2023 Field Trial of Matrices and Formulations for Extended-release Oxalic Acid

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The extended-release method for oxalic acid (OAE) is becoming widely used throughout the world, notably where mites have developed resistance to the overused synthetic miticides. This year we ran a field trial to compare the efficacy of various application methods, matrices, and ratios of OA to glycerin.

See my associated announcement in this issue about EPA allowing us to use generic oxalic acid for varroa control. While we were waiting for an answer, I continued my research on extended-release application (OAE).

A large field research project

I receive a lot of questions from beekeepers about using OAE in their hives. I'm often unable to give definitive answers, since this is still experimental research in progress, with updates in this journal, and at my website.[1] Five questions continue to pop up:

1 What is the cheapest and/or most efficacious matrix to use?
2 What is the best formulation (ratio) of oxalic acid to glycerin?
3 Is it better to use pads laid flat on the top bars, or strips hung between the frames?
4 Does it help to replace the pads or strips after a month?
5 When is the best time of season to apply the strips?

This summer we ran a large field trial to attempt to help answer the first four questions.

Materials and methods

Experimental design

Due to the inherent variability between colonies in any field trial, in order to tease out "the signal from the noise" one must use a large "n" of test hives for each test group, and replicate the trial in different yards. This is especially the case with varroa treatments, since there are often substantial hive-to-hive differences in the efficacy of exactly the same treatment.

So I decided to run a large-scale trial in five different apiaries (for replication), and to test each of five different matrices with three different ratios of oxalic acid to glycerin, degree of saturation of the matrix, and whether the treatment was applied only once or replaced at 30 days. My plan was to then use analysis of variance (ANOVA) to tease out the differences between each variable. For example, by testing each formulation ratio on five different matrices, I could look for consistencies for the efficacy of the ratio independent of the matrix (and vice versa). This design would hopefully allow me to get the most informative results from a single trial.

I've written previously about my testing of various absorbent application matrices,[2] looking for matrices that were biodegradable and non-contaminating, easy to prepare, apply and remove, efficacious in delivery, ***and inexpensive*** (perhaps the most important consideration for commercial beekeepers). My favorite to date were the Swedish sponges sold as "If You Care" brand,[3] which I used as a "known" positive control (since at the 1:1 ratio it was a treatment combination that had a history of working well for us).

In some previous trials, I had tested chipboard hung strips from New Zealand, where the recommendation has been to use a 1:1.5 OA:gly ratio, and to replace them after a month (since the hung strips tend to "dry out" and be chewed away by the bees; Figure 1). I was curious as to (1) whether a different ratio might be more efficacious, and (2) whether the amount of benefit from replacement was worth the cost of the labor involved.

Fig. 1 *Freshly-prepared strips to the left; to the right strips removed 65 days after application. These strips tended to "dry out" more quickly than did the other matrices (perhaps having something to do with gravity), but may have continued to dispense oxalic acid onto the bees during the process of chewing and removal. I thank the manufacturer of the strips (Beequip, New Zealand[4]) for generously donating the raw strips for this project.*

MATRICES AND FORMULATIONS TESTED						
	Hung strips	Pads laid flat				
	OXALIC ACID					CITRIC
	NZ hung strips	Swedish sponges	Corrugated cardboard	Kingzak cloth	Chipboard flat	Swedish sponges
w:w ratio of OA:glycerin:water	1:1	1:1	1:1	1:1	1:1	1:1
	1:2	1:2	1:2	1:2	1:2	1:2
	1:2:1	1:2:1	1:2:1	1:2:1	1:2:1	1:2:1

Table 1 *The variables were the two acids, the five matrices, and the three formulations, resulting in 18 different treatments to test.*

Based upon preliminary experimentation, I also tested using citric acid rather than oxalic. Citric acid provides roughly the same amount of total acidity gram-for-gram as does oxalic, but it is not as strong an acid. Citric has the huge advantage of being on EPA's Minimal Risk Pesticide list.

Table 1 shows the 18 different matrix, acid, and formulation ratios tested.

Surface area of the treatments

I've previously determined that the number of square inches of matrix surface area is critical for efficacy.[5] But it's really the amount of acid ***on that surface*** — as opposed to any hidden deep within the matrix — that allows for distribution by the bees and transfer to the mites.

Important note: All flat-laid treatments for this trial were cut into two pads having a total surface area equal to that of the Swedish sponge: **60 square inches**. Surprisingly, the recommended application rate of 6 hung New Zealand strips provides nearly twice as much exposed surface area: **112.5 square inches**. And that doesn't take into account that most of the surface area of the flat-laid pads is blocked by the top bars, whereas the bees are exposed to both sides of the hung strips. I know — this observation is completely contrary to "common sense," so go figure!

Again surprisingly, I found in previous testing that if that large amount of saturated chipboard is instead laid flat, that it can overdose the bees, and cause serious agitation, bearding, and brood kill.

Justification for the ratios tested

Our "positive control" formulation was the 1:1 (weight:weight) ratio that we've used for years. Maggi[6] used a 1:2.5 ratio, so I split the difference between it and the preferred New Zealand 1:1.5 formula, and tested a 1:2 ratio. In addition, Kanelis[7] suggested using a 1:2.7:1.7 (OA:gly:$H_2$0) ratio. This solvent-heavy ratio would have put very little oxalic acid into the matrices, so I included a 1:2:1 ratio, which results in a "drier" feeling matrix, similar to the first ratio that I used with shop towels.[8]

The various formulations resulted in a range of the amount of total oxalic acid applied per hive (Table 2), although with the more absorbent matrices, plenty of OA remained in the matrices at the end of the trial 65 days later.

The test colonies

An added difficulty for me is that (as I described in September[9]) some of the colonies that I had at my disposal turned out to be mite resistant (what a bummer), and thus would not be suitable for testing the efficacy of a treatment against varroa. So I could only use colonies which exhibited a buildup in their mite levels.

Experimental limitation: It's nearly impossible to equalize the mite infestation rates of a starting group of colonies, since over half the mites are typically in the brood, and the mite population and reproduction dynamics can vary greatly from hive to hive. I dealt with this issue by using a randomized block design to assign treatments (stratified assignment by their mite counts after 3 ½ months of mite buildup), thus not only minimizing the effect of this variable, but also allowing us to test the effect of treatment over a range of infestation rates.

I could only scrounge up enough colonies with high enough mite counts to allow me an "n" (number of replicates) of 12 hives per treatment (the minimum that I'll use), but since each matrix was applied to 36 hives, and each formulation ratio to 72 hives, I hoped that this repetition would allow me to tease out useful findings. So we applied treatments to 18 x 12 = 216 hives in total (Figure 2).

Fig. 2 *We numbered all the test hives in the five yards, sorted them by starting mite count, and randomly assigned treatments to each tier of 18 (a randomized block design). At each yard, we spread out the 18 tubs, and very carefully applied the assigned treatment to each hive. Here Brooke is acting as the supervisor "Hawk," tracking and recording confirmation that Rose took each treatment from the right tub and placed it on top of the correct hive for insertion.*

Since the three formulations for each matrix looked similar, we had to be very careful, and actually (due to a possible error in proper application) in two yards replaced all of two treatments the following day (I'm a stickler for getting everything exactly right). The colonies varied in strength, but all had bees in both the upper and lower boxes.

The ladies did a great job at distributing and applying the treatments, but they weren't quite up to lifting 216 heavy upper brood chambers (Figure 3).

Fig. 3 *It was dang hot as we set up the trial (Figure 4), and I supplied the muscle to crack open each hive for inspection and insertion of the treatment. The surfaces of our dark hive covers reached over 150˚F, and just resting them against my bare arm when I tipped them up would send a flash of heat through my body. I had to pause from time to time from lifting the heavy boxes to avoid heatstroke! Thank goodness that we keep gentle bees that don't require us to wear much protection.*

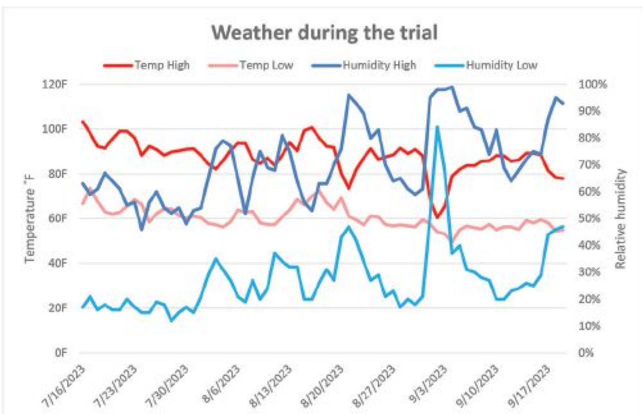

Fig.4 *The weather started out very hot and dry as we were performing mite washes and applying treatments, then cooled off a bit. Daytime humidity was low early on, but briefly got high in early September (there was no rain during the trial). Data from KCAGRASS50 (2523 ft elevation).*

The matrices

In order to introduce you to the matrices that we tested, I took photos of Rose applying them to some hives (Figures 5 through 10).

Following recommendations from New Zealand, I'd planned on replacing the hung strips in half the test hives with fresh ones at 30 days. However, when the time came to do so, I decided to also replace **all** the pads in the other treatment groups in the same yards as well (in order to equalize that variable for all test groups). That then doubled the number of treatment groups to compare to a count of 36!

Fig. 5 *Our "positive control" was a Swedish sponge (If You Care brand) cut in half. All flat-laid treatments were laid on the top bars of the lower brood chamber, to the front and the rear of the hive, centered on the cluster. This enabled us to feed pollen sub to encourage brood rearing during the dearth (to allow for the mites to continue to reproduce).*

Fig. 6 *I had dismissed corrugated cardboard during previous exploratory testing, since the adhesive of the pieces that I tried fell apart in the hot oxalic solution. But I recently noticed a shipping box made from double-layer, eco-friendly corrugated cardboard[10] and gave it a quick test, which suggested that its glue might work for us. I confirmed that the double-corrugation board could absorb a lot of oxalic solution, so I included it in the trial, since as a matrix it would be readily available to penny-pinching beekeepers.*

Fig. 7 *Unfortunately, we found that after a couple of days in the tub, the corrugated pads tended to delaminate, making them a bit difficult to apply and to later remove. But the price is right!*

Fig. 8 *I ordered chipboard sheets (similar to that used for the New Zealand hung strips) from Uline.[11] They took some time to absorb the solution, but were easy to install and remove. You may have noticed that we've shifted from using nitrile gloves to food-handling gloves, which not only provide adequate protection, but are much easier to get on and off sweaty hands. And because they are so inexpensive we don't hesitate to remove (and properly dispose of) them any time that we want our hands to be free of gloves (or acid).*

Fig. 9 *At the suggestion from a beekeeper whose name I've sadly misplaced, I tried King Zak biodegradable towels.[12] These towels, although thin, were surprisingly absorbent, and remarkably strong (they held together for removal after 65 days). They look promising for further experimentation.*

Dose applied to the bees

This brings us to the indirect dispersion (by transfer by the bees) of the active ingredient — oxalic acid — and its exposure to the mites (the intended target) (Figure 11).

A teaser: I've been using chemical titration to track the actual amount of acid residues on the bees' bodies resulting from various application methods of oxalic acid (and have presented my preliminary findings at conventions and on Zoom). I hope to soon publish my findings.

Practical application: Glycerin absorbs nearly half its weight in water at cluster humidity (which runs at about 50-60% independent of ambient humidity). My sons and I have learned to check for a pad's potential for acid transfer from its surface by touching them lightly with a fingertip, and then tasting that finger for acidity.[14] So long as the pad's surface remains moist and sour tasting, it can continue to disburse acid onto the bees.

Fig. 10 *For hung matrices, we applied Beequip's 1 ¼" x 15" chipboard strips at the rate of 3 strips per brood chamber.[13] As pointed out previously, this results in a far greater amount of surface area exposure to the bees than with the flat-laid pads. The hung strips, although a bit more tedious to apply, work well for treating nucs or single brood chamber hives.*

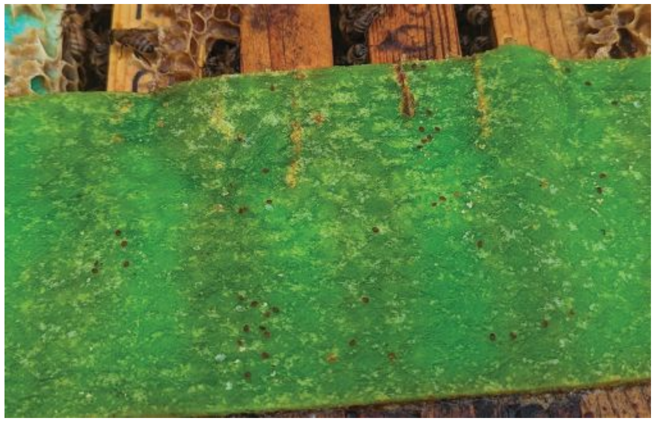

Fig. 11 *Although we're happy to see dead mites on the pads, in actuality it's unlikely that they died from direct contact — it's the amount of acid that gets distributed from the pads onto the bodies of the bees that apparently does the trick. The moist surface of this sponge is due to the absorption of atmospheric moisture within the cluster by the glycerin humectant.*

Results

So how much total acid would the applied treatments contain? I weighed them as we prepared them to see (Table 2).

So just how important is the total amount of acid in a pad or strip? This would be considered as the "dose per hive," but doesn't account for how much acid degradation occurs[15] or never gets dispersed before the pad is removed by the beekeeper or the bees (I've confirmed that oxalic acid degrades fairly rapidly when in contact with organic materials, but some matrices maintained substantial acidity (by the taste test) on their surface for over two months in my environment).

Although the total amount of OA contained in the matrix does not necessarily reflect the amount that makes it onto the bees, ***it did correlate with performance*** (Figure 12). The 1:1 sponges and NZ strips held the most acid (66 & 62 g respectively; both performed well), and the 1:2:1 King Zak cloth and chipboard the least (12 & 18 g respectively; both performed poorly).

APPROXIMATE GRAMS OF ACID APPLIED PER HIVE							
OXALIC ACID						CITRIC ACID	
Ratio	NZ strips	Sponge	Corrug cardboard	KingZak cloth	Chipboard	Ratio	Sponge
1:1	62	66	42	30	33	1:1:1	46
1:2	44	43	23	22	19	1:2	31
1:2:1	32	32	18	12	18	1:2:1	33

Table 2 *The amounts shown in red are the total applied dose per hive. Note that at higher solvent (glycerin and water) ratios, less total acid is contained in the matrix. And the addition of water results in there being less glycerin as a humectant (and a drier surface).*

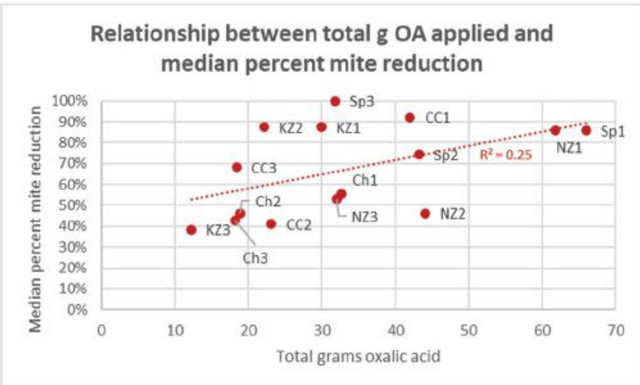

Fig. 12 *There appeared to be a weak correlation between the gross amount of oxalic acid in the pads or strips, and the resulting percent mite reduction. This could simply be because there was more acid on the surface upon application, or more of a reservoir of acid to diffuse to the surface over time. Note that the highest doses were no more efficacious than half the dose, notably with the sponges, King Zak cloth, and corrugated cardboard — the matrix appeared to be more important than the dose! Surprisingly, the sponges with the 1:2:1 ratio performed very well in all test hives but two.*

In my analyses, I used median values rather than means. Means can be greatly skewed by a single hive with a very high mite count. Medians more reflect what a beekeeper is interested in — the midpoint, with half the hives exhibiting lower, and half higher values. Statistically, one includes the standard deviation or a box plot. But with this large data set, I found that simply showing the raw data in blue and red tells us all we need to know[16] (Figure 13).

Summary of my interpretation of the chart

Important note: The chart does not indicate "efficacy" of treatment, but only reduction (in most cases) from the starting count. In any hive in which its red column is no taller than its blue column, its mite infestation rate did not increase (in most cases it decreased). I stacked the columns in this chart for better visibility — if you see any blue at all, that colony's mite count went down. Any column showing *mostly* blue would indicate high *efficacy*, but I could not calculate the value, since we didn't have negative controls.

Formulations: As far as formulation, the 1:1 ratio performed the best overall (the most blue), with a few other surprise showings. Its consistent performance across a variety of matrices is telling.

Matrices: Unfortunately, chipboard laid flat was unimpressive, even at the repeated 1:1 ratio. However, when hung in the New Zealand strips, it performed well. ***Swed-***

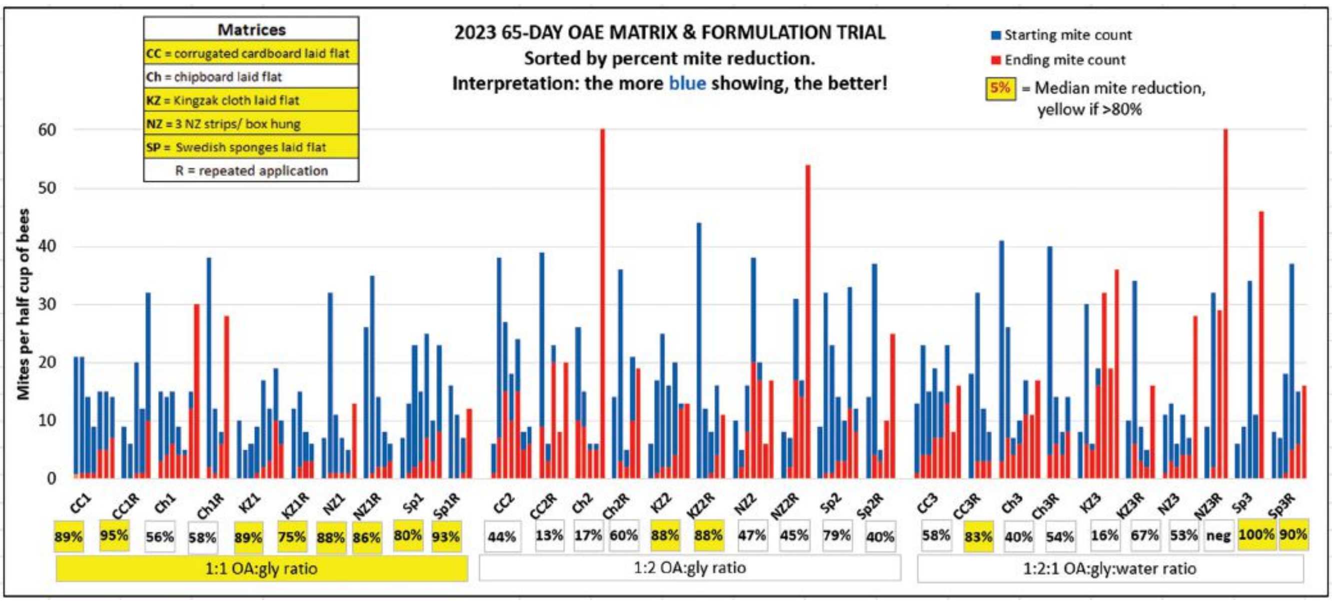

Fig. 13 *Results 65 days after application of the treatments. The more* **red** *per treatment group, the poorer the performance; the more* **blue** *the better. I highlighted the best performers in yellow.*

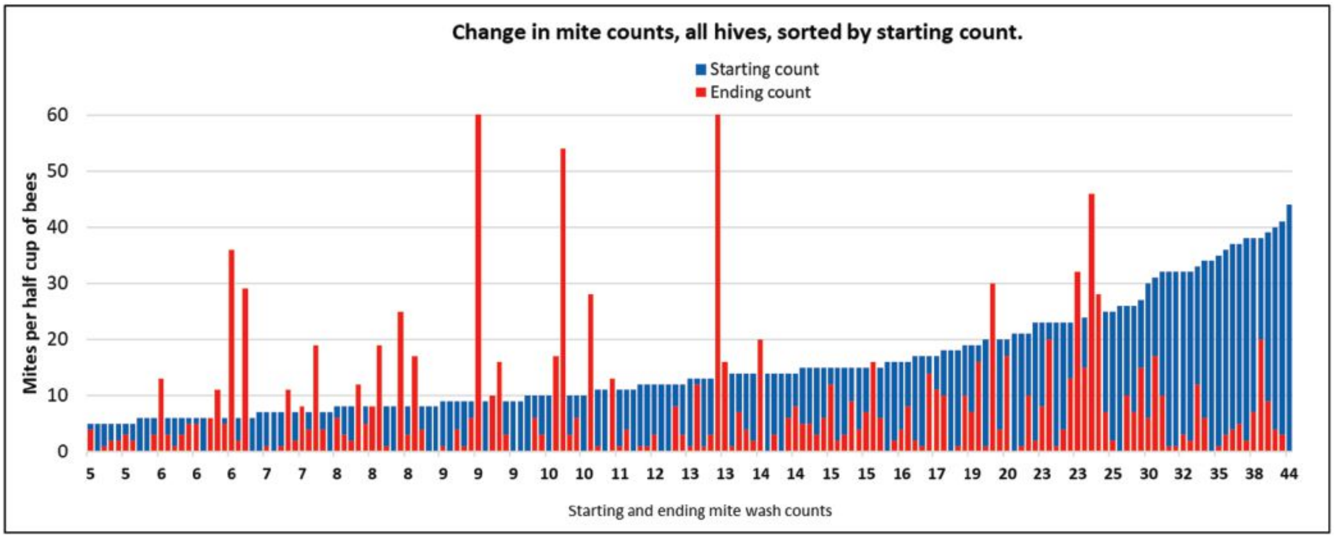

Fig. 14 *Note that there are proportionally more* **increases** *in mite counts in those hives starting with lower counts (to the left) than in those with higher starting counts (to the right). I'm not clear as to why this is, but it was again apparent in this trial.*

ish sponges were the most consistent performers overall, followed closely by the ***New Zealand strips***, ***double-corrugated cardboard*** (notably at the 1:1 ratio), and the surprise showing by the thin ***King Zak towels*** (other than with the 1:2:1 ratio). I did not test Maximizer pads,[17] since they've performed very similarly to Swedish sponges in previous trials.

Repeating the application: Surprisingly, replacing the treatments at 30 days didn't improve their performance for any test group other than the King Zak towels. I'm not clear on why the Kiwis think that it is of benefit with the hung strips.

The citric acid treatments: I didn't show the results of the citric acid treatments, since at our spot monitoring at the trial midpoint, many of their mite counts were exploding, so we removed them from the trial and treated them with oxalic. Bummer, since I had high hopes for citric.

Discussion

Reduction vs. efficacy: This was a ***comparative trial*** of the matrices and formulations, in which we could ***compare the percent mite reductions*** by the various treatments, but not determine ***efficacy***, which would have also required an untreated negative Control group.

For example, if in a negative Control group the mite count quadruples over the two months of a trial (a realistic increase), a colony that maintained its original starting infestation rate would exhibit a percent mite ***reduction of zero***, but an ***efficacy of*** **75%** (relative to the Control) by the Henderson-Hilton calculation.

Practical application: Based upon the major increases in the infestation rates of the citric treatments and the other poor performers, we can conclude that the *efficacies* of most of the 1:1 treatment groups were actually quite high.

Another thing to notice in the chart is that you tend to see proportionally more red in colonies that started with low mite counts, compared to those that started with high mite counts. This is something that I've noticed before with other miticide treatments[18] – that you get your greatest amount of "treatment failures" in colonies starting with lower counts. I've reworked the data in Figure 14 to show the pattern.

Practical application: Mite treatments in general tend to exhibit a greater percent reduction (and thus calculated efficacy) when applied to high-mite hives than to low-mite hives. I wish that I could explain why!

Musing on other studies

There are a number of really good studies on OAE, but I must take the results of a number of others with a grain of salt. Some do not understand that although the treatment causes elevated mite drop during the first week, that it may also cause an *increase* in the infestation rate for the first month, taking two full months to attain full efficacy (perhaps due to additional modes of action other than acute toxicity). And as shown above, it makes a difference whether the test colonies start with low or high mite infestation rates (so starting with low-count hives may result in more confounding outliers).

In addition, as evidenced by this trial:

- It's not only about the total dose applied.
- The ratio of oxalic acid to glycerin makes a big difference.
- As does the delivery matrix used.
- The degree of saturation of the matrix ("sloppy" matrices may work better than "dry" ones).
- The amount of surface area of the of the pads or strips is critical.
- As well as is their placement — bees apparently must make contact in order to distribute the acid to the mites.
- The surprising observation that hung strips require a greater amount of surface area than do pads laid flat across the top bars (at least when applied to double brood chamber hives).
- Whether the surface of the delivery matrix remains moist and acidic.
- The fact that we're not yet clear on the modes of action that oxalic treatment has upon the mites (it's not just acute toxicity — watch this space!).
- And add the effect of the starting infestation rate, as well as
- The large differences in hive-to-hive performance in the same yard.

Confusing? Yes. Does OAE work? Yes. Do we still have a lot to learn? Yes!

Final Notes

The best use of this treatment appears to be ***application at the beginning of the honey flow***. We need more research on its efficacy at other times of the season.

Repeated application of oxalic acid without rotation of miticides with other modes of action may well select for the evolution of resistant mites (don't bet against evolution). Thymol is a great follow-up after you've pulled your honey. Formic acid works well in the springtime. You can also rotate in Hopguard or a synthetic miticide. And of course our goal is to use resistant bee stock that may only require a single treatment a year!

A request: Please don't write me for details on application until you've read this instructions page (which I will try to keep updated):
https://scientificbeekeeping.com/instructions-for-extended-release-oxalic-acid/

Acknowledgements

Thanks to my helpers Rose Pasetes, Brooke Molina, and Corrine Jones.

Citations and notes

1 **https://scientificbeekeeping.com/instructions-for-extended-release-oxalic-acid/**
2 **https://scientificbeekeeping.com/extended-release-oxalic-acid-progress-report-2/** (First published in *American Bee Journal*, October 2017)
https://scientificbeekeeping.com/extended-release-oxalic-acid-progress-report-3/ (First published in *American Bee Journal*, January 2018) **https://scientificbeekeeping.com/extended-release-oxalic-acid-progress-report-4/** (First published in *American Bee Journal*, November 2018
https://scientificbeekeeping.com/extended-release-oxalic-acid-progress-report-2019/ (First published in *American Bee Journal*, December 2019)
https://scientificbeekeeping.com/2022-extended-release-oxalic-update-part-3/ (First published in *American Bee Journal*, May 2022)
https://scientificbeekeeping.com/testing-cotton-matrices-for-oae/ (First published in *American Bee Journal*, January 2023)
3 Available on Amazon, or much cheaper if purchased in bulk lots of 500 or 1000 (contact Oliver Weiss, **oliver@ossipee.biz**).
4 **https://beequip.nz/**
5 **https://scientificbeekeeping.com/7701-2/** (First published in *American Bee Journal*, March 2022)
6 **Maggi, M, *et al.* (2016).** A new formulation of oxalic acid for *Varroa destructor* control applied in *Apis mellifera* colonies in the presence of brood. *Apidologie 47*: 596-605.
7 **Kanelis, D, *et al.* (2023).** Evaluation of oxalic acid with glycerin efficacy against *Varroa destructor* (Varroidae): a four year assay. *Journal of Apicultural Research*, DOI: 10.1080/00218839.2023.2169368
8 **https://scientificbeekeeping.com/extended-release-oxalic-acid-progress-report/** (First published in *American Bee Journal*, July 2017)
9 Selective Breeding Progress Report 2023
10 Pratt Eco Options, in a box from Home Depot. I've now tried a few brands, and none really hold together long.
11 **https://www.uline.com/Product/Detail/S-18997/Corrugated-Pads/8-1-2-x-11-Chipboard-Pads-050-thick**
12 From Amazon: "Eco Friendly Reusable Cleaning Cloths, Reusable Paper Towel Cloth, 30 Sheets, All Purpose Clothe, Biodegradable." Thank you to the beekeeper who suggested these, and whose name I've misplaced.
13 For the occasional weaker colony, we applied strips proportionally, in order to maintain consistent exposure.
14 I of course am not recommending that you do this. But keep in mind that a single serving of spinach may contain a full gram of oxalic acid.
15 These figures are the acid content immediately after preparation. I've not yet performed titrations to determine how rapidly the acid degrades after preparation (it's on my to-do list!).
16 I know — without a calculated p-value, trusting our eyes and brain to pick out a pattern would never pass peer review. But I often trust my eyes more than I trust convoluted statistics.
17 2022 Extended-Release Oxalic Update Part 1 **https://scientificbeekeeping.com/7701-2/** (First published in *American Bee Journal*, March 2022)
18 **https://scientificbeekeeping.com/mite-control-while-honey-is-on-the-hive-part-2/** (First published in American Bee Journal, December 2020)

Randy sees beekeeping through the eyes of a biologist. He's kept bees for over 50 years, and with his sons runs around 1500 hives in the California foothills. He closely follows bee research, engages in some himself, and enjoys sharing what he's learned with others.