ATTTA
Atlantic Tech Transfer Team for Apiculture

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# Atlantic Canada regional survey on the prevalence of Varroa mites (*Varroa destructor*) in honey bee (*Apis mellifera*) colonies, and the efficacy of amitraz for treatment

## Atlantic Tech Transfer Team for Apiculture 2024 Report

### Background

High Varroa mite (*Varroa destructor*) levels remain a top reason for colony loss in the winter according to the Canadian Association of Professional Apiculturists (CAPA) Winter Loss Survey 2023-2024 (CAPA, 2024). Six out of nine provinces listed Varroa mites as a top three reason for colony winter loss in the winter of 2023-2024. This does not include Newfoundland and Labrador as they remain Varroa mite free. Additionally, provinces that list Varroa mites as a top reason for winter loss also experience a higher percentage of winter loss.

Apivar® (3.3% amitraz as the active ingredient) is a synthetic miticide widely used to manage Varroa mites. Currently, Apivar® is the only recommended synthetic miticide for Varroa mites in Canada. There are other products available with active ingredients fluvalinate (Apistan®) and flumethrin (Bayvarol®), but, due to extensive and exclusive use of these products, widespread reduced efficacy occurred (Rinkevich, 2020). That is why it is essential to maintain the efficacy of Apivar® by practicing integrated pest management. This includes testing for Varroa mites at least monthly during the beekeeping season (pre- and post-treatment), and only treating when levels are above the economic threshold. Beekeepers should also implement cultural and physical controls to reduce the need for chemical treatment and alternate treatment of Apivar® with other non-synthetic treatments.

Reduced efficacy occurs when a product, such as Apivar®, is not used correctly. The misuse of Apivar® leads to individuals in a mite population demonstrating reduced amitraz susceptibility. Those mites that are not killed by Apivar® can reproduce and provide new individuals with their same genetic traits (Van Leeuwen and Dermauw, 2016; Whalon et al. 2008). This can lead to a larger population of mites with reduced susceptibility to treatment until the treatment is no longer economically effective. A population is unlikely to become 100% resistant to a product, but there comes a point when the percentage of mite mortality is no longer sufficient to justify the products use. A product is considered mostly effective when it kills greater than 90% of the population.

There are various mechanisms of reduced efficacy, including: enhanced detoxification (Field et al. 2001), target-site insensitivity (Rinkevich et al. 2013; Fournier, 2005; González-Cabrera et al. 2013) and reduced cuticular penetration (Balabanidou et al. 2018). The precise mechanisms causing the reduced efficacy to amitraz in Varroa mites are still unknown (Maggi et al. 2009; Maggi et al. 2011).

Amitraz has been used to control Varroa mite populations for more than 20 years in the USA. Since that time there have been many reported incidents of reduced efficacy of amitraz (Elzen, 2000; Maggi, 2010; Kamler, 2016). However, Varroa has maintained susceptibility to amitraz despite a long and extensive use history (Rinkevich, 2020). Since amitraz is currently the only synthetic active ingredient still viable to treat Varroa mites, it is important to continually test the efficacy of amitraz against mite populations worldwide.

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Since 2017, the Atlantic Tech Transfer Team for Apiculture (ATTTA) has evaluated the efficacy of Apivar® against Varroa mites in the Maritimes through field and lab-based studies. Results of studies done by ATTTA demonstrate that Apivar® is still a product with high efficacy, ranging from 89% to 98%, in Atlantic Canada. This may not be the case with other areas in Canada, as reduced efficacy of Apivar® is being reported elsewhere in Canada (Currie et al. 2010). To learn more about past efficacy studies on Apivar® completed by ATTTA, read "Summary of Miticide Efficacy for *Varroa destructor* Management in the Maritimes 2024" available at perennia.ca

For the past two summers, ATTTA has assessed the efficacy of amitraz against Varroa mites through lab-based studies. Lab experiments offer controlled environments where specific variables can be manipulated and more detailed investigations into the efficacy of active ingredients like amitraz can be conducted.

## Objectives

1. Determine Varroa mite levels across the Maritime region at three important time points during the 2024 beekeeping season;
2. Collect Varroa mites for miticide efficacy testing;
3. Establish temporal measurements for annual comparison of Varroa burden for the Maritime region;
4. Create a stored bank of honey bee samples for possible future testing

## Materials and Methods

### Regional Varroa Mite Survey

At three different time points during the beekeeping season, sampling supplies for honey bees were delivered to beekeepers across the Maritimes. Sampling supplies included: three ventilated, plastic, 250mL sampling bottles (containing a small amount of fondant) (Economy Wide-Mouth Plastic Bottle, Cole-Palmer®, Canada; Fondant, Ambrosia®, Canada), each labeled with a unique hive identification code; hive labels that corresponded to sampling bottles; three strips of Parafilm (Parafilm, Bermis Company, USA); Canada Post prepaid return packaging; and instructions for collecting honey bee samples. The ATTTA team collected samples directly from beekeeping operations in close proximity.

Beekeepers were instructed to randomly choose three representative colonies in their apiary. They placed a label on each of the selected hives for the duration of the study. To collect the sample, beekeepers used the collection bottle with the corresponding number to the hive label. Using a frame from the center of the brood nest, they ensured the queen was not on the selected frame. Samples were collected by angling the bottle at 45 degrees from the frame surface and gently dragging the bottle lip downward over the bees, causing them to roll into the bottle. This step was repeated until the amount of bees reached the marked fill line on the bottle (~300 bees or ½ cup of bees). Then the cover was secured onto the bottle and Parafilm was wrapped around the outer edge. This procedure was repeated with all three selected colonies. All collected samples were shipped to ATTTA as soon as possible.

Upon receiving the samples, bees were placed into the CO2 Varroa tester (CO2 Varroa tester, Swienty®, Denmark). Then CO2 (CO2 16g threaded cartridges, Impeccable Culinary Objects, Canada) was added to the cylinder containing the bees for a duration of four seconds, and then researchers continuously shook the Varroa tester for approximately one minute. Researchers then collected all fallen mites, which had been knocked off by the CO2, into the separate cylinder chamber and placed them into a labeled 20mL glass vial for amitraz efficacy testing to follow (20mL glass screw cap vials, Sigma-Aldrich®, Germany).

At this point, 20 honey bees from each sample were placed into a 50mL falcon tube (Cole-Palmer®) for long-term storage for future testing. The remaining bees were then placed into an alcohol wash shaker (Varroa shaker, Dancing Bee Equipment, Canada) and submerged in 70% ethanol (Ethanol, Reliable Maintenance Products, Canada). Researchers shook the bees for two minutes. After shaking, the jars were oriented vertically to let the alcohol and dislodged mites flow into the bottom jar while the bees remained in the upper jar. Researchers counted the number of mites in the bottom jar.

When providing information about economic thresholds to each beekeeper, researchers used the total number of mites (CO2 drop plus alcohol wash). The total number of honey bees per sample was also counted for determining economic thresholds. Once the number of mites per sample was calculated, the ATTTA team informed the respective beekeepers of the results for ongoing mite management.

### Amitraz efficacy testing

The baseline information about the lethal concentration of Varroa mites to amitraz was determined from a variety of studies from 2008 to 2020 (Table 1).

**Table 1. Lethal concentrations of amitraz to 50% or 90% of *Varroa destructor* populations, as determined from multiple lab-based studies.**

| Study | $LC_{50}$ (95% CI) | LC90 (95% CI) |
|---|---|---|
| Almecija *et al.* 2020 | 0.046 (0.034 – 0.061) µg/mL | 0.39 (0.2979 – 0.50789) µg/mL |
| Kamler *et al.* 2016 | 0.251 (0.167 – 0.36) µg/mL/vial | 1.417 (0.918 – 2.693) µg/mL/vial |
| Maggi *et al.* 2008 | 0.1 (3.25 e-002 - 0.15) µg/dish | NA |
| Rinkevich *et al.* 2020 | NA | 0.014 (0.010 – 0.017) µg/ vial |
| Rinkevich *et al.* 2020 | NA | 0.031 (0.021 – 0.045) µg/ vial |
| Rinkevich *et al.* 2020 | NA | 0.053 (0.037 – 0.077) µg/ vial |
| Rinkevich *et al.* 2020 | NA | 0.021 (0.017 – 0.025) µg/ vial |
| Rinkevich *et al.* 2020 | NA | 0.180 (0.082 – 0.394) µg/ vial |
| Rinkevich *et al.* 2020 | NA | 0.076 (0.042 – 0.138) µg/ vial |
| Rinkevich *et al.* 2020 | NA | 0.106 (0.085 – 0.132) µg/ vial |
| Rinkevich *et al.* 2020 | NA | 0.063 (0.049 – 0.080) µg/ vial |
| Rinkevich *et al.* 2020 | NA | 0.050 (0.036 – 0.066) µg/ vial |
| Rinkevich *et al.* 2020 | NA | 0.026 (0.021 – 0.033) µg/ vial |
| Rinkevich *et al.* 2020 | NA | 0.014 (0.007 – 0.025) µg/ vial |

To test the efficacy of amitraz for Varroa mite treatment, a lab-based study by Rinkevich (2020) was adapted. To start, solutions of amitraz (Amitraz, Sigma-Aldrich®, Germany) dissolved in profession grade acetone (Acetone, Solvable®, Canada) were prepared at concentrations of 2 ng/µL, 1 ng/µL, 0.2 ng/µL, 0.02 ng/µL, 0.002 ng/µL and 0 ng/µL. Then, using a micropipette, researchers applied 500 µL of each solution to a labeled 20mL vial. To evenly coat the inside of the vials with solution, researchers placed the vials on a roller (Stackable roller, Biolynx Inc., Canada) and, with the cap off, rolled the vials for two minutes at a speed of one rotation per minute. After allowing all acetone to evaporate, the final concentrations were 1 µg/vial, 0.5 µg/vial, 0.1 µg/vial, 0.01 µg/vial, 0.001 µg/vial and 0 µg/vial.

Immediately after preparing each vial, between 7 and 16 mites were transferred into a vial. The number of mites per vial was dependent on how many mites were available from the collection methods, where there was a target sample size of 10 mites per vial. Each vial was then covered with Parafilm and small air holes were punctured with a needle.

Vials containing mites were then placed in an incubator (Digital mini-incubator, VWR International, Canada) at 33 ± 1 °C for 24 hours. After 24 hours, mortality of all mites was assessed by probing mites with a paintbrush and checking for movement.

## Statistics

The mortality percentage for each concentration assessed, for each replication, was determined by dividing the number of dead mites in a vial by the total mites in a vial and then multiplying by 100. To calculate the lethal concentration of 50% of the mite population ($LC_{50}$) at 24 hours, a Probit test was performed using R version 4.0.3 (R Core Team, 2020). Then, researchers compared the $LC_{50}$ of the tested mite population to the $LC_{50}$ of an amitraz-sensitive USDA Lab population, which provided a resistance ratio (RR) (RR = (Tested Population $LC_{50}$) / (amitraz-sensitive Population $LC_{50}$)). The $LC_{50}$ of the amitraz-sensitive population was 0.008 µg/vial.

A Kruskal-Wallis Rank Sum Test, followed by a Dunn's Test for Multiple Comparisons, was performed to determine if concentration had a significant impact on mite mortality. These tests were chosen as the data is not normally distributed (Shapiro-Wilk Test for Normality).

## Results

### Regional Varroa Mite Survey

The ATTTA team sampled from a total of 23 different commercial beekeepers from New Brunswick, Nova Scotia and Prince Edward Island. These beekeepers represent a significant portion of Maritime honey bee colonies, where 17% of Nova Scotia colonies are represented and between 25 to 30% of all New Brunswick colonies managed are represented. All sampling occurred between May 11, 2024 to September 24, 2024. The survey included samples from 25 apiaries, 81 colonies, and included a total of 167 samples. For details on Varroa mite load, which is determined using an alcohol wash, please refer to Table 2 and Figure 1.

**Table 2. Summary of results for ATTTA Varroa Mite Survey between May 11, 2024 and September 24, 2024.**

| Sampling Period | Samples Positive for Varroa Mites | Samples Above the Economic Threshold Requiring Treatment |
|---|---|---|
| Trial 1 (Pre-Pollination)<br>May 11, 2024 – June 26, 2024 | 3 / 42 (7%) | 1 / 42 (2%) |
| Trial 2 (Mid-Season)<br>July 3, 2024 – August 22, 2024 | 20 / 75 (27%) | 4 / 75 (5%) |
| Trial 3 (Late-Season)<br>September 9, 2024 – September 24, 2024 | 34 / 50 (68%) | 5 / 50 (10%) |
| Combined sampling at three time points | 57 / 167 (34%) | 10 / 167 (6%) |

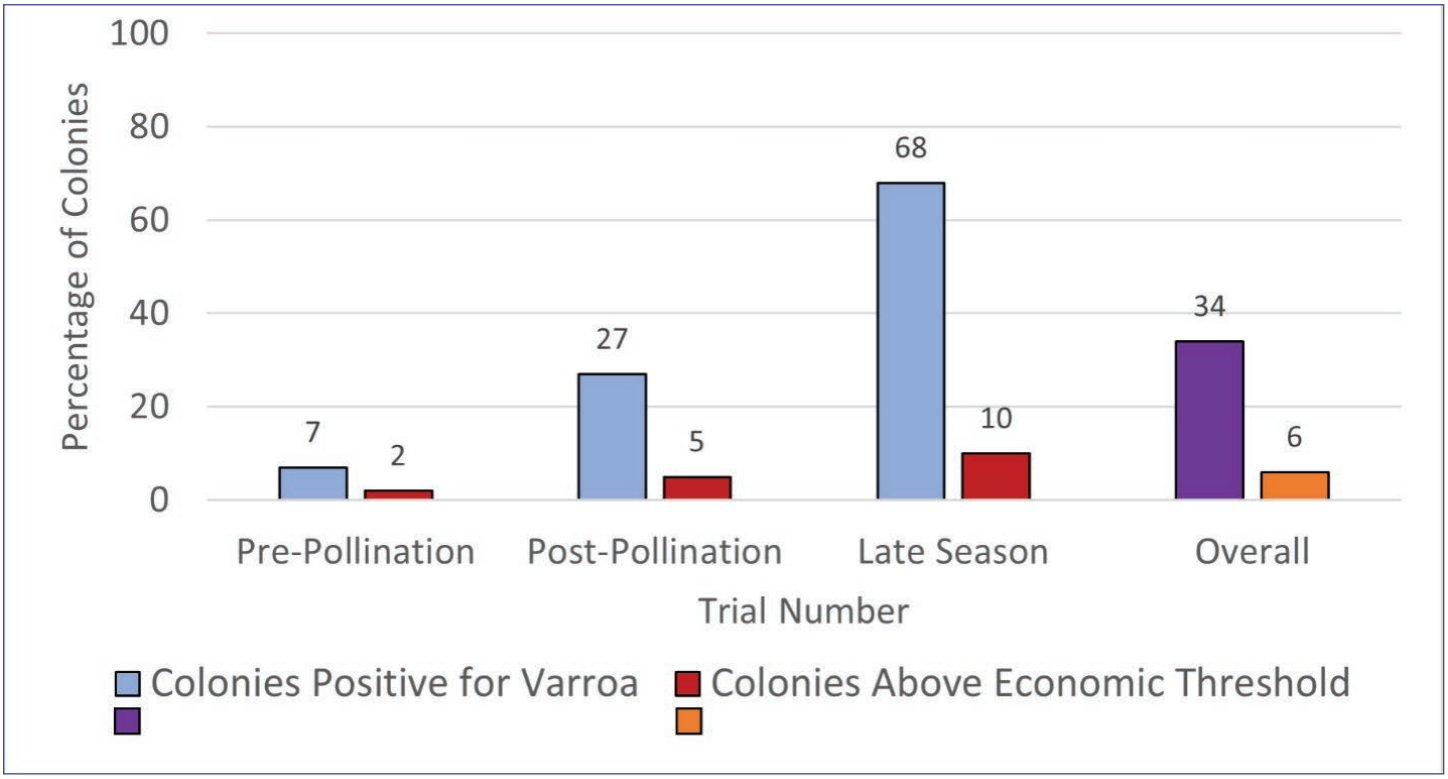



**Figure 1. Analysis of Varroa mite (*Varroa destructor*) levels for 81 Maritime colonies, from 23 different beekeepers, at three time points throughout the beekeeping season in 2024 (between May 11 and September 24).**

The number of samples positive for Varroa mites increased by 61% from early-season (7%) to late-season (68%) (Table 2 and Figure 1). The number of colonies above the economic threshold increased by 8% from early-season (2%) to late-season (10%) (Table 2 and Figure 1). The average number of bees sampled per colony was 312 (target number = 300).

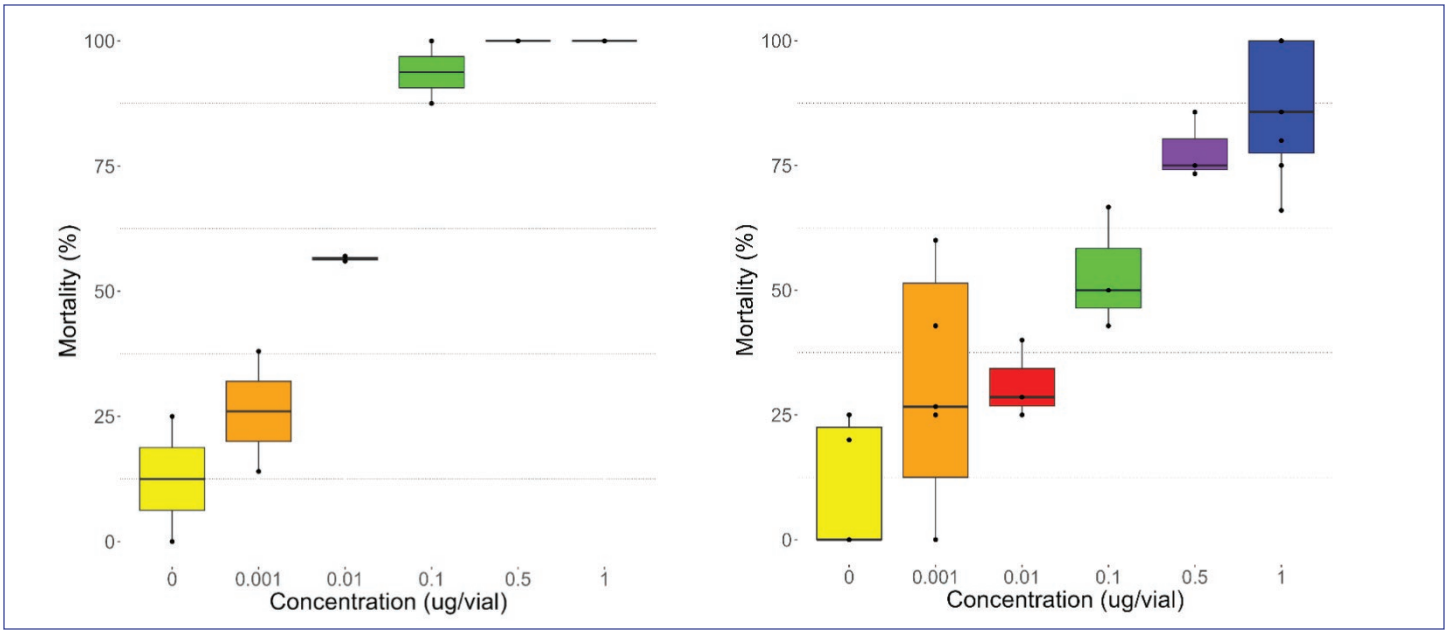
### Amitraz efficacy testing

It was determined that the $LC_{50}$ at 24 hours for the tested population of mites was 0.03 µg/vial ± 0.0077 µg/vial. Between the six tested concentrations, mortality ranged between 0 and 100% (Figure 2).

The resistance ratio was calculated to be 3.75. High amitraz resistance is considered a resistance ratio greater than 10 and low amitraz resistance is considered a resistance ratio less than 5 (Rinkevich, 2020).

**Figure 2. Analysis of the percent mortality of *Varroa destructor* when exposed to six different concentrations of amitraz in a 20mL vial for an incubation period of 24 hours at 33 ± 1 °C. (A) Analysis of the 2024 data collection, where sample size varies between 7 and 16 mites. (B) Analysis of the 2023 data collection, where sample size varies between 3 and 15 mites.**

**Results of the Kruskal-Wallis Rank Sum Test indicate that the concentration of amitraz does not have a statistically significant impact on mite mortality (*p-value* = 0.06831).**




## Discussion

The team determined the Varroa mite levels across the Maritime region at three important time points during the 2024 beekeeping season, where there was an increase in mites sampled between each trial. Researchers collected over 200 mites (138 were used for amitraz efficacy testing) and were able to assess the efficacy of amitraz against Varroa mite populations in the Maritimes. The team established temporal measurements for annual comparison of Varroa mite levels in the Maritimes and will be able to compare any changes to the temporal measurements starting next season. Finally, researchers prepare 167 samples of honey bees for storage, which will be used for possible future testing.

### Varroa mite levels across the Maritime region and annual comparison for Varroa burden

The results of the 2024 survey determined Varroa mite levels across the Maritime region increase throughout the beekeeping season as expected, where there is an increase in mites sampled between each trial. This makes early spring monitoring and treatment for Varroa mites crucial because populations can quickly get well beyond the economic threshold if waiting until the fall to treat colonies again. Beekeepers need to be vigilant with monitoring for mites each month to ensure that colonies will be strong and healthy by the time winter preparations occur. If a beekeeper is seeing mites during the early part of the beekeeping season, then they should be prepared to provide treatment by fall. There is also the potential that the number of mites will exceed the economic threshold prior to the fall if colonies are not treated in the spring, in which case a mid-season knock down treatment may be indicated to keep the mite population under control until fall treatments can occur.

Conventionally, with monthly monitoring, treatment is only indicated when the mite population is at or above the economic threshold. However, beekeepers should remember that current established economic thresholds need to be contemplated against the changing climate and lengthening of seasons. Also, treatments should be applied in consideration of the mite populations across an apiary and not individual colonies. Beekeepers in the Maritimes report having low levels of mites across an apiary with the exception of one or a small number of hives. This makes frequent and representative monitoring important for beekeepers. In recent years, the changing beekeeping season in the Maritimes has increased the challenge of managing Varroa mites.

### Amitraz efficacy testing

The results of the amitraz efficacy study for 2024 suggest low reduced efficacy of amitraz for a limited number of mites that were assessed (n = 138; RR = 3.75). These results differ from the previous year's study where the $LC_{50}$ at 24 hours for the tested population of mites was 0.283 µg/vial ± 0.0539 µg/vial, and the resistance ratio was calculated to be 35.4 for the limited number of mites assessed (n = 206; RR = 35.4). Multiple factors could have impacted both the 2023 and 2024 results, and the results should be interpreted with caution. One notable limitation is the relatively small sample size of mites that were included in the study, which may not be representative of the entire mite population. Additionally, the data was collected from just six beekeepers in 2023, and only two beekeepers in 2024, which further limits the generalizability of the findings. The reason only two beekeepers were included within the testing for 2024 is that most beekeepers did not have enough mites present to set-up a proper experiment replicate. Furthermore, there was variability in the sample sizes for each concentration of amitraz that was tested, which can introduce biases into the results. Given these limitations, it would be premature to make a conclusion about any level of reduced efficacy to amitraz over the past two years, and the study needs to be replicated with a larger sample size.

On a national level, a study conducted in Ontario, Canada in 2022 (Morfin et al. 2022) demonstrated that Apivar was mostly effective (90-97%) as an acaracide using the Pettis test (Pettis et al. 1998). The results of the 2024 Varroa mite survey also demonstrate that Apivar remains mostly effective in the Maritime region.

The results of the 2023 study concluded that the beekeeper has no significant impact on mite mortality, or reduced efficacy of amitraz. Therefore, no single beekeeper demonstrated a higher or lower level of reduced efficacy of amitraz in their apiary compared to others. This finding can be reassuring, as it indicates that the variation in mite mortality and amitraz effectiveness is not strongly associated with the specific practices of any of these beekeepers. Given that mites were only collected from two beekeepers in 2024, no statistical tests could be performed to determine if the beekeeper/operation had any significant impact on mite mortality in 2024.

Additionally, the results of the 2024 study were deemed inconclusive as to the effect of amitraz concentration on mite mortality. The sample size (n = 2 experimental replicates) is too small to have enough statistical power to have a statistically significant interaction. However, the general trend observed is that with increasing concentration of amitraz there is increased mortality. These results are comparable to the previous year's study where it was concluded that the concentration of amitraz does significantly impact mite mortality. In other words, higher concentrations of amitraz are more effective at killing the mites, suggesting a dose-response relationship between the amitraz concentration and mite mortality.

### Creating a stored bank of honey bee samples

Researchers have stored a sample of 20 honey bees from each of the 167 samples collected throughout the season. These stored samples will allow for future testing of indicators of honey bee health, such as testing for the presence of Tracheal mites (*Acarapis woodi*). No formal study has taken place in the Maritimes to test for Tracheal mites since the National Honey Bee Health Survey in 2017, and the ATTTA team feels a survey is warranted for our industry.

In the 2016 Canadian National Bee Health Survey, tracheal mites were not detected in any samples from NS, PE or NL, but were detected in 1 of 11 colonies sampled in NB by PCR methodology (Polymerase Chain Reaction). In the 2017 Survey, tracheal mites were not detected in any samples from NS or NL, but were detected in 1 of 8 colonies sampled in NB, and 1 of 7 colonies sampled in PE by PCR methodology.

### Summary

ATTTA plans to continue this survey for the next two years to compare the trend in Varroa mite levels over a time span. This will allow the industry to better understand when levels are peaking throughout the season, if the timing is changing and better plan for key times to monitor and treat for Varroa.

The team also plans to assess how the efficacy of amitraz changes year to year and the goal is to test a larger number of mites from more Maritime beekeepers. Having a larger sample size will allow researchers to draw more definitive conclusions on potential reduced efficacy of mite populations to amitraz. It is critically important to extend the effectiveness of amitraz-based products to control Varroa mites by practicing integrated pest management. There are many reported incidents of reduced efficacy of amitraz in the global beekeeping industry (Elzen, 2000; Maggi, 2010; Kamler, 2016). Therefore, it is the job of all beekeepers to manage Varroa mites using an integrated pest management plan.

Overall, the results of the survey indicate that beekeepers are doing a good job at managing mites in the Maritimes and that current treatments continue to be effective within the Atlantic region.

Finally, having stored samples of honey bees each year the study continues will allow researchers to complete future testing on honey bee health, such as testing for the presence of Tracheal mites. Testing for Tracheal mites is important right now for the industry as no formal study has taken place in the Maritimes to test for Tracheal mites since the National Honey Bee Health Survey in 2017.

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