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NOTES AND COMMENTS

A method for rapidly marking adult varroa mites for use in brood inoculation experiments

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A wide variety of markers are used to mark insects and other animals for several types of biological studies (Hagler and Jackson, 2001). Due to varroa (*Varroa destructor*) mites being small, finding an ideal marker has proved difficult. Yet marking is crucial in identifying test mites that have been inoculated into brood, for example when brood is multiply infested or if daughters are present. Harris (2001) developed a technique for marking individual varroa mites using pieces of polyester glitters attached to the dorsal side of the idiosoma with super glue (cyanoacrylate), a variation of the method of Schulz (1984) who used cyanoacrylate with fluorescent pigments.

We explored a method for marking varroa mites using correction fluid (PRESTO!^{TM} Jumbo Correction Pen, Pentel Co.; Ltd., Japan). Individual mites were placed on a piece of nylon mesh (165 mesh) to prevent them from moving during marking. A tiny droplet of correction fluid was transferred onto the mite's dorsum close to the posterior end (Fig. 1) using a piece of nylon fishing line (diameter = 0.30 mm) attached to an applicator stick. It was ensured that no fluid ran onto the mite's rectum as this may be harmful. The mites were then inoculated into mapped brood comb cells containing newly sealed honey bee larvae obtained from two colonies having low levels of varroa infestation (Trial 1- Russian: 40 marked and 40 unmarked mites, Trial 2 - Italian: 50 marked mites). In addition to the unmarked mites in Trial 1, 50 brood cells opened and closed without mite inoculation and 66 cells not manipulated (Trial 2) served as control groups. Test brood cells were examined after nine days. As the removal of mites from sealed brood disrupts their normal reproductive cycle (Kirrane *et al.*, 2011), it was only noted whether test mites produced progeny or not.

Overall, 47% of marked mites were removed compared with only 15% of unmarked mites. This observation indicates that marking with correction fluid increased the likelihood of removal (c^2 = 13.5, df = 2, P < 0.01) possibly due to the detection of the fluid's odours. Nevertheless, our results were similar to that of Harris (2001) who recovered 55% (40 out of 73) of the mites marked with glitters. Overall, the reproduction of marked mites did not differ significantly (c^2 = 0.15, df = 2, P > 0.05) from that of the unmarked mites; 63.4% of marked mites reproduced compared with 52% of unmarked mites. Hence, reproduction of varroa was not affected by marking them with correction fluid. The mortality of marked mites was about 12%. This mortality was not, however, as dramatic as the results of Harris (2001) when 15% and 20% of mites painted with Testors enamel paints died after 24 h and 36 h, respectively.

Fig. 1. Varroa mite with correction fluid marking on honey comb

The use of correction fluid has advantages for marking varroa mites. It is a one step process that can be performed quickly and with relative ease for marking a group of mites with a single mark. The glue and glitter method (Harris, 2001) is a tedious two step process that might best be reserved for experiments requiring the marking of two or more treatment groups. Correction fluid also dries relatively quickly which reduces the danger of the marking droplet running and damaging the mite, which is a common problem with the acrylic paint method of Schulz (1984). On the other hand, enamel paints can cause increased mortality to the mites (Harris, 2001). The correction fluid is inexpensive and readily accessible. Being white, correction fluid marks are very noticeable to the naked eye during recovery.

We did not determine how long the mark lasts, but we found at least three marked mites in non-test brood about two weeks after the test. These mites probably came from the inoculated brood and were freed when bees removed some of the inoculated brood during our test. This discovery suggests that at least some of the marks are long-lasting and that some mites released by hygienic behaviour eventually find a synchronous host and reproduce.

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References

HAGLER, J R; JACKSON, C G (2001) Methods for marking insects: current techniques and future prospects. *Annual Review of Entomology* 46: 511–543, http://dx.doi.org/10.1146/annurev.ento.46.1.511

HARRIS, J W (2001) A technique for marking individual varroa mites. *Journal of Apicultural Research* 40: 35-37.

KIRRANE, M J; DE GUZMAN, L I; RINDERER, T E; FRAKE, A M; WAGNITZ, J; WHELAN, P M (2011) Asynchronous development of honey bee host and *Varroa destructor* (Mesostigmata: Varroidae) influences reproductive potential of mites. *Journal of Economic Entomology* 104: 1146-1152, http://dx.doi.org/10.1603/EC11035

SCHULZ, A E (1984) Reproduction and population dynamics of the parasitic mite *Varroa jacobsoni* Oud. and its dependence on the brood cycle of its host *Apis mellifera* L. [in German]. *Apidologie* 15: 401-420, http://dx.doi.org/10.1051/apido:19840404