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The efficacy of Formic Pro™ and 65% liquid formic acid against varroa mite (*Varroa destructor*) in honey bee (*Apis mellifera*) colonies in autumn in Nova Scotia, Canada

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ABSTRACT

The efficacy of Formic Pro™ and 65% liquid formic acid to reduce infestations of varroa mite (*Varroa destructor* Anderson and Trueman) in colonies of honey bees (*Apis mellifera* L.) was tested in autumn 2017 in Nova Scotia, Canada. A total of 36 colonies were divided into three treatment groups. One treatment group (n=12) 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 (n=12) or two consecutive rounds of a single strip, each for 10 days (n=12). The 65% liquid formic acid was used as a standard to compare the efficacy of Formic Pro strips. 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. The majority of mite drop occurred during the first two days of product application. Based on this trial, Formic Pro appears to be an effective tool for varroa mite control in Nova Scotia.

RÉSUMÉ

Nous avons évalué l'efficacité de Formic ProMD et de l'acide formique liquide à 65 % pour réduire les infestations de varroa (*Varroa destructor* Anderson et Trueman) dans les colonies d'abeilles domestiques (*Apis mellifera* L.) à l'automne 2017 en Nouvelle-Écosse, au Canada. Au total, 36 colonies ont été divisées en trois groupes de traitements. Le premier groupe (n = 12) a reçu cinq doses d'acide formique liquide à 65 % réparties sur 20 jours, alors que les deux autres groupes ont été traités au moyen de bandes Formic Pro; dans un cas deux bandes ont été installées pendant 14 jours (n = 12), et dans l'autre cas une bande a été installée pendant 10 jours, puis a été remplacée par une autre bande laissée en place durant 10 jours (n = 12). Le traitement d'acide formique liquide à 65 % a été utilisé comme témoin pour comparer l'efficacité des bandes Formic Pro. Les trois traitements ont permis de réduire les infestations de varroa, mais les traitements de Formic Pro ont entraîné un taux de mortalité plus élevé chez le varroa au cours de la période de traitement. L'efficacité s'est chiffrée à 62,0 % pour le traitement d'acide formique liquide à 65 %, à 89,4 % pour le traitement de Formic Pro de 14 jours et à 82,4 % pour le traitement de Formic Pro de 20 jours. La majeure partie des varroas sont morts au cours des deux jours suivant l'application du produit. D'après cet essai, le produit Formic Pro semble représenter un outil efficace contre le varroa en Nouvelle-Écosse.

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INTRODUCTION

Several chemical products designed to reduce infestations of the invasive varroa mite (*Varroa destructor* Anderson and Trueman) (Acari: Varroidae) are registered for use in Canada. Canadian beekeepers typically rotate between the use of organic acid treatments (e.g., formic acid and oxalic acid) and synthetic miticide treatments (e.g., amitraz and fluvalinate) to reduce the risk of miticide resistance developing from frequent and widespread application (Currie et al. 2010; Rosenkranz et al. 2010; Pernal and Clay 2013).

Formic acid is commercially available in Canada in bulk liquid form with a 65% concentration which can be soaked into cotton Dri-Loc® pads and placed on the top bars of frames within hives (Pernal and Clay 2013; NOD Apiary Products 2018a). Several rounds of liquid formic acid application are recommended to achieve maximum efficacy (OMAFRA 2017). This requires beekeepers to frequently return to and open their hives, which is labour intensive, time consuming, and disruptive to colonies.

Formic acid as a varroa treatment is also commercially available in Canada as Mite Away Quick Strips® (MAQS®) (NOD Apiary Products, Frankford, ON). MAQS are pre-divided strips containing a formulation of 46.7% formic acid. These strips can be applied in one or two rounds over a shorter treatment period than liquid formic acid, which requires multiple rounds of application (NOD Apiary Products 2018a). A new product, Formic Pro™ (NOD Apiary Products, Frankford, ON), is an amended version of MAQS that features an extended 24-month shelf life. Initial testing has shown Formic Pro™ to be 83-97% effective at controlling mites parasitizing adult honey bees and mites developing underneath brood cell cappings (NOD Apiary Products 2018b). Due to their slow release properties, both MAQS and Formic Pro are permitted for use while honey supers are in place and are appropriate late-summer and early-autumn treatments when ambient temperatures are usually within the recommended range of 10° to 29.5° C (NOD Apiary Products 2018a,b). Applications of 65% liquid formic acid are not permitted for use when honey supers are in place (NOD Apiary Products 2018a). The objective of this experiment was to evaluate the efficacy of Formic Pro and 65% liquid formic acid as treatments for autumn varroa management in Nova Scotia.

MATERIALS AND METHODS

Test Colonies

The experiment was conducted in the autumn of 2017 in five apiaries managed by the same beekeepers in Colchester County, Nova Scotia. The apiaries were separated on average by 11.8 km (range 2.78 - 21.0 km). All but two apiaries were greater than 5 km apart. Colonies of honey bees (*Apis mellifera* L.) were housed in wooden Langstroth hive boxes. Following recommendations on the Formic Pro product label, only colonies whose populations of adult bees covered at least six frames were included in the trial (NOD Apiary Products 2017). The number of seams of bees (i.e., spaces between frames containing adult bees) were counted as an assessment of this colony strength threshold (Nasr et al. 1990). Colony feeding was carried out by the hosting beekeepers. According to the label, in-hive feeding should be avoided during Formic Pro treatment, but since colonies needed to be fed, hive top feeders were placed on all test hives the week of 16 October 2017 (midway through experiment). Only colonies whose varroa mite levels met the August economic threshold for treatment (i.e., minimum 3 mites per 100 bees) were included in the experiment, as at this infestation level and higher, colonies could experience lost honey production and fall bee losses (Currie and Gatien 2006; Currie 2008; OMAFRA 2017). Pre-treatment varroa mite levels were quantified using an alcohol wash three days prior to treatment (De Jong et al. 1982; Vandervalk et al. 2014). At this time, presence of a laying queen and brood in colonies was confirmed.

At each apiary, temperature probes (Watchdog B-series Button Loggers, Spectrum Technologies, Aurora IL) were placed in small, sealable plastic containers, out of direct sunlight, to record ambient temperature. Small holes were perforated into the containers to permit airflow. Temperature data were recorded every hour throughout the treatment period.

Experimental Design

The experiment was a randomized block design with each of the 36 test colonies randomly assigned to one of three equally proportioned treatment groups, regardless of colony strength. Bee yard location (n = 5) was used as a random blocking factor in all analyses. Although all treatments were present in each study yard, the design was unbalanced. Colonies in the first treatment group were each treated with two strips of Formic Pro which were left in each hive for 14 days. Colonies in the second treatment group were each treated with one strip of Formic Pro for ten days, which was replaced with another strip for nine additional days. The third group was treated with five rounds of 65% liquid formic acid (Formic Acid 65%, Medivet Pharmaceuticals, High River, AB) every four to five days, following industry recommendations (OMAFRA 2017; Lafrenière 2018). No untreated control group was included in the experiment due to the risk of mite populations reaching dangerous levels in untreated hives, which could have led to mite drift (Jay 1965) and compromised the experiment and the overall health of the host beekeeping operation.

Treatment Protocol

Colony strength was measured before and after the treatment period by recording the number of seams of bees in each hive. 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 was placed underneath each hive to allow mites to fall through the screen and down onto a file folder (hereafter referred to as 'sticky board'), which was inserted and removed below the screen without disturbing the colony. Sticky boards (31 cm x 43 cm) covered the entire bottom of the colony, and were covered in a thin layer of petroleum jelly (Vaseline®, Unilever, London, UK/ Rotterdam, Netherlands) to ensure falling mites were immobilized upon landing. Sticky boards were replaced two days later from each test colony and wrapped in a thin layer of plastic wrap (Cling Wrap®, Glad, Oakland, CA) to prevent mites from falling off the boards. The sticky boards were labeled and returned to the lab for quantification of fallen mites.

Formic Pro and 65% liquid formic acid were applied to the respective colonies on the first day with the screens in all bottom boards closed. Sticky boards were replaced twice per week throughout the experiment. After the treatment period, Apivar® (amitraz, Véto-pharma, Villebon sur Yvette, France), a miticide with a known high efficacy, was applied to each test colony as a "clean-up" treatment at the appropriate product label dosage for 14 days. This ensured any remaining mites in test colonies were treated to optimize overwintering success of the colony.

Statistical Analyses

Analysis of variance using a general linear model in Minitab 17 (Minitab 2018) was used for all statistical analyses. For initial varroa mite infestation from alcohol washes, and for treatment efficacy, treatment was used as a fixed effect and bee yard location was used as a random blocking factor. Overall treatment efficacy (mite mortality) (%) was calculated by dividing the total number of mites dropped during the treatment period by the total number of mites dropped during the treatment period and the Apivar clean-up period, and multiplying by 100. Analysis of colony strength data was carried out using treatment, time, and the interaction between treatment and time as fixed factors, and bee yard location as a blocking factor. Starting infestation of varroa mites was used as a covariate to determine the effect, if any, of starting infestation of varroa mites on colony strength at the end of the experiment.

Assumptions of normality of error terms and constant variance of residuals were verified for all analyses and independence was assumed through randomization. Post hoc analyses of treatment effects were carried out using Tukey tests.

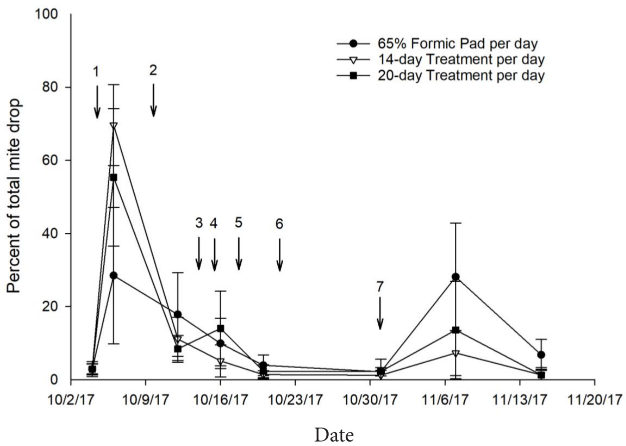
RESULTS

There was no significant difference among treatments in the mean level of varroa mite infestation (±SD) before treatment application ($F_{2,29} = 0.44$; $P = 0.65$; 12.0 ± 6.59%, 13.3 ± 6.55%, and 12.3 ± 7.52% for the 65% liquid formic acid, 14-day Formic Pro, and 20-day Formic Pro treatments respectively). There was a significant difference in varroa mite levels among bee yards ($F_{4,29} = 3.27$; $P = 0.025$): mean percent varroa infestation (across all treatments) was 16.3%, 16.0%, 11.4%, 9.9%, and 6.6% for yards 1-5, respectively. Initial mite infestation for all treatments was higher than the economic threshold for treating in NS (NS follows treatment recommendations from Ontario) of 3% infestation (OMAFRA 2017) and therefore treatment was warranted. There was a significant difference in efficacy among treatment groups ($F_{2,29} = 11.89$, $P < 0.001$) but not between bee yard location ($F_{4,29} = 1.26$, $P = 0.31$). Post hoc testing of treatment effects showed no significant difference in efficacy between the 14-day Formic Pro treatment (mean 89.4%, SD = 8.3%, range = 69.7-98.6%), and the 20-day Formic Pro treatment (mean 82.4%, SD = 14.1%, range = 58.6-99.1%). The 65% liquid formic acid treatment was significantly less efficacious (mean 62.0%, SD = 18.6%, range = 33.5-77.8%) than both Formic Pro treatments. Ambient temperature during the treatment period remained largely within the recommended range of 10 ° - 29.5 °C for all five test apiaries (NOD Apiary Products 2018a,b).

Mite drop was monitored multiple times throughout the treatment period (Figure 1). The highest mite drop was observed two days after the treatments were applied, and both of the Formic Pro treatments seemed to result in a higher mite drop compared to the 65% liquid formic acid treatment. Mite drop declined over time, except there was a small spike for the 20-day Formic Pro treatment after the second application was applied. During the clean-up treatment, more mites dropped from hives in the 65% liquid formic acid treatment compared to either of the Formic Pro treatments.

There was a significant reduction in colony strength

Figure 1. Mean varroa mite drop per day (±SD) for each of three treatments: 65% liquid formic acid, 14-day Formic Pro, and 20-day Formic Pro. Numbers correspond to specific activities in honey bee colonies in autumn in Nova Scotia, Canada: 1) application of all treatments, 2) second liquid formic application, 3) third liquid formic application, 4) 20-day Formic Pro replacement, 5) fourth liquid formic application, 6) fifth liquid formic application, and 7) removal of formic pads and application of Apivar.



during the treatment period ($F_{1, 61} = 25.17$, $P < 0.001$), however, there was no significant effect of treatment ($F_{2, 61} = 0.65$, $P = 0.53$), nor the interaction between treatment and period ($F_{2, 61} = 0.17$, $P = 0.84$) on colony strength. There were strength differences among bee yards ($F_{4, 61} = 0.17$, $P < 0.001$). Initial varroa mite infestation did not significantly impact colony strength ($F_{1,61} = 0.43$, $P = 0.51$). Mean hive strength (seams ± SD) at the start of the experiment (11.5 ± 3.83) was significantly greater than at the end of the experiment (7.7 ± 3.77). Observations at the end of the trial found no queens died in any of the treatment groups throughout the experiment.

DISCUSSION

The 14-day Formic Pro treatment had the highest efficacy against varroa mites (89%), although not significantly higher than the 20-day Formic Pro treatment (82%). The liquid formic acid treatment resulted in the lowest efficacy (62%), slightly lower than recorded efficacy in Western Canada (78%) (Vandervalk et al. 2014) but higher than in New York State (56%) (Calderone and Nasr 1999). The 14-day Formic Pro treatment had the smallest range of efficacy and standard deviation, while the 65% liquid formic acid treatment had the lowest efficacy, highest standard deviation, and the largest range (33%-78%). Despite five applications of the 65% liquid formic acid treatment, this product was comparatively less effective and varroa control was more variable in comparison to the two Formic Pro treatments.

Both Formic Pro and liquid formic acid have their greatest effects the first few days of application, with the largest mite drop in our study occurring the first two days after treatment, although mites continued to drop over the course of the treatment period. The concentration of formic acid vapours is highest for several hours after application, which can cause heavy mite drop. Spikes in varroa mite drop were observed after formic acid applications several times during our study, supporting the 'flash' treatment reputation of formic acid application. Giovenazzo and Dubreuil (2011) also noticed this effect during spring treatment of formic acid in Quebec. In the case of the 65% liquid formic acid treatment, liquid formic acid is presoaked into cotton filled Dri Loc® pads and volatilizes relatively quickly, resulting in the 'flash' phenomenon (rapid release and high concentration of formic vapours) that occurs, causing heavy mite drop soon after application (Giovenazzo and Dubreuil 2011). Other formic acid products such as Mite Away Quick Strips and Formic Pro produce these high concentrations soon after application, but the delivery of formic acid is more controlled over time. This is due to the acid being impregnated into a polysaccharide gel strip, allowing the active ingredient to remain more stable over time (Giovenazzo and Dubreuil 2011; NOD Apiary Products. 2018b).

After the first application of formic acid and Formic Pro, the 14-day treatment group experienced the largest initial mite drop. This is to be expected as two strips are placed in the hives simultaneously as opposed to two rounds of a single strip at a time. Mite drop levelled off relatively consistently across all three treatments as the treatment period progressed. Similarly, the 20-day treatment spiked after it was replaced on 16 October (Figure 1). It is likely the 65% liquid formic acid treatment experienced the highest mite drop on 7 November (clean-up treatment) as it had the lowest efficacy overall, resulting in more mites remaining for the clean-up treatment. Both Formic Pro treatments had a higher efficacy than the 65% liquid formic acid treatment which resulted in fewer mites remaining at the end of the trial during the clean-up treatment.

Despite the late-season initiation of the trial, temperatures remained relatively warm and daytime temperatures remained within the recommended temperature guidelines for Formic Pro. Our study provides a more controlled observation of the efficacy of formic acid against varroa mites than previous work performed in Western Canada, where daily ambient temperatures frequently dipped below the recommended lower limit (Vandervalk et al. 2014). Our results support applying formic acid products under recommended treatment temperatures, particularly during the first few days of treatment application, as this is when the greatest mite drop was observed. During our study, adult bees in all treatment colonies remained active - foragers were regularly seen departing and returning to their hives. In order for formic acid to effectively reduce varroa infestations, adult bees must remain active enough to fan the fumes produced as the formic acid in Formic Pro strips or soaked into the cotton pads evaporates (NOD Apiary Products 2019).

As expected with the seasonal timing of the trial, almost every colony decreased in strength over the course of the treatment period. Due to the lack of an untreated control group, it is difficult to discern whether the decrease in colony strength can be fully attributed to a natural autumn decrease in colony population, or whether a treatment effect on colony strength occurred. Hive strength varied among yards at the end of the trial, but this was not due to initial varroa infestation. Potential causes for varying hive strength among yards could be availability, quantity, and quality of floral resources in each yard, as well as landscape factors such as wind breaks.

Due to the high risk of colony mortality and mite drift, untreated control colonies were not used in this trial (Jay 1965; Guzman-Novoa et al. 2010; Degrandi-Hoffman et al. 2016). However, the comparison of natural mite drop to treatment efficacy from formic acid has been documented by others. Calderone (1999) found 51% efficacy from 65% liquid formic acid against varroa mites in New York, compared to 33% efficacy in natural drop (control). Similarly, the efficacy of natural mite drop was 19% - 26% compared to 57% - 73% using 60% liquid formic acid in Central Italy (Pietropaoli & Formato 2018).

Due to its slow release formula, Formic Pro is suitable to use during the honey flow while supers remain on hives (NOD Apiary Products 2018b). At the request of the hosting beekeepers, the trial was initiated after the removal of the honey supers, which remained on the hives relatively late in the season due to an autumn honey flow. Mite levels in test colonies were relatively high at the initiation of the trial and on average were well above the late-summer economic threshold (OMAFRA 2017). It is possible mite levels reached these high levels due to a delay in mite treatment. Drift from foraging bees from neighbouring colonies carrying phoretic mites can also contribute to high varroa populations in the autumn (Degrandi-Hoffman et al. 2016). Phoretic varroa mites shift their preference to parasitize older forager bees as opposed to nurse bees in the autumn as a possible means of dispersal (Kuenen et al. 1997).

All products tested provided some level of efficacy against varroa mites, but Formic Pro products were more effective and efficient. Although Formic Pro products are more expensive than liquid formic acid itself, there are potential benefits such as decreased labour cost (for liquid formic acid, beekeepers need to create their own treatment pads and apply multiple rounds of homemade formic acid treatment pads to colonies) and less disruption (Formic Pro products require fewer applications than traditional liquid formic acid pads). Formic Pro is also safer for beekeepers to handle, and can be used while honey supers are on colonies. In summary, both Formic Pro products were more effective at controlling mite infestations in this trial than 65% liquid formic acid. The 14-day treatment had the highest efficacy, although not significantly different than the 20-day treatment. This experiment demonstrates the high efficacy of Formic Pro, particularly the 14-day treatment option in which mites are exposed to a relatively high initial dose of formic acid.

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