

Evaluating the Efficacy of Oxalic Acid Vaporization and Brood Interruption in Controlling the Honey Bee Pest *Varroa destructor* (Acari: Varroidae)

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Abstract

A successful Integrated Pest Management approach to *Varroa destructor* Anderson and Trueman control in managed colonies of western honey bees *Apis mellifera* Linnaeus (Hymenoptera: Apidae) must be an improvement over conventional control methods and include cost-effective treatments that can be readily employed by beekeepers. Herein, we tested the efficacy of oxalic acid (OA) vaporization and brood interruption as *Varroa* controls. Sixty experimental colonies were randomly assigned to one of six treatment groups with 10 colonies per group. The six treatments were: 1) OA applied once, 2) OA applied three times, 3) brood interruption, 4) OA applied once + brood interruption, 5) OA applied three times + brood interruption, and 6) no OA or brood interruption. The OA was applied via vaporization, with each application being 1 g OA applied through the hive entrance (label rate), on the bottom board. Brood interruption was accomplished by caging a colony's queen in a queen cage for a period of 24 d. An additional 10 colonies were treated with amitraz (Apivar - positive control). *Varroa* levels were estimated before, during, and after treatment applications using sticky boards left in colonies for 3 d. Our data suggest that queen caging to achieve brood interruption during the fall season can negatively impact colony strength and survival. We observed high colony mortality in some treatments, despite diligent colony management to alleviate the side effects of the treatments. Colonies treated with amitraz were healthier and had better survival than those treated with OA vaporization. In conclusion, OA and/or brood interruption did not provide sufficient *Varroa* control.

Key words: *Apis mellifera*, *Varroa destructor*, oxalic acid, vaporization, brood interruption

Varroa destructor is an ectoparasitic mite that causes significant harm to managed colonies of western honey bees (*Apis mellifera* L.). Current control technologies and practices employed by beekeepers to maintain *Varroa* populations below an economic threshold are inadequate (Rosenkranz et al. 2010). This is, in part, due to the overuse and mismanagement of chemical treatments that have caused the mites to develop resistance to many once-effective treatments (Elzen and Westervelt 2002, 2004; Pettis 2004; Maggi et al. 2009, 2010; Gonzalez-Cabrera 2016). Mechanical manipulations, such as screen bottom boards, can provide partial control but are rarely sufficient as stand-alone treatments (Ellis et al. 2001, Delaplane et al. 2005). Furthermore, mechanical manipulations are often time-consuming and thus impractical for most commercial beekeeping operations. Therefore, an effective Integrated Pest Management (IPM) approach to *Varroa* control must improve upon existing control methods and

include new cost-effective treatments that can be employed by commercial beekeepers.

Oxalic acid (OA) is a natural compound that has been used to control *Varroa* effectively for several decades (Popov et al. 1989), with no reports of mite resistance to date (Maggi et al. 2017). Despite its extensive use history in Canada and Europe (Johnson et al. 2010), OA was only registered in the United States for control against *Varroa* in 2015. Beekeepers commonly treat their colonies by dissolving OA into a sugar solution and spraying the solution directly on the frames of bees or trickling the solution between frames (Charriere and Imdorf 2002, Rademacher and Harz 2006). Some beekeepers, typically those in temperate climates, sublimate OA crystals with heat inside a hive during the late season broodless periods so that the hives do not need to be opened (Rademacher and Harz 2006). Recent studies have produced contradicting results regarding

which method of OA application is most effective at controlling *Varroa* (Al Toufaily et al. 2015, Gregorc et al. 2016, Papežíková et al. 2017).

Oxalic acid is generally considered to be most effective during broodless periods (Charriere and Imdorf 2002, Gregorc and Planinc 2001, Gregorc et al. 2016, Gregorc et al. 2017), as the chemical will not kill mites that are inside capped cells. However, some beekeepers treat with OA once a week for up to 3 wk when brood is present in the hive (Gregorc and Planinc 2001), believing that most mites will be exposed to the chemical at least once during the treatment period. Oxalic acid may kill *Varroa* via contact (Aliano et al. 2006, Aliano and Ellis 2008, Papežíková et al. 2017), though the chemical mode of action is not well understood. The chemical is also effective at dislodging mites by increasing honey bee grooming behavior (Schneider et al. 2012). Negative impacts on honey bee development, behavior, physiology, and longevity have been observed when the bees are exposed to OA (Higes et al. 1999, Schneider et al. 2012, Rademacher et al. 2017).

Varroa reproduction is closely associated with honey bee reproduction as *Varroa* can only reproduce in capped brood cells containing honey bee pupae (Rosenkranz et al. 2010). Therefore, mite reproduction can be halted in a colony by caging the queen and interrupting the honey bee brood rearing cycle. The absence of brood in a colony forces all mites onto the bodies of adult bees and prevents them from reproducing. Brood interruption by removal of capped brood or caging honey bee queens is popular in several European countries and has been shown to reduce *Varroa* populations in colonies significantly (Maul et al. 1988, Calis et al. 1999, Wagnitz and Ellis 2010, Nanetti et al. 2011, Pietropaoli et al. 2012, Lodesani et al. 2014, Giacomelli et al. 2016, Gregorc et al. 2017).

Sublimation was observed to be the most effective OA application method in the United Kingdom (Al Toufaily et al. 2015) and is currently gaining in popularity in the United States. Herein, we use the word ‘vaporize’ when discussing the heating as oxalic acid dihydrate, as it melts to a liquid before turning to a vapor (ILO 2009). In contrast, pure oxalic acid sublimates (Budavari 1989). It is unclear in most occurrences in the literature which form of OA the investigators used. Thus, we use ‘vaporize’ to describe what happened in our study and the term used by the respective authors (either vaporize or sublimate) when citing their manuscripts.

As beekeepers search for sustainable *Varroa* control options, it is necessary to determine whether successes found in one region of the world can be replicated in other regions. Italian beekeepers have reported success at controlling *Varroa* populations for several years by combining the mechanical approach of brood interruption via queen caging with OA application (Nanetti et al. 2011, Pietropaoli et al. 2012, Lodesani et al. 2014, Giacomelli et al. 2016). Despite claims of success from Italian beekeepers, there are only two published records of queen caging and OA application ever being tested in the United States (Wagnitz and Ellis 2010, Gregorc et al. 2017), but neither applied OA via vaporization. To our knowledge, there has yet to be a published study testing the efficacy of OA vaporization combined with brood interruption.

The main objective of this project was to determine whether brood interruption and OA vaporization can be used in concert to control *Varroa*. These methods are already thought to be useful individual treatments for *Varroa* but may potentially have a greater effect when used together (Wagnitz and Ellis 2010, Gregorc et al. 2017). The second objective was to evaluate the effects of these treatments on colony health by measuring various colony health

parameters. Given the literature for both treatments, we hypothesized that brood interruption coupled with OA vaporization would be an effective *Varroa* control treatment.

Materials and Methods

Experimental Design

In September 2016, 70 honey bee colonies of European-derived honey bee stock were managed at the University of Florida Honey Bee Research and Extension Laboratory (Entomology and Nematology Department, Gainesville, FL, 29°37'38" N 82°21'23" W). All colonies were infested with *Varroa* and managed in 10-frame Langstroth hives consisting of a single deep hive body, a medium honey super and a solid bottom board. Colonies were equalized over several weeks prior to the start of the experiment to ensure that each colony was of similar size and strength (~10 frames of bees and seven frames of brood). Once the experiment was started, no brood combs were shared between colonies, even within treatments, to prevent the transfer of *Varroa*; otherwise, all colonies were managed according to standard management practices for the region. Each colony was randomly assigned to one of six treatment groups such that there were 10 colonies per group (Table 1). An additional 10 colonies served as positive controls and were treated with Apivar strips (active ingredient: amitraz) per label directions.

Oxalic Acid Vaporization

Oxalic acid was administered to treated colonies as a vapor per the label instructions (U.S. EPA 2015). The average high temperature during the time of treatment in Florida, United States was between 30 and 33°C. This process of vaporization involves heating a metal plate (vaporizer) using a 12-volt car battery, thus causing the OA dihydrate to vaporize inside the honey beehive. We used the commercially available Varrox-Vaporizer (OxaVap LLC, Manning, SC) apparatus to vaporize 1 g of OA dihydrate (Sigma Aldrich, St. Louis, MO) per brood chamber, per label instructions. The vaporizer was inserted into the hive entrance and the OA was vaporized as the plate heated for 2.5 min. During vaporization, the hive entrance and all cracks around the hive were sealed with tape to limit the escape of the OA vapor. After the 2.5-min vaporization period, the vaporizer was removed from the hive entrance, but the hive remained sealed for an additional 10 min to ensure sufficient exposure to the chemical. Applicators were properly equipped with personal protective equipment, including protective eyewear and respirators with cartridges to filter organic vapors, all per label instructions.

Table 1. Treatment groups in a 3 × 2 full factorial design

	Oxalic acid (one application on day 24)	Oxalic acid (three applications— days 8, 16, and 24)	No oxalic acid
Brood interruption (queen caged 24 d)	BI-OA-1	BI-OA-3	BI
No brood interruption (queen not caged)	OA-1	OA-3	Neg. Control

BI = Brood interruption, achieved by caging the queen for 24 d. OA = Oxalic acid, administered to treated colonies as a vapor per label instructions (1 g OA/brood chamber per application).

Application of Treatments

Brood interruption colonies (BI-OA-1, BI-OA-3, BI—see Table 1 for an explanation of the treatment codes) had their queens placed into a plastic, in-frame Var-Control cage (Api-Mo.Bru, Campodoro, Italy) within the brood nest for a period of 24 d as per Lodesani et al. (2014). The queens were released after the 24-d period. Colonies receiving one OA application (BI-OA-1, OA-1) were treated 24 d after the queen had been caged (i.e., upon her release) while those receiving three OA treatment applications (BI-OA-3, OA-3) were treated 8, 16, and 24 d after the queen was initially caged. Positive control colonies had Apivar strips added at the start of the experiment and removed after 35 d (Table 2). Some beekeepers prefer to treat with OA vaporization while brood is present in colonies once per week for 3 wk. Others prefer to treat only once while the colony is broodless (Gregorc et al. 2016). Thus, our staggered treatment schedule allowed us to compare both methods.

Varroa Level and Colony Strength Parameters

Colony *Varroa* levels were determined using mite fall by placing sticky boards (Mann Lake, Product # DC-681, Minnesota, United States) at the bottom of each hive for a 72-h period. *Varroa* levels were determined in all treatments prior to treatment application, at the time of OA applications, midway and at the end of the experiment (Table 2). Colony strength was evaluated using visual estimates of adult bees, brood, honey and pollen as described by Delaplane et al. (2013). Briefly, colonies were opened and a single observer visually estimated the percentage of comb surface covered by bees, brood cells, honey cells or pollen cells. The percentage of surface covered by bees was converted into an area (cm²) covered by bees using the surface area of deep (880 cm²) or medium (665 cm²) frames used in the United States. Then, the cm² bees was converted into the appropriate bee density (1.38 bees/cm²). Similarly, the percentages of brood, honey and pollen cells were also converted into an area estimate and converted to an estimate of number of cells using the average cell density of 3.7 worker cells/cm². Strength estimates were made prior to the start, midway and at the end of the experiment (Table 2). Colony mortality was noted at the end of the experiment (Table 2).

Statistical Analyses

The effect of treatment (amitraz and one, three or no OA applications) and/or queen status (caged or not caged) on *Varroa* levels and colony strength parameters were determined using generalized

linear mixed models methodology as implemented in SAS PROC GLIMMIX (SAS/STAT 14.1; SAS Institute, Cary, NC). We used a distribution function and default link function appropriate for the response variable in question, i.e., negative binomial for count data and binomial for proportions. Because of the treatment timing component, appropriate comparisons were made by linear contrasts among treatment × timing means.

Specific comparisons using the generalized linear mixed models methodology were made at each time period to determine the effect of treatment on mite fall. The comparisons made at each time period were as follows:

- 1) all treatments compared to one another;
- 2) amitraz versus other treatments;
- 3) amitraz versus other treatments, brood interruption treatments versus non-brood interruption treatments, BI-OA-1 versus OA-1, BI-OA-3 versus OA-3;
- 4) amitraz versus other treatments, brood interruption treatments versus non-brood interruption treatments, BI-OA-1 versus OA-1, BI-OA-3 versus OA-3, BI versus BI-OA-1, neg. control versus OA-1;
- 5) amitraz versus other treatments, brood interruption treatments versus non-brood interruption treatments, BI-OA-1 versus OA-1, BI-OA-3 versus OA-3, BI versus BI-OA-1 and BI-OA-3, neg. control versus OA-1 and OA-3, BI-OA-1 versus BI-OA-3, OA-1 versus OA-3;
- 6) amitraz versus other treatments, brood interruption treatments versus non-brood interruption treatments, OA-1 versus OA-3, BI-OA-1 versus BI-OA-3;
- 7) amitraz versus other treatments, brood interruption treatments versus non-brood interruption treatments, OA-1 versus OA-3, BI-OA-1 versus BI-OA-3;
- 8) amitraz versus other treatments, brood interruption treatments versus non-brood interruption treatments, OA-1 versus OA-3, BI-OA-1 versus BI-OA-3.

Using a generalized linear model, we could not detect significant differences in the survival of colonies at the end of the experiment between the different treatments ($P > 0.05$), likely due to the sample size. Therefore, odds ratios were used to interpret colony survival and compare the relative odds of the occurrence of colony survival or mortality given a specific treatment. An odds ratio measures the association between an exposure and an outcome by dividing the log odds of one treatment by each of the remaining treatments (Szumilas

Table 2. Summary of the timing of events for this experiment

Time period	Time relative to start of experiment	Description of events
1	day -4	Baseline data were collected for <i>Varroa</i> levels and colony strength for all colonies.
2	day 0	Apivar strips were added to the negative control colonies. Queens were caged in all colonies receiving brood interruption. <i>Varroa</i> levels were measured in all colonies.
3	day 8	Colonies receiving an OA application three times received their first application. <i>Varroa</i> levels were measured in all colonies.
4	day 16	Colonies receiving an OA application three times received their second application. <i>Varroa</i> levels were measured in all colonies.
5	day 24	All queens placed in cages for brood interruption were released. Colonies receiving an OA application three times received their final application and colonies receiving a one-time OA application were treated. <i>Varroa</i> levels were measured in all colonies.
6	day 31	<i>Varroa</i> levels and colony strength parameters were measured for all colonies.
7	day 35	ApiVar strips were removed from amitraz colonies and <i>Varroa</i> levels were measured in all colonies.
8	day 62	<i>Varroa</i> levels and colony strength parameters were measured for all colonies. Colony mortality was noted.

Bold letters indicate the start (day 0), mid-point (day 31), and end (day 62) of the experiment.

2010). Herein, we compared the neg. control treatment against all other treatments.

Results

Colony Survival

The colonies treated with amitraz via Apivar experienced no colony mortality by the end of the experiment (day 62). To calculate the log odds for the group, we labeled their survival as 99.9% rather than 100% (Table 3). Using the odds ratio for survival, a colony in the amitraz treatment is 42.4× more likely to survive than one in the negative control treatment group (Table 3). We observed the highest colony mortality when brood interruption was the only method implemented for *Varroa* control (BI, Table 3). Using the odds ratio for mortality, BI colonies are 21× less likely to survive than colonies in the negative control group (Table 3). In all cases, more colonies died when brood interruption was implemented.

Varroa Levels

The effects of the various treatments on the 72-h *Varroa* levels are summarized in Fig. 1. Prior to treatment (day -4), there were no significant differences in mite fall across any treatments ($P = 0.445$).

Effect of OA Application Rate

After the final application of OA on day 24, significantly higher *Varroa* fall was observed in the BI-OA-1 and BI-OA-3 treatment colonies than in the BI treatment colonies ($P < 0.001$), but not the OA-1 and OA-3 treatment colonies compared to the negative control colonies ($P = 0.262$). Mite fall in colonies receiving one application of OA was not significantly different than mite fall ($P > 0.05$) in colonies receiving three OA applications in either the presence or absence of brood.

Effect of Brood Interruption

All caged queens were still alive when released on day 24. In general, colonies with caged queens had statistically lower mite fall levels than colonies with uncaged queens starting from day 24 ($P = 0.032$) and continuing throughout the rest of the experiment (days 31, 35, and 62— $P < 0.001$). However, we did not observe any significant differences in mite fall during the OA treatment periods (days 8, 16, and 24) between colonies with caged and uncaged queens that were treated one (BI-OA-1 and OA-1) or three (BI-OA-3 and OA-3) times ($P > 0.05$).

Effect of Amitraz

Colonies treated with amitraz had significantly higher mite fall than did colonies with any other treatment immediately after application

(day 0— $P < 0.0001$). Furthermore, mite fall in amitraz-treated colonies was significantly different from that in colonies receiving any other treatment for the second half of the experiment (days 31, 35, and 62— $P < 0.001$). The average mite fall in amitraz-treated colonies was significantly higher than that in all other treatment colonies on days 31 and 35, but significantly lower than that of all other treatment colonies a month later at the end of the experiment (day 62).

Colony Strength

Bees

The numbers of bees present in colonies were not significantly different between treatments prior to the start of the experiment (6140.2 ± 179.6 ($N = 70$), mean \pm SE bees per colony, N number of colonies) (day -4— $P = 0.999$) but were by the end of the experiment (2483.8 ± 210.2 ($N = 40$)) (day 62— $P < 0.001$) (Fig. 2). Colonies with caged queens had significantly fewer adult bees both midway (2108.6 ± 258.2 ($N = 22$)) and at the end of experiment (1644.9 ± 344.7 ($N = 11$)) when compared to colonies where brood interruption was not applied ($P < 0.01$) midway (4308.2 ± 243.2 ($N = 38$)) and at the end of the experiment (2801.9 ± 224.6 ($N = 32$)). There were no significant differences in the number of bees between colonies whose queens were not caged ($P > 0.05$).

Brood

The numbers of brood cells present in colonies were the same across treatments prior to the start of the experiment (3587.0 ± 152.0 ($N = 70$), mean \pm SE cells/colony, N number of colonies) (day -4— $P = 0.999$) but differed significantly midway (2479.2 ± 211.6 ($N = 60$)) (day 31— $P = 0.0014$) and at the end of the experiment (1225.6 ± 86.2 ($N = 60$)) (day 62— $P = 0.0041$) (Fig. 2). Colonies, where brood interruption was applied, had no or very little brood midway through the experiment (645.84 ± 93.1 ($N = 22$)) and the amount of brood they did have was significantly less than that for colonies for which brood production was not interrupted (3214.4 ± 277.3 ($N = 38$)) (day 31— $P < 0.001$). However, by the end of the experiment, only the BI-alone treatment (850.1 ± 0 ($N = 11$)) had significantly fewer brood cells than did colonies where brood interruption was not applied (1329.5 ± 141.5 ($N = 32$)) (day 62— $P = 0.015$). All colonies where brood interruption was not applied had statistically similar amounts of brood throughout the experiment ($P > 0.05$).

Honey

The numbers of honey cells present in colonies were not significantly different across treatments prior to the start (5290.0 ± 270.6 ($N = 70$), mean \pm SE cells per colony, N number

Table 3. Colony survival for all treatments was compared to that of the negative control colonies

Treatment	Survival	Log odds	Odds ratio (survival)	Odds ratio (mortality)
Neg. control	0.7	2.3	-	-
OA-1	0.5	1.0	0.4	2.3
OA-3	0.7	2.3	1.0	1.0
BI	0.1	0.1	.05	21.0
BI-OA-1	0.4	0.7	0.3	3.5
BI-OA-3	0.6	1.5	0.6	1.6
Amitraz	0.999 ^a	999.0	42.4	0.02

^aTo calculate odds ratios, survival cannot equal 1. Thus, 100% survival is presented as 0.999. OA = oxalic acid applied via vaporization, BI = brood interruption achieved by caging the queen, Amitraz = amitraz applied via Apivar strips, 1 = one application, 3 = three application, neg. control = negative control.

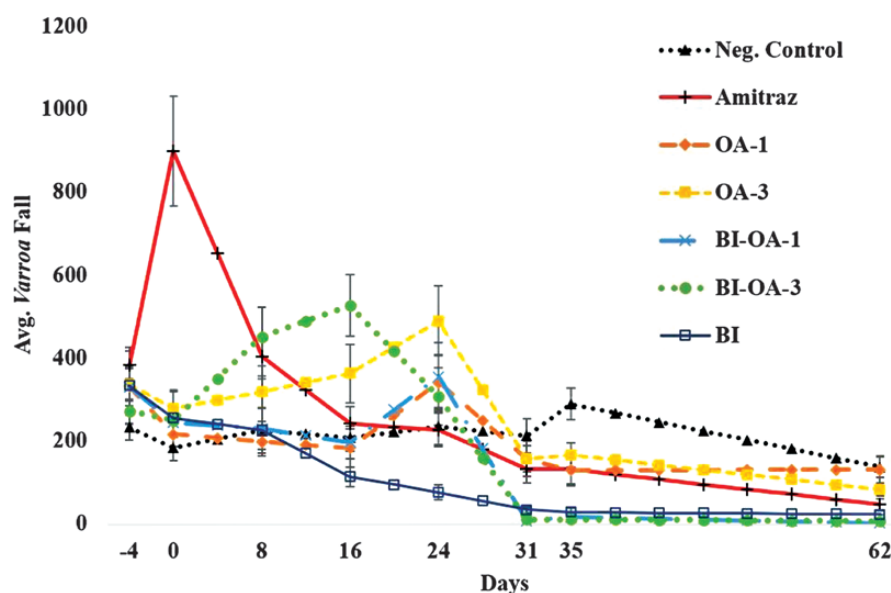


Fig. 1. Average mite levels across all treatments for the duration of the study. OA = oxalic acid applied via vaporization, BI = brood interruption achieved by caging the queen, Amitraz = amitraz applied via Apivar strips, 1 = one application, 3 = three applications. Error bars represent the standard error. Data points without error bars are not mite fall measurements, but continuation of lines. $N = 10$ for all treatment groups at day -4.

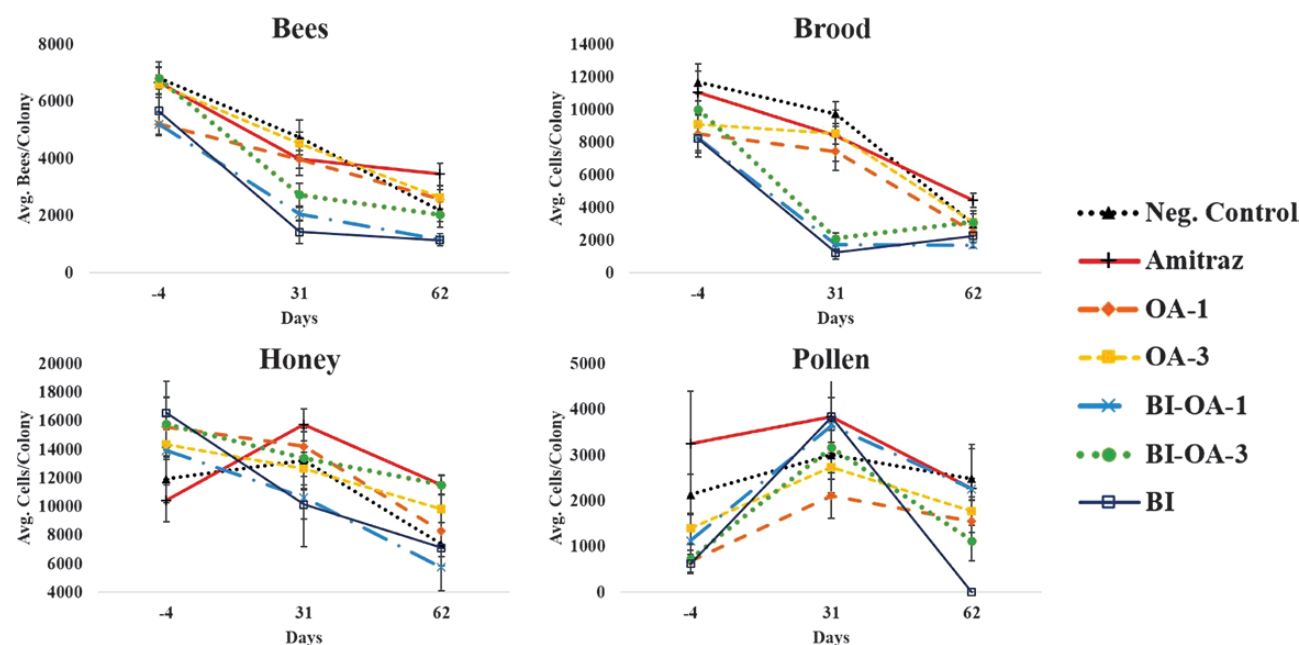


Fig. 2. The average number of bees and cells containing brood, honey or pollen at each colony evaluation period. Error bars represent the standard error. $N = 10$ for all treatment groups at day -4.

of colonies) (day -4— $P = 0.997$) or midway (4908.1 ± 273.3 ($N = 60$)) (day 31— $P = 0.945$) through of the experiment. However, by the end of the experiment, colonies in the BI-alone treatment had significantly fewer honey cells than did colonies from any other treatment group (2671.7 ± 0 ($N = 1$)) (day 62— $P = 0.013$) (Fig. 2).

Pollen

The number of pollen cells present in colonies was never significantly different for any treatment group at any evaluation period during the experiment ($P > 0.05$) (Fig. 2).

Discussion

To our knowledge, this is the first study in which the combined impact of brood interruption and OA vaporization on *Varroa* levels has been determined. We found that OA vaporization at the current label rate in the United States of 1 g per brood chamber is ineffective at controlling *Varroa*. Furthermore, our results indicate that caging the queen as a method of brood interruption in the early fall can be detrimental to colony health and survival. We observed high colony mortality in many treatments, despite diligent colony management to alleviate the side effects of the treatments. As colony populations began to decline, they were fed sugar syrup and had

entrance reducers placed on their hive entrances to reduce robbing. Furthermore, small hive beetle (*Aethina tumida*) traps were added to all colonies to reduce the effects of beetle damage. Colonies receiving amitraz were generally healthier and survived better than those treated with OA vaporization by itself or in combination with brood interruption.

The efficacy of OA for *Varroa* control has been well documented (Lodesani et al. 2014, Giacomelli et al. 2016, Gregorc et al. 2017, Maggi et al. 2017). Sublimation is regarded by some to be the best method of OA application as it is quick, effective, and causes little harm to the colony (Al Toufaily et al. 2015). Multiple applications of OA sublimation at 1 g were effective at controlling *Varroa* in Poland (Gregorc et al. 2016), but we observed that colonies receiving three applications of OA never had significantly lower *Varroa* levels than colonies only receiving one application of OA. Furthermore, colonies receiving three applications of OA still had high mortality rates and colony strengths similar to those of untreated colonies. Our inability to control *Varroa* effectively regardless of OA treatment suggests that the current labeled dose of 1 g per brood chamber was ineffective, at least under the conditions we maintained in our study.

All of the published research conducted on OA sublimation has been conducted in Europe and very few of those studies are written in peer-reviewed journals (Rademacher and Harz 2006). Rademacher and Harz (2006) reviewed many unpublished European studies and reported that 1 g OA applied via sublimation is sufficient for treating small colonies maintained in small hives, but 2 g is needed for larger colonies maintained in larger hives, such as the standard deep Langstroth hive. The lowest effective dose of OA sublimation in the United Kingdom was found to be 2.25 g (Al Toufaily et al. 2015), which is more than twice the label rate in the United States. Therefore, researchers should focus future efforts on determining the effective dose for OA vaporization and seek changes to legislation allowing beekeepers to apply the effective dose.

In this study, we vaporized OA dihydrate with a commercially available OA vaporizer. The device used to sublimate pure OA is an important item to consider in terms of effective *Varroa* control. Oxalic acid must reach a temperature of 157°C to sublimate. At a temperature of 189.5°C, OA decomposes to formic acid, carbon dioxide, carbon monoxide, and water (Budavari 1989, Rumble and Haynes 2017). Some vaporizers will get too hot too quickly and possibly decompose rather than sublimate the OA. Currently, there are no regulations in place in the United States or Europe to ensure that commercially available vaporizing devices reach the desired temperature without leading to OA decomposition.

Other researchers have observed increased efficacy in *Varroa* control when brood interruption was combined in conjunction with OA treatments (Wagnitz and Ellis 2010, Pietropaoli et al. 2012, Lodesani et al. 2014, Gregorc et al. 2017); however, we did not observe any benefit of brood interruption during our experiment. In fact, in all cases in this experiment, more colonies died when brood rearing was interrupted. To our knowledge, all other published experiments combining OA treatment and brood interruption applied the OA via the trickling method rather than vaporization. It is possible that the amount of OA dihydrate that we have vaporized may not be enough to kill *Varroa* effectively, even when forcing all the mites onto adult bees during broodless conditions. A higher dose of OA dihydrate would possibly increase mite fall during broodless conditions and should be further investigated.

We experienced high levels of colony mortality by the end of this experiment. Colonies in the brood interruption groups had

especially low survival rates, even though all of the queens in these treatments survived the 24-d caging period. It is likely that these colonies were not given enough time to recover from the long period of broodlessness before entering the fall season. A honey bee queen's egg production and laying are reduced in fall in preparation for winter (Fukuda and Sekiguchi 1966, Winston 1987). Florida has prolonged warm seasons and very mild winters; therefore, we hypothesized that brood interruption would be a safe treatment even during the early fall. However, based on the high level of colony mortality, we recommend that brood interruption only be attempted during the summer months or possibly not at all.

In general, colonies treated with Apivar had higher mite fall at the start of the experiment (Fig. 1) and were strong throughout, which likely resulted in the high survival rates observed for this group. Beekeeper dependence on amitraz has, unfortunately, led to isolated reports of resistance to the acaricide worldwide (Elzen et al. 2000, Rodriguez-Dehaibes et al. 2005, Maggi et al. 2010, Kamler et al. 2016); though we feel that more widespread resistance is inevitable if current *Varroa* treatment practices are not changed. Here, we only observed 30% mortality in the non-treated control colonies, which surprisingly was the same level as the colonies treated with three OA applications. Additionally, 40% of colonies treated with brood interruption and three applications of OA died by the end of the study. This demonstrates the lack of efficacy of OA at the labeled rate and brood interruption during the fall.

Beekeepers around the world are desperate for an effective IPM approach to control *Varroa*. Though the combination of brood interruption and the application of OA has demonstrated promise in Italy and the United States (Wagnitz and Ellis 2010; Nanetti et al. 2011, Pietropaoli et al. 2012, Lodesani et al. 2014, Giacomelli et al. 2016, Gregorc et al. 2017), our data suggest that neither is effective at controlling *Varroa* at current labeled rates (OA) or recommended uses (brood interruption). Nevertheless, research on both treatment options should continue. Due to the growing popularity of OA vaporization, future studies should concentrate on regional optimization of the method and identifying safe, efficacious application rates.

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