

ECOLOGY, BEHAVIOR AND BIONOMICS

Uncapping Activity of *Apis mellifera* L. (Hymenoptera: Apidae) towards Worker Brood Cells Infested with the Mite *Varroa destructor* Anderson & Treuman (Mesostigmata: Varroidae)

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Atividade de Desoperculação de *Apis mellifera* L. (Hymenoptera: Apidae) em Células de Crias de Operárias Infestadas com o Ácaro *Varroa destructor* Anderson & Treuman (Meostigmata: Varroidae)

RESUMO - A varroosis, doença causada pelo ácaro *Varroa destructor* Anderson & Treuman, é responsável pela mortalidade de milhares de colônias de *Apis mellifera* L. em várias partes do mundo. Contudo, o dano causado por esse ácaro varia com a raça de abelhas e condições climáticas. A varroa causa poucos danos nas colônias de abelhas africanizadas no Brasil, os níveis de infestação são relativamente baixos e estáveis. Foi avaliado o comportamento higiênico (desoperculação e remoção de crias mortas ou parasitadas) em colônias de abelhas africanizadas altamente higiênicas usando favos infestados naturalmente com o ácaro *V. destructor*. A taxa diária de células desoperculadas, medida em oito colônias durante seis dias, foi 3,5 vezes maior em favos infestados com varroa comparados quando com favos não infestados. Os resultados mostram que as abelhas africanizadas reconhecem e removem crias parasitadas naturalmente com o ácaro *V. destructor* o qual é um mecanismo de tolerância da praga varroosis.

PALAVRAS-CHAVE: Comportamento higiênico, varroosis, infestação natural, abelha africanizada

ABSTRACT - Varroosis, a disease caused by the mite *Varroa destructor* Anderson and Treuman has killed hundreds of thousands of *Apis mellifera* L. colonies in various parts of the world. Nevertheless, the damage caused by this mite varies with the type of bee and climate conditions. Varroa causes little damage to Africanized bee colonies in Brazil, as the infestation rates are relatively stable and low. We evaluated the hygienic behavior (uncapping and removal of brood) of highly hygienic Africanized bees using combs with worker brood cells infested (naturally) and no infested with *V. destructor*. The daily uncapping rate, measured in eight colonies during six days, was 3.5 fold higher in the combs infested with varroa compared to no infested combs. The results show that the Africanized bees are able to recognise and remove brood cells naturally infested with *V. destructor* what is an important mechanism for tolerance against varroa.

KEY WORDS: Hygienic behavior, varroosis, natural infestation, Africanized honey bee

The mite *Varroa destructor* (Anderson & Trueman), a parasite first observed in the Asian bee, *Apis cerana* Fabr. (Hymenoptera: Apidae), became known to beekeepers only after it infesting the Western bee, *Apis mellifera* L. Few years after becoming established on its new host, it caused an enormous impact on the world apiculture due to its rapid dispersion and the drastic effects of varroosis (De Jong 1984).

In Europe and in some parts of South America, *A. mellifera* colonies are killed by the mites. In Brazil and Paraguay, the infection levels of varroosis are low and cause

no serious damage to bee colonies (De Jong *et al.* 1984).

Various factors affect the population dynamics of *V. destructor*. The mite causes more problems in temperate climates than in tropical regions (Moretto *et al.* 1991, De Jong & Soares 1997). Nevertheless, it is also known that the type of bee also affects the development of this parasite. African bees and their hybrids are more tolerant to mite infestation than are the European races (De Jong 1984, Moretto *et al.* 1991, Medina & Martin 1999).

The tolerance degree to the mite among *A. mellifera* bee races seems related to the reproductive success of female

varroa on worker brood. The number of descendants per female adult mite that parasitizes worker brood is greater in European bees than in African bees and their hybrids (Camazine 1986, Moretto *et al.* 1991, Medina & Martin 1999). Harbo & Harris (1999) found that the reproductive capacity of female varroa varies among European honey bee colonies.

It is known that the hygienic behavior (uncap and remove dead brood from combs) in *A. mellifera* is an important control mechanism against various diseases (Rothenbuhler 1964, Gillian *et al.* 1988). Spivak & Reuter (1998, 2001) found that lines of European honey bees selected for hygienic behavior were also less affected by the mite *V. destructor*. We examined whether highly hygienic Africanized honey bees preferentially uncapped and removed varroa-infested brood.

Material and Methods

Africanized honey bee colonies classified as highly hygienic were used to determine whether combs infested with the mite *V. destructor* were preferentially uncapped. A pin-killed brood method developed by Gramacho (1995) was used to select for highly hygienic colonies. This classification was given to colonies that uncapped and removed at least 85% of the dead brood within 24h.

Uninfested colonies were obtained by confining the queen, removing all of the brood and treating the colonies with two strips of Bayvarol®, which is a commercially available bee mite preparation used in part of the world where the varroa mite is considered a dangerous pest. This acaricide was imported from Germany only for this purpose. After 15 days, when the colonies were examined by using methodology developed by Stort *et al.* (1981), no varroa mites were present in all colonies tested. Then the queens were released and egg laying started.

Brood combs free of mites were obtained from these colonies. Brood combs infested with varroa were obtained from untreated colonies. As the brood infestation rates in Brazil are normally low, the colonies were manipulated to increase infestation. The queens were confined during 15 days in order to concentrate most of the mites on the adult bees. After the queen is liberated, the new brood becomes highly infested. These pieces of combs were evaluated for varroa infestation before they were introduced into the hygienic colonies. Those that had no varroa in 100 manually uncapped brood cells were considered free of the mite.

In order to register the number of the capped and uncapped cells in each piece of combs to be introduced into hygienic colonies, all broods were removed from uncapped cells. The infested and uninfested combs (both from colonies other than the receiving colony) were introduced into each hygienic colony at the same time. Subsequently, the combs were examined and the number of uncapped cells counted every 24h during six days.

The daily uncapping rate was calculated for infested and uninfested combs by dividing the number of cells uncapped during 24h by the number of capped cells present during the same period. The Student-*t* test was used to compare the

mean daily percent of cells that were uncapped in each of the two types of comb.

Results and Discussion

The percentage of uncapped brood cells in the infested combs was higher than in combs free of the mite *V. destructor* ($t = 7.42$; $P < 0.0001$). In the infested combs, $1.7 \pm 1.44\%$ (mean and standard deviation) of the sealed cells were uncapped per day. In the uninfested combs, only $0.5 \pm 0.12\%$ of the cells were uncapped per day (Table 1).

Guerra *et al.* (2000) found that Africanized honey bees increased their uncapping activity when worker brood was artificially infested with varroa mites. We found that the uncapping rate was nearly four times higher in combs naturally infested with varroa, when compared to uninfested ones. This higher frequency observed in uncapping of brood combs naturally or artificially infested with varroa leads us to conclude that Africanized honey bees must have mechanisms that allow them to detect the mite within sealed brood cells.

European races of *A. mellifera* are generally less hygienic and less tolerant to varroa than are Africanized honey bees (Guerra *et al.* 2000). In European bees, however, hygienic behavior affects the development of *V. destructor*; the more hygienic colonies have lower varroa infestation levels (Spivak & Reuter 2001).

At least part of the progeny of the mite *V. destructor* produced in *A. mellifera* brood can reach the adult stage before the workers and drones emerge. All the parasites die if they have not attained the adult stage when the bees emerge, or when they are removed from the cells. Although the brood removal rate infested with *V. destructor* is greatly influenced by climate conditions, such as pollen flow (Janmaat & Winston 2000), the real effect of hygienic behavior on varroa infestation in *A. mellifera* colonies is unknown. However, a higher rate of brood uncapping in

Table 1. Mean daily percentages of uncapping of worker brood cells by Africanized bees in combs infested or not with the mite *V. destructor*.

Colony	with varroa	without varroa
1	3.3	0.9
2	2.2	0.7
3	0.7	0.5
4	1.5	0.2
5	1.9	0.2
6	1.0	0.1
7	2.0	0.9
8	1.0	0.1
Mean	1.7	0.47
Standard deviation	1.44	0.12
Coefficient of variation	0.84	0.26

infested combs should affect the population dynamics of the mite due to the deleterious effects on the varroa progeny.

In this work we used only highly hygienic colonies. However, the variability of the uncapping tendency (based on the coefficient of variation) was over three times higher in the infested combs, when compared to that found for the uninfested combs (Table 1). When we checked the combs of infested colonies for mites, we were not able to precisely determine the infestation rates. Consequently, we could not determine if the variation in uncapping activity was related to the number of infested brood cells and/or to the number of mites in each brood cell. Nevertheless, the greater variability of uncapping rates for the infested combs suggests variations in infestation rates in these combs. Other experiments will be necessary to test this hypothesis.

Although this work did not quantify the effect of hygienic behavior on the population dynamics of the mite *V. destructor* in Africanized bee colonies, the results showed that this character, together with the low reproductive capacity of this parasite on worker brood (Corrêa-Marques *et al.* 2003, Martin & Medina 2004) and grooming behavior (Moretto 2002), influences the tolerance of Africanized bees against varroosis.

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