Journal of Apicultural Research 40(1): 35–37 (2001)

Notes and comments

A TECHNIQUE FOR MARKING INDIVIDUAL VARROA MITES

JEFFREY W HARRIS*

Honey Bee Breeding, Genetics and Physiology Laboratory, USDA-ARS, Baton Rouge, LA 70820, USA

(Received 4 July 2000,
accepted subject to revision 9 October 2000,
accepted for publication 8 November 2000)

Keywords: *Varroa destructor*, varroa, mites, marking mites, glitter tags, cyanoacrylate glue

One hindrance to a fuller understanding of the biology of *Varroa destructor* (Anderson & Trueman, 2000) is the difficulty in observing individual mites through multiple reproductive cycles under natural conditions. Most attempts to study the reproduction of individual mites has involved the transfer of mites from either brood cells or from adult bees to host larvae in newly capped brood cells (Ifantidis, 1983; Schultz, 1984; Ruijter, 1987; Steiner *et al.*, 1994). These artificially induced 'cell invasions' may disrupt the normal reproductive biology of the mites. Moreover, it is often desirable to monitor a group of mites through time.

This report provides a method of marking mites. There are but few other studies where marked mites were used. One of these studies (Schultz, 1984) used cyanoacrylate with fluorescent pigment. The marking technique described here involves the attachment of a tiny flake of polyester glitter (Poly Flake; Glitterex Corporation; Cranford, NJ, USA) to the dorsal idiosoma of a mite using a small droplet of no-run super glue (Quick Gel; Loctite Corporation; Rocky Hill, CT, USA). This technique is a modification of that used by Schultz (1984) because the no-run super glue is a cyanoacrylate ester. The marking procedure requires two steps: (1), application of a tiny drop of Quick Gel to the mite; and (2), pressing a single flake of glitter onto the adhesive.

All procedures were performed beneath a dissecting stereo-microscope with magnification × 20–40. Mites were placed on a textured surface (such as thick paper or a cloth) so that their feet could grip and anchor the mite. A tiny drop of Quick Gel was placed on the mite using a spatula formed by mounting a minute pin to an applicator stick. The glue was pressed firmly onto the idiosoma so as to penetrate the setae that cover a mite's dorsum. Next, a thick hair from a pig was used to pick up a flake of glitter and place it on the drop of glue. The end of the hair was dampened in a drop of water before touching it to the flake of glitter. This wet surface stuck to the glitter and held it in place while it was positioned over the glue. It was best to wait 15–20 s after placing the glue on the mite before attaching the flake of glitter so that the glue had thickened. The

*jharris@ars.usda.gov

FIG. 1. Close-up view of a varroa mite wearing a glitter tag that was attached using no-run super glue as adhesive (magnification × 25).

glitter was firmly pressed against each mite and in a few seconds the tag was permanently attached (fig. 1). Mites usually responded to the pressure by 'crouching' or moving forward.

Although the use of glitter tags was a two-step process, the method has several advantages over other methods tested, such as the use of enamel paint or melted beeswax. For these trials drops of enamel paints were applied to mites using a human eyelash as a paintbrush. Drops of melted beeswax were applied using a heated wire (heated by mounting it to an electric soldering iron).

The advantages of glitter tags were three-fold. First, Quick Gel was not toxic to mites. No mites died within 24 h (0 out of 55 mites) or 36 h (0 out of 73 mites) of being tagged with glitter (2 separate tests). Mites were held within Petri dishes with bee pupae for food during these tests (incubator environment: RH = 60% and temperature = 34.5°C). In contrast, 15% of mites (13 out of 84 mites) died within 24 h and 20% (16 out of 78 mites) within 36 h (2 separate trials) of being painted with Testors enamel paints (Testor Corporation; Rockford, IL, USA). Mortality from this paint also suggests the possibility of sub-lethal effects on the reproduction of varroa mites. Mortality to the method of attaching beeswax was not determined.

Secondly, the properly secured tag was not removed from the mites whereas enamel paints often peeled away from mites. In several experiments, paint marks were found on the metathoracic legs of some worker bees after they had been caged with painted mites for 3–5 days within an incubator. In contrast, worker bees did not remove any glitter tags in several experiments (n = 208 mites) where mites were caged with 100–150 worker bees within the incubator (3 separate tests). Melted beeswax also stuck to individual mites better than paint, but it was difficult to control the size of the wax droplet, and wax often cooled before being attached to a mite. Heavy drops of wax were uncomfortable to mites, as was demonstrated by their agitated locomotor activity.

Third, the glitter-marked mites were easily seen with the unaided eye on adult bees because the glitter shines when illuminated by sunshine or artificial bright light. Marks of beeswax were difficult to see on mites without a microscope, and some colours of paints were also difficult to see without a microscope. White and yellow paints could usually be seen with the unaided eye.

For these reasons, the use of glitter is a preferred method for marking mites. Experiments were also performed to test whether the reproduction of mites was affected by the use of glitter tags. In one test, 73 mites were marked with glitter and held in a Petri dish with bee pupae for 36 h before they were placed into a mite-free colony of bees. The colony contained about 3000 bees and a queen on five standard deep combs.

Three Apistan (fluvalinate) strips had been placed in the colony for 26 days to remove varroa. At this time the strips were removed, and the queen was caged for nine days. There was only capped brood in the colony at the end of this period. A single patch of 400–500 eggs (aged about 18 h) on a brood comb was placed in the colony, and the 73 tagged mites were added to the colony. The queen remained caged until the end of the test. The frame that had contained the eggs was removed after another 15 days. At this time, the worker bee pupae had tan coloured bodies. Only 40 of the original 73 mites were recovered, and 37 of those mites had laid eggs. They averaged 3.450 ± 0.75 progeny, which is not different from the 3–4 progeny expected from unmarked mites found on host pupae at the same age (Ifantidis, 1983). The families produced by marked mites appeared normal (most had adult males, protonymphs, deutonymphs, and adult daughters). No attempt was made to locate tagged mites on the adult bees at the end of the test.

Although the recovery of mites was not 100%, this experiment demonstrates that mites can be tagged, released into a colony of bees and recovered in capped brood cells after a two-week period. These methods could be used in many different tests where individual mites or a cohort of mites are being investigated. If more than one group of mites needs to be followed in an experiment, at least seven different colours of glitter are available.

REFERENCES

ANDERSON, D; TRUEMAN, J W H (2000) *Varroa jacobsoni* (Acari: Varroidae) is more than one species. *Experimental and Applied Acarology* 24: 165–189.

RUIJTER, A DE (1987) Reproduction of *Varroa jacobsoni* during successive brood cycles of the honeybee. *Apidologie* 18: 321–326.

IFANTIDIS, M D (1983) Ontogenesis of the mite *Varroa jacobsoni* in worker and drone honeybee brood cells. *Journal of Apicultural Research* 22: 200–206.

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; English summary). *Apidologie* 15: 401–420.

STEINER, J A; DITTMANN, F; ROSENKRANZ, P; ENGELS, W (1994) The first gonocycle of the parasitic mite (*Varroa jacobsoni*) in relation to preimaginal development of its host, the honey bee (*Apis mellifera carnica*). *Invertebrate Reproduction and Development* 25: 175–183.

Journal of Apicultural Research 40(1): 37–40 (2001)

COMPARISON OF TWO COMMERCIAL KITS FOR BIOCHEMICAL CHARACTERIZATION OF *PAENIBACILLUS LARVAE LARVAE* IN THE DIAGNOSIS OF AFB

WIM DOBBELAERE*[1,2]; DIRK C DE GRAAF[1]; JOHAN E PEETERS[1]; FRANS J JACOBS[2]

[1]Veterinary and Agrochemical Research Centre, Groeselenberg 99, B-1180 Brussels, Belgium

[2]University of Ghent, Laboratory of Zoöphysiology, Krijgslaan 281 S33, B-9000 Ghent, Belgium

Keywords: American foulbrood, *Paenibacillus larvae larvae*, diagnosis, biochemical characterization, BBL CRYSTAL, API 50CHB, carbohydrate metabolism, identification

The use of commercial kits for the biochemical characterization of bacteria is a general practice for quick and reliable identification of a wide range of bacterial species. In the case of *Paenibacillus larvae* subsp. *larvae*, Carpana *et al.* (1995) described the use of the API 50CHB kit (BioMérieux, Brussels, Belgium) for the diagnosis of American foulbrood (AFB). Here we describe the use of another kit, i.e. BBL CRYSTAL (Becton Dickinson, Aalst, Belgium) and compare the results with those obtained by using the API 50CHB kit. We have chosen to test the BBL CRYSTAL kit for its suitability to identify *P. l. larvae* because in the Veterinary and Agrochemical Research centre (Brussels, Belgium) this kit is the standard test kit for biochemical characterization of bacteria. It was found to be more user-friendly then the API kits and it provides results in 24 h, whereas the API kit requires 48 h.

The BBL CRYSTAL kit was tested on 11 *P. l. larvae* field strains (outbreaks from 1997 and 1998) in addition to three *P. l. larvae* reference strains from the LMG-collection (Laboratory of Microbiology Ghent, University of Ghent) (i.e. LMG 14425, LMG 14426 and LMG 15969). The API 50CHB kit was tested on the same three *P. l. larvae* reference strains and six field strains from the outbreaks of 1997. Two *P. alvei* reference strains (i.e. LMG 13253 and LMG 13258) and two *Bacillus licheniformis* field strains were used in both kits as negative control. In addition to this, reference strains of *B. cereus* (LMG 6923), *B. megaterium* (LMG 7127), *B. subtilis* (LMG 7135), *B. thuringiensis* (LMG 7138), *P. validus* (LMG 9817), *P. lautus* (LMG 11157), *P. polymyxa* (LMG 13296), *P. l. pulvifaciens* (LMG 15974) and *P. apiarius* (LMG 17433) were tested with the BBL kit in order to evaluate the discriminatory power of the kit between species related to *P. l. larvae*. The BBL

*Corresponding author: widob@var.fgov.be