

## The ectoparasite mite *Varroa destructor* Anderson and Trueman in southeastern Brazil apiaries: effects of the hygienic behavior of Africanized honey bees on infestation rates

[O ácaro ectoparasita *Varroa destructor* Anderson e Trueman em apiários do sudeste brasileiro: efeito do comportamento higiênico de abelhas africanizadas nas taxas de infestação]

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### ABSTRACT

In Brazil, the ectoparasitic mite of bees *Varroa destructor* Anderson and Trueman (Acari: Varroidae) remains at low levels of infestation causing no major damage. However, with the introduction and possible dominance of a new haplotype (*K*) of the mite, usually found in areas with high infestation rates (IR), it is necessary to monitor and select beehives that are resistant to the pest in order to avoid future problems. Several factors are listed as potentially being responsible for the dynamics of mite infestation, among which hygienic behavior (HB) stands out. In this context we sought to evaluate the HB of Africanized honey bees *Apis mellifera* L. (Hymenoptera: Apidae) compared with the mite IR in apiaries of two municipalities of southeastern Brazil (Taubaté and Viçosa). For the municipality of Taubaté, the average IR was 4.9% (3.4 to 5.8%), while the HB averaged 98.6% (96 to 100%). In Viçosa, the average mite IR was found to be 10.0% (5.4 to 21.0%) with an average value for HB of 57.7% (0 to 79.0%). Results from this research show that IR and HB were negatively correlated ( $R = -0.9627$ ,  $P < 0.01$ ), suggesting that hives with higher HB have lower IR.

Keywords: Acari, Apidae, bee pathology, pest management, Varroidae

### RESUMO

No Brasil, o ácaro ectoparasita de abelhas *Varroa destructor* Anderson e Trueman (Acari: Varroidae) se mantém em índices de infestação baixos, não causando grandes prejuízos. Todavia com a introdução e possível predominância de um novo haplótipo (*K*) do ácaro, geralmente encontrado em regiões com altas taxas de infestação (TI), faz-se necessário o monitoramento e seleção de colônias resistentes à praga, evitando problemas futuros. Vários fatores são relacionados como possíveis responsáveis pela dinâmica de infestação do ácaro, dentre os quais se destaca o comportamento higiênico (CH). Nesse contexto buscamos avaliar o CH de abelhas africanizadas *Apis mellifera* L. (Hymenoptera: Apidae) correlacionando com as TI do ácaro em apiários de dois municípios do sudeste brasileiro (Taubaté e Viçosa). Em Taubaté, o valor médio da TI foi de 4,9% (3,4 a 5,8%), enquanto que o CH foi em média 98,6% (96 a 100%). Já em Viçosa, a TI média do ácaro foi de 10,0% (5,4 a 21,0%) com valor médio de CH de 57,7% (0 a 77,0%). Resultados dessa pesquisa demonstraram que as variáveis TI e CH foram negativamente correlacionadas ( $R = -0,9627$ ;  $P < 0,01$ ), sugerindo que colônias que possuem CH mais elevado possuem menores TI.

Palavras-chave: Acari, Apidae, manejo de pragas, patologia apícola, Varroidae

### INTRODUCTION

In Brazil, apiculture is increasing in importance due to the increase in consumption of its products along with favorable environmental conditions for apiculture, and professionalization

of beekeepers, yielding products of better quality (Perosa *et al.*, 2004). With the increase in demand for hive products, more research is needed to improve productivity and to find potential pathogens that may threaten the beehives or cause losses (Perosa *et al.*, 2004).

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The emergence of several diseases and phenomena, such as Colony Collapse Disorder (CCD) has been the cause many losses to this business (Cox-Foxter *et al.*, 2007). Many factors are mentioned as causing CCD, among which parasitism by the mite *Varroa destructor* Anderson and Trueman (Anderson and Trueman, 2000) may be included.

*Varroa destructor* is an ectoparasite of adult bees and post-capped brood. It feeds from its host's hemolymph, which may disrupt development and causes death of the entire hive in high infestation rates (IR) (Rosenkranz *et al.*, 2010). The mite was initially characterized as being a parasite of *Apis cerana* F. (Hymenoptera: Apidae) in Asia (Rosenkranz *et al.*, 2010). However, after being exposed to *Apis mellifera* L. (Hymenoptera: Apidae), it rapidly spread to several countries (Bailey and Ball, 1991; Rosenkranz *et al.*, 2010). The dispersion in beehives of *A. mellifera* has deeply impacted the apiculture business causing high hive mortality rate and the need to use acaricides for mite control (Bailey and Ball, 1991; Rosenkranz *et al.*, 2010).

The intensity of the IR of the mite and the losses caused to the hives changes regionally. In Europe, the mite usually causes significant loss and acaricides are widely used (Lodesani *et al.*, 2008). However, in countries such as Brazil the IR remains at lower levels, eliminating the need to use acaricides (De Jong and Gonçalves, 1998). In Brazilian territory, the mite was first observed during the 70s in the apiaries of Ribeirão Preto (São Paulo). In the beginning high IR were reported, but after a few years the parasite - host interaction seemed to have stabilized, as the average IR was around 3%, not causing significant losses to the beehives (De Jong and Gonçalves, 1998).

One of the possible reasons that explain the resistance of *A. mellifera* to the varroa in Brazil is the use of Africanized honey bees, which possibly have characteristics that favor lower mite IR (De Jong, 1996; Rosenkranz *et al.*, 2010). Several studies have indicated that the mite IR and damage is multifactorial. Biotic and abiotic factors have been shown to be significant in the infestation dynamic of the varroa in the beehives. Among these factors, one may highlight temperature (Moretto, 1997; Pinto *et al.*, 2011a), bee race (Moretto *et al.*, 1991;

Rosenkranz *et al.*, 2010), size of brood comb cells (Message and Gonçalves, 1995; Ellis *et al.*, 2009; Maggi *et al.*, 2010), mite genetic variations (haplotype) (Anderson and Trueman, 2000; Calderón *et al.*, 2010) and hygienic behavior (HB) (Peng *et al.*, 1987; Spivak, 1996; Harbo and Harris, 2009).

In general, HB is defined as the opening and removal of sick, parasitized or dead broods (Spivak, 1996; Rosenkranz *et al.*, 2010). Colonies with high HB will tend to dispose of the broods parasitized by the mite, and thus interrupt the mite's development cycle which consequently will stop the IR from increasing. HB is also highly influenced by climatic conditions (Boecking and Spivak, 1999; Harris, 2008). In this context, we evaluated the levels of HB for the Africanized honey bees in apiaries of two Brazilian municipalities (Taubaté and Viçosa), allowing the observation of the influence of this variable on the IR of the mite *V. destructor*.

## MATERIAL AND METHODS

The samples used for this study were obtained from the apiary of the Universidade de Taubaté (Taubaté, Vale do Paraíba, São Paulo, Brazil) and from the apiary of the Universidade Federal de Viçosa (Viçosa, Zona da Mata, Minas Gerais, Brazil). Five hives with a similar population were selected from each apiary, from which the samples were collected during the spring of 2009 and 2010. This season was selected for sample collection because it is characterized by a high flow of nectar and pollen availability in both municipalities.

In order to evaluate the IR, 150 adult bees were harvested from the brood area and were stored in a vessel with 98° alcohol (method adapted from De Jong *et al.*, 1982). The solution (bees + alcohol) was then agitated so that the mites would be released from the bees' bodies. Finally, the actual number of bees and mites were counted and recorded.

For the evaluation of the hives' HB, a frame with capped brood comb cells from each hive was selected. With entomological pins, 100 capped broods were carefully pin-killed without overflow of the body fluids. After this procedure the sampled area was labeled and the frame was

placed back inside the beehive in its original position. Twenty-four hours later, the labeled frames were checked and the number of clean cells were counted and recorded, then the hive HB was calculated (adapted from Newton and Ostasiewski, 1986). Pearson's Correlation was performed between HB and IR of the mite in adult bees.

## RESULTS AND DISCUSSION

The average value for IR of *V. destructor* in adult bees in apiaries of Taubaté was 4.9%, while the average value for HB was 98.6%. In Viçosa, the mean IR was 10.0% and the HB values ranged from 0 to 79% (Table 1). The hive that did not present HB had a high IR (21.0%) (Table 1). The correlation coefficient between HB and IR was  $R = -0.963$  ( $P < 0.01$ ) (Figure 1). IR values for the mite reflect the parasite - host relationship inside the beehive. Resistant bees maintain the mite population at low levels, keeping the IR from reaching values that might cause any loss to the beehives (Rosenkranz et al., 2010; Pinto et al., 2011a).

We believe that an important factor that favored the quick adaptation of the Africanized honey bees in Brazil was the initial prevalence of the haplotype *J* of the mite, usually found in regions with less damage by varroa (Anderson and Trueman, 2000; Strapazzon et al., 2009; Rosenkranz et al., 2010). Garrido et al. (2003) observed a predominance of the haplotype *K* in beehives of the municipality of Ribeirão Preto (São Paulo, Brazil). This fact may be a warning for a change in the reproductive potential of the mite, which may cause future problems to Brazilian apiculture. However, Pinto et al. (unpublished data) found IR values for the mite that varied from 0 to 5.5% in 16 municipalities from Vale do Paraíba and Serra da Mantiqueira (São Paulo, Brazil). These values are similar to those found for Taubaté (3.4 to 5.8%). It becomes evident then, that the Africanized honey bees are able to keep hive IR at low levels, as compared to other countries, even with the presence of the haplotype *K* of the mite in state of São Paulo (Brazil) (Garrido et al., 2003). However, there is the need of further research to verify the current situation of the predominant haplotypes in the region.

Table 1. Mean ( $\pm$  Standard Error) infestation rate (IR) of the mite *V. destructor* and hygienic behavior (HB) of Africanized honey bees (*A. mellifera*) in apiaries of the municipalities of Taubaté (São Paulo, Brazil) and Viçosa (Minas Gerais, Brazil) (minimum-maximum value)

	Taubaté	Viçosa
IR (%)	4.9 $\pm$ 0.42(3.4-5.8)	10.0 $\pm$ 2.84(5.4-21.0)
HB (%)	98.6 $\pm$ 0.75(96.0-100.0)	57.7 $\pm$ 14.71(0.0-79.0)

The regional variation of the reproductive potential of *V. destructor* is also one of the factors that may explain the IRs. Garrido et al. (2003) observed differences in mite fertility between Ribeirão Preto (São Paulo, Brazil) and Florianópolis (Santa Catarina, Brazil). These values were confirmed by Strapazzon et al. (2009) who found similar levels of fertility (71%) in southern Brazil, with a predominance of the haplotype *K*, which may explain the increase in fertility levels in recent years. However, the increase of reproductive potential does not seem to be enough to increase IRs of *V. destructor* in Brazil. Defense mechanisms, such as hygienic and grooming behavior tend to increase as the number of offspring decreases

and the number of mites increases in adults (Junkes et al., 2007).

Contrary to what was observed for Taubaté, we found considerably high values for IR in the municipality of Viçosa. Similar values were found by Bacha Júnior et al. (2009), who evaluated IR values for apiaries located in three municipalities of the same micro-region (Zona da Mata) where Viçosa is located. For one of the sampled municipalities, the IR varied from 4.7 to 20.2% (Bacha Júnior et al., 2009). These authors warned of a possible influence of the abundance of beehives on the dynamics of mite infestation. Regions with high concentrations of beehives, with superposing apiaries, are more prone to the mite infestation.

The availability of food can also affect the dynamics of mite infestation in the colony. The flow of nectar and pollen is closely related to the rainy periods ranging from one region to another. For example, Pinto *et al.* (2011a) observed the highest levels of mite infestation on adult bees in dry seasons. The increase in infestation of adult bees in periods of low feed can be related to lack of brood, which favors the increase of the phoretic period of the mite (Rosenkranz *et al.*, 2011). The extension of the period of parasitism because of phoresis may aggravate the effects on host fitness. Pinto *et al.* (2011b) observed decreases in the average cell area in 11% of hypopharyngeal glands of bees infested during the pupae stage. Adding long periods of parasitism in adults can affect the production of royal jelly, development of tasks and consequently the survival of the colony (Rosenkranz *et al.*, 2011, Pinto *et al.*, 2011b). However, most tropical/subtropical regions like Brazil have food available almost all year to the colonies (Barreto *et al.*, 2006), which favors the mites in the reproductive phase and during phoresis.

Several studies on IR of *V. destructor* and its reproductive potential have shown that its infestation dynamic is conditioned to several factors. Among them one may highlight HB as one of the most important (Rosenkranz *et al.*, 2010). Aimed at finding non-chemical methods to control varroa in regions with high levels of infestation, new lines of highly hygienic bees have been tested successfully. Ibrahim and Spivak (2006) analyzing highly tolerant colonies called SMR (Suppression of Mite Reproduction) found high levels of HB and mite infertility compared with common hygienic colonies. Bees with high HB are able to remove broods parasitized with the varroa, interrupting the mite's development cycle (Spivak, 1996). This might be one of the reasons for the resistance of the Africanized honey bees to the mite *V. destructor* in Brazil (Spivak, 1996; Côrrea-Marques, 1998). Africanized honey bees became adapted to Brazilian climatic conditions, spreading out to almost the entire country, except for southern Brazil due to the temperate climate (Moretto *et al.*, 1991). However, because of the

high genetic variability, the high HB might not be a characteristic that is found in all hives of *A. mellifera* in Brazil. Within the same apiary one will find large variability in HB as shown by the results presented for Viçosa (0 to 79%). The beehives evaluated in Taubaté presented the highest levels of HB; these values are considered very homogeneous as compared to those from Viçosa. This fact may be explained by the management practices applied to that apiary such as periodical queen replacement for those that were highly selected genetically to perform a high HB level.

## CONCLUSIONS

HB appears to influence the IR of varroa in the sampled hives. In the apiary of Taubaté, where the HB levels were as high as 96 to 100%, the IR varied from 3.4 to 5.8%. In Viçosa, both HB and mite IR presented high variability. Hives from the apiary of Viçosa, however, did not have queens that were genetically selected; this fact may explain the high variability, along with the abiotic factors that might also have played an important role to the levels of HB for that region. Besides the fact that the beehives own the ability to develop HB through genetic background, some sort of stimulus is still necessary for the expression of it. For this case, a small IR that would evolve to high HB for non-selected bees will launch high HB in beehives with genetically selected bees. The periodical substitution of the queens for those with high HB along with other desirable characteristics is recommended. This practice will yield in better productivity and can also be considered a preventative step against the need for acaricide applications that may contaminate hive products.

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