Final Report: Apiguard® efficacy for controlling *Varroa destructor* in honey bee (*Apis mellifera*) colonies in Canada

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Certification

This report represents a true and accurate record of all data obtained.

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INTRODUCTION

Apiguard® is a registered trademark of VITA Bee Health - United Kingdom (https://www.vita-europe.com/beehealth/) that is available in Europe and the USA but not in Canada. Apiguard® is a varroacide composed of a gel containing 25% thymol, a natural compound found in thyme and in some honeys such as linden honey. The incorporation of thymol in a gel has the advantage of allowing the gradual release of the vapours in the hive under treatment.

Apiguard® has two complementary modes of action: 1) thymol vapours spread in the colony with the help of ventilating bees and acts against varroa by respiration; 2) the workers transport and spread the gel in the colony by physical contact and trophallaxis and acts against varroa by contact. Thymol is considered to be of low toxicity to humans and the European Union tolerates concentrations of 50 mg / kg in food. Apiguard® has been the subject of many trials worldwide since 1995 and has proven efficacy (reference: https://www.vita-europe.com/beehealth/products/apiguard/). Efficacy is superior when applied in the absence of brood but in can be used during brood production periods. It is not recommended to treat during periods of honey flow. The tolerance of bees to treatment is good but it produces a slight agitation of the colony during the days following application.

The object of this work was to conduct an efficacy trial of Apiguard® under typical Canadian apicultural conditions. Trials were realized at apicultural service of the Centre de recherche en sciences animales de Deschambault's (CRSAD).

METHODOLOGY

Duration of trial: August 2018 to June 2019

Colonies

The trials were conducted with experimental colonies of the Centre de recherche en sciences animales de Deschambault (CSRAD; 46˚ 40026.8500 N, 71˚ 54054.3900 W), Quebec, Canada. All colonies were distributed in three apiaries: *Leclerc* (N 46˚ 78421 N, 71˚ 85974 W), *Pagé* (46˚ 68955 N, 71˚ 71410 W) and *Picard* (46˚ 71735 N, 71˚ 64564). Each colony was housed in a Langstroth commercial hive consisting of a single brood chamber (10 frames) above a screened bottom board allowing the varroa mites to fall through to sticky traps.

The treatments were applied September 12, 2018, using a completely randomized design (Table 1). Two weeks before the treatment, 48 colonies were evaluated for strength (number of frames covered with bees), queen status and overall colony health. In order to balance colony strength and initial varroa infestation levels between groups, colonies were ranked and randomly assigned to one of the treatments: 1) negative control (no treatment), 2) Apiguard® dosage 100g/colony/6 weeks (2 consecutive applications of 50 g), 3) Apiguard® dosage 75g/colony/6 weeks (3 consecutive applications of 25 g), and 4) positive control commercially available registered standard (single application of Thymovar®).

Table 1: Description of the various experimental groups (n=12 colonies per group).

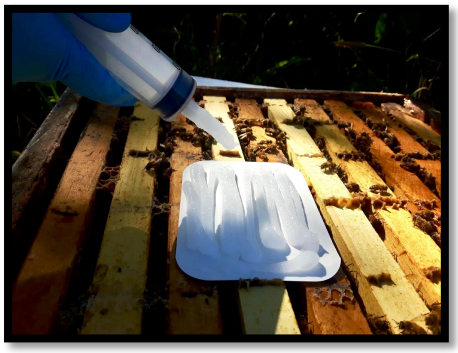
Groups	Fall treatments (starting on September 12, 2018)
1	Negative control, (*No treatment*)
2	Apiguard®, 2 consecutive applications, Total dosage 100g/colony - Application 1 *(12-24/09/18)*: 50 g/colony/2 weeks - Application 2* *(25/09/18 – 24/10/18)*: 50 g/colony/4 weeks
3	Apiguard®, 3 consecutive applications, Total dosage 75g/colony: - Application 1 *(12-24/09/18)*: 25 g/colony/2 weeks - Application 2* *(25/09/18 – 09/10/18)*: 25 g/colony/2 weeks - Application 3* *(10/10/18 – 24/10/18)*: 25 g/colony/2 weeks
4	Positive control Thymovar® (one strip per colony)

*Any remaining product from the previous application was removed from the hive

A follow-up treatment was performed, on October 24, 2018, on all the colonies, using Apivar® (active ingredient: amitraz; 2 strips/colony). Here again, mite drop was monitored with sticky boards once a week throughout the duration of the treatment (42 days).

Method of Apiguard® administration (as written on label)

" Open the hive. Place a piece of cardboard (approximately 4" x 4") centrally on top of the brood frames. Stir [product] well before each use. Remove the dosing syringe from its sealed packaging. Insert the syringe nozzle fully into the gel; ensuring no air is drawn into the syringe. Slowly pull the plunger back on the syringe to draw up 51 ml (equivalent to 1.76 oz/50 g) of Apiguard gel. Remove the syringe from the gel. Gently push the plunger downwards to release the gel within the syringe onto the dosing tray (Figures 1 and 2). Flatten out the gel with a hive tool if necessary. Ensure that there is a free space of at least 1/4 inches between the top of the tray and the hive cover board, for example, by placing an empty super on top of the brood box. Close the hive."



Figures 1 & 2: Application of the Apiguard® gel with a syringe on a piece of cardboard placed over the frames of the brood box.

Varroa Control Assessment

Fallen varroa mites (Figure 3) were counted on sticky boards placed on the bottom boards of all hives. Sticky boards covered all the bottom surface of hives and were replaced weekly during the following periods: pre-treatment (23-31/08/18), treatment period

(12/09/18 – 24/10/18), follow-up treatment (24/10/18 – 03/12/18), and following spring (May 2019).

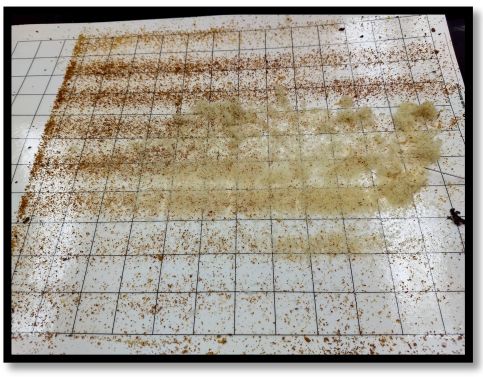


Figure 3: Sticky-board covering all the bottom board and showing fallen varroa and hive debris.

Treatment efficacy

The efficacy of the various fall varroa treatments was calculated for each colony using the formula:

- % Efficacy = (total number of mites killed during fall treatment per colony x 100) / (total number of mites killed during fall treatment + total number of mites killed during follow-up treatment with Apivar®).

Colony Performance Assessment

Honey bee brood population: The number of immature honey bees (eggs + larvae + capped brood) in each colony was evaluated by measuring the area (width and length) on each side of every brood frame. The rectangular surface obtained was multiplied by 0.8 to compensate for the elliptic form of the brood pattern. These values were added, in order to calculate the total brood surface in each colony. A factor of 25 worker cells per 6.25 cm^2 (i.e., a square inch) was used to convert the area to the number of immature worker honey bees. These measures are carried out before treatment applications (August 23, 2018) and the following spring (May 2019).

Colony strength and overwintering survival: for each colony, the size of the cluster of bees was measured before (November 7, 2018) and after wintering (April 20, 2019) by opening each hive and counting the number of frames occupied by the bee cluster around the brood as viewed from above and the number of frames as viewed from below. The index varies between 0 (dead colony) and 10 and is calculated using the following formula: (number frames with bees and brood viewed from above + number frames with bees and brood viewed from below) / 2.

Outdoor Temperature

Ambient temperatures during experimental treatments were recorded with a portable weather station placed in (Onset - Hobo® *Data logger temp/RH/ext channel* U12-012) each experimental apiary.

Statistical analysis

Varroa mite drop dynamics were analysed using the proc mixed procedure in JMP® PRO Software (14.1.0). Data was divided in groups: pre-treatment period, treatment period, and post treatment period. Then, for each group, a repeated measures analysis of variance (RM-ANOVA) was performed in order to compare the effect of treatments, time and their interaction on the numbers of weekly fallen varroa mites. Significant parameters were analysed using contrasts to compare the weekly mite drop between: 1) negative control (no treatment), 2) Apiguard® label dosage 100g/colony/6 weeks (2 applications of 50 g), 3) Apiguard® test dosage 75g/colony/6 weeks (3 applications of 25 g), and 4) Thymovar®. A log10 transformation was applied to normalize the data distribution. Results were then back-transformed and presented in the figures. Treatment efficacy was calculated as a percentage for each colony and colony performances were compared between groups using non-parametric analyses, using the Kruskal-Wallis test, followed by the Wilcoxon–Mann–Whitney test. Significance was defined as $P < .05$ for all analyses.

RESULTS

** The Thymovar®) was applied in a single dosage (one Thymovar® strip in each hive instead of two strips) and its efficacy is used only for comparison purposes (appearing in grey). It is not considered in the discussion.*

Initial evaluation on experimental colonies shows that various groups started with similar varroa infection levels and brood strength (Table 2).

Table 2: Daily varroa drop and brood strength before treatments.

Group	**Colony Strength (# Brood cells/colony)**		**Pre Varroa Drop (# Varroas/day)**	
	Mean	**SE**	**Mean**	**SE**
1. Negative Control	23 272	4 708	2	1.9
2. Apiguard® (2x50 g)	23 196	4 621	2	1.9
3. Apiguard® (3x25 g)	23 330	4 849	2	2.0
4. Thymovar®	23 187	4 759	2	2.0
Statisitics	$(F_{(3,44)} = 0.37)$ P = .78		$(F_{(3,44)} = 0.29)$ P = .83	

Temperature

Maximum daily temperatures for each apiary (Figure 4) are between 25˚C and 40˚C (yellow zone) and over the recommended temperature during the first week of treatments. The maximum temperatures during weeks 2, 3, and 4, were between 15˚C and 25˚C in the "ideal zone" (green) for maximum efficacy. For weeks, 5 and 6, most of the maximum temperatures were below 15˚C and under the recommended temperature (red zone).

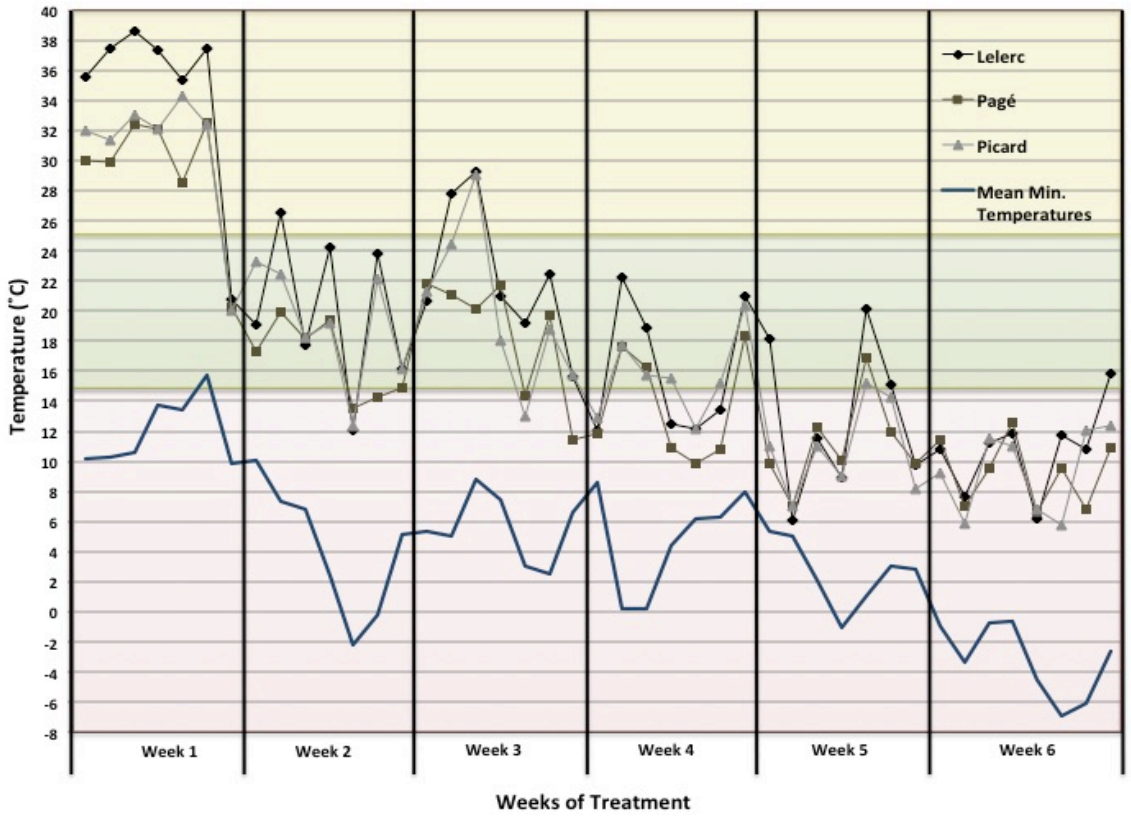


Figure 4: Graph showing the maximum temperature (mark) reached daily in apiaries. The blue line represents the average minimum temperature measured in apiaries. The green section represents the optimal temperature range for Apiguard® efficacy (15˚C to 25˚C); the yellow section is above the optimal rage (between 25˚C and 40˚C) and the red zone is below the optimal range (< 15˚C).

Varroa Drop During Treatment

All groups confounded started with a varroa drop of 21 ± 3 (mean ± SE) / week; Figure 5). Statistical analyses demonstrated an interaction between groups and treatment period ($F_{(10,161)} = 10.76$; $P < .0001$). After the first treatment application (week 1), there was an average varroa drop of 240 ± 76 / week for the Apiguard® (2x50 g) group 2 and 157 ± 50 / week for the Apiguard® (3x25 g) group 3, while only 33 ± 10 / week for negative control group 1. There was a varroa drop decrease after the second treatment week in all groups. At the third week, varroa drop increased for both Apiguard® groups (95 ± 30 [group 2]; 106 ± 34 [group 3]). Afterwards, during week 4, 5 and 6, varroa drop gradually decreased for all groups.

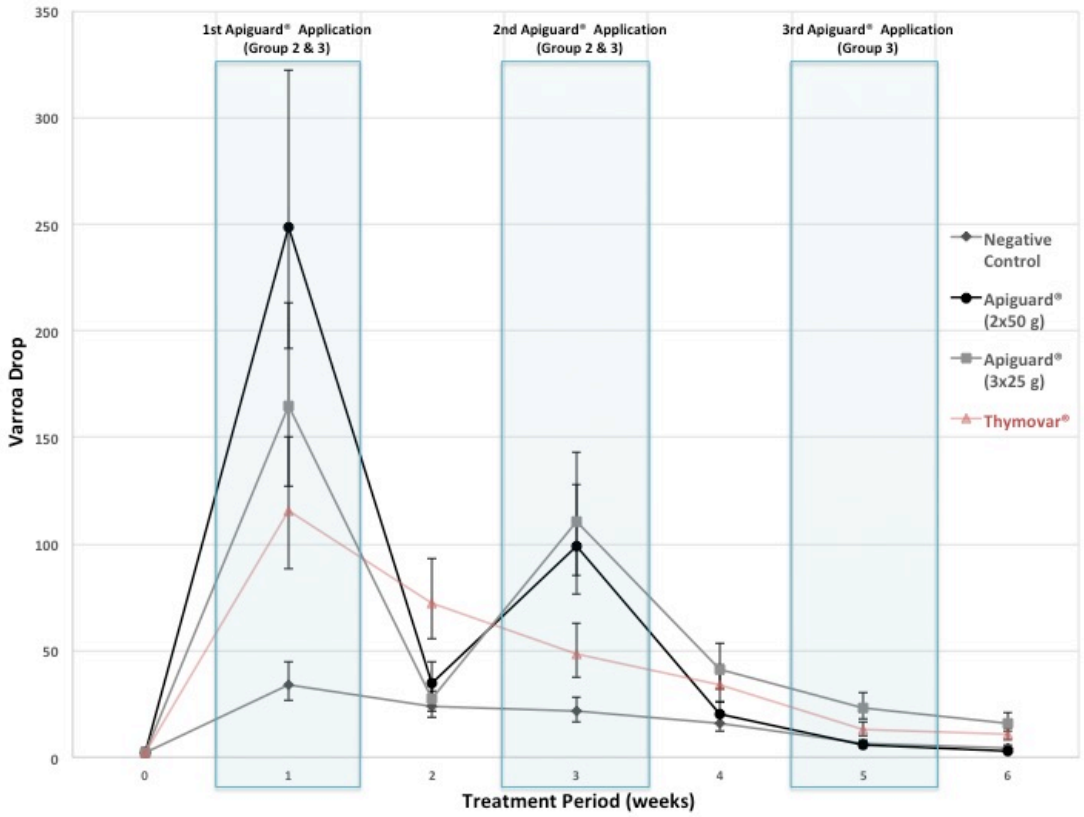


Figure 5: The average varroa drop per colony per week in various experimental groups (Mean ± SE; $F_{(10,161)} = 10.76$; $P < .0001$). The treatment period was between 12/09/18 and 24/10/18 and each week counted 7 days (for a total of 42 days of treatment). The blue zones represent periods of Apiguard® applications for the groups 2 and 3. Treatment period "0" is the initial varroa drop, before the application of treatments (evaluated between 23 and 31 of August, 2018).

Efficacy of treatments in various experimental groups

There was a significant difference of treatment efficacy between groups (Mean ± SE; $X^2 = 241.8$; $P < .0001$) (figure 6). Group 2 Apiguard® treatment (2x50 g) had the highest efficacy (89.8 % ± 0.8) while group 3 Apiguard® treatment (3x25 g) had a slightly lower efficacy (83.1 % ± 1.2). Efficacy of the negative control group 1 was 15.6 % (± 0.30).

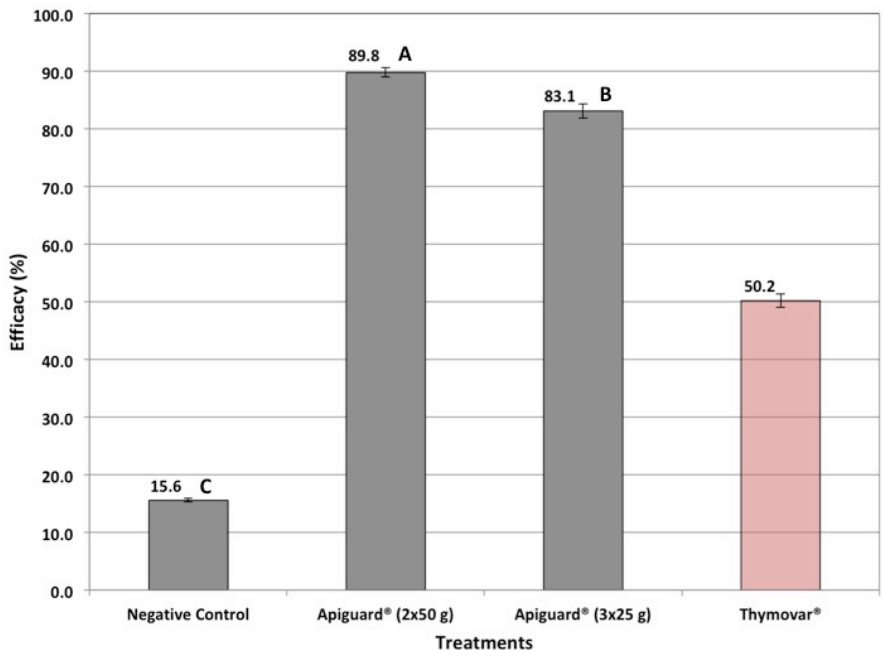


Figure 6: Efficacy for each treatment (Mean ± SE; $X^2 = 241.8$; $P < .0001$). The efficacy is measured by dividing the total number of mites killed during fall treatment with the total number of mites killed during fall treatment and the Apivar® follow-up treatment (as explained in the methodology). Different letters indicate a significant difference.

Colony strength and health status

There was no significant difference between various groups for colony strength (cluster size) after treatment applications (Figure 7; $F_{(3,44)} = 0.401$; $P = .753$).

There was a significant difference of colonies winter survival between group 3 and the other groups ($X^2 = 9.6$; $P < .05$). Three colonies from group 3 did not survive to winter (Table 3). Two of them were too weak to survive winter, while the other died of starvation. Chalkbrood symptoms were observed in each group (group 1: 2/12 colonies; group 2: 4/12 colonies; group 3: 4/12 colonies). One colony of the group 2 Apiguard® (2x50 g) group did not survive because of queen infertility (Table 3).

Colony cluster size in early spring was different between various groups (Figure 8; $F_{(3,44)} = 6.38$; $P < .001$). Colonies of group 1 had a significantly highest cluster strength (7.0 ± 1.4 frames of bees) compared to groups 2 and 3 treated with Apiguard® (4.2 ± 1.6 and 6.0 ± 1.4 frames of bees, respectively). For the Spring brood development (Figure 9), the negative control and the group 3 have an average of 8387 ± 4206 and 8171 ± 3270 of worker cells,

while the group 2 had a fewer number (5109 ± 3465 brood cells). However, we did not observe any statistical difference for the brood development ($F_{(3,44)} = 1.97$; $P = 0.136$).

Table 3: Colony health status in various experimental group.

Group	**Disease (Fall 2018)**	**Colony survival (Nov 2018)**	**Cause of Death (Nov 2018)**	**Colony survival (Apr 2019)**	**Cause of Death (Apr 2019)**
Negative Control	Chalkbrood (n=2)	100% (12/12)	-	100% (12/12)	-
Apiguard® (2x50 g)	Chalkbrood (n=2)	92% (11/12)	Drone-laying queen	100% (11/11)	-
Apiguard® (3x25 g)	Chalkbrood (n=2)	100% (12/12)	-	75% (9/12)	Weak (n=2) Starved (n=1)
Thymovar®	Chalkbrood (n=2)	100% (12/12)	-	100% (12/12)	

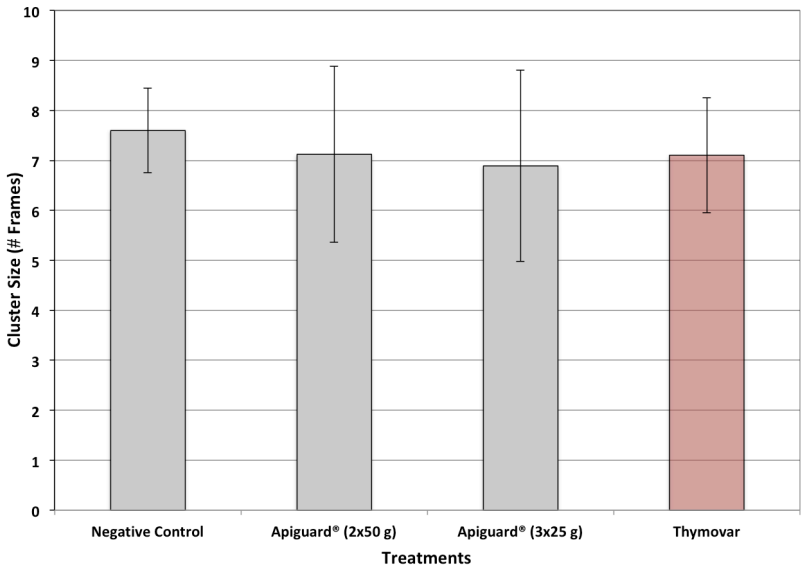


Figure 7: Colony strength (cluster size) after treatments (Mean ± SE: $P = .753$) in October 2018. The colony strength is measured by the number of frames covered by the bee cluster (see Methodology section for details). There is no difference between groups ($P > 0.05$).

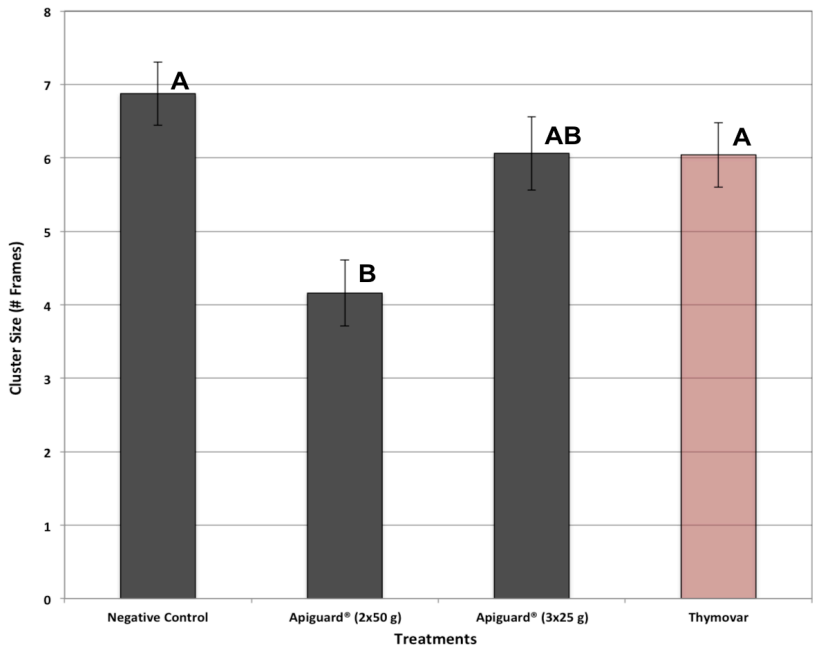


Figure 8: Colony strength (cluster size) of each treatment group (Mean ± SE) after wintering in April 2019 . The colony strength is measured by the number of frames covered by the bee cluster. There is a significant difference between groups ($P > 0.001$; different letters)

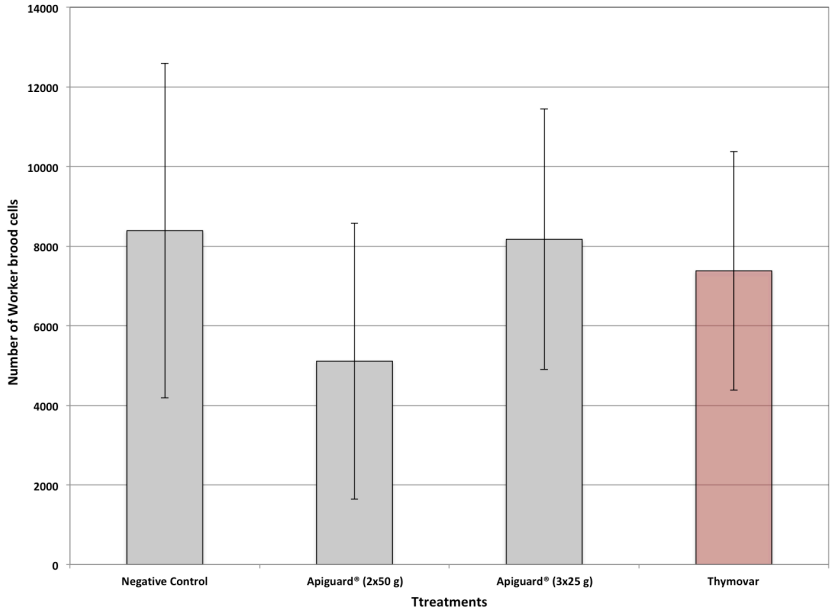


Figure 9: Colony strength (brood population) of treatment groups (Mean ± SE) three weeks after wintering (early May 2019). There is no difference between groups ($P = 0.136$).

Varroa infestation after wintering in May 2019

We measured an overall colony varroa drop (all groups confounded) of 1 to 3 varroa per week . No significant difference was observed between groups (Figure 10, $F_{(3,44)} = 2.297$; $P = 0.092$).

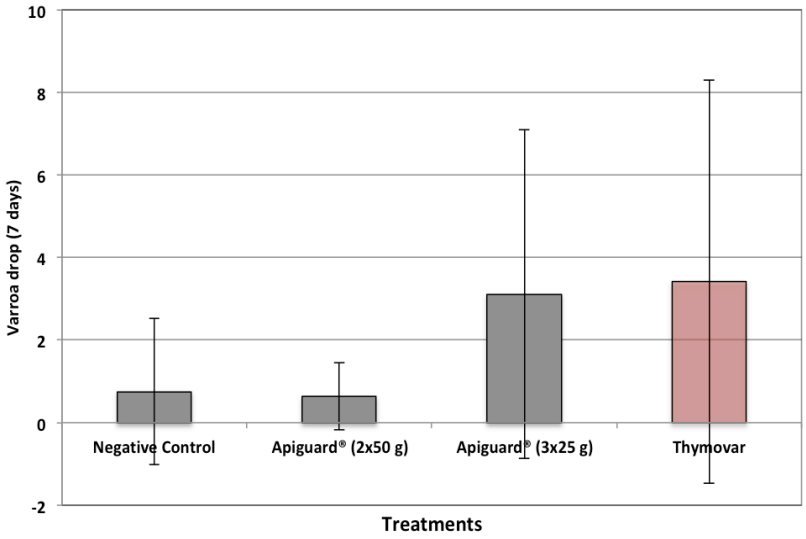


Figure 10: Varroa drop per colony (7 days) after wintering in May 2019 (Mean ± SE). There is no difference between groups ($P = 0.092$).

DISCUSSION

The goal of our research was to conduct an efficacy trial of Apiguard® in typical north-eastern Canadian apicultural climates and beekeeping management conditions. Our results will give valuable efficacy data for its registration by the Health Canada Pest Management Regulatory Agency (PMRA) who is responsible for pesticide regulation in Canada. The Apiguard® varroa fall treatment is currently available in Europe and the USA. Our results show that Apiguard® is an effective fall varroa treatment when used under the conditions of this Northeastern Canadian trial.

Treatment Dosages and Temperatures

The application of an Apiguard® treatment causes an increased varroa drop during the first and second application. The third application in group 3 (25 g Apiguard®), did not significantly increase varroa drop. Many factors can explain these results. Thymol quantities are double in group 2 Apiguard® 50 g compared to group 3 Apiguard® 25 g thus reducing the amount of active ingredient that is in contact with the bees. Secondly, most of the maximum temperatures measured in the last two treatment weeks (5 and 6) were in the red zone (below 15˚C) (figure 1). As mentioned on the Apiguard® label, maximum efficacy is attained between 15 ˚C and 25˚C. This also explains the lower mite fall during the third application in group 3 (Figure 2; week 5). In a climate where daily temperatures are near the minimum efficacy range, a slight temperature change can greatly influence the treatment efficacy. Temperatures (Figure 1) measured during treatment varied greatly and it is difficult to find the optimal moment to treat during September and October. However, if both Apiguard® applications of 50 g are administered at the beginning of treatment (as realized in group 2), cold autumn temperatures can definitely be avoided.

Efficacy of treatments

Apiguard® treatments tested were effective to reduce varroa populations in colonies. The 2x50 g Apiguard treatment, group 2, gave the highest efficacy (89.8 % ± 0.8). According to the Vita Bee Health (reference: https://www.vita-europe.com/beehealth/products/apiguard/), the average efficacy for 10 countries across

Europe, Middle East and North America are approximately 93% (Countries: Italy, France, Finland, Switzerland, Germany, Belgium, Morocco, Algeria, Tunisia, Israel and Jamaica). Out of theses 10 countries, Switzerland and Belgium had the lowest efficacy (86% and 90%, respectively) and were comparable to our Apiguard® efficacy results (89.8 % ± 0.8 and 83.1 % ± 1.2). If we take a closer look to the ambient temperature of every country, the countries with the highest efficacy measured, except Finland, are located in the subtropical climate, where the average temperature in September and October are higher.

Colony Survival

Three colonies died during winter in group 3 (3 x 75g Apiguard®). There was a significant difference between groups. However, this difference could be explained by something other than the treatment. One colony died because of a limited nutritional resource. This factor is not related to the treatment. Also, the two remaining colonies that died during winter, was due to the colony strength. They started the trial with a low quantity of brood cells (12740 and 18860 brood cells), too weak to survive winter. Moreover, they were infected by the chalked brood, increasing the risk of mortality.

Spring Development

Apiguard® treatments did not have an effect on colony strength before wintering. However, the colony cluster after wintering of the group 2 treated with Apiguard® (2 x 50g) was smaller than the negative control. If we compare the colony clusters before and after wintering, group 2 lost approximately three bee frames, while the other groups, 1 and 3, lost an average of one bee frame. On the other hand, Apiguard® treatment did not have an effect on spring colony development (May brood production). This indicates that Apiguard® treatment may cause a reduction of the winter cluster size but that they can rapidly recover after a few weeks of spring build up.

Varroa Count

Finally, the spring varroa drop weekly count was between 1 varroa (group 1 and 2) and 3 fallen varroas (group 3). According to the management thresholds given by Ministère de l'Agriculture des Pêcheries et de l'Alimentation (MAPAQ, 2014), no varroa spring

treatment is recommended when varroa drop is lower than 1 every 2 days. Therefore, the efficacy of the Fall treatment was sufficient to maintain varroa infestation below the recommended spring treatment threshold and thus avoid an additional spring treatment.

CONCLUSION

Our efficacy trials show that Apiguard® is an effective fall varroa treatment that can be used in typical north eastern Canadian apicultural climates and beekeeping management conditions. Apiguard® is relatively easy to use and would be a valuable alternative for varroa control in Canada. We measured an optimal efficacy with 2 consecutive Apiguard® applications (2 x 50g/colony at 2-week interval). This application is interesting for beekeepers because only two applications are needed and application does not extend during October when temperatures are often below the maximum efficacy of the product.

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