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NOTE

Surveillance of synthetic acaricide efficacy against *Varroa destructor* in Ontario, Canada

Nuria Morfin[1]*, Devan Rawn[2], Tatiana Petukhova[3], Paul Kozak[4], Les Eccles[5], Jim Chaput[4], Tim Pasma[4], and Ernesto Guzman-Novoa[1]

[1]School of Environmental Sciences, University of Guelph, 50 Stone Road East, Guelph, Ontario, N1G 2W1, Canada, [2]Ontario Bee Breeders' Association, 490 York Road A102, Guelph, Ontario, N1E 6V1, Canada, [3]Department of Population Medicine, University of Guelph, 50 Stone Road East, Guelph, Ontario, N1G 2W1, Canada, [4]Ontario Ministry of Agriculture, Food and Rural Affairs, 1 Stone Road West, Guelph, Ontario, N1G 5NW, Canada, and [5]Technology Transfer Program, Ontario Beekeepers' Association, 520 Highway 6 North, Guelph, Ontario, N1H 6J2, Canada
*Corresponding author. Email: nmorfinr@uoguelph.ca

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Abstract

The parasitic mite *Varroa destructor* (Mesostigmata: Varroidae) is the main culprit of honey bee (Hymenoptera: Apidae) colony mortality in Ontario, Canada. Most beekeepers use synthetic acaricides to control *V. destructor* infestations in their colonies, but the use of these products increases the risk of *V. destructor* developing resistance to the active ingredients, which is a growing concern. This study sought to determine the efficacy of three synthetic acaricides approved for use in Canada for control of *V. destructor* infestations, amitraz (Apivar™), tau-fluvalinate (Apistan®), and flumethrin (Bayvarol®), using the Pettis test. The mite mortality rate in populations of *V. destructor* differed for the acaricides tested. Amitraz caused a significantly higher mite mortality rate (92%) than flumethrin (78%) and tau-fluvalinate (72%) did. Amitraz was classified as "mostly effective" (90–97%), whereas flumethrin and tau-fluvalinate were classified as "minimally effective" (< 80%) for *V. destructor* control in the mite populations studied. Variation in mite mortality due to acaricide exposure was observed between apiaries and regions. The results highlight the importance of periodically assessing the varroacidal activity of acaricides to inform decisions to control the parasite when implementing integrated pest management strategies.

Varroa destructor (Mesostigmata: Varroidae) is the most damaging parasite that the beekeeping industry has faced since its introduction to North America, and it is the main culprit of honey bee (Hymenoptera: Apidae) colony losses during winter in Canada (Currie *et al.* 2010; Guzman-Novoa *et al.* 2010). Because of the high prevalence of *V. destructor* infestations in North America, which may exceed 90% of the colonies (Traynor *et al.* 2016), beekeepers must regularly monitor and control *V. destructor* parasitism in their colonies to help prevent extreme damage to or death of honey bee colonies. The beekeeping industry has limited options to control *V. destructor* infestations. The use of synthetic acaricides provides an effective and practical way of controlling the parasite. Commonly used acaricides for *Varroa* control are the synthetic pyrethroids tau-fluvalinate (Apistan®; Wellmark International, Bensenville, Illinois, United States of America) and

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flumethrin (Bayvarol®; Bayer, Ontario, Canada), which act by inhibiting gated sodium channels in the mite's central nervous system (Davies *et al.* 2007). Amitraz (Apivar™; Véto-pharma, New York, United States of America), another commonly used synthetic acaricide, is a formamidine that acts by interacting with octopamine receptors in the mite's central nervous system (Sanchez-Bayo 2012). Unfortunately, mite populations have developed resistance to these chemicals because synthetic acaricides have been widely used to control *V. destructor* infestations in hives for many years (Pettis *et al.* 1998; Elzen *et al.* 2000; Elzen and Westervelt 2002; Pettis 2004; Sammataro *et al.* 2005).

Resistance is the acquired ability of *V. destructor* to tolerate synthetic acaricides, resulting in the mites' increased survival after exposure to the chemicals (Coles and Dryden 2014). Because of this, *V. destructor* resistance to synthetic acaricides can compromise bee health and colony survival. Although alternative methods of *V. destructor* control, such as organic acids and essential oil compounds, are available, synthetic acaricides are still regularly used by beekeepers because formulations of organic or naturally derived chemicals show ample variability in miticidal efficacy, and some can be harmful to the bees (Gashout *et al.* 2018, 2020; Sabahi *et al.* 2020).

Resistance of *V. destructor* to synthetic acaricides has been previously reported in Canada (Currie *et al.* 2010). However, little recent information exists about the efficacy of synthetic acaricides used for the control of *V. destructor* infestations in Canada. The objective of this study was to measure the efficacy of three commonly used acaricides (amitraz, tau-fluvalinate, and flumethrin) for *V. destructor* control in honey bee colonies in Ontario, Canada.

Ontario has 2856 beekeepers registered, with a total of 101 989 colonies (Ontario Ministry of Agriculture, Food, and Rural Affairs 2020). Commercial beekeepers (with 50 or more managed colonies) were recruited to participate in the study on a volunteer basis through the Ontario Animal Health Network (Guelph, Ontario, Canada). All beekeepers who volunteered participated in the study. Colonies were selected based on levels of mites required to perform the Pettis test (Pettis *et al.* 1998). Twelve commercial beekeeping operations in Ontario provided bees and mites for the study. Participating beekeepers managed their colonies with no restrictions in the timing and type of treatment used for *V. destructor* control. Worker honey bees and *V. destructor* mites were collected from honey bee colonies from 12 apiaries located in five different regions: east, central, Niagara, northwest, and southwest. Bees and mite samples were collected from late August to early September 2019. This sampling period represents the time of the year when mite populations increase in colonies.

The efficacy of the three synthetic acaricides was determined using the protocol described by Pettis *et al.* (1998), with modifications by the Ontario Beekeepers' Association, Technology Transfer Program (Eccles *et al.* 2013). First, colonies with *V. destructor* infestations equal to five mites per 300 bees or higher were identified, which is a requirement for the test. *Varroa destructor* infestation levels were initially determined in 130 colonies using the alcohol wash method (Shimanuki and Knox 1991; Eccles *et al.* 2013), and colonie from 12 different apiaries met the criteria. These colonies were used for the assessments.

From each of the 22 colonies that met the criteria to be used in the Pettis test, 16 samples of approximately 150 bees each (1/4 cup of bees) were collected from the brood chamber. These included four samples for each acaricide tested and the control (the control consisted of bees not exposed to acaricides). Each sample of bees was placed inside a 250-mL mason jar that was closed with a modified lid that had a #8-size wire mesh. A 2-cm^2 piece of the plastic strip formulation of each synthetic acaricide to be tested – amitraz (Apivar™; Véto-pharma), tau-fluvalinate (Apistan®, Wellmark International), or flumethrin (Bayvarol®; Bayer) – was placed inside the jar. The bees were kept in the jars for 24 hours at room temperature (22–28 °C), after which the bottoms of the jars were stroked three times with the palm of one hand (with the screen facing down) to allow dead mites to fall onto a piece of white filter paper. The mites that fell onto the paper were counted and recorded. Then each sample was washed in alcohol as per Eccles *et al.* (2013) to dislodge the remaining live mites from the bees, which

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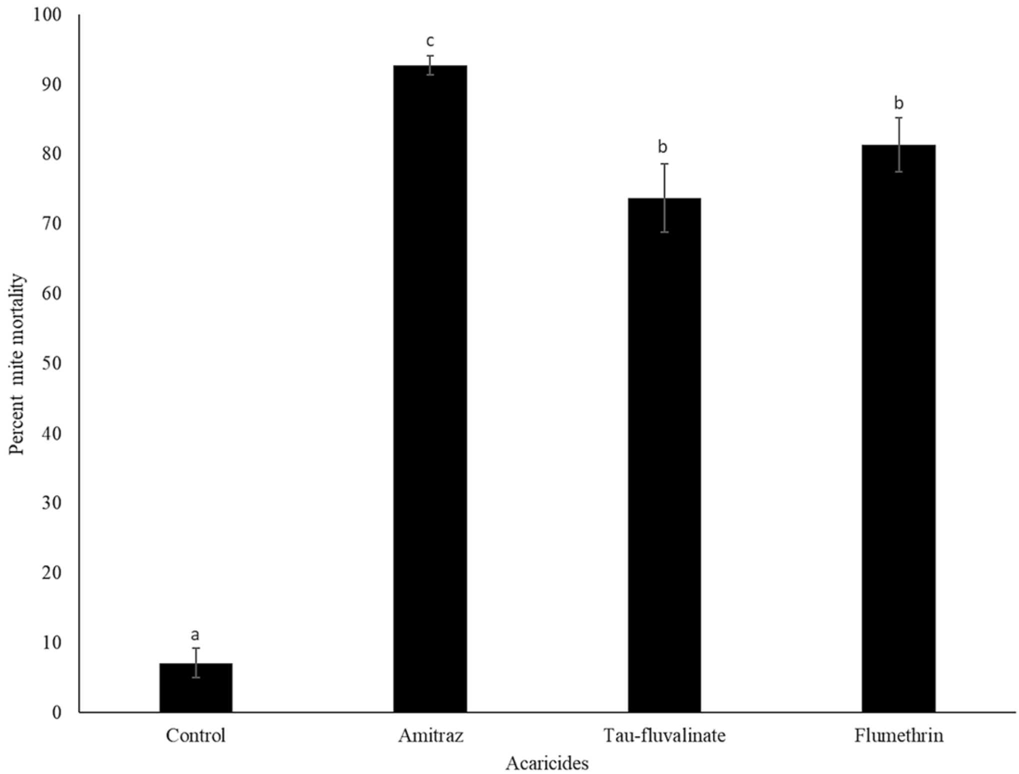

Fig. 1. Percent *V. destructor* mortality (± standard error) 24 hours post-exposure to synthetic acaricides. Different letters above the bars represent significant differences based on Kruskal–Wallis and Conover–Iman tests (α of 0.05; $n = 336$).

were also recorded. Four repetitions were conducted for each of the three treatments and the control for each colony, making for a total of 352 Pettis tests.

The miticidal efficacy rate of the acaricides tested was calculated by dividing the number of mites that died during the 24 hours of acaricide exposure by the total number of mites in the jar, and then multiplying the result by 100, as follows:

$$\%\,\text{miticidal efficacy} = \left(\text{no. mites killed in 24 h}/\text{total no. mites in jar}\right) \times 100$$

$$\text{Total no. mites in jar} = \text{no. mites killed by the acaricide} + \text{no. mites from alcohol wash}$$

Based on the results of the Pettis tests, the efficacy of the synthetic acaricides against *V. destructor* was classified as per Rinkevich (2020) into “highly effective” (> 97%), “mostly effective” (90–97%), “somewhat effective” (80–90%), and “minimally effective” (< 80%). In addition, the Kruskal–Wallis test was used to compare the acaricides for their miticidal efficacy rate because the data did not comply with normality based on the Shapiro–Wilk test. When significance was detected, multiple pairwise comparisons of medians were performed using the Conover–Iman procedure. The interactions between beekeeping operations and treatments, as well as between regions and treatments, on mite mortality were also analysed with an analysis of variance of aligned rank transform and multiple pairwise comparisons using an aligned rank transform procedure. The statistical analyses were performed using RStudio, version 1.1.456 (RStudio Team 2016) and XLSTAT 2019 (Addinsoft 2019), with the significance level set at $P < 0.05$ (α of 0.05).

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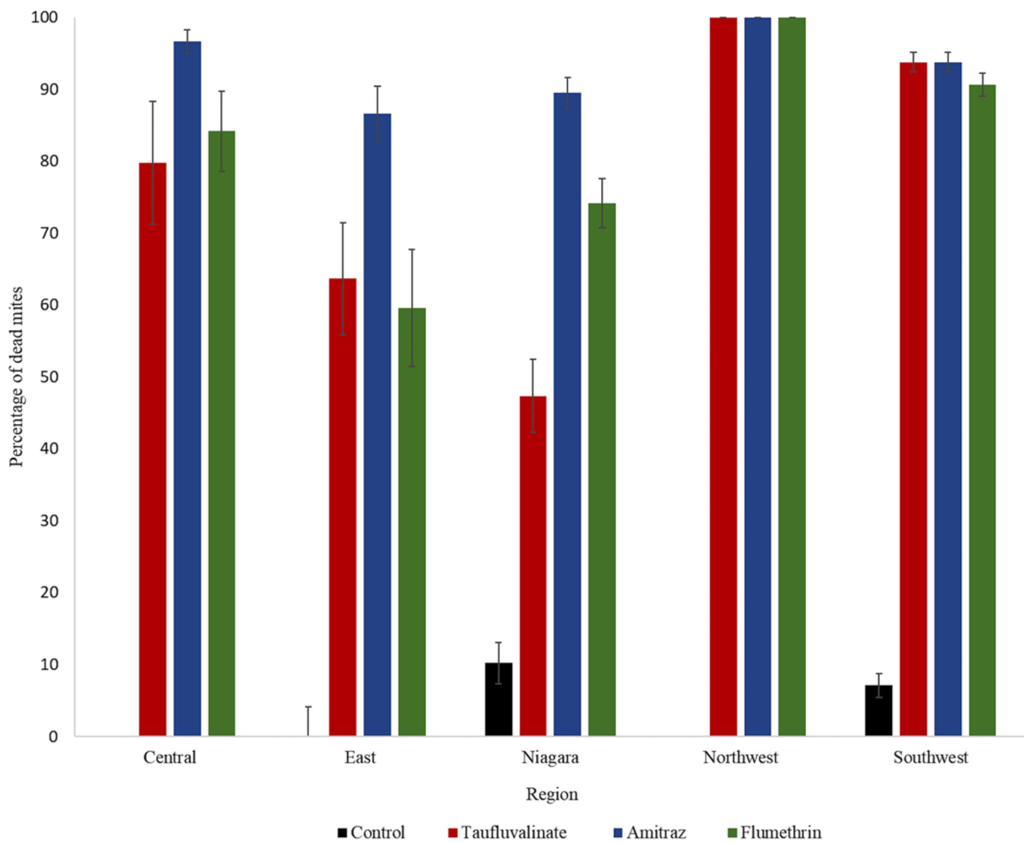

Fig. 2. Percent mite mortality in the control group (bees not exposed to any treatment) and in the groups exposed to synthetic acaricides (amitraz, tau-fluvalinate, and flumethrin) in five regions of Ontario.

Differences between the treatments were found for rate of mite mortality ($\chi^2 = 208.18$, $df = 3$, $P < 0.001$; Fig. 1). The three acaricides differed significantly from the control for mite mortality ($P < 0.01$), but amitraz killed a significantly higher percentage of mites (92%) than tau-fluvalinate (72%) and flumethrin (78%) did. Tau-fluvalinate and flumethrin did not differ for the proportion of mites killed ($P = 0.16$).

A significant interaction between apiary and treatment on percent mite mortality was found ($F_{33,\ 320} = 7.38$, $P < 0.01$). Similarly, a significant interaction between region and treatment on percent mite mortality was observed ($F_{12,\ 332} = 11.26$, $P < 0.01$; Fig. 2). *Varroa destructor* populations from colonies located in the central, northwest, and southwest regions had higher percentages of mites killed by the acaricides compared to those in the east and Niagara regions.

The results suggest that amitraz continues to be mostly effective against *V. destructor* populations in Ontario. Conversely, in the United States of America, low efficacy of amitraz against *V. destructor* was reported in 2000 from Minnesota (Elzen *et al.* 2000), and a recent study reported differences in amitraz efficacy across beekeeping operations in Louisiana (Rinkevich 2020), indicating variability in tolerance of *V. destructor* populations to amitraz, something not found in the present study. Based on the efficacy thresholds proposed by Rinkevich (2020), the present study showed that amitraz was "mostly effective" (90–97%), whereas flumethrin and tau-fluvalinate showed "minimal effectiveness" (< 80%) in the *V. destructor* populations assessed in this study. This classification is practical but arbitrary,

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and future studies should therefore seek to determine the lower limit of varroacidal efficacy at which an acaricide could still be considered useful without compromising colony health.

The results also suggest that *V. destructor* resistance to flumethrin may be occurring in Ontario. This is an unexpected result, given that Bayvarol® and its active ingredient, flumethrin, were recently registered for use in Canadian honey bee colonies (in spring 2017, two years before this study was conducted). However, flumethrin belongs to the same chemical class as tau-fluvalinate (synthetic pyrethroid), and the development of resistance of *V. destructor* populations to tau-fluvalinate was identified in Ontario nearly two decades ago (Skinner *et al.* 2003). Therefore, cross-resistance between tau-fluvalinate and flumethrin as a result of a prolonged exposure of Ontario varroa mites to tau-fluvalinate (Mitchell 1996) cannot be discounted to explain the results found for flumethrin in the present study.

Low efficacy of synthetic varroacides has been reported previously in North America and other parts of the world (Elzen *et al.* 2000; Rodríguez-Dehaibes *et al.* 2011; Kamler *et al.* 2016), demonstrating the potential risk that *V. destructor* populations can develop resistance to synthetic acaricides. In the present study, differences in the percentage of mite mortality due to exposure to the synthetic acaricides tested were noted between different regions of Ontario. Environmental conditions are known to influence the ability of pests, including mites, to develop resistance to acaricides. For example, mite resistance to acaricides can be accelerated by changes in climate patterns (Pu *et al.* 2020) and as a result of management practices, such as migratory beekeeping (Rodríguez-Dehaibes *et al.* 2011). The Niagara region, one of the regions in which acaricides had lower efficacy against mite populations in the present study, is characterised by having commercial beekeeping operations that provide migratory beekeeping-for-pollination services. In eastern Ontario, nucleus colonies are introduced from other regions in the province, with some colonies possibly coming from operations having acaricide-tolerant mites. This might explain the low efficacy of some acaricides found there. However, further studies are needed to confirm this hypothesis.

The variation in the acaricidal efficacy of tau-fluvalinate and flumethrin among apiaries and regions underscores the importance of periodically assessing the efficacy of synthetic acaricides against *V. destructor* to integrate the information of these assessments in mite management protocols. Integrated pest management strategies are used to decrease the risk of pests developing resistance to synthetic acaricides (Ehler 2006; Ruffinengo *et al.* 2014). Assessing the efficacy of synthetic miticides in varroa mite populations periodically should be a key component of integrated pest management strategies. Information on synthetic acaricide efficacy would help beekeepers to select the best plan to control varroa mite levels by allowing them to reconsider the use of synthetic acaricides when the substances lose efficacy. In addition, studies examining larger sample sizes and in other regions of Ontario are needed to assess the value of the different synthetic acaricides used for the control of *V. destructor* infestations in honey bee colonies. Moreover, further studies on management practices, nucleus introductions, and other stressors contributing to the development of mite resistance should be conducted to better understand the influence of beekeeping management on the efficacy of synthetic miticides used for *Varroa* control.

Strategies to decrease the development of resistance, such as rotation of acaricides (Milani 1999) and the use of alternative treatments with different modes of actions (*e.g.,* oxalic acid and thymol), should be considered to successfully control *V. destructor* infestations in the coming years.

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