

Apivar[®] and Bayvarol[®] suppress varroa mites in honey bee colonies in Canadian Maritime Provinces

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ABSTRACT

Two synthetic miticides were tested against varroa mites in honey bee (*Apis mellifera*) colonies in the Maritime Provinces of Canada in 2017 and 2018. We found no significant difference between Apivar® (a.i., amitraz) and Bayvarol® (a.i., flumethrin) in controlling varroa mites. Apivar caused 99.8% mortality of varroa mites and Bayvarol caused 96.5% mortality of varroa mites, on average, although Bayvarol demonstrated greater variability in mite mortality. Our results are encouraging due to the widespread reliance on Apivar in the Maritimes, suggesting that Bayvarol could be an effective alternative treatment.

RÉSUMÉ

Nous avons évalué l'efficacité de deux acaricides de synthèse contre le varroa dans des colonies d'abeilles domestiques (*Apis mellifera*) dans les provinces des Maritimes du Canada en 2017 et en 2018. Nous n'avons observé aucune différence entre les produits Apivar® (m.a., amitraze) et Bayvarol® (m.a., fluméthrine) quant à leur efficacité contre le varroa. En effet, Apivar a causé un taux de mortalité moyen de 99,8 % chez le varroa, et Bayvarol, de 96,5 %, mais le taux de mortalité présentait une plus grande variabilité dans le cas de Bayvarol. Compte tenu de la grande dépendance à l'égard d'Apivar dans les Maritimes, nos résultats sont encourageants puisqu'ils laissent croire que Bayvarol pourrait constituer un traitement de remplacement efficace.

INTRODUCTION

Varroa mites (*Varroa destructor* Anderson & Trueman) are widely considered one of the greatest challenge beekeepers face (Currie et al. 2010; Guzman-Novoa et al. 2010; Ferland et al. 2018). Beekeepers currently combat varroa mites through the use of miticides in both synthetic and organic formulations. Varroa mite development of resistance to older synthetic products has been well documented, including resistance to Checkmite+° (a.i., coumaphos) and Apistan° (a.i., fluvalinate) (Pettis 2004; Currie et al. 2010). The current synthetic miticide industry standard in Canada is Apivar° (a.i., amitraz). It is recommended that this product is rotated with other treatments including formic and oxalic acid to manage mites throughout the season (i.e., the same synthetic miticide is only used once per twelve-month period to retain efficacy). Bayvarol° (a.i., flumethrin) was registered for use in Canada in 2016 (Health Canada 2016) against varroa mites. Beekeepers wish to understand the efficacy of this product in comparison to Apivar. We tested varroa mite mortality from Apivar and Bayvarol in honey bee colonies at locations in New Brunswick, Nova Scotia, and Prince Edward Island in 2017 and 2018. These two products were compared to an untreated control group. We expected Apivar to

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cause high mite mortality across this region, and expected mite mortality from Bayvarol might vary in different

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beekeeping operations, depending on historical use of Apistan. The active ingredients of Bayvarol and Apistan are both pyrethroids (class 3A insecticides), and cross-resistance has been documented between these two products, potentially lowering the efficacy of Bayvarol against varroa mites (Lafreniere and Ostermann 2017). Apistan was commonly used by Maritime beekeepers approximately 10 years ago, but beekeepers readily shifted to Apivar once that product was registered.

MATERIALS AND METHODS

Experimental Design

Miticide testing was conducted in the summer of 2017, and the spring and summer of 2018. Testing was conducted with 153 colonies located in 8 different apiaries across the Maritimes: 4 apiaries and 103 colonies in Nova Scotia (NS); 3 apiaries and 33 colonies in Prince Edward Island (PE); and, 1 apiary with 17 colonies in New Brunswick (NB) (Table 1). In NS, the 4 apiaries tested belonged to 4 separate commercial beekeepers, while in PE, all 3 apiaries belonged to the same commercial beekeeper. All 17 colonies in 1 apiary in NB belonged to the same beekeeper. Colonies were randomly assigned to each of the three treatment groups: Apivar, Bayvarol, or untreated control, in a completely randomized block design, with all treatments being tested at each apiary.

Specialized miticide efficacy test kits following a modified Pettis test (Pettis 2004; BC Ministry of Agriculture 2015) were supplied by Bayer CropScience (North Carolina, United States) in 2017. The kits contained modified containers used for miticide efficacy testing and specially designed bags that served as incubation chambers. The modified containers used for the Pettis tests were plastic transparent cups 85 mm in diameter and 95 mm in height, with eight 4 mm holes drilled in the cup to allow air exchange. The cup was inverted and placed into an 85 mm screw-on ring used for glass jars with 6.4 mm wire mesh cut to size and glued on the bottom of the ring. The mesh was small enough that bees could not escape but still large enough to allow any mites removed from bees to drop through the mesh and be collected on an 85 mm diameter piece of sticky board placed on the bottom of the container. Each kit contained a section of a strip of either Apivar, Bayvarol, or brown corrugated cardboard as a procedural control.

A sample of approximately 300 bees (1/2 cup) was collected from each hive and placed randomly into one modified plastic container. Bent paper clips were used to hang the treatment miticide strip (either a strip of cardboard cut 25 mm x 40 mm, a strip of Apivar cut 25

Table 1. Description of miticide testing in honey bee colonies in the Maritime Provinces, Canada, 2017-2018.

Year	Province	Apiary location (County)	No. of bee samples			No. of colonies
			Apivar	Bayvarol	Control	tested in apiary
2017	NB	Albert	8	5	4	17
2017	NS	Colchester	4	4	4	12
2017	NS	Pictou	4	4	4	12
2017	NS	Cumberland	4	4	4	12
2018	NS	Hants	26	28	13	67
2018	PE	Kings	4	4	4	12
2018	PE	Kings	4	4	4	12
2018	PE	Queens	4	3	2	9
No. of	colonies in	trial				153

mm x 40 mm, or a strip of Bayvarol cut 30 mm x 35 mm) in each test container, about 50 mm from the top. The strip dimensions were based off of equivalent doses at label rate. Although the sample size of each treatment was different at each apiary, all three treatments were tested at each apiary (Table 1). In PE, 4 samples of bees per treatment were used, but 2 control samples of bees and 1 Bayvarol sample of bees across the 3 apiaries were omitted because no mites were present in the sample. In NB, 8 samples of bees for the Apivar treatment, 5 samples of bees for the Bayvarol treatment, and 4 samples of bees for the control treatment were collected. Four samples of bees for each of the treatment groups were collected for 3 of the 4 apiaries used in NS, while 13 control bee samples, 26 Apivar bee samples, and 28 Bayvarol bee samples were collected from the remaining apiary. Hives tested in 2017 were not again tested in 2018.

Immediately after the bees were placed into the test container, a circular piece of sticky board (diameter = 85 mm) was secured to the bottom of the glass jar ring and the wire mesh. Once all of the samples were collected and placed into their respective containers, the containers were placed in incubation bags and were incubated for 6 hours with minimal disturbance. The bees were held in incubation bags on laboratory bench tops in the dark under ambient conditions. Following the incubation period, the pieces of sticky boards that were placed below the containers to catch the fallen mites were removed and the number of varroa mites killed by the miticide strips were counted. The bees were then chilled on ice until multiple alcohol wash applications (DeJong et al. 1982) were performed to remove and quantify any of the mites that were not killed by the miticide. The number of mites that were killed as a result of the miticide and the number of mites that remained on the bees during the incubation period were used to calculate mite mortality.

Statistical Analysis

A linear mixed effect model with treatment as the independent variable, mite mortality as the dependent variable, and bee yard as a blocking factor was used to determine if mortality differed among treatment groups. We also examined the interaction effect of treatment X location. Mite mortality (%) was calculated by dividing the total number of mites dropped during the incubation period and collected on the sticky board by the total number of mites on the sticky board, plus the mites that were collected during the multiple alcohol washes after the incubation period. Samples were pooled across years and apiaries as we were interested in the overall mite mortality from these products in the Maritimes, rather than mite mortality from products in individual years or provinces.

Assumptions of normality of error terms and constant variance of residuals were verified and independence was assumed through randomization. Multiple means comparison was performed using Tukey's means separation. All statistics were conducted using Minitab version 18 (Minitab 2018).

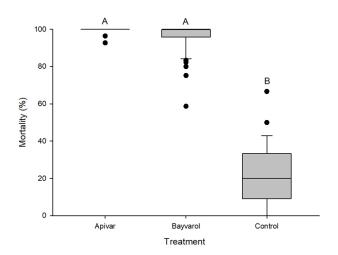
RESULTS

There was a significant interaction between treatment and location (apiary site) ($F_{14,129} = 2.01$, P = 0.022). This significant interaction arose due to the magnitude of difference among the treatments among apiary sites. There was no difference in varroa mite mortality between Apivar or Bayvarol treatments in any location, however, there was a significant difference in mite mortality between the treatment groups and the control group, regardless of location. Apivar demonstrated an average mite mortality of 99.8% (sd = 1.06, range = 92.7-100, n = 58) and Bayvarol demonstrated an average mite mortality of 96.5% (sd = 7.72, range = 58.7-100, n = 56), compared to only 21.2% mite mortality in the untreated control group (sd = 16.3, range = 0-66.6, n = 39) (Figure 1).

DISCUSSION

Our testing demonstrated that both Apivar and Bayvarol work well to control varroa mites in the colonies that were tested. Apivar caused the highest varroa mite mortality, although not significantly higher than Bayvarol. The consistently high mite mortality from Apivar is an encouraging result since most beekeepers in the Maritime

Figure 1. Box plot demonstrating comparison of varroa mite mortality from Apivar®, Bayvarol®, and a procedural control in honey bee colonies in 2017 and 2018 in Canadian Maritimes. Letter groupings that differ show significant differences. Boxplots display interquartile range, median, and outliers.



Provinces still rely on this product, particularly as a spring mite treatment option. Notably, our study reveals higher mite mortality from Apivar than a study conducted on the Canadian Prairies, where average mite mortality of Apivar was 87% in the spring and 75% in the fall (Vandervalk et al. 2014), although our research was done under more controlled conditions (i.e., incubation method) and not done in a field experiment. In Vandervalk et al. (2014), mite mortality was conducted at the yard level where colony population, weather, temperature, location of cluster, etc. may have impacted mortality results, whereas our study was controlled using an incubation method and a smaller number of bees. Using the incubation method, the strip was placed in a manner that stimulated the bees to cluster on the strip, encouraging the spread of the miticide in a short time frame. Our doses followed label directions in a controlled space. For in-hive testing, bees have more room to avoid contact with the mite strip, potentially influencing the overall mite mortality results. Further in-hive testing is needed for varroa mite products to gain a better understanding of mite management under field conditions.

There was greater variably in mite mortality from Bayvarol compared to Apivar, potentially due to cross-resistance from the historically-used and closely related product, Apistan (Lafreniere and Ostermann 2017), however, we could not access or evaluate historical Apistan use in the participating beekeeping operations. Somewhat troubling with respect to the distribution of

the data in Figure 1 are the outliers shown in the box-plot for Bayvarol. These outliers show that for some colonies, low mite mortality was found for a few Bayvarol samples, with one sample as low as 59% mortality. Low mortality values (e.g., 59% and 75%) both came from the NB apiary tested, while two values (82% and 84%) came from NS apiaries. No mortality values below 80% were detected in NS. One 83% mortality value was detected in PE. Although a few Bayvarol samples had lower than 80% mortality, this was not the case for the majority of the samples collected. Our results suggest beekeepers in the Maritimes can rely on either product (Apivar or Bayvarol) for controlling mites, although mite mortality may vary based on treatment history within each operation.

Although significantly fewer mites dropped in the control group compared to both synthetic miticide treatments, mean mite mortality was still 21% and there was a fairly large range of mite mortality. Ideally, mite mortality in the control group should be under 10% (D. Rogers, personal communication). It is possible that the mean mite mortality in the control group was slightly elevated because we had to collect samples in remote locations and the travel of the samples in the vehicle may have influenced mite drop.

Our results demonstrate that Apivar and Bayvarol are effective miticides for Maritime beekeepers. As these two synthetic products are from different insecticide classes, beekeepers could be rotating varroa mite treatments with these miticides among years, potentially prolonging the efficacy for each product and reducing the risk of resistance. By conducting studies such as ours across Canada and the global beekeeping community, beekeepers and researchers alike can gain a better understanding for treatments that are working and impending resistance. Miticide screening should continue in the Maritimes (and beyond) at a larger scale to measure the efficacy of miticides, an important tool in the beekeepers' toolbox, especially the longer a particular product is in use.

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