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Cover picture: A convenient water source is crucial for brood rearing during hot summer days.

Photo by James Campbell, Stonewall, MB

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Canadian Honey Council Report

Rod Scarlett, Executive Director, CHC

I am writing this in late June, prior to the release of the Canadian Association of Professional Apiculturalists final overwintering numbers for 2022-23. Certainly, there are going to be lower than last year's 45.5% average national losses, but I still expect the losses to be quite high. On the surface, I heard that generally, those that did well last year didn't fair so well this year, and those who did poorly last year did much better. There were some disasters and some successes and while varroa is certainly a primary concern, I am beginning to hear rumblings from beekeepers across the country that other issues such as viruses could be playing a major role in the high losses. More research needs to happen, and it should be a priority. There is an interesting side note on stock replacement numbers this year compared to last. By the end of April this year, 46,151 one kg package equivalents of honey bees arrived in Canada, down from 54,941 packages arriving by the same time last season. On the other hand, 118,232 queens were imported by the end of April compared to the 55,825 we had received last year for the same time.

Two significant announcements from the Canadian Honey Council highlighted the first half of 2023. First, an on-line training program for apiary workers is now available and can be accessed on the CHC website. There is more information on the program in this edition of Hivelights. Second, for the first time in many years, the CHC has joined with CAPA to hold a stand-alone national conference and tradeshow. BeeTech will be held February 8-10, 2024 at the Deerfoot Inn and Casino in Calgary. Information on the conference can also be found on the CHC website and details of the event will be forthcoming but there will not only be scientific presentations, but several beekeeper panels and discussion on issues such as stock replacement, labour, honey adulteration and pricing, economics and pollination. Please mark it down in your calendar and make plans to attend.

Commercial beekeepers who export to Japan recently received some bad news despite efforts from the Canadian Food Inspection Agency and the Japan Honey Fair Trade Council as the Japan Ministry of Health, Labour and Welfare will not consider setting an MRL for quinclorac in honey until Canada sets it own MRL. This decision jeopardizes a multi million-dollar market and could have severe impacts on the honey market in Canada. The CHC has urged the registrant, BASF, to immediately begin the process of setting a MRL for honey. The extraordinary fact is this would be the world's first MRL for quinclorac in honey despite the fact that there are numerous other food items with quinclorac MRL's.

The CHC has a booth at Apimondia 2023 in Santiago, Chile September 4th to 8th. If you are going, please stop by. Not only are we promoting Canadian honey and bee products, but we are also securing and promoting business relationship with Chilean bee exporters. In addition, work will be done with Apimondia's honey committee to improve the definition, testing and trade of honey throughout the world. ■

Regional Reports

Atlantic

Rodney Reid

The summer season is shaping up in Atlantic Canada after a mixed bag of spring weather. Overall, I am hearing that hives are doing well and beekeepers are busy. Many parts of NS had drought to start the season in April and a wet May and June. NL spring temperatures were below seasonal and wet. In the Avalon region, there were many RDF (rain, drizzle, and fog) days which kept many people out of their hives. NB and PE also have had a mix of weather with more rain but things did improve. Hopefully, the weather is a little more consistent this summer.

Reports are that blueberry pollination went well this year and there was no frost during the pollination. No frost should mean a good berry yield. Cranberry pollination comes after blueberry and starts early around the first of July. With the wet chilly spring, this year's flower set is looking to be 2-3 weeks behind.

There have been some changes in directors in both NS and NL since the last report. I am now the past president of the NLBKA, and I wish the new directors all the best in putting the industry forward under the leadership of Donna House. NS as well has had some executive changes and Duncan Wentzel is the new President of the NSBA. Taking time to volunteer on a board takes dedication and sacrifice but can be very rewarding. Being part of an industry association for me has provided me with many rewarding opportunities for professional development, relationship building, knowledge mobilization and more. If you have the opportunity to and time, I recommend putting your name forward.

My next report will be coming from the Apimondia 48th Apicultural Congress in Chile. I look forward to representing the CHC and look forward to sharing the experience with everyone. As the season goes on, hopefully, the weather continues to improve and there will be good honey flows for all.

Québec

Maggie Lamothe Boudreau

During the winter of 2023, Quebec beekeepers experienced varying outcomes in hive survival. Although overall hive losses were lower compared to last year, some beekeepers once again reported poor winter survival this year.

In recent years, Quebec has been swept by the dandelion challenge, an initiative that encourages people to keep dandelions on their lawns until they finish blossoming to help feed pollinators. This movement selected dandelions as the flagship flower due to their bright colors and early flowering, providing early food for bees. Consequently, beekeepers in Quebec have significantly increased their production of dandelion honey.

However, our provincial veterinarians have issued warnings about the presence of varroa mites, signaling a potential threat. The early heat spells across the province may have increased the risk of early mite reproduction, potentially leading to a drastic population growth earlier this year.

As summer arrived, it brought with it erratic weather patterns, causing challenges for beekeepers in northern Quebec. The region was plagued by imposing fires, forcing beekeepers to navigate the smoke and make adjustments in their management practices and transportation between pollinations.

Initially, some beekeepers faced difficulties in securing blueberry pollination contracts. Nonetheless, as the season progressed, all beekeepers managed to rent most, if not all, of their hives for pollination, earning an average rate of $225 per hive or more.

At present, beekeepers are actively transporting their colonies for cranberry pollination. This year, preparations for this move were more complex for some beekeepers due to the necessity of removing excess blueberry honey.

On a positive note, provincial veterinarians are closely monitoring the presence of small hive beetles, and fortunately, no alarming infestations have been declared. With this encouraging news, I wish every one of your hives a great summer and a successful winter.

……..

En général, l'hivernage 2023 au Québec a été marqué par un bon taux de survie des ruches. Cependant, certains apiculteurs ont tout de même connu des résultats variables et signalé des pertes considérables.

Ces dernières années, le défi pissenlit, initié par Miel&co, a connu un grand succès au Québec en encourageant les gens à laisser les pissenlits sur leur pelouse jusqu'à ce qu'ils aient fini de fleurir, fournissant ainsi une source de nourriture aux pollinisateurs. Le choix du pissenlit comme fleur emblématique s'explique par ses couleurs vives et sa floraison précoce, offrant aux abeilles une source de nourriture précoce en début de saison. D'ailleurs, les apiculteurs québécois ont constaté une augmentation considérable de la production de miel de pissenlit, qui peut probablement être partiellement attribuée à ce mouvement.

Nos vétérinaires provinciaux ont émis des avertissements concernant la présence et l'évolution du Varroa dans les ruches. Les

épisodes de chaleur précoce dans toute la province ont probablement augmenté le risque de reproduction précoce des acariens, entraînant potentiellement une croissance drastique de leur population, dépassant ainsi le seuil critique plus tôt cette année. De plus, les conditions météorologiques erratiques, notamment les incendies importants touchant la région, ont posé des défis aux apiculteurs québécois, les obligeant à faire face à la fumée et à adapter leurs pratiques de gestion et de transport entre les pollinisations.

En ce qui concerne la pollinisation, certains apiculteurs ont rencontré des difficultés initiales pour obtenir des contrats de pollinisation des bleuets. Cependant, au fur et à mesure que la saison avançait, les apiculteurs ont réussi à décrocher des contrats et à louer la plupart, voire la totalité, de leurs ruches pour la pollinisation, obtenant ainsi un tarif moyen de 225 $ par ruche, voire plus.

À l'heure actuelle, les apiculteurs sont activement en train de transporter leurs colonies pour la pollinisation des canneberges. Cette année, les préparatifs de ce déplacement ont été plus complexes pour certains apiculteurs en raison de la nécessité de retirer l'excès de miel de bleuet.

Sur une note positive, nos vétérinaires provinciaux surveillent de près la présence de petits coléoptères de la ruche, et heureusement, rien d'alarmant n'a été répertorié pour le moment. Avec ces nouvelles encourageantes, je souhaite à chacune de vos ruches un bel été et un hiver réussi.

Ontario

John Van Alten

After a very dry spring, June has been abnormally wet in southern Ontario. The rain is pelting down as I am writing this report. Generally speaking beekeepers are optimistic about the 2023 honey crop.

The blueberry pollination season is behind us now, but it will be the subject of many conversations and concerns for the foreseeable future. Some of the larger operations that provide pollination services to eastern Canada were found to have cases of EFB. (European Foulbrood). EFB is a reportable disease, and those large apiaries were not allowed to move. With millions of dollars at stake, tensions were understandably escalated. Our inspection program is now under the Animal Health department of the Ontario Ministry of Agriculture, Food and Rural Affairs. (OMAFRA). The focus of our P.A. seemed to be enforcement with no consideration for economic impacts, or suggestions for solutions.

Those of us who have been around for a number of years have seen EFB on occasion and have usually been able to overcome the disease when the stress of early spring eases and nutritional resources become available. Sometimes treatments with antibiotics are required as well. The relatively new Blueberry pollination opportunities for Ontario beekeepers seem to have increased the prevalence of EFB. We need to work at finding practical solutions to help our members overcome this issue. If we don't get past this, it will have huge impacts on not only the profitability of beekeeping in Ontario, but also the blueberry industry. We are already seeing large numbers of bumblebees from outside of Canda being imported for use in blueberry fields. Will bees on comb be next?

Manitoba

Osee Podolsky

Spring has been fairly normal across Manitoba, although there have been some areas of poor germination due to a lack of moisture. With the favorable conditions and early seeding this spring the flowering crops matured quickly and have begun blooming quite early. Part of the early bloom is due to high heat and a lack of moisture through June.

The bees have come along nicely in Manitoba, beekeepers have reported very good overwinter losses coming into this spring followed by a good spring build up. For the most part this has enabled operations to replace the last of their colony deficit from last season and have full numbers to run this season during honey production.

Manitoba has approved an Argri-recovery program for Manitoba Beekeepers to offset the extraordinary overwinter losses incurred spring 2022. The program uses a simple calculation based on registered colony numbers going into Winter 2021, less surviving colonies spring 2022 to provide the percentage of colonies lost over winter. The first 30% is not eligible to file in the claim as these are not extraordinary losses, only losses following the initial 30% deductible are eligible. The program will pay $35 per eligible queen purchase, $140 per eligible package purchase, and $210 per eligible Colony purchase. This will be a help to operations which didn't have the bee mass to split hives to make much of an increase spring 2022.

Saskatchewan

Jake Berg

Honey flow is coming on strong about 10 days to two weeks earlier in Saskatchewan. This is due to early planting and unusually warm temperatures throughout the spring. I can't remember a year that canola has started to blossom, in my local area, this early before. Hopefully we will get a few more rains in the next couple of weeks to extend the flowering length of these crops.

As the labour committee chair, I'm very happy to announce that the CHC Online Training for Apiary Workers Course package is finally available for purchase on the CHC website (chclearning.ca). The full course can be purchased for $75 and was developed by the CHC and the Agricultural Human Resources Council. This course set is an excellent tool to train new employees as well as a brush up for existing employees.

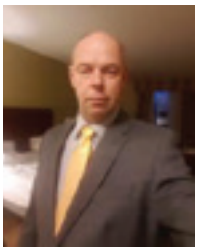
I am looking forward to the The National Beekeeping Convention and Tradeshow that is being organized by CHC along with CAPA. It will be held February 8 to 10, 2024 in Calgary,Alberta at the Deerfoot Inn and Casino. Watch for conference updates on the CHC website. I hope you will consider coming to participate in this event.

General beekeeper moral is overall better this year as bee health has improved and is better this year than it was last year. There are still some residual viruses and other bee diseases that still seem to

be elevated. But most beekeepers are confident they will get them under control in the next year.

The SBDC held our annual field day at Moyen's Honey farm on June 24. It was one of the largest field days by attendance that we've had in recent years. It was wonderful day of information, visiting with all the beekeepers, networking and enjoying each other's company.

Alberta



Ron Greidanus

"Sow your seed in the morning, and at evening let not your hands be idle, for you do not know which will succeed, whether this or that, or whether both will do equally well." Eccl. 11.

What a Spring it has been. Late. Disappointing. Drought. Strikes. Smoke and Wildfires. Timely rains. Every day the greeting is the same. "Hi Ron, how are you doing?" The biggest lie that we as humans utter is the anticipated response – "Fine".

I have come up with some novel responses to this typical greeting.

"If making money was easy, everyone would be doing it"

"My fire is small and my irons are many"

"Just when I am convinced that my life sucks,
someone comes along and shows me
I am just an amateur at the sport"

This spring started with a lot of promise and high expectations. All importers from all sources (Aus, NZ, Chile, Italy) reported having waiting lists for packages. And the packages came – a record amount. All the waiting lists were filled. There were a few hiccups.

Hiccup number one.

One of the queen exporters in California, was found to have a positive finding on queens that were subjected to a required DNA test. The test, though not subspecies specific, tests for Africanized genetics. The test, being positive, precluded this breeder from shipping queens to Canada this season. The finding also suspended the export permits from a number of other breeders in California.

Through the diligent persistence of the CHC, along with CAPA, the issue was resolved by CFIA permitting a derogation to the protocol in place, by reducing the Africanised free radius to be reduced for the season to 30 miles from 50 miles. This would allow the other affected breeders, whose genetic tests returned negative to be permitted to ship queens to Canada for this season. The season would have been very different if no legal avenue to import queens from California had been established.

Hiccup number two.

Australia is the first continent that really kicks off package season in Canada. Canada can only import packages from two different parts of Australia. Tasmania and Western Australia, two very isolated parts of the continent. Tasmania is separated from the Continent by 100 miles of open ocean. Western Australia is separated from the populated east by 4000 km of the Gibson's desert (very interesting story of how it got its name – buy me a beverage and I'll tell you the story).

Australia is very mindful of its beekeeping industry and the peril that invasive pests, if unchecked, could have. One year ago, Varroa were discovered in sentinel hives in the port of NewCastle in New South Wales. To date the Aus Gov has spent over $10 million trying to stamp out varroa. Tasmania and Western Australia remain the only two significant beekeeping regions that do not have small hive beetle or Varroa. Australia wants to keep it that way.

Having said that, this spring one sentinel hive in the port of Devonport on the northern shore of Tasmania was found to have a small hive beetle. CFIA cancelled all export permits for Packages from Australia immediately. One importer had two pallets of packages in the air at the time, enroute from Tasmania to Sidney. These packages could not be returned to Tasmania – strictly forbidden to import bees into Tasmania. They could not proceed to Canada and there was no domestic market to sell them to. This represented a significant loss to the Exporter/Importer as 2/3 of the packages did not survive.

Again CHC through diligent persistence was able to assist CFIA to resolve the issue and resume importation of package bees from Tasmania and Western Australia into Canada. The Small hive beetle that set off the alarm, was a suspected hitchhiker on a shipment of mangos from the mainland into Tasmania. No other discoveries of Small Hive beetle have been found on Tasmania since, despite extensive testing.

Hiccup number three.

Beekeepers across Canada wanted to rebuild from the devastating losses spring 2022. Many more queens and packages came to Canada from Chile and Italy this spring (Almost no packages made it from Ukraine). One of the projects that CHC had been working on with Air Canada and the importers was a Standard Operating Practices for importing packages by air freight. Unfortunately, those SOP's were not being followed by Air Canada employees in Italy. A significant percentage of the pallets of bees that were shipped from Italy had significant heat damage when delivered to the beekeeper.

These problems point to more work that needs to be done between industry and Government – CFIA. We need a plan in place for what to do if…. Although well intentioned, knee jerk reactions of 'Shut it down" by government hurt our industry. A free for all is not in the Canadian beekeeping Industry's best interest, neither is 'on/off' situation we currently have. Honest hardworking responsible individuals affected by CFIA's decisions are too often faced with complying with regulation, resulting in economic harm; or risking irregular activity and being able to pay the bills.

Canadian Industry and CFIA need to develop a better strategy in anticipation that some nasty unwanted invasive risk may be found in country that supplies stock to Canada. This is what the CHC stock replacement Committee will be working on for the foreseeable future.

Hoping that you all were able to restock your hives, that you have a bountiful crop, and that whatever you put your hand to succeeds.

Alberta

Jeremy Olthof

As our spring efforts on CHC give way to the busy season I will try to give a quick overview of the work I was involved with.

As one of the tech team liaisons my primary focus was to work towards federal funding. As with many things on a national level this took a lot of back and forth and debate over whether our push should be for sustainable funding to keep each provincial tech team viable or funding based on national priorities that each provincial tech team would then be involved with. There is still a lot of work to do, a proposal is currently making its rounds and we wait to hear back.

Alberta and Manitoba recently announced they have received Agri Recovery support which will help many producers recover from the devastating losses suffered in the spring of 2022. This was the result of a significant effort from the Alberta Beekeepers Commission and Manitoba association.

As a member of the ABC I went to Ottawa in April to testify in front of the Senate Standing Committee on Agriculture and Agri-Food on Bee Mortality. My main goal was to highlight the fact that the working relationship between the industry, CAPA and CFIA is broken especially when it comes to stock replacement. CFIA lacking expertise relies heavily on CAPA as a resource and to provide recommendations. As an industry we need to demand more transparency and cooperation from both. My point was that CFIA must have expertise in house with knowledge of not only honey bees but the beekeeping industry as well. While this may not solve our issues it would be a start. In my opinion CFIA should be coming to industry meetings and debating and defending the decisions they make, especially when these decisions have an enormous economic impact on our industry. CAPA should engage with industry not only at the national level but provincial as well.

I have tabled a motion to once again request that CFIA pursue another risk assessment for importing package bees from northern California, specifically from the established quarantine zone utilized for importing queens. In addition, the motion requests that CFIA take the time to examine the established protocols that are currently in place to minimize the identified risks to acceptable levels. On a side note has anyone seen a risk assessment for importing packages from New Zealand or Australia? Does it exist? I'm not saying I'm going to push for that but the blatant bias against the US industry drives me insane.

I look forward to the National meeting CHC is hosting next spring as the plan is to have roundtable discussions. A few topics I am hoping will come up that need industry discussion are: What role should CAPA play in making CFIA decisions? Should there be more industry involvement within CAPA? What is the real risk of Africanized genetics to our industry? Looking at the U.S., what has been the impact of Africanized honey bees over the past 2 decades? Are the current genetic tests being used to determine Africanized genetics telling us anything useful? I have more but just a few to ponder. I hope that everyone will make an effort to come to Calgary to help shape the future of our industry, National meetings don't happen very often in Canada.

British Columbia

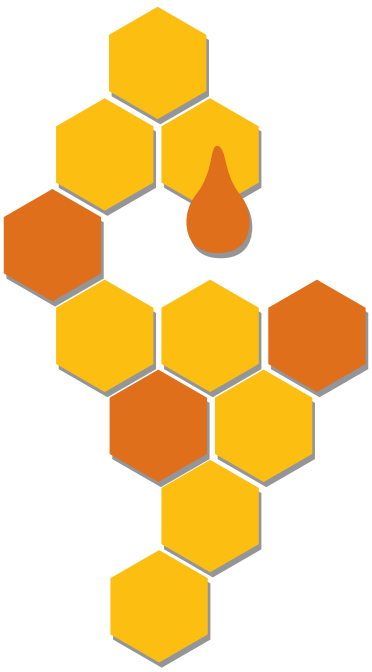
Stan Reist

It looks like summer has arrived, the bees are finally doing alright there's nectar coming in and we are not feeding this year. Right now we are looking at July conditions for the fireweed, never seen it this far advanced this early. Could be a very interesting year for the honey crop. Talking about Honey, it's looking like the sale of honey is dropping off, as it is luxury item on the grocery list. Our Qualicum farmers market is still doing great with better than average sales. We have one farmers market that is half of normal and to small grocery stores that are down by half. In talking to the managers, they say it's all price point right now the smaller sizes are moving but not with vigor. The meat sales are less than half of last year. The prediction of a recession might not be far off the mark.

The BCHPA applied for a grant from the Vancouver Foundation and we were approved. We now have a little bit better finances to operate our tech Transfer program and the beauty is it's not government money. We will have a chance to work with it. Now If the CHC's proposal comes through that would be even better. So thanks to Kerry Clark and Nuria for this and I hope Rod's project gets approved.

The BCHPA has received a request from a group of university students in the interior UBCCO iGEM requesting funding to create a vaccine for DWV. There was some debate as to whether they could be asked to create a vaccine for EFB which was thought to be of a higher importance. My thoughts were if they can make a vaccine to help with DWV then it might not be that much different to make one for EFB. I could be way out in left field on this one but the end result was the approval of $2,000 for the project.

There are probably things that could expand on however, I have a few projects on the go that I have to get done. Namely cutting hay and bailing it before it's too late. We have people wanting to pick it up off the field, so that's it for now.



Field Trials of a New Acaricidal Compound Against *Varroa destructor* in Honey Bee Colonies

Year 2 Report

Stephen Pernal (Agriculture and Agri-Food Canada, Beaverlodge, Alberta)
Erika Plettner (Simon Fraser University, Burnaby, British Columbia)
Project Term: June 20, 2021 – March 31, 2024

Objectives and General Methods:

The objectives of this study are to perform a set of full-scale field trials of the new acaricidal compound 3c{3,6}, to compare the activity of the new compound to a known acaricide of similar physical properties, and to understand partitioning of the compound within bee hives. These factors will be evaluated for both fall and spring applications of 3c{3,6}.

To accomplish these objectives, identical field trials were conducted in Beaverlodge, Alberta and the lower mainland of British Columbia during the fall of 2021, the fall of 2022 and will be in the spring of 2023.

In this report, we will outline the results of the most recently completed fall 2022 experimental trials. Both the Alberta and British Columbia experiments were modified from 2021 to include 40 colonies, with 5 treatments and 8 replicate colonies per treatment. We tested two new 3c{3,6} applicator designs (one wood and the other cardboard), having greater surface area than those used in the 2021 trials. Additionally, the dosage of 3c{3,6} was increased from 4 g in 2021 to 8 g in 2022, and the treatment phase was extended from 4 to 6 weeks.

Progress:

This year, filed experiments to evaluate our experimental compound 3c{3,6} against the honey bee parasitic mite, Varroa destructor, started in September 2022 at Beaverlodge Research Farm and in July 2022 in Surrey, BC. Robert Lu continued as a Masters' student in the Department of Biological Sciences at the University of Alberta and was resident in Beaverlodge during the fall of 2022 to conduct the experiment at that location, with assistance from AAFC technician Abdullah Ibrahim. Field work in the lower mainland of BC was led by Jorge Enrique Macias-Samano of the Plettner lab with the assistance of beekeeper Carolyn Essaunce.

The generalized timelines and activities for our 2022 experiments can be seen in Fig 1. The exact start date of each experiment (Day 0) was dependent on colonies having appropriate phoretic mite levels, that being approximately 3 mites per 100 adult bees, considered to be the fall treatment threshold. Experimental Day 0 was 14 July 2022 in Surrey, BC and 13 Sep 2022 in Beaverlodge, AB.

In both of the parallel trials in AB and BC, there were five experimental treatments applied to single brood chamber colonies: 1) 3c{3,6}, impregnated on 3 wooden strips hung between the central brood frames of each colony; 2) 3c{3,6}, impregnated on 3 cardboard strips hung between central brood frames of each colony; 3) a negative solvent control applied to 3 wooden strips; 4) a negative solvent control applied to 3 cardboard strips; and 5) Thymovar® applied as per label instructions. Each treatment was replicated eight times for a total of 40 colonies in each location. Wooden strips (24.0 cm L × 5.0 cm W × 0.5 cm thick) and corrugated cardboard strips (24.0 cm L × 5.0 cm W × 0.3 cm thick) were suspended between frames by a fourth notched strip placed across the top bars. The 3c{3,6} compound was applied in layers to the release devices, with drying between applications. Strips were coated with the compound in isopropanol with 2% glycerol, while control strips were treated with solvent only. The total dose applied used was 8 g of 3c{3,6} per colony, evenly divided across all release devices, and left in the colonies for 42 days. The thymol treatments consisted of two successive, single wafer, applications of Thymovar® to the top bars of colonies, where the wafer was replaced with a new one after 21 days. As such, the total thymol content applied each colony in this treatment was 30 g.

Prior to the application of experimental treatments, colonies were equalized with regard to brood and food stores, as well as mite loads. Two days prior to the start of the experiment in BC, or five days prior in Beaverlodge, natural mite drops were counted by installing a sticky board under each colony. The board was replaced on the first experimental day when the treatment devices were installed (Day 0). Also on Day 0, we performed an alcohol wash of workers to establish pho-

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retic mites on adult bees, and examined 100 cells of capped brood to determine infestations of varroa on pupae and levels of mite reproduction. In addition, we estimated areas of pollen, honey, open and sealed brood, and adult bees. After 42 days of experimental treatment, the devices were retrieved, a second alcohol wash was taken and the same set of assays performed. Apivar strips were installed for the clean-up treatment, lasting for an additional 42 days, and the phoretic mite infestations were again determined when the strips were removed. Efficacy of the mite treatments was assessed by comparing "mite fall" on sticky boards during the period while experimental treatments were being applied, divided by this plus mite fall during the Apivar clean-up period.

Findings to Date:

1. Field Experiments

On Day 0, the average mite infestation level in the BC apiary was 2.6 ± 0.3 (mean ± SE) mites per 100 adult bees, while in AB this level was 4.1 ± 0.7 mites per 100 bees.

Figure 2 shows detailed mite fall data from the experiment in Beaverlodge, AB, while Figure 3 shows data from the experiment in Surrey, BC. From these collective data, 3c{3,6} demonstrated noticeably higher mite killing efficacy compared with the results from our 2021 trials. This was especially true in the case of the Beaverlodge experiment, where the efficacy of both wood (94.5 ± 2.6) and cardboard (94.6 ± 2.7) applicators was comparable to that of the Thymovar positive control (94.5 ± 1.6).

In Beaverlodge (Fig. 2), there were higher numbers of mites on the sticky boards in both 3c{3,6} treatments, compared with the controls, with the cardboard applicators initially appearing to outperform the wooden applicators, though overall their performance proved almost identical. Both 3c{3,6} treatments outperformed Thymovar early in the experimental period, though an increase in mite fall is seen when the second Thymovar wafer was applied on Day 21. In the Fraser Valley (Fig. 3), the wooden applicators for 3c{3,6} initially seemed to outperform the cardboard applicators, however final efficacy calculations showed the cardboard applicators to be slightly more effective. Thymovar appeared to have a slightly delayed effect, then matched the performance of 3c{3,6} on wooden applicators by Day 3, with no later spike in mite fall on Day 22 when the Thymovar wafers were replaced. It is important to note that in BC, during the second half of the experimental treatment, two of the Thymovar-treated colonies died and one became so weakened that it died a later date. Temperatures during the second half of the exper-

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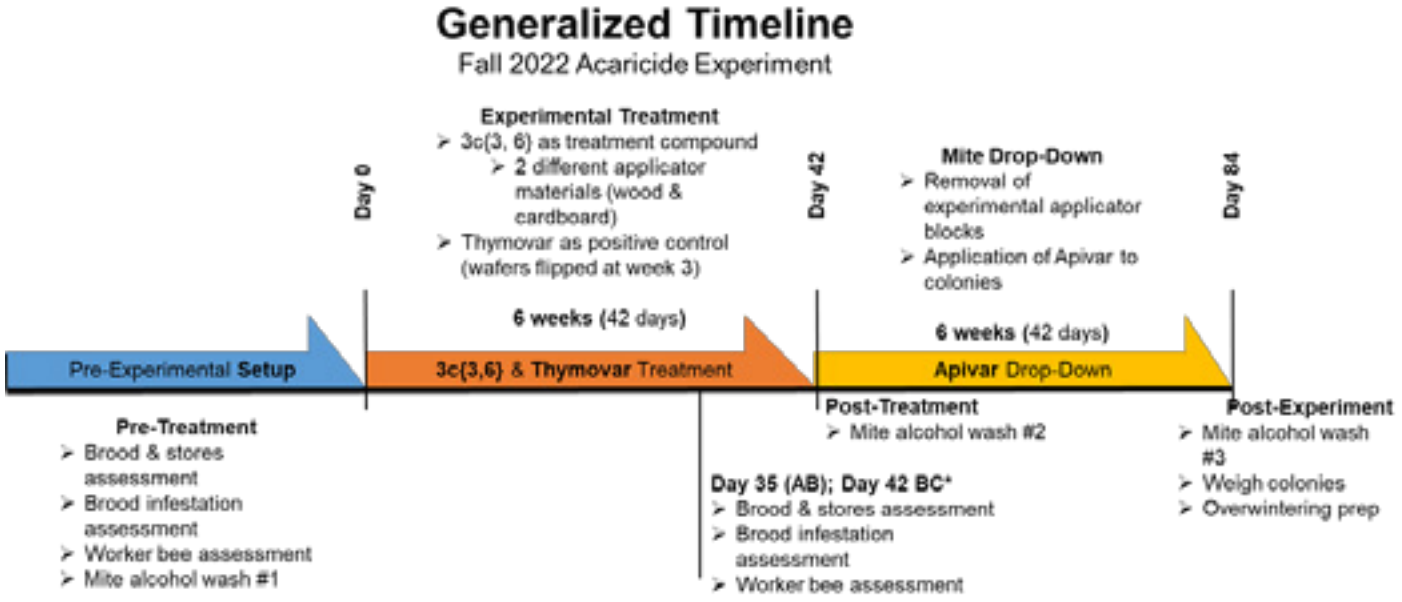
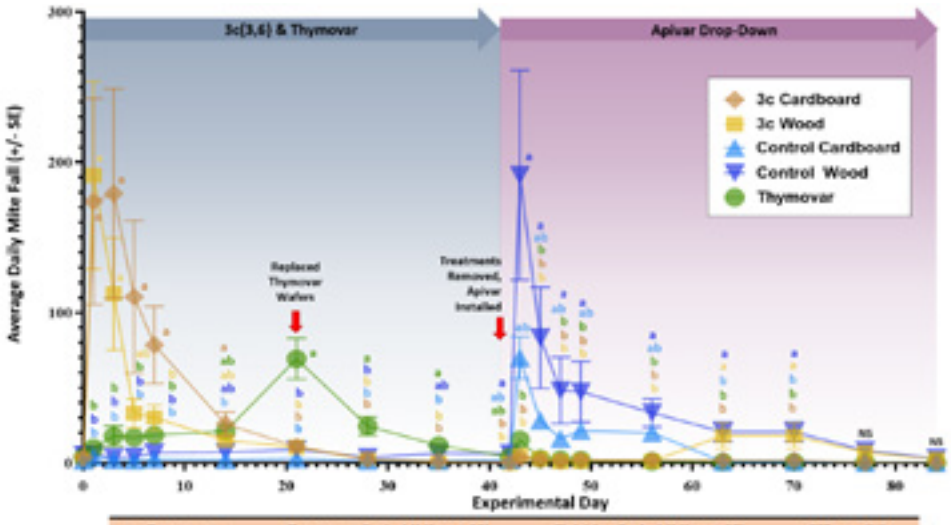
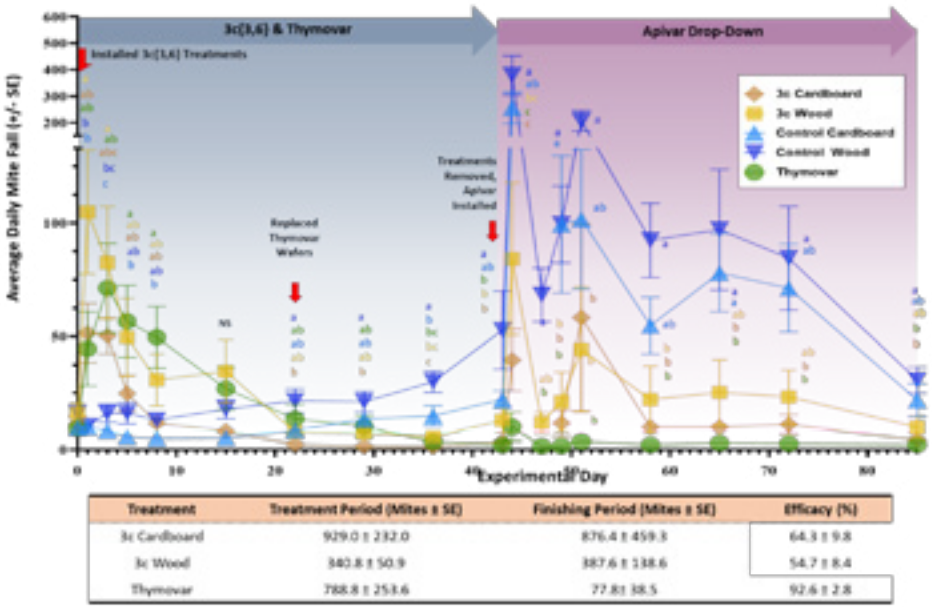


Figure 1. Generalized experimental timelines for fall 2022 experiments located in Beaverlodge, AB and Surrey , BC.



Treatment	Treatment Period (Mites ± SE)	Finishing Period (Mites ± SE)	Efficacy (%)
3c Cardboard	1196.1 ± 433.1	57.1 ± 26.6	94.6 ± 2.7
3c Wood	741.8 ± 198.8	36.8 ± 16.6	94.5 ± 2.6
Thymovar	1031.0 ± 151.5	49.5 ± 10.7	94.5 ± 1.6

Figure 2. Mite fall plot from acaricide experiment in Beaverlodge, AB. Points represent means ± SE of 8 replicates. Means with different letters are significantly different within time points (Tukey-HSD; P<0.05).



Treatment	Treatment Period (Mites ± SE)	Finishing Period (Mites ± SE)	Efficacy (%)
3c Cardboard	929.0 ± 232.0	876.4 ± 459.3	64.3 ± 9.8
3c Wood	340.8 ± 50.9	387.6 ± 138.6	54.7 ± 8.4
Thymovar	788.8 ± 253.6	77.8 ± 38.5	92.6 ± 2.8

Figure 3. Mite fall plot from acaricide experiment in the Fraser Valley, BC. Points represent means ± SE of 8 replicates. Means with different letters are significantly different within time points (Tukey-HSD; P<0.05).

imental treatment were high (≥ 27°C) and it was unusually dry, so colonies may have been over-dosed with Thymovar when the wafers were changed. These differences in temperature and seasonality may have contributed to increased deleterious effects of Thymovar against bees as seen in the Fraser Valley colonies. However, the mite-killing efficacy did not appear as heavily impacted, as the efficacy of Thymovar remained as high as in Alberta.

Table 1 (pg. 15), captures many of the other parameters recorded in these experiments to date.

On Day 0, there were no differences in phoretic mite loads among the five treatments, per location, whereas on Day 42, reductions in phoretic mite loads appear to reflect the relative efficacy of the 3c{3,6} and Thymovar treatments. Similar patterns are reflected in mite infestation densities in brood comparing Days 0 and 42. In addition, there were no differences among the five treatments in

Table 1. Data for mites on adult bees (washes), in brood, and areas of brood and food stores (means ± SE).

Location	Treatment	Alcohol Washes (Mites per 100 bees)			Brood Infestation (Mites per 100 cells)		Brood (Frame sides)				Food (Frame sides)			
							Day 0		Day 35 (AB) / 42 (BC)		Day 0		Day 35 (AB) / 42 (BC)	
		Day 0	Day 42	Day 84	Day 0	Day 35 (AB) / Day 42 (BC)	Capped	Open	Capped	Open	Honey	Pollen	Honey	Pollen
AB	3c Cardboard	4.0 ± 1.3	0.2 ± 0.1^{a}	0.2 ± 0.1^{a}	9.1 ± 3.5	0.4 ± 0.3^{a}	2.91 ± 0.51	0.52 ± 0.17	1.01 ± 0.18	0.18 ± 0.12	6.96 ± 0.88	2.84 ± 0.75	14.05 ± 0.63	2.24 ± 0.37
	Control Cardboard	4.1 ± 1.6	3.9 ± 1.2^{ab}	0.5 ± 0.2^{a}	5.5 ± 1.1	10.9 ± 2.6^{ab}	3.22 ± 0.44	0.40 ± 0.15	0.98 ± 0.11	0.11 ± 0.07	8.46 ± 1.16	0.93 ± 0.53	13.51 ± 0.82	2.31 ± 0.74
	3c Wood	4.1 ± 1.5	0.1 ± 0.04^{a}	0.2 ± 0.1^{a}	4.3 ± 0.8	1.8 ± 0.6^{abc}	2.53 ± 0.31	0.56 ± 0.15	0.87 ± 0.29	0.12 ± 0.04	7.43 ± 0.89	2.33 ± 0.84	14.14 ± 0.54	1.73 ± 0.74
	Control Wood	4.0 ± 1.5	7.3 ± 2.4^{b}	2.0 ± 0.7^{b}	7.8 ± 3.5	12.6 ± 4.9^{b}	3.09 ± 0.34	0.86 ± 0.24	1.03 ± 0.18	0.40 ± 0.14	5.81 ± 0.94	2.56 ± 0.80	13.47 ± 0.88	1.99 ± 0.75
	Thymovar	4.2 ± 1.7	0.3 ± 0.1 ^{a}	0.1 ± 0.1^{a}	6.6 ± 2.0	1.0 ± 0.4^{c}	2.88 ± 0.45	0.38 ± 0.09	0.81 ± 0.16	0.33 ± 0.08	7.72 ± 0.78	2.35 ± 0.90	13.61 ± 0.83	2.13 ± 0.56
BC	3c Cardboard	1.7 ± 0.7	1.2 ± 0.5^{a}	1.7 ± 0.6	3.6 ± 1.6	4.3 ± 2.1^{a}	2.59 ± 0.57	0.93 ± 0.22	1.25 ± 0.24	0.54 ± 0.10	3.33 ± 0.55	0.86 ± 0.18	5.09 ± 0.45	0.89 ± 0.28
	Control Cardboard	2.5 ± 1.0	4.7 ± 1.2^{ab}	6.2 ± 2.7	2.5 ± 0.5	18.1 ± 4.7^{ab}	3.15 ± 0.48	1.10 ± 0.50	1.84 ± 0.20	0.54 ± 0.14	3.46 ± 0.66	1.19 ± 0.33	4.38 ± 0.72	0.76 ± 0.31
	3c Wood	3.7 ± 0.7	2.3 ± 0.6^{a}	8.5 ± 5.4[1]	4.8 ± 1.7	13.7 ± 5.3^{ab}	2.82 ± 0.38	1.42 ± 0.38	1.29 ± 0.42	0.50 ± 0.16	3.92 ± 0.75	0.93 ± 0.33	5.09 ± 1.28	1.13 ± 0.41
	Control Wood	2.5 ± 0.5	8.5 ± 2.0^{b}	7.8 ± 4.0	4.4 ± 1.4	23.5 ± 5.3^{b}	3.45 ± 0.51	1.09 ± 0.29	2.37 ± 0.33	0.98 ± 0.23	3.95 ± 0.78	1.41 ± 0.28	5.53 ± 1.03	0.82 ± 0.18
	Thymovar	2.6 ± 0.6	0.3 ± 0.1^{a}	0.5 ± 0.3[2]	3.63 ± 0.9	0.80 ± 0.6^{a}	2.40 ± 0.55	0.63 ± 0.24	1.11 ± 0.42	0.78 ± 0.28	3.43 ± 0.43	0.80 ± 0.13	3.52 ± 1.11	0.56 ± 0.09

[1] Note: there was one outlier with a 35% mite load. Without the outlier, the wash result was 3.2 ± 1.2.

[2] Three colonies had perished by this time.

Unique letters following treatment means denote significant differences, for each dependent variable, within sites and time periods (Tukey-HSD; P<0.05).

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terms of brood areas (capped or open), or between honey and pollen stores at the time points during which these were measured, suggesting no deleterious effects on colony health.

During the course of the experimental treatment in Alberta, there was a significant decrease in the amount of brood and a significant increase in the quantity of honey stored. These are both indications that the colonies had stopped growing late in the fall, and also reflect the stored sugar syrup content in colonies, as they were fed in preparation for wintering. This period was also unseasonably warm for northern Alberta.

For the Beaverlodge colonies, the mite wash immediately following the removal of Apivar on Day 84 revealed minimal mite levels in most colonies, with all treatments except the control wood exhibiting phoretic mite loads of ≤ 0.5 mites per 100 bees. Furthermore, while worker bees from the control wood colonies had an average mite load of 2.0 ± 0.7 (greater than other treatments at this time point), levels remained low enough to avoid any additional treatment. A potential explanation for higher mite level in this treatment may be due to the overwintering clusters localizing away from the Apivar strips during the bulk of the 42-day Apivar treatment phase which, in conjunction with the relatively high Day 42 post-treatment infestation rate of the control wood colonies, resulted in comparatively elevated Day 84 mite loads.

In BC, during the 42-day Apivar clean-up phase, and well past the end of the experimental period, the weather was unseasonably summer-like. Thus, the third alcohol washes in BC on Day 84 still indicated high levels of varroa (Table 1), which necessitated continuation of mite control treatments in preparation for winter. This supplemental treatment was introduced one week after the end of the experiment, as an oxalic acid drizzle (7% w/v in heavy sugar syrup, 5 mL per comb interspace occupied by bees), in addition to a second set of two Apivar strips.

Overwintering evaluation of colonies in the Fraser Valley was completed in February 2023 (Fig. 4). From the results of the ongoing mortality assessments, the negative controls experienced the highest mortality, with the wood and cardboard controls experiencing a relative decline of 6 and 4 colonies, respectively, from the beginning of winter. Both Thymol- and cardboard 3c{3,6}-treated colonies experienced zero relative mortality from the start of winter, while the wood 3c{3,6}-treated colonies had 3 colonies die since October.

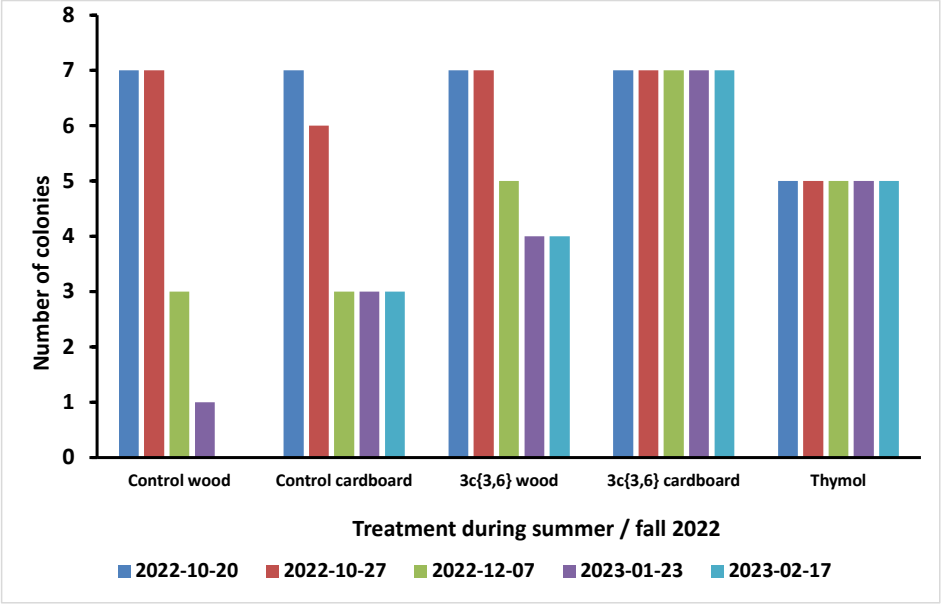

Figure 4. Survival of the Fraser Valley colonies, with five checks performed across the fall and winter. Each treatment initially contained 8 colonies. Number of living colonies per treatment is shown by date.

Overall, our applications of compound 3c{3,6} proved to have success in both locations. High efficacy in Alberta was likely contributed to by the very warm fall temperatures during the application period. Somewhat lower efficacy in BC was attributable to application in mid-summer during a period of high brood production and during very hot temperatures. We will also endeavour to examine other applicator materials more homogenous than wood, but not as consumable by bees as corrugated cardboard.

2. Residue Analyses

2.1 3c{3,6} Release Rates

During the fall 2022 experiments, we inserted Porapak Q sorbent columns on the bottom boards of each colony to evaluate temporally-produced airborne volatiles 3c{3,6} treatments within hives.

Chemical analyses of Porapak devices and spent release devices retrieved from hives in BC have begun by graduate student Ms. Xinyi Feng, in the Plettner lab. Initial results from the Porapak devices indicate that during the first 21 days ~ 230 ng/day were captured by the devices (Fig. 5). Later in the experimental treatment, the amount captured per day decreased. Porapak device #4 was in the hives after the experimental treatment had ended, so the capture of the compound by these devices indicates that the compound had fumigated the hive and was still present in the first ~ 20 days of post-experimental treatment with Apivar. The fifth set of Porapak devices used in BC was taken out after overwintering, and no detectable compound was present (limit of detection: 17-27 ng total per Porapak device, or 1- ~ 1.5 ng/day for a 14-day period). This indicates that the compound can slowly evaporate and vent from a strong sorbent such as Porapak Q over the course of the winter. Notably, there was no difference between wood and cardboard in terms of the levels of 3c{3,6} detected in the Porapak devices from BC. Therefore, data shown in Fig. 5 are pooled.

In Alberta, Porapak devices were sampled as in BC, except for the fifth set, which was taken out at the end of the treatment with Apivar. Results are shown in Figure 6. Wooden and cardboard devices released equal amounts during the first week, and then wooden devices released significantly more material in the following sampling round (Porapak 2) than cardboard ones. Amounts released from wooden devices decreased as the treatment proceeded. Porapak 4 was installed when the experimental treatment ended and Porapak 5 was installed during the Apivar treatment, to the end of the experiment. Detection of compound 3c{3,6} in those cases indicates that some of the compound was still lingering in the colonies but was decreasing.

Colonies treated with the cardboard devices showed steady levels of ~ 200 ng/day, as detected with the Porapak devices. The pattern seen for Porapak 4 and 5 (Fig. 6) is difficult to explain, unless the pipettes labelled as Porapak 5 were installed at the time the ones labelled Porapak 4 should have been.

In summary, fumigation of colonies with compound 3c{3,6} was seen in both provinces, and the levels of the compound de-

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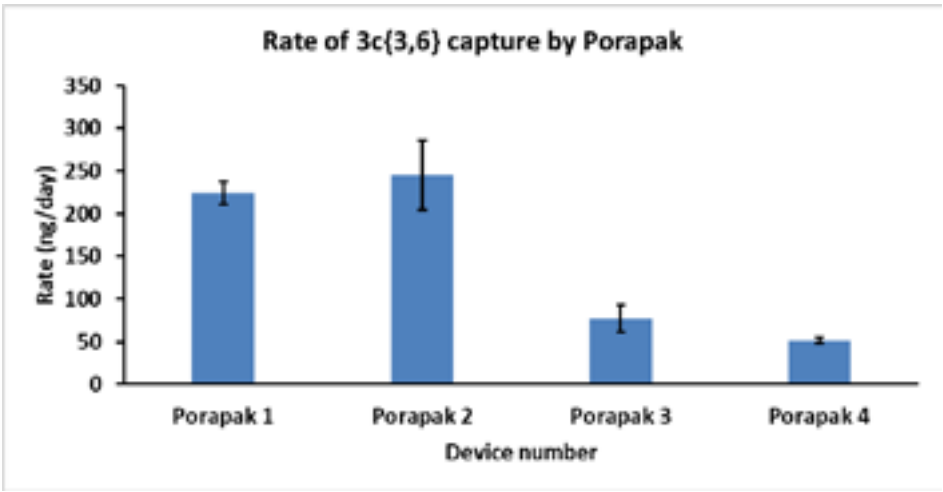

Figure 5. Average capture rates per day of Pasteur pipettes filled with Porapak Q, a sorbent for volatile compounds. These data are for colonies treated in BC. Porapak 1 was inserted into colonies when treatments were applied on Day 0 and stayed in the colonies 8-9 days; Porapak 2 replaced the first Porapak and stayed in for 14 days. Porapak 3 replaced the second device and stayed in for 21 days. Porapak 4 replaced the third device on the day the experimental treatment ended and stayed in for 19 - 20 days. Average levels per day are pooled over 8 treatments with wooden release devices and 8 treatments with cardboard devices.

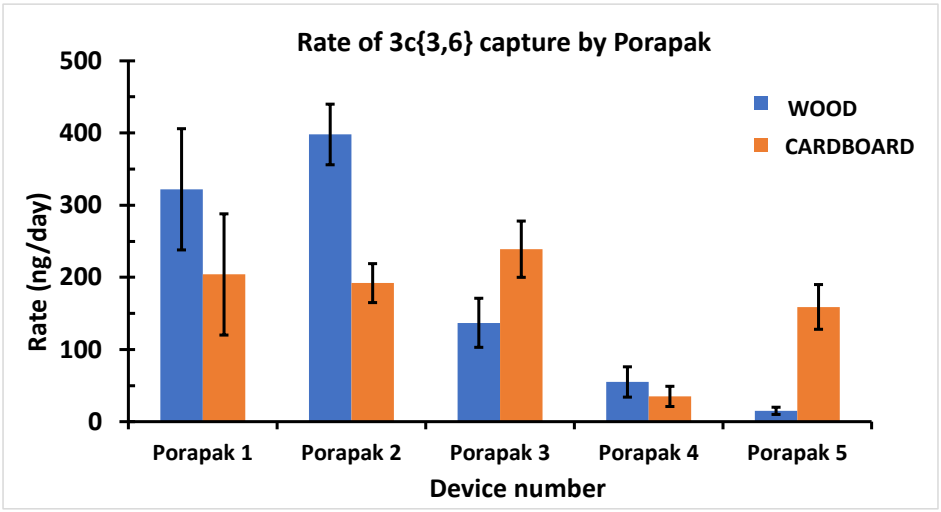

Figure 6. Average daily rates of capture of 3c{3,6} by sorbent (Porapak Q) in Pasteur pipettes, placed in the colonies being treated, in AB. The dates for installation and removal were: Porapak 1 Sept 13 – Sept 20, Porapak 2 Sept 20 – Oct 4, Porapak 3 Oct 4 – Oct 25, Porapak 4 Oct 25 – Nov 8, Porapak 5 Nov 8 – Dec 5. Bars represent mean ± SE (n = 8) for colonies treated with wooden devices (blue) or with cardboard devices (orange).

tected per day in Porapak devices decreased over time, once the release devices with the compound had been removed. Extraction of the wooden release devices that were recovered from the colonies indicated that, in both provinces and also in one experiment done with a collaborator in the US, most of the material had been released during the 42-day treatment. This translated to a release rate of ~ 190 mg/day/colony.

2.2 Residues in wax and Honey

To address the question of residues in 2022, we collected a full set of wax samples immediately after 3c{3,6} application, and will do so again after wintering, during the spring of 2023. We will also collect honey and wax samples during the 2023 honey production season from the same colonies. We were unable collect honey and wax samples in the summer of 2022 for Beaverlodge colonies treated in the fall of 2021, as too few colonies survived winter due to the high mite loads before treatment.

A method for extraction of wax and analysis of the extract by GC-MS was also developed by Dr. Plettner during the summer of 2022. One challenge with wax analysis is that there is a natural compound in wax, possibly a lignan, with a m/z 192 ion, the same as the molecular ion of compound 3c{3,6}. It was necessary to optimize the GC method to at least partly separate compound 3c{3,6} from the natural one, and we routinely now use the new method for all analyses (including ones presented here). Ms. Feng has now synthesized the compound we suspect is the natural isomer of compound 3c{3,6}, and is exploring GC-MS runs with various mixtures of the active (artificial compound 3c{3,6}) and the natural isomer. After this methodological development work is completed, analysis of incurred wax and honey samples can commence.

Accomplishments:

- Conducted an additional two full-scale field trials in two different geographic locations.
- Evaluated modified release devices for 3c{3,6}.
- Development of a new GC-MS technique for the detection of 3c{3,6} from wax
- Ongoing training of graduate students at AAFC and SFU.
- Additional field trial conducted by Dr. Steve Cook (USDA-ARS) in the summer of 2022, at the Beltsville Bee Laboratory (not reported herein).
- Submission of a peer-reviewed manuscript regarding 3c{3,6} discovery and testing.

Summary:

In conclusion, we have completed two additional large-scale field trials in 2022 that are producing valuable data for evaluating the efficacy of compound 3c{3,6}. Our results appear to show consistency in the utility of using compound 3c{3,6} for varroa mite control, which is comparable or exceeds that of thymol-based treatments. We also continue to find no negative effects of compound 3c{3,6} on the size or health of honey bee colonies and are examining deposition of the compound in honey and beeswax.

Our work is on track and is positioned to produce data to support the registration of compound 3c{3,6} in both Canada and the U.S. as a novel compound for controlling varroa mites.

We thank the Canadian Bee Research Fund for support of this project.

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Field assays (or "Pettis tests") as a tool to detect miticide resistance – development, benefits and considerations

By Dr. Ulrike Marsky, Technical Manager, Véto-pharma

Field assays can be a valuable tool for beekeepers to determine whether varroa mite populations in their apiaries are sensitive towards a specific miticide or not. Several factors of the assay protocol such as ambient temperature during the exposure, exposure time to the tested miticide, and treatment history of the sampled hive can affect the outcome of assays. To obtain meaningful results, it is important to consider these when developing a protocol. Field assays were originally developed as an easy and quick method to detect varroa mite resistance in the field, not as an exact measure of potential treatment efficacy.

Introduction

Not very long after the first miticides were authorized as a response to the spread of Varroa destructor, beekeepers worldwide experienced the phenomenon of miticide resistance in their apiaries.[1,2,3] In other words, the treatments they had been using successfully for several years were not efficient anymore. In some cases, treatments failed spectacularly, with a demonstrated field efficacy as low as 4% - referring to the percentage of varroa mites killed during the treatment period.[4] Keeping in mind that 90% (for organic miticides) to 95% (for chemical miticides) efficacy is considered the minimum necessary treatment efficacy to control varroa successfully in the field[5], numbers like this are devastatingly low.

But how does resistance develop, and how can we tell it apart from "low efficacy" of a varroa treatment caused by a different reason?

Resistance develops after repeated contact of varroa mites with a specific active ingredient (chemical substance) over time.[6] The chemical substance, killing the mites, imposes a high selection pressure on the varroa population. This means, finding a way to become less sensitive, or resistant, against an active ingredient is literally a question of life or death to the mites. Those mites that are less sensitive towards the active ingredient are more likely to survive a treatment and reproduce. As a result, these mites are more likely to pass on their genes (including those responsible for resistance development against a specific active ingredient) to the next generation. At least for as long as the mite population continues to be exposed to this active ingredient, carrying the genes that are causing resistance is beneficial, and they will spread in the population.[6]

Telling resistance apart from other causes of low efficacy or treatment failure is not always easy. Observing and monitoring honey bee colonies throughout the season and focusing especially on the development of the mite infestation level is crucial in this context. Knowing mite infestation levels throughout

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the season can help identify "super infested colonies" early on. This is important to prevent the spread of high varroa mite numbers across the whole apiary by treating soon enough. Comparing mite infestation levels before and right after a varroa treatment is an important element of monitoring treatment success in the field, but factors such as re-infestation, migration, or pre-treatment infestation play into the mix, as well.

Re-infestation from other colonies can affect treatment efficacy significantly – even when mite populations are sensitive. Key questions for beekeepers to ask themselves include: Was this treatment used before in this apiary? If yes, how often? Resistance can only develop after repeated contact of the mite population with a miticide. Was the treatment applied as recommended by the label? Or was the treatment possibly underdosed / overdosed / removed too early / kept in the hive for too long / applied incorrectly? Asking these questions and contacting local apiary inspectors, as well as the producer of the miticide, about a failed treatment is strongly recommended.

The global development of varroa mite resistance against different active substances has quickly led beekeepers and researchers to the question: How can beekeepers recognize, with a simple test in the field, whether the varroa mite populations in certain colonies are resistant to one or the other active ingredient?

The genesis of bioassays in beekeeping

In the United States, the idea of a quick and easy tool for beekeepers to detect resistance in their mite populations in the field first came up in the 1990s when fluvalinate resistance was suspected and later confirmed.[3] Dr Jeff Pettis from the ARS's Bee Research Laboratory, in Beltsville, Maryland and colleagues published a protocol for a field bioassay for beekeepers in 1998.[7]

The idea behind the assay was to develop a tool that was user-friendly, did not require specialized equipment, and enabled beekeepers to detect resistance before applying varroa treatments – allowing them to increase the survival rate of their colonies as well as to save money and apply a different (efficient) treatment, in case their mite populations were resistant towards fluvalinate.[8] In the case of fluvalinate, an assay result of less than 50% mite mortality would indicate resistance, below 25% indicates full resistance.[8] So, instead of an "exact science" approach, exposing varroa mites to chemical substances in the laboratory, the goal was to provide beekeepers a simplified, quick tool for the apiary.

The original "Pettis test" protocol for recognizing fluvalinate resistance was later adapted to help beekeepers detect coumaphos resistance in their colonies.[9]

The basic principle of field bioassays is this: Beekeepers bring some equipment, including a jar or other container, a piece of the strip of the miticide to be tested, possibly a cup measure, a bucket, white paper, alcoholic solution, and other tools to the apiary. They take bee (and thus attached varroa mite) samples from the brood area of their colonies, taking care that they do not include the queen in the sample. The sample is then exposed to a piece of said miticide strip for a pre-defined period of time. After the incubation time, dead mites are counted on a white bucket or piece of paper. An alcohol wash is performed with the bee sample to detect all mites that were not killed during the incubation time. The mortality rate of mites during the incubation time (exposure to the miticide) can now be calculated. It is assumed to represent the percentage of sensitive mites in the sample (vs. resistant = surviving mites that were not killed during the incubation time).[7-9]

Field bioassays to detect amitraz resistance

In recent years, amitraz resistance in varroa mites has been reported in some regions of France and the United States.[10] Amitraz treatment failure has occurred in a less widespread and more patchy pattern compared with the relatively fast spread of pyrethroid or coumaphos resistance in the past. Nevertheless, researchers in North America and Europe have since worked on the development of a field bioassay for beekeepers that specifically fits this molecule. Most of them have been using the authorized amitraz strip treatment, Apivar, to expose mites in a bee sample to amitraz. Different exposure periods of varroa mites towards the active ingredient (from 2 hours up to 24 hours) have been tested. Different sizes of the piece of the Apivar strip and different methods to fix the strip inside the container with the bee (and mite) sample have been explored.[11-12]

Some general factors to consider in bioassay protocols include all factors revolving around expiry date and packaging of the testing strips used in the assays. All strip treatments against varroa mites with different ingredients (amitraz, flumethrin, coumaphos) have a specific shelf-life, and each produced batch / lot of the product has an expiry date. Of course, these expiry dates should not only be respected when applying strips as a varroa treatment in honey bee colonies, but also when they are being utilized to detect resistance in the apiary.

Even though the original packaging of the strips has to be opened in order to access them and cut them in smaller pieces for a field assay, shipments of strips or pieces of strips in a different packaging than the original product should be avoided. As soon as the original, sealed packaging of a product is opened, the degradation process due to exposure to air (more precisely, oxygen) and sunlight begins.

In addition to that, the timing of the field bioassays should be considered wisely. For the benefit of colony health, resistance testing before the treatment season is recommended. Beekeepers can thus estimate the sensitivity

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Microbial Myths

True or False? If microbes don't colonize, they aren't effective.

It's true that some beneficial microbes, such as Lactobacilli in probiotics, are aimed at replenishing native bacteria that colonize (or continue to populate) the gut. However, many bacteria have demonstrated their ability to impart positive effects even though they are transient and do not remain in the digestive tract or the rectum for extended periods.

You may have wondered why probiotics for humans are often recommended daily. This is because beneficial microbes that do not colonize the gut need to be replenished. Even bacteria that do colonize the gut face overwhelming circumstances that often disrupt their natural balance. For example, when honey bees face several environmental stressors such as transportation, dearth, supplemental feeding, pathogens, parasites, exposure to toxins and antibiotics, these all negatively impact a flourishing microbiome.

When good bacteria are diminished, it leaves holes or gaps for harmful bacteria to move in. Imagine your honey bee's gut is a big neighborhood; we want our society to have plenty of helpful neighbors that shield our community when hard times come. Unfortunately, when environmental stressors come along, some of the helpful neighbors (bacteria) will die or dwindle in numbers until they can no longer take up residence like they used to. When this happens, it leaves a hole or a gap, like a vacant house. That empty space is an opportunity for pathogens to take up residence. This is why it's crucial before, during, and after stressful times like pollination, when your bees face a deluge of stressful circumstances, that their gut's microbiome is fortified with good neighbors (bacteria) to help them get through the tough time ahead.

of varroa mite populations in their apiaries towards a specific molecule before they pick a varroa treatment, **but not predict the exact field efficacy of an authorized treatment.**

Bioassay protocols to detect amitraz resistance have been proposed by Pettis (2019)[11], Higes et al. (2020)[12] and others. The different protocols vary mainly in three methodological details:

1. The consistency or lack thereof of ambient temperature during the assay.
2. The exposure period during which mites are exposed to the active substance (amitraz)
3. The sampling of complete bee samples (including varroa mites) vs. sampling of varroa mites only (from the bee brood and to be transferred in petri dishes or glass vials).

Temperature matters

Pettis (2019) has found a lower mite mortality in assays carried out at temperatures of 70F (21°C) vs. assays carried out at 90F (32°C). The protocol of Higes et al. (2020) is relying on a more controlled approach by exposing mites to amitraz at a constant temperature of 34°C (93.2F). The protocol suggested by Rinkevich (2020)[13] does not suggest any type of temperature control of the bee (and mite) samples during exposure towards amitraz. Of course, one may argue that the requirement to keep bee (and mite) samples at a constant temperature reduces practicability in the field.

However, the Ministry of Agriculture in British Columbia already published a Pettis test protocol for amitraz in 2015.[14] This protocol suggests several alternatives to provide at least some control of ambient temperature in the field:

"Place the jars in an incubator or a warm room, in the dark, for 6 hours. Alternatively, place jars in a picnic cooler with a couple of hot water bottles. Refill the bottles with hot water after the first 3 hours. Make sure the lids of the jars are not covered so that the bees have air." The protocol, similar to the original purpose of field bioassays proposed by Pettis, includes the following disclaimer: "**This assay is intended to screen for resistant mites and is not intended to indicate the exact level of resistance.**"[14]

Exposure time: 3 or 6 hours?

With regards to exposure time, Pettis (2019) and the Ministry of British Columbia (2015) suggest 6 hours of exposure of the bee sample towards amitraz. Shorter exposure times are usually known from laboratory bioassays[15-16] or mixtures of laboratory and field assays such as the protocol suggested by Higes et al. (2020).[12] The protocol by Rinkevich (2020)[13] is the only field bioassay protocol (working with bee samples taken from the testing colonies, exposing the samples to strip treatments, and not controlling for ambient temperature) that recommends a shorter exposure period of three hours.[13]

Figure 1 - Material used in the Rinkevich protocol (2020)

Figure 2 - Material used in the British Columbia protocol

The relevance of exposure periods of bioassays has been discussed amongst experts as well. Whilst the possibility of obtaining false negative results (= varroa populations falsely classified as "sensitive") with longer exposure times has been suggested by some, the main factor determining a cut-off time of the exposure period in the field is probably the viability of varroa mites. Looking at the exposure periods suggested in different assays (field and laboratory assays), exposure times usually range between 1 and 6 hours. In laboratory assays, when varroa mites may not have access to nutrition (bees or larvae), viability may play a more important role, and exposure times are usually shorter. However, in field assays with varroa mites being attached to and feeding on their hosts, their natural probability of survival (without exposure towards the active substance) after 3 hours may not necessarily differ significantly from survival after 6 hours. Whether or not an exposure period of 6 instead of 3 hours would significantly increase mite mortality for reasons other than the exposure towards the active ingredient, remains to be demonstrated. As it is the primary goal of a field bioassay to determine sensitivity or resistance towards active substances, the observation of false positive results (= varroa population falsely classified as "resistant") should be avoided by choosing long enough exposure periods.[11-14]

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Figure 3 - Material used by the Véto-pharma team

Sampling: complete bee sample or varroa mites only?

In the protocol of Higes et al. (2020), instead of exposing the whole bee sample, varroa mites are sampled individually and exposed to a piece of an amitraz strip in a petri dish in groups of 15.12 The exposure in assays like this is much less dependent on the activity of the bee sample. The exposure of varroa mites towards the active ingredient is more immediate, as mites directly get into contact with a strip treatment or a petri dish, coated with the active substance. Of course, sampling individual mites and exposing them to amitraz at a controlled temperature is less convenient for beekeepers in the field. However, it does allow for a more standardized procedure under laboratory conditions.

Conclusion

Overall, researchers, technicians and beekeeping experts working on the development of field bioassays to be recommended for broad use in beekeeping, must be aware of the responsibility to weigh accuracy against user-friendliness and practicality in the field. The expectation of beekeepers to carry out quick resistance tests in the field must be recognized, if possible, without discarding methods that may slightly complicate the protocol, but could significantly improve the reliability of assay results in the field.

This is especially important for bee inspectors and/or beekeeping advisors and/or technicians who may recommend conducting bioassays as a diagnostic tool to detect the presence of resistance in an apiary. Such a careful evaluation of bioassay results includes the distinction between mite sensitivity (sensitive vs. resistant) and field efficacy of an authorized treatment, as one is not equal to the other. In fact, the classification of differing percentages of varroa mite mortality in bioassays as "efficient", "mostly efficient" or "resistant" is well-intentioned, but somewhat arbitrary.17 Continued efforts are required to investigate field bioassay protocols for the detection of amitraz resistance in the apiary and to obtain the best possible balance between satisfying the needs of beekeepers and respecting methodological standards required for reliable assay results.

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