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Varroa-tolerant Italian honey bees introduced from Brazil were not more efficient in defending themselves against the mite *Varroa destructor* than Carniolan bees in Germany

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ABSTRACT. In Europe and North America honey bees cannot be kept without chemical treatments against *Varroa destructor*. Nevertheless, in Brazil an isolated population of Italian honey bees has been kept on an island since 1984 without treatment against this mite. The infestation rates in these colonies have decreased over the years. We looked for possible varroa-tolerance factors in six Italian honey bee colonies prepared with queens from this Brazilian island population, compared to six Carniolan colonies, both tested at the same site in Germany. One such factor was the percentage of damaged mites in the colony debris, which has been reported as an indicator of colony tolerance to varroa. A mean of 35.8% of the varroa mites collected from the bottoms of the Italian bee colonies were found damaged, among which 19.1% were still alive. A significantly greater proportion of damaged mites were found in the Carniolan bees (42.3%) and 22.5% were collected alive.

The most frequent kind of damage found was damaged legs alone, affecting 47.4% of the mites collected from debris in Italian bees, which was similar to the amount found in Carniolan colonies (46%). The mean infestation rate by the varroa mite in the worker brood cells in the Italian bee colonies was 3.9% in June and 3.5% in July, and in drone brood cells it was 19.3% in June. In the Carniolan honey bee colonies the mean infestation rates in worker brood cells were 3.0 and 6.7%, respectively in the months of June and July and 19.7% in drone brood cells in June. In conclusion, the 'Varroa-tolerant' Italian honey bees introduced from Brazil produced lower percentages of damaged mites (*Varroa destructor*) in hive debris and had similar brood infestation rates when compared to 'susceptible' Carniolan bees in Germany. In spite of the apparent adaptation of this population of Italian bees in Brazil, we found no indication of superiority of these bees when we examined the proportions of damaged mites and the varroa-infestation rates, compared to Carniloan bees kept in the same apiary in Germany.

Key words: *Apis mellifera ligustica*, *Apis mellifera carnica*, Host-parasite relationship, Natural selection, *Varroa jacobsoni*

INTRODUCTION

In Germany, as in other temperate climate regions of the world, the mite *Varroa destructor* (formerly, *Varroa jacobsoni*, see Anderson and Trueman, 2000) is a lethal parasite of honey bee colonies (*Apis mellifera*). However, in Brazil varroosis does not cause any significant problems for apiculture, apparently because of the development of tolerance by Africanized honey bees (De Jong, 1997; De Jong and Gonçalves, 1998). Several mechanisms that may be responsible for this tolerance have been investigated, including reduced reproduction by the mites (Ritter and De Jong, 1984), greater efficiency in removing infested brood (Corrêa-Marques and De Jong, 1998; Guerra et al., 2000), and increased grooming behavior (Moretto et al., 1991, 1993). One of the phenomena that apparently signals effective bee attacks on the mites is dead and damaged mites found on the bottom board of the colonies (Ruttner et al., 1984; Morse et al., 1991). Some studies on the percentage of damaged mites in the hive debris and varroa infestation rates have indicated an inverse relationship (Büchler, 1993; Moosbeckhofer, 1997; Arechavaleta-Velasco and Guzmán-Novoa, 2001), suggesting that bee-inflicted mite damage can promote tolerance to this parasite. However, the use of this criterion for the selection of tolerant bees has been questioned (Liebig, 1997; Rosenkranz et al., 1997; Corrêa-Marques et al., 2000).

Climate apparently has an important role in creating the conditions necessary for the development of tolerance to varroa. In Brazil even European colonies can be maintained without treatment (Engels et al., 1986), but it is difficult to make objective observations of this phenomenon in European colonies in Africanized areas, because the European bee colonies remain small and do not thrive when they have to compete with the tropically adapted Africanized honey bees. However, there is an island 360 km off the coast of Brazil where Italian bees can produce large colonies. An isolated Italian honey bee population was established on the Brazilian island of Fernando de Noronha (3º south latitude) in 1984. The original colonies were already

infested with the mite *Varroa destructor* from Pernambuco State, Brazil, and have been kept naturally without chemical treatments ever since. There are now about 100 colonies (half in managed hives), and they are strong, normally producing units. Even though this is a relatively small number of colonies, there has been a gradual decrease in the mean level of infestation with the varroa mite (from 26 mites per 100 bees in 1990 to 14 in 1996), demonstrating an apparent natural selection towards tolerance (De Jong and Soares, 1997).

We decided to test and compare the tolerance to varroa of these apparently adapted Italian bees from Brazil with local honey bees in an apiary in Germany, a location where honey bee colonies would normally die if they are maintained without treatment (Ritter, 1981).

METHODOLOGY

Six local Carniolan (*Apis mellifera carnica*) colonies and six Italian (*Apis mellifera ligustica*) honey bee colonies were set up at the Apiculture Institute of Hohenheim University, in Stuttgart, Germany. The Italian colonies were headed by queens produced and mated on Fernando de Noronha island in Brazil. These queens were transported to Germany and introduced into dequeened Carniolan bee colonies in early May, 1997. The colonies were kept in standard Zander hives. We placed removable, screen-protected trays at the bottom of the colonies to collect debris, including naturally fallen mites. The initial infestations were quite low, due to efficient chemical controls in the previous year, so we introduced pieces of infested drone brood removed from other colonies in the region during a two-week period. About 15 days after the last such introduction we began to collect mites from the colony debris (from late June to the end of July). These mites were collected during intervals of 24 h. Longer intervals can affect the percentage mites damaged and the types of damage, in part due to damage or removal by predators such as wax moths and ants (Bienefeld et al., 1999). One hundred mites per colony were analyzed with a microscope in order to identify and classify those that were damaged (Corrêa-Marques et al., 1994, 2000). We investigated the level of infestation with varroa mites in the colonies, in worker brood cells in late June (just before we started collecting fallen mites) and in late July, and in drone brood cells in June (in July drone rearing had ceased). The level of infestation was determined as the number of cells infested by the varroa mite among 100 cells. The results were analyzed with a Student *t*-test or with a chi-square test.

RESULTS

The percentage of damaged mites among those found in the debris of the colonies was significantly lower in the Italian than in Carniolan colonies (chi-square test, $P = 0.02$, Table 1). Among these damaged mites about 40% were young (lightly colored) adult females and the rest were mature (dark colored) adult females; the proportion of young damaged mites was significantly higher ($P<0.05$) in the Carniolan bee colonies (Table 1). The proportion of live damaged mites was about 20% of the total number of damaged mites and there was no significant difference in this proportion between the two types of bees ($P>0.5$, Table 1). The most frequent kind of damage was damaged legs (total or partial loss of one or more legs) representing about 47% in the Italian bees, which was not significantly different from that found in the Carniolan colonies ($P = 0.8$, Table 2). The mean level of infestation in worker brood cells in June month was 3.9% in the Italian bees and 3.0% in the Carniolans. In July these means were 3.5 and 6.7%, respectively. These infestation rates were not

Table 1. Frequency (%) of damaged *Varroa destructor* collected in the debris of Carniolan and Italian honey bees (*Apis mellifera)* colonies, and percentages of mites that were "new", lightly colored adult females and that were found alive among those found damaged.

	Carniolan bees	Italian bees
Damaged mites	42.3	35.8
New adults	44.5	36.8
Alive	22.4	19.1

Table 2. Classification and quantification of the kinds of damage to *Varroa destructor*, collected in the debris of Carniolan and (Brazilian) Italian honey bee (*Apis mellifera)* colonies.

Kinds of damage	Carniolan bees (%)	Italian bees (%)
Damaged legs	46.0	47.4
Indentation or hollow in the dorsal shield	42.1	36.7
Carcass - empty dorsal shield	3.5	5.2
Damaged shields + damaged legs	2.0	5.6
Hollow in the dorsal shield + damaged legs	4.7	2.3
Damaged shields	1.6	2.8

Damaged legs = total or partial loss of one or more legs; Empty dorsal shield = mites that lacked all legs and all or almost all of the ventral shields, generally only the dorsal shield remained; Damaged shields = loss of ventral shields, fissures in and loss of pieces of the dorsal shield.

significantly different (*t*-test, P>0.5). The mean level of infestation of drone brood cells was similar in Italian and in Carniolan bees, in June (19.4 and 19.8%, respectively, P>0.8, Table 3). Similar percentages of the infested worker brood cells in Italian honey bee colonies and Carniolan colonies were infested by a single adult varroa mite (P>0.5, Table 3). In drone brood cells the proportions of singly infested cells were also similar for these two types of bees (P>0.7, Table 3) and were significantly lower than in infested worker brood cells (P<0.05, Table 3). Multiple infestation (more than one original invading mite in each brood cell) was significantly more common in the drone brood cells.

Table 3. Invasion rates of female adult mite *Varroa destructor* in worker and drone brood cells in Italian and Carniolan honey bee (*Apis mellifera)* colonies.

	Worker		Drone	
	Italian	Carniolan	Italian	Carniolan
Colonies (N)	6	6	6	5
Brood cells (N)	1200	1200	600	406
Cells with varroa (N)	53	68	116	80
Cells with 1 mite (%)	74	87	51	56
Cells with 2 mites (%)	25	10	32	19
Cells with 3 mites (%)	2	3	9	18
Cells with 4 mites (%)	0	0	5	4
Cells with 5 or more mites (%)	0	0	3	4

DISCUSSION

We had expected that the Italian bees from Fernando de Noronha island in Brazil would be more tolerant to varroa than the Carniolan bees when we tested these two kinds of honey

bees together in Germany. Nevertheless, there were no consistent differences in infestation rates, in the percentage of damaged mites found in the debris of the colonies or in the proportions of different kinds of damage to the mites (Tables 1 and 2). Initial infestation rates were low, but they increased considerably in both groups of colonies.

The approximately 36-42% damaged mites among those found in the debris of these European honey bee colonies (Table 1) was similar to the 37% found in Africanized bees in Brazil (Corrêa-Marques et al., 2000). This is surprising, as varroa is not a problem in Brazil and the Africanized bees can be considered truly tolerant since the colonies are maintained year after year without treatment (De Jong et al., 1982; Corrêa-Marques and De Jong, 1998). Some sort of difference would be expected if this character (percentage damaged mites) really represents the degree of tolerance of honey bees to varroa. Therefore, even though some studies have demonstrated an inverse relationship between percent damaged mites and mite infestation rate (Büchler, 1993; Arechavaleta-Velasco and Guzmán-Novoa, 2001) this character cannot explain the tolerance of Africanized bees in Brazil, when we compare them with European bees in Germany.

The mites found on the hive floor may be dead or alive, and they vary in the degree of damage. Corrêa-Marques et al. (1994, 2000) classified the types of damages into six categories, showing that mites with damaged legs were more frequent than the other kinds. According to Ruttner and Hänel (1992) damaged legs are most indicative of an active defense against varroa. We found similar percentages of mites with only damaged legs (about 47%) in the Carniolan and the Italian bees we examined in Germany. The total frequency of mites with damaged legs, including those that also had damaged or deformed dorsal shields, was about 54% in the Italian and Carniolan colonies. However, in a study of Africanized bees in Brazil about 72% of the mites had damaged legs (Corrêa-Marques et al., 2000). Thus, the percentage of damaged mites that had leg injuries was significantly greater in the tolerant Africanized bees in Brazil than in the apparently susceptible European bees we studied in Germany (Table 2, the present study and Table 3, Corrêa-Marques et al., 2000; chi-square test, $P<0.01$). As the mite damage analysis was made by the same person under the same conditions in both studies, we conclude that there is a real difference in the percentage of damaged mites that have damaged legs. The "tolerant" Africanized honey bee colonies in Brazil had a greater percentage of fallen damaged mites with leg injuries than did the European colonies (both Carniolan and Italian) in Germany.

Another strong difference in the damage in these two studies was the percentage of damaged mites that had only an indentation (or hollow) in the dorsal shield. This class comprised 37-42% of the damaged mites in the European colonies we examined in Germany (Table 2) but less than 16% of the damaged mites taken from Africanized colonies in Brazil (Corrêa-Marques et al., 2000) (chi-square test, $P<0.01$).

In conclusion, the Italian bees from the island of Fernando de Noronha in Brazil and the German Carniolan bees were similarly affected by varroa infestations when these two types of bees were tested together in Germany. Whatever tolerance mechanisms these bees may have developed in Brazil (De Jong and Soares, 1997) they were not effective in Germany. The percentage damaged mites among those found on the hive floor does not appear to be a good indicator of tolerance levels, since similar percentages of damage were found in the European bees in Germany when compared to Africanized bees kept in Brazil, even though varroa kills virtually all untreated colonies in Germany, and is relatively innocuous in Brazil. However, differences in the rates of specific types of damage to the mites, such as damaged legs, may be indicative of tolerance capabilities by the bees.

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REFERENCES

Anderson, D. and **Trueman, J.W.H.** (2000). *Varroa jacobsoni* (Acari: Varroidae) is more than one species. *Exp. Appl. Acarol. 24*: 165-189.

Arechavaleta-Velasco, M.E. and **Guzmán-Novoa, E.** (2001). Relative effect of four characteristics that restrain the population growth of the mite *Varroa destructor* in honey bee (*Apis mellifera*) colonies. *Apidologie 32*: 157-174.

Bienefeld, K., Zautke, F., Pronin, D. and **Mazeed, A.** (1999). Recording the proportion of damaged *Varroa jacobsoni* Oud. in the debris of honey bee colonies (*Apis mellifera*). *Apidologie 30*: 249-256.

Büchler, R. (1993). Rate of damaged mites in natural mite fall with regard to seasonal effects and infestation development. *Apidologie 24*: 492-493.

Corrêa-Marques, M.H. and **De Jong, D.** (1998). Uncapping of worker bee brood, a component of the hygienic behavior of Africanized honey bees against the mite *Varroa jacobsoni* Oudemans. *Apidologie 29*: 283-289.

Corrêa-Marques, M.H., Issa, M.R. and **De Jong, D.** (1994). Estudo dos danos causados pelas abelhas africanizadas ao ácaro *Varroa jacobsoni*. *Anais do IV Congresso Iberolatinoamericano de Apicultura*, Córdoba, 97-100.

Corrêa-Marques, M.H., Issa, M.R.C. and **De Jong, D.** (2000). Classification and quantification of damaged *Varroa jacobsoni* found in the debris of Africanized honey bee colonies as criteria for selection? *Am. Bee J. 140*: 820-824.

De Jong, D. (1997). Varroa and other parasites of brood. In: *Pests, Predators and Diseases of Honey Bees* (Morse, R.A., ed.). 3rd edn. A.I. Root Co., Medina, OH, USA, pp. 231-279.

De Jong, D. and **Gonçalves, L.S.** (1998). The Africanized bees of Brazil have become tolerant to Varroa. *Apiacta 33*: 65-70.

De Jong, D. and **Soares, A.E.E.** (1997). An isolated population of Italian bees that has survived *Varroa jacobsoni* infestation without treatment for over 12 years. *Am. Bee J. 137*: 742-745.

De Jong, D., Roma, D.A. and **Gonçalves, L.S.** (1982). A comparative analysis of shaking solutions for the detection of *Varroa jacobsoni* on adult honey bees. *Apidologie 13*: 297-306.

Engels, W., Gonçalves, L.S., Steiner, J., Buriolla, A.H. and **Issa, M.R.C.** (1986). Varroa-Befall von Carnica-Volkern in Tropenklima. *Apidologie 17*: 203-216.

Guerra, J.C.V., Gonçalves, L.S. and **De Jong, D.** (2000). Africanized 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. *Genet. Mol. Biol. 23*: 89-92.

Liebig, G. (1997). Breeding aim, Varroa resistance - More than a beekeeper's wishful thinking? *Am. Bee J. 137*: 657-659.

Moosbeckhofer, R. (1997). Observations on reproduction rate of *Varroa jacobsoni* and the occurrence of mutilated mites in *Apis mellifera carnica* colonies. *Apidologie 28*: 193-195.

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

Moretto, G., Gonçalves, L.S. and **De Jong, D.** (1993). Heritability of Africanized and European honey bee defensive behavior against the mite *Varroa jacobsoni*. *Rev. Bras. Genet. 16*: 71-77.

Morse, R.A., Miksa, D. and **Masenheimer, J.A.** (1991). Varroa resistance in U.S. honey bees. *Am. Bee J. 131*: 433.

Ritter, W. (1981). Varroa disease of the honeybee *Apis mellifera*. *Bee World 62*: 141-153.

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

Rosenkranz, P., Fries, I., Boecking, O. and **Stürmer, M.** (1997). Damaged *Varroa* mites in the debris of honey bee (*Apis mellifera* L) colonies with and without hatching brood. *Apidologie 28*: 427-437.

Ruttner, F. and **Hänel, H.** (1992). Active defense against Varroa mites in a Carniolan strain of honeybee (*Apis mellifera carnica* Pollmann). *Apidologie 23*: 173-187.

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