	2441	100.0	26 (21.1-30.9)	29.0 (24.4-33.2)
Montana	26	73.1	51637	0.2	50.6 (34.4-66.8)	20.4 (14.4-28)
Nebraska	16	81.3	95800	0.1	29.5 (16.9-42.1)	29.3 (25.9-32.9)
Nevada	6					
New Hampshire	84	97.6	902	98.7	55.4 (47.1-63.6)	31.4 (25.2-38.2)
New Jersey	110	90.0	2366	29.7	30.9 (24.1-37.6)	28.7 (25.3-32.2)
New Mexico	24	95.8	188	97.3	19 (5.0-32.9)	11.7 (6.8-19.3)

Table 1 Cont'd. The number of operations and colonies contributing to the percentage of average and total losses by state (also summarized in Fig. 1 and Fig. 2) and the percentage of operations and colonies in each state that operated exclusively in that state. Operations reporting managing colonies in more than one state have had all of their colonies counted in all states in which they reported managing colonies. Results for states with fewer than nine respondents are not presented.

State	No. Operations	Operations exclusively in state (%)	Total No. Colonies	Colonies exclusively in state (%)	Average Loss mean (95 % CI)	Total Loss mean (95 % CI)
New York	217	92.2	11737	18.4	44.1 (39.3-48.8)	58.5 (55.0-62.0)
North Carolina	616	96.4	7939	74.9	25.7 (23.2-28.1)	24.7 (23.1-26.3)
North Dakota	21	38.1	162799	0.3	33.9 (20.4-47.3)	24.6 (20.2-29.5)
Ohio	242	100.0	1449	100.0	38.6 (33.9-43.2)	42.6 (38.6-46.7)
Oklahoma	31	93.5	793	98.5	29.2 (16.8-41.5)	16.5 (8.4-29.9)
Oregon	179	92.2	20138	5.8	29.7 (24.7-34.6)	9.5 (0.08-10.7)
Pennsylvania	431	98.4	9056	42.3	49 (45.2-52.7)	67.1 (64.2-69.8)
Rhode Island	66	95.5	226	94.7	46.2 (35.9-56.5)	48.6 (40.3-57.2)
South Carolina	81	88.9	3741	18.0	20.1 (15.2-24.9)	39.1 (34.9-43.3)
South Dakota	18	77.8	92218	0.1	22.9 (7.8-38.0)	31.7 (30.7-32.8)
Tennessee	90	94.4	732	83.6	21.7 (15.5-27.9)	22.3 (17.3-28.3)
Texas	76	85.5	138338	1.2	20.3 (14.6-25.9)	25.8 (23.6-28.2)
Utah	117	98.3	5389	45.1	32.9 (26.9-38.9)	29.5 (26.6-32.4)
Vermont	119	95.8	1591	93.8	41.2 (34.6-47.8)	26.7 (21.8-32.3)
Virginia	406	95.8	4450	57.7	33.7 (30.3-37.2)	31.1 (28.8-33.5)
Washington	150	94.7	27472	5.3	41.6 (35.6-47.6)	23.4 (20.2-27)
Washington, D.C.	3					
West Virginia	55	90.9	588	82.7	49.2 (38.7-59.7)	54.8 (45.2-64.0)
Wisconsin	126	96.0	3665	97.5	57.7 (51.3-64.1)	66.3 (61.6-70.7)
Wyoming	8					

(31.6%; 95% CI: 23.4 – 39.8%; n = 79) was not significantly different from beekeepers whom did not (38.9 %; 95 % CI: 37.9 – 40.0%; n = 4,931; P = 0.77).

Only 1.7% of responding beekeepers indicated that they had transported a majority of their colonies across state lines during the preceding year. Beekeepers who moved their colonies lost, on average, fewer colonies (32.9%; 95% CI: 25.1 – 40.7%; n = 88), but the difference was not significant (P = 0.74) when compared to those that did not move colonies (38.9%; 95% CI: 37.9 – 40.6; n = 4,914).

One of the defining characteristics of CCD is the complete absence of dead bees in the hive or apiary (vanEngelsdorp *et al.*, 2009). This survey was not meant to differentiate between colonies lost to CCD and other conditions that may cause colony loss with this symptom. Of those respondents who experienced at least some loss, and answered this question, 23% (of n = 3,610 respondents) indicated that at least some of their dead colonies were found without dead bees in the hive or apiary. Those reporting losses in addition to the no dead bees symptom reported higher average losses (62.3%; 95% CI: 60.2 – 64.4%; n = 828) when compared to those who reported losses without the symptom of no dead bees (56.5%; 95% CI: 55.4 – 57.7%; n = 2,782; P < 0.0001). In all, of the 114,118 colonies reported to have died over this survey period, an estimated 26.3% (n = 30,135) died with the symptom "no dead bees in the hive or apiary". Of beekeepers who reported suffering losses, commercial beekeepers were 2.6 and 1.4 times more likely to report having some of their dead colonies die with an absence of dead bees than were backyard and sideline beekeepers (χ^2 = 19.0; P < 0.001 and χ^2 = 18.7; P < 0.001, respectively).

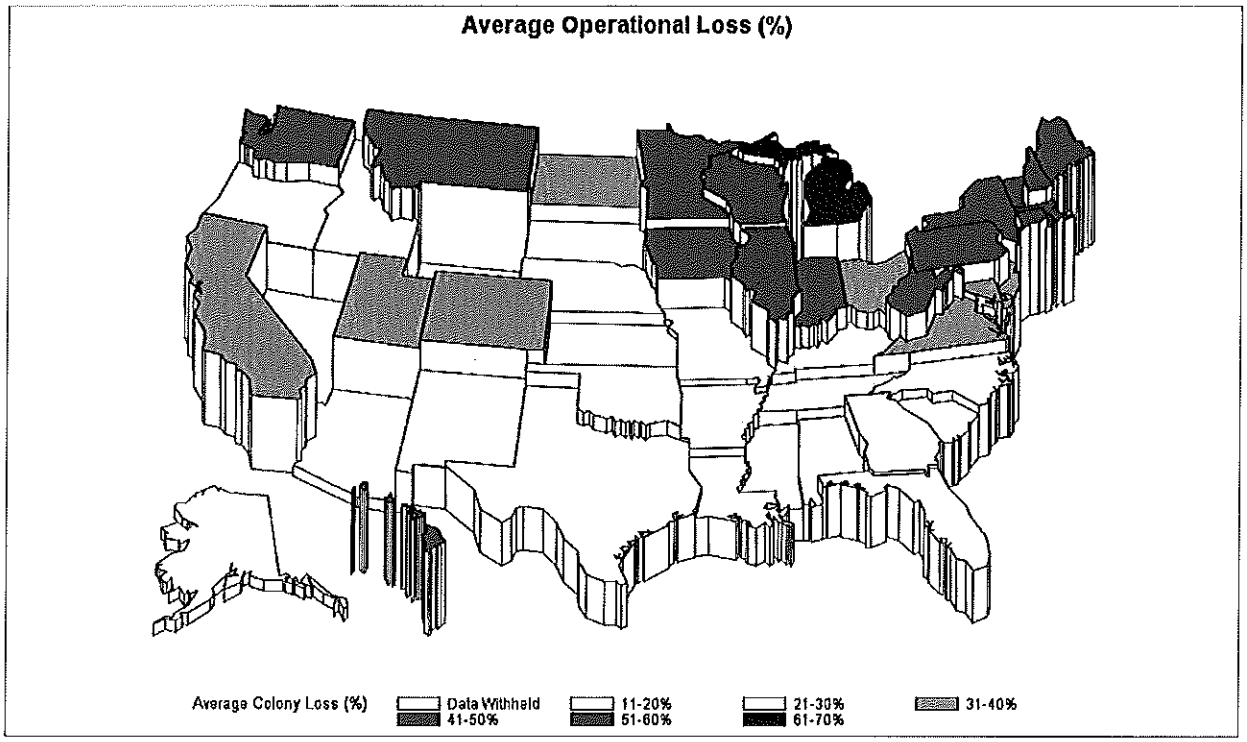

Fig. 1. Average percentage of loss in each operation by state. Operations who reported managing colonies in more than one state had their losses included in all of the states in which they reported managing colonies (see Table 1). States which had fewer than nine respondents (data withheld) are not included.

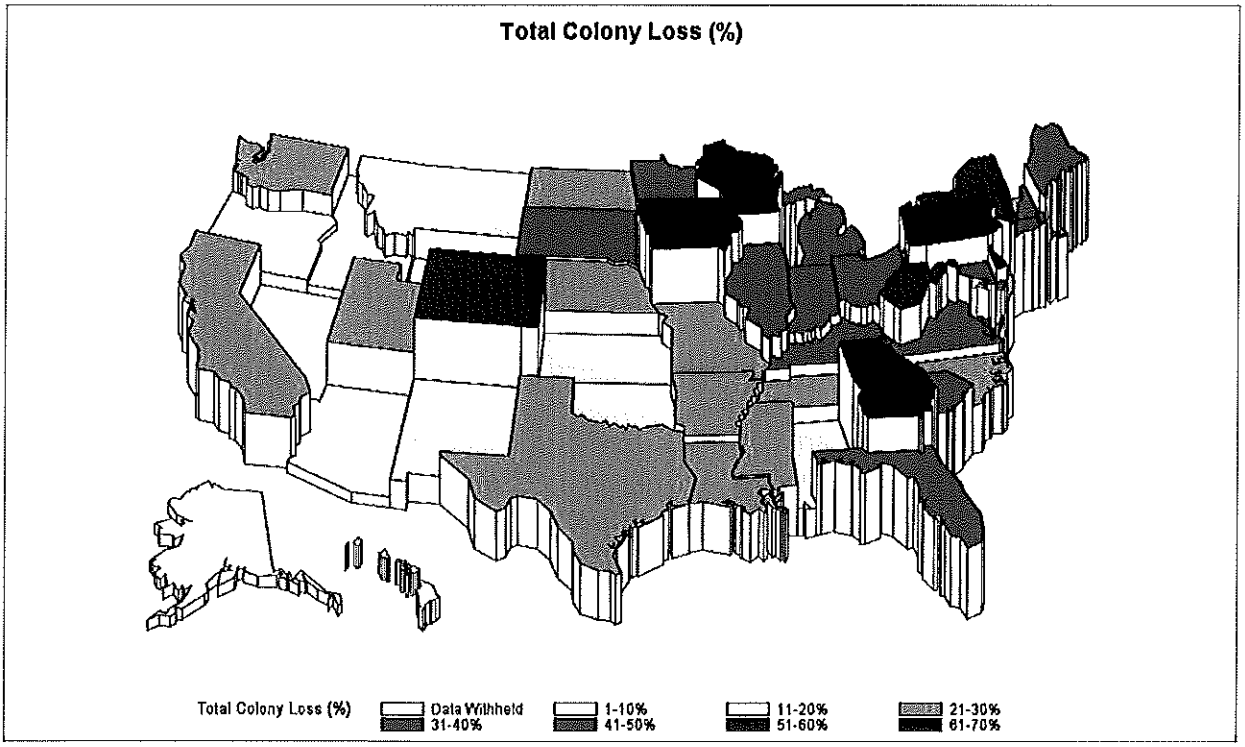

Fig. 2. Total percentage of colony loss by state. Operations who reported managing colonies in more than one state had their losses included in all of the states in which they reported managing colonies (see Table 1). States which had fewer than nine respondents (data withheld) are not included.

Table 2. Average losses suffered by beekeepers grouped by the size of their operation.

Operation Type	Respondents	Average Loss Mean (95% CI)
Backyard	5220	38.5 (37.5 -39.5)
Sideline	163	37.4 (31.7 - 43.2)
Commercial	58	28.3 (18.7 – 38.0)

Table 3. Average losses reported by beekeepers who listed one or more factors as the leading cause of mortality in their beekeeping operation as compared to responding beekeepers not listing that particular cause as important. *Excludes those who indicated they suffered no loss as well as those who indicated they did not know which factors contributed to their losses.

		Factor Listed			Not Listing Factor*		Kruskal Wallis Rank SumTest	
Factor	*n*	Avg Loss % (95%CI)		*n*	Avg Loss % (95%CI)		X^2	P
Starvation	1053	53.7 (51.8–55.7)		1629	54.4 (52.8–56.0)		0.16	0.6822
Weak in the fall	921	52.8 (50.7-54.9)		1761	54.8 (53.3-56.4)		1.78	0.1840
Poor winter	833	64.3 (62.2-66.5)		1849	49.7 (49.1-51.0)		118.8	0.0001
Queen	655	47.5 (45.0-50.0)		2027	54.4 (52.8-56.0)		37.5	0.0001
Varroa	534	59.5 (56.8-62.3)		2148	52.8 (51.4-54.2)		18.8	0.0001
Nosema	317	55.9 (52.3-59.5)		2365	53.9 (52.6-55.3)		1.14	0.2843
CCD	199	65.1 (60.6-69.6)		2483	53.3 (52.0-54.5)		23.6	0.0001
Pesticides	125	58.9 (53.1-64.6)		2557	53.9 (52.7–55.2)		2.51	0.1134
Small hive beetle	96	63.7 (57.1-70.2)		2586	53.8 (52.5-55.0)		8.29	0.0040

Acceptable losses

Surveyed beekeepers were asked "What percentage of loss, over this time period, would you consider acceptable?" On average, responding beekeepers (n = 4,425) reported that a winter loss of 13.2% (95% CI: 12.7 - 13.7%) was considered acceptable. Sixty percent of responding beekeepers experienced actual losses higher than they considered acceptable. The average losses experienced by this group were higher than the average losses experienced by those who had losses below what they considered acceptable (60.0%; 95% CI: 59.1 – 61.0% *vs.* 4.0%; 95% CI: 2.5 -5.3%, respectively; P < 0.0001).

Perceived causes of losses

A total of 4,781 respondents answered the question "To what do you attribute the cause of death for the colonies that died?" Of these, 70% experienced at least some loss. Twenty-one percent of these 3,389 beekeepers indicated that they did not know the cause of death of the colonies in their operation that had died. Beekeepers who indicated that they did not know the cause of mortality in their operation lost, on average, 64.4% (95% CI: 61.9 – 66.7%; n = 707), more than those who lost colonies and identified at least one reason for their loss (54.1%; 95% CI: 52.4 –55.3%; n = 2,682). Among beekeepers who experienced losses and indicated at least one reason why they lost colonies, the top five most frequent reasons given, in order, were: starvation; weak colonies in the fall; poor wintering conditions; poor queens; and varroa mites (Table 3). Respondents who suspected varroa mites, small hive beetles, poor wintering conditions, and / or CCD as responsible for their losses experienced higher average losses when compared to beekeepers who suspected other factors. Conversely, those respondents who suspected poor queens as the major cause of their losses suffered lower average losses than those who did not suspect queens as responsible for their losses (Table 3).

Discussion

This survey records the fifth consecutive year of overwintering colony losses well above the level US beekeepers consider acceptable. Survey respondents reported total colony losses of 29.9% and average operational losses of 38.4%. This is the fifth year that average losses of 30% or more have been recorded. Should these survey results be representative of national losses, between 782,560 and 814,720 colonies were lost in the US over the winter of 2010-11. Caution should however be used when interpreting this projection, as

this survey cannot be considered to be representative of all beekeepers. The email solicitation of beekeeper respondents probably biased participation to the subgroup of beekeepers that are internet literate. As no comprehensive census of US beekeepers exists, we have no way to quantify and adjust for this potential bias.

Larger operations were more likely to report having some of the colonies in their operation die with the symptom of "no dead bees in the hive or apiary". This symptom is one of the defining characteristics of CCD, and as in previous years, those losing some of their colonies to this condition experienced greater total losses than those not reporting the condition.

In summary, this national survey effort, in its fifth consecutive year, recorded high rates of mortality in overwintering colonies in the US. Losses suffered by smaller-sized operations were higher than the losses suffered by larger operations, even though larger operations were more likely to report having some of their losses occur in the absence of dead bees in the hive or apiary; a defining symptom of CCD. These results all point to the continuing need to record colony losses on an annual basis. These continuing efforts should also strive to improve survey methods to ensure a more representative beekeeping population is sampled and accounted for. Concentrated efforts aimed at understanding the underlying causes of these losses are also needed.

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