ATTTA
Atlantic Tech Transfer Team for Apiculture

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

Atlantic Tech Transfer Team for Apiculture 2025 Report

Background

Varroa mites (*Varroa destructor*) remain the most widespread and economically damaging pest of the western honey bee (*Apis mellifera*)[1]. Varroa mites parasitizes both the adult bee and developing brood by feeding on both hemolymph and fat bodies[1]. Additionally, Varroa mites function as a viral vector and are responsible for the transmission of various honey bee viruses such as deformed wing virus, Israeli acute paralysis virus and acute bee paralysis virus[1]. In Canada, high Varroa mite 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[2], and the pest continues to present significant challenges to the Canadian beekeeping industry. To manage Varroa mites in Canada, Apivar® (3.3% amitraz as the active ingredient) is the only recommended synthetic miticide available.

Since 2017, the Atlantic Tech Transfer Team for Apiculture (ATTTA) has been testing the efficacy of the synthetic miticide Apivar®. The consensus across the Maritime region and supported through ATTTA's research is that Apivar® remains a product with good efficacy for the management of Varroa mites. However, it is becoming increasingly prevalent how important it is for the Maritime region to maintain the efficacy of Apivar®, by practicing integrated pest management and continuing to assess the efficacy of the product, as there are reports of amitraz resistance across the globe and within Canada.

Over the past 20 years, amitraz-resistant mite populations have been confirmed in the United States[3,4], France[3,5], Spain[6], Argentina[7], Mexico[8], Czech Republic[9], Portugal[10] and Algeria[11]. The issue of amitraz-resistance is wide spread and presents a threat to the global beekeeping industry.

Within Canada, there have been reports of decreasing Apivar® efficacy over the past decade. In 2022, a Canadian study detected reduced Apivar® efficacy in apiaries across Alberta, with product efficacy ranging from 22% to 92%[12] by using a Pettis test technique[13]. The majority of included apiaries showing below 55% efficacy. The study also demonstrated that Apivar® efficacy has decreased since 2020, when similar research was conducted[14].

Other Canadian provinces do not report reduced efficacy of Apivar®. Previous Canadian studies show high product efficacy of Apivar® (>90%) in Nova Scotia, Prince Edward Island, New Brunswick15 and Ontario16. Additionally, within the Maritime region, the Atlantic Tech Transfer Team for Apiculture has evaluated the efficacy of Apivar® from 2017 to 2024 and has demonstrated that Apivar® is still a product with good efficacy, ranging from 89% to 98%.

It is important to recognize that a population of Varroa mites is unlikely to become 100% resistant to a product such as Apivar® but there comes a point when the percentage of mite mortality is no longer sufficient to justify the product's use. A product is considered mostly effective when it kills greater than 90% of the population.

Current research provides a greater understanding of how

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amitraz resistance or reduced efficacy can occur within a population of Varroa mites. Amitraz acts as an octopamine receptor agonist, which means it initiates a physiological response when bonded to the receptor. When amitraz bonds to the octopamine receptor it causes constant excitation and paralysis of the Varroa mite and causes the mite to drop from the honey bee's back[17]. Secondarily, Varroa mites die due to starvation. Specific genetic mutations within the octopamine receptor, such as N87S and Y215H, have been associated with reduced efficacy of amitraz in Varroa mite populations in both France and the United States[3,4]. In Spain, another mutation (F290L) was found to be associated with reduced efficacy of amitraz in Varroa mites[6].

In Canada, Alberta researchers also examined the mechanism of reduced efficacy for Varroa mites and found that the majority of sampled mites carried the 2Y15H mutation[12]. The study concluded that the mutation Y215H is associated with amitraz reduced efficacy and it is widely distributed across Alberta[12]. This is the first study to evaluate the presence and prevalence of mutations associated with amitraz reduced efficacy in Canadian bee-keeping operations[12].

The ATTTA team has been assessing the efficacy of amitraz against Varroa mite populations within the Maritime region through laboratory-based methodology for three beekeeping seasons. Laboratory 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. This testing is one indication of the efficacy of Apivar® but needs to be complimented with other assessments to fully understand the efficacy of Apivar® within the region.

As part of this research, the ATTTA team has also been assessing Varroa mite population levels across the Maritime region throughout the beekeeping season. Understanding the current Varroa mite population trends will allow researchers and beekeepers to recognize when population levels deviate, which could occur with changes in Apivar® efficacy as well climate-related changes.

Objectives

1. Determine Varroa mite levels across the Maritime region at three important time points during the 2025 beekeeping season;
2. Establish temporal measurements for annual comparison of Varroa mite burden for the Maritime region;
3. Create a stored bank of honey bee samples for possible future testing;
4. Collect Varroa mites for miticide efficacy testing.

Materials and Methods

Regional Varroa Mite Survey

At three important time points during the beekeeping season (prior to pollination, mid-season and late season), sampling supplies were delivered to beekeepers across the Maritime region (Table 1). Sampling supplies included: three to six 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 to six strips of Parafilm (Para-film, Bermis Company, USA); prepaid return packaging; and instructions for collecting honey bee samples. The ATTTA team collected samples directly from some beekeeping operations.

Table 1. Timing, number of participants and number of samples per trial for ATTTA Maritime Regional Varroa Mite Survey 2025.

Trial	Date	Participants	Samples
1	Apr. 30 to May. 28	7 NS 9 NB 4 PE	71
2	Jul. 3 to Aug. 6	9 NS 7 NB 7 PE	76
3	Aug. 28 to Sep. 26	8 NS 6 NB 1 PE	58

Beekeepers were instructed to randomly choose three 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 (approximately 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 to six selected colonies. All collected samples were shipped to ATTTA as soon as possible.

Upon receiving the samples, bees were placed into the CO_2 Varroa tester (CO2 Varroa tester, Swienty®, Denmark). Then CO_2 (CO_2 16g threaded cartridges, Impeccable Culinary Objects, Canada) was added to the cylinder containing the bees until the activity of the bees slowed 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 CO_2 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).

Twenty honey bees from each sample were placed into a 50mL falcon tube (Cole-Palmer®) for long-term storage and 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. All collected mites were saved in 1.5mL vials (Labcon, United States) with 70% ethanol for future molecular testing.

When providing information about economic thresholds to each beekeeper, researchers used the total number of mites (CO_2 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 2).

Table 2. Lethal concentrations of amitraz to 50% or 90% of *Varroa destructor* populations, as determined from multiple lab-based studies.

Study	LC_{50} (95% CI)	LC_{90} (95% CI)
Almecija et al. 2020[18]	0.046 (0.034 – 0.061) µg/mL	0.39 (0.2979 – 0.50789) µg/mL
Kamler et al. 2016[19]	0.251 (0.167 – 0.36) µg/mL/vial	1.417 (0.918 – 2.693) µg/mL/vial
Maggi et al. 2008[20]	0.1 (3.25 e-002 - 0.15) µg/dish	NA
Rinkevich et al. 2020[21]	NA	0.014 (0.010 – 0.017) µg/ vial
Rinkevich et al. 2020[21]	NA	0.031 (0.021 – 0.045) µg/ vial
Rinkevich et al. 2020[21]	NA	0.053 (0.037 – 0.077) µg/ vial
Rinkevich et al. 2020[21]	NA	0.021 (0.017 – 0.025) µg/ vial
Rinkevich et al. 2020[21]	NA	0.180 (0.082 – 0.394) µg/ vial
Rinkevich et al. 2020[21]	NA	0.076 (0.042 – 0.138) µg/ vial
Rinkevich et al. 2020[21]	NA	0.106 (0.085 – 0.132) µg/ vial
Rinkevich et al. 2020[21]	NA	0.063 (0.049 – 0.080) µg/ vial
Rinkevich et al. 2020[21]	NA	0.050 (0.036 – 0.066) µg/ vial
Rinkevich et al. 2020[21]	NA	0.026 (0.021 – 0.033) µg/ vial
Rinkevich et al. 2020[21]	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)[21] was adapted. To start, solutions of amitraz (Amitraz, Sigma-Aldrich®, Germany) dissolved in profession grade acetone (Ace-tone, 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 at a speed of one rotation per minute until all the ace-tone had evaporated. 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.

After preparing each vial, between four and nine mites were transferred into a vial. The number of mites per vial was dependent on how many mites were available from the collection methods. 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

All statistical tests were performed using R version 4.4.0 (R Core Team, 2024).

Varroa mite loads were determined by counting the total number of mite per total number of bees in a sample and the value is presented as the number of mites per 100 bees (or percentage of mites). To assess if there is a significant difference in average mite loads between trials, a Kruskal-Wallis Rank Sum test, followed by a Dunn's Test for Multiple Comparisons, was performed. These tests were chosen as the data is not normally distributed (Shapiro-Wilk Test for Normality).

To assess if there is a significant difference in average mite load per trial between 2024 and 2025, a Wilcoxon Rank Sum test was performed. This test was selected as the data is not normally distributed (Shapiro-Wilk Test for Normality).

To calculate the lethal concentration of 50% of the mite population (LC_{50}) at 24 hours, a Probit test was performed. 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 LC50 of the amitraz-sensitive population is 0.008 µg/vial21. Low reduced efficacy is identified as an RR value less than five-fold, medium reduced efficacy is an RR value between five and 10-fold, and high reduced efficacy is an RR value greater than 10-fold[21].

Additionally, the data was fitted to a linear model to assess if there is a significant relationship between the concentration of amitraz and Varroa mite mortality.

Results

Regional Varroa Mite Survey

The results of the regional survey demonstrate that Varroa mite levels significantly increase throughout the beekeeping season (Figure 1; Figure 2; Table 3). The average Varroa mite levels in 2024 and 2025 are below the economic threshold of requiring treatment (1%) early to mid-season but increase above the economic threshold of treatment by late-season (Figure 1). In 2025, the average Varroa mite load for trial one was 0.02%, for trial two the average had increased to 0.16% and by trial three the average had increased to 1.17% (Figure 1).

When analyzing individual trials, there is not a significant difference in average Varroa mites loads between 2024 and 2025 (Figure 1; Figure 2; Table 4).

The average number of bees per sample in 2025 was 304 and the average number of bees per sample in 2024 was 312.

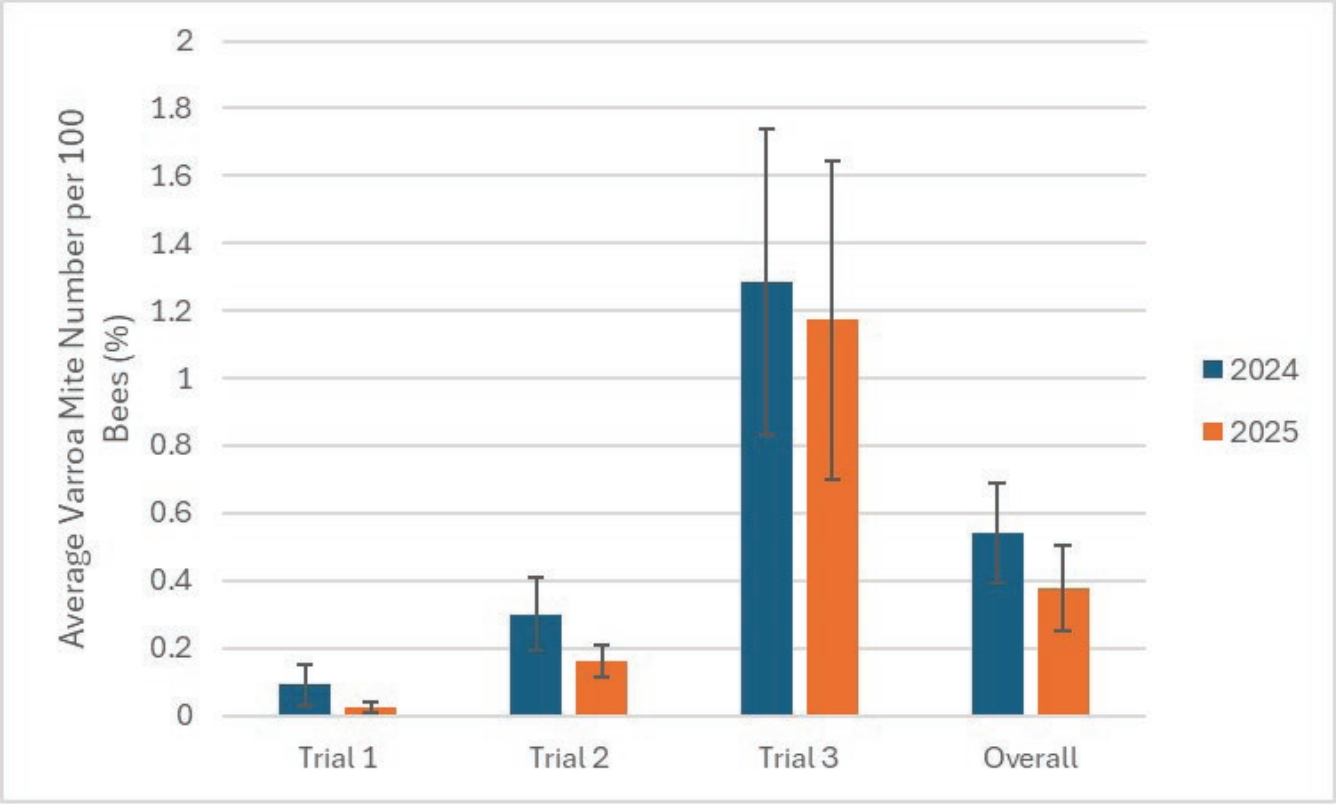
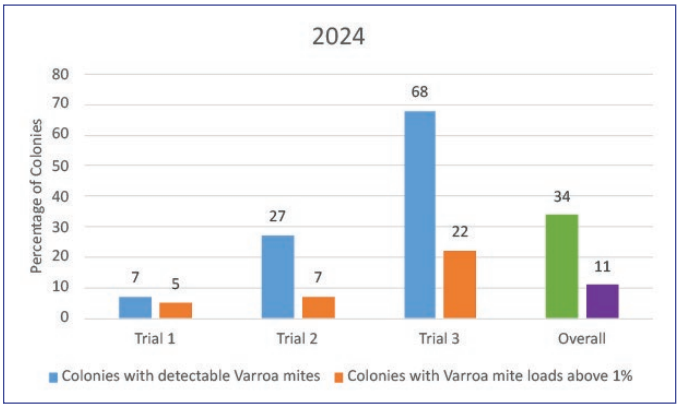


Figure 1. Average Varroa mite (*Varroa destructor*) number per 100 honey bees (%) sampled in the Maritime region across the beekeeping season with a total of 23 commercial beekeepers represented in 2024 and 2025. Trial one occurred during April through to June, trial two occurred during July and August and trial three occurred during August and September. Error bars represent standard deviation.



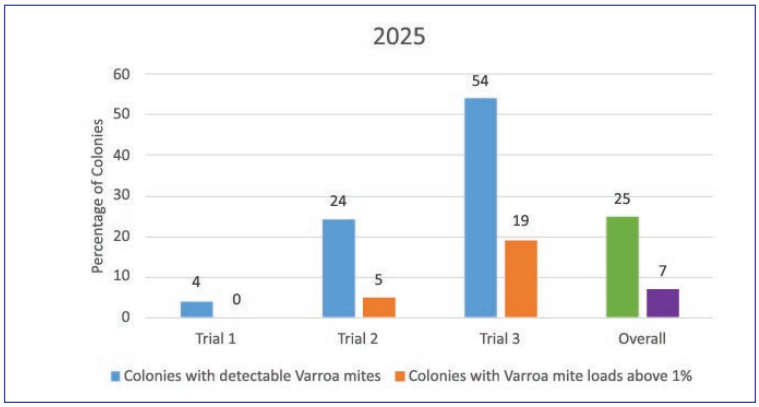


Figure 2. Comparison of Varroa mite (*Varroa destructor*) loads in the Maritime region between 2024 and 2025 at three important time periods in the beekeeping season (prior to pollination, mid-season and late season), as well as overall averages for the combined three trials across the sampling period. Trial one occurred during April through to June, trial two occurred during July and August and trial three occurred during August and September.

Table 3. Results of Dunn's Test for Multiple Comparisons to assess if there is a significant difference in Varroa mite (Varroa destructor) loads in the Maritime region between trials in 2024 and 2025. Significant p-Values (< 0.05) are in bold.

Year	Trial Comparison	p-Value
2024 and 2025 combined	Trial 1 and Trial 2	**p = 0.0013257**
2024 and 2025 combined	Trial 1 and Trial 3	**p < 0.05**
2024 and 2025 combined	Trial 2 and Trial 3	**p < 0.05**
2024	Trial 1 and Trial 2	p = 0.05530322
2024	Trial 1 and Trial 3	**p < 0.05**
2024	Trial 2 and Trial 3	**p < 0.05**
2025	Trial 1 and Trial 2	**p = 0.01030172**
2025	Trial 1 and Trial 3	**p < 0.05**
2025	Trial 2 and Trial 3	**p < 0.05**

Table 4. Results of Wilcoxon Rank Sum tests to compare average Varroa mite (*Varroa destructor*) loads in the Maritime region per trial between 2024 and 2025. Significant p-Values (< 0.05) are in bold.

Trial Number	p-Value
1	0.5062
2	0.6358
3	0.2126

When Varroa mites were collected through sampling methods, the Carbon Dioxide (CO_2) wash was only able to collect a portion of the total mites (Table 5), with the 70% alcohol wash frequently collecting additional mites within the sample.

Table 5. The average percentage of Varroa mites (*Varroa destructor*) collected from a Carbon dioxide (CO_2) wash when followed by an additional 70% alcohol wash in 2024 and 2025 when Varroa mites were collected from the sample.

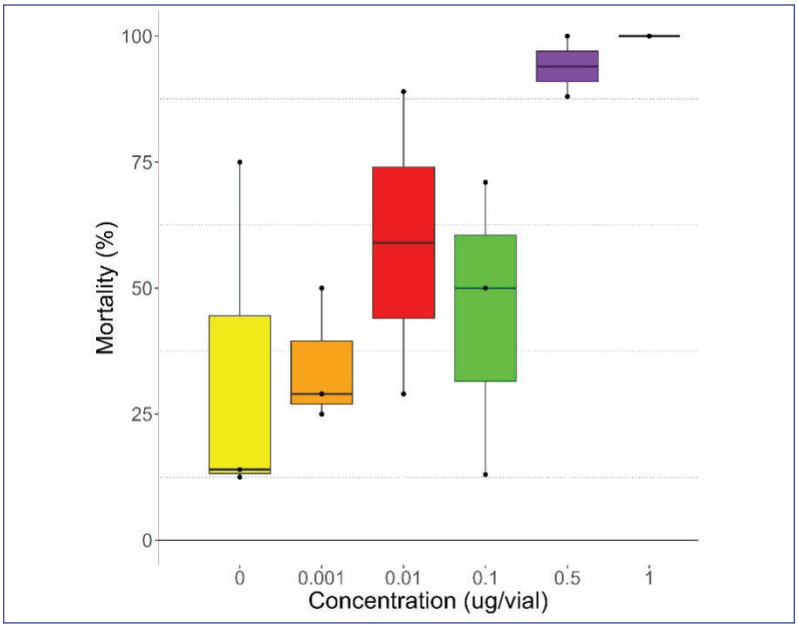
Year	Average percentage of Varroa mites collected from CO2 wash (1^{st} wash)	Average percentage of Varroa mites collected from additional 70% alcohol wash (2^{nd} wash)
2024	44% ± 7.8	56% ± 7.8
2025	19% ± 6.5	81% ± 6.5
Combined 2-year average	33% ± 5.6	67% ± 5.6

NB: samples with 0 collected Varroa mites were not included in the averages.

Amitraz efficacy testing

In 2025, researchers collected 252 Varroa mites of which 133 were used for amitraz efficacy testing.

For 2025 amitraz efficacy testing, there is a significant relationship between the concentration of amitraz and Varroa mite mortality (Figure 3; Table 6), where increased concentration results in higher mortality. Researchers also investigated all amitraz efficacy data, including data from 2023, 2024 and 2025, and found that across the three years, the concentration of amitraz does have a significant impact on mite mortality (Figure 4; Table 6).



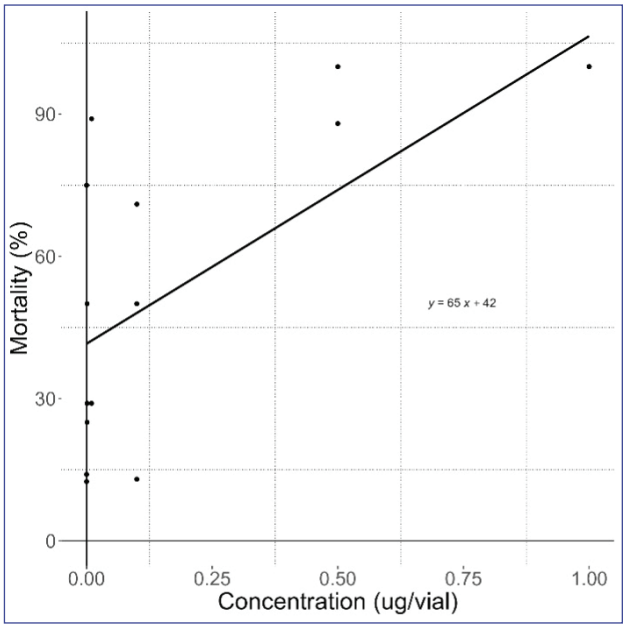
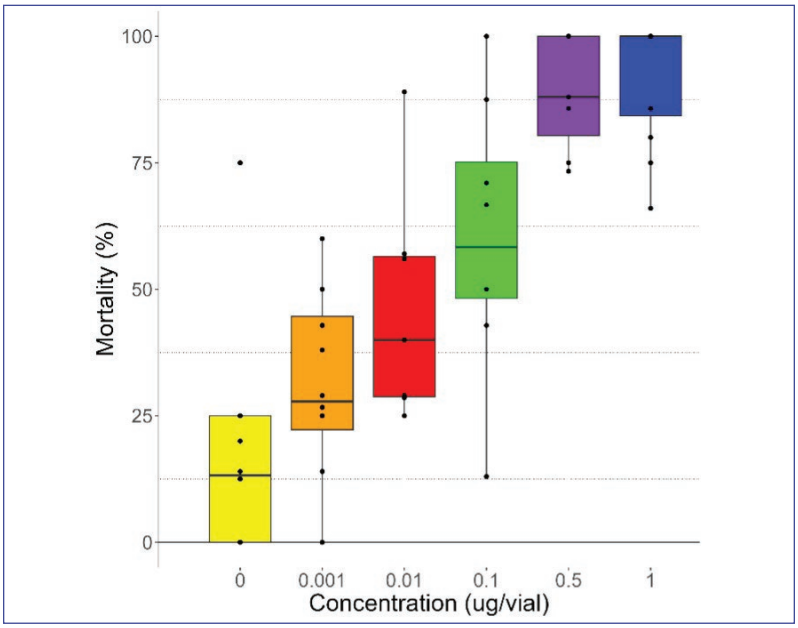


Figure 3. Analysis of the percent mortality of Varroa mites (*Varroa destructor*) when exposed to six different concentrations of amitraz in a 20mL vial for an incubation period of 24 hours at 33 ± 1°C. Varroa mites were collected across the 2025 beekeeping season in the Maritime region and between four and nine mites were tested per treatment group with the experiment being replicated three times (n = 3).



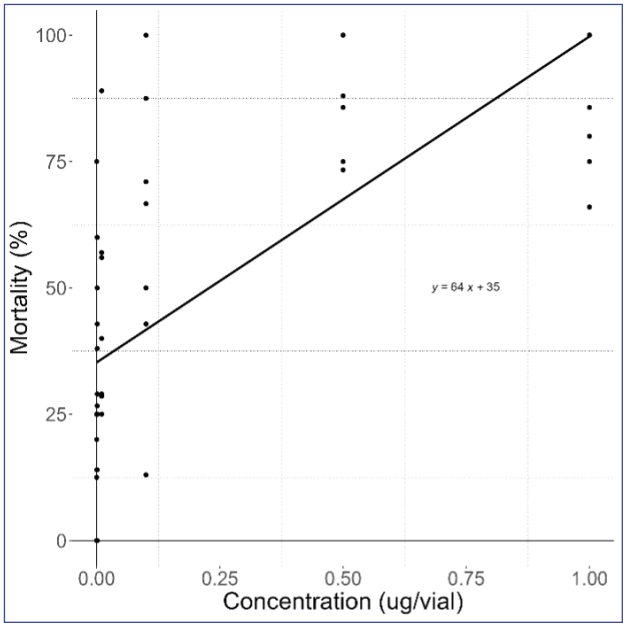


Figure 4. Analysis of the percent mortality of Varroa mites (Varroa destructor) when exposed to six different concentrations of amitraz in a 20mL vial for an incubation period of 24 hours at 33 ± 1°C. Varroa mites were collected across three beekeeping season (2023 – 2025) in the Maritime region and between three and 16 mites were tested per treatment group with the experiment being replicated 12 times (n = 12).

Table 6. Results of fitting linear models to assess if there is a significant relationship between concentration of amitraz and Varroa mite (*Varroa destructor*) mortality in a laboratory setting, where mites were collected within the Maritime region. Significant p-Values (< 0.05) are in bold.

Year	p-Value
2025 data	**0.001119**
2023, 2024 and 2025 data	**p < 0.05**

For 2025 only, it was determined that the LC_{50} at 24 hours for the tested population of Varroa mites was 0.1 µg/vial ± 0.04 µg/vial. Between the six tested concentrations, mortality ranged between 12.5% and 100% (Figure 3). The resistance ratio was calculated to be 12.5.

When analyzing all data from 2023 to 2025, it was determined that the LC_{50} at 24 hours for the tested population of Varroa mites was 0.16 µg/vial ± 0.03 µg/vial. Between the six tested concentrations, mortality ranged between 0% and 100% (Figure 4). The resistance ratio was calculated to be 20.

The calculated LC_{50} and RR value for each year of this study has varied (Table 7).

Table 7. Summary of results for laboratory efficacy testing of amitraz against Varroa mites (*Varroa destructor*) collected within the Maritime region between 2023 and 2025.

Year	Number of mites per treatment group	Number of replicates	Calculated LC_{50}	Resistance ratio (RR)	Category of reduced efficacy
2023	3 - 15	7	0.28 µg/vial ± 0.05 µg/vial	35	High
2024	7 - 16	2	0.03 µg/vial ± 0.008 µg/vial	3.75	Low
2025	4 - 9	3	0.1 µg/vial ± 0.04 µg/vial	12.5	High
2023/24/25	3 - 16	12	0.16 µg/vial ± 0.03 µg/vial	20	High

Discussion

Objective one - Determine Varroa mite levels across the Maritime region at three important time points during the 2025 beekeeping season

Researchers determined the Varroa mite levels across the Maritime region at three important time points during the 2025 beekeeping season, where there was a significant increase in Varroa mites sampled between each trial. In 2025, the average Varroa mite levels are below the economic threshold of requiring treatment (1%) early to mid-season but increase above the economic threshold of treatment by late-season. This makes early spring monitoring and treatment for Varroa mites crucial, because the population can quickly get beyond the economic threshold if waiting until the fall to treat colonies again. It is also important that beekeepers start late-summer/early-fall mite treatments as soon as possible when levels are at or exceeding 1% to help ensure healthy winter bees with low virus loads.

Varroa mite levels increase throughout the beekeeping season as mites can grow their population along with honey bee brood production and since Varroa mite treatments are typically not applied during the summer months, when beekeepers are focused on honey production, there are no chemical treatments actively knocking down their population until fall treatments occur.

The other reason why an increase in Varroa mites is seen throughout the beekeeping season is due to sampling methods. This study used alcohol washes to assess Varroa mite levels which is only targeting phoretic mites living on adult bees. This type of sampling only indirectly represents the percentage of reproductive mites which are living in honey bee brood cells. Honey bee brood production increases during the spring and peaks during the summer, which means there is an increase of mites existing in brood cells rather than on adult bees during this period of time. Brood production slows down towards late-summer and early-fall which means there is an increase of mites in their phoretic life stage and more mites will be collected during sampling.

A recently published study which took place in Ontario beekeeping operations (2015 – 2019) demonstrated a similar seasonal pattern in Varroa mite populations[22]. This seasonal pattern can be described by an initial spike in early spring around hive opening, followed by a sharp decline due to initial spring treatments, succeeded by a gradual population increase over the summer, leading to exponential growth in the mite population in early fall. This seasonal trend is consistent with previous knowledge on Varroa mite population dynamics and global observations based on reported mite levels to the World Organization for Animal Health[23].

Additionally, it is important that beekeepers understand that 0% Varroa mite load, as determined by an alcohol wash, does not mean that no Varroa mites are present within the colony. There is always a background population of Varroa

mites within a single colony and without frequent and representative monitoring, undetected Varroa mite populations can quickly grow beyond treatment thresholds.

Within the context of this study, researchers determined that the first wash on a sample of bees does not collect 100% of the potentially collected mites and that frequently a second wash will collect additional mites. This study did perform two different Varroa washes (Carbon dioxide and 70% alcohol), which have different levels of efficacy for collecting mites, but it can be recognized that the second wash collected between 56 to 81% additional mites. It can be inferred that some amount of inaccuracy and variability exists for mite monitoring methods such as an alcohol wash. Therefore, beekeepers should be conscientious of their sampling techniques. It is recommended that beekeepers monitor frequently and representatively to have the best understanding of Varroa mite levels across their operation.

Objective two - Establish temporal measurements for annual comparison of Varroa mite burden for the Maritime region

Per individual trial there was no significant difference in the average Varroa mite load between 2024 and 2025, indicating that these assessed levels may be within the normal range for the Maritime region and that no significant change has occurred in Varroa mite population dynamics between 2024 and 2025. These annual differences are likely to reflect seasonal influences (weather, winter losses, etc.) resulting in variation in population per year. A main priority of this research is to establish a baseline of the region's mite levels, and this will be better understood after a third season of data collection.

Understanding the current Varroa mite population trends in the Maritimes will allow researchers and beekeepers to recognize when population levels deviate. Varroa mite population trends may change as a result of reduced efficacy to Apivar®, which was assessed within this study, but also could occur due to changes in climate. Significant deviations from the average temperature within a region for prolonged durations may impact honey bee brood production, which also impacts Varroa mite reproduction. For example, having increased daily temperatures throughout the fall would allow increased brood production, which would also allow for Varroa mite population growth. Having increased Varroa mite pressure late into the beekeeping season puts honey bee colonies at risk of dying throughout the winter months as a result of winter bees developing in a high Varroa mite environment.

Objective three - Create a stored bank of honey bee samples for possible future testing;

Researchers have stored a sample of 20 honey bees from each of the 205 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) or honey bee virus profiles and viral loads.

Objective four - Collect Varroa mites for miticide efficacy testing

Researchers collected 252 Varroa mites (133 were used for amitraz efficacy testing) and were able to perform testing to help assess the efficacy of amitraz against Varroa mite populations in the Maritimes.

Overall, there is a significant correlation between the concentration of amitraz and Varroa mite mortality, where increased amitraz concentration results in higher Varroa mite mortality. This correlation was observed in 2025, and when looking at the combined results from 2023, 2024 and 2025. This correlation demonstrates that the methodology is achieving expected trends in the data and it is an option for helping to assess amitraz efficacy.

Amitraz efficacy is directly related to the efficacy of Apivar®. The study by Rinkevich (2020)[21] assessed the relationship between amitraz efficacy and Apivar® efficacy by using a Pettis test[13]. Their research determined that a resistance ratio less than five (low reduced efficacy) correlates to an Apivar® efficacy of greater than 90%, a resistance ratio between five and 10 (medium reduced efficacy) correlates to an Apivar® efficacy of approximately 85 to 90% and a resistance ratio greater than 10 (high reduced efficacy) correlates to an Apivar® efficacy of less than approximately 85%.

It is important to understand that this laboratory testing is only one option available to beekeepers and researchers and there are other factors and testing to be considered before making any conclusions on Apivar® efficacy.

To fully understand if reduced efficacy of Apivar® exists within an operation, beekeepers and researchers need to investigate the efficacy of the product at various levels, which includes Varroa mite monitoring before and after treatment to understand if products are effectively knocking down mite populations, the Pettis test[13] (field-based testing), laboratory testing on product efficacy[21] and molecular testing for resistant genotypes3-10. All of these options together can start to provide a strong understanding of Apivar® efficacy.

The results of the amitraz efficacy study for 2025 suggest high reduced efficacy of amitraz for a limited number of mites

that were assessed (n = 107; RR = 12.5). An amitraz efficacy value of 12.5 would correlate to an Apivar® efficacy of approximately 80%[21]. These results differ from the previous year's study where it was suggested low reduced efficacy of amitraz for a limited number of mites tested (n = 138; RR = 3.75). Furthermore, in 2023, the results suggested high reduced efficacy of amitraz (n = 206; RR = 35.4). Multiple factors could have impacted the results of the study over the past few years and given the large amount of variation, all re-sults should be interpreted with caution.

One notable limitation is the relatively small sample size of Varroa 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 bee-keepers in 2023, two beekeepers in 2024, and five beekeepers in 2025, which further limits the generalizability of the findings. The reason only a small number of beekeepers were included within the testing 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 three years and the study needs to be replicated with a larger sample size.

To help address the issue of not collecting enough Varroa mites for amitraz efficacy testing, researchers need to increase the percentage of mites being collected from the CO_2 wash. On average, the CO_2 wash collects between 19 to 44% of the total collected mites from the samples when mites are found to be present. This leaves an additional 56 to 81% of the collectable mites unusable for amitraz efficacy testing. Researchers are currently investigating improvements to the mite collection methodology. One way to increase the number of mites collected would be to expose the bees and mites to a more consistent flow of CO_2. Rather than using the current CO_2 Varroa tester (CO2 Varroa tester, Swienty®, Denmark), it would be beneficial to set-up a CO_2 tank that allows for a set air flow rate. Additionally, rather than manually shaking the bees it would be beneficial to use an automatic shaker that controls how vigorously the bees are shook to increase mite drop. Both the application of CO_2 and consistency and speed of shaking samples of bees have an impact on Varroa mite collection[24].

To compare the preliminary results of this research to other amitraz efficacy studies within Canada, research demonstrates that amitraz reduced efficacy exists within some regions of Canada. Apiaries in Alberta have found Apivar® efficacy ranging from 22% to 92%12 by using a Pettis test technique13 and this reduced efficacy was determined to be associated with the mutation of Y215H within the octopamine receptor. In contrast, a study conducted in Ontario[16] demonstrated that Apivar was mostly effective (90-97%) as an acaracide using the Pettis test[13].

Conclusion

The ATTTA team plans to continue this survey for another season to establish a baseline of the region's Varroa mite levels. This will allow the industry to better understand Varroa mite populations dynamics across the season, recognize significant annual changes and better plan for key times to monitor and treat for Varroa mites.

Researchers also plan improved mite collection methods so that a larger sample of mites can be assessed for amitraz efficacy. Having a larger sample size will allow researchers to draw more definitive conclusions on potential reduced efficacy of Varroa mite populations to amitraz.

Overall, this study demonstrates the importance of beekeepers frequently and representatively monitoring mite levels within their operation to know when levels are increasing and allow for early intervention of treatment when levels are at or exceeding 1%.

Additionally, given recent reports of reduced efficacy of Apivar® across the globe and within Canada, it is essential that Maritime beekeepers practice all aspects of integrated pest management to main the efficacy of the product. 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 (1%). Beekeepers should also implement cultural and physical controls to reduce the need for chemical treatment, follow all manufacturer instructions when applying treatments and alternate treatment of Apivar® with other non-synthetic treatments. The Maritime beekeeping industry is at high risk of losing the efficacy of Apivar® if integrated pest management is not practiced.

The results of the 2025 Maritime regional survey on the prevalence of Varroa mites (*Varroa destructor*), combined with other indicators, suggests an overall, unquantified, slippage in efficacy of amitraz for the control of Varroa destructor. The mite resistance ratio calculation for amitraz, based on our limited sample size, is categorized as high. Although, it cannot be stated with confidence that the efficacy of Apivar® has dropped below the critical 80% level (minimally effective) for the Maritime region. Ongoing survey work and active-ingredient testing will calculate better the changes in overall efficacy. The recommendation from this season's research is for beekeepers in the Maritimes to continue using Apivar® along with increasing reliance on alternative acaricides. Vigilant surveillance is needed to ensure honey bee health

and effective mite control. Successful beekeepers will regularly monitor mite populations and transition away from the reliance on a single acaricide product.

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