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Africanized honey bees (*Apis mellifera* L.) are more efficient at removing worker brood artificially infested with the parasitic mite *Varroa jacobsoni* Oudemans than are Italian bees or Italian/Africanized hybrids

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Abstract

Africanized honey bees are more tolerant of infestations with the mite *Varroa jacobsoni* than are honey bees of European origin. The capacity of these bees to detect and react to brood infested with this mite could be one of the factors determining this tolerance. We tested colonies of Africanized bees headed by queens from swarms collected in Ribeirão Preto, São Paulo State. The Italian colonies had queens imported directly from the USA, or from the Brazilian Island of Fernando de Noronha, where varroa-infested Italian colonies have been maintained, untreated, since 1984. Recently sealed worker brood cells were artificially infested by opening the cell capping, inserting live adult female mites and resealing the cells. Control cells were treated in the same way, but without introducing mites. The ability of the Africanized honey bees to recognize and remove this artificially infested brood was compared with that of first generation Italian/Africanized hybrid bees, and with the two groups of "pure" Italian bees, in three separate experiments. Africanized colonies removed a mean of 51% of the infested brood, while Italian/Africanized hybrid colonies removed 25%. Africanized colonies also removed a significantly greater proportion of infested brood than did Italian colonies, headed by queens from the USA (59 vs. 31%, respectively). Similarly, when Africanized colonies were compared with colonies of Italian bees from Fernando de Noronha, the former were found to be significantly more efficient at removing infested brood (61 vs. 35%, respectively), even though the population of Italian bees on this island has been exposed to and survived varroa infestations (without treatment) for more than 12 years. Only the Africanized honey bees removed a significant proportion of varroa-infested brood, when the data was corrected for brood removal from control cells.

INTRODUCTION

In Brazil the mite parasite *Varroa jacobsoni* does not cause serious damage to apiculture (Shimanuki *et al.*, 1991). The infestations are so low, usually less than 3% of adult worker bees infested, that no treatment is necessary. Initially, when the mite was first discovered in many regions of this country in the early 1980s, infestations were considerably higher. So it appears that Africanized honey bees have adapted to this mite (De Jong, 1997). The (Africanized) honey bees used commercially in Brazil are more resistant to varroa than are the European races or hybrids between these two types of bees (Moretto *et al.*, 1991b; Guerra Jr. and Gonçalves, 1992). Climate is also an important factor. In tropical and subtropical regions of South America, the varroa infestation levels are lower than in cooler regions (De Jong *et al*., 1982, 1984; Moretto *et al*., 1991b).

Several apparent resistance factors have been found in honey bees. The reproductive rate of this mite is lower in Africanized bees in Brazil than in European bees (Ritter and De Jong, 1984; Camazine, 1986; Issa *et al*., 1993). Africanized bees also have a greater ability to remove the mites from their bodies (Moretto *et al*., 1991a, 1993).

Even though they cannot survive infestations by this mite in Europe and North America (De Jong, 1997), Italian bees can survive varroa infestations under tropical conditions in Brazil. A small population of Italian colonies was established in 1984 on Fernando de Noronha Island, near Recife, Brazil. The colonies there survive *V. jacobsoni* parasitism without the need for treatment, and infestation levels have steadily decreased (De Jong and Soares, 1997). Possibly these Italian bees have some of the tolerance mechanisms we find in Africanized bees.

The original host of *V. jacobsoni*, *Apis cerana*, can selectively remove varroa-infested worker brood, and thus greatly reduce the reproduction of this mite (Peng *et al*., 1987; Rath and Drescher, 1990; Rosenkranz *et al*., 1993). Similar brood nest cleaning behaviors have also been found, to a lesser degree, in *Apis mellifera* (Boecking and Drescher, 1991; Boecking *et al*., 1992; Ruttner and Hänel, 1992). The removal of infested brood could limit the growth of varroa populations in the colonies by mortality of immature mites, interruption of mite brood production and/or mortality of the original breeding females (Fries *et al*., 1994). It is not known how the bees determine that brood is infested, inside the wax-capped brood cells. Acoustical signals may be involved (Boecking, 1994). There is also evidence that hygienic bees are more responsive to olfactory signals associated with diseased brood (Masterman *et al*., 1998). The high degree of tolerance of Africanized bees in Brazil to varroa disease prompted us to determine if they have an enhanced capability to remove mite-infested brood, in comparison with European (Italian) honey bees, and with Italian/Africanized hybrids.

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MATERIAL AND METHODS

Africanized bee colonies originated from natural swarms were maintained in the university apiary in Ribeirão Preto, SP, Brazil. Observations were made on Africanized and hybrid Italian/Africanized colonies (N = 9, each) from May to October 1991 and on Africanized and "pure" Italian colonies (N = 6, each) from January to May 1992. The bees were kept in standard Langstroth four-frame nucleus colonies, containing three frames with brood, honey and pollen, and an internal feeder supplied occasionally with sugar syrup. Italian queens, instrumentally inseminated with Africanized drones, headed the hybrid colonies. The original queen mothers were from commercial stocks imported from the USA. Imported queens headed the "pure" Italian colonies (also from the USA). A third series of tests was made in 1997 with three Africanized colonies, tested twice each, in comparison with three Italian colonies, headed by queens from Fernando de Noronha island, PE, Brazil (De Jong and Soares, 1997).

Sealed worker brood cells in the prepupal or last larval phase were selected. Live adult female mites were removed from infested brood cells, maintained on worker pupae in an incubator at 34ºC for 24-48 h, and then used in the experiment. A small opening in the edge of the cell capping was made with an insect pin and a live mite introduced with the aid of a fine-tipped brush. A piece of capping from a neighboring brood cell was then removed with a forceps and immediately pressed over the opening in order to reseal the perforated brood cell. The control cells (in the second and third experiments) were treated in the same way, but without introducing a mite. The cells were marked by placing a pin in the brood cell immediately to the right. Eight days after introduction the combs were examined to determine if the artificially infested brood was differentially removed. Data analysis was done using a median test ($\alpha = 0.05$; Daniel, 1978).

Table I - Proportion (number removed/number infested) of worker pupae artificially infested with *Varroa jacobsoni*, removed by the bees in Africanized (A) and Italian/Africanized hybrid (H) colonies.

Africanized bees		Italian/Africanized hybrids	
Colony		Colony	
1A	3/8	1H	0/5
2A	1/8	2H	1/7
3A	6/7	3H	1/6
4A	7/14	4H	2/9
5A	4/10	5H	2/12
6A	17/38	6H	11/20
7A	22/31	7H	3/8
8A	8/15	8H	2/10
9A	10/15	9H	7/15
Mean % ± SD	51 ± 21		25 ± 17

SD = Standard deviation.

Table II - Proportion (number removed/number infested) of worker pupae artificially infested with *Varroa jacobsoni*, removed in Africanized (A) vs. Italian (It.) colonies, with respective controls (cells opened but no mites introduced).

Africanized bees			Italian bees		
Colony	Infested	Control	Colony	Infested	Control
10A	7/10	3/10	1It.	2/9	1/10
11A	7/11	4/12	2It.	3/11	2/11
12A	5/11	2/11	3It.	2/10	1/10
13A	7/11	3/11	4It.	5/12	2/12
14A	6/11	4/11	5It.	4/11	4/12
15A	7/13	4/13	6It.	5/13	6/13
Mean % ± SD	59 ± 8.9	29 ± 6.3		31 ± 9.1	22 ± 14

SD = Standard deviation.

RESULTS AND DISCUSSION

In the first experiment, in 1991, the Africanized colonies removed the infested brood at about double the rate of the Italian/Africanized hybrids (Table I). The Africanized bees were significantly more efficient than the hybrid Italian/Africanized bees at removing the infested brood.

In the comparison between Africanized colonies and Italian bees from the USA, the former removed a significantly greater proportion of infested brood than the latter (Table II). Thakur et al. (1997) found that Carniolan bees in Germany removed 26-30% of varroa-infested worker brood, which is similar to our findings for Italian bees (Table II). The Africanized colonies in our experiment also removed a greater proportion of the control (opened, resealed, but not artificially infested) pupae, than did the Italian colonies, though this difference was not significant. When the rates at which bees removed pupae from infested cells were corrected for the rate of removal of the control pupae in each colony (Table II), the resultant figures were also significantly greater for the Africanized bee colonies compared to the Italian colonies. The Africanized bees removed significantly more infested than control pupae. However, in the Italian colonies, there was no significant difference between the rates with which bees removed infested vs. control pupae ($P > 0.25$).

Africanized bees were also significantly more efficient at removing varroa-infested brood than the Italian bees from Fernando de Noronha (Table III). Even though these Italian bees have survived varroa infestations on the island for many generations (De Jong and Soares, 1997), they were significantly inferior in their capacity to react to varroa-infested brood, when compared to Africanized honey bees, kept under the same conditions. The Africanized colonies also removed a greater proportion of the control (opened, resealed, but not artificially infested)

Table III - Proportion (number removed/number infested) of worker pupae artificially infested with *Varroa jacobsoni* removed in Africanized (A) bees vs. Italian bees from the Island of Fernando de Noronha (It. FN), Brazil, with respective controls (cells opened but no mites introduced).

Africanized bees			Italian bees		
Colony	Infested	Control	Colony	Infested	Control
16A	11/16	5/15	1It. FN	6/15	4/15
16A	7/13	4/13	1It. FN	6/14	3/13
17A	6/11	5/12	2It. FN	4/11	2/11
17A	7/12	3/12	2It. FN	4/13	4/13
18A	7/10	4/11	3It. FN	3/10	2/10
18A	9/15	6/15	3It. FN	5/16	3/16
Mean % ± SD	61 ± 7.0	35 ± 6.2		35 ± 5.4	22.9 ± 14

SD = Standard deviation.

pupae, than did the Italian colonies, though this difference was not significant (Table III). When the rates at which bees removed pupae from infested cells were corrected for the rate of removal of the control pupae, the resultant figures were also significantly greater for the Africanized bee colonies (26%), compared to the Italian colonies (12%). The Africanized bees removed significantly more infested than control pupae. In the Italian colonies, there was no significant difference between the rates with which bees removed infested vs. control pupae ($P > 0.20$).

The Africanized colonies were able to selectively remove brood infested with *V. jacobsoni*, and were significantly more efficient at this task than the Italian colonies and the Italian/Africanized hybrid colonies, kept under the same conditions. Moreover, only the Africanized honey bees removed a significant percentage of varroa-infested brood, when the data was corrected for brood removal from control cells (opened and resealed, without introducing mites). Increased efficiency in the removal of infested brood could be one of the mechanisms which has permitted Africanized honey bee colonies in Brazil to maintain low, stable infestation rates without interference by the beekeepers (De Jong, 1997; Corrêa-Marques and De Jong, 1998).

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RESUMO

Abelhas africanizadas são mais tolerantes à infestação com o ácaro *Varroa jacobsoni* do que abelhas de origem européia. A capacidade destas abelhas de detectar e reagir à cria infestada com este ácaro pode ser um dos fatores chaves que determina esta tolerância. Células de cria de operária foram infestadas artificialmente, abrindo o opérculo, inserindo ácaros fêmeas adultas vivas e selando as células. A habilidade das abelhas africanizadas de reconhecer e remover esta cria infestada artificialmente foi comparada com a de abelhas híbridas italianas/africanizadas e com dois grupos de abelhas italianas "puras", em três experimentos distintos. Rainhas italianas já fecundadas foram importadas dos EUA ou foram obtidas da ilha de Fernando de Noronha, Brasil, onde abelhas italianas são mantidas desde 1984. Colmeias africanizadas removeram em media 51% da cria infestada, enquanto colmeias híbridas italianas/africanizadas removeram 25%. Colmeias africanizadas também removeram uma proporção significativamente maior de cria infestada do que colmeias "puras" italianas com rainhas dos EUA (59 vs. 31%, respectivamente). Da mesma maneira, quando colmeias africanizadas foram comparadas com colmeias de abelhas italianas da ilha de Fernando de Noronha, as africanizadas foram significativamente mais eficientes na remoção de cria infestada (61 vs. 35%, respectivamente), apesar de que a população de abelhas italianas desta ilha foi exposta a varroa e elas sobreviveram sem tratamento durante mais de 12 anos. Somente as abelhas africanizadas removeram uma proporção significante da cria infestada com *V. jacobsoni*, quando os dados foram corrigidos pela taxa de remoção da cria das células controles.

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