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3 # Risk assessment and predation potential of *Stratiolaelaps*
4 # *scimitus* (Acari: Laelapidae) to control *Varroa destructor*
5 # (Acari: Varroidae) in honey bees

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8 Sabrina Rondeau[1*,#a], Pierre Giovenazzo[2], Valérie Fournier[1]

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10 [1] Département de phytologie, Université Laval, Québec City, Quebec, Canada

11 [2] Département de biologie, Université Laval, Québec City, Quebec, Canada

12 [#a] Current Address: School of Environmental Sciences, University of Guelph, Guelph,
13 Ontario, Canada

14

15 * Corresponding author

16 Email: sabrina.rondeau.1@ulaval.ca (SR)

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Abstract

The biocontrol of the honey bee ectoparasite *Varroa destructor* is an underexploited but promising avenue that would benefit from being integrated in a *Varroa* management program. Our study aimed to investigate the potential of the predatory mite *Stratiolaelaps scimitus* to control *Varroa* infestations in honey bees. Tests on safety and predation were carried out to: (1) assess the risk of predation of the honey bee brood by *S. scimitus* under laboratory conditions and within the colony, and (2) evaluate the predation potential of *S. scimitus* on phoretic *Varroa* mites. Under laboratory conditions, *S. scimitus* was able to feed upon free *Varroa* mites, but also attacked every unprotected honey bee brood stages with a strong preference for bee eggs. When introduced inside colonies, however, *S. scimitus* does not have negative effects on the survival of the bee brood. Moreover, observations made in the laboratory revealed that *S. scimitus* does not attack *Varroa* mites when they are attached to the body of bees. However, all *Varroa* mites that had naturally fallen from the bees were predated upon by *S. scimitus* and died in less than 24h. This study provides evidence that *S. scimitus* does not represent a significant threat to the bee brood, but also suggests that its effect in *Varroa* control will probably be limited as it does not attack phoretic *Varroa* mites. Our results represent a first step in assessing the potential of *S. scimitus* to control *V. destructor* and provide novel information about the predator's behavior inside the honey bee colony.

40 # Introduction

41 The ectoparasitic mite *Varroa destructor* Anderson & Trueman (Acari: Varroidae)
42 is considered as the most damaging honey bee (*Apis mellifera* L.) pest worldwide [1, 2].
43 Since its introduction in Europe in the 1970s and in North America in the 1980s [3], the
44 *Varroa* mite has caused major damages and economic losses to the beekeeping industry [4,
45 5]. In North temperate regions of America and much of Europe, the pest is also a key factor
46 of high winter colony losses [6-8]. Through direct physical damages to honey bees [3, 9]
47 and transmission/activation of many honey bee viruses [10-12], an untreated infested
48 colony will most likely die within months [13].

49 Controlling *Varroa* mite populations in honey bee colonies is challenging as there
50 exists no one-fits-all approach to get rid of the pest. Even though synthetic acaricides have
51 been successfully used for *Varroa* control in the past years [14], the development of mite
52 resistance now limits their use [15-17]. As alternative treatments, some "natural chemicals"
53 such as organic acids and essential oils are increasingly used by beekeepers but also have
54 disadvantages such as variable toxic effect on bees [18-22], possible contamination of wax
55 and honey [23, 24] and an effectiveness dependent on environmental conditions [25]. Thus,
56 Integrated Pest Management (IPM), which combines non-chemical and chemical methods
57 with *Varroa* infestation thresholds, is currently considered as the best approach to control
58 the *Varroa* and aims to reduce beekeepers' reliance on synthetic acaricides [3, 26, 27].

59 The biocontrol of *Varroa* mites is an underexploited but promising avenue that
60 could enhance an IPM strategy. Despite all the known benefits of the biological pest
61 control, little research has been done on the use of living organisms to control *Varroa* mites.

62 In addition to be lethal for *Varroa* mites, a good candidate biocontrol agent should have:
63 (1) the ability to operate under the physical conditions of a honey bee colony, (2) the ease
64 of targeting against the *Varroa*, and (3) the potential for mass production [28]. According
65 to Chandler et al. [28], as *V. destructor* seems to be relatively free of natural enemies, its
66 biocontrol is likely to require natural enemies from other hosts. Likewise, the absence of
67 identified specialist enemies of *Varroa* mites [29] brings us to consider generalist predators
68 as potential biocontrol agents.

69 Due to its ecology and specific characteristics, the predatory mite *Stratiolaelaps*
70 *scimitus* (Womersley) (Acari: Laelapidae), formerly known as *Hypoaspis miles* (Berlese),
71 appears to be particularly promising as a biocontrol agent against *Varroa* mites.
72 *Stratiolaelaps scimitus* is a polyphagous soil-dwelling mite naturally occurring throughout
73 the Northern hemisphere [30]. It preys upon many soil organisms such as thrips nymphs,
74 nematodes, phorid and sciarid fly larvae and several species of mites and other
75 invertebrates [31-33]. The predatory mite thrives in hot and humid environments and can
76 survive temperatures up to 32°C [34], which suggests its adaptability to the conditions
77 observed within a honey bee colony. Already mass-reared and commercially available in
78 North America and Europe [32], *S. scimitus* has proven to be useful in the biocontrol of
79 fungus gnats and thrips of protected crops [35-39] and is now known to reduce infestations
80 of the poultry red mite on chicken livestock in small cages [40]. More recently, the pet
81 industry has also started using *S. scimitus* as a means to control parasitic mites on reptiles
82 in captivity [41] although little data is available on the actual effectiveness of this practice.

83 Nowadays, some beekeepers in the United States, Canada and Europe are using *S.*
84 *scimitus* for *Varroa* mite control in honey bee colonies but to date, no scientific study has

85 shown the effectiveness of the investigated biocontrol agent to control *Varroa* populations
86 *in situ*. A team of researchers from Texas (USA) has recently demonstrated, using *in vitro*
87 trials, that *S. scimitus* indeed attacks and feeds upon free *Varroa* mites [42]. However, little
88 is known about its effectiveness in the hive and while some anecdotal observations made
89 in Ontario (Canada) suggest that *S. scimitus* would reduce *Varroa* mite populations when
90 introduced in honey bee colonies [43], a similar field experiment resulted in ineffective
91 *Varroa* control [42]. Despite these contradictory results and the lack of experimental proof
92 of effectiveness, some biocontrol suppliers are now selling *S. scimitus* for *Varroa* control.
93 Considering that effective *Varroa* control is a key factor for honey bee colony survival
94 [44], the use of a method whose real effectiveness is unknown could have detrimental
95 consequences for the apiarists' bee stocks and the beekeeper's perception of biocontrol.

96 Before demonstrating the impact of *S. scimitus* in *Varroa* biocontrol inside the
97 honey bee colony, it is judicious to test its safety and predation effectiveness in lab
98 bioassays. Indeed, as previously put forward by Chandler et al. [28], there is a significant
99 risk that any generalist predator introduced in a colony as a means of *Varroa* control would
100 consume bee eggs. Another important factor to consider is that to be effective, the predator
101 must be able to attack phoretic *Varroa* mites and not just the free mites. Free *Varroa* mites
102 are not common in a bee colony as the mites are found either attached to the body of an
103 adult bee (phoretic stage) or parasitizing a pupa in a capped brood cell (reproductive stage)
104 [5, 45]. Therefore, as *S. scimitus* cannot reach reproducing *Varroa* mites because they are
105 protected by a wax cap, it must attack those parasitizing adult bees for the treatment to be
106 effective.

107 Our study aimed to investigate the potential of *S. scimitus* to control *Varroa* mite
108 infestations in honey bees. The specific objectives of this paper were: (1) to assess the risk
109 of predation of honey bee brood by *S. scimitus* under both laboratory conditions and within
110 the colony, and (2) to evaluate the predation potential of *S. scimitus* on phoretic *Varroa*
111 mites. According to what we know from the literature, we hypothesized that the use of *S.*
112 *scimitus* in *Varroa* biocontrol would not be a threat to the honey bee brood. In fact, the bee
113 brood does not correspond to the type of prey typically consumed by *S. scimitus* [34, 39].
114 We also believe that *S. scimitus* is a potential predator of phoretic *Varroa* mites. This
115 hypothesis is supported by the use of the predatory mite to control hematophagous mites
116 in infested animals [40, 46] and the few anecdotal reports by beekeepers of *Varroa*
117 population reductions. Assessing both the risk and the predation potential of *S. scimitus* to
118 control *Varroa* mites is a very important step in the study of this biocontrol agent in
119 beekeeping.

120 Materials and methods

121 Livestock sources and maintenance

122 *Stratiolaelaps scimitus* was obtained from Applied Bio-nomics Ltd. (British
123 Columbia, Canada). Mites were supplied in a mixture of vermiculite and peat in 1L bottles
124 with mold mites (*Tyrophagus putrescentiae*) as a food source. The predatory mites were
125 stored in their original containers, lying on their side in complete darkness at 15°C, and
126 were regularly checked for predator vitality (i.e., normal activity, vigour and abundance
127 when observed under a stereomicroscope) and the presence of prey.

128 Adult female *Varroa* mites were collected from infested hives located in apiaries
129 of various beekeepers near Quebec City (Quebec, Canada) following the “Icing Sugar”
130 method described in Dieteman et al. [1]. Briefly, we collected approximately 300 bees (125
131 ml) from brood frames and placed them in a 500 ml Mason jar whose lid had been replaced
132 by a 2 mm hardware mesh. Powdered sugar (15 ml) was added through the mesh and the
133 jar was rolled to cover the bees with sugar. After letting the jar stand for one minute, it was
134 turned upside down and shake over a white plastic cardboard until the mites stopped falling.
135 The mites were collected with a fine paint brush and brought to the lab. They were then
136 maintained alive by confining them by groups of five on a drone pupa in a 1 ml Eppendorf
137 tube pierced with two holes for ventilation and kept in an incubator (32.0 ± 0.5°C, ≈70%
138 RH, complete darkness). *Varroa* mites were successfully kept this way for up to one week.

139 Honey bee (*Apis mellifera*) brood was sampled from a single hive located in the
140 city of Levis (46°44'56.02"N, 71°10'2.17"O), 15 km from our laboratory at the Université
141 Laval. Eggs and larvae were gently sampled with a small paintbrush and transferred in a
142 small Petri dish (50 x 12 mm) containing a moistened filter paper. Capped pupae cells were
143 carefully cut with a scalpel directly from brood frames and transferred to the same Petri
144 dish. Only worker brood was used. Samples were quickly transferred into an incubator and
145 maintained under controlled conditions (32.0 ± 0.5°C, ≈70% RH, complete darkness) until
146 their transfer in the arenas.

147 Adult worker bees were collected from the livestock of a bee research facility in
148 Quebec (Centre de recherche en sciences animales de Deschambault, CRSAD,

149 46°43'6.00"N, 71°33'5.79"O) and were used immediately following their collection.
150 Similarly, all the colonies used in our study were operated by the CRSAD.

151 *In vitro* assessment of *S. scimitus* predation upon *V. destructor* 152 and bee brood

153 The tests took place between July 21 and September 1, 2016. There were six
154 treatments representing potential prey for *S. scimitus*: 1) adult female *Varroa* mite; 2)
155 honey bee egg; 3) 1st or 2nd bee larval instar (L1-L2); 4) 3rd or 4th bee larval instar (L3-L4);
156 5) 5th bee larval instar (L5); and 6) capped bee pupa. Honey bee larval instars were
157 estimated from visual assessment of the space occupied by the larva in the brood cell
158 according to Human et al. [47], allowing for a rough estimate of age (two-instar overlap).

159 Experimental arenas consisted of small glass vials (5 ml) filled with 1 cm of pre-
160 autoclaved vermiculite and moistened with 0.3 ml of tap water. Only adult female predators
161 were used, and each one was starved individually for 48h in small portion containers (1 oz)
162 with a piece of moistened tissue paper prior to their transfer in the arenas. Twenty starved
163 predators were transferred to each arena with a fine paintbrush. Then, one single prey was
164 added according to the treatment. Vials were closed with a piece of Nitex® synthetic nylon
165 screening (105 μm) and a rubber band, allowing for ventilation while blocking mite escape.
166 Arenas were held in an incubator (32.0 ± 0.5°C, complete darkness) throughout the
167 duration of the tests. A saltwater pool helped to maintain the desired humidity in the
168 incubator, which varied from 48 to 76% RH.

169 After 12 h, each prey was observed using a stereomicroscope and was scored as
170 follows: alive without predation, dead without predation, alive with predation, dead with
171 predation or fully consumed. The presence of visible wounds or missing parts (legs,
172 antennae, cuticle parts) were considered as signs of predation. Prey viability was
173 determined by the presence of movements when touched with a fine paintbrush. If
174 predation did not take place after 12 h, the prey was replaced by a fresh one. Arenas were
175 then returned to the incubator for an additional 12 h and the prey were checked one last
176 time. At the end of the test, a count of living and dead predators was done to ensure that a
177 reasonable number of predators was still in the arena. For each treatment, a control arena
178 (with a prey but without predators) allowed us to observe the normal appearance of the
179 prey in absence of predation. For each trial period (block), all six treatments and their
180 paired control counterparts were tested simultaneously according to a randomized block
181 design and each treatment was repeated 20 times.

182 Prey preference test

183 In order to determine if the predatory mite will more likely attack honey bee eggs
184 or *Varroa* mites in the first place, a prey preference test was conducted using the same
185 experimental arenas as described above. The experiment took place in the laboratory on
186 August 5, 12 and 19, 2016 and included 10 replicates for each date (for a total of 30
187 replicates). Ten starved predatory mites were transferred to each arena with one honey bee
188 egg and one female *Varroa* mite added simultaneously. For each arena, the order of prey
189 introduction was randomly determined. Once closed, arenas were held in an incubator (32.0
190 ± 0.5°C, 51-75% RH, complete darkness) throughout the duration of the test. Prey were

191 observed under a stereomicroscope every hour for signs of predation and the test ended as
192 soon as predation was detected. The first prey attacked was considered as a choice. In the
193 case where both prey would have been attacked in the same one-hour observation interval,
194 the choice would have been recorded as "equal".

195 *In vivo* assessment of *S. scimitus* predation upon bee brood

196 An in-hive predation experiment was also conducted in an apiary of the CRSAD
197 (46°47'50.09"N, 71°43'42.50"O) on colonies of equivalent strength and having sister
198 queens of known descent. Each colony was housed in a Langstroth commercial hive
199 consisting of a single brood chamber (10 frames) supporting two or three honey suppers
200 over a queen excluder. Prior to the trial, visual inspections were performed to ensure that
201 all colonies were healthy and without signs of brood diseases. On August 9, 2017, honey
202 bee colonies were randomly assigned to two groups with five colonies per treatment: Group
203 1) colonies inoculated with *S. scimitus,* and Group 2) untreated colonies (control). For each
204 colony, the queen was caged on a frame with empty combs for 48h and allowed to lay eggs
205 as described in Human et al. [47]. Then, each queen was removed from the exclusion cage
206 and reintroduced in its colony. The position of every comb cell containing an egg was
207 marked using a permanent marker on a transparent sheet of acetate placed on each side of
208 the frame. Each frame was placed back to the exclusion cage to prevent further oviposition
209 by the queen and was replaced in the middle of the brood chamber. Colonies were then
210 inoculated by pouring 500 ml (≈ 12,500 *S. scimitus* individuals) of the biocontrol
211 commercial product (Group 1) or the same amount of pre-autoclaved vermiculite (Group
212 2) on top of the queen excluder. For both groups, the respective substrate was poured

213 parallel to the brood frames, so that it was partially retained by both the queen excluder
214 and the top of the frames (S1 Fig). Some substrate inevitably fell to the bottom of the hive
215 during inoculation, but in a negligible amount. We used 500 ml of the commercial product
216 containing *S. scimitus*, which is twice the dose currently recommended by biocontrol
217 suppliers [42, 43]. In doing so, we wanted to make sure that we used enough predators to
218 detect a predation effect, if any, while still using a realistic amount of product as it is likely
219 to be used in honey bee hives. Six days later, brood cells of each frame were observed for
220 a second time by checking with previous acetates if the larvae (L4-L5) were present. Cells
221 with a missing larva were marked with a permanent marker of another color before the
222 combs were returned to the hives. This was repeated four days later (capped pupa). At each
223 period, cells with brood were counted to determine the percentage of eggs and larvae that
224 survived until cell capping. At each of the three periods of brood monitoring, hive floor
225 and frames were also visually checked to ensure that the predatory mites remained in the
226 hives. Observing five to ten mites during a visual inspection was considered satisfactory.
227 At the end of the trial, a sample of debris (≈ 60 ml) was collected at the bottom of the hive
228 for further screening under the stereomicroscope.

229 The number of experimental units (bee colonies) used in this trial is rather low given
230 certain constraints related to the equipment availability and handling time. If resources are
231 available, a better statistical power could be obtained in further studies by increasing the
232 number of colonies under study. The full protocol is available at protocols.io
233 (http://dx.doi.org/10.17504/protocols.io.unaevae).

234 ## *S. scimitus* predation of phoretic *Varroa* mites

235 The experiment was conducted in the laboratory at two distinct periods, each one
236 included half of the replicates. The first part of the trials started on July 10, 2017, while the
237 other one started on August 9, 2017. Modified plastic pill bottles (34 mm diameter; 63 mm
238 high) served as experimental arenas in which a hole was cut in the lid and was then covered
239 with a glued piece of Nitex® synthetic nylon screening (105 µm). A hole was cut in the
240 lowest quarter of each bottle allowing for the insertion of a 0.5 ml Eppendorf tube pierced
241 with three small holes and serving as a bee feeder. Paraffin film was used to ensure
242 tightness. Bottles were filled with 5 ml of pre-autoclaved vermiculite moistened with 2 ml
243 of tap water. In a completely randomized design, twenty starved adult female *S. scimitus*
244 were transferred to each treated arena (n=40) whereas control arenas (n=40) received no
245 predators.

246 Using a fine paintbrush, one freshly collected adult female *Varroa* mite was
247 transferred to the body of each adult worker bee used in this trial. Then, a parasitized bee
248 was introduced in each arena and was fed daily with a 50% (w/v) sucrose solution. Arenas
249 were held in a growth chamber (30.0 ± 0.5°C, 75 ± 2% RH, complete darkness) throughout
250 the duration of the test (i.e., from 1 to 14 days according to *Varroa* survival time). Once a
251 day, honey bees and *Varroa* mites were observed and recorded as dead or alive. If the
252 honey bee was dead but the *Varroa* was still alive, the bee was changed by a new one and
253 the *Varroa* was transferred back on its body. For each arena, observations ended as soon
254 as the *Varroa* was recorded dead and the latter was then observed under a stereomicroscope
255 for evidence of predation. Here again, a count of living and dead predatory mites was done

256 at the end of the test to ensure that a reasonable amount of living predators was still in the

257 treated arenas.

258 Data analyses

259 Descriptive statistics of *in vitro S. scimitus* predation upon *Varroa* mites and bee

260 brood are given as proportions ± 95% confidence intervals. To test whether higher prey

261 mortality occurred even in absence of apparent signs of predation, the status of the prey

262 (dead or alive) was compared between treated replicates and their matched controls using

263 the McNemar mid-p test [48] in the R software [49]. The occurrence of predation among

264 treatments (type of prey) after 12 and 24h was compared using Fisher's exact test followed

265 by pairwise comparisons with Benjamini-Hochberg adjustment to control the false

266 discovery rate (FDR). True difference between predation choices was investigated using a

267 binomial two-sample test of proportions in R. Data of the *in vivo* predation test were

268 analyzed using the proc mixed procedure in SAS® University Edition [50]. The normality

269 of residuals was achieved, so a repeated measures analysis of variance (ANOVA) with

270 autoregressive correlation structure was performed to compare differences of brood

271 survival (number of eggs and surviving larvae and pupae) due to treatment, brood stage

272 (post-oviposition time) and their interaction. Results are presented as percentages of brood

273 survival (number of surviving larvae or pupae x 100 / initial number of eggs). Regarding

274 *S. scimitus* predation assessment of phoretic *Varroa* mites, a log-rank Kaplan-Meier

275 survival analysis was carried out to compare the survival curves of the *Varroa* in the

276 presence or the absence of the predatory mite (survival package in R). *Varroa* death events

277 that occurred on the same day as their respective bee death were considered as right
278 censored data. Significance was defined as $p \leq 0.05$ for all analyses.

279 Results

280 *In vitro* assessment of *S. scimitus* predation upon *V. destructor*
281 and bee brood

282 Predation occurred on all types of prey offered to *S. scimitus* (Fig 1). Only the prey with
283 obvious signs of predation were recorded as having been predated upon (Table 1). This
284 includes live observations of predation or attack, eggs fully consumed, liquefied larvae and
285 *Varroa* mites with obvious missing appendages and damaged cuticle. Obvious predation
286 events (stylet inserted into the body of the prey) were observed in real time at least twice
287 for each type of prey (Fig 2). At the end of the experiment, an average of 15 ± 3 (mean ±
288 SD) predatory mites were still alive in each arena.

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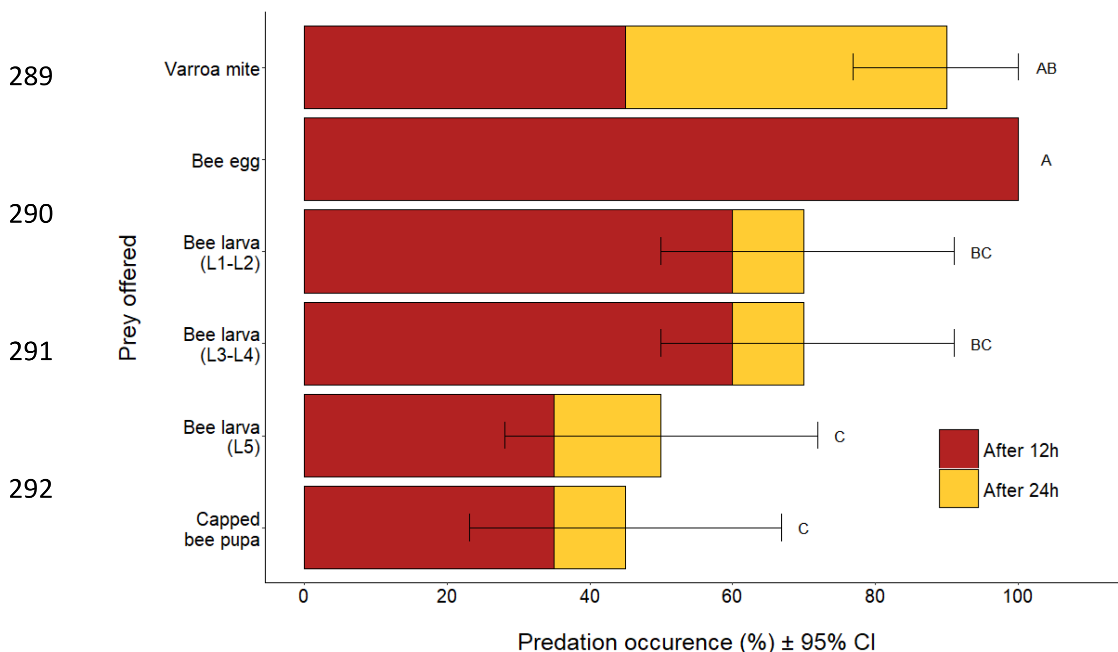

293 **Fig 1. Occurrence of predation of *Varroa destructor* (female adults) and five different
294 honey bee brood stages by the predatory mite *Stratiolaelaps scimitus*, after 12h and
295 24h of confinement in experimental arenas.** Each arena (n=20 per type of prey)
296 contained 20 starved female predatory mites and a single prey. Error bars show the 95%
297 confidence intervals after 24 h. Different letters represent significant differences ($p \leq 0.05$,
298 Fisher's exact test followed by pairwise comparisons with Benjamini-Hochberg
299 adjustment) in predation occurrence at the end of the test.

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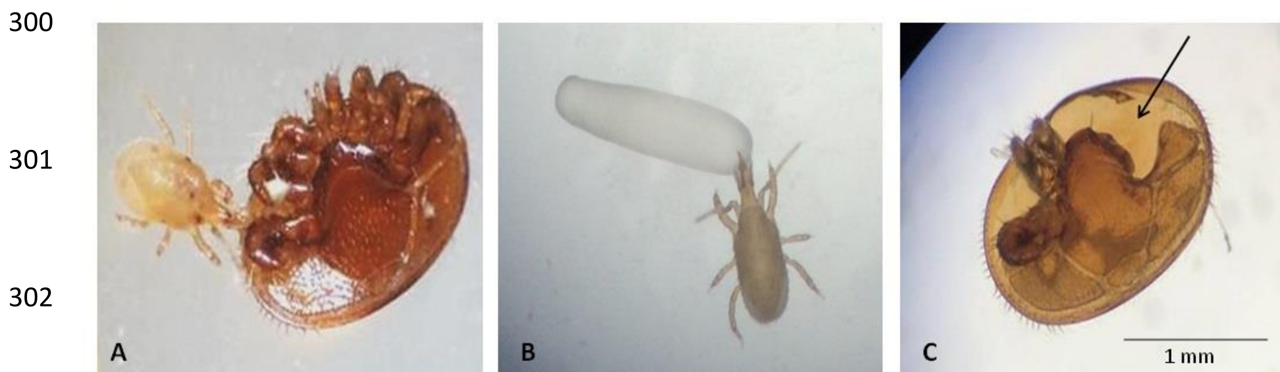

303 **Fig 2. The predatory mite *Stratiolaelaps scimitus* feeding on a female *Varroa* mite (A)
304 and a honey bee egg (B) under laboratory conditions. After being attacked by *S.*
305 *scimitus*, the *Varroa* showed characteristic signs of predation (C) such as missing legs
306 and holes in the cuticle (arrow).** (Photos: Sabrina Rondeau, 2016)

307

308 **Table 1. Status of *Varroa destructor* (female adults) and five different honey bee brood
309 stages after a maximum of 24h of confinement with *Stratiolaelaps scimitus* under
310 laboratory conditions.** Each arena (n=20 per type of prey) contained 20 starved female
311 predatory mites and a single prey.

Prey /state	Number of observations (n)				
	Fully consumed	Alive with predation	Alive without predation	Dead with predation	Dead without predation
Varroa mite	0	2	0	16	2
Bee egg	20	0	0	0	0
Bee larva (L1-L2)	0	0	1	14	5
Bee larva (L3-L4)	0	1	4	13	2
Bee larva (L5)	0	6	10	4	0
Capped bee pupa	0	1	4	8	7

312

313 All *Varroa* mites in the control group were still alive at each observation period.
314 Similarly, all honey bee eggs in the control group were still present and intact after 12h,
315 while the eggs of the group treated with *S. scimitus* were all fully consumed at that same
316 time. Analysis of the status of honey bee larvae and pupae between treated replicates and
317 their matched controls revealed that mortality of honey bee brood likely occurred more
318 often in presence of *S. scimitus*, regardless of the presence (mid-p < 0.001, McNemar test)
319 or the absence (mid-p $= 0.013$, McNemar test) of apparent signs of predation (S1 Table).
320 In this analysis, data of all instars of bee larvae and pupae have been pooled together to
321 obtain a larger sample size for statistical purposes.

322 During the first 12h of confinement with *S. scimitus*, obvious predation events
323 occurred significantly more often for honey bee eggs than for the other groups of prey
324 (Fisher's exact test, $p < 0.001$; FDR adjusted $p < 0.010$). At the end of the test, the overall
325 occurrence of predation differed significantly between the type of prey offered to *S.*

326 *scimitus* (Fisher's exact test, $p < 0.001$; Fig 1), with the bee eggs and the *Varroa* mites

327 being predated more frequently. The 5th bee larval instar and the capped bee pupae showed

328 the lowest occurrences of predation, which were significantly less than those of bee eggs

329 (FDR adjusted p's ≤ 0.002) and *Varroa* mites (FDR adjusted p's ≤ 0.050) although not

330 significantly different from L1-L2 and L3-L4 larvae (FDR adjusted p's ≥ 0.353). The

331 occurrence of predation in L1-L2 and L3-L4 larvae differed significantly only from that of

332 bee eggs (p's = 0.050).

333 Prey preference test

334 When both prey were offered simultaneously, *S. scimitus* individuals first predated

335 upon the bee egg (n=28) over the *Varroa* mite (n=2) significantly more often (Fig 3;

336 binomial test, n=30, $p < 0.001$). In most cases (25/28), the bee egg was consumed during

337 the first hour while the predation upon the *Varroa* only occurred after 4 or 5 hours. In this

338 last scenario, the bee egg remained untouched while the *Varroa* was dead and showed

339 evident signs of predation (multiple missing appendages). Predation of both prey never

340 occurred during the same one-hour observation interval.

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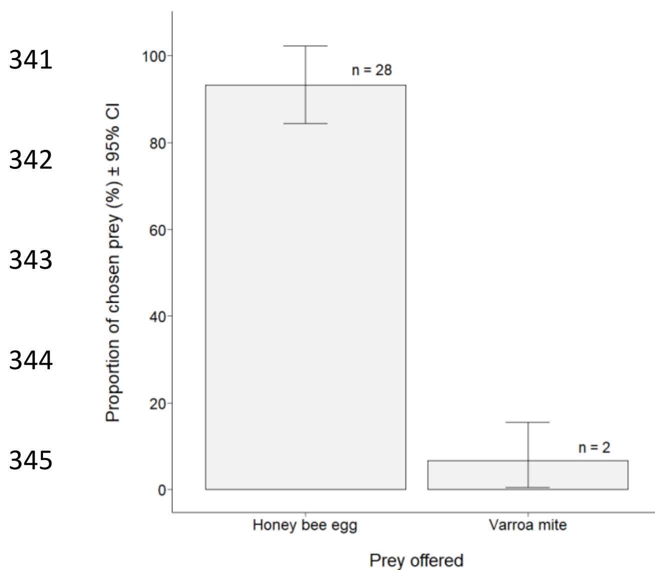

346 **Fig 3. Proportion of honey bee eggs and *Varroa* mites first chosen by *Stratiolaelaps***

347 ***scimitus* during a preference test where both prey were offered simultaneously**

348 **(n=30) to ten starved *S. scimitus* individuals.**

349 *In vivo* assessment of *S. scimitus* predation upon bee brood

350 Two colonies in the control group were rejected from the analysis due to abnormally

351 low brood survival (0 and 23%) between the first two periods of data collection (i.e., before

352 reaching the L4-L5 larval stage). On average, 1800 ± 111 (mean ± SE) eggs were marked

353 in each colony and monitored over time. The initial number of eggs did not differ between

354 groups (two sample t(6) = 0.103, p = 0.922). The repeated measures ANOVA revealed no

355 interaction between treatment and time ($F_{(2,\ 12)} = 0.05$, $p = 0.956$) and there was no

356 significant effect of the treatment ($F_{(1,6)} = 0.03$, $p = 0.864$) on the bee brood survival . Only

357 the time had an effect on the brood survival ($F_{(2,\ 12)} = 21.92$, $p < 0.001$) with an average

358 survival (mean ± SE) of 79.7 ± 8.3% and 80.9 ± 4.9% of the L4-L5 larvae and 76.3 ± 8.2%

359 and 76.3 ± 4.4% of the pupae for the control and the treated colonies respectively (Fig 4).

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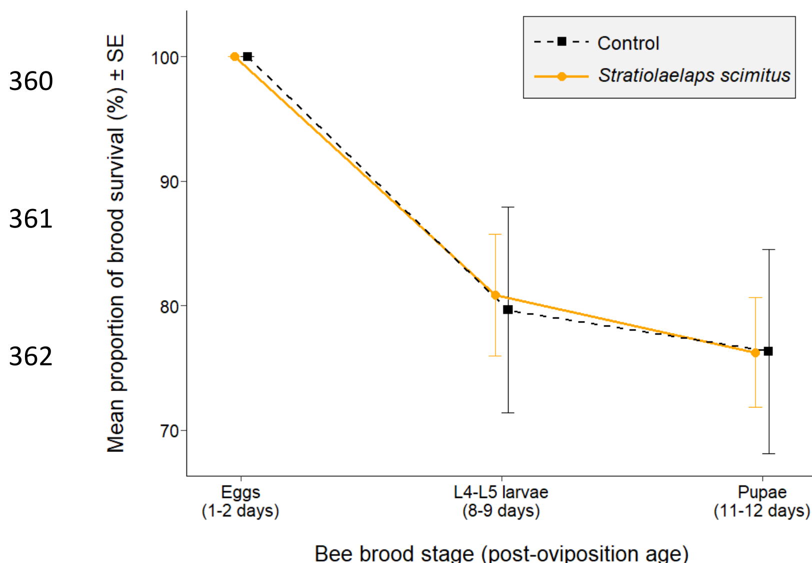

363 **Fig 4. Effect of the inoculation of honey bee colonies (n=5) with ≈ 12,500 *Stratiolaelaps***
364 ***scimitus* individuals on the mean proportion of bee brood survival from the eggs to**
365 **the pupae in comparison with untreated colonies (control; n=3).** On average, 1800 ±
366 111 (mean ± SE) eggs have been marked in each colony and monitored over time (August
367 09 to 21, 2017). There was no effect of the treatment on the bee brood survival (repeated
368 measures ANOVA; $F(1,6) = 0.03$, $p = 0.864$).

369 ## *S. scimitus* predation of phoretic *Varroa* mites

370 Some *S. scimitus* individuals escaped and were found in two control arenas which
371 were rejected. The log-rank Kaplan-Meier survival test showed a significantly lower
372 survival rate of *Varroa* mites when *S. scimitus* was present (Fig 5; $p < 0.01$). Mortality of
373 90% of phoretic *Varroa* in control and treated arenas occurred after ten days and eight days
374 respectively. No *Varroa* mite survived longer than nine days in the presence of the
375 biocontrol agent. On the other hand, we stopped monitoring the survival of the last *Varroa*
376 mite in the control group after 14 days and artificially killed it by freezing (right censoring).
377 Within the treated group, all *Varroa* mites that were found dead showed signs of predation
378 (missing legs or mouthparts, holes in the cuticle, etc.). An average of 9 ± 4 (mean ± SD)
379 predatory mites were still alive in each treated arena at the end of the test.

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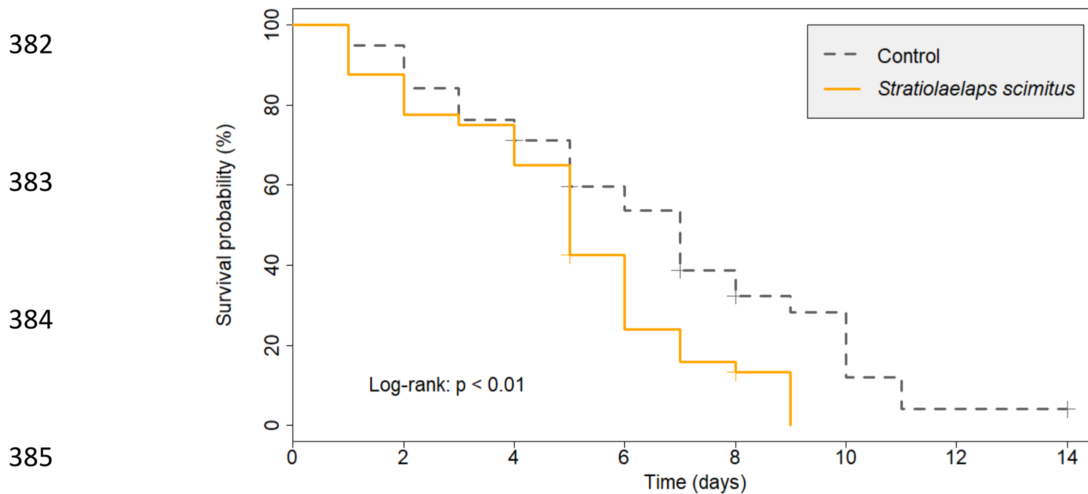

386 **Fig 5. Kaplan-Meier survival curves of the phoretic *Varroa* mites when confined in**
387 **experimental arenas with 20 starved *Stratiolaelaps scimitus* individuals (n=40) or none**
388 **(control; n=38).** Each arena consisted of a modified plastic pill bottle and contained one
389 worker bee parasitized by a single *Varroa* mite. Death events of both the *Varroa* and the
390 bee have been recorded once a day and *Varroa* death events that occurred on the same day
391 as their respective bee death were considered as right censored data.

392 # Discussion

393 Our experiment indicates that, under controlled conditions, *S. scimitus* attacks and
394 feeds upon *Varroa* mites when no other food choice is given. Despite the relatively smaller
395 size of *S. scimitus* compared to the *Varroa* (Fig 2), the predator still succeeded in killing
396 them. This is not surprising considering that *S. scimitus*, like the other mites of the family
397 Lealapidae, is an aggressive edaphic predator [51]. Typically, *Varroa* mites that had been
398 attacked by *S. scimitus* showed many missing legs and large holes in their cuticle. This is
399 typical of the attack of many mesostigmatan mites that strive at the leg joint of large

400 arthropods until the hemolymph flows [51]. In the experimental arenas, the predators were
401 constantly on the move, searching for prey. However, as they are used to live in the soil,
402 they were mainly active and searching in the vermiculite at the bottom of the vial, climbing
403 the walls only from time to time. On the opposite, most of the time the *Varroa* remained
404 hidden on the piece of Nitex® cloth that served as a cover. This could explain why half of
405 the predation events occurred only after 12 hours despite the small size of the arena and
406 the relatively high number of starved predators it contains. We observed some group attack
407 events, but attacks by a single mite were also common. During a group attack, the *Varroa*
408 mite was first found and targeted by a single *S. scimitus* individual before being rapidly
409 surrounded by others and assailed with quick jabs with the chelicerae. Then, the *Varroa*
410 was presumably drained of its fluids (considering the feeding behaviour of *S. scimitus*) and
411 the cuticle, apparently empty, was left behind.

412 Under these same restrictive laboratory conditions, *S. scimitus* was able to feed
413 upon every honey bee developmental stages from egg to pupa. This goes against our
414 predictions, which were based on the facts that predation of sciarid eggs and pupae by *S.*
415 *scimitus* rarely occurs as the predatory mite is thought to prefer mobile stages and smaller
416 prey [34, 52]. In fact, the 4th instar larvae of the sciarid flies are not always attacked by *S.*
417 *scimitus* because they are presumably too large (up to 7 times the size of the adult mite),
418 as postulated by Wright and Chambers [34]. These are similar in size and weight to the
419 honey bee 2nd or 3rd larval instar [47, 53], which in the present case were repeatedly
420 attacked. All bee eggs were completely consumed by *S. scimitus* while the larvae were
421 almost exclusively attacked at their body ends (head or anus). Some pupae were also
422 attacked despite being protected by a sealed wax cell. However, we do not know whether

423 these cells had been previously damaged during their sampling, allowing the mites to enter
424 the cell through small openings, or if the predators punctured the wax by themselves. Group
425 feeding was the norm for all types of prey. Usually, the prey was initially attacked by a
426 single mite before others joined it and began to feed. Here, chemical cues could be involved
427 [34].

428 In hindsight, our results are not so surprising if we consider the specific and highly
429 restrictive conditions of our test. In fact, a single prey was given to multiple highly
430 polyphagous predators that had been starved for 48 hours, without alternative food sources.
431 These conditions were put in place specifically to ensure that any potential predation by
432 the predator would be detected, even though these are unrealistic of in-hive conditions. The
433 biggest difference between the conditions of both environments was the accessibility of
434 prey. Within the bee colony, eggs and larvae are found in cells and are cared for and
435 protected by worker bees. On the opposite, in our experiment, the brood was unprotected
436 and offered to the predatory mites in a restricted environment so that their presence was
437 easily detectable. Thus, these results should be taken with caution as predation tests
438 conducted in the colony prove to be more realistic and revealing of the predation behavior
439 of *S. scimitus* and the non-target effects that might ensue.

440 When a choice is given under controlled conditions, *S. scimitus* first predates upon
441 the unprotected honey bee egg over the free *Varroa* mite. Since these two prey were the
442 most consumed in the previous trial, it was relevant to assess the predator's preference
443 when both prey are present, as this is the case in a bee colony. In many cases, even if a prey
444 has been contacted by a predator, the decision to attack may be influenced by the

assessment of relative risks and costs compared with the nutritional benefits brought by the prey at hand [54]. Here, the smaller size of the bee egg and its soft body certainly make it easier for *S. scimitus* to attack compared with the *Varroa*. The *Varroa* ability to flee the predator also plays a role. Indeed, this escape behavior might explain why the time elapsed before the predation event was much longer when the *Varroa* was predated first than when the bee egg was.

Interestingly, when introduced inside colonies, *S. scimitus* does not have negative effects on the survival of the honey bee brood. This suggests that the predatory mite does not feed upon the bee brood inside the colony. Here, there are two possible explanations. First, the tendency of *S. scimitus* to seek and stay in the vermiculite or other debris and the protection provided by the worker bees may be sufficient to prevent the predator from attacking the brood. Indeed, the ecology of *S. scimitus* (i.e., soil-dwelling predator) leads us to believe that the predator rather tends to search for prey at the bottom of the hive, where the debris are, than at the center of the bee cluster where the brood is. Observations made in the colonies three days after the introduction of the predator seem to confirm this behavior since several predators were found at the bottom of the hive while very few were observed walking on the brood frames. The displacement of vermiculite by the bees in the hive certainly contributed to the mites' dispersal since much of the vermiculite was moved to the bottom of the hive over time. Presumably, this propensity to seek debris may also limit the predator's ability to attack *Varroa* mites within colonies, as the adult parasites are mainly phoretic or in the brood cells. We know that *S. scimitus* remained in the colony for at least ten days, since we observed its presence in the debris at the bottom of the hive and confirmed it under magnification. Moreover, the invasion of a brood cell by *S. scimitus* is

468 likely to result in the removal of the mite by worker bees during routine maintenance duties,
469 preventing the brood from being predated [28]. A second explanation for the absence of
470 bee brood predation in the colony would be the presence of other food sources. During our
471 observations, we collected debris in the bottoms of hives for screening purposes. In
472 addition to *Varroa* mites, we recorded the presence of various species of mites and spiders,
473 springtails, ants, nitidulid beetles and wax moth larvae. There were also plenty of mold
474 mites (presumably *Tyrophagus putrescentiae*) which were most likely introduced with the
475 biocontrol agent since they are supplied as food with the predatory mite during the transit
476 and in storage. Thereby, the presence of multiple alternative food sources might prevent
477 non-target effects on the bee brood, while also reducing the efficiency of *S. scimitus* to
478 target the *Varroa*.

479 Assessing the risk of honey bee brood predation by *S. scimitus* is a step that should
480 be taken seriously, considering the deleterious impacts that this predation could have on
481 the strength and the survival of the colony. Based on previous observations conducted in
482 Canada, biocontrol suppliers currently suggest using 150 to 200 ml of the *S. scimitus*
483 mixture for *Varroa* control [42, 43]. In our *in-vivo* trial, we used 500 ml of this mixture
484 (12,500 individuals) and considering the voracity of the predator, we believe this must be
485 enough to detect a predation effect if there is any. We acknowledge that the number of
486 replicates used in this trial would have benefited from being higher. Nevertheless, our
487 results correspond to those obtained using observation hives, which reinforce the reliability
488 of our findings (S1 Appendix). In these undescribed tests, we introduced hundreds of
489 starved *S. scimitus* individuals in observation hives containing a single frame of brood and
490 we observed their behavior for several hours, using a red light in the dark. When worker

491 bees were absent, most of the mites remained in the vermiculite poured on top of the frame
492 but some of them occasionally walked on the comb. Some mites were observed entering
493 brood cells containing a bee egg, but predation was rarely observed. Moreover, when
494 worker bees were present in the observation hives, the mites did not climb on the frames
495 at all and no brood predation was observed. In addition to corroborating the absence of
496 significant predation risk of the bee brood by *S. scimitus* within colonies, these observations
497 also support the role of worker bees in brood protection.

498 Observations made in laboratory revealed that *S. scimitus* individuals do not attack
499 *Varroa* mites when they are attached to the body of bees. Indeed, even when the predatory
500 mites were deposited carefully with a small paint brush on the body of an adult worker bee,
501 these did not adhere to the insect body and fell to the slightest bee movement. Moreover,
502 *S. scimitus* has never been recorded to be phoretic, as most of the lealapid mites [55]. Even
503 if the predatory mite is known to be able to feed upon phoretic hematophagous mites in
504 infested birds and lizards [41, 46], it seems that it only attacks the parasites when they are
505 off their host body [40].

506 Since the biocontrol agent under study is not able to attack phoretic *Varroa* mites,
507 it is unlikely that it will be effective enough to be used alone in *Varroa* control. When ready
508 to reproduce, the female *Varroa* mite leaves its honey bee host to invade a worker cell
509 approximately 20h before its capping [56] and the entire reproductive cycle takes place
510 into that cell. Thus, the effective period for *S. scimitus* to enter into the brood cell in tandem
511 with the *Varroa* is short, which makes it unlikely that the predador will impact significantly
512 neither on reproductive adult *Varroa* mites nor on *Varroa* eggs or larvae [28]. After this

513 period of time, reproductive *Varroa* mites are blocked by the brood cell cap and only the
514 phoretic parasites remain accessible to *S. scimitus*. Thereby, to be at least partially
515 effective, the biocontrol agent must be able to search bee bodies for adult *Varroa* mites and
516 attack them. Likewise, most of the chemicals used in *Varroa* control only kill the phoretic
517 mites, except for formic acid which effectively kills *Varroa* mites in sealed brood cells
518 [57].

519 In our trial, however, all *Varroa* mites that had fallen from their bee host body were
520 predated upon by *S. scimitus* and died in less than 24h. It strongly suggests that *S. scimitus*
521 only predates upon *Varroa* mites that naturally fell from the bees. In fact, a certain
522 percentage of mites in a colony simply lose their grip and fall to the bottom of the hive
523 over time. Moreover, in order to avoid parasitism by *V. destructor*, honey bees often exhibit
524 defensive behaviors such as "grooming" which involves self-removal of phoretic *Varroa*
525 mites on the body of adult bees [58]. When effective, this behavior leads to the removal of
526 the parasite which is more likely to fall on the hive floor. In our experiment, the reduced
527 probability of survival recorded for the phoretic *Varroa* mites from the treated group is due
528 to the fact that the *Varroa* were instantly attacked by *S. scimitus* after a natural fall from
529 their host body. In the control group, fallen *Varroa* mites survived longer and even had a
530 chance to return on their host body.

531 As previously mentioned, *S. scimitus* is very unlikely to provide effective *Varroa*
532 control if used alone. However, in future assessments, it might be interesting to test its
533 potential when combined with other existing methods or new avenues in a context of
534 integrated pest management. We demonstrated that instead of attacking phoretic *Varroa*

535 mites, *S. scimitus* is more likely to predate upon the mites that fall on the bottom of the
536 hive. In doing so, the biocontrol agent might have a similar effect to that of screen bottom
537 boards or might increase their effectiveness in a similar way than sticky sheets [59]. We
538 know that about 50% of the *Varroa* are still alive and very active when they fall on the hive
539 floor [59]. Thereby, screen bottoms boards that allow *Varroa* to fall through it are often
540 used to prevent the living fallen mites from returning to the colony. Even if not reliable as
541 a single control technique, the use of these screen boards could reduce about 20% of the
542 mite population over the season and increase the degree of *Varroa* control obtained with
543 soft chemicals and other cultural practices [27, 60, 61]. In parallel, Reinbacher et al. [62]
544 recently showed that the entomo-pathogenic fungus *Metarhizium anisopliae*, in addition to
545 its lethal effect on *Varroa* mites, is repelling the parasite from attaching to bees.
546 Interestingly, the fungus is known to be harmless to *S. scimitus* and the combination of *M.*
547 *anisopliae* and *S. scimitus* have been shown to improve the efficacy of the predator against
548 pupating western flower thrips in container studies [63]. Therefore, assessing the combined
549 effect of both agent in *Varroa* control might be an avenue of interest. Whether the
550 introduction of *S. scimitus*, alone or in combination with *M. anisopliae,* would be more
551 effective, convenient or cheaper than the combined use of bottom boards and sticky sheets
552 is, however, uncertain and is worth more consideration.

553 In a recent study [42], Rangel and Ward showed, using *in vitro* assays, the capacity
554 of *S. scimitus* in attacking free *Varroa* mites but they raised questions regarding the overall
555 ability of the predator and whether it could prey on honey bee brood. Here, not only did
556 we bring answers to several of their questions, but we provided additional, crucial
557 information on *S. scimitus* as a biocontrol agent of *Varroa* mites. For instance, by using

more realistic conditions under which we conducted our *in vitro* predation tests (32°C; 70% RH vs 29.5°C; uncontrolled humidity in [42]), we showed that *S. scimitus* can survive and be active within the range of temperature and humidity conditions of a honey bee colony [64]. Since free *Varroa* mites are uncommon in the hive, our study also provides a better understanding of the limitations of *S. scimitus* in controlling *Varroa* mites under more realistic conditions.

In summary, our study provides evidence that *S. scimitus* does not represent a significant threat to the honey bee brood but suggests that its effect in *Varroa* control will probably be limited as it does not attack phoretic *Varroa* mites. Our results represent an important step in assessing the potential of *S. scimitus* to control *V.* destructor and provide novel information about the behavior of the predator inside the honey bee colony. Nevertheless, the actual efficacy of the predatory mite to control *Varroa* populations in honey bee colonies still needs to be investigated in greater depth. As *S. scimitus* is highly polyphagous, assessing the predator's ability to control other honey bee pests found on the hive floor, such as wax moth and small hive beetle larvae, should also be considered.

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References

1. Dietemann V, Nazzi F, Martin SJ, Anderson DL, Locke B, Delaplane KS, et al. Standard methods for varroa research. J Apic Res. 2013;52(1):1-54. doi: 10.3896/IBRA.1.52.1.09.

2. Nazzi F, Le Conte Y. Ecology of *Varroa destructor*, the Major Ectoparasite of the Western Honey Bee, *Apis mellifera*. In: Berenbaum MR, editor. Annual Review of Entomology, Vol 61. Annual Review of Entomology; 2016. p. 417-432. doi: 10.1146/annurev-ento-010715-023731.

3. Rosenkranz P, Aumeier P, Ziegelmann B. Biology and control of *Varroa destructor*. J Invertebr Pathol. 2010;103 Suppl 1: S96-119. doi: 10.1016/j.jip.2009.07.016.

4. Emsen B, Guzman-Novoa E, Kelly PG. Honey production of honey bee (Hymenoptera: Apidae) colonies with high and low *Varroa destructor* (Acari: Varroidae) infestation rates in eastern Canada. Can Entomol. 2014;146(2): 236-240. doi: 10.4039/tce.2013.68.

5. Sammataro D, Gerson U, Needham G. Parasitic mites of honey bees: Life history, implications, and impact. Annu Rev Entomol. 2000;45: 519-548. doi: 10.1146/annurev.ento.45.1.519.

6. Dainat B, Evans JD, Chen YP, Gauthier L, Neumann P. Predictive Markers of Honey Bee Colony Collapse. Plos One. 2012;7(2): e32151. doi:10.1371/journal.pone.0032151.

612 7. vanEngelsdorp D, Meixner MD. A historical review of managed honey bee
613 populations in Europe and the United States and the factors that may affect them.
614 J Invertebr Pathol. 2010;103: S80-S95. doi: 10.1016/j.jip.2009.06.011.
615
616 8. Desai SD, Currie RW. Effects of Wintering Environment and Parasite-Pathogen
617 Interactions on Honey Bee Colony Loss in North Temperate Regions. Plos One.
618 2016;11(7): e0159615. doi: 10.1371/journal.pone.0159615.
619
620 9. Koleoglu G, Goodwin PH, Reyes-Quintana M, Hamiduzzaman MM, Guzman-
621 Novoa E. Effect of *Varroa destructor*, Wounding and Varroa Homogenate on
622 Gene Expression in Brood and Adult Honey Bees. Plos One. 2017;12(1):
623 e0169669. doi:10.1371/journal.pone.0169669.
624
625 10. McMenamin AJ, Genersch E. Honey bee colony losses and associated viruses.
626 Current Opinion in Insect Science. 2015;8: 121-129. doi:
627 10.1016/j.cois.2015.01.015.
628
629 11. Nazzi F, Brown SP, Annoscia D, Del Piccolo F, Di Prisco G, Varricchio P, et al.
630 Synergistic Parasite-Pathogen Interactions Mediated by Host Immunity Can Drive
631 the Collapse of Honeybee Colonies. PLoS Path. 2012;8(6): e1002735.
632 doi:10.1371/journal.ppat.1002735.
633
634 12. Bowen-Walker PL, Martin SJ, Gunn A. The transmission of deformed wing virus
635 between honeybees (*Apis mellifera* L.) by the ectoparasitic mite *Varroa jacobsoni*
636 Oud. J Invertebr Pathol. 1999;73(1): 101-106. doi: 10.1006/jipa.1998.4807.
637
638 13. Le Conte Y, Ellis M, Ritter W. Varroa mites and honey bee health: can Varroa
639 explain part of the colony losses? Apidologie. 2010;41(3): 353-363. doi:
640 10.1051/apido/2010017.
641
642 14. Cabras P, Floris I, Garau VL, Melis M, Prota R. Fluvalinate content of Apistan(R)
643 strips during treatment and efficacy in colonies containing sealed worker brood.
644 Apidologie. 1997;28(2): 91-96. doi: 10.1051/apido:19970206.
645
646 15. Milani N. The resistance of *Varroa jacobsoni* Oud. to acaricides. Apidologie.
647 1999;30(2-3): 229-34. doi: 10.1051/apido:19990211.
648
649 16. Elzen PJ, Baxter JR, Spivak M, Wilson WT. Control of *Varroa jacobsoni* Oud.
650 resistant to fluvalinate and amitraz using coumaphos. Apidologie. 2000;31(3):
651 437-441. doi: 10.1051/apido:2000134.

652
653 17. Maggi MD, Ruffinengo SR, Damiani N, Sardella NH, Eguaras MJ. First detection
654 of *Varroa destructor* resistance to coumaphos in Argentina. Exp Appl Acarol.
655 2009;47(4): 317-320. doi: 10.1007/s10493-008-9216-0.
656
657 18. Giovenazzo P, Dubreuil P. Evaluation of spring organic treatments against *Varroa*
658 *destructor* (Acari: Varroidae) in honey bee *Apis mellifera* (Hymenoptera: Apidae)
659 colonies in eastern Canada. Exp Appl Acarol. 2011;55(1): 65-76. doi:
660 10.1007/s10493-011-9447-3.
661
662 19. Gregorc A, Smodis Skerl MI. Toxicological and immunohistochemical testing of
663 honeybees after oxalic acid and rotenone treatments. Apidologie. 2007;38(3):
664 296-305. doi: 10.1051/apido:2007014.
665
666 20. Schneider S, Eisenhardt D, Rademacher E. Sublethal effects of oxalic acid on
667 *Apis mellifera* (Hymenoptera: Apidae): changes in behaviour and longevity.
668 Apidologie. 2012;43(2): 218-225. doi: 10.1007/s13592-011-0102-0.
669
670 21. Vandervalk LP, Nasr ME, Dosdall LM. New Miticides for Integrated Pest
671 Management of *Varroa destructor* (Acari: Varroidae) in Honey Bee Colonies on
672 the Canadian Prairies. J Econ Entomol. 2014;107(6): 2030-2036. doi:
673 10.1603/ec14048.
674
675 22. Alayrangues J, Hotier L, Massou I, Bertrand Y, Armengaud C. Prolonged effects
676 of in-hive monoterpenoids on the honey bee *Apis mellifera*. Ecotoxicology.
677 2016;25(5): 856-862. doi: 10.1007/s10646-016-1642-x.
678
679 23. Bonvehi JS, Coll FV, Martinez JAR. Residues of essential oils in honey after
680 treatments to control *Varroa destructor*. J Essent Oil Res. 2016;28(1): 22-28. doi:
681 10.1080/10412905.2015.1076741.
682
683 24. Floris I, Satta A, Cabras P, Garau VL, Angioni A. Comparison between two
684 thymol formulations in the control of *Varroa destructor*: Effectiveness,
685 persistence, and residues. J Econ Entomol. 2004;97(2): 187-191. doi:
686 10.1603/0022-0493-97.2.187.
687
688 25. Al Naggar Y, Tan Y, Rutherford C, Connor W, Griebel P, Giesy JP, et al. Effects
689 of treatments with Apivar® and Thymovar® on *V-destructor* populations, virus
690 infections and indoor winter survival of Canadian honey bee (*Apis mellifera* L.)

691 colonies. J Apic Res. 2015;54(5): 548-554. doi:
692 10.1080/00218839.2016.1186917.
693
694 26. The Honey Bee Health Coalition. Tools for Varroa Management: A Guide to
695 Effective Varroa Sampling & Control. Honey Bee Health Coalition; 2017. p. 1-
696 25. Available from: https://honeybeehealthcoalition.org/varroa/.
697
698 27. Delaplane KS, Berry JA, Skinner JA, Parkman JP, Hood WM. Integrated pest
699 management against *Varroa destructor* reduces colony mite levels and delays
700 treatment threshold. J Apic Res. 2005;44(4): 157-162. doi:
701 10.1080/00218839.2005.11101171.
702
703 28. Chandler D, Sunderland KD, Ball BV, Davidson G. Prospective biological control
704 agents of *Varroa destructor* n. sp., an important pest of the European honeybee,
705 *Apis mellifera*. Biocontrol Sci Technol. 2001;11(4): 429-448. doi:
706 10.1080/09583150120067472.
707
708 29. Pernal SF, Clay H. Maladies et organismes nuisibles de l'abeille domestique, 3e
709 edition (version française). 3ieme ed. Beaverlodge (Alberta): Association
710 canadienne des professionnels de l'apiculture (ACPA); 2015.
711
712 30. Walter DE, Campbell NJH. Exotic vs endemic biocontrol agents: would the real
713 *Stratiolaelaps miles* (Berlese) (Acari : Mesostigmata : Laelapidae), please stand
714 up? Biol Control. 2003;26(3): 253-269. doi: 10.1016/s1049-9644(02)00171-8.
715
716 31. Hoy MA. Agricultural acarology introduction to integrated mite management.
717 Boca Raton: CRC Press; 2011.
718
719 32. Cabrera AR, Cloyd RA, Zaborski ER. Development and reproduction of
720 *Stratiolaelaps scimitus* (Acari : Laelapidae) with fungus gnat larvae (Diptera :
721 Sciaridae), potworms (Oligochaeta : Enchytraeidae) or *Sancassania aff.*
722 *sphaerogaster* (Acari : Acaridae) as the sole food source. Exp Appl Acarol.
723 2005;36(1-2): 71-81. doi: 10.1007/s10493-005-0242-x.
724
725 33. Navarro-Campos C, Wackers FL, Pekas A. Impact of factitious foods and prey on
726 the oviposition of the predatory mites *Gaeolaelaps aculeifer* and *Stratiolaelaps*
727 *scimitus* (Acari: Laelapidae). Exp Appl Acarol. 2016;70(1): 69-78. doi:
728 10.1007/s10493-016-0061-2.
729

730 34. Wright EM, Chambers RJ. The biology of the predatory mite *Hypoaspis miles*
731 (*Acari: Laelapidae*), a potential biological control agent of *Bradysia paupera*
732 (*Dipt.: Sciaridae*). Entomophaga. 1994;39(2): 225-235. doi:
733 10.1007/bf02372360.
734
735 35. Chambers RJ, Wright EM, Lind RJ. Biological control of glasshouse sciarid flies
736 (*Bradysia* spp.) with the predatory mite, *Hypoaspis miles*, on cyclamen and
737 poinsettia. Biocontrol Sci Technol. 1993;3(3): 285-293. doi:
738 10.1080/09583159309355283.
739
740 36. Castilho RC, de Moraes GJ, Silva ES, Freire RAP, Da Eira FC. The predatory
741 mite *Stratiolaelaps scimitus* as a control agent of the fungus gnat *Bradysia*
742 *matogrossensis* in commercial production of the mushroom *Agaricus bisporus*. Int
743 J Pest Manage. 2009;55(3): 181-185. doi: 10.1080/09670870902725783.
744
745 37. Wu S, Gao Y, Xu X, Wang E, Wang Y, Lei Z. Evaluation of *Stratiolaelaos*
746 *scimitus* and *Neoseiulus barkeri* for biological control of thrips on greenhouse
747 cucumbers. Biocontrol Sci Technol. 2014;24(10): 1110-1121. doi:
748 10.1080/09583157.2014.924478.
749
750 38. Wu S, Zhang Z, Gao Y, Xu X, Lei Z. Interactions between foliage- and soil-
751 dwelling predatory mites and consequences for biological control of *Frankliniella*
752 *occidentalis*. BioControl. 2016;61(6): 717-727. doi: 10.1007/s10526-016-9762-z.
753
754 39. Berndt O, Meyhofer R, Poehling HM. The edaphic phase in the ontogenesis of
755 *Frankliniella occidentalis* and comparison of *Hypoaspis miles* and *Hypoaspis*
756 *aculeifer* as predators of soil-dwelling thrips stages. Biol Control. 2004;30(1): 17-
757 24. doi: 10.1016/j.biocontrol.2003.09.009.
758
759 40. Lesna I, Sabelis MW, van Niekerk TGCM, Komdeur J. Laboratory tests for
760 controlling poultry red mites (*Dermanyssus gallinae*) with predatory mites in
761 small 'laying hen' cages. Exp Appl Acarol. 2012;58(4): 371-383. doi:
762 10.1007/s10493-012-9596-z.
763
764 41. Mendyk RW. Preliminary Notes on the Use of the Predatory Soil Mite
765 *Stratiolaelaps scimitus* (Acari: Laelapidae) as a Biological Control Agent for
766 Acariasis in Lizards. J. Herpetol. Med. Surg. 2015;25(1-2): 24-27. doi:
767 10.5818/1529-9651-25.1.24.
768

769 42. Rangel J, Ward L. Evaluation of the predatory mite *Stratiolaelaps scimitus* for the
770 biological control of the honey bee ectoparasitic mite *Varroa destructor*. J Apic
771 Res. 2018;57(3): 425-432. doi: 10.1080/00218839.2018.1457864.
772
773 43. The Niagara Beeway [Internet]. Niagara: The Niagara Beeway; c2018 [cited 2018
774 Apr 17]. Varroa Mite 2014 Report. Available from:
775 http://www.niagarabeeway.com/varroa-mite.html.
776
777 44. Jacques A, Laurent M, Ribiere-Chabert M, Saussac M, Bougeard S, Budge GE, et
778 al. A pan-European epidemiological study reveals honey bee colony survival
779 depends on beekeeper education and disease control. Plos One. 2017;12(3):
780 e0172591. doi: 10.1371/journal.pone.0172591.
781
782 45. Boot WJ, Calis JNM, Beetsma J. Invasion of *Varroa jacobsoni* into honey bee
783 brood cells - a matter of chance or choice. J Apic Res. 1993;32(3-4): 167-174.
784 doi: 10.1080/00218839.1993.11101302.
785
786 46. Ali W, George DR, Shiel RS, Sparagano OAE, Guy JH. Laboratory screening of
787 potential predators of the poultry red mite (*Dermanyssus gallinae*) and assessment
788 of *Hypoaspis miles* performance under varying biotic and abiotic conditions. Vet
789 Parasitol. 2012;187(1-2): 341-344. doi: 10.1016/j.vetpar.2012.01.014.
790
791 47. Human H, Brodschneider R, Dietemann V, Dively G, Ellis JD, Forsgren E, et al.
792 Miscellaneous standard methods for *Apis mellifera* research. J Apic Res.
793 2013;52(4): 1-53. doi: 10.3896/IBRA.1.52.4.10.
794
795 48. Fagerland MW, Lydersen S, Laake P. The McNemar test for binary matched-pairs
796 data: mid-p and asymptotic are better than exact conditional. BMC Med Res
797 Methodol. 2013;13(91). doi: 10.1186/1471-2288-13-91.
798
799 49. R Core Team (2016) R: A language and environment for statistical computing.
800 Vienna: R Foundation for Statistical Computing. Available: https://www.R-
801 project.org/.R.
802
803 50. SAS Institute Inc. (2017) SAS/STAT® 14.3 User's Guide. Cary, NC.
804
805 51. Walter DE, Proctor HC. Mites ecology, evolution & behaviour : life at a
806 microscale. Dordrecht: Springer; 2013.

52. Berndt O, Poehling HM, Meyhofer R. Predation capacity of two predatory laelapid mites on soil-dwelling thrips stages. Entomol Exp Appl. 2004;112(2): 107-115. doi: 10.1111/j.0013-8703.2004.00185.x.

53. Berg MP. Mass-length and mass-volume relationships of larvae of *Bradysia paupera* (Diptera : Sciaridae) in laboratory cultures. European Journal of Soil Biology. 2000;36(3-4): 127-133. doi: 10.1016/s1164-5563(00)01055-4.

54. Van Driesche RG, Hoddle M, Center TD. Control of pests and weeds by natural enemies: an introduction to biological control. 1st ed. Malden, MA: Blackwell Pub; 2008.

55. Berndt O, Meyhofer R, Poehling HM. Propensity towards cannibalism among *Hypoaspis aculeifer* and *H. miles*, two soil-dwelling predatory mite species. Exp Appl Acarol. 2003;31(1-2): 1-14. doi: 10.1023/b:appa.0000005108.72167.74.

56. Boot WJ, Calis JNM, Beetsma J. Differential periods of *Varroa* mite invasion into worker and drone cells of honey bees. Exp Appl Acarol. 1992;16(4): 295-301. doi: 10.1007/bf01218571.

57. Fries I. Treatment of sealed honey bee brood with formic acid for control of *Varroa jacobsoni*. Am. Bee J. 1991;131(5): 313-314.

58. Pritchard DJ. Grooming by honey bees as a component of varroa resistant behavior. J Apic Res. 2016;55(1): 38-48. doi: 10.1080/00218839.2016.1196016.

59. Webster TC, Thacker EM, Vorisek FE. Live *Varroa jacobsoni* (Mesostigmata : Varroidae) fallen from honey bee (Hymenoptera : Apidae) colonies. J Econ Entomol. 2000;93(6): 1596-1601. doi: 10.1603/0022-0493-93.6.1596.

60. Harbo JR, Harris JW. Effect of screen floors on populations of honey bees and parasitic mites (*Varroa destructor*). J Apic Res. 2004;43(3): 114-117. doi: 10.1080/00218839.2004.11101120.

61. Pettis JS, Shimanuki H. A hive modification to reduce varroa populations. Am. Bee J. 1999;139(6): 471-473.

62. Reinbacher L, Fernández-Ferrari MC, Angeli S, Schausberger P. Effects of *Metarhizium anisopliae* on host choice of the bee-parasitic mite *Varroa destructor*. Acarologia. 2018;58(2): 287-295. doi: 10.24349/acarologia/20184241.

847

848 63. Saito T, Brownbridge M. Compatibility of soil-dwelling predators and microbial
849 agents and their efficacy in controlling soil-dwelling stages of western flower
850 thrips *Frankliniella occidentalis*. Biol Control. 2016;92: 92-100. doi:
851 10.1016/j.biocontrol.2015.10.003
852
853 64. Jones JC, Myerscough MR, Graham S, Oldroyd BP. Honey bee nest
854 thermoregulation: Diversity promotes stability. Science. 2004;305(5682): 402-
855 404. doi: 10.1126/science.1096340.

856