ATTTA
Atlantic Tech Transfer Team for Apiculture

JULY 2024

Report – Summary of Miticide Efficacy for Varroa destructor Management in the Maritimes 2024

Varroa destructor

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 2022 - 2023. There were seven out of nine provinces that listed Varroa mites as a top three reason for colony winter loss in the winter of 2022 to 2023. This does not include Newfoundland 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.

Figure 1. Varroa destructor on the back of a honey bee (Apis mellifera)

Mite treatments

Apivar®, with the active ingredient amitraz, came into use in 2009 and is currently still a viable option in Atlantic Canada for Varroa mite treatment. However, the industry needs to be cautious of resistance development like previous products. For example, Apistan® came into use in 1995, and then ceased use in 2001 due to resistance development (Rinkevich, 2020). CheckMite® came into use in 2002, and then ceased use in 2005 due to resistance development (Rinkevich, 2020).

Figure 2. Apivar® strips being placed in a hive.

Multiple past studies done in Atlantic Canada demonstrate that Apivar® is still a product with high efficacy ranging from 89% to 98%, with studies done over the last 7 years by the Atlantic Tech Transfer Team for Apiculture (ATTTA). This may not be the case with other areas in Canada, as reduced efficacy of Apivar® is being reported across the country. This makes maintaining the efficacy of Apivar® crucial to our industry, as currently this is the only recommended synthetic product for use.

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Prince Edward Island Wild Blueberry Growers Association
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According to the CAPA Winter Loss Survey 2022 - 2023, Apivar® is currently the most used chemical treatment for Varroa mites used in Canada, with six out of ten provinces listing Apivar® as their top treatment. This just highlights how reliant on Apivar® we are in Canada, and the need to maintain its efficacy.

To prevent reduced efficacy beekeepers need to practice integrated pest management. This includes testing for Varroa mites at least monthly (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. Also, remember to follow all product labels to ensure the amount, timing and frequency of the product is safe for honey bees. Not following product labels can be damaging to bees.

Reduced efficacy

Reduced efficacy occurs when a product, such as Apivar®, is not being used correctly. The misuse of Apivar® leads to some individuals in a mite population becoming unsusceptible to the active ingredient amitraz. 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 unsusceptible to treatment until the treatment is no longer effective. A population is unlikely to become 100% resistant to a product, but there comes a point when only a small percentage of the population is being killed by the product, and there appears to be no population knock down, which is when the term resistant is appropriate to use. Generally, a treatment is no longer effective when the cost of the product is greater than the benefit of the treatment, and a product is considered mostly effective when it kills 90% or more of the population.

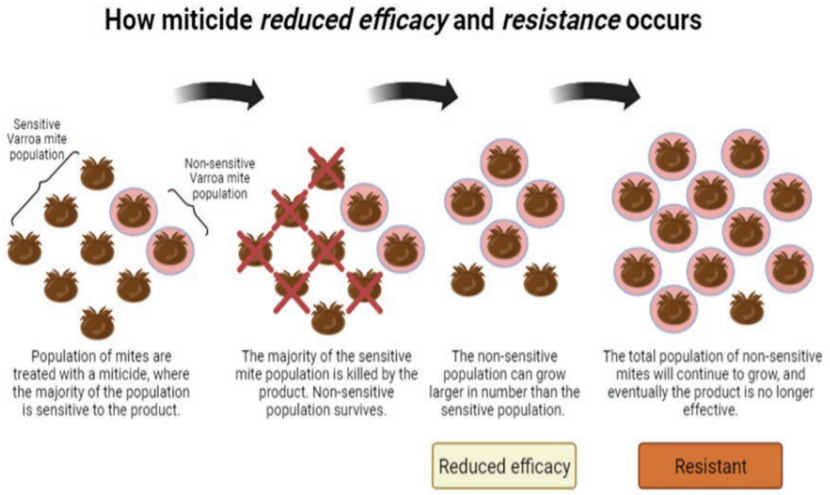

Figure 3. Schematic of how reduced efficacy and resistance occurs within a population of mites (Biorender).

Physiological mechanisms for resistance in Varroa mites can be split into five different categories (Mitton et al. 2022). Mechanisms can be inherited which reduce or prevent the penetration of miticides into the body of the mites (Balabanidou et al. 2018). Mites have enzymes and proteins in their body which can bind to molecules in miticides and transfer them away from the targeted site to the fat body or hemolymph for storage (Mitton et al. 2022). Behaviors can be developed by mites to avoid toxic compounds (Mitton et al. 2022). Mites can increase the level of enzymes in their body, which increases the rate of metabolism, and breaks down miticides to less toxic forms (Field et al. 2001). Also, the target site for the miticides can be altered, which results in the miticide being less toxic (Rinkevich et al. 2013; Fournier, 2005; González-Cabrera et al. 2013; Mitton et al. 2022). It is important to understand that resistance can also be caused or enhanced due to poor management practices of beekeepers.

When trying to control Varroa mites, a variety of factors can contribute to reduced efficacy including the type of miticide, the amount and concentration applied, the mode of action of the miticide to mites, the persistence of previous treatments, and the number of applications.

Once mites develop resistance, they can migrate from one hive to another, across operations and even geographic regions. Resistant mites will weaken a hive and the remaining bees may relocate to nearby colonies.

Also, repeated usage of these miticides may cause accumulation and persistence of chemicals in the hive products such as wax and honey. Long term use of these hive products, and accumulation of chemicals has the potential to reduce the efficacy for treatment against Varroa mites through chronic low-level exposures.

Table 1. Mode of action for various Varroa destructor chemical treatments.

Product	Active Ingredient	Mode of Action
Apivar®	Amitraz	Octopamine receptors
Bayvarol®	Flumethrin	Voltage-gated sodium channel
Apistan®	Fluvalenate	Voltage-gated sodium channel
Thymovar®	Thymol	Octopamine receptors, Tyramine receptors and GABA receptors
Formic Pro, formic acid and MAQS	Formic acid	Mitochondrion disruption
Oxalic acid	Oxalic acid	Mitochondrion disruption

Mode of action

Amitraz® works by acting on the octopamine receptors of mites (Chen et al. 2007). It leads to constant excitation and paralysis, followed by mite drop from the bee's back. Secondarily, Varroa mites die due to starvation because of this paralysis. Amitraz acts by contact only.

Bayvarol® and Apistan® work by producing an over-excitation of the nervous system of the mite. In particular, they bind to the voltage-gated sodium channel, present on the membrane of neurons (Dong et al. 2014). The inability of the channel to close and reset the neuron to the resting state leads to paralysis and death of the mite (Dong et al. 2014).

Some essential oils, such as thymol, appear to have neurotoxic effects by binding and affecting the function of octopamine receptors (Enan, 2005). In addition, thymol binds to tyramine receptors (Enan, 2005). There is further evidence that thymol affects the function of GABA receptors in insects, which are important for nerve signal transmission (Priestley et al. 2003). The presence of multiple targets for thymol makes it more difficult for resistance to occur.

Formic acid and oxalic acid work by disrupting the mitochondria, and then the cells of the mite cannot function resulting in death (Song and Scharf, 2009).

Synthetic products, such as Apivar®, are absorbed by the mite and tend to affect one single protein target, such as the voltage-gated sodium channel (flumethrin and fluvalinate) and octopamine receptors (amitraz). This specificity on single targets makes it highly likely that the mites will develop resistance by mutations in those targets, as has indeed been reported. In addition, mites can also develop resistance with detoxification enzymes that degrade or get rid of these chemicals from the body.

The mechanism of reduced efficacy to pyrethroids (Apistan®) in Varroa destructor is well known. It is associated with mutations at the voltage-gated sodium channel (González-Cabrera et al. 2018; González-Cabrera et al. 2016; Hubert et al. 2014). On the other hand, the precise mechanisms causing the reduced efficacy to amitraz, in V. destructor are still unknown (Maggi et al. 2009; Maggi et al. 2011).

Is there reduced efficacy of treatments in Atlantic Canada?

Studies done by the Atlantic Tech Transfer Team for Apiculture (ATTTA) over the last 7 years demonstrate that our region still has a variety of products with high efficacy against Varroa mites.

A study done by ATTTA in 2017 and 2018 assessed the efficacy of Apivar® and Bayvarol® (Olmstead et al. 2019). Miticide testing was conducted with 153 colonies located in 8 different apiaries across the Maritimes.

The testing was done using specialized miticide efficacy test kits following a modified Pettis test. Full details on the methodology can be found within the publication by Olmstead et al. (2019)

The study found that Apivar® caused 99.8% mortality of Varroa mites and Bayvarol caused 96.5% mortality of Varroa mites, although Bayvarol demonstrated greater variability in mite mortality (Figure 4).

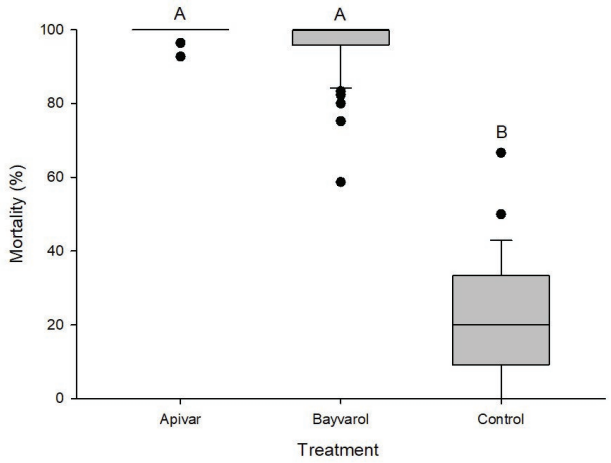

Figure 4. Box plot demonstrating comparison of Varroa mite (Varroa destructor) mortality from Apivar®, Bayvarol®, and a procedural control in honey bee colonies (Apis mellifera) in 2017 and 2018 in Canadian Maritimes. Letter groupings that differ show significant differences. Boxplots display interquartile range, median, and outliers (Olmstead et al. 2019).

In 2017, a second study involving Apivar® was conducted by the ATTTA team. There were 44 hives from three different apiaries in Nova Scotia sampled for their Varroa mite levels. Apivar®, Apistan®, and one commercial 'natural' miticide alternative – Thymovar® were each placed in 12 – 14 of the 44 hives following label recommended application rates.

Prior to the initiation of treatment, mite levels were assessed with an alcohol wash. After the recommended treatment period, the miticides were removed from the hives in early June and mite levels were assessed again.

In addition to assessing pre- and post-treatment Varroa levels with an alcohol wash, the relative strength of each colony was assessed by counting the number of "seams of bees", defined as the spaces in between frames within a hive mostly or entirely occupied by worker honey bees.

The average mite levels for all three miticides were significantly reduced after treatment (Figure 5). Most of the colonies included in the trial increased in size and strength over the treatment period (Figure 5).

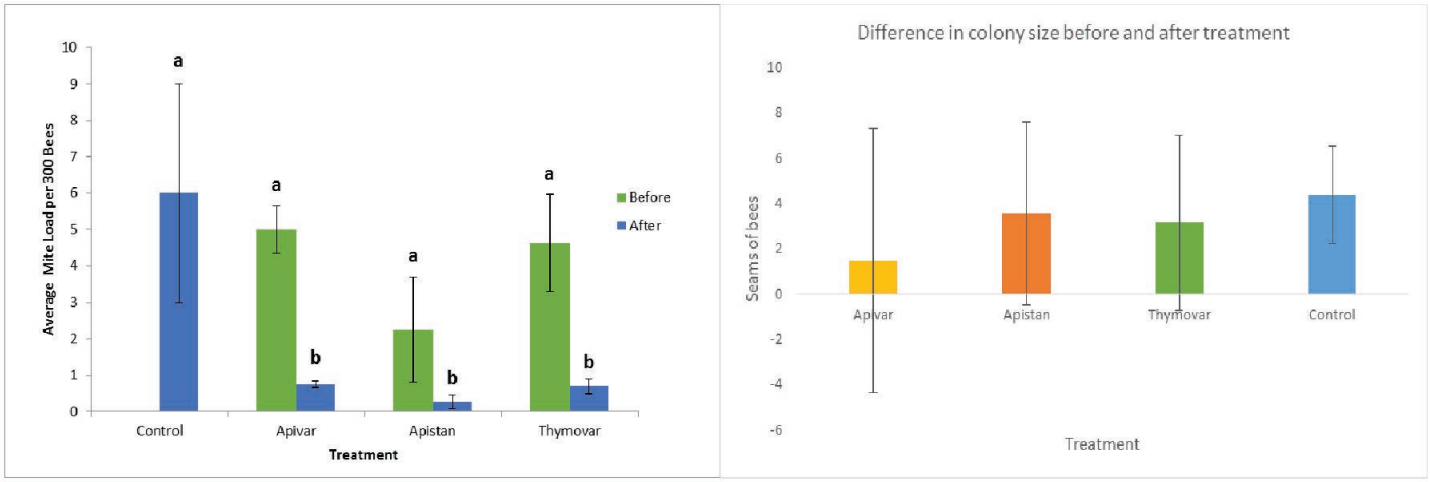

Figure 5. A) Comparison of Varroa mite levels before and after treatment with Apistan® (fluvalinate), Apivar® (amitraz), and Thymovar® (thymol) (bars labeled with different letters are significantly different from each other). B) Net colony change in colony size over the course of the treatment period for each treatment group.

Despite documented resistance to fluvalinate in Canada, Apistan® was effective at controlling Varroa mites in the hives included in this trial. This is likely due to a gap of several years since the product was last used in the operation.

Of notable interest is the fact that Thymovar® controlled mites as effectively as the other two synthetic miticides, particularly considering many of the spring days during the trial were cool and below the optimal ambient temperature for treatment recommended by the manufacturers (i.e. 13 °C – 30 °C).

As expected, Apivar® was effective and continues to be the industry standard in Canada for Varroa mite treatments.

A final study done by ATTTA in 2017, tested the efficacy of Formic Pro™ and 65% liquid formic acid to reduce infestations of Varroa mites in colonies of honey bees in Nova Scotia (Menzies et al. 2019). A total of 36 colonies were divided into three treatment groups. One treatment group received five separate doses of 65% liquid formic acid across a 20-day period, while the other two treatment groups were treated with strips of Formic Pro, either with two strips for 14 days or two consecutive rounds of a single strip, each for 10 days. The natural rate of mite mortality in each test colony was monitored for two days

prior to the beginning of the experiment. A screened bottom board and sticky board was used to collect and quantify fallen mites during the treatment period.

All three treatments reduced mite infestations, but the Formic Pro treatments resulted in the greatest mite mortality during the treatment period. Percent efficacies for the treatments were 62.0%, 89.4%, and 82.4% for the 65% liquid formic acid, 14-day Formic Pro, and 20-day Formic Pro treatments, respectively.

In 2022, a different study by ATTTA tested the efficacy of Apivar® and Api Life Var® using honey bees from 72 colonies. Samples of bees were collected from nine apiaries across the Maritime provinces. Four apiaries were in New Brunswick, four in Nova Scotia and one in Prince Edward Island. To collect samples with high levels of Varroa mites, colonies were assessed via an alcohol wash prior to starting the experiment.

Hives were first sampled just following wild blueberry pollination, and then resampled late summer. To collect the bees, brood frames covered in honey bees were shaken into a plastic dish washing tub. A ½ cup measuring cup was used to scoop approximately 300 bees into each jar. Samples were brought to the laboratory to assess miticide efficacy of Apivar® and ApiLife Var® using a modified Pettis test. Each sample was then treated with an alcohol wash to identify any remaining mites after testing. Efficacy was calculated by dividing the number of dead mites counted during the knock down by the total number of mites counted in each sample.

The efficacy of Apivar® against varroa mites in this study was 74.13% in round one and 89.15% in round two. The use of the modified Pettis test in the ApiLife Var® treatment did not provide credible results for this volatile compound.

More recently, in 2023, ATTTA aimed to test the efficacy of amitraz for Varroa mite treatment using a lab-based study. The lab-based study is a quantifiable way to assess the amount of amitraz required to kill a Varroa mite population, and with repeated years of this study the efficacy of amitraz can be monitored.

This study involved the collection of 225 mites from a total of 5 different beekeepers between Nova Scotia and New Brunswick, 5 apiaries, and 13 colonies of honey bees. For each beekeeper, 1 drone frame was placed in 3 or 4 different colonies. Drone frames were placed within the brood nest of each colony. The drone frames were left for 3-weeks before researchers returned to collect them. To collect mites, every capped cell on the drone frame was opened with forceps and inspected for the presence of mites.

To test the efficacy of amitraz for Varroa mite treatment, ATTTA adapted a lab-based study by Rinkevich (2020). The efficacy of amitraz against mites was assessed using six different concentrations. Vials were coated with amitraz dissolved in acetone, and then mites were placed into the vial for a 24-hour incubation period prior to the researcher assessing mite mortality. The goal was to establish the lethal concentration (or the LC50) that results in a 50% mortality rate among the mite population within a 24-hour period. The LC50 represents the concentration at which half of the mite population succumbs to the treatment, as determined by monitoring mite mortality across a range of concentrations. The LC50 is a statistical model.

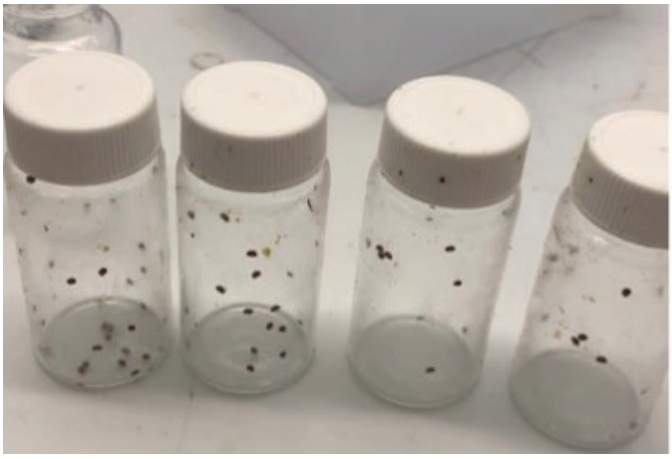

Figure 6. Varroa mites (Varroa destructor) in 20mL glass vial coated with amitraz dissolved in acetone for lab-based efficacy testing of amitraz.

The results of the 2023 study demonstrated that mite mortality is dependent on the concentration of amitraz, and that with increasing concentration mortality increases (Figure 7). The team demonstrated that the lab-based study is an effective way to measure the efficacy of amitraz. However, due to the limited number of mites collected it would be premature to make a conclusion about potential reduced efficacy from the small number of mites tested. A consistent issue with testing miticide efficacy in Atlantic Canada is a lack of mites collected from beekeepers voluntarily providing honey bee samples. This means beekeepers are managing Varroa but presents a challenge for conducting experiments.

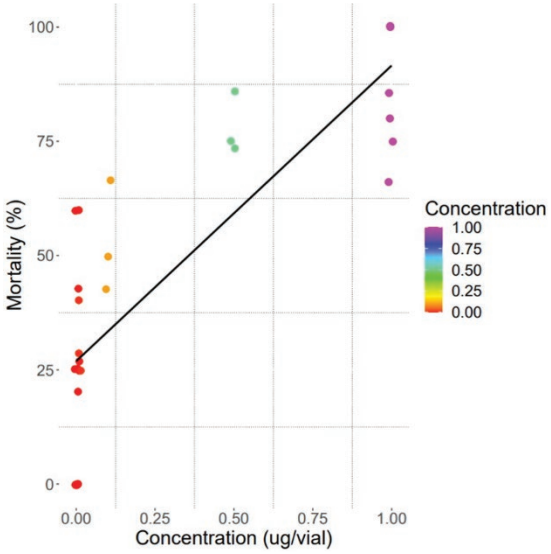

Figure 7. Relationship between the concentration of amitraz in a 20mL glass vial and the mortality percentage of Varroa mites (Varroa destructor) in a 24-hr period of time.

ATTTA plans to continue this study 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. Effectively managing Varroa mites is essential to have low and consistent winter losses of honey bees which allows beekeepers to support the pollination industry each year.

What Beekeepers Need to Know

Reduced efficacy occurs when products such as Apivar® are not being used correctly. This involves both over- and underuse of the product. Using more or less Apivar® strips than recommended will impact the dose of amitraz the mites will receive. The recommended number of strips is the exact amount needed to effectively kill the mite population. Too few strips will not kill a sufficient number of mites, and the ones that survive will be the most tolerant of the population, which contributes to reduced efficacy. More strips than recommended will overexpose the mites to amitraz. Too many strips may result in residual product remaining in the colony for longer, and this prolonged exposure leads to reduced efficacy.

Also using Apivar® strips for more or less time than recommended will impact how long mites are exposed to amitraz. The recommend time (56 days) is the exact amount of time needed to effectively bring down the mite population. If strips are not left in long enough the product will not kill enough mites in the population, and once again the most tolerant mites will survive contributing to reduced efficacy. If the strips are left in too long the mites will be overexposed to low concentration of amitraz leading to resistant populations.

Finally, the frequency of treatment can contribute to reduced efficacy. If Apivar® is being used too frequently in an operation this once again is overexposing mites to amitraz, which contributes to reduced efficacy. This is why integrated pest management is always the best management practice. Beekeepers should alternate between treating with a synthetic miticide such as Apivar®, and a non-synthetic product such as formic acid, oxalic acid, Thymovar®, or Hopguard®.

Beekeepers should test for Varroa mites at least monthly during the season (pre- and post-treatment), and only treat when levels are above the economic threshold. Beekeepers should also implement cultural and physical controls to reduce the need for chemical treatment. It is good practice to rotate out old comb approximately every 4 years to prevent a buildup of product residues.

Finally, it is essential to follow all product labels to ensure the amount, timing and frequency of product is safe for honey bees. Not following product labels can be damaging to bees.

Monitoring for Varroa Mites

Part of integrated pest management is monitoring for mites before and after treatment. It is important to remember that mite infestation levels indicated by sampling typically remain fairly stable throughout spring and summer but dramatically increase in fall. This is because mites move from brood cells to a phoretic stage on adult bees as brood production decreases. This will result in increased numbers of mites seen when monitoring, especially in washes.

Potential methods to monitor include alcohol wash, ether roll, sugar shake and sticky board. Check out the ATTTA factsheet “Summer Disease and Pest Monitoring in Honey Bees” for more information on the different mite monitoring methods.

Integrated pest management is the best approach for managing Varroa, and following IPM practices is essential to prevent reduced efficacy or resistance of miticide products. For any questions regarding monitoring and treating for Varroa mites please reach out to the ATTTA team.

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