EFFICACY OF DIFFERENT METHODS OF OXALIC ACID APPLICATION

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INTRODUCTION

Varroa destructor remains one of the primary causes of honey bee decline worldwide. Beekeepers continue to look for alternative methods to control this parasite. Varroa mites can quickly evolve resistance to synthetic acaricides, such as coumaphos and fluvalinate (Elzen et. al. 2002; Milani et. al. 1999). Residues of these synthetic acaricides are usually found in wax and pollen, increasing the frequency of bees exposed to these toxic chemicals. Thus, natural compounds that have low toxicity to bees and do not accumulate in wax are being used more often to control varroa mites (Imdorf et al., 1997). These compounds include organic acids, such as oxalic and formic acid, and essential oils, such as a thymol. Both formic acid (active ingredient in Mite-Away Quick Strips®) and thymol (active ingredient in Apiguard® and ApiLifeVar®) have been legal in the US for quite a few years, but their efficacy is highly dependent upon ambient temperature. Moreover, these chemicals can have negative effects on brood and queen health. Oxalic acid (OA) has been used for many years by beekeepers in Europe and Canada, but was legalized in the US only in 2015. OA is an organic acid commonly found in plants and honey, and it is employed in the domestic sector as a household cleanser or wood bleach. Its efficacy against varroa mites can reach 90%, it is naturally found in honey (Bogdanov et al 2002), and due to its hydrophilic properties, it is not expected to accumulate in beeswax (Rademacher and Harz 2006). All of these characteristics make OA a desirable chemical for mite control.

The mode of action for OA is not completely understood. Some studies suggest that OA exhibits lethal effects on varroa mites primarily via direct physical contact with the substance (Aliano et. al. 2006; Aliano et. al. 2008). Because reproductive varroa mites are nestled under the brood capping, they do not come into direct contact with OA when applied. Therefore, its high efficacy (>90%) is only reached when applied to broodless colonies, such as bee packages, swarms or during winter months. During periods with brood, efficacy drops to 60% (Nanetti 2005). The two most common modes of OA applications are the (1) *trickle* or *dribble method*—OA dihydrate is mixed in a sugar solution and dribbled onto the bees in between the frames, and (2) s*ublimation* or *vaporization method*—OA dihydrate crystals are evaporated via a heating apparatus (vaporizer) that is inserted at the entrance of the colony (Charrière et.al. 2004) (Photo 1).

Because OA has only recently been registered for use against varroa mites in the US, most studies on its efficacy against ectoparasites have been conducted in Europe and Canada. Here in the US, Dr. Marion Ellis research group, from University of Nebraska-Lincoln, has done extensive work quantifying OA toxicity on honey bees and mites (Aliano et. al. 2006; Aliano and Ellis 2008, 2009). In the January 2017 volume of *American Bee Journal*, Randy Oliver presented results about the effectiveness of oxalic acid and glycerin strips and found this combination to be very effective against varroa mites. However, we have limited information regarding the success of traditional methods of application in controlling in-hive varroa mite populations in the US, and whether these applications can improve colony survival. The goal of our experiment was to compare the efficacy of two common methods of OA application, trickle and

Photo 1. Honey bee hive being treated with oxalic acid using the sublimation method. ***Photo K.C. Evans***

sublimation, on number of varroa mite dropping and over-winter survival. We discuss our results in the context of the importance of incorporating OA into IPM management programs.

METHODS

Twenty-three colonies near State College, PA were used to assess the efficacy of the trickle and sublimation methods. Each colony was in a 10-frame Langstroth hive. Colonies were randomly assigned to three treatment groups: control (no OA applied) (n=9), trickle method (n=7), and sublimation (n=6) (Table 1). All hives had a minimum of two deep hive bodies, and 1-3 medium hive bodies depending on colony size. We used the recommended concentration of 3.5% for the trickle method (Gregorc et. al. 2001; Nasr et al.2001), and 2g of OA dehydrate per colony for the sublimation method (Imdorf et al. 2003; Rademacher and Harz 2006). OA was applied to all colonies on November 30 (2016) under a recorded temperature of 55°F.

Trickle method: The recommended application per hive is a maximum of 50 ml solution per colony. The OA solution contains 35 g of OA dihydrate in 1 liter of 1:1 sugar:water (weight:volume) (Table 1). A 50 ml syringe was used to trickle 5 ml of the OA solution per frame, directly applying it onto the bees clustered between the frames.

Sublimation: Each colony was treated with 2g OA dihydrate in crystalline form, which was heat-evaporated or "sublimated" using a commercial vaporizer (Heilyser) (Table 1). The vaporizer was placed inside the colony for two minutes, allowing the crystals to fully evaporate, and all entrances were sealed with a wet cloth. Upon removal of the vaporizer, all holes were sealed for 10 minutes, including the entrance to the colony. As an additional safety precaution, the operators wore approved respirator masks and nitrile gloves (Photo 2).

Photo 2. Necessary protective gear for oxalic acid application using the sublimation method. *Photo K.C. Evans*

Varroa mite counts: We measured efficacy of treatments by comparing the number of varroa mites that dropped after OA application. Stickyboards (coated with petroleum jelly) were placed under the screened bottom board of each colony for two days. Varroa mites were counted both before treatment (28-November-2016) and after treatment (until 16-December-2016; Figure 1) following the method developed by Ostiguy and Sammataro (2000). Statistical differences between the numbers of varroa mite drops with three different methods were investigated with an ANOVA.

Control	No treatment
Trickle	Oxalic acid, 1 application 1:1 sugar:water (weight:volume) 50 ml dripped per colony
Sublimation	Oxalic acid, 1 application 2g OA dihydrate crystals 2 minutes per colony

Table 1. Detailed description of each treatment group

RESULTS

We observed a significantly larger number of varroa mite drops in colonies treated with the sublimation method (26.55 ± 41.61) than untreated colonies (11.02 ± 21.02) or colonies treated with the trickle method (4.17 ± 4.31) (Figure1). The greatest varroa mite drop occurred during the first five days following the sublimation treatment, after which numbers levelled out as expected. The total varroa mite drop in the control (no treatment) and the trickle method did not differ significantly. Control colonies did however show a lower survival rate (28%) in comparison with both the trickle method (56%) and sublimation method (66%).

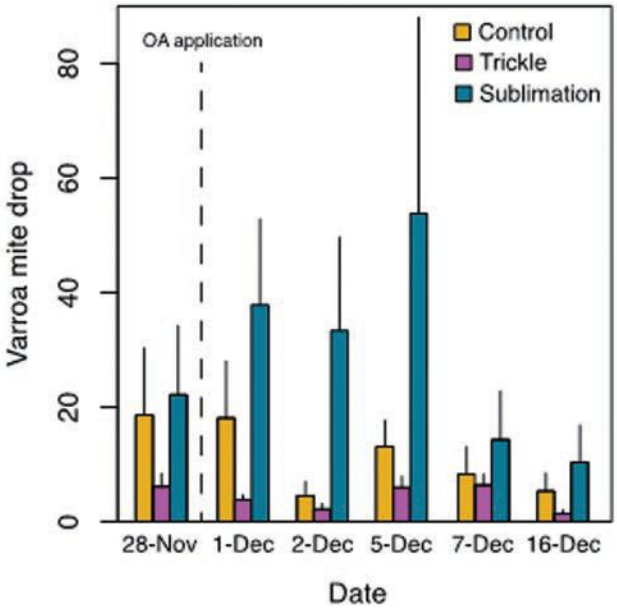

Figure 1. Mean varroa mite drop per time period in untreated (control) and oxalic acid treated colonies.

DISCUSSION

Our results suggest that the application of OA via sublimation is potentially a more effective method to reduce varroa mite numbers during periods with little to no brood. However, the trickle method did not have a significant effect on varroa mite counts contrary to what we expected. This could have stemmed from several factors, including that we treated later in the season (November 2016). OA is typically applied during the fall when there is little brood, the bees are still active and there is minimal clustering. It is possible that bees had already started clustering when we applied the OA treatment, as the colonies had already experienced several days of temperatures below 50 °F. Therefore, dense bee clustering could have prevented bee-to-bee contact in the colonies treated with the trickle method, while during sublimation OA vapor could have penetrated the cluster and spread across the colony (Aliano et. al. 2006; Aliano et. al. 2008).

In addition, there are other pros and cons for each method. While we found sublimation to cause greater varroa mite drop than the trickle method, the former method is more expensive. The cost of the vaporizer, personal protective equipment—including respirators, eye-protection and nitrile gloves—would cost a beekeeper about $160-200, but it is a one-time investment. Another important point about the sublimation method is that it poses the greatest hazards to the applicator, and we cannot overemphasize enough the need to use protective apparel when using the vaporizer with OA (Photo 2). However, the higher costs and greater hazard of OA sublimation applications are compensated by its high efficacy reducing varroa mites and the short time necessary for its application. Sublimation is also the only feasible method of varroa mite control during inclement weather (e. g., cold temperatures or rain) when opening a colony is not advisable.

While honey bees are known to exhibit high tolerance to OA, its toxicity to honey bees is largely unknown. In particular, there

is limited research on the effects of OA exposure on queen health, brood rearing and colony survival, but there are some indications of long-term negative effects on both brood amount and queen health (Higes et al. 1999). Therefore, high doses of OA, which can occur from multiple OA treatments within a season, might impair brood development, overwintering survival and queen health (Higes et. al. 1999; Nanetti 2003; Toufailia et al. 2015). Varroa mite resistance to OA is also of concern as the use of this organic acid becomes more widespread.

We conclude that application of OA via sublimation is an effective method of varroa mite control with potential for widespread use in the US. More research is needed to compare the trickle and sublimation methods during other periods, in other regions, and to quantify longer-term benefits and hazards. To help prevent rapid development of varroa mite resistance to OA, we would like to stress that beekeepers should follow recommended dosage and use it responsibly. It is also important to use the appropriate protective equipment to ensure beekeeper's safety. With these considerations in mind, fall treatment using OA could play an important role within an IPM management strategy to control varroa mite and strengthen colonies.

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CITATIONS

Aliano, N. P., Ellis, M. D., & Siegfried, B. D. (2006). Acute contact toxicity of oxalic acid to *Varroa destructor* (Acari: Varroidae) and their *Apis mellifera* (Hymenoptera: Apidae) hosts in laboratory bioassays. *Journal of Economic Entomology*, 99, 1579–1582. doi:10.1603/0022-0493-99.5. 1579

Aliano, N., & Ellis, M. (2008). Bee-to-bee contact drives oxalic acid distribution in honey bee colonies. *Apidologie* 39, 481-487.

Aliano, N., & Ellis, M. (2009). Oxalic acid: A prospective tool for reducing Varroa mite populations in package bees. *Experimental and Applied Acarology* 48, 303–309.

Charrière J.D., Imdorf A., & Kuhn R. (2004). Bienenverträglichkeit von Varroabehandlungen im Winter [Bee tolerance of different winter Varroa treatments]. *Schweizerische Bienen-Zeitung* 127, 19-23.

Elzen, P., & Weservelt, D. (2002). Detection of coumaphos resistance in *Varroa destructor* in Florida. *American Bee Journal*, 142, 291–292.

Gregorc A, Planinc I. (2001). Acaricidal effect of oxalic acid in honey bee (*Apis mellifera*) colonies. *Apidologie* 32, 333–340.

Higes, M., Meana, A., Suárez, M., & Llorente, J. (1999). Negative long-term effects on bee colonies treated with oxalic acid against Varroa jacobsoni Oud. *Apidologie* 30, 289–292. doi:10.1051/apido:19990404

Imdorf A., Charrière J.D., & Bachofen B (1997). Efficiency checking of the *Varroa jacobsoni* control methods by means of oxalic acid. *Apiacta* 32, 89–91.

Imdorf, A., Charriere, J.D., Kilchenmann, V., Bogdanov, S., & Fluri, P. (2003). Alternative strategy in central Europe for the control of Varroa destructor in honey bee colonies. *Apiacta* 38, 258-285

Milani, N. (1999). The resistance of *Varroa jacobsoni* Oud. to acaricides. *Apidologie* 30, 229–234.

Nasr, M.E., Servos, D., Bannister, R., & Wilson, G. (2001). Efficacy of three miticides (Oxalic acid, Formic acid, Apilife Var) on *Varroa destructor* and *Acarapis woodi* in honey bee colonies in Canada. European Group for Integrated Varroa Control, York, [online] **http://www.apis.admin.ch/host/ varroa/york.htm**.

Nanetti, A. (2003). Oxalic acid treatments for varroa control (a review). [online] **http://docplayer.net/21155569-Oxalic-acid-treatments-for-varroa-control-review.html**

Ostiguy N., Sammataro, D. (2000). A simplified technique for counting *Varroa jacobsoni* Oud. on sticky boards. *Apidologie* 31, 707-716.

Rademacher, E, & Harz, M. (2006). Oxalic acid for the control of varroosis in honey bee colonies – a review. *Apidologie* 37, 98–120.

Toufailia, H.A., Scandian, L., & Ratnieks, F.L.W. (2015). Towards integrated control of varroa: 2) comparing application methods and doses of oxalic acid on the mortality of phoretic *Varroa destructor* mites and their honey bee hosts, *Journal of Apicultural Research*, 54, 108-120, DOI: 10.1080/00218839.2015.1106777.