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

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J. Econ. Entomol. 101(4): 1057–1061 (2008)

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

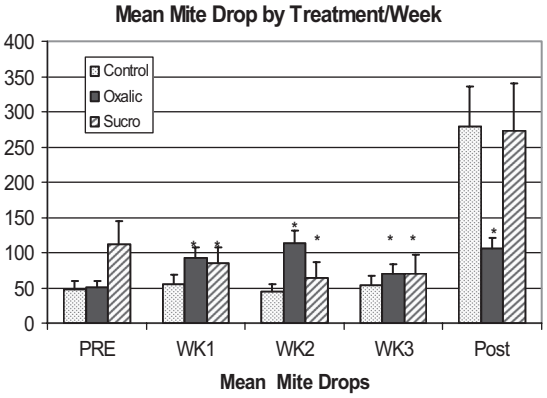

Fig. 1. Mean ± SE varroa mite drop onto sticky boards in control colonies and colonies treated with oxalic acid or Sucrocide before treatment (PRE), in each of 3 wk during treatment (WK1-3), and after treatment (Post) when Apistan was applied. An asterisk indicates a significant difference in the change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

Acknowledgments

This research was supported by the National Honey Board and the Almond Board of California. Special thanks

to George Hanson; Steve Gibson; Drs. G. DeGrandi-Hoffman, A. Nanetti, J. Skinner, and R. Alarcon; D. vanEngelsdorp; and all the beekeepers who supported this project. Thanks also to the anonymous reviewers whose comments improved this paper.

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Received 3 October 2007; accepted 25 February 2008.