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NOTES AND COMMENTS

Electronic monitoring of feeding behaviour of *Varroa* mites on honey bees

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Introduction

The *Varroa* mite (*Varroa destructor* Anderson and Trueman) is an ecto-parasite and the most devastating pest of the honey bee (*Apis mellifera* L) (Sammataro *et al,* 2000). Because *Varroa* mites are small and feed on adult bees between body segments, or on larvae and pupae inside brood cells, it is difficult to observe their feeding behaviour. Donzé and Guerin (1994) reported that *Varroa* mites fed 0.81–1.49 hr on honey bee worker pupae during a 24-hr period. Their criterion for identifying feeding bouts was based on observing mites on the pupa (the feeding site) rather than on actual feeding activity. We decided to test whether it is possible to monitor the feeding behaviour of *Varroa* mites using electronic means. The Electronic Monitor of Insect Feeding (EMIF) has been widely used to study feeding behaviour of leafhoppers and aphids on plants (Zehnder *et al,* 2001). In a typical setting, an insect and the potting soil in which a plant is cultured are both attached with electrodes. When the insect touches the plant in any fashion, the circuit is completed and a current flows through the insect and the plant. This current can be amplified and recorded as voltage changes. When the insect inserts its mouthparts into the plant, the resistance becomes smaller and hence results an elevation of voltage in the recorder. We thought it might be possible to use the same machine to monitor *Varroa* mite feeding behaviour because when a mite sucks heamolymph from a bee, resistance might be lower than when the mite is walking or resting on a pupa. In our study, we used an alternate current Insect Feeding Monitor (IFM) manufactured by the Elaine Backus laboratory, University of Missouri (Columbia, MO, USA). The IFM output voltage was 80 mV and frequency 4,000 Hz. The IFM was connected to a personal computer which ran WINDAQ (Dataq, Akron, OH, USA). The IFM can monitor four channels (mites) simultaneously.

Combs containing brood cells of bees were taken out of colonies infested with *Varroa* mites. After several adult *Varroa* mites were collected from drone brood cells, each mite was attached to a 1.27 nm diameter gold wire that was 30–60 mm long using a silver conducting paint (#60805, Ladd Research, USA). Mites were cold anaesthetized on ice to reduce their movement and facilitate adherence. The gold wire was then attached to the input electrode that was connected to the IFM. A pupa at purple-eye stage was removed from a cell and placed on a thin plastic sheet that was bent so that it has both a horizontal and a vertical surface. The plastic sheet was made from a re-closable plastic (Ziploc) bag (Inteplast Group, Ltd, USA). A horizontal slit was cut in the vertical surface and the gold wire with the mite attached passed through the slit so that the movement of the mite was restricted. This was done to prevent the mite from touching the other electrode directly, causing a "short-circuit" (circuit formed but bypassing the bee pupae). The electrodes were made of copper. Three electrodes were used for each mite; one was attached to the gold wire, another attached to the bee pupa (by having the pupae lying next to the electrode), and the third was a reference electrode to reduce interference (hanging in the air, not contacting bees or mites). The experiment was done June to August 2004 under natural room light conditions and ambient temperatures of ca 26° C.

After comparing the waveforms on the computer and mite behaviours under the microscope or on a television monitor, four types of waveforms were identified: resting, walking, feeding and probing (Fig. 1). The "resting" waveform is a relatively straight line without spike, exhibiting no voltage changes compared to the baseline. The "walking" waveform is spiky and characterized by median frequency (5.51 ± 1.7 Hz, mean ± SD) spikes with the highest voltage changes (0.03 to 0.17 volt) among all three non-resting waveforms. The feeding waveform is characterized by a sudden increase in voltage (about 0.06 volt), indicating that the mite has pierced the pupa and electrical resistance is rapidly reduced. This low resistance state was maintained until the mouthparts were withdrawn, after which the voltage returned to the baseline level. During the feeding, the waveform is characterized by symmetrical spikes in both directions (increase or decrease in resistance) that are of the highest frequency (10.67 ± 1.59 Hz) among all waveforms. Finally, the probing

waveform resembles many short feeding spurts, with voltages reaching the same level as the feeding wave, but then returning quickly to the baseline This pattern is repeated at a frequency of 1.45 ± 0.40 Hz.

After identifying the waveforms, we attempted to record 12 *Varroa* mites feeding on worker pupae continuously for 24 hr and to analyze their time distribution in the four behaviours using these waveforms. Most of the mites escaped, but the remaining 3 mites showed that they fed only during the daytime (6:00 – 21:00 hr) and not at night (21:00 – 6 hr), and the total feeding time was short (0.35 ± 0.12 hr per 24 hr). These results should be viewed cautiously as the feeding monitoring occurred under artificial conditions (outside brood cells, at room temperature and florescent lighting during daytime) and might not represent those under natural conditions. We also compared the feeding behaviour of mites when they fed on worker (n=7) or drone pupae (n=6) using an average recording duration of 8 hr. Mites feeding on drone pupae were more active than those on worker pupae and spent significantly less time resting than those on worker pupae (6.55 ± 1.09 [drones] *vs* 7.54 ± 0.35 hr [workers], t = 2.23, P = 0.047). They also spent more time feeding on drone pupae, but the difference was not significant (0.59 ± 1.13 [drones] *vs* 0.079 ± 0.13 [workers], t = -1.20, P = 0.25). Walking and probing times were not statistically different on the two types of hosts. It is not clear whether the difference represents naturally occurring differential feeding behaviours toward drone and worker pupae or represents an artifact due to the fact that all mites were obtained from drone brood and then monitored on different hosts.

Because this is the first time an EMIF was used to study the feeding behaviour of *Varroa* mites, there are still many problems to be solved. For example, the gold wire was not insulated which required us to restrict mite movement so that no short circuit would occur. It was also weak and many mites escaped after breaking the wires. However, our data show that it is feasible to use an EMIF device to study *Varroa* mite feeding behaviour. With this device, it may be possible to perform long-term monitoring of mites without human observation. It also may be possible to study mite host choice preferences (*eg,* a young bee *vs* a forager) or subtle behavioural changes due to sublethal pesticide effects. We anticipate many uses for the EMIF system in studies of *Varroa* mite feeding behaviour.

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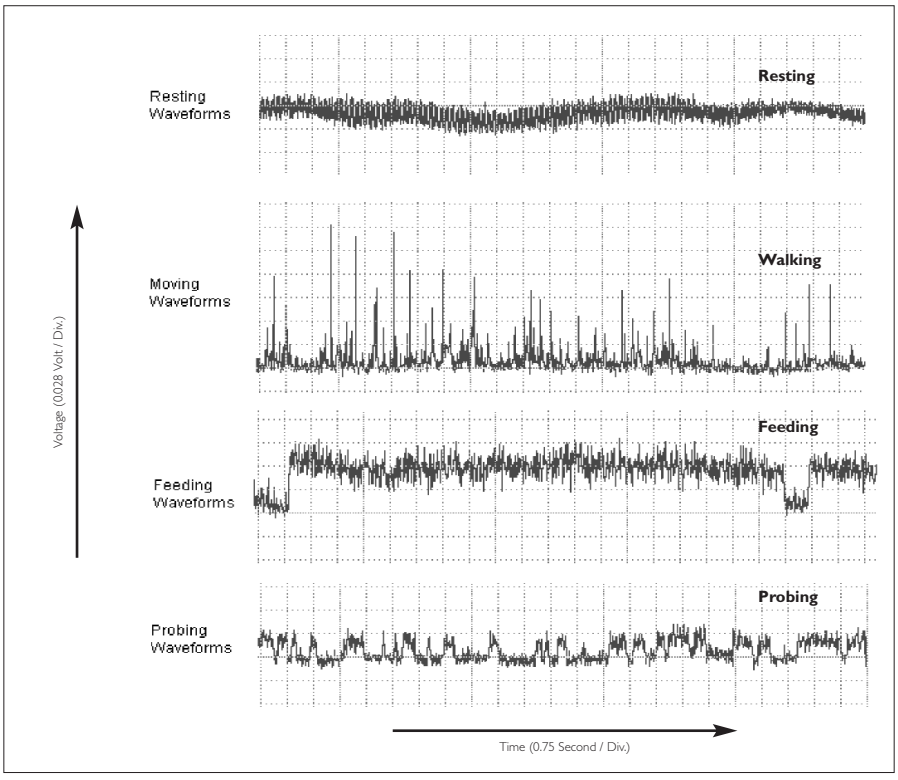

Fig. 1. Different waveforms produced by the Insect Feeding Mornitor (IFM) resulting from different mite behaviours on honey bee drone pupae. Arrows indicate the position of baseline on each wave form, which has a voltage value of zero.