Apidologie (1995) 26, 381-386

Original article

Effect of the size of worker brood cells of Africanized honey bees on infestation and reproduction of the ectoparasitic mite *Varroa jacobsoni* Oud

D Message [1], LS Gonçalves [2]

[1] *Depto Biologia Animal, Universidade Federal de Viçosa, 36571-000 Viçosa, MG;*
[2] *Depto Biologia, FFCLRP, USP, 14049-901 Ribeirão Preto, SP, Brazil*

(Received 28 October 1993; accepted 28 March 1995)

Summary — An investigation was made of the influence of honey bee worker brood cell size on infestation by the mite *Varroa jacobsoni*. Pieces of combs constructed by Africanized (small cells) and pure Italian (large cells) honey bees were placed within the same frame and eggs were laid by a single queen in each trial. Two queens, one Africanized and the other Italian, were mated with Africanized drones, and their respective colonies were used in 16 trials. The frequency of infested brood cells was higher in the large worker brood cells built by European races than in the small worker brood cells built by Africanized bees (15.7 *vs* 7.3%, respectively), though other factors, including egg origin and colony, were equal. The same was true for frequency of cells with female deutonymphs (which represent effective reproduction) (11.9 *vs* 4.7%, respectively).

***Apis mellifera* / *Varroa jacobsoni* / Africanized bees / mite reproduction / brood infestation**

INTRODUCTION

Although the ectoparasite *Varroa jacobsoni* Oud has maintained itself in equilibrium for a long time with its original host *Apis cerana* Fabr, in *A mellifera* L this adaptation has not been established and the mite normally causes serious losses for the beekeeping industry (De Jong *et al*, 1982a). However, in Brazil, where most beekeeping is based on Africanized bees, infestation has not reached alarming levels, with no reports of colony losses exclusively attributable to varroosis. In contrast, in Argentina, where European bees predominate, the infestation has reached high levels (De Jong *et al*, 1984).

This difference observed between European bees and African or Africanized bees has stimulated a great deal of research to determine what factors influence *Varroa* populations. The race of honey bees

(Camazine, 1986; Moretto *et al*, 1991a) and climatic conditions (Moretto *et al*, 1991b) appear to play a crucial role in the resistance of bees in Brazil, though Rosenkranz *et al* (1984) did not attribute the low rate of reproduction to the high tropical ambient temperatures. Hygienic and grooming behavior, the shorter post-capping phase of the brood and the higher rate of infertile females are some racial factors that could contribute to this higher resistance of African and Africanized bees (Moritz and Hänel, 1984; Ritter and De Jong, 1984; De Jong, 1990; Moritz and Mautz, 1990; Moretto *et al*, 1991a). Increase in worker infestation has also been observed in reared cells independently of the race of bees (De Jong and Morse, 1988; De Ruitjer and Calis, 1988).

In the present study, the influence of the smaller worker cells built by Africanized bees in relation to larger cells built by European races on the infestation and reproduction of the mite *V jacobsoni* was investigated.

MATERIALS AND METHODS

We used specially built (mosaic) combs containing 2 pieces of comb each about 7 x 10 cm, inserted into the center, one made up of cells built by Africanized bees (small cells) and the other with cells built by *A m ligustica* (large cells). The smallest width and the volume (measured by filling the cells with water from a calibrated pipette) of these cells varied from 4.5 to 4.6 mm and from 175 to 195 μl, respectively, in small cells, and from 4.9 to 5.1 mm and from 240 to 260 μl in large cells.

Two queens and their respective colonies were utilized for egg laying and brood development. One was a wild-type Africanized honey bee queen and the other an Italian queen, which was open mated in an Africanized bee region, therefore producing F_1 hybrid Africanized/Italian workers. Combs with the 2 types of cells with eggs of one queen were placed in the same colony or in the colony of the other queen, and *vice versa*.

Egg laying by the queens in the 2 types of cells occurred with only a small variation in time, so that brood of practically the same age and from the same queen was available in each of the composite combs. The brood cells were analyzed individually at 17–18 d, for the presence of adult mite females, deutonymphs and other offspring (eggs, female protonymphs and males at the various developmental phases) of *Varroa*. Sixteen trials were made for a total of 1 572 pupae developed in small cells and 1 642 pupae in large cells. The data were analyzed statistically by the Student's *t*-test for paired samples.

RESULTS AND DISCUSSION

No significant effect of the queen or colony was found ($P > 0.4$), and so the data were pooled. The *Varroa* infestation rate was 2-fold higher in brood developed in large cells in relation to brood developed in small cells (table I). The significantly ($P < 0.01$) higher brood weight observed in large cells (average of 108.2 mg) in relation to the small cells (average 99.2 mg) should promote differential feeding. Worker brood developed in large cells should receive more visits from nurse workers, increasing the probability of the mites transferring to the cells to infest the brood. Fuchs (1990) found a decrease in the intensity of the attraction of the drone larvae when they are nursed less intensively towards the end of drone production. Besides physical stimuli, larvae from these cells could also contribute with other stimuli, such as kairomones. Le Conte *et al* (1989) have isolated and identified some esters (*eg,* methyl palmitate) promoting a strong attractive response in the *Varroa* female. This substance was isolated from drone brood but is also present in worker brood in smaller amounts. The prolonged and intensive capping activity (Fuchs, 1990; Wieting and Ferenz, 1991) should be related to the phoresis hypothesis, due to the large amount of time that nurse workers spend near the brood during this activity, increasing the chance for mites to move from cell capping workers to the host brood.

During the experiments we did not observe any drone brood in the colonies, and so the mites had only the worker cells available. There were slightly more original adult females in large infested cells than in infested small cells (table II). We found 29 cells with 2 adult females and 7 cells with 3 adult females among large cells, whereas among small cells only 6 cells had 2 adult females and none had 3 or more.

The number of female deutonymphs produced did not differ significantly between infested large and small cells (table I). However, a greater percentage of all large cells had deutonymphs (table II). If we consider that the female deutonymphs encountered in this experiment on 17–18 d pupae are effective reproductions, the large cells contributed 2.6-fold more to the population increase of *Varroa*.

In this experiment we also observed a high proportion of original adult females that entered into the cells with brood and left no offspring (tables I and II). This proportion was the same for both type of cells. This suggests that the infertility rate is not due to the size of cells built by European or Africanized bees, but to other factors. A high rate of mite infertility has been shown in Africanized bees (De Jong *et al*, 1982b; Ritter and De Jong, 1984; Camazine, 1986) and in European bees in South America (Ruttner *et al*, 1984; Marcangeli *et al*, 1992).

We conclude that small comb cell size affects the infestation and reproduction of *V jacobsoni* in worker brood of Africanized bees in Brazil. Other factors observed in Africanized bees, including shorter development time (19–20 d) of worker brood (Message, 1986), grooming and hygienic behavior; climatic conditions (Moretto *et al*, 1991a, b) and higher frequency of temperature peaks during the thermal regulation of the brood chamber of Africanized bees (Le Conte *et al*, 1990) also contribute to the greater resistance of Africanized bees to *V jacobsoni* in Brazil and other tropical and subtropical countries compared with European races of bees.

Table I. Mean and standard deviation (SD) of the rate of infestation and reproduction of the mite *V jacobsoni* in *A mellifera* in small cells (built by Africanized bees) and large cells (built by European bees) of worker brood.

	Small cells		*Large cells*		
	Mean	*SD*	*Mean*	*SD*	*P(t)*
Infested cells	0.073	0.042	0.157	0.100	$P < 0.01$
Number of deutonymphs in cells with offspring	1.15	0.49	1.40	0.43	NS
Number of other descendants in cells with offspring	1.93	0.43	2.24	0.67	NS
Rate of infested cells without offspring	0.433	0.270	0.434	0.225	NS

NS = non-significant ($p > 0.05$).

Table II. *Varroa* infestation and reproduction data of large and small worker bee brood cells (the data in parentheses indicate their relative frequency (%) in relation to the total number of cells analysed).

	Small cells	*Large cells*
Total cells analyzed	1 572 (100)	1 642 (100)
Total cells infested	115 (7.3)	258 (15.7)
Number of cells infested with offspring	65 (4.1)	145 (8.8)
Number of cells infested without offspring	50 (3.2)	113 (6.9)
Total of adult *Varroa* infesting worker cells	120 (7.6)	298 (18.2)
Total of female deutonymphs	74 (4.7)	195 (11.9)
Total offspring (not including deutonymphs)	140 (8.9)	310 (18.9)

ACKNOWLEDGMENTS

We wish to thank D De Jong for providing useful discussions, reviewing the manuscript and for writing the summary. This work was funded by CNPq (Brazil) and NSF (USA).

Résumé — Effet de la taille des cellules de couvain d'ouvrières d'abeilles africanisées sur l'infestation et la reproduction de l'acarien parasite *Varroa jacobsoni*. La parasitose par *Varroa jacobsoni* est un problème grave pour l'apiculture. Cependant, au Brésil, où l'apiculture est basée sur l'utilisation de l'abeille africanisée, l'épidémie n'a pas atteint un niveau alarmant, et il n'y a pas eu de pertes de colonies dues exclusivement à l'acarien. Puisque l'abeille africanisée est plus petite que l'abeille européenne, à la fois au stade adulte et aux stades larvaires, nous avons analysé l'influence de la taille des cellules de couvain d'ouvrières d'abeilles africanisées sur l'infestation et la reproduction du parasite. Des morceaux de rayon construits par des abeilles africanisées (petites cellules) et de race italienne (grandes cellules) ont été placés dans le même cadre. Une seule reine a pondu au cours de chacun des 16 essais. La fréquence des cellules de couvain infestées (tableau I) a été significativement plus élevée dans les grandes cellules (15,7%) que dans les petites (7,3%). Le nombre de femelles de *Varroa* a été légèrement supérieur dans les grandes cellules (298/258 = 1,15 femelle adulte par cellule) que dans les petites (120/115 = 1,04 femelle adulte par cellule). Le nombre de deutonymphes a été 2,6 fois plus grand dans les grandes cellules. Le poids plus grand des nymphes élevées dans les grandes cellules (108,2 mg) par rapport à celles élevées dans les petites cellules (99,2 mg) suggère que les ouvrières nourrices s'occupent davantage des premières, ce qui augmente donc

la probabilité d'apporter des acariens au contact des larves. Les données obtenues dans ce travail, sur l'effet de la taille des cellules, le nombre de cellules infestées et le temps de développement du couvain d'ouvrières d'abeilles africanisées, aussi bien que les données de la littérature concernant le comportement de nettoyage, les conditions climatiques et la régulation thermique de la colonie, contribuent à expliquer la plus grande résistance des abeilles africanisées à *Varroa jacobsoni* au Brésil et dans d'autres régions tropicales et sub-tropicales, par rapport aux races européennes.

***Apis mellifera* / *Varroa jacobsoni* / abeilles africanisées / reproduction de *Varroa* / infestation du couvain**

Zusammenfassung — Einfluß der Größe von Arbeiterinnenbrutzellen der afrikanisierten Honigbiene auf Befall und Reproduktion der ectoparasitischen Milbe *Varroa jacobsoni* Oud. Durch *Varroa jacobsoni* ist für die Bienenhaltung ein gefährliches Problem entstanden. In Brasilien werden afrikanisierte Bienen gehalten und dort hat der Milbenbefall nicht so alarmierende Raten erreicht wie zB in Europa. Es gibt keine Verluste von Bienenvölkern, die nur auf dem Befall durch *Varroa jacobsoni* beruhen. Afrikanisierte Bienen sind kleiner als Bienen der europäischen Rassen. Größenunterschiede finden sich auch bei den Arbeiterinnenbrutzellen. Deshalb wurde der Einfluß der Zellgröße auf den Befall und die Reproduktion der Milbe *Varroa jacobsoni* untersucht.

Wabenstücke, die entweder von afrikanisierten (kleine Zellen) oder von reinen Italienerbienen (große Zellen) gebaut waren, wurden gemeinsam in einen Rahmen eingeschnitten. Die Eier stammten in allen 16 Versuchen von derselben Königin.

Die größeren Zellen der Italienerbienen waren statistisch gesichert häufiger befallen (15,7%) als die kleinen der afrikanisierten Bienen (7,3%, Tabelle I). Es befanden sich geringfügig mehr Muttermilben in großen befallenen Zellen (298/258 = 1,15 Muttermilben/Zelle) als in den kleinen Zellen (120/115 = 1,04 Muttermilben/Zelle). Die Anzahl der Deutonymphen in Zellen mit Nachkommen zeigte keine größenabhängigen statistischen Unterschiede. Insgesamt aber befanden sich in großen Zellen 2,6 mal mehr Deutonymphen. Der signifikante Unterschied im Gewicht der 17 bis 18 Tage alten Brut in großen und kleinen Zellen läßt vermuten, daß unterschiedlich häufig gefüttert wird und damit auch die Wahrscheinlichkeit unterschiedlich ist, daß eine Milbe in die Zelle getragen wird.

Die hier gewonnenen Daten über den Einfluß der Zellgröße, die Anzahl der befallenen Zellen ohne Reproduktion (43%) und die kürzere Entwicklungsdauer (19–20 Tage) der afrikanisierten Arbeiterinnenbrut tragen im Zusammenhang mit Literaturdaten über Putzverhalten, über Klimabedingungen und über häufiges Vorkommen von Temperaturpeaks bei der Thermoregulation zur Erklärung der höheren Resistenz der afrikanisierten Bienen in Brasilien und anderen tropischen und subtropischen Ländern im Vergleich zu europäischen Rassen bei.

Zellgröße / Arbeiterinnenbrut / *Varroa jacobsoni* / afrikanisierte Honigbienen

REFERENCES

Camazine S (1986) Differential reproduction of the mite, *Varroa jacobsoni* (Mesostigmata: Varroidae), on Africanized and European honey bees (Hymenoptera: Apidae). *Ann Entomol Soc Am* 76, 801-803

De Jong D (1990) Potencial produtivo das abelhas africanizadas em relação ao das abelhas européias. *Anais da 27ª Reunião Anual da Sociedade Brasileira de Zootecnia*, FEALQ, Piracicaba, SP, 577-587

De Jong D, Morse RA (1988) Utilization of raised brood cells of the honey bee, *Apis mellifera* (Hymenoptera: Apidae), by the mite, *Varroa jacobsoni* (Acarina: Varroidae). *Entomol Gen* 14, 103-106

De Jong D, De Jong PH, Gonçalves LS (1982a) Weight loss and other damage to developing worker honeybees from infestation with *Varroa jacobsoni*. *J Apic Res* 21, 165-167

De Jong D, Morse RA, Eickwort GC (1982b) Mite pests of honey bees. *Annu Rev Entomol* 27, 229-252

De Jong D, Gonçalves LS, Morse RA (1984) Dependence on climate of the virulence of *Varroa jacobsoni*. *Bee World* 65, 117-121

De Ruitjer A, Calis J (1988) Distribution of *Varroa jacobsoni* female mites in honey bee worker brood cells of normal and manipulated depth (Acarina: Varroidae). *Entomol Gen* 14, 107-109

Fuchs S (1990) Preference for drone brood cells by *Varroa jacobsoni* Oud in colonies of *Apis mellifera carnica*. *Apidologie* 21, 193-199

Le Conte Y, Arnold G, Trouiller J, Masson C, Chappe B, Ourisson G (1989) Attraction of the parasitic mite *Varroa* to the drone larvae of honey bees by simple aliphatic esters. *Science* 245, 638-639

Le Conte Y, Arnold G, Desenfant PH (1990) Influence of brood temperature and hygrometry variations on the development of the honey bee ectoparasite *Varroa jacobsoni* (Mesostigmata: Varroidae). *Environ Entomol* 19, 1780-1785

Marcangeli JA, Eguaras MJ, Fernandez NA (1992) Reproduction of *Varroa jacobsoni* (Acari: Mesostigmata: Varroidae) in temperate climates of Argentina. *Apidologie* 23, 57-60

Message D (1986) Aspectos reprodutivos do ácaro *Varroa jacobsoni* e seus efeitos em colônias de abelhas africanizadas. PhD Thesis, Departamento de Genética, Faculdade de Medicina de Ribeirão Preto, USP, Brazil, 146 p

Moretto G, Gonçalves LS, De Jong D (1991a) Africanized bees are more efficient at removing *Varroa jacobsoni*. Preliminary data. *Am Bee J* 131, 434

Moretto G, Gonçalves LS, De Jong D, Bichuette MZ (1991b) The effects of climate and bee race on *Varroa jacobsoni* Oud infestations in Brazil. *Apidologie* 22, 197-203

Moritz RFA, Hänel H (1984) Restricted development of the parasitic mite *Varroa jacobsoni* Oud in the Cape honeybee *Apis mellifera capensis* Esch. *Z Ang Ent* 97, 91-95

Moritz RFA, Mautz D (1990) Development of *Varroa jacobsoni* in colonies of *Apis mellifera capensis* and *Apis mellifera carnica*. *Apidologie* 21, 53-58

Ritter W, De Jong D (1984) Reproduction of *Varroa jacobsoni* Oud in Europe, the Middle East and tropical South America. *Z Ang Ent* 98, 55-57

Rosenkranz P, Tewarson NC, Engels W (1984) Optimal host selection by reproductive female *Varroa jacobsoni*. *In: Advances in Invertebrate Reproduction 3* (W Engels, ed), Elsevier Publishers, Amsterdam, New York, Oxford, 628

Ruttner F, Marx H, Marx G (1984) Beobachtungen über eine mögliche Anpassung von *Varroa jacobsoni* an *Apis mellifera* L in Uruguay. *Apidologie* 15, 43-62

Wieting J, Ferenz H (1991) Behavioral study on the invasion of honey bee brood by the mite *Varroa jacobsoni* on wax combs and ANP combs. *Am Bee J* 117-118