Short Communication

The mite *Varroa jacobsoni* does not transmit American foulbrood from infected to healthy colonies

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ABSTRACT

The present study was conducted to determine whether *Varroa jacobsoni* can transmit American foulbrood (AFB), caused by the bacterium *Paenibacillus larvae* to healthy colonies by the surface transport of spores. Five two-storey Langstroth colonies of *Apis mellifera ligustica* were infested by placing a sealed brood comb, with 10% *Varroa* prevalence, between the central brood combs of each colony. Two months later the colonies were inoculated with *P. larvae* by adding brood comb pieces with clinical signs of AFB (45 ± 5 scales per colony). After 60 days the brood area was completely uncapped by means of dissecting needles and tweezers, separating the *Varroa* mites from the larvae and the collected mites were introduced at a rate of 51 per colony into four recipient hives placed in an isolated apiary. Twenty female *Varroa* specimens were separated at random and observed by SEM. *Paenibacillus larvae* spores were found on the dorsal shield surface and on idiosomal setae. All colonies died after 4–5 months due to a high incidence of varroosis. No clinical AFB symptoms or *P. larvae* spores were observed in microscopic preparations. It is concluded that *Varroa jacobsoni* does not transmit AFB from infected to healthy colonies; it does, however transport *P. larvae* spores on its surface.

Key words: American foulbrood, *Paenibacillus larvae*, *Varroa jacobsoni*, spores, varroosis, honey bees, *Apis mellifera*, SEM.

The mite *Varroa jacobsoni* Oud. (Acari: Varroidae) is an agent which transmits several pathogenic microorganisms to *Apis mellifera* L. It has been demonstrated that *Varroa* can transmit both sacbrood virus (SBV) (Bailey, 1991) and acute paralysis virus (APV) (Ball, 1985, 1988; Ball and Allen, 1988) by inoculating virus particles into the haemolymph of honey

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bees. By means of SEM studies, Puerta *et al.* (1990) found spores of *Ascosphaera apis* (Maasen ex Clausen) Olive and Spiltoir and *Aspergillus flavus* (Link ex Fries), on the cuticle of adult female *Varroa*. The mite is also one of the vectors of *Hafnia alvei* Møller, which causes septicaemia (Strick and Madel, 1988) and of several other bacteria (Glinski and Jarosz, 1990a, 1992).

On the other hand, when varroosis and American foulbrood (AFB) develop simultaneously, colony death is accelerated (Grobov, 1977). SEM and cultural studies have shown that *V. jacobsoni* carries viable spores of *Paenibacillus larvae* (White) Ash, Priest and Collins (Ash *et al.*, 1993) which causes AFB (Alippi, 1992). However, there is no information concerning the transmission of this disease from colonies with AFB to healthy colonies. The purpose of the present work was to determine whether *V. jacobsoni* can transmit AFB to healthy colonies by the surface transport of *P. larvae* spores.

Five two-storey standard Langstroth colonies of *Apis mellifera ligustica* L. were used. The selected hives had four brood combs each of which were treated with Fluvalinate (Apistan®, manufactured by Sandoz-Zöecon) 40 days before the experiment was started in order to ensure the absence of *Varroa*. At the beginning of the austral summer (23 December 1993), a sealed brood comb with 10% *Varroa* prevalence (approximately 150 mites) was placed in the centre of the four brood combs of each colony. Two months later, the five colonies were inoculated with *P. larvae* by adding brood combs with clinical signs of AFB. Four comb pieces of 126–144 cells each on both sides containing a total of 45 ± 5 AFB scales were used (each colony received 40–50 scales). These were placed at the four corners of each central brood comb (A. M. Alippi, G. N. Albo, D. Leniz, I. Rivera, M. Zanelli and A. E. Roca, unpublished manuscript). The colonies were examined weekly to determine disease development. Clinical symptoms of AFB were observed after 35 days in the central comb and in both contiguous sides of the near combs.

In mid-autumn (13 April 1994) the brood area was completely uncapped by means of dissecting needles and tweezers, separating *Varroa* mites from the larvae. In the cells with larvae which had advanced AFB symptoms, either there were no *Varroa* present, or those observed were dead.

The mites collected were introduced, at a rate of 51 *Varroa* per colony, into four recipient colonies placed in an isolated apiary in La Plata area. These colonies had similar quantities of brood, adult bee population and food (pollen and honey). To ensure the absence of mites, the receptive colonies had been treated with fluvalinate 40 days before the beginning of

the experiment. Honey samples taken from the brood chambers were analysed for *P. larvae* (Alippi, 1991; Hornitzky and Clark, 1991; A. M. Alippi, submitted). None had *P. larvae* spores, and there were no clinical symptoms of AFB disease.

Before introducing the mites to the receptive colonies, 20 female *Varroa* (five from each colony) were separated at random and examined to determine, by SEM observations, if they had *P. larvae* spores on their surfaces. The mites were fixed in 2% glutaraldehyde in Sorensen buffer (pH 7.5) for 1 h at 4°C, then washed in the same buffer (three times, 15 min each) and dehydrated in a series of ethanol solutions of increasing concentration: 20, 30, 50 and 70% (10 min each), 96% (30 min) and the last two in absolute ethanol (30 min each). Mites were mounted on stubs with diphase tape, vacuum metallized with a 200 Å width gold layer and observed by a Jeol scanning microscope JSM-T 100, scanning the whole surface of each mite. *Paenibacillus larvae* spores were found on the dorsal shield surface (Figs 1 and 2) and on idiosomal setae (Fig. 3). Eighty per cent of the examined mites carried *P. larvae* spores, but only one mite

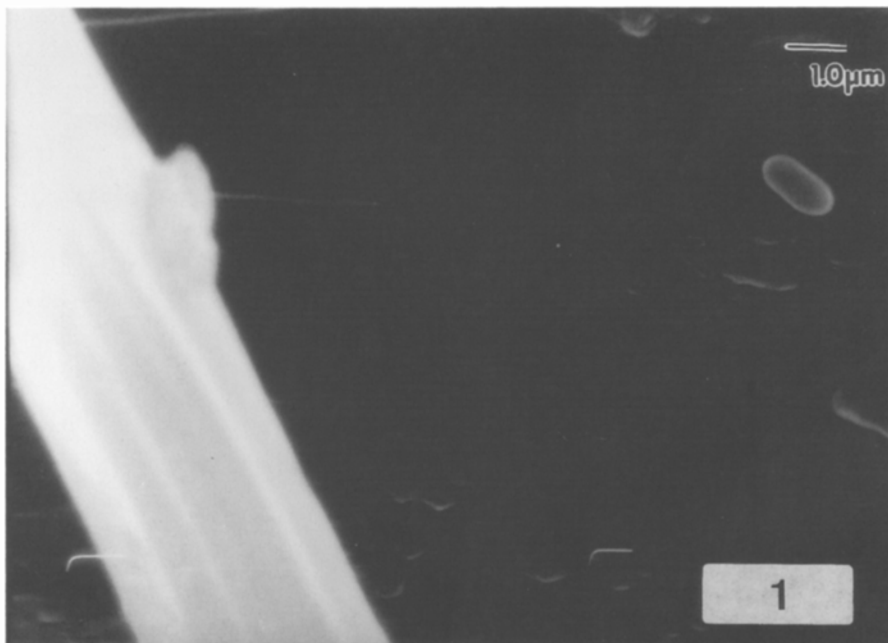

Fig. 1. Scanning electron micrograph of *P. larvae* spores on the dorsal shield surface of a female *V. jacobsoni*.

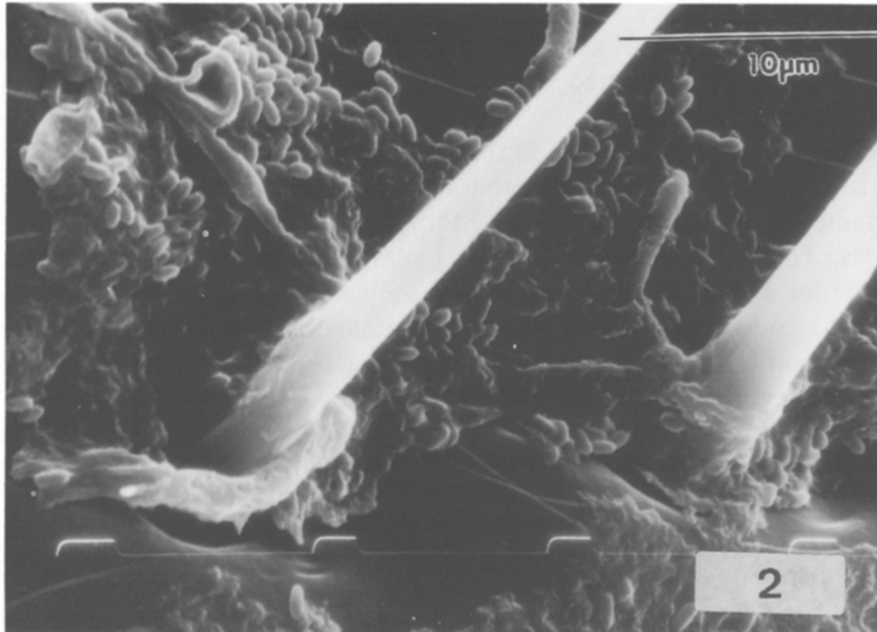

Fig. 2. Scanning electron micrograph of *P. larvae* spores in the dorsal shield surface of a female *V. jacobsoni.*

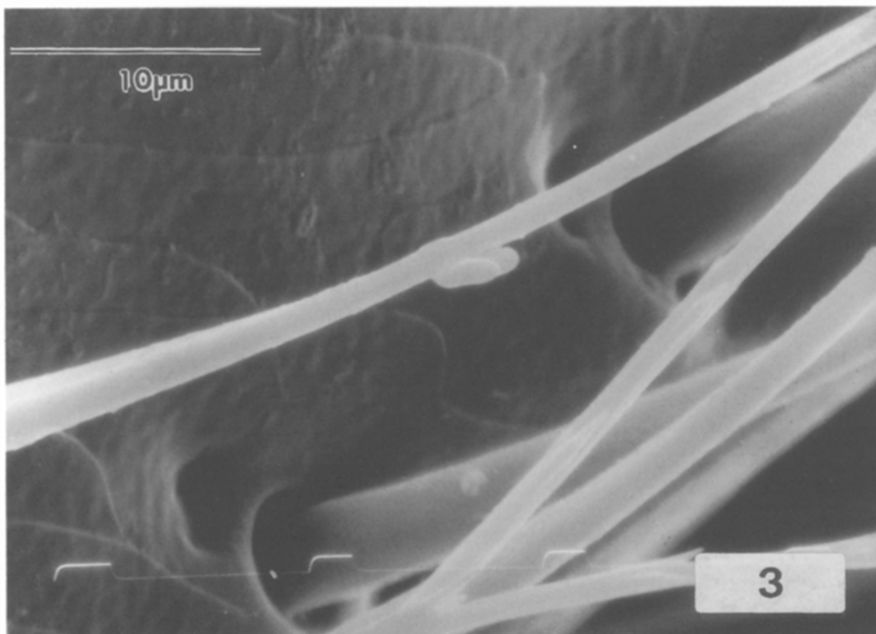

Fig. 3. *Paenibacillus larvae* spores on an idiosome seta of *V. jacobsoni.*

showed a considerable number of spores (Fig. 2) while, the mean density of spores on the body surfaces of the rest were 3 ± 1 per mite.

The colonies were evaluated every 15 days to detect AFB clinical symptoms. Three colonies died 4 months after the mites had been introduced due to a high incidence of varroosis, while the remaining colony died after the fifth month. These colonies died because they were not able to bear the high parasitic mite levels. This phenomenon is more common in winter when the brood area is smaller and, consequently, most cells are parasitized. As a result of this, some bees had malformed wings and others were wingless. These symptoms, which are caused by *V. jacobsoni* have been previously observed in local 'Creole bees' (*A. m. mellifera* × *A. m. ligustica*) (Marcangeli *et al.*, 1992).

Two to three cells with suspected symptoms of foulbrood were found in a brood area of approximately 209 average cells per comb side; they were examined by use of the modified hanging drop (Shimanuki and Knox, 1991) and nigrosin colouration methods (Gochnauer *et al.*, 1979), applied to the material collected from inside the cells. Vegetative forms resembling *Melissococcus pluton* (White) Bailey, the causal agent of European foulbrood (EFB), were observed in nigrosin preparations, as well as spores of *Paenibacillus alvei* (Cheshire & Cheyne) Ash *et al.* and *Bacillus laterosporus* Laubach, microorganisms commonly associated with this disease. Although no spores similar to those of *P. larvae* were found, isolations in J medium (Gordon *et al.*, 1973) and in J medium supplemented with 6 $\mu g\ ml^{-1}$ nalidixic acid were made following previously described techniques (Hornitzky and Karlovskis, 1989; Alippi, 1991; Hornitzky and Clark, 1991). Only *P. alvei* and *B. laterosporus* colonies were obtained on plates with J medium.

Larvae with chalkbrood symptoms were also found in the colonies, with approximately five larvae per colony. The presence of EFB and chalkbrood might be due to weakening of the colonies caused by the high incidence of *V. jacobsoni*. This conclusion is based on the observation that at the beginning, no colony presented clinical symptoms of these diseases, which are endemic in the area where the experiment was carried out. Both these diseases are known to be related to the weakening of colonies.

It is thus concluded that *V. jacobsoni* does not transmit AFB from infected to healthy colonies; however, it does transport *P. larvae* spores on their surfaces as observed by SEM (see also Alippi, 1992).

The number of *P. larvae* spores that the mites carry on their surfaces and that can be introduced into receptive colonies is probably not sufficient to trigger AFB. Normally, the mode of transmission of *P. larvae* spores which develop in AFB infections is by ingestion of larval brood

food. However, because *Varroa* is a blood-sucking ectoparasite of larvae and adults, the probability that the mite can contaminate the larval food by contact is very low.

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