In vivo assessment of S. scimitus predation upon bee brood

Version 2

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ABSTRACT

In order to investigate the potential of predatory mites to control *Varroa* infestations in honey bees, it is important to assess the risk of predation of the honey bee brood by the predators under realistic conditions (i.e. within the honey bee colony). Here, we provide a protocol to evaluate this risk of brood predation by the soil-dwelling predatory mite *Stratiolaelaps scimitus*.

EXTERNAL LINK

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PROTOCOL STATUS

Working

GUIDELINES

Upon reception of the commercial product containing *Stratiolaelaps scimitus*, it is important to check the product for predator vitality under a stereomicroscope (normal activity, vigour and abundance) and the presence of prey. The product must be used as soon as possible after reception and maintained in appropriate conditions until its use (according to the supplier instructions).

The experiment must be performed according to a completely randomized design, where all colonies are located in the same apiary. If more than one apiary is used (blocks), the number of colonies in each apiary must be sufficient to provide enough degree of freedom for statistical analyses and to account for the site variations (e.g. flower resources).

IMPORTANT: Prior to the experiment, it is primordial to ensure that all colonies are strong, healthy and without signs of brood diseases (chalkbrood, American and European foulbrood). This could be done by visual inspections of all frames in all colonies. Any colony with suspected brood disease or other abnormalities (e.g. low egg laying by the queen, scattered brood pattern, low honey and pollen reserves, etc.) must be excluded. Abundant flower resources must be available near the apiary to decrease the risk of brood cannibalisms by the worker bees in case of starvation.

Ideally, the brood monitoring must be performed in a cool and shady place to prevent the brood (especially the eggs and young larval instar) to dry. For this same reason, monitoring (brood marking) must be performed as quickly as possible. Additional precautions such as placing the frames on a wet towel during marking must also be taken.

MATERIALS TEXT

Stratiolaelaps scimitus can be obtained by various biocontrol suppliers. Mites are usually supplied in a mixture of vermiculite and peat in 1L bottles with mold mites (*Tyrophagus putrescentiae*) as a food source. Pre-autoclaved vermiculite is used as a control.

All honey bee colonies used must be of equivalent strength. Ideally, these colonies must have sister queens of known descent. It is preferable to use hives with a single brood chamber.

Experimental design

1 **A**) Randomly assign each hive to a treatment (control or treated) and identify the hives.

Note: Refer to both the Guidelines and the Materials sections for more information about colony and apiary requirements.

Egg laying monitoring

2 As described in **Human et al. (2013). doi: 10.3896/IBRA.1.52.4.10** :

A) For each colony, place an empty comb with worker cells in an exclusion cage with the queen.

B) Place the cage in the brood chamber of the hive and allow the queen to lay eggs for 48h.

C) Remove the queen from the exclusion cage and reintroduced her in its colony.

D) Using a permanent marker and a transparent sheet of acetate, mark the position of every comb cell containing an egg (for both sides of the frame). This step must be performed quickly to prevent the eggs from drying. Additional precautions are needed (see the Guidelines section).

E) Place back the frame in the exclusion cage to prevent further oviposition by the queen and replace the cage in the middle of the brood chamber.

Introduction of the predatory mites

3 **A**) Pour the desired amount of the biocontrol commercial product containing *Stratiolaelaps scimitus* (treated group) or the same amount of pre-autoclaved vermiculite (control group) on top of the queen excluder (we used 500 ml in our experiment). The product must be poured parallel to the brood frames. In doing so, it is partially retained by both the queen excluder and the top of the frames.

Note: Refer to the Guidelines section for requirements regarding quality of the biocontrol product.

Monitoring of brood survival

4 **A**) Six days after the introduction of the predatory mite in the colonies, use the previous acetate (step 2) to check for a second time each brood cells where an egg had been previously laid. Verify if the larvae (L4-L5 stage) are present.

B) For each frame, mark the cells with a missing larva with a permanent marker of another colour.

C) Return the combs to the hives.

D) Repeat steps A, B and, C four days later (capped pupae).

Notes: As mentioned in step 2, the monitoring must be performed quickly. In our experiment, the time required to mark both sides of each frame never exceeded 45 minutes. Younger larvae (L1-L2) are too small and difficult to be observed in the cells, which results in a longer monitoring period. This is the reason why we did not monitor the survival of this larval instar.

Monitoring the presence of the predatory mites in the colonies

5 **A**) At each period of brood monitoring, perform visual examinations of the hive floor and the frames to ensure that the predatory mites remain in the hive throughout the experiment.

B) At the end of the trial, collect a sample of debris at the bottom of the hive for further screening and mite identification under the stereomicroscope.

Note: To avoid important disturbance of the mite behaviour in the colony, we considered observing five to ten mites during a visual inspection as satisfactory.

Data analyses

6 **A**) For each period of brood monitoring, use the acetate sheets to count the number of cells with brood and determine the percentage of eggs and larvae that survived until cell capping.

B) If the assumptions of the test are met, a repeated measures analysis of variance (ANOVA) with autoregressive correlation structure could be performed to compare differences of brood survival (number of eggs and surviving larvae and pupae) due to treatment, brood stage and their interaction. We recommend to use the proc mixed procedure in SAS® University Edition.

Notes: a significant difference in brood survival due to treatment is considered as the result of significant predation by the predatory mites. However, other causes could be responsible for missing brood (e.g. brood diseases, starvation, etc.). This is the reason why multiple precautions must be taken before starting the experiment. See the Guidelines section for more details.