A note on the use of dead *Varroa* mite in the study of the removal behaviour of the honey bee.

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Abstract: *Varroa destructor* is a parasite of the honey bee *Apis mellifera*. *Varroa* infests both the adult bee and its brood, although Varroa can only reproduce in the latter. The removal behaviour of worker bees towards worker brood cells infested by *Varroa* might be considered as an important trait in order to select *Varroa* tolerant bees. Thus, we need to study this removal behaviour so as to develop some techniques which could measure it. In this article we will prove that bees can detect and remove brood cells which have been artificially infested with dead mites, and that they do so in the same extent as when these cells have been infested with alive *Varroa* (22.42% and 20.55%, respectively). Considering these results we think that to artificially infest brood cells with 3 dead *Varroa* mites and to register how many of them are removed in a 24 hour-period, could be an interesting technique to value the removal behaviour of honeybees.

Keywords: *Apis mellifera*, *Varroa destructor*, honeybee, tolerance, hygienic behaviour, removal behaviour

Resumen. *Varroa destructor* es un parásito de la abeja de la miel *Apis mellifera*. Varroa parasita tanto a las abejas adultas como a la cría, aunque sólo se reproduce en esta última. La limpieza, por parte de las abejas adultas, de las celdillas que contienen cría parasitada es considerado un carácter interesante para la selección de abejas tolerantes al parásito. Por ello, es necesario estudiar el comportamiento higiénico de las abejas frente a la cría parasitada y desarrollar técnicas para medirlo. En este artículo demostramos que las abejas pueden localizar y limpiar celdillas de cría que han sido artificialmente infestadas con parásitos muertos y que lo hacen en igual medida que si fueran parásitos vivos (22.42% y 20.55% respectivamente). Considerando estos resultados, pensamos que infestar celdillas con varroas muertas y registrar la respuesta de las abejas frente a ellas en 24 horas puede ser una técnica útil para estudiar el comportamiento higiénico de las abejas.

Palabras clave: *Apis mellifera*, *Varroa destructor*, abejas, tolerancia, comportamiento higiénico

1. Introduction

Varroa destructor (Anderson and Trueman, 2000) is a recent mite of the honeybee *Apis mellifera*. *Varroa* infests both adult bees and its brood but it can only reproduce in the latter. This parasitosis is considered as the most important problem in Western beekeeping. Chemical treatments have prevented great losses in apiaries, but they have generated some problems like residues in the bee products (reviewed by Wallner, 1999) and some mite resistance problems to chemical treatments (reviewed by Milani, 1999).

Varroa comes from the Asian bee *Apis cerana* Fabr. As this bee is tolerant to the mite it is unnecessary to treat the colonies. This tolerance is partly due to the bees' removal behaviour (reviewed by Rath, 1999). *A. mellifera* also show this behaviour, although with a lower intensity. Nevertheless this behaviour is being thoroughly studied so as to use it in the selection of mite-tolerant bees (reviewed by Boecking and Spivak, 1999).

The techniques mostly used to evaluate the removal behaviour of bees against *Varroa* are usually based on the artificial infestation of the cells with living mites, thus evaluating the response of the bees during a long period (seven-ten days) (reviewed by Boecking and Spivak, 1999). This raises two essential problems: Varroa is usually host to some other diseases of virical, bacteriological or fungical ethiology which varroa will transmit to the bee brood (Ball, 1997); diseases which

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can be responsible both for the removal of part of such (Vandame *et al.*, 1998), and for the lack of control on the mite reproduction, which could generate some offspring and thus increase the response of the bees (Boot *et al.*, 1995; Flores *et al.*, 2001). Both circumstances can alter the results. In some preliminary research we pointed out the possibility of avoiding these problems by artificially infesting the worker cells with two or three dead mites and evaluating the response of the bees during shorter periods of time (Flores *et al.*, 2001). The aim of this new article is to corroborate the possibility of using dead *Varroa* in order to study the removal behaviour of the bees.

2. Materials and methods

The tests were carried out along 1999, in Córdoba (Spain), between March 21st and December 23rd. We used nine colonies of *A. mellifera* placed in Langstroth hives. During this whole period adult bees filled between seven and ten combs in the hives.

Worker brood cells seven days after the sealing were used. So as to know the age of the brood and to be able to locate it after on, we marked on a transparent plastic sheet those which were sealed in 24 hour-periods. The varroa were obtained using powdered sugar from bees from highly infested colonies. The mites thus obtained were introduced in the cells through incisions made in the caps, which were later on carefully closed (De Ruitjer, 1987; Boecking and Ritter, 1993; Flores *et al.*, 2001).

Four treatments were carried out:

1.- Cells artificially infested with three living *Varroa*.

2.- Cells artificially infested with three dead *Varroa*. The mites were captured and frozen (-18ºC) 24 hours before the tests were carried out, and they were defrosted two hours before they were introduced in the cells.

3.- Control A: cells which were opened and closed without introducing any mites.

4.- Control B: non-manipulated cells.

In each treatment, we used ten worker brood cells. The number of repetitions carried out on each hive is shown in Table 1. The removal behaviour of the bees was evaluated 24 hours later, registering the number of removed cells.

The inspection of the caps (stereoscopy microscope x20) of the non-removed cells let us know wether they had been opened, examined and resealed by the bees during the experiments (Boecking and Spivak, 1999).

Data obtained were evaluated statistically by descriptive parameters; analysis of variance (ANOVA) ($p<0.05$) and "Tukey Honest Significant Difference (HSD) Test" ($p<0.05$) (Statistic 5.0, 1995).

3. Results and discussion

No significant differences were registered between controls A and B. But we did register significant differences between these controls and the infested cells either with three living *Varroa* or three dead *Varroa*. No significant differences were registered against infested cells both with three living mites or with three dead mites (Table 1). The inspection of the caps of the non-removed control cells (controls A and B) let us check that they had not been opened and resealed in almost any case by the bees during the experiments.

Rath and Drescher (1990) proved that *A. cerana* behaved identically towards infested worker brood cells either with dead or with living *Varroa*. In *A. mellifera* very few studies have been carried out using dead *Varroa* in order to test the removal behaviour, and the results obtained are contradictory. Boecking and Drescher (1994) found no response while Aumeier and Rosenkranz (2001) and us (Flores *et al.*, 2001) did find out that bees could locate and remove the contents of these cells. The results we are now publishing confirm those we had previously obtained, supporting that *A. mellifera* showed a response against infested cells with three dead mites, and that this response did not differ significantly from the one obtained against cells infested with three living *Varroa* (Tabla 1).

Our results also prove that the removal response of the bees had not been elicited by the manipulation of the cells, as it is proved by the fact that only a 0.43% of the manipulated but not infested cells (control A) were removed, while a 20.55% and a 22.42% of the cells infested either with living or dead mites respectively were removed.

Table 1. Removal behaviour of *A. mellifera* towards worker brood cells with 4 experimental treatments: cells artificially infested with three living Varroa and with three dead *Varroa*, un-infested opened and closed cells (control A), and non-manipulated cells (control B). Data are expressed as an average amount of the %±s.e. (n=23).

		Treatment 1	Treatment 2	Control A	Control B
	N° of Test	Cells infested with 3 living mites.	Cells infested with 3 dead mites.	Cells opened and closed Without mites	non-manipulated cells
Colony 1	6	35.19±13.29	32.04±11.31	0.00±-----	0.00±-----
Colony 2	3	6.67±6.67	6.67±6.67	0.00±-----	3.33±3.33
Colony 3	3	6.06±6.06	10.74±6.43	0.00±-----	3.33±3.33
Colony 4	3	11.11±11.11	3.70±3.70	0.00±-----	0.00±-----
Colony 5	2	45.00±5.00	55.00±5.00	0.00±-----	10.00±0.00
Colony 6	2	20.00±20.00	10.00±10.00	5.00±5.00	0.00±-----
Colony 7	2	0.00±0.00	30.00±10.00	0.00±-----	0.00±-----
Colony 8	1	20.00±------	60.00±-----	0.00±-----	0.00±-----
Colony 9	1	40.00±-----	20.00±-----	0.00±-----	0.00±-----
Mean		20.55±4.98	22.42±4.65	0.43±0.43	2.17±0.88

Boecking and Spivak (1999), Aumeier and Rosenkranz (2001) and Flores *et al.* (2001) proved that in some occasions bees visited infested cells, opened them, removed the mites and resealed them. These altered cells' caps were easily distinguishable, a distinct change in the silk/waxstructure of the inner cell cap can be observed (reviewed by Boecking and Spivak, 1999). This fact let us check that practically all the control cells which had been manipulated (control A), were not opened and examined by the bees. Thus, we have to eliminate the possibility of worker bees opening systematically any manipulated cells and removing just those in which they found any mites.

The obtained results are also useful in order to clarify some other doubts related to the removal behaviour of the bees. On the one hand, Boecking and Spivak (1999) pointed out the controversy about whether the mites are removed by the bees or they leave the cells on themselves when the worker bees open the cell. In our study, as we introduced some dead mites, we proved the active role bees play in the removal of the mites. On the other hand, the fact that bees remove to a same extent the cells which had been infested both with dead or living *Varroa*, supports the results obtained by Aumeier and Rosenkranz (2001), which stated that the removal behaviour of the bees does not depend on the movement of the mites.

We must also take into consideration that the use of dead mites versus living mites, reduces perceptibly the time needed to introduce them in the cells. This fact is important if we aim to develop some techniques so as to evaluate the removal behaviour in a great number of colonies.

Lastly, the results we have explained here are to be considered as a step further in the study of the removal behaviour of bees towards *Varroa*. It is important to continue this research, specially the research concerning the removal behaviour and the final number of the mites in the hives.

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