Assessing Repeated Oxalic Acid Vaporization in Honey Bee (Apis mellifera) Colonies for Control of the Ectoparasitic Mite Varroa destructor

Jennifer A. Berry, Lewis J. Bartlett, Selina Bruckner, Christian Baker, S. Kris Braman, Keith S. Delaplane, Geoffrey R. William

Note: Jennifer Berry presented “Wrap Up of Multiple Research Projects Testing the Efficacy of Oxalic Acid for Controlling Varroa destructor in Honey Bee Colonies” during OSBA's 2021 Fall Conference. She has provided the following article, only portions of which are reprinted here, to share with OSBA membership. The portions not reprinted, including methods, are nonetheless vital and reflect a crucial part of science process, which by nature is collaborative and requires the ability to replicate experiments. The entire article can be found in Journal of Insect Science, Volume 22, Issue 1, available at: doi.org/10.1093/jisesa/ieab089.

Abstract

The American beekeeping industry continually experiences colony mortality with annual losses as high as 43%. A leading cause of this is the exotic, ectoparasitic mite, Varroa destructor. Integrated Pest Management (IPM) options are used to keep mite populations from reaching lethal levels, however due to resistance and/or the lack of suitable treatment options, novel controls for reducing mites are warranted. Oxalic acid for controlling V. destructor has become a popular treatment regimen among commercial and backyard beekeepers. Applying vaporized oxalic acid inside a honey bee hive is a legal application method in the USA, and results in the death of exposed mites. However, if mites are in the reproductive stage and therefore under the protective wax capping, oxalic acid is ineffective. One popular method of applying oxalic is vaporizing multiple times over several weeks to try and circumvent the problem of mites hiding in brood cells. By comparing against control colonies, we tested oxalic acid vaporization in colonies treated with seven applications separated by five days (35 days total). We tested in apiaries in Georgia and Alabama during 2019 and 2020, totaling 99 colonies. We found that adult bees, and developing brood experienced no adverse impacts from the oxalic vaporization regime. However, we did not find evidence that frequent periodic application of oxalic during brood-rearing periods is capable of bringing V. destructor populations below treatment thresholds.

Introduction

The western honey bee, (Apis mellifera L.), is a ubiquitously used insect pollinator of many agricultural crops around the world (Allsopp et al. 2008, vanEngelsdorp and Meizner, 2010) and the economic services provided by these managed bees has become increasingly important as world population expands (Degrandi-Hoffman et al. 2019). However, in recent years, populations of A. mellifera have seen a gradual, yet steady decline (Kulhanek et al. 2017, Potts et al. 2010, Spivak et al. 2011, vanEngelsdorp et al. 2017) with the American beekeeping industry experiencing annual losses of 43.7% (Bruckner et al. 2020). There are a number of drivers involved in colony loss, with the ectoparasitic mite Varroa destructor among the most important (Guzmán-Novoa et al. 2010, Le Conte et al. 2010, Rosenkranz et al. 2010).

To date there are three synthetic acaricides (amitraz, coumaphos and fluvalinate) approved for use against V. destructor in the USA (US EPA, 2016), but due to sub-lethal effects on
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Southwestern Oregon
Eric McEwen—541.415.5171; beetruehoneybees@gmail.com

North Willamette Valley
Jeremy Mitchell—503.580.1464; info@flyingbeeranch.net

South Willamette Valley
Tim Wydronek—541.740.4127; timwydronek@comcast.net

AFFILIATED REGIONAL ASSOCIATIONS

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Website: wvbahive.org

OSBA OFFICERS

President
John Jacob—541.582.2337; oldsolbees@gmail.com

Vice President
Joe Maresh—503.703.5060; joemaresh@bctonline.com

Secretary
Barbara Derkacht—503.631.3063; bderkacht@yahoo.com

Treasurer
Becca Fain—541.997.3792; rfain18@gmail.com

Past President
Harry Vanderpool—503.399.3675; shallotman@yahoo.com
The Kingbee of the Humptulips
Natt Noyes Dodgeme

Note: A while back, Dirk Olsen sent copies of three issues of the American Bee Journal from Oliver Petty's collection. This article, reprinted with kind permission from ABJ, is from one of them—December 1929, pages 590–593. Dirk marked it with a post-it: “Even though this is about Washington to our north, it's close enough to show what impressive honey crops were had . . .” Indeed! Yet, it also speaks: to the pace of change—the majestic forests that graced this region such a short while ago as well as all that has since occurred in our culture and technologies; to opportunity—our ongoing efforts to parse out safe, adequate, essential nutrition for bees (and people) everywhere; and to creativity—working from possibilities opened up when we choose to maintain perspective.

Forced slowly backward by the plow of the “stump farmer” and by the gradual retreat into the rugged foothills of the logging operations which, by its very nature, it must follow, the fireweed, famous honey plant of the Pacific Northwest, is gradually giving place to the more common nectar producers, the clovers, berries, and other plants of cultivation. Among the most heavily fortified of its strongholds, and one in which its legion of flame-tinted blossoms is destined to wave in glorious profusion long after the alders and bracken have crowded it from less favored regions, is the Olympic Peninsula of western Washington. Here, where are the headwaters of the Wynoochee, the Wiskah, and the Humptulips, the Queets, the Skokomish, and the Hoh are still protected by forests of virgin cedar and fir, where the deer, elk, and bear roam unmolested, and the shrill toot of the “whistle punk” and the reverberating crash of a falling monarch of the forest have never echoed through the stillness of the woods, lies the future fireweed pasture of the North Pacific Coast.

Even now engineers are surveying a route from Aloha to the Hoh River for a railroad which will “open up” this lumber land (see accompanying map), and within three years fresh timber cutting activities will be in progress, and, with the burning of slashings and debris piled in the wake of axe and saw, foundations for new fireweed pastures will be laid.

Approximately half way between the thriving city of Aberdeen, famous lumber center of the southwestern Washington, and beautiful, wild Lake Quinault, tourist Mecca of the Peninsula, is the sturdy cabin home of Fred Brittain, veteran bee man of Grays Harbor County. Here for 10 years his bees have roared through the misty Washington summers, colonies of immense strength because of the long, warm springs with their steady nectar flows from salal, the maples, cascara, and the wild berries. Logging operations with their following burns have provided an immense pasture of the luxuriant fireweed, and the reputation of the clear, mild honey from the Brittain apiary has spread the length and breadth of the county.

Fred Brittain is a large man: tall stature, big of heart, and broad of mind. As is the nature of many big men, he is modest to the point of shyness. His crops, sometimes averaging more than two hundred pounds to the colony, are due entirely to the fine location; any man with the same pasture might have done as well; the “breaks” have all been in his favor. Thus he explains his success as a honey producer. But there is a story behind these simple statements.

Fred Brittain knows bees. His self-deprecating remarks lead the listener to think him a novice with a wonderful run of good luck, for it is his nature to let the other man do the boasting as he keeps his own counsel. However, once he becomes engaged in an argument in which the finer points of beekeeping are under discussion, the listener will begin to realize the depths of observation and keen thinking of which this man is capable. Ideas to him are theories until proved. Once established to his satisfaction as facts, he puts them into immediate practice. So has it been with the bees, for it is the bees that gather the nectar, and in the cool, moist climate of the Peninsula, breathed upon by the misty winds from the nearby Pacific, there are many days of spring and summer when the bees hardy strains of bees prefer to remain within the shelter of the hives. “Front porch sitters,” Mr. Brittain calls them, and at once proceeds to weed
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them ruthlessly from his yards. For years he experimented with various strains, purchasing queens from breeders the country over, and, although he has achieved success, as his enormous crops testify, he is still on the lookout for a better bee. Because of their superior hardiness and the refusal to allow cool, cloudy weather to interfere with their out-of-door labors, Mr. Brittain prefers the darker strains of Italians, popularly known as the leather colored. Not all of these are both prolific and industrious, so he is constantly weeding—selecting and discarding in an effort to maintain the highest possible standard of production among his entire four hundred colonies, three hundred of which are kept together in one yard, the home location.

Fred Brittain’s ability as a beekeeper is not, strange as it may seem, the characteristic which makes him a leader among the Peninsula beekeepers, although his counsel and advice in matters of honey production are sought by bee men from miles around. That is but a mark of confidence and esteem with which he is held. He does not, as many, look up on other beekeepers as competitors, but regards them as colleagues interested in the same method of making a living, striving for the same goal, hoping for the same reward. Because of his attitude, in which he is most sincere, it has been possible for him to accomplish much in which other men have failed.

For some years after Fred and his brother, Charlie, first established bees in the valley of the Humptulips River, fortune smiled upon their efforts. Hundreds of acres of cut-over land carpeted the hills with fireweed, and the citizens of the towns and villages of the county furnished a ready market for the honey. But gradually more and more beekeepers began to locate in the vicinity, drawn there by the stories of the wonderful fireweed pasture available. Fred did not resent their coming, but welcomed them to this land of plenty. As the population of the region increased and the potential buying power of the centers grew, honey from other sections found its way into the grocery stores of the towns. Prices slumped until even the immense crops of the Brittain apiary returned little profit. Instead of blaming other beekeepers, or wasting energy and fighting the importation of honey, Fred Brittain began to think. He knew the possibilities of the Peninsula as a honey-producing region, for with hundreds of square miles of timberland awaiting the logger’s axe, and the thousands of burns yet to be made, it would be many years before the vast fields of fireweed faded from the hills. The problem, then, lay in finding an outlet for the ever increasing crops of honey which future production would bring.

In western Washington and northern Oregon are five of the major cities of the Northwest: Portland, Olympia, Aberdeen–Hoquiam, Tacoma, and Seattle. Here was a vast market for the minty-flavored, white fireweed honey of the Peninsula, but one man with his relatively small crop could not expect to enter this market in competition with the carloads of honey brought in from the Yakima Valley and the vast alfalfa and sweet clover sections of the Rocky Mountain states. It was in 1926 that Fred Brittain conceived the idea of forming a cooperative marketing association among the beekeepers of Grays Harbor County. With the assistance of his brother, Charles, whose experience in the lumber industry had developed a keen business judgment, and because of the trust and high regard with which he was held among the beekeepers of the Peninsula, Fred Brittain succeeded in organizing the beekeepers and establishing the Western Washington Beekeepers’ Association, with headquarters and bottling plant at Aberdeen. Charles Brittain was appointed as its manager. Many other names, such as William Cox, John Walker, Fred Mandary, Helen Stein, A. R. Ovenell, Julian Joubert, Charles Schader, H. N. Paul, and W. H. Higgins, many of them outside of Grays Harbor County, have become well known in the organization, which soon outgrew its quarters in Aberdeen and in August 1927 established a bottling plant in the heart of Seattle’s wholesale district. As the association gathered strength and other leaders came forward to carry on the work which he had started, Fred Brittain was content to go back to his bee yards, where he enjoys wrestling with the problems of production, now that a channel “to the outside” has been opened for his crops and those of other beekeepers of the Peninsula.

In the spring of 1928, the Western Washington Beekeepers’ Association affiliated with the Mountain States Honey Producers’ Association. The Seattle bottling plant became known as the Pacific Slope Honey Company, sales subsidiary of the two associations. As manager of this company and as president of the Mountain States Honey Producers’ Association, Charles Brittain has carried the family name high in the world of commercial honey marketing.

Each fall several truckloads of honey, the cases neatly marked with the number “2,” leave the valley of the Humptulips for the Seattle bottling plant and warehouse. Fred Brittain’s honey is going to market. In the bee yard a tall, lank man clad in overalls leans against a hive and contemplates the results of the past season and prospects for the new as he looks over the hives ready for the evergreen winter of the Olympic Peninsula. Perhaps a neighbor beekeeper is visiting him, and he is telling in his slow, easy drawl of the passing of the fireweed and how,
within a year or two, he will have to move his location closer to the firing line of the logging camps, where more recent burns have paved the way for the luxuriant growth of the ruddy queen of nectar-producing flowers. Few would think of this unimposing man as the Kingbee of the Humptulips, and perhaps his retiring disposition does not give him a clear title to this imaginary position—he might be better termed the power behind the throne.

Northwest Apiculture Fund for Honey Bee Research, Extension, and Education

In addition to our current campaign to raise funds for the OSU Honey Bee Lab (www.gofundme.com/f/100year-anniversary-help-us-save-the-bees-event) is OSBA’s Northwest Apiculture Fund for Honey Bee Research, Extension, and Education.

Making your check out only as described ensures that your donation is correctly applied to the appropriate Endowment and not to any other program.

For questions regarding details of the fund or how to donate, please contact Jan Lohman, Chair of the Grants and Foundations Committee, at 541.567.3209; 541.980.0304.

To Donate to the Fund

- Make your check out to: The OSU Foundation
- On the memo line, take care to write: The Northwest Apiculture Fund for Honey Bee Research, Extension, and Education
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- Mail to: Oregon State University Foundation at 4238 SW Research Way, Corvallis OR 97333
It is still spring in May, and Oregon spring weather is known for changing quickly. The old saying, “If you do not like the weather, wait a minute” applies. Honey bees are ready for good weather; they want to forage and gather lots of nectar and pollen for their colony. Colonies grow quickly when foraging is good. This can lead to swarming, something that beekeepers like to control so that they do not lose production and/or colonies.

This time of year, colonies can also be more susceptible to robbing. Larger colonies will use up stores more quickly and can find it easier to take from smaller colonies that may have few defenders to protect food supplies.

If several bad weather days occur in a row, colonies may run out of food. As bees die and fall to the bottom board, the entrance can become blocked. When this happens, the bees may be confined to the hive even when the weather improves. If the beekeeper does not intervene soon enough, the colony will starve. After I lost one of my largest spring colonies to this problem, I now make sure my bees have an upper entrance. I also check and clean entrances often.

Entrances can become busy in spring as colonies grow. A busy entrance may also be caused by invaders from other colonies, robber bees. Look closely at the behavior of the bees at the entrance. Are bees coming and going normally, or is there fighting at the entrance? You can also see dead bees at the entrance or in front of the hive when the activity is created by robber bees. If you suspect robbing, you can reduce the entrance of the colony being invaded. However, the best solution may be to move the colony under attack. Remember, a new location must be at least 3 miles away.

Contact with other beekeepers can be very helpful when new locations are needed for whatever reason. A good place to meet other beekeepers can be beekeeping classes, bee lectures, and association meetings. Bee problems are not restricted to new beekeepers. Having contacts and talking with others is good for all of us. So, join associations and go to meetings (both local and state); also read your newsletters.

Where you locate a colony is a good question to solve before your bees arrive. Bees like a sunny location, floral and water sources nearby.

If you will have the bees where neighbors are close, it is best to discuss with your neighbor before the bees arrive. Respect the fear that they may have of bees. Also remember that bee dropping can be a problem for neighbors close by.

A jar or two of honey can be helpful in many situations.

Note: Reprinted from the May 2021 issue of The Bee Line.

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**Apiary Registration with ODA**

Every person who owns, or is in charge of, five or more colonies of bees located within the state or Oregon, must register each year with the Oregon Department of Agriculture. The form needed to register colonies is located at: apps.oregon.gov/SOS/LicenseDirectory/LicenseDetail/606 or obtained in person by visiting: 635 Capitol Street NE, Salem OR 97301.

The current cost of apiary registration is $10 with an additional charge of $0.50 per colony for five or more hives. After July 1, the registration fee will increase to $20. The fee per hive remains at $0.50 per colony for five or more hives. The number of colonies that must be registered is equal to the highest number of full-strength colonies managed within the state at any point during the previous year, prior to the registration deadline of June 1.

All money collected from apiary registration shall be spent on research at the OSU Honey Bee Lab predominantly focused on honey bees (honeybeelab.oregonstate.edu).

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**BEEKEEPER EVENTS**

May 20: World Bee Day. *Information:* internationaleventday.com/event/world-bee-day/


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The North Willamette Valley experienced some wild weather conditions in the middle of April. The freezing temperatures brought snow to much of the area breaking 80-year old records according to the National Weather Service–Portland. These cold temperatures made installing nucs challenging for new beekeepers. Decisions had to made between keeping nucs cramped in their cardboard nuc boxes, or braving the cold weather to get them transferred into their new hives. The weather is always an interesting part of keeping bees in Oregon.

We saw a lot of swarming activity in April, and there is more to come this month. The prime season for catching swarms in the Willamette Valley is April–June. If you’re interested in catching a swarm of bees, make sure you are subscribed to the Swarm Call List for the OSBA and your local association. You can also try luring in a swarm of bees by placing swarm traps outside this time of year. Swarm traps can be made from unused brood boxes or old nuc boxes with some empty frames in them. The idea is to offer scout bees the ideal home site by providing the right amount of space. The bees like it when you furnish the inside for them. When selecting furniture for the bees, I like to use 1 or 2 frames of drawn comb. I prefer older and darker comb for this purpose. I think the bees can smell it better. I then fill the rest of whatever box I am using with frames without any drawn comb. I like to fill the swarm traps with new foundations in my frames in order to take advantage of having the swarms draw new honeycomb. They are the best at drawing new comb. Try to find a spot that is easily accessible and off the ground to set your trap out. In addition to the old smelly comb, I have good luck using swarm lures like Swarm Commander, available in local beekeeping supply shops, which is applied inside the trap.

Catching swarms is always fun, but what about your bees? Most beekeepers don’t want their bees swarming away, so here are some tips to prevent swarming. Start by adding more space to your hive. If you still have one brood box, you can add a second. If you haven’t already added a honey super, add one now. Colonies that overwintered strong may be ready for a second honey super in May. Try adding slatted racks to the bottom of your hives. Slatted racks help your colony utilize all of the available space for brood laying on your frames. You might also try “checkerboarding” your frames. Checkerboarding refers to alternating frames in your brood box with drawn, but empty frames of comb between brood frames. This gives the queen more space to lay and keeps the bees busy in the nursery. Giving your bees more space to work keeps them from feeling crowded and reduces the tendency to swarm.  

Jeremy Mitchell

Central Oregon has been continuing with a confusing spring. Our poor bees were awakened first back in February, followed by a significant cold spell, then again with more unseasonable warm weather in March, followed by another couple weeks of cold spell in April. Those colonies that were light on food or adults had some pretty good brood loss. Now it’s no longer getting as cold, and the local fruit trees are mido-bloom, so all is, hopefully, right with the world . . . although there are still good chances for freezing, low irrigation water levels, and a forecast for an active fire season.

We had a successful beginner bee school back in February with a planned field day in April. Because of the aforementioned cold snap, it has been rescheduled into early May. We’re now also having association meetings in a hybrid fashion, both for those who want to meet face to face as well as those who aren’t yet comfortable with it.

Our meetings are on the 3rd Tuesday of most months at 6 pm when in person at the Environmental Center in Bend, 16 Kansas Avenue. Please feel invited to attend.

Douglas County Bees

We started our bee season early this year. The first cut-out was completed on March 30. A double extraction was completed on April 2. Our weather here in the Roseburg area has been tricky to say the least, and we have to watch it carefully so that we can adjust accordingly for what the bees might be doing. The very next scheduled cut-out ended up being a rain-out! We will stop by our booth and say “hello!”

Beau Miakinkoff

Spring is coming on strong again this year in the southern Willamette Valley. April provided lots of pollen and nectar sources for bees along with mild temperatures. Favorable conditions should continue into May. Our colonies are getting a good kick start for the year, judging by member’s reports and the number of early swarm calls to the association.

The best news for our group is a resumption of in-person meetings starting this month (May). Returning to in-person meetings should boost participation and interactions with new beekeepers. We are hoping to offer a virtual meeting option for members still timid about large group gatherings.

Another part of our new normal will be delivering a presentation on “Pollinators in My Garden” at the Eugene Public Library on June 23 during National Pollinator Week. We expect a receptive crowd with lots of questions during this event. Keeping with a garden theme, the association will be auctioning off a supply of native seed mix packets from Territorial Seed Company during our May meeting. Proceeds
The Bee Line

will be given to the OSU bee lab to help fund research. Finally on April 21-22, GloryBee in Eugene will be handing out ordered bees, where the association typically staffs a booth to answer questions. This year the association chose to simply make a contact list of members available for anyone with questions. Happy spring!  

Brian McGinley

Oregon Central Coast Beekeepers

We had a well-attended, in-person meeting last month. There was good discussion on how members’ bees are doing. Unfortunately I was not able to attend as I’m recovering from knee surgery. Vice President Jim Parish took the reins and led the meeting.

We have an event planned next month with the Florence Garden Club. We will have a table with displays at their Plant Sale. Our volunteers will answer questions about bees and pollination.

As far as how our bees are doing, I can only report on my own. Still have three hives that survived thus far. I started feeding sugar syrup and removed the moisture boxes. One hive I did a quick check on the day it was 70 degrees and found a beautiful tight batch of capped brood. Hoping for some warmer weather soon.  

Pat Wackford

Portland Metro Beekeepers

In the Portland Metro Area, it seems like spring has come and gone, and come again, and sort of left, then crept back in some places.

The short bursts of great weather were enough to help with a spectacularly successful queen grafting experiment for PMBA, with better-than-expected numbers of developing queens to offer to association members in need of queens for overwintered colonies. Our annual nuc sale went smoothly thanks in large part to the numerous volunteers who helped hand out nucs and direct traffic. The association was also very excited about our spectacularly successful queen grafting experiment for PMBA, the blackberry nectar flow, coming late May into June. We are raffling off the Cape Meares nuc delivery to the 23rd.

But spring will come, and we will be excited to install 150 nucs into our apiaries.

Bee Informed Partnership on best practice management for small beekeepers. Out of 72 practices identified for successful overwintering, they identified the top 4 which made the most difference. Two were what you’d expect: Treat for Varroa and start new colonies from nucs or splits (as opposed to packages). But the two others were unexpected: How you treated your deadout and comb culling. Overwintering was more successful when people immediately re-used their boxes after a deadout versus storing them for the next season. Researchers aren’t sure why this is the case, and it needs more study. This isn’t practical when your deadout occurs in January, but the advice was that if you’re able to reuse it immediately—try it out. The last item was about treating comb, and she found freezing frames as a hygienic practice was as effective as not reusing them (which had the highest rate of overwintering success).

It’s hard to think about overwintering when our season is just starting, but we know everything we do for these next few months matters and her research bears that out.

Our monthly association meetings will remain online, and no membership is required so join us some time!  

Jessica Anderson

Tillamook Beekeepers

Last month, I wrote about wishing spring’s arrival. This morning when I awoke, it was 37 degrees and there was white stuff all over my yard. We were planning on having 150 nucs delivered this Saturday, but the weather looks like low 40s and possible snow again on Friday night. That said, we have pushed back our nuc delivery to the 23rd.

But spring will come, and we will be excited to install 150 nucs into our apiaries.

We are also looking forward to the annual Tillamook Home and Garden Show to be held at the Tillamook County Fairgrounds on April 30 and May 1. We are raffling off the Cape Meares 10-frame double-deep, two supers, Langstroth hive complete with frames and foundations, stand, bottom board, inner cover, and telescopic lid. The hive was designed and painted by two of our new beekeepers, Chris and Patsy Weber. As always, we will have an observation hive for the public to see honey bees up close and in action. There will be lots of educational opportunities with the public as well. This is traditionally one of our best public outreach events each year.

The best news for us right now is that we applied for and received a $3,000 Grant from the Tillamook Peoples Utility District. This money will go towards our 2nd annual tree
planting program. Last year, we planted $2,500 worth of bee friendly flowering trees in the cities of Tillamook and Manzanita. This year, we are expanding the program to include Tillamook, Garibaldi, Bay City, Nehalem, Manzanita, and the Port of Tillamook Bay. We hope to plant $6,000 or more in new trees in these areas. One good flowering tree is worth an acre of flowers.

Brad York

Tualatin Valley Beekeepers
Tualatin Valley Beekeepers Association has had a super busy spring thus far with all-new teachers and curriculum for our three-session March bee school, plus two well attended, in-person, hands-on field day sessions. We are super grateful for the expert teachers in our group and for Dr. Dewey Caron who supports us in so many ways. We organized another annual successful nuc and mite treatment buy delivered on April 16. Wild cold wet spring weather in early April as we submit this text is a bit worrisome for the virgin queens’ success after early swarms action. Fingers crossed, that’s life in western Oregon! Our board members are working to implement hybrid technology (in-person plus online via Zoom) for member meetings. Our presenters for our last Tuesday member meetings are John Rockroher in May and Jim Tew in June.

Debby Garman

Willamette Valley Beekeepers
Willamette Valley Beekeepers Association (WVBA) has had its challenge this winter in trying to find a new meeting site. Chemeketa Community College isn’t working out anymore. We met at a church-owned community center; however, the meeting rooms are just not big enough. Last month we met at a different church. We think this one will work out great. The meeting room is the “old sanctuary.” It has lots of room and we can have refreshments there. Terry Holm continues to be our refreshment coordinator. She does a fantastic job. There is also plenty of parking. The church is offering the room for free. What a blessing. We plan to donate some funds to the church. We had over 50 members at the last meeting. Several new members are joining. Our membership numbers are looking much better than I thought they would be at this time.

The American Bee Journal selected one of Elaine Timm’s photos for the cover of the April issue. Elaine is a WVBA member. The photo is a swarm in a holly shrub. Congrats to Elaine.

This year we have several members who are selling nucs to the members. We have had those fine days where the bees have been out to work the blooming maple. Other major plants in bloom are the wild mustard, plums, prunes, and Scotch broom is just beginning to bloom. Although WVBA didn’t have a bee school this year, we hope to have one next year. We are thinking of having a bee day in May.

May your bees elude the viruses and flourish this year.

Richard Farrier

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Oxalic Acid — Continued from page 1

honey bees along with rapidly evolving resistance in *V. destructor* (Berry et al. 2013, Elzen et al. 2000, Mathieu and Faucon, 2000, Rodriguez-Dehaibes, 2005, Rinkevich, 2020, Sammataro et al. 2005, Thompson et al. 2002), there is a need for additional efficacious active ingredients. Because of this, beekeepers have employed integrated pest management (IPM) techniques instead of relying on a single method of control. Beekeepers embracing IPM use a variety of approaches to try and keep colonies from succumbing to the detrimental effects caused by *V. destructor* (Delaplane et al. 2005). However, adequate control of this pest remains a serious challenge for many US beekeepers and chemical acaricides are still necessary as part of IPM frameworks in this system.

Non-synthetic compounds such as formic acid and thymol are effective at controlling *V. destructor*; however, their effectiveness is dependent on ambient conditions. For example, they are not effective when temperatures are too low and may kill adult and developing bees when temperatures are too high (US EPA 2019, US EPA, 2020). Another widely adopted natural compound is crystalline oxalic acid (OA) dihydrate. This organic acid naturally occurs in nectar, has putatively low likelihood of inducing *V. destructor* resistance on account of no resistance yet being observed in treated populations compared to naïve ones despite years of continuous use (Maggi et al. 2017), and has high efficacy against *V. destructor* (Adjlane et al. 2016, Bogdanov et al. 2002, Rademacher and Harz, 2006, Al Toufailia et al. 2015) in certain circumstances. Widely used for decades in Europe, (Popov et al. 1989), OA has only recently been popularized in the US and wasn’t registered for legal use until 2015 (US EPA 2015). One method for applying OA is to heat the crystals using a vaporizer, creating gaseous OA that permeates the colony (Rademacher and Harz 2006, US EPA 2015). Even though these treatments are highly effective at killing *V. destructor* on contact, OA does not penetrate the wax-capped brood cells where the majority of *V. destructor* reside (Rademacher and Hartz, 2006, Rosenkranz et al. 2010). Therefore, the best time to apply OA and reduce *V. destructor* populations is when colonies are broodless, without developing larvae (Charrière and Imdorf, 2002, Gregorc and Planinc, 2001, Gregorc et al., 2016, Gregorc et al., 2017), rendering all mites phoretic on adult bees and vulnerable to the fumigant (Rademacher and Harz, 2006). However, brood-free intervals are brief or absent altogether in some warm latitudes, raising the need for alternative treatment schedules.

For treating during periods of brood rearing, instructions for one commercial vaporizer, the ProVap 110, calls for four treatments with five days between each treatment. The rationale for this 19-day interval being that this schedule exposes an entire cohort of mites bound in worker brood as the mites successively emerge with their parasitized hosts. This multiple treatment regimen has gained popularity in commercial and hobby beekeeping operations. However, the protocol has not been shown effective. The objective of this study was to test the efficacy of a regimen of repeated OA applications against *V. destructor* during periods of brood rearing. A secondary objective was to determine if these repeated OA applications are measurably detrimental to adult bees and brood (proxies for colony viability). We hypothesized that a repeated OA treatment regimen would have a negative effect on *V. destructor* abundance while having no negative effect on *A. mellifera* colony strength, in agreement with prior demonstrations of its relative safety (Rademacher and Harz 2006).

**Oxalic Acid Application**

OA application was administered to colonies by crystal vaporization according to label instructions of the registered product (US EPA 2015) and the user manual for the ProVap 110 Vaporizer (OxaVap, Manning, SC). Prior to vaporization and to ensure that vaporized gas would not leak from the hives, colony entrances were sealed with blue shop towels or duct tape, and screened bottom boards were sealed using corrugated plastic boards. Powered by a Champion 2000-watt gasoline generator, the vaporizer device, a Pro VAP 110, was inverted and the chamber bowl heated to 230°C. One gram of solid OA dihydrate crystals per deep brood box was placed into the separated Teflon lid and inserted into the chamber. Turning the device right side up caused the OA crystals to fall into the heated vaporizer chamber thereby generating gaseous OA. The nozzle of the device was inserted into the entrance of each UGA colony or into a pre-drilled hole in the bottom brood box of each Auburn colony, where it remained for 30 seconds to ensure that the full dose was vaporized and delivered into the colony. Once completed, the device was removed and shop towels and plastic corrugated boards left in place for an additional 10 minutes per hive. For the safety of all persons applying the OA, full face respirators with OV/P100 cartridges were worn.

**Results**

For testing the efficacy of repeated Ig oxalic acid sublimation in treating for Varroa destructor, we analyzed data from all sites across both years using a linear mixed model with ΔPMI as the response variable, treatment as a fixed effect, year as a standalone random effect and site and apiary as nested random effects to reflect the spatial structuring of the field trials. We found a significant difference in ΔPMI values between control and treated colonies (F1,88.99 = 9.16, p = 0.003); across the study (35 days) a typical control colony showed an increase in PMI of 4.4 (±2.6 SE), whereas a typical OA treated colony showed a very small decrease in PMI of -0.7 (±2.5SE). As shown in Figure 1, treated colonies remained at the same PMI after treatment as before (no significant change in PMI, see prior quoted effect size estimates with standard errors spanning zero).

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As there was no meaningful difference in mite loads between pre- and post- treatment treated colonies (Figure 1 – UGA 2020), there is no confounding by which V. destructor control indirectly improved colony health by masking or compensating for toxic effects of oxalic acid sublimation. For testing the effects of oxalic acid vaporization on overall colony health, we therefore present a detailed analysis of the UGA 2020 data (see Figure 2) as we did not gather detailed colony health data for the UGA 2019 trial data set, and the Auburn 2020 data set was more confounded by a difference in mite control between the two treatments (which will impact colony health, masking possible negative effects of the oxalic acid which compromises assessing safety) compared to the larger UGA 2020 data. Furthermore, this data set was the most replicated experiment, across the three apiaries, and represented the majority of the data.. We used a mixed modelling framework as above where response variables were either change in brood area, change in bee population, or change in honey stores, fixed effect was treatment, and random effect was apiary (yard). We found no significant differences in changes in brood (F1, 52.06 = 0.39, p = 0.534), bees (F1, 51.23 = 0.20, p = 0.653), or honey stores (F1, 51.20 = 2.30, p = 0.136) based on treatment.

**Discussion**

On average, after 35 days colony V. destructor numbers were significantly higher in non-treated controls compared to OA-treated colonies. However, this effect is wholly explained by a small V. destructor increase in controls while V. destructor levels remained unchanged in OA-treated colonies (Figure 1). OA did not reduce V. destructor numbers; at best, it held them static. This effect is similar to those found by Jack et al. (2020, 2021) in which one and three applications of 1g of vaporized OA / super were also ineffective at significantly reducing V. destructor infestation levels while brood was present. Additionally, we observed that multiple treatments vaporizing with OA had no significant effects on overall A. mellifera adult bees, brood, or stored honey quantity.

Until now, there has only been anecdotal evidence that the recommended vaporizing with OA four times, 5 days apart, results in controlling V. destructor. Other studies that have examined the effect of repeated applications of the labeled rate of OA, either by liquid trickling or vaporization, have not shown OA to be effective during the brood rearing season (Gregorc et al. 2017, Jack et al. 2020, 2021). Studies that examined vaporizing with OA during broodless periods have documented good control of V. destructor (Rademacher and Harz, 2006), and for higher doses of 2.25g permissible outside the US we point to Al Toufailia et al. (2015, 2018) who also demonstrated efficacy of OA in the absence of brood by trickling, spraying and vaporizing. Correspondingly, our first question was simply whether V. destructor infestation levels would be affected by the repeated OA vaporization treatment despite brood being present. Figure 1 depicts how change in percent mite infestation remained static, hovering around zero (ΔPMI in treated colonies -0.7 (±2.5 SE)). As expected, V. destructor levels in control colonies did increase +4.4 (±2.6 SE); hence there was a significant difference between the control colonies and those treated, with OA. However, for a V. destructor treatment to be successful, especially when treating colonies that have exceeded the treatment threshold as part of an IPM approach, V. destructor infestation must be lowered significantly and not simply remain the same. This raises the question, if colonies are treated with vaporized OA, multiple times, well before V. destructor levels reach the treatment threshold, can suitable V. destructor control be achieved (explaining anecdotal evidence from beekeepers). It is also a question whether 1 g OA / super is an effective dose. Al Toufailia et al. (2015) working in the United Kingdom found that vaporizing with 4 times the US-label rate of 1 g per brood box resulted in a 98.2% reduction in V. destructor levels. Recently, Jack et al. (2021) demonstrated in Florida that colonies vaporized with 4 g of OA while brood is present had significantly lower infestation levels of V. destructor than those vaporized with only 1 g per brood box. Future studies could investigate the efficacy of increased doses of OA on reducing V. destructor population levels.

It was already widely known that the most desirable time to treat with OA is when colonies are broodless (Charriere & Imdorf, 2002, Gregorc & Planinc, 2001, Gregorc et al., 2016, Gregorc et al., 2017). Unfortunately, vaporizing with OA does not penetrate the wax capping of the brood cell where V. destructor is reproducing (Rademacher and Harz, 2006, Rosenkranz et al. 2010) and likely accounts for much of the variance in reported success with OA. Broodless periods naturally occur when the queen.
seasonally stops laying eggs. Future research should investigate the practicality and effectiveness of forcing or exploiting brood breaks as part of management (Jack et al. 2020). This may be possible by caging the queen, and may be a promising avenue of future research, but this is not always convenient or possible for many beekeepers especially at commercial scales. It may also be possible to exploit brood breaks that occur incidentally as part of normal management such as making splits or requeening.

Our second objective was to determine if multiple applications of OA in a colony have measurable effects on adult bees, brood, and stored honey amounts. The overall higher exposure of OA to the colony could plausibly lead to detrimental effects, especially for developing brood (Gregorc et al. 2004, Hatjina et al. 2005, Higes et al. 1999, Terpin et al. 2019). Our results showed no significant differences in changes in adult bees, brood or stored honey when colonies were exposed to OA. This supports previous studies with gaseous OA (Jack 2020, 2021, Al Toufailia et al. 2015). Our UGA 2020 data set is well suited for inferring the safety (or lack thereof) as the unchanging \( V. destructor \) parasitism levels in control and treated populations (Figure 1) removes potentially confounding effects of OA mitigating effects of the parasite. We consider our results here to be among the strongest demonstrations of the relative safety of OA to \( A. mellifera \). Perhaps, future experiments may want to explore the long-term effects and over wintering ability of colonies after being treated with oxalic acid.

Based on our results, we do not recommend employing this method for controlling \( V. destructor \) when brood are present, especially as a summer or fall treatment option when infestation levels are at or above the treatment threshold in an IPM framework. Even though there was a difference between control and treated groups, colonies vaporized with OA multiple times did not experience a reduction in \( V. destructor \) infestation levels, and so treatment was ineffective by common standards. It is important for beekeepers to adopt reliable and effective treatment regimes along with realistic, IPM approaches to sustainably reduce infestation levels of \( V. destructor \). In 2020 and 2021, two studies which vaporized OA, were successful in significantly reducing \( V. destructor \) populations. Büchler et al. (2020) vaporized with 2g of OA while incorporating a brood break and Jack (2021) vaporized with increased amounts of OA (2g & 4g) while brood was present. Because of these results, one future study could be to investigate vaporization with increased doses of OA, in conjunction with and without a brood-break.
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The Bee Line

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Please send news about your bees and your experiences in keeping them, as well as events, corrections, comments, questions, photographs and stories, interviews, recipes, points of view—and ads/advertising—to: Rosanna Mattingly, The Bee Line, 4207 SE Woodstock Blvd Ste 517, Portland OR 97206; e-mail: osba.newsletter@gmail.com. It’s your newsletter—we want to hear from you!

The next issue to be printed will be the June 2022 issue. The deadline for submitting copy is May 10, 2022. Please let me know if you find difficulties with the deadline so we can work out the space and timing for the material.

May all be well!

“Books are the bees which carry the quickening pollen from one to another mind.”
—James Russell Lowell

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