Negative behavioral effects from Oxalic Acid Vaporization on honey bee colony vitality, brood, and honey production in various sized hives containing VSH queens.

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Summary

Repeated application of Oxalic acid (OA) in the form of three treatments equally applied over 21-days to active, growing colonies of honey bees resulted in noticeable changes to hive vitality compared to hives receiving no treatment. Most significant was the reduction in egg laying and subsequent brood production. Nectar gathering and honey stores were also affected suggesting a negative impact on worker activity, as a notable decrease occurred in two of the three groups tested. Varroa mite loads were inordinately low at the beginning and after seven days following the end of the test period, and with few exceptions, at all inspection points during the test. It is unclear whether VSH queens and offspring tested had an influence on overall mite count or control.

Introduction

Varroa Destructor, the ubiquitous, opportunistic, parasitic mite and the bane of beekeepers everywhere, is considered the leading cause of colony morbidity and *the* major factor responsible for colony losses worldwide.[1] The use of Oxalic dehydrate, a natural organic acid is aimed at controlling mite populations thus reducing bee injury, compromised immune systems, and the diseases, viruses and bacterial infections Varroa mites vector in the honey bee. OA treatment is a common hive management practice. It is shown to be most effective in hives during broodless periods, and to a lesser extent in hives containing capped brood.[2] Although Oxalic acid has a low acute toxicity to honey bees and a high acute toxicity to mites,[3] negative long-term effects of the treatment on honeybees are to be expected with a notable decrease in worker activity, nursing behavior and longevity.[4]

Evidence has shown honey bees are quite tolerant to repeated moderate doses of Oxalic acid either through direct application in solution (OA) or by vaporization (OAV). With that in mind, a small, short-term study was designed to observe and determine the extent of specific secondary or sub-lethal effects from Oxalic acid on active, growing hives during the summer when there is an abundance of capped brood. Since the effectiveness of the treatment is limited to exposed mites and ineffective on those contained in capped larva cells, three successive applications of OAV were performed at seven-day intervals to ensure a majority of the brood cycle was exposed.

Materials and methods

Six subject Langstroth style hives were selected for the study each consisting of 2, 10-frame deep brood boxes. Two strong hives each had an approximate starting population of 30-40,000 bees; 2 moderately strong hives each had an approximate starting population of 15-25,000 bees; and 2 small hives each had an approximate starting population of 8-15,000 bees (Table 1). Subject populations consisted of *Apis mellifera carnica* with Varroa Sensitive Hygiene (VSH), overwintered queens. Each hive was identified by number permanently affixed to the landing board. The region where the test was performed was in the upper Midwest (USDA Plant Hardness Scale 5a).

Table 1

Subject hive identification. Hives treated with OAV in grey.

	Approximate Starting Bee Populations		
Hive #	**Strong**	**Moderate**	**Small**
7	30-40,000		
8	30-40,000		
9		15-25,000	
10			8-15,000
11			8-15,000
12		15-25,000	

Fig 1

Odd numbered hives were treated at seven-day intervals over a three-week period, for a total of three treatments spanning twenty-one days (Day 0, Day 7, and Day 14). 2 g 99.6% Oxalic acid dehydrate crystals were applied as is the recommended threshold for 2 story hives.[5] Application was by sublimation of crystals on a VARROX 12V, 15W pan vaporizer (Fig. 1), inserted through the center point of the hive entrance. Screened bottom boards were closed off with corrugated plastic mite/debris boards and entrances closed off using a wet dishcloth. Upper entrances were sealed closed with duct tape. Honey supers (if any) were removed prior to treatment and returned to their respective hives 24 hours post treatment.


Fig 2

The pan vaporizer was connected to a 12V truck battery with engine running to ensure consistent current (Fig 2). Current was connected for 2 minutes and 30 seconds to allow the pan to heat sufficiently and vaporize the crystals. The pan remained internal to the hive for 2 additional minutes with no current, to ensure sublimation of any residual crystals. The pan was then removed from the hive and quenched in a bucket of cool water and prepared for the next hive application. Entrances remained closed off on each treated hive for an additional 10 minutes before reopening all entrances. This process was repeated at seven-day intervals throughout the 21-day study. Even numbered hives served as the control receiving no Varroa mite treatment.

Mite counts were performed and recorded on all subject hives the morning of the first treatment via the 'sugar shake' method per operating instructions from the University of Minnesota[6], using their *Gizmo* device. Mite/debris boards were indelibly labeled with the corresponding hive number, sprayed with a light coating of vegetable oil, and inserted into their respective screened bottom boards of each subject hive prior to OAV treatments. Mite-drop counts were performed and recorded on all subject

Fig 3

hive mite boards, whether treated or not, 24 hours post treatment application, then cleaned, oiled, and reinserted. Mite-drop counts were performed and recorded on all subject hive mite boards again at 48 hours post treatment application (Fig 3). Mite boards were then cleaned and stored, and prepped as above the day of the next treatment.

On the morning of each OAV application, each frame in each hive was inspected (Side A, south side facing and Side B, north side facing) for the number of frames with brood; using a counting template made of clear Lexan® inscribed with 1 inch square grid lines. (1^2 inch = 30 cells) (Fig 4). Results were field recorded on individual hive worksheets and the data later transferred to a Microsoft Excel file. Side A and Side B of each frame containing nectar and capped honey from each subject hive was also counted, field recorded, and summed to the nearest 0.5 frame.

Mite load estimates on each hive were conducted after 28 days (sugar shake method). Excel files were analyzed to determine changes in mite counts, brood production and nectar/honey stores on control hives vs. OAV treated hives.

Fig 4

Results

Hives treated with OAV resulted, in general, in a dramatic decrease in the amount of brood present seven days after each treatment in two of the three groups; the strong hives and the small hives. This was most clearly evident in hives in the strong populations (#7 and #8). The two hives started with very similar colony sizes and brood populations – differing by only 1,500 brood cells. However, successive weekly OAV applications to hive #8 resulted in significant decreases in brood week-to-week, with a 34% decrease a week after the first application; an additional 32% decrease a week after the second application, and another 53% decrease over the previous week, a week after the third and final application, suggesting a cumulative negative effect on the queen's egg laying ability.

Mite loads in all hives, as evidenced by the initial sugar shake mite load count, were minimal at the onset with 0-1 mite/300 bees. The final mite load taken on day 21, 7 days from the final OAV treatment, were also well within manageable range 0-4 mites/300 bees. Mite counts taken at 24 hours and 48 hours after each treatment were widely variable and at times indiscernible from hives that did not receive OAV treatment, suggesting little or no residual effect. Hive #8, a Strong, OAV treated hive, contained the highest overall mite-drop count during treatment with 50 mites. This is a reasonable result given it was the most populous hive.

A decrease in nectar collection and subsequent honey production in the OAV treated hives compared to hives that went untreated was also observed in the strong hives and the small hives. The moderately-strong treated vs. non-treated hives each collected a comparable amount amount of nectar and produced about the same amount of honey.

STRONG HIVES

Comparing the two similar strong hives, the decrease in brood was a precipitous 81% over the course of the 21-day experiment. The control hive (#7), receiving no OAV treatment, gained a small but steady increase in brood week-to-week with a 16% increase in brood overall by the 21st day. (Fig. 5).

Initial mite-load counts performed by sugar shake method for both the control and treated hive were extremely low given their colony size and time of year. At each inspection point the treated hive contained more dead mites on the mite/debris board than the non-treated hive. (Table 2 & Fig. 6). For the treated hive, more mites were found after the 24-hour post-treatment inspections than after the 48-hour inspection/count, suggesting that the effects of OAV are immediate. The control hive mite drop counts were fewer in number than the treated hive in all cases, but their numbers appear random at each inspection point. The cumulative mite drop for all inspection points during OAV treatment was 50 mites on the OAV treated hive and 18 on the untreated hive, a difference of 177%, suggesting considerable effectiveness. The final mite counts on day 21, after sugar shakes were performed, were low and comparative as in the beginning, before OAV treatments were administered.

Fig. 5

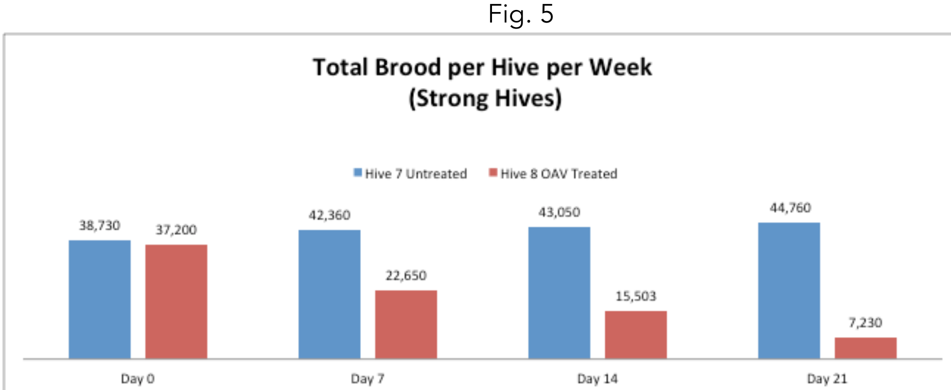
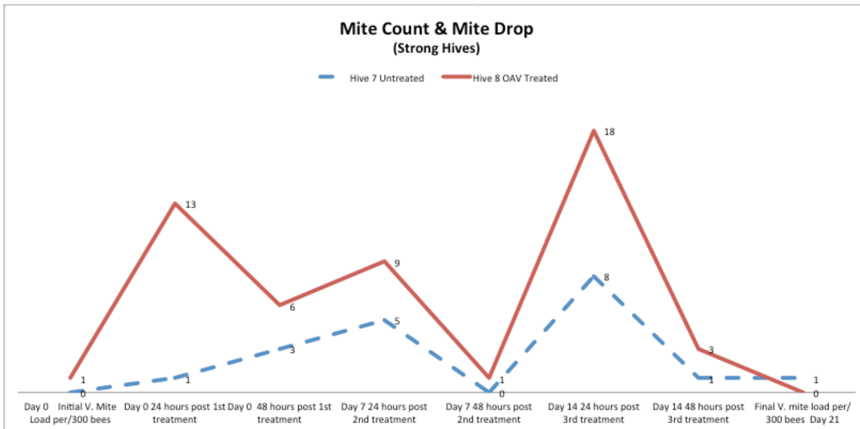


Table 2

Numerical comparison of Varroa mite loads and mite drops (Strong Hives)

	Day 0 Initial V. Mite Load per/300 bees	Day 0 24 hours post 1st treatment	Day 0 48 hours post 1st treatment	Day 7 24 hours post 2nd treatment	Day 7 48 hours post 2nd treatment	Day 14 24 hours post 3rd treatment	Day 14 48 hours post 3rd treatment	Final V. mite load per/300 bees Day 21
Hive 7 Untreated	0	1	3	5	0	8	1	1
Hive 8 OAV Treated	1	13	6	9	1	18	3	0

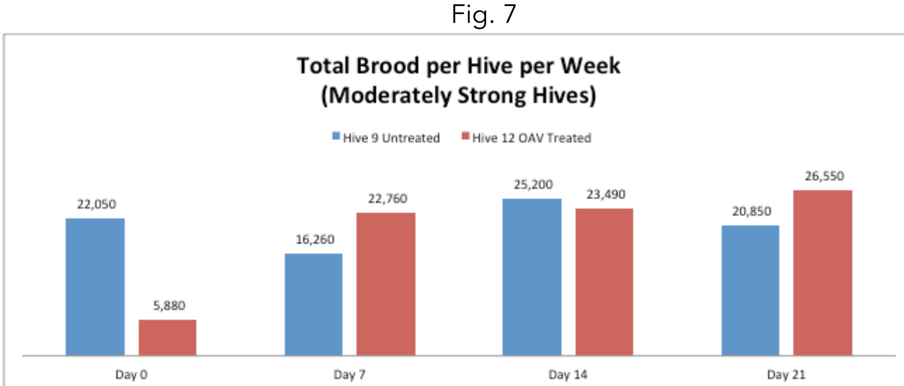
Fig. 6



MODDERATELY STRONG HIVES

Results for the two moderately strong hives were not as clear-cut. Hive #9 (untreated) swarmed at an undetermined time prior the start of the test. Brood production modulated with an overall decrease of 5% over the course of the 21-day test. Hive #12 (treated) was made from a Spring split whose queen only began laying the week before the start of the experiment. OAV treatments on this hive appeared to have seemingly no negative effect on brood production, which soared to an overall increase of 351% over the course of 21 days. However, the laying pattern became increasingly ununiformed week-to-week. (Fig. 7).

Fig. 7



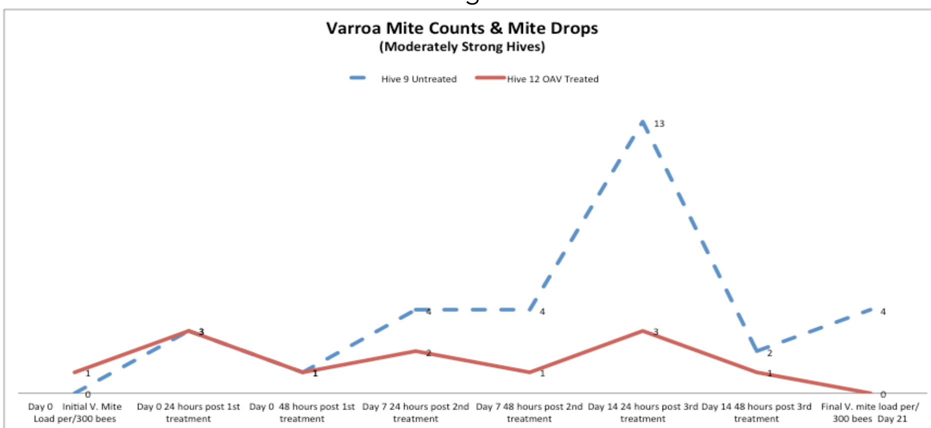
Like the strong hive group, the initial Varroa mite load counts performed by sugar shake method for both the control and treated hive were extremely low and relatively comparable. However, at each subsequent inspection point the *untreated* hive contained more dead mites on the mite/debris board than the treated hive. (Table 3 & Fig. 8). This may be attributed to the fact that hive #12 was a recent split and the brood cycle had been interrupted. The untreated hive (#9) demonstrated varying levels of brood. It should be noted this hive swarmed again on day 21 of the experiment and the drop in brood count from day 14 to day 21 may be attributed to the colony's swarm preparations. Final mite counts on day 21 revealed a considerable difference in mite loads with the untreated hive #9 at 4/300 bees and the treated hive with 0/300 bees. Given #12 had a brood-break, the 0 count is not surprising.

Table 3

Numerical comparison of Varroa mite loads and mite drops (Moderately Strong Hives)

	Day 0 Initial V. Mite Load per/300 bees	Day 0 24 hours post 1st treatment	Day 0 48 hours post 1st treatment	Day 7 24 hours post 2nd treatment	Day 7 48 hours post 2nd treatment	Day 14 24 hours post 3rd treatment	Day 14 48 hours post 3rd treatment	Final V. mite load per/300 bees Day 21
Hive 9 Untreated	0	3	1	4	4	13	2	4
Hive 12 OAV	1	3	1	2	1	3	1	0

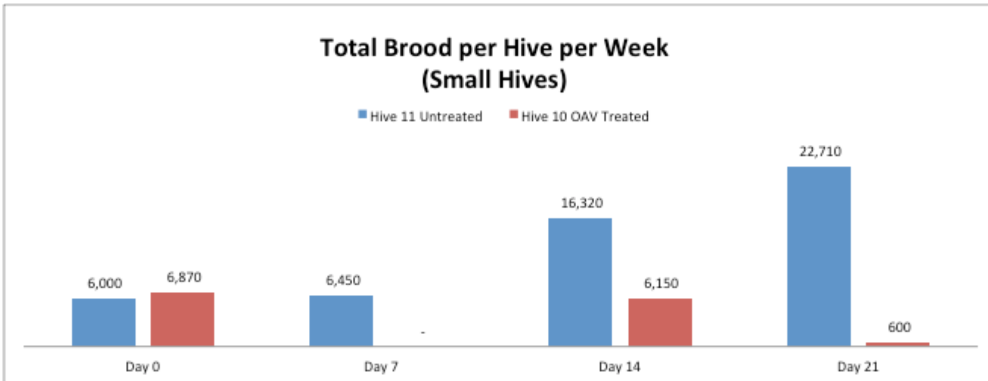
Fig. 8



SMALL HIVES

Results for the two small hives were muddled. Hive #11 was a captured swarm from my apiary 9 days prior to the start of the test. Hive #10 swarmed 2 days before the start of the test and had several sealed queen cells but no emerged queen at Day 0, only capped brood. While the control hive (#11) was slow to start, brood production eventually accelerated during the second and third weeks to an overall increase of 278% (Fig. 9). The OAV treated hive (#10) showed evidence of emerged queen cells but failed to produce a laying queen. It is undetermined if OAV treatment was a contributing cause. Even after the introduction of two frames of brood from a nucleus colony after Day 7, the hive failed to materialize a viable queen. At the conclusion of the test, this hive was merged with the hive #8 whose percentage of brood was the most decimated as a result of OAV treatments.

Fig. 9



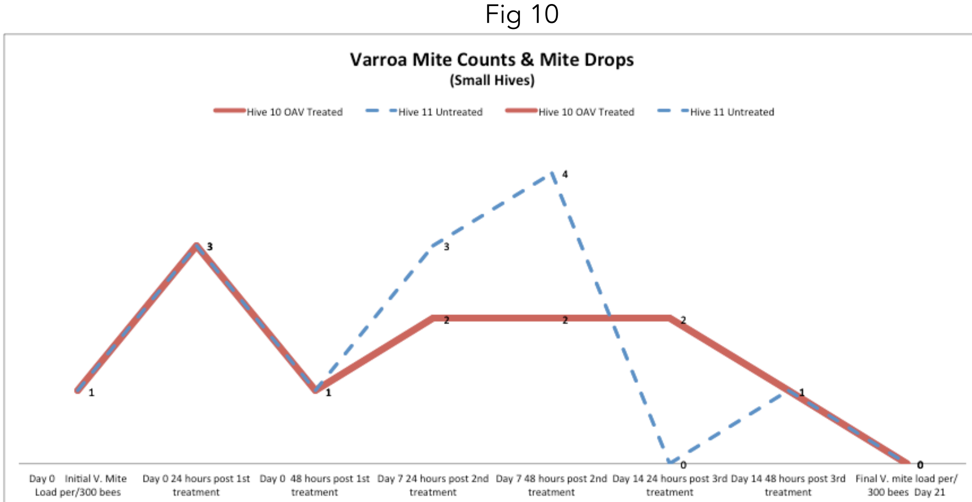
Varroa mite loads were identically low before the start of the test and at the conclusion of the test. Mite drop counts were considerably lower than the other 2 groups due to their small colony size and low brood cell counts at the onset, combined with the fact that the brood cycle in each hive had been recently interrupted. (Table 4 & Fig 10).

Table 4

Numerical comparison of Varroa mite loads and mite drops (Small Hives)

	Day 0 Initial V. Mite Load per/300 bees	Day 0 24 hours post 1st treatment	Day 0 48 hours post 1st treatment	Day 7 24 hours post 2nd treatment	Day 7 48 hours post 2nd treatment	Day 14 24 hours post 3rd treatment	Day 14 48 hours post 3rd treatment	Final V. mite load per/300 bees Day 21
Hive 11 Untreated	1	3	1	3	4	0	1	0
Hive 10 OAV	1	3	1	2	2	2	1	0

Fig 10

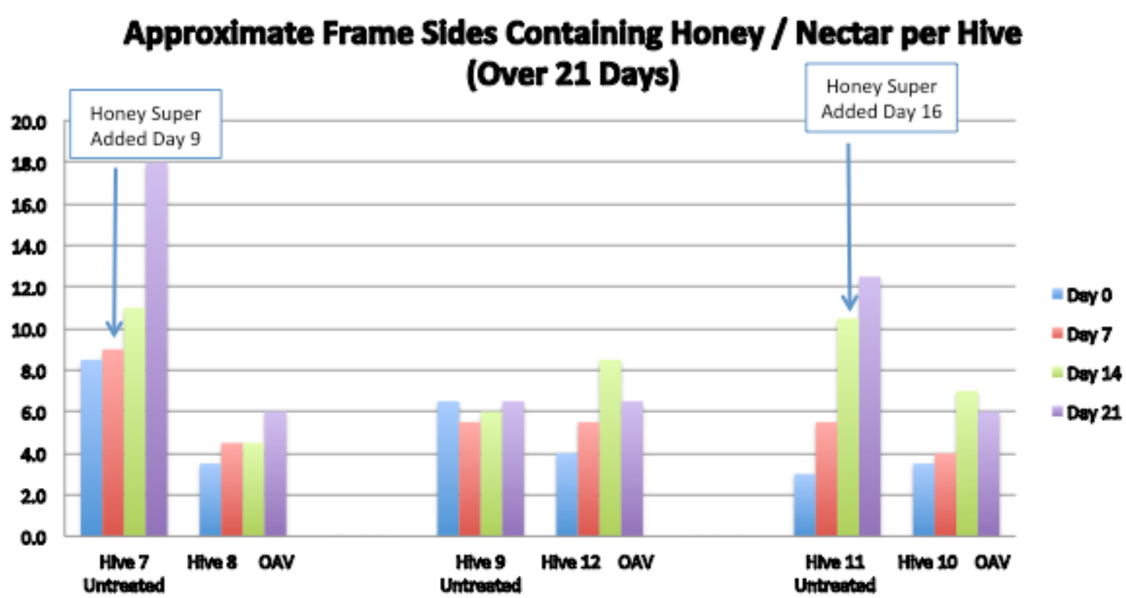


Nectar Collection / Honey Production

The strongest hives showed the greatest disparity in nectar collection and honey production in untreated vs. treated hives with the untreated hive (#7) far exceeding all other subject hives in the test in total volume. Beginning with 8.5 frame sides and ending with 18 frame sides with the addition of a honey super added on day 9, the overall increase in nectar / honey in hive #7 was 112% over 21 days. The OAV treated hive (#8) had considerably less nectar / honey by comparison at the start and failed to accelerate going from 3.5 frame sides to 6 frame sides over the course of 21 days, a 71% overall gain. Even though the colony population was comparable to hive #7, hive #8 generated 37% less food stores.

Hive #9 only managed to maintain a relatively steady level of nectar / honey. As noted earlier, this hive swarmed shortly before the test began taking with it a considerable population of foraging bees. In general, #12, a young colony and OAV treated hive, made gains in nectar collection and honey production commensurate with its colony population growth from 4 frame sides at the outset to 6.5 frame sides after 21 days, a 63% gain.

Hive #11 (untreated) began with only 2.5 frame sides of nectar / honey and quickly accelerated as the burgeoning house-bee population matured to foragers. A honey super was added on Day 16 to avoid becoming honey-bound but by Day 21, only the equivalent of 1 frame-side of nectar had been deposited in the super. The overall hive gain was 9.5 frame sides in 21 days, up 317%. This represents the greatest overall gain for all hives in the experiment. Hive #10, with little – and at times - no brood to care for, and with a gain in an aging-up population, increased its nectar / honey stores by 71%, most of which remained uncapped nectar.



Discussion

While the strong hives reflect consistency with previous published studies, overall reported results are only moderately consistent with expectations as some subject hives were unstable. A larger sample size, equalized hive strength, a retest or a parallel test site would be necessary to confirm trends and make more definitive conclusions. Beginning hive strength variability was not a consideration of the original protocol but was necessitated due to subject availability and time. The propensity of A. *milifera carnica* to swarm more frequently than A. *milifera ligustica* would seem to indicate that the latter be a better choice subspecies for this test.

Mite loads pre- and post-test were far less than expected. Mite counts post-treatment were far below what was expected. It is undetermined whether VSH queen genetics played any supporting role. Recent communications with my local bee club and State inspector provide strong evidence that a significant decrease in mite loads has occurred this season compared to previous years all throughout our area of the state. Consideration must also be given to technique, accuracy and reliability of the sugar shake method to determine mite loads. Consideration, too, must be given to the quality and potency of the Oxalic acid crystals, and to the timing of treatment. It is unknown whether a consistent seven-day interval is optimal. Higher mite count drops were expected. In hind site, a sugar shake test should have been performed prior to each OAV application to gain finer detail and determine weekly efficacy. This was an unintentional oversight.

The clearest results of the test were in the form of nectar collection inhibition and reduced honey production. There is an indication from this test that worker activity and nursing was negatively impacted. Egg laying became less patterned and more arbitrary in one treated hive, #12, raising the question as to whether OAV interfered with the queen's ability to maximize brood density patterns, or if she was failing as a result of other reasons. Additionally, several dead and dying bees were observed on the inner covers and screened bottom boards of the treated hives than the control hives although their numbers were not significantly different.

CONCLUSIONS

Due to the limited sample size, hive variability and time constraints, definitive conclusions of this study are limited. Notably, Oxalic acid vaporization is an effective tool in an overall strategy for the control of Varroa mites. It worked more effectively on hives with little or no capped brood than on hives with significant capped brood, consistent with previous published results. OA significantly affected brood production in two of the three groups tested. It also appeared to affect worker bee behavior to the extent that it resulted in decreased foraging and subsequent honey stores in two of three groups tested, and altered laying pattern in one of the three treated hives. The ramifications of these attributes resulted in a decrease in nectar collection and honey production. Accuracy and reliability of the sugar shake methodology for determining mite loads is suspect, at least as far as this experiment shows, as too much variability in mite drops, and mite counts. There is no clear indication that VSH queens attributed to any meaningful reduction in mite loads as this was beyond the scope of the test. Further study with more focused protocols and less variability should be examined.

[1] Martin, S.J., Highfield, A.C., Brettell, L., Villa-lobos, E.M., Budge, G.C., Powell, M., Nikaido, S., and Schroeder, D,C. (2012) Global honey bee viral landscape altered by a parasitic mite. Science. 336: 1304-1306.

[2] Gregoric, A., & Planinc, I., The Control of *Varroa destructor* Using Oxalic Acid, The Veterinary Journal, Volume 163, Issue 3, May 2002, Pages 306-310

[3] Aliano, N.P., Ellis, M.D.;Siegfried, D., 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*, Volume 99, Issue 5, 1 October 2006, Page 1579

[4] Schneider, S., Eisenhardt, D. & Rademacher, Sublethal effects of oxalic acid on *Apis mellifera* (Hymenoptera: Apidae): changes in behaviour and longevity, E. Apidologie (2012) 43: 218. https://doi.org/10.1007/s13592-011-0102-0

[5] Rademacher, E., & Harz, M., Oxalic acid for the control of varroosis in honey bee colonies - a review, Free University of Berlin, Dept. of Biology/Chemistry/Pharmacy, Neurobiology, Königin-Luise-Str. 28-30, 14195 Berlin, Germany. Apidologie 37 (2006), p.116.

[6] Reuter, G. S., Lee, K., Spivak, M., *Gizmo Operating Instructions, Method to accurately measure 300 bees and determine the infestation of varroa mites,* University of Minnesota Instructional Poster #171, Department of Entomology, University of Minnesota, www.extension.umn.edu/honeybees