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NOTES AND COMMENTS

A laboratory technique to study the effects of *Varroa destructor* and viruses on developing worker honey bees

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Many studies of honey bee, *Varroa destructor* mite and virus interactions can be facilitated if honey bees and mites can be reared in the laboratory. *In vitro* rearing of honey bees has been successfully demonstrated by several researchers (e.g. Peng *et al.,* 1992 and citations therein; Crailsheim *et al.*, 2013). However, their methods did not use single, tight confinement chambers which *V. destructor* needs for successful reproduction. The full life cycle of *V. destructor* in the laboratory has also been achieved but with minimal success (Dietemann *et al.*, 2013). In 1994, Nazzi and Milani showed that 62% of the mites inoculated into newly sealed larvae (NSL) placed inside gelatin capsules (6 mm diameter) reproduced successfully with 3.5 progeny per female. However, this method involves manipulation of NSL. In this study, we assessed the use of larvae (L4 or NSL) naturally developing in a comb as mite-and virus-inoculation hosts.

In Trial 1, a frame of brood containing L4 larvae and NSL obtained from a colony was used. Each test brood received one foundress *V. destructor* collected from NSL. Thereafter, brood cells (L4 larvae = 48 cells; NSL = 30 cells) were individually sealed with a gel cap. The bottom portion of clear gelatin capsules (size 0 or 00) (Solaray; Park City, UT, USA) were cut to fit the rim of a cell (diameter = 5 mm) and then gently pressed onto the comb (Fig. 1.). Mite-inoculation of the NSL group followed the techniques of Kirrane *et al.* (2011). In brief, one foundress mite was introduced into a small opening created at the edge of a capped brood. Thereafter, the capping was pressed back and sealed with a gel cap. Thirty un-manipulated NSL (no gel cap) served as controls. Trial 2 used L4 larvae (sealed with gel caps) as hosts with the following treatments: a) one mite (n = 31); b) two mites (n = 27); c) no mites and fed 2 µl deformed wing virus (DWV, n = 30); d) one mite and fed 2 µl DWV (n = 30); and control (no mites or DWV, n = 26). Inoculum mites were collected from NSL. DWV lysate was prepared by grinding 10 bees with deformed wings in 10 ml of PBS. Presence of DWV in the lysate was confirmed by qRT-PCR (Nazzi *et al.*, 2012). Thereafter, test brood frames were placed in an incubator (34ºC, 60-70% relative humidity). After nine days, each brood cell was examined for mite reproduction. Each pupa was then placed in a 0.5 ml Eppendorf vial with a small hole through the cover and allowed to develop to adulthood in an incubator. Individual bees were weighed at emergence.

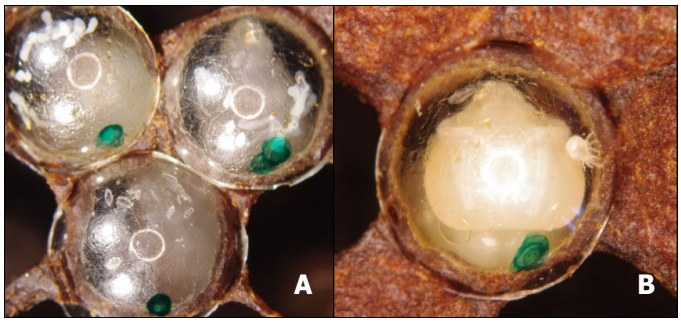

Fig. 1. Developing brood showing mite faeces and cocoon (A), and immature mite (B) on gel caps.

Overall, about 80% of the *V. destructor* reproduced. In Trial 1, analysis of variance (ANOVA) showed no difference in fecundity was detected between mites inoculated in L4 (4.0 ± 0.3 progeny) and NSL (3.7 ± 0.4) groups (t_{76} = 0.41, P = 0.682). Adult bee weights differed significantly ($F_{2,93}$ = 75.23, P < 0.0001) among treatment groups. Workers from the control group (108.81 ± 0.91 mg) were heavier than those inoculated as NSL (96.2 ± 1.6 mg) which were in turn heavier than those inoculated at the L4 stage (79.1 ± 2.1 mg).

In Trial 2, brood inoculated with two mites supported more progeny per infested cell (5.8 ± 0.6 progeny) than did brood with one mite (3.1 ± 0.4) or one mite and DWV (3.2 ± 0.4) ($F_{2,85}$ = 5.10, P = 0.008). Bee weights also differed significantly ($F_{4, 136}$ = 11.88, P < 0.0001): control bees (101.2 ± 1.9 mg) ≥ bees having one mite (94.6 ± 3.5 mg) ≥ bees that had DWV (91.8 ± 2.6 mg) > bees with DWV and one mite (80.9 ± 3.4 mg) and bees with two mites (75.2 ± 2.5 mg). Overall, bee weights correlated negatively with the number

of mites per infested cell (r = - 0.376, P < 0.0001). Also, out of the 166 mite- or DWV- inoculated L4, 3.6% died as larva or pupa, and 30.1% reached adulthood with wing deformities probably as a result of mite-feeding or viral infection. *V. destructor* are vectors of DWV (Chen and Siede, 2007). However, viral levels of both mite- and DWV-inoculated brood were not confirmed. Neither mortality nor deformity was recorded from the control group.

V. destructor introduced in brood sealed with gel caps successfully reproduced. On average, one female mite produces about three progeny in suitable hosts (de Guzman *et al*., 2008; Kirrane *et al*., 2011). Here, we recorded 3-4 progeny per foundress. Our results agreed well with those of de Jong *et al*. (1982) who documented that newly emerged uninfested bees weigh about 95 mg and that weights decrease with increasing number of mites per infested cell. Since no dead or deformed bees were observed in the control group (Trial 2) and control bees weighed about 100 mg, sealing L4 with gel caps does not interfere with bees' development.

This technique allows for the study of individual worker bee's responses to inoculations with mites and viruses without the need to manipulate larvae, provision larval food or transfer brood to pupation plates. Loss of brood through hygienic behaviour of bees is also avoided since test brood is kept in an incubator. This method allows simplified tracking of individual bees. Gel caps can be labelled directly making a map of the test brood unnecessary.

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