Sabrina Rondeau
Centre de recherche et d'innovation sur les végétaux,
Université Laval
2480 boul. Hochelaga,
Québec (Qc), Canada, G1V 0A6
Email: sabrina.rondeau.1@ulaval.ca

The use of the predatory mite *Stratiolaelaps scimitus* (Mesostigmata: Laelapidae) to control *Varroa destructor* (Mesostigmata: Varroidae) in honey bee colonies in early and late fall

Sabrina Rondeau[1], Pierre Giovenazzo[2] and Valérie Fournier[1]

[1] Département de phytologie, Université Laval, 2480 boul. Hochelaga, Québec, Quebec, Canada, G1V 0A6

[2] Département de biologie, Université Laval, 1045, av. de la Médecine, Québec, Quebec, Canada, G1V 0A6

Abstract

The ectoparasitic mite *Varroa destructor* Anderson & Trueman is a major pest of the honey bee *Apis mellifera* L. and its control is one of the most important challenges that beekeepers have to face. In this study, we investigated the use of the predatory mite *Stratiolaelaps scimitus* (Womersley) for the biological control of varroa mites in Eastern Canada, as part of an integrated pest management strategy. Our study aimed to evaluate the effectiveness of *S. scimitus* in controlling varroa populations in early and late fall in comparison with untreated colonies and two currently used organic treatments: Thymovar® and oxalic acid. Performing weekly mite drop monitoring, we first compared the effectiveness of two introduction rates of *S. scimitus* (≈ 6,250 or 12,500 mites/colony) during a fall treatment (September) and, as we detected no differences of effectiveness between these two treatment types, we used the dosage currently recommended by biocontrol suppliers (≈ 6,250 mites) in a complementary treatment test (November). Results showed that *S. scimitus* did not succeed in controlling varroa populations in honey bee colonies when introduced either in early or in late fall according to current suppliers' recommended rates and application method. On the other hand, our results demonstrated that Thymovar® and oxalic acid remain effective options for controlling varroa mite populations during fall in Quebec, Canada.

Keywords

Apis mellifera, biological control, oxalic acid, thymol, varroa mite

Introduction

For more than a decade, winter losses of honey bee (*Apis mellifera* L.) colonies have remained at levels considerably higher in North America and Europe than rates identified as acceptable by beekeepers (van der Zee et al. 2012, Ferland et al. 2017, Kulhanek et al. 2017), raising concerns about the future of crop pollination services performed by managed honey bee colonies. In fact, while the global demand for commercial pollination services by honey bees is increasing (Aizen et al. 2008), the ongoing high rates of colony losses threaten the production of many vegetables, fruits, nuts and seeds (Klein et al. 2007, Potts et al. 2010). In an attempt to mitigate the negative effects of colony losses and to rebuild bee stocks in the spring, beekeepers strive to replace their dead colonies year after year (vanEngelsdorp and Meixner 2010). However, these manipulations are labor intensive and involve important financial costs (Kulhanek et al. 2017), which threaten the long-term sustainability of commercial beekeeping operations and related pollination services.

Although honey bee colony mortality is known to be driven by multiple interacting factors, the scientific consensus consider the parasitic mite *Varroa destructor* Anderson & Trueman (Acari: Varroidae) as the main culprit of winter colony losses (Rosenkranz et al. 2010, McMenamin and Genersch 2015, van der Zee et al. 2015). Since its shift from its original host *Apis cerana* Fabr. to the Western honey bee *A. mellifera* in the middle of the 20th century, the ever-increasing widespread distribution and prevalence of the parasite have had detrimental effects to the apiculture industry, making *V. destructor* the most important pest that beekeepers have never had to face worldwide (Boecking and Genersch 2008, Rosenkranz et al. 2010). Through direct parasitism of bees and transmission of viruses and secondary infections (Yang and Cox-Foster 2007, Nazzi et al. 2012), the varroa is known to have strong deleterious effects on overall colony

71 health (Sammataro et al. 2000, Rosenkranz et al. 2010). As a consequence, highly infested
72 colonies that are kept untreated typically exhibit reduced colony growth, honey production and
73 winter survival (Le Conte et al. 2010, Emsen et al. 2014, Desai and Currie 2016).

74 Several acaricides have been developed over the last decades as an attempt to control varroa
75 infestations, especially in temperate regions where the absence of periodic treatment results in
76 rapid colony collapse (Rosenkranz et al. 2010). For instance, synthetic acaricides have been
77 successfully used in the past but their repeated use has rapidly led to the development of
78 pesticide-resistant mites (Milani 1999, Elzen et al. 2000, Maggi et al. 2009). In response,
79 Integrated Pest Management (IPM) strategies have been developed (Delaplane et al. 2005,
80 Rosenkranz et al. 2010), encouraging timely use of appropriate chemical and non-chemical
81 tools. Alternative strategies to control varroa mites include the use of soft chemicals such as
82 organic acids (formic acid or oxalic acid) and essential oils (thymol), genetic selection, as well as
83 cultural and physical methods (Rosenkranz et al. 2010). However, none of these strategies are
84 sufficiently effective to be used alone (Delaplane et al. 2005).

85 In Eastern Canada, varroa control based on IPM is performed at multiple key moments
86 throughout the year (Eccles et al. 2016). If needed, a first treatment takes place during early
87 spring. A summer treatment may be needed in late July to early August, while a preventive fall
88 treatment is always strongly recommended and should occur no later than mid-September.
89 Finally, for fall treatments using formic acid or thymol, a complementary treatment with oxalic
90 acid must be carried out at the beginning of November, just before winter. This very last
91 treatment is crucial because formic acid or thymol alone does not get rid of all the mites
92 (Gregorc and Planinc 2012, Coffey and Breen 2013, Gregorc et al. 2016) and their effectiveness
93 is strongly dependent on environmental conditions (Al Naggar et al. 2015). Moreover, these

chemical treatments may have toxic effects that affect colony productivity and survival (Gregorc and Smodis Skerl 2007, Giovenazzo and Dubreuil 2011, Schneider et al. 2012, Vandervalk et al. 2014, Alayrangues et al. 2016). For example, external temperatures influence thymol evaporation, affecting its effectiveness considerably at low temperatures (< 15°C) and increasing bee mortality above 30°C (Imdorf et al. 1995, Imdorf et al. 1999). Organic acids are also toxic to humans (Rademacher and Harz 2006, Canadian Honey Council 2010) and most beekeepers would rather not use them if safer alternative measures were available. For all these reasons, the development of alternative methods of varroa control continues to stand as a research priority for the beekeeping industry (Dietemann et al. 2012, Nazzi and Le Conte 2016).

Despite its importance, the use of biological control agents as an important aspect of IPM remains underexploited. In fact, it is not easy to find a living organism that would control varroa mite numbers without increasing the mortality of the bees themselves (Chandler et al. 2001). Nevertheless, a new candidate, the predatory mite *Stratiolaelaps scimitus* (Womersley) (Acari: Laelapidae), has been put forward in recent years as being particularly promising to control varroa mites. In a previous study (Rondeau et al., unpubl. data), we showed that in addition to being able to feed upon free varroa mites under laboratory conditions (Rangel and Ward 2018), this generalist soil-dwelling predator can survive and be active under the physical conditions of a honey bee colony and does not represent a significant threat to bee brood. However, to date, few scientific data are available on the effectiveness of the investigated biocontrol agent to control varroa populations in situ. Although preliminary observations from Ontario (Canada) suggest the predator's effectiveness in lowering varroa numbers in honey bee colonies (Scott 2014), a recent study revealed no effectiveness of the predator in field colonies (Rangel and Ward 2018). Therefore, we urgently need to further investigate the effectiveness of the predator in the field.

The main objective of this study was to investigate whether the introduction of the predatory mite *S. scimitus* into honey bee colonies could effectively be used as a fall IPM strategy against varroa mites in the cold temperate climate of Quebec, Canada. More specifically, this project aimed to evaluate and compare the effectiveness of *S. scimitus* in controlling varroa populations when used: 1) in the early fall in comparison with Thymovar®, and 2) in replacement of oxalic acid to complement a standard fall treatment in November. Performing mite drop monitoring, we first compared the effectiveness of two introduction rates of *S. scimitus* during a fall treatment and, as we detected no differences of effectiveness between these two treatment types, we used the dosage currently recommended by biocontrol suppliers in our complementary treatment test.

Methods

Honey Bee Colonies

The trials were conducted in experimental apiaries of the Centre de Recherche en Sciences Animales de Deschambault (CSRAD) located in the province of Quebec, Canada. All colonies used in each trial had sister queens of known descent. 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 last time the colonies were treated was in fall 2016 with organic acids. The colonies were fed sugar syrup after honey suppers were removed on September 11, 2017.

Predatory Mite Sources

The biocontrol agent *S. scimitus* was supplied by Applied Bio-nomics Ltd. (British Columbia, Canada) in a mixture of vermiculite and peat in 1L bottles with mold mites (*Tyrophagus*

putrescentiae (Schrank)) as a food source. The product was used within two days following its receipt and was checked for predator vitality before its use. Upon receipt, the product was stored in its original containers, lying on their side in complete darkness at 15°C.

Fall treatment

Field trials to assess the effectiveness of *S. scimitus* as a varroa treatment were performed according to the COLOSS BEEBOOK recommendations (Dietemann et al. 2013). The trials were conducted between August 28 and November 27, 2017, using a completely randomized design. Three weeks before the treatment, 28 colonies were evaluated for strength (number of frames covered with bees), queen status and overall colony health. From then, natural mite drop from the colonies was monitored once a week (7-day intervals) until the end of the test, using home-made sticky boards consisting of corrugated plastic sheets coated with vegetable shortening. This allowed to obtain weekly mite drops before, during, and after the treatments. In order to balance colony strength and initial varroa infestation levels between groups, colonies were first ranked before being randomly assigned to one of the four treatments, with seven colonies per group: 1) negative control (no treatment), 2) treatment with a low number of predatory mites (≈ 6,250 mites; 250 ml/colony), 3) treatment with a high number of predatory mites (≈ 12,500 mites; 500 ml/colony), and 4) positive control treatment (application of one wafer of Thymovar®/colony as per label). The two predatory mite rates were chosen based on supplier recommendations and previous anecdotal observations (Scott 2014). Colonies were treated on September 11, 2017, either by sprinkling 500 ml of pre-autoclaved vermiculite (group 1) or the corresponding amount of vermiculite-based medium containing *S. scimitus* (groups 2 and 3) over the top bar of the brood frames, or by using Thymovar® according to label directions (group 4). Each wafer of Thymovar® contained 15 g of thymol. The wafer was cut in half and

placed on top of the combs of the top brood chamber on either side of the edge of the brood. Thymovar® wafers were removed after four weeks and we continued counting the mites for one additional week to allow for possible residual effect. By that time, the mite drop had returned to pre-treatment levels.

In order to quantify the number of varroa mites remaining in the colonies and to calculate the effectiveness of each treatment, a follow-up treatment was performed on October 16, 2017, on all the colonies, using Apivar® (active ingredient: amitraz; 2 strips/colony) according to label directions. The natural mite drop was monitored with sticky boards once a week throughout the duration of the treatment (42 days). The effectiveness of each fall treatment was calculated as follows (Dietemann et al. 2013): % Effectiveness = (total number of mites killed during fall treatment x 100) / (total number of mites killed during fall treatment + total number of mites killed during follow-up treatment with Apivar®).

Complementary treatment

The 21 colonies used in this trial were located in one single apiary – not the same as from the previous experiment – and each colony had been previously treated with Thymovar® (one wafer/colony) on September 11, 2017, according to label directions. The wafers were removed after four weeks of treatment. On October 31, 2017, these colonies were also evaluated for strength (number of frames covered with bees), queen status and overall colony health. We then started monitoring natural mite drop once a week (7-day intervals) using sticky boards. Colony strength and initial varroa infestation levels between groups were balanced using the same method as previously described and seven colonies were randomly assigned to each of the three treatment groups. Colonies were treated two weeks later according to the following treatments: 1) negative control (no treatment; 250 ml of pre-autoclaved vermiculite), 2)

186 treatment with predatory mites (≈ 6,250 mites; 250 ml/colony), and 3) positive control
187 treatment (oxalic acid dihydrate in sucrose solution). The vermiculite (group 1) and the medium
188 containing the predatory mites (group 2) were introduced in colonies by pouring the substrate
189 over the top bar of the brood frames, while oxalic acid was applied following the standard
190 trickling method procedures according to label directions (Canadian Honey Council 2010).
191 Thereby, the oxalic acid solution was trickled directly onto the bees (5 ml in each occupied bee
192 space) and the total dose did not exceed 35 ml per colony. The solution was prepared by
193 dissolving 35 g of Oxalic Acid Dihydrate (99.65%) in 1 liter of 50% sucrose solution (w/v).

194 All colonies were moved to a building kept at 4 ± 1°C for indoor overwintering on November 23,
195 2017. At that time, we continued monitoring mite drop weekly until the numbers had returned
196 to pre-treatment levels. The last monitoring of the fall was conducted on December 11, 2017,
197 four weeks after the treatment application.

198 On April 20, 2018, the hives were taken out of the overwintering building and subsequently
199 evaluated for survival and strength (number of frames covered with bees) three days later. At
200 that time, we observed the frames and the floor of the hives in search of *S. scimitus* individuals.
201 A follow-up treatment was performed on April 24, 2018, on all the colonies, using CheckMite+®
202 (active ingredient: coumaphos; 2 strips/colony) according to label directions. Once again we
203 monitored the natural mite drop with sticky boards weekly through the duration of the
204 treatment, which lasted 43 days. The effectiveness of each treatment was calculated as
205 previously described: % Effectiveness = (total number of mites killed during the complementary
206 treatment x 100) / (total number of mites killed during the complementary treatment + total
207 number of mites killed during follow-up treatment with CheckMite+®).

Screening of predation evidences

Counts of fallen varroa mites on sticky boards were made in the laboratory. For every week of monitoring following treatment with *S. scimitus*, varroa "shells" (≥10 per sticky board) were observed under the stereomicroscope and screened for signs of predation. Following an attack by *S. scimitus*, varroa mites typically show many missing legs and large holes in their cuticle (Rondeau et al., unpubl. data). The appearance of the varroa shells from the colonies treated with the predatory mites were compared to those from control colonies.

Temperature records

For both experiments, ambient temperature records were obtained from the nearest weather station (Donnacona2, QC) of Environment and Climate Change Canada. The station was located at 3 and 13 km from the apiaries of our fall and complementary experiments respectively. Since temperature can influence the effectiveness of thymol as well as the behaviour of *S. scimitus*, the potential effect of the daily minimal and maximal temperatures was accounted for in the discussion section.

Statistical analysis

For both experiments, varroa mite drop dynamics were analysed using the proc mixed procedure in SAS® Univrsity Edition (SAS Institute Inc. 2017). Data were first divided in groups: pre-treatment period, treatment period, and, if applicable, follow-up treatment period. Then, for each group, a repeated measures analysis of variance (RM-ANOVA) with autoregressive correlation structure was performed on log-transformed data in order to compare the effect of treatments, time and their interaction on the numbers of weekly fallen varroa mites. Significant parameters were then analysed using planned contrasts to compare the weekly mite drop

between: 1) positive control and other treatments, 2) negative control vs *S. scimitus*, and, for the fall treatment only, 3) low vs high rates of *S. scimitus*. Concerning the effectiveness calculations of the fall treatment experiment, two colonies were removed from the analysis due to missing data, reducing to six the number of colonies used for group 2 (low rate of *S. scimitus*) and group 4 (Thymovar®). For both experiments, treatment effectiveness was calculated as a percentage for each colony and log-transformed for normalization prior to statistical analyses. Using the R software (R Core Team 2016), differences in means between groups were assessed using a one-way ANOVA followed by the same planned contrasts described above. Significance was defined as $p \leq 0.05$ for all statistical tests.

Results

Colonies were infested with an average of 1,228 varroa mites per colony (fall treatment) and 888 varroa mites per colony (complementary treatment). These values are well above the minimum infestation level of 300 varroa mites per colony recommended for the purpose of effectiveness assessments using mite drop (Dietemann et al. 2013). Similarly, the infestation level of all colonies remained below the damage threshold (< 4,200 mites per colony) identified by Delaplane and Hood (1999). For each colony, the infestation level corresponds to the sum of the total number of mites killed before and during the treatment, as well as during the follow-up treatment.

Fall treatment

The treatment application was carried out during a sunny morning of 16°C. Throughout the treatment, the daily ambient temperatures ranged from -3.2 to 31.8°C (Table 1).

Prior to the treatment, the average (± SE) weekly varroa mite fall was 32 ± 3 mites per colony and did not significantly differ between treatment groups (RM-ANOVA, $F = 0.17$; $df = 3, 24$; $p = 0.916$). During the treatment period, there was only an effect of the monitoring time on the mite drop ($F = 6.56$; $df = 4, 90$; $p < 0.001$) while the effect of the treatments ($F = 1.12$; $df = 3, 27$; $p = 0.357$) and the overall effect of the interaction between treatments and time ($F = 0.88$; $df = 12, 89$; $p = 0.573$) were not significant. However, when treatment means were compared within each week using contrasts, varroa mite drop was significantly higher in colonies treated with Thymovar® compared to other treatments during the first ($p = 0.001$) and the second ($p = 0.033$) week of treatment. The average number of fallen varroa mites subsequently declined over the following weeks. Throughout the 5-week treatment period, however, the average mite drop in colonies treated with *S. scimitus* never differed from those in untreated colonies and there was no difference between both rates of *S. scimitus*. Similarly, during the follow-up treatment period, neither the effect of treatments on the mite drop nor the overall effect of the interaction between treatments and time were significant ($F = 0.90$; $df = 15, 102$; $p = 0.567$). However, as a result of higher varroa mortality with Thymovar®, the average number of fallen varroa mites was significantly lower in this group during the first week after the application of Apivar® ($p = 0.028$), when data were compared within each week using contrasts. On the other hand, the average varroa mite drop in the colonies treated with the predatory mite remained similar to those of control colonies (Fig 1).

The average effectiveness of each fall treatment is given in table 2. In control colonies, the natural mite reduction ranged from 9.3% to 25.8%, which was really similar to the effectiveness of *S. scimitus* at both low (12.2% to 22.1%) and high (11.6% to 27.3%) rates. Thymovar® was the most effective treatment, with a calculated effectiveness ranging from 37.8% to 77.6%. There was a significant difference of effectiveness between treatments (ANOVA, $F = 32.4$; $df = 3, 22$; p

< 0.001) and subsequent contrast analyses showed a significantly higher effectiveness of Thymovar® compared with other treatments ($p < 0.001$), but no difference in effectiveness between the control group and the use of *S. scimitus* ($p = 0.803$) or between the two tested rates of *S. scimitus* ($p = 0.295$).

Complementary treatment

The temperature (2°C) was much cooler when the complementary treatment was applied in November. At that time, the nocturnal temperatures were under 0°C. Throughout the treatment, the daily ambient temperatures ranged from -16.0°C to 5.7°C (Table 1).

Here again, the average ($\pm$ SE) weekly varroa mite fall per colony (54 ± 8) did not significantly differ between treatment groups prior to the treatment (RM-ANOVA, $F = 0.23$; $df = 2, 18$; $p = 0.753$). During the treatment period, there was a significant interaction between treatment and time ($F = 5.01$; $df = 6, 52$; $p < 0.001$). Our contrast analysis revealed significantly higher numbers of fallen varroa mites with oxalic acid during the entire treatment period (Fig 2). On the contrary, the varroa mite drop was not different between untreated colonies and colonies treated with *S. scimitus* at any time during treatment. During the follow-up treatment period, both treatment ($F = 3.4$; $df = 2, 18$; $p = 0.036$) and time ($F = 44.23$; $df = 5, 77$; $p < 0.0001$) had a significant effect on the weekly mite drop but no interaction was detected. As a result of higher varroa mortality with oxalic acid, the average number of fallen varroa mites was significantly lower in this group during the first week after the application of CheckMite+® ($p = 0.031$), when data were compared within each week using contrasts. As for varroa mite drop in the colonies treated with the predatory mite, it continued to share a similar dynamic with control colonies throughout the follow-up treatment period (Fig 2).

All 21 colonies survived through winter and there was no difference in strength (6 frames of bees on average) between colonies of each group (ANOVA, $F = 0.11$; $df = 2, 18$; $p = 0.899$) after the wintering period. During the spring evaluation, no *S. scimitus* individual was observed neither on sticky boards nor in the hives.

The average effectiveness of each complementary treatment is shown in table 3. The natural mite reduction in control colonies of the complementary treatment was comparable to those of the fall treatment and ranged from 3.7% to 22.4%, which was similar to the effectiveness of *S. scimitus* (4.2% to 35.6%). Of the three treatments, oxalic acid was the most effective, with a calculated effectiveness ranging from 83.6% to 92.0%, with an outlier colony that had an effectiveness of only 45.4%. This last data was kept in the analysis as it reflects the existing difficulty of obtaining reliable treatment effectiveness time after time. In this case, the median (87.0%) serves as a better indicator of the real oxalic acid effectiveness. The calculated effectiveness differed significantly between treatments (ANOVA, $F = 195$; $df = 2, 18$; $p < 0.001$) and subsequent contrast analyses showed a significantly higher effectiveness of oxalic acid compared with other treatments ($p < 0.001$), but no difference in effectiveness between the control group and the use of *S. scimitus* ($p = 0.499$).

Discussion

Our study showed that the use of *S. scimitus* did not succeed in controlling varroa populations in honey bee colonies when introduced either in early or in late fall according to current suppliers' recommended rates and application method. The dosage currently recommended by biocontrol suppliers is about 150 ml to 200 ml per hive (≈ 3,750 to 5,000 mites), which correspond to the lowest rate used in this trial. However, neither this dosage (our low application rate) nor the

double of it (our high application rate) increased varroa mortality when compared to untreated colonies. For both experiments, the calculated average natural varroa mortality in the control colonies during treatment period (16.5% and 14.6%) is similar to the 17.8% mortality reported by Coffey and Breen (2013) and slightly lower than the 23% reported in Stanghellini 2004. The calculated effectiveness of both rates of *S. scimitus* reported in our study does not exceed this natural varroa mortality. Higher rates of introduction could potentially increase the level of varroa control using *S. scimitus*. However, if it was the case, the use of the biocontrol agent would be rather expensive, considering that a treatment with 500 ml of the product containing *S. scimitus* (high rate) currently details approx. $15.00 CAD per colony. According to Canadian reference, this is three times the price of a treatment with Thymovar® ($4.50/colony) and more than 100 times the price of a treatment with oxalic acid (less than $0.15/colony).

Our data showed similar varroa mite mortality dynamics between colonies treated with *S. scimitus* and untreated ones. In both trials, the predatory mite did not cause higher initial varroa mite mortality following treatment application, which suggests that multiple introductions would not be more efficient. Similarly, a field experiment conducted at Texas A&M University in fall 2014 and spring 2015 recorded no significant difference of varroa population reductions with weekly inoculations of 2,500 *S. scimitus* individuals (100 ml) during a six-week period (Rangel and Ward 2018). Moreover, even if repeated introductions of *S. scimitus* could cause higher varroa mite mortality, such labor intensive treatment schedule would probably not be adopted by commercial beekeepers with substantial numbers of colonies.

In a recent study, Rangel and Ward (2018) detected no significant effect of *S. scimitus* treatment on lowering varroa populations in colonies compared to an untreated control group. However, their study was performed in different field settings (lower rate of *S. scimitus*, multiple

342 introductions, spring treatment) and did not include the calculation of the predator's
343 effectiveness (%) to reduce varroa populations in hives. Nevertheless, our results corroborate
344 the first findings of these authors, which reinforce the effectiveness improbability of the
345 predatory mite in varroa control.

346 Our results contradict the anecdotal but promising observations of varroa control with *S.*
347 *scimitus* reported by the Niagara Beeway in Ontario, Canada (Scott 2014). The lack of details for
348 methods and related results of the preliminary investigation they conducted makes the
349 comparison with our study difficult. However, our results do not support their observation of
350 similar varroa control levels obtained with *S. scimitus* and chemicals. This is important because
351 many biocontrol suppliers and honey bee professionals cite the research done at the Niagara
352 Beeway as a reference for *S. scimitus* potential to fight the varroa. Using an ineffective varroa
353 treatment may have highly detrimental effects on colony health and survival. Therefore,
354 beekeepers and bee professionals should be aware that the field effectiveness of the predatory
355 mite must be confirmed by peer reviewed experimental data. Our study, on the contrary,
356 provides evidence of the ineffectiveness of *S. scimitus* in varroa control, at least under the
357 conditions and region within which we conducted the experiments.

358 In our fall treatment experiment, Thymovar® was the most effective treatment to reduce varroa
359 mite populations. However, the effectiveness percentage of Thymovar® calculated in the
360 present study (64.7%) is lower than those reported in previous ones. For example, Coffey and
361 Breen (2013) and Vandervalk et al. (2014) reported an average effectiveness of 84% and 89% for
362 Thymovar® in the cool climate of Ireland and Western Canada respectively. These differences in
363 effectiveness probably reflect different treatment methods, climatic conditions, geographic
364 emplacement and hive management practices. For instance, while we used only one wafer of

Thymovar® per hive for four weeks in our study, both of the previous studies used two wafers for a longer period of time. Considering the specific climatic conditions encountered in Quebec, Thymovar® is traditionally used during a shorter period and its use is followed by a complementary treatment with oxalic acid. Moreover, it is suggested by the Health Canada Pest Management Regulatory Agency (2010) that the effectiveness of Thymovar® may be reduced if it is applied during the feeding period due to increased ventilation by bees. This could explain the lower effectiveness obtained in our study as we performed both at the same time. However, this practice is commonly used by beekeepers in order to reduce the number of manipulations required during fall hive management. Thus, we consider that our results accurately reflect Thymovar® effectiveness obtained in the realistic hive management conditions of Eastern Canada.

The calculated effectiveness of Thymovar® to kill varroa mites varied strongly at the colony level, going up to double between the lower (37.8%) and the higher (77.6%) obtained percentages. This is not surprising since thymol typically shows inconsistent degrees of varroa control and great variability between studies, localities and environmental conditions (Floris et al. 2004, Coffey and Breen 2013, Leza et al. 2015). In fact, it seems that the amount of thymol delivered in hives decreases at low temperatures and high humidity (Emsen et al. 2007). Considering the high variability of our results, the median (73.1%) is probably a better indicator of Thymovar® effectiveness than the mean. Moreover, as seen in our study, moderate varroa infestations typically allow the use of less effective control treatments when multiple IPM strategies are used together. If we consider that the fall treatment is to be followed by a complementary treatment in November, the use of Thymovar® remains an adequate IPM tool for varroa control.

388 Based on varroa mite mortality dynamics, oxalic acid provided significant varroa control as a
389 complementary treatment. This organic acid is typically reserved as a late-season treatment
390 when there is little or no brood production, to complement a fall treatment with soft chemicals
391 (e.g. formic acid or thymol). In late fall, as a result of broodless colonies, the entire population of
392 varroa mites parasitizes adult bees (phoretic stage). In such conditions, many studies have
393 demonstrated the effectiveness of oxalic acid in varroa control. For example, using the same
394 trickling method to apply oxalic acid as we used in this study, Charriere and Imdorf (2002)
395 reported > 97% varroa control effectiveness in late fall. Similarly, Stanghellini and Raybold
396 (2004) reported 92% mite mortality in the Northern temperate climate of New Jersey (USA). This
397 is slightly higher than the effectiveness obtained in our study (mean: 82.1%, median: 87.0%),
398 which nevertheless confirms the effectiveness of oxalic acid under the testing conditions. At the
399 opposite, a previous study conducted at our laboratory showed that *S. scimitus* does not attack
400 varroa mites when they are attached to the body of adult bees (Rondeau et al., unpubl. data).
401 This finding is consistent with the poor varroa biocontrol achieved in the present study and
402 probably constitutes the main explanation for the predator ineffectiveness.

403 Under the stereomicroscope, very few evidence of predation was noted on varroa shells fallen
404 on sticky boards. During the first two weeks following the early fall introduction of *S. scimitus* in
405 hives, we recorded some *S. scimitus* individuals walking on the sticky boards (< 10
406 individuals/board). From the third week, no more *S. scimitus* were found. Similarly, in previous
407 trials we observed the presence of the predatory mite in the hive for at least 10 days during
408 summer. However, in the complementary treatment experiment, we never recorded its
409 presence on boards, even during the first week following its introduction. It is important to note
410 that the sticky boards used in our study for varroa monitoring did not trap the predatory mites,
411 as they easily move over the vegetable shortening layer covering the corrugated plastic sheets.

412 On the other hand, dozens of mold mites (presumably *Tyrophagus putrescentiae*) were seen on
413 our boards during the first weeks of treatment of both experiments. Most likely, these mites
414 were introduced along with *S. scimitus* since they are supplied with the predatory mite as a food
415 source during the transit and in storage. Similarly, no *S. scimitus* individuals were recorded in
416 the hives on the following spring although we observed many mold mites and other mite species
417 on the hive floor of several colonies. These observations suggest that the biocontrol agent may
418 have left the hives soon after its introduction in November or, at least, that it did not stay in the
419 hives throughout the winter. In any case, more data on the behavior and movements of *S.*
420 *scimitus* within bee colonies would be needed to fully understand its ecological dynamics and
421 related biocontrol potential against varroa mites under the characteristic conditions of the
422 honey bee hive.

423 Environmental conditions, especially temperature, may have played an important role in the
424 results obtained. For instance, the field effectiveness of thymol based products is known to be
425 reduced under 15°C (Imdorf et al. 1995). For better results, fabricant recommendations for
426 Thymovar® include a daily maximum temperature above 12°C. This recommendation was met in
427 our study, since the maximum daily ambient temperature never ran under 16°C during the 4-
428 week treatment period with Thymovar®. From mid-October, however, ambient temperatures
429 decreased significantly, justifying the use of oxalic acid in late fall, which remains effective at
430 cool temperatures. Of course, living organisms are also sensitive to temperature conditions. It is
431 known that the predator *S. scimitus* can develop and reproduce between 15 °C and 30°C, with
432 an optimum temperature of 25°C (Ydergaard et al. 1997). Under 12°C, the predatory mite can
433 no longer complete its developmental cycle (Wright and Chambers 1994) but adults may still
434 survive for several weeks at 10°C (Steiner et al. 1999). Although we did not monitor the
435 temperatures at the hive floor – where *S. scimitus* is most likely to be found – it is evident that

436 the conditions were milder during the fall experiment than during the complementary one. This
437 would explain why we observed some predators walking on sticky boards only in early fall. One
438 of the most plausible explanation would be that the predator had rapidly returned to the
439 ground, its natural environment, to escape the cool weather.

440 Even in the two weeks during which the predator stayed in the hive, *S. scimitus* had no effect on
441 varroa mortality, indicating that other factors than just climatic conditions have played a role in
442 the predator's inability to control varroa populations. Both the inability of *S. scimitus* to attack
443 phoretic varroa mites and the presence of multiple food sources in the hive have been put
444 forward in our previous study as potential barriers likely to reduce the efficiency of *S. scimitus* to
445 target the varroa. Acaricide residues accumulated in hive materials could also have prompted *S.*
446 *scimitus* to escape from its new environment. However, we do not think that the concentration
447 of these residues was high enough to kill the predatory mite since we noticed the presence of
448 other mite species on the sticky boards throughout the varroa monitoring process. Summer
449 would probably be a more appropriate season to introduce *S. scimitus* into colonies considering
450 the warmer ambient temperatures. Yet, even if summer conditions could increase varroa
451 mortality induced by *S. scimitus*, treatment with the predator would be likely more effective if
452 used in combination with other varroa control strategies. However, this is tricky since the
453 biocontrol agent, which is a mite just like the varroa, could not be used at the same time of any
454 chemical acaricides. For this reason, we think that the integration of *S. scimitus* in an IPM
455 approach would be very difficult and has few chances of success.

456 In light of the results obtained, we believe that the predatory mite *S. scimitus* does not show
457 promise as a viable alternative for the control of varroa mites under the cold temperate climate
458 of Eastern Canada. Along with our previous study (Rondeau et al. unpubl. data), our results

provide strong evidence that the use of *S. scimitus* is not an effective means of varroa control when introduced in the fall. Thus, we discourage the use of the predator as a replacement for a varroa treatment of known effectiveness, at least until new scientific evidence is shown. This recommendation is also most probably valid in many cold temperate climate areas. On the other hand, our study confirmed that Thymovar® and oxalic acid, two widely used organic varroacides, remain effective options for controlling varroa mite populations during fall in Quebec. Considering the numerous disadvantages of the use of chemicals in beekeeping, research on less damaging alternative avenues for varroa control remain necessary.

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Figure captions

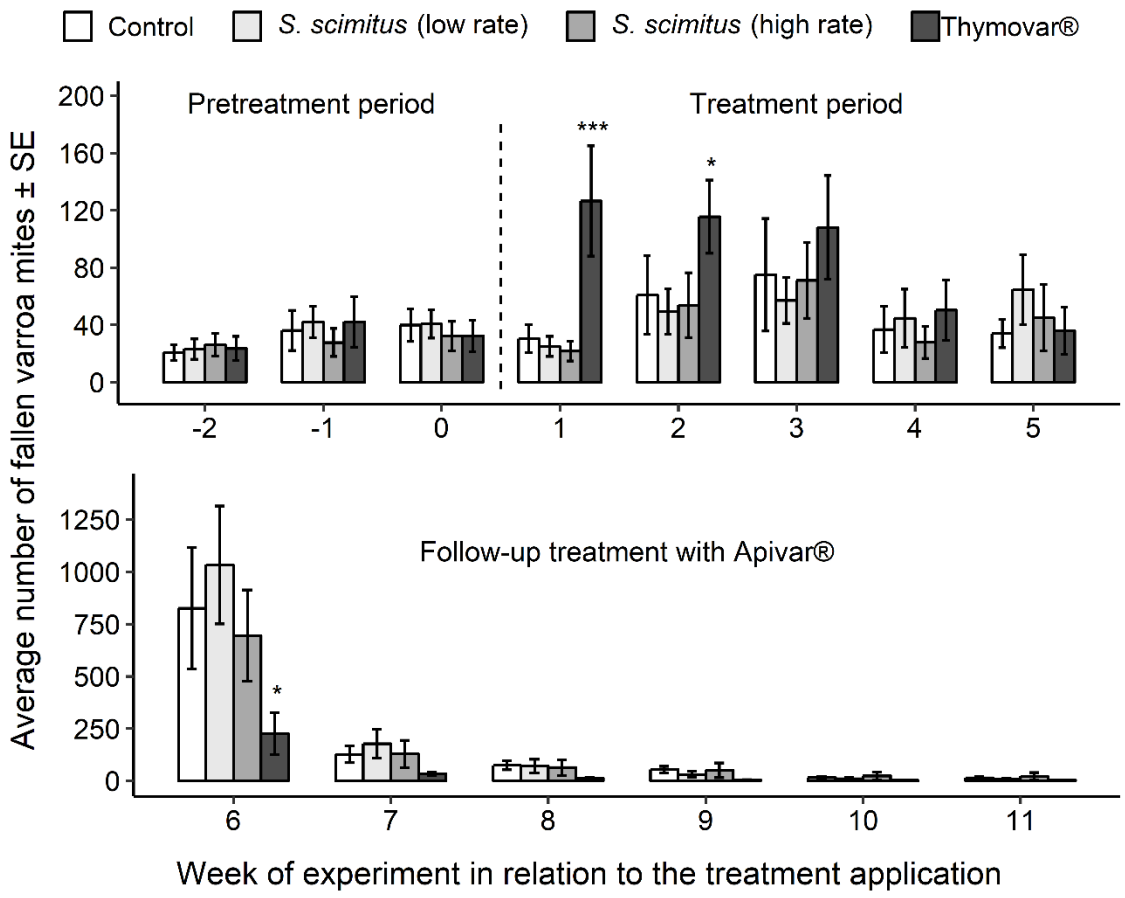
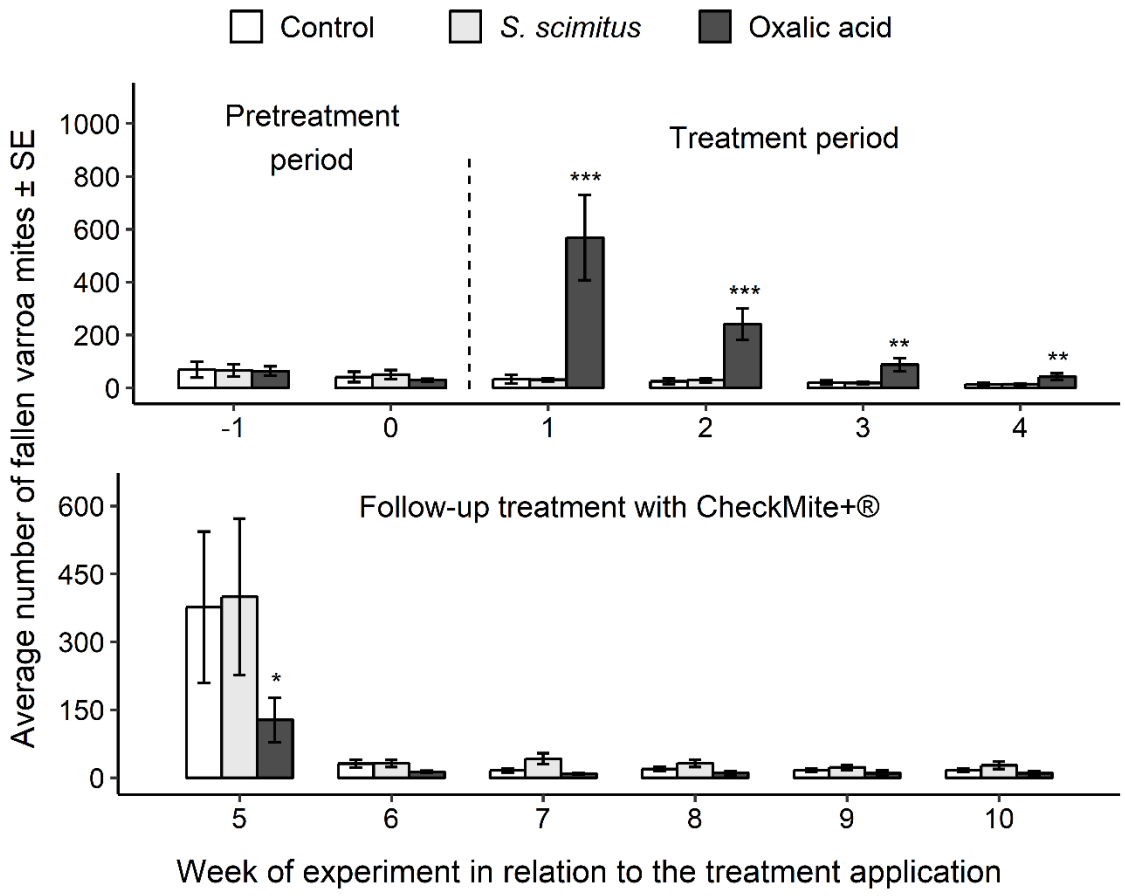


Figure 1 Average (± SE) weekly number of fallen varroa mites in honey bee colonies before and during the fall treatment period, as well as during the follow-up treatment with Apivar®. The effect of two rates (low = 6,250 mites/colony; high = 12,500 mites/colony) of the predatory mite *Stratiolaelaps scimitus* was compared to that of untreated colonies (control) and Thymovar®. The application of treatments was made on September 11, 2017 (week 0), in Quebec (Canada).

683 Within each week, asterisks indicate significant differences (* $p < 0.05$; *** $p < 0.001$; Repeated
684 measures ANOVA followed by contrasts).

685



686

687 **Figure 2.** Average (± SE) weekly number of fallen varroa mites in honey bee colonies before and
688 after the application of complementary varroa treatments (November 13, 2017; week 0) in
689 Quebec Canada, as well as during a follow-up treatment with CheckMite+® (April 24, 2018; week
690 5). The effect the predatory mite *Stratiolaelaps scimitus* (≈ 6,250 mites/colony) was compared
691 to that of untreated colonies (control) and oxalic acid. Within each week, asterisks indicate
692 significant differences (* $p < 0.05$; ** $p < 0.001$; *** $p < 0.001$; Repeated measures ANOVA
693 followed by contrasts).

694

695

696 Tables

697 **Table 1.** Daily ambient temperatures recorded during the treatment of honey bee colonies
698 against varroa mites between September 11 and October 16, 2017 (fall experiment) and
699 between November 13 and December 11, 2017 (complementary treatment). Temperature
700 records were obtained from a weather station of Environment and Climate Change Canada
701 located near both apiaries.

Treatment week	Daily ambient temperature (°C)		
	Mean (SD)	Minimum	Maximum
Fall experiment			
Week 1	16.6 (2.2)	3.3	27.4
Week 2	17.9 (2.4)	6.1	30.1
Week 3	13.5 (7.3)	-3.2	31.8
Week 4	11.3 (3.0)	-0.3	23.5
Week 5	9.1 (3.3)	-3.2	17.6
Complementary experiment			
Week 1	-2.2 (2.6)	-11.7	5.7
Week 2	-2.3 (5.5)	-16.0	4.0
Week 3	Indoor temperature* : 4.0 ± 1.0 °C		
Week 4			

702 * On November 23, 2017, colonies were moved in a wintering building for indoor overwintering.

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710 **Table 2.** Effectiveness of two rates (low = 6,250 mites/colony; high = 12,500 mites/colony) of the
711 predatory mite *Stratiolaelaps scimitus* to reduce varroa mite populations in honey bee colonies
712 during fall, in comparison with untreated colonies (control) and Thymovar®, and total numbers
713 of fallen varroa mites used to calculate these (mean ± SE). Treatment took place on September
714 11, 2017, in Quebec (Canada).

Treatment	n	Total number of fallen varroa mites			Effectiveness (%)
		Before treatment (3 weeks)	During treatment (5 weeks)	During follow-up treatment (6 weeks)	
Control (untreated)	7	97 ± 30	237 ± 97	1110 ± 349	16.5 ± 2.0[a]
S. scimitus (low rate)	6	101 ± 26	220 ± 66	1222 ± 371	15.4 ± 1.7[a]
S. scimitus (high rate)	7	82 ± 28	220 ± 83	983 ± 340	18.6 ± 2.1[a]
Thymovar®	6	69 ± 20	343 ± 90	184 ± 34	64.7 ± 6.6[b]

715 Means followed by different letters are significantly different at α = 0.05 (ANOVA followed by
716 contrasts).

717

718 **Table 3.** Effectiveness of the predatory mite *Stratiolaelaps scimitus* (≈ 6,250 mites/colony) to
719 reduce varroa mite populations in honey bee colonies in late fall, in comparison with untreated
720 colonies (control) and oxalic acid, and total numbers of fallen varroa mites used to calculate
721 these (mean ± SE). Treatment took place on September 11, 2017, in Quebec (Canada).

Treatment	n	Total number of fallen varroa mites			Effectiveness (%)
		Before treatment (2 weeks)	During treatment (4 weeks)	During follow-up treatment (6 weeks)	
Control (untreated)	7	111 ± 50	93 ± 42	477 ± 175	14.6 ± 2.4[a]
S. scimitus	7	119 ± 40	94 ± 20	556 ± 202	20.0 ± 4.7[a]
Oxalic acid	7	93 ± 20	941 ± 251	180 ± 65	82.1 ± 6.2[b]

722 Means followed by different letters are significantly different at α = 0.05 (ANOVA followed by
723 contrasts).