

ASSESSING THE TOXIC EFFECTS OF ACARICIDES BY MONITORING THE GRAVITATIONAL REFLEX OF VARROA DESTRUCTOR*

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A free-moving *Varroa destructor* mite performs an accurate walking behaviour inside and outside the honeybee brood cell. In an attempt to study the neuronal control of walking we noticed a rhythmic flexion of the legs well-coordinated with an expansion of the sternal shield, initiated when *Varroa* was placed in a dorsal side down position. The fact that this rhythmic expansion of the sternum could be recorded for over 24 hours, or for as long as the *Varroa* mite was alive, stopping when the mite was placed in a normal standing position, is an indication of a gravitational reflex. This is an attempt of *Varroa* to return to its normal position, dorsal site up, a reflex observed in all invertebrates and vertebrates.

The accurate monitoring of the force generated during the expansion of the sternum was used as an indication of the vitality of the *Varroa* preparation. For the initiation and maintenance of such a behavioural motor pattern, the proper function of the chain of proprioceptors, interneurons, motoneurons, neuromuscular junctions and muscles of *Varroa* is required. Any deleterious effect of synthetic chemical or natural compounds (acaricides) may have a direct effect in one or more of these links disturbing or even inhibiting the reflex.

To monitor the force generated by either the rhythmic flexion of the legs or the movement of the sternal shield, a sensitive isometric force displacement transducer (FT-03C, Grass Instrument Company, USA) was used. The transducer was mounted on a micromanipulator, while its probe was extended to a micropin. The transducer was gently attached on the surface of the sternal shield. The analog signal of the transducer was amplified 70,000-fold, using the proper preamplifier and an AC/DC Neurolog NL 106 amplifier (Digitimer, England, UK).

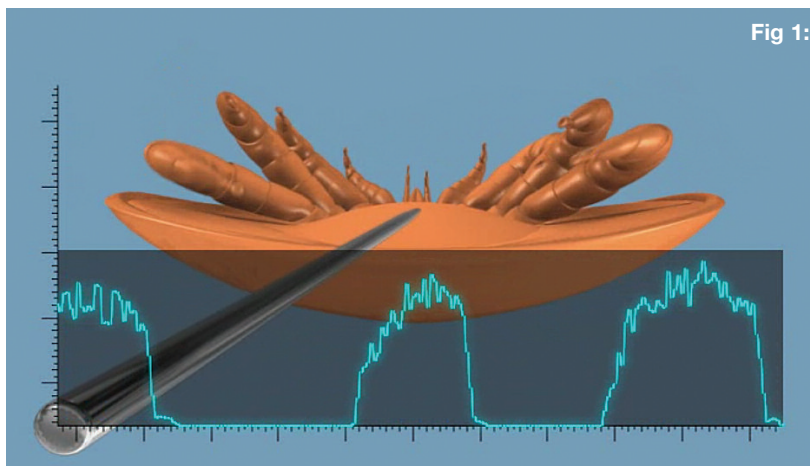


Fig 1:

The amplified signal from the transducer was initially filtered (Neurolog NL115), then digitized (1,000 Hz) using a data-acquisition card interface (Keithley KPCI-3102, Keithley Instruments, Cleveland, OH, USA) and analyzed using the proper software (Labview 5.1, National Instruments USA). The entire experimental procedure used to obtain recordings from the sternum via the transducer is presented in Figure 1.

The pulses generated by the force of the rhythmic expansions had an average duration of 3.11 s, force (amplitude) of 73 μ N, and frequency of 0.228 Hz. These parameters remained constant for the first 10 h of recording, whilst significant changes occurred after 15 h. The rhythmic sternal expansion is an indication of a *Varroa* mite's gravitational reflex, or attempt to return to an upright position; this reflex is observed in all invertebrates and vertebrates. The sternal expansion can be recorded for over 20 h, or for as long as the *Varroa* mite remains alive, and the expansion stops as soon as the mite is placed in a normal, upright position.

Topical application of 1.81 μ g/mite amitraz completely inhibited the gravitational reflex within 60-70 minutes. The volatile acaricides formic acid thymol crystals and Apiguard[®] eliminated the reflex within 10-20min. Crystalline thymol had a faster inhibition than Apiguard[®], as was expected, since Apiguard[®] is a thymol-based slow release matrix gel. However, overall Apiguard[®] induced higher mite mortality than thymol crystals. In addition to the mortality rate and time, sub-lethal effects were assessed.

This bioassay could be a useful tool for accurate assessment of the acaricidal action of numerous compounds under laboratory conditions, saving money and time before field trials.

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