

Analysis of Mortality in Africanized Honey Bee Colonies with High Levels of Infestation by *Varroa destructor*

by

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ABSTRACT

The mite *Varroa destructor* (Anderson & Treuman 2000) is one of the world's most important plagues of apiculture. In Brazil this mite does not encounter good conditions for parasitism because weather conditions are not ideal for its maintenance, and some strains of Africanized honey bees are resistant to the parasite. This status is reflected in the low number of dead colonies caused by varroatosis and also the stability of infestation levels. The aim of this study was to evaluate the damage caused by mite infestations in hives with higher levels of infestation than the ones considered normal for Brazilian apiaries. The level of infestation in each colony was determined and the mortality rates of parasitized bees during development were periodically recorded. The G Test of Independence and a Test of Proportions were used to compare the data. The rates of mortality of pupae and larvae were mostly proportional to the level of infestation in each colony. All colonies showed mortality rates significantly higher than the control one. In Africanized honeybee colonies with high rates of infestation by *Varroa destructor* mortality rates varied from 19.27% to 23.28% in pupae ($\bar{X} = 21.27\%$) and from 15.71% to 16.15% in larvae ($\bar{X} = 15.93\%$), against 3.85% and 3.74% in the control colony, respectively. In the parasitized colonies the average rates of mortality caused by the hurtful effects of the mite were, respectively, 5.52 and 4.26 times greater in those two developmental stages. Thus it can be concluded that even in tropical regions, like Brazil, it is necessary to give special attention to the levels of mite infestation (IR), particularly where the IR tends to be higher.

Keywords: Africanized honeybees, *Apis mellifera*, *Varroa destructor*, mortality analysis, development.

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INTRODUCTION

Varroa jacobsoni Oudemans was described as an ectoparasitic mite of the Eastern honeybee, *Apis cerana* Fabricius. This parasite is incapable of reproducing on *Apis mellifera* brood (Anderson 1994, Anderson & Sukarsih 1996). The mite that parasitizes the Western honeybees (*Apis mellifera* Fabricius) presents size variations and is a different species, renamed as *Varroa destructor* (Anderson & Trueman 2000).

This new species started causing serious damages to these bees and consequently to apiculture (Ilfantidis & Rosenkranz 1988, Erickson *et al.* 1994, Donze' & Guerin 1994, Anderson & Trueman 2000). The extensive damage caused by *Varroa destructor* is often related to the brief period of coevolution between this parasite and the new host, since, except for African and Africanized honeybees, the other races of *Apis mellifera* did not develop an effective defense behavior against the mite (De Jong 1984, De Jong *et al.* 1984, Moretto *et al.* 1993, De Jong 1997, Boecking & Genersch 2008).

The varroatosis, as the parasitism is called, causes serious harm to the developing bees as well as the adults (Delfinado-Baker *et al.* 1992, De Jong 1997, Beestma *et al.* 1999). As a consequence of parasitism during development, newly emerged honeybee workers present reduced weight, wing deformations and changes in several other appendices (mainly the legs), besides a significant decrease in the size of the abdomen. Other signs, such as hypopharyngeal gland malformation and decreased life span (in adults) are also commonly found (De Jong *et al.* 1982, Schneider & Drescher 1987, Schatton-Gadelmayer & Engels 1988, Beestma *et al.* 1989, Bowen-Walker & Gunn 2001, Romero-Vera & Otero-Colina 2002, Garedew *et al.* 2004, Genersch 2005, Kralj & Fuchs 2006). Furthermore, the mites act as a vector for the transmission of some viruses, for example, DWV (Deformed Wing Virus) (Ball 1988, Martin 1998, Bowen-Walker *et al.* 1999, Martin 2001, Tentcheva *et al.* 2006).

Some researchers relate the *Varroa* mite to CCD (Colony Collapse Disorder) and van Engelsdorp *et al.* (2009) suggested that this syndrome originates from an interaction between pathogens and other factors that cause stress to the colony, whereas the mite could suppress some immune responses of its host. According to Le Conte *et al.* (2010) the hypothesis that CCD is due to the parasitic mite is feasible and, indeed, is reinforced by studies carried

out by van Engelsdorp *et al.* (2009). CCD was first reported in colonies of *A. mellifera* in the U.S.A. Interestingly, at the time of collapse, the infestation levels of *Varroa* had not reached levels known to cause economic damage or declining populations (van Engelsdorp *et al.* 2009). Considering this information, we may conclude that *Varroa* can play an important role as a cause of CCD even in Brazil, where the infestation rates by the mite are not commonly high.

Considering this worldwide scenario of damages, controlling the mite has become necessary. But the use of acaricides has shown no satisfactory results because of important drawbacks such as contamination of bee products and resistant mite lineages (Milani 1995, 1999, Hillesheim *et al.* 1996, Jacobs *et al.* 1997, Elzen *et al.* 1998, 2000, Wallner 1999, Bogdanov 2006, Martel *et al.* 2007).

African honeybees and their hybrids (Africanized Honey Bees - AHB) have greater tolerance to *Varroa destructor* infestations, with few reports of major damage (De Jong *et al.* 1984, Montiel & Piola 1976, Gusman-Novoa *et al.* 1999).

In Brazil, where AHB are commonly used, the levels of infestation have remained stable since 1978 (between 2% and 3%, reaching 5% in some apiaries) (Gonçalves 1986, Rocha & Almeida-Lara 1994), but as shown by Mattos & Chaud-Netto (2011) even at those levels of infestation the death rates of developing bees could be 2.28 times greater (for pupae) to 2.65 times greater (for larvae) than those found in less-infested colonies.

So, it is apparent that new information and research about the relationship between the mite and its host, such as the harm caused by the parasite, are very important to the development of new methods of controlling and preventing varroatosis.

The aim of this study was to quantify the damages caused by mites in colonies with high levels of infestation, in relation to those considered common in tropical areas of Brazil (2-5%), by registering the loss of individuals during development.

MATERIAL AND METHODS

To quantify the level of infestation in each colony the method of Stort *et al.* (1981) was used: frames covered by bees were removed from colonies

of the apiary from UNESP Campus at Rio Claro, and the workers swept with a brush into containers load with 150 ml of 96 % alcohol (volume corresponding to about 300 bees). Next the samples were placed in a shaker for 30 minutes so that the mites still attached to the bees could be separated. The samples were then transferred to plastic containers fitted internally with a fine metallic net which separates bees and mites. All bees (NB) and mites (NM) were counted and the Infestation Rate (IR) was calculated: $IR = (NM/NB) \times 100$. This experimental procedure was repeated three times with one week interval between samples collected. Then an average of three data obtained was calculated in order to determine the final degree of parasitism in each colony, making possible the establishment of a rank of infestation.

Three colonies were chosen from the rank of degree of infestation, two of which had levels of infestation higher than values usually found in Brazilian apiaries (between 2% and 5%). The colony named C1 presented $IR = 15.91\%$, while the colony C11 had $IR = 10.96\%$. The colony that showed the lowest degree of infestation (C20: 0.20%) was used as a control.

The experiment began with the introduction of an empty comb with a demarcated area in each colony. That area contains approximately 500 brood cells. After the queens performed postures in the demarcated area, the combs were periodically inspected in order to follow the bees' development. The technique described by Garófalo (1977) was used to quantify the loss of developing bees.

The results were analyzed using the software BioStat 5.0 (Ayres *et al.* 2007). The binomial statistical test (Two Proportions) and the G Test of Independence were used to compare the data.

RESULTS AND DISCUSSION

The results showed that the rate of mortality of pupae was proportional to the degree of infestation in each colony, i.e., colonies with major rates of infestation presented higher frequencies of individuals died in the pupa stage (Fig. 1). The G Test of Independence indicated a significant interaction between the mortality of pupae and the infestation rates (Test G = 117.33; $p < 0.0001$; Test-G (Williams) = 117.04; $p < 0.0001$).

In the colony used as control (C20) the experiment was performed during three complete cycles of development of *Apis mellifera* workers (Table 1; Fig.

3). In this period 1220 pupae were observed, but 47 did not complete their development, which represents 3.85% loss.

Colony C11 was studied by a period of two cycles for worker brood (Table 2; Fig. 4). From 638 pupae observed in the experiment, 123 did not survive (19.27%). The test of proportions indicated a significant difference between the mortality rates of pupae obtained for the control colony (C20) and colony C11 ($Z = 10.95$; $p < 0.0001$).

In colony C1, with the highest infestation rate, a proportional death rate for pupae was observed (Table 3; Fig. 5). During three cycles of development 1224 pupae were observed, and 285 of them did not complete the cycle (23.28%). A significant difference between the mortality rates of pupae obtained for the control colony (C20) and the colony C1 ($Z = 14.02$; $p < 0.0001$) was detected.

The tests concerning the death of larvae showed a similar trend to those of pupae, i.e., the colony with the higher rate of infestation showed the higher

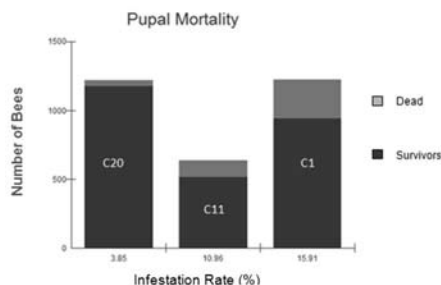


Fig 1. Number of dead pupae (grey) recorded in Africanized honeybee colonies infested by *Varroa destructor*.

Table 1. Percentage survival during each immature stage in colony C20.

		1° Cycle	2° Cycle	3° Cycle
No. Eggs		386	414	420
Days	0-1	100%	100%	100%
	1-2	98.7%	98.55%	100%
	2-3	99.47%	94.85%	99.52%
	3-4	99.2%	100%	99.76%
Survival		97.4%	93.47%	99.28%
Larvae		376	387	417
Days	0-1	100%	99.48%	100%
	1-2	99.73%	97.4%	99.52%
	2-3	96%	99.2%	98.79%
	3-4	99.72%	99.46%	99.26%
Survival		95.47%	95.6%	97.12%
Pupae		359	370	405
Days	0-12	95.26%	98.91%	94.09%
No. Adults produced		342	366	379
Survival		88.6%	88.4%	93.58%

frequency of dead larvae (Fig. 2). The G test of independence confirmed the existence of a significant interaction between the mortality of larvae and the

number of mites (Test G = 73.85; $p < 0.0001$; Test-G (Williams) = 73.64; $p < 0.0001$).

In colony C20 (control group), 1229 worker larvae were followed for three cycles of development (Table 1; Fig. 3). In this period, 46 dead larvae were recorded, corresponding to a mortality rate of 3.74%.

In C11 439 larvae were registered and 69 died before completing their development (15.71%). The test of proportions indicated a significant difference between the mortality rates of larvae obtained for C11 and C20 colonies ($Z = 7.39$; $p < 0.0001$). (Table 2; Fig. 4).

In C1 984 larvae were observed (Table 3; Fig. 5), but 159 of them did not survive (16.15%). A significant difference between the mortality rates of larvae obtained for C1 and C20 colonies ($Z = 8.72$; $p < 0.0001$) was also detected.

Survival tables were made with basis on the total number of eggs laid in the observation area (Garófalo 1977). Tables 1, 2 and 3 show the percentage of survivors during the stages of development in each colony. In those tables day 3 – 4

was included in the egg stage and day 5 – 6 was included in the larval stage in order to show the mortality in these periods (considering that the mor-

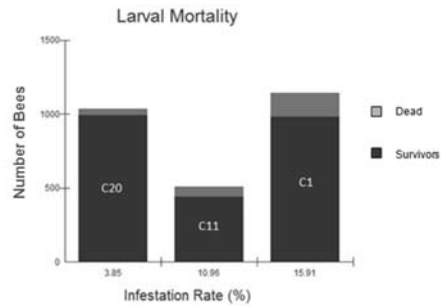


Fig. 2. Number of dead larvae (grey) recorded in colonies infested by *Varroa destructor*.

Table 2. Percentage survival during each immature stage in colony C11.

		1° Cycle	2° Cycle
No. Eggs		240	494
Days	0-1	100%	100%
	1-2	98.75%	99.79%
	2-3	100%	99.59%
	3-4	100%	99.79%
Survival		98.75%	99.19%
Larvae		237	490
Days	0-1	97.04%	100%
	1-2	95.65%	97.75%
	2-3	91.81%	99.58%
	3-4	96.53%	98.95%
Survival		82.17%	95.71%
	Pupae	189	469
Days	0-12	48.14%	94.66%
	No. Adults produced	91	444
Survival		37.91%	89.87%

phological alterations to the next stage had not already occurred) (Garófalo 1977). The duration of the pupae stage was considered to be 12 days, as Garófalo (1977) said it is the most common duration. In all tables the frequency of survival in each period of 24 hours was calculated, so that a general survival frequency was calculated in each stage of development. At last the number of adults produced was compared with the number of eggs laid and the final frequency of survival was calculated. Figs. 3, 4 and 5 show the survival curves during those stages, in each colony.

The G Test of independence was used to verify if there was

Table 3. Percentage survival during each immature stage in colony C1.

		1° Cycle	2° Cycle	3° Cycle
No. Eggs		487	373	364
Days	0-1	98.35%	99.73%	100%
	1-2	98.74%	100%	97.52%
	2-3	99.15%	98.65%	99.15%
	3-4	100%	99.18%	99.71%
Survival		96.3%	97.58%	96.42%
Larvae		469	364	351
Days	0-1	100%	99.17%	100%
	1-2	89.97%	98.33%	97.43%
	2-3	94.07%	96.05%	93.56%
	3-4	98.99%	98.24%	97.18%
	4-5	96.69%	99.7%	100%
Survival		81.02%	89.54%	88.6%
Pupae		380	334	311
Days	0-12	74.47%	78.14%	63.02%
	No. Adults produced		283	261
Survival		58.11%	69.97%	53.84%

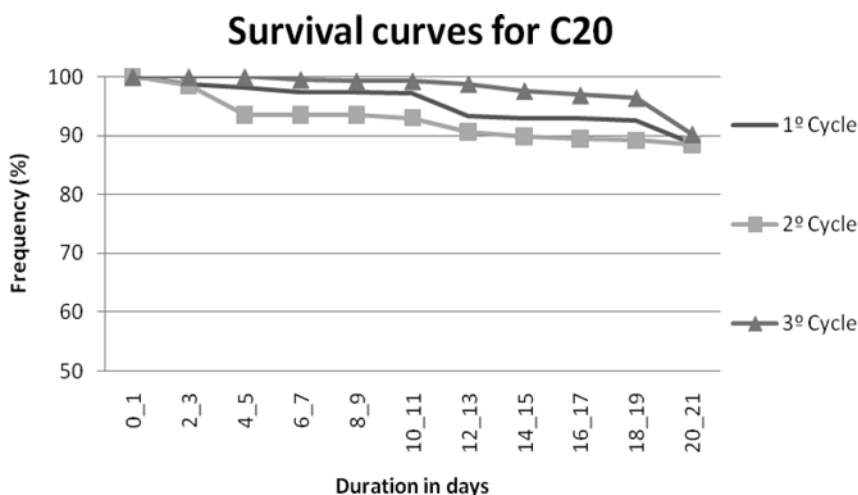


Fig. 3. Survival Curves for immature stages in colony C20.

any relationship among the total number of dead larvae and the number of larval deaths that occurred between the 3rd and 4th days of larval life. This interval of days was characterized by Laidlaw *et al.* (1956) as the period of main effects of inbreeding. The statistical analysis indicated no significant

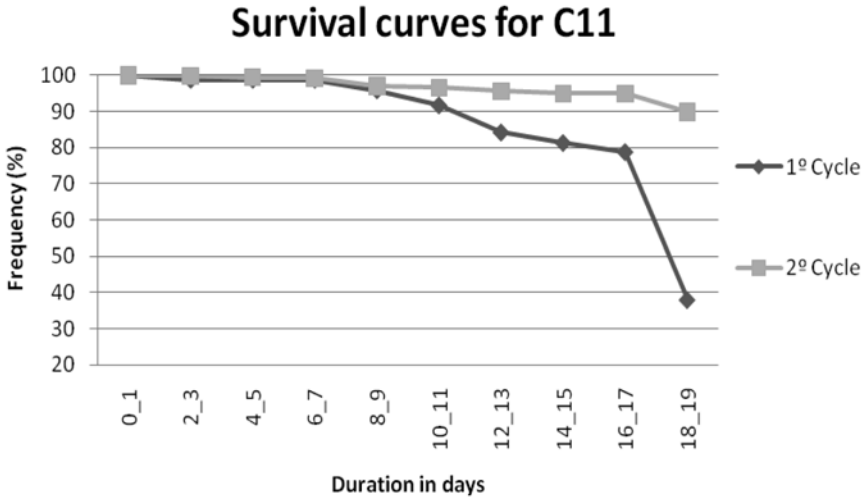


Fig. 4. Survival Curves for immature stages in colony C11.

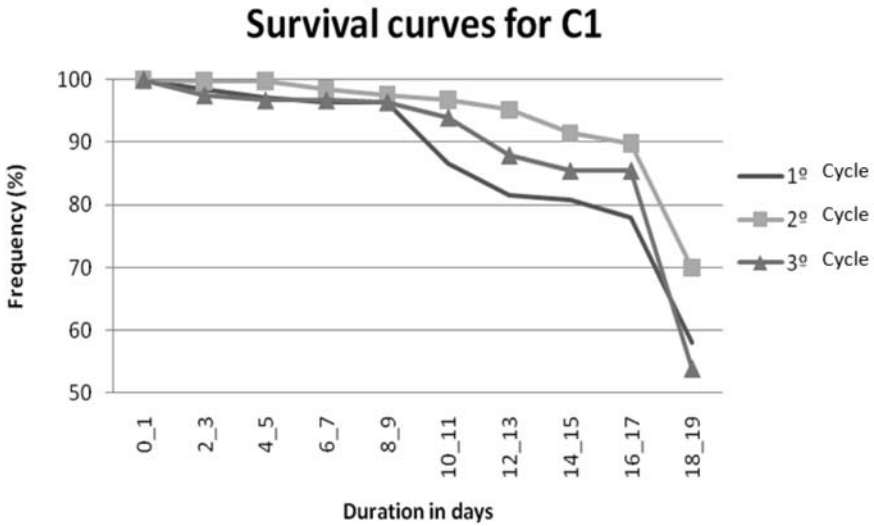


Fig. 5. Survival Curves for immature stages in colony C1

interaction among the parameters tested (G Test= 11.86, P= 0.1050; G Test (Williams) = 11.21, P= 0.1297).

Laidlaw *et al.* (1956) observed that in colonies with low genetic variability (major inbreeding) the developing bees show higher rates of mortality on the 3rd and 4th days of larval life. Mattos & Chaud-Netto (2011) have shown similar correlation on data obtained for lower IR of AHB (between 2% and 5%). In the present research the G Test of Independence did not indicate a significant difference between the number of dead larvae and the number of larvae which died on the 3rd and 4th days of life (inbreeding effect). So it can be concluded that the deaths occurred during the period cited by Laidlaw *et al.* (1956) did not contribute significantly to the total number of larval deaths.

CONCLUSION

The results obtained in this research revealed that in AHB colonies parasitized by *Varroa destructor* mortality rates of larvae under conditions of high infestation ranged from 15.71% to 16.15% ($\bar{X} = 15.93\%$). In the case of pupae, the frequencies of dead bees varied from 19.27% to 23.28% ($\bar{X} = 21.27\%$). Considering that in the control group the rate of dead bees was 3.74% in the larval stage and 3.85% in the pupal stage, it can be deduced that in the infested colonies the average rates of mortality caused by the harmful effects of the mite were, respectively, 4.26 times and 5.52 times greater in those two developmental stages. This implies a significant loss of developing bees in the colony and consequently a lower number of adults produced, which could be reflected directly on hive productivity. It proves that high IR could be significantly detrimental for beekeeping.

Thus it can be concluded that even in tropical regions, like Brazil, it is necessary to devote special attention to the levels of mite infestation (IR), particularly when the IR tends to be higher such as in winter, or in the case of susceptibility to the mite or weakening of the colony (caused by any reason).

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