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***Varroa destructor* infestation levels in Africanized honey bee colonies in Brazil from 1977 when first detected to 2020**

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Abstract – The objectives of this review article were to examine the dynamics of *Varroa destructor* infestation levels in Africanized honey bees (AHB) in Brazil, since this parasitic mite was first detected in 1977. Data from published research articles, conference proceedings, congress abstracts, and unpublished data obtained from academic researchers was included. Although mite infestations varied significantly along the years, there were no indications that varroa negatively impacted Brazilian apiculture. The mean infestation levels have remained around 4.5 mites per 100 adult bees, with a median of 3.8, during the last 45 years. Adult bee and worker brood infestation rates were found to be similar, though with some geographical variation, including a tendency for higher infestations in the southern regions of the country. Various researchers have suggested that the low infestation levels could be a consequence of the tropical and subtropical climate, honey bee hybridization, grooming and hygienic behaviors, honey bee and mite genetic factors, low nutritional stress, management practices, low migratory stress, and environmental conditions. The lack of a need for chemical treatment of varroa infestations facilitates apiary management and favors organic beekeeping throughout the country. However, though AHB colonies and beekeeping in Brazil thrive without the need for treatment measures, more research should be conducted to better assess the impact that the low varroa mite infestations have on AHB colony health and productivity.

Apis mellifera / varroa / climate / nutrition / management

1. INTRODUCTION

Fifty years ago, the only major honey-producing areas not infested by varroa mites were the USA, Canada, Australia, and New Zealand (De Jong et al. 1982a). In 2020, only Australia was reportedly free of *Varroa destructor* infestation (Iwasaki et al. 2015; Roberts et al. 2017; Australian Government 2019; Brettell et al. 2020). Except for winter losses, this cosmopolitan mite is considered the main cause of honey bee colony decline for beekeeping in Europe and North America. *Varroa destructor* is also responsible for the transmission of viruses that cause diseases affecting honey bees at individual and colony levels (Genersch et al. 2010; Genersch and Aubert 2010).

Varroa was apparently introduced from Japan to Paraguay in 1971 (De Jong et al. 1982b; Traynor et al. 2020), arriving in Brazil in 1972 (De Jong et al. 1982b; Thoms et al. 2019), unwittingly transported by a beekeeper who was interested in the bees from Japan. The first infestation in Brazil was officially reported in 1977 (Morse and Gonçalves 1979), becoming a matter of concern for researchers and beekeepers.

Due to hybridization of existing populations of European honey bees (EHB) with imported African honey bees (*Apis mellifera scutellata*) beginning in 1957 (Kerr 1967), a

new poly-hybrid Africanized honey bee (AHB) emerged (Gonçalves 1974). Compared to the EHB, this new bee had many advantages, including better adaptation to the environment and greater robustness, disease resistance, and productivity (De Jong 1996). Probably the most important advantage of the AHB is tolerance to infestation by *V. destructor* (De Jong et al. 1982b; Medina-Flores et al. 2014; Oddie et al. 2018).

Various factors favorable for AHB but adverse for varroa infestation in Brazil have been studied, such as climate and bee strain (Moretto et al. 1991a), grooming (Guzman-Novoa et al. 2012; Invernizzi et al. 2015), hygienic behavior (Wielewski et al. 2012; Spivak and Danka 2021), mite haplotype (Calderón et al. 2010), shorter capped brood cell duration (Moretto et al. 1995; Rosenkranz 1999), infested brood cell inspection and re-capping (Corrêa-Marques and De Jong 1989; Martin et al. 2020), smaller comb cell dimensions, and low mite fertility in AHB worker brood cells (Calderón et al. 2010; Maggi et al. 2010).

Iwasaki et al. (2015) noted that Australia's varroa-free status is an opportunity for the research community to understand honey bee dynamics prior to varroa invasion. Likewise, the long-term low infestation levels in Brazil merit further study, as this situation provides

an opportunity to investigate the interactions between managed honey bees and *V. destructor* without interference from chemical controls.

The objectives of this review article were to examine the dynamics of *V. destructor* infestation levels (*VIL*) in Brazil from its discovery in this country in 1977 to 2020, including variability of these levels across climate regions and on adult bees versus worker brood of AHBs in Brazilian apiculture.

2. MATERIALS AND METHODS

Data were retrieved from published papers ($n = 26$), congress abstracts ($n = 32$), and unpublished studies ($n = 13$) about *V. destructor* infestations in diverse locations throughout Brazil (Figure 1), totaling 361 data sources. A database was created with the following information: sampling year, latitude and longitude, Brazilian geographic region,

and ontogenetic phase (adult bee or sealed brood). Data (Table S.M. 1 – Supplementary Material) were gathered and validated for analysis from information in publications and by contacting researchers (Figure 1).

The method used to separate and count varroa mites from adult honey bees was noted when documents were assessed; the method developed by Stort et al. (1981) was the most common method used nationwide. In brief, mites are separated from honey bees in a solution of 70% ethanol, followed by a final visual inspection to confirm complete separation of the parasites. The infestation levels were calculated by dividing the number of mites by the number of honey bees in each sample.

Varroa mites in worker brood were examined according to Gonçalves et al. (1982), by counting the number of adult female mites in brood cells uncapped with forceps in initial studies. Methods proposed by Medina and Martin (1999) and by

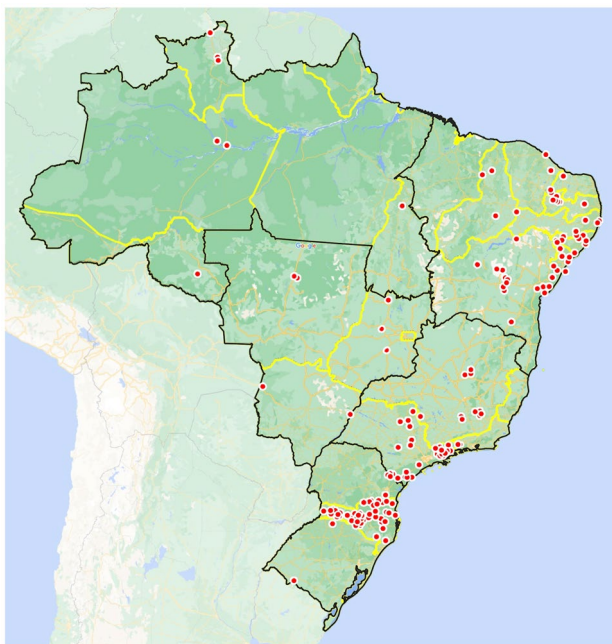


Figure 1. GPS locations where Africanized honey bee samples were collected to investigate infestation by *Varroa destructor* (red dots) from previous research (published and unpublished data). Black lines = national and regional borders. Yellow lines = state borders. Source: Cartographic base: Google Maps, 2019. Modified by authors with survey data.

Dietemann et al. (2013) were also used in later studies.

Apiaries were located by Global Positioning System (GPS), highlighted on a map (Figure 1), and described in detail (Table S.M. 1—Supplementary Material) where the 361 samples were collected from. Apiary coordinates were usually cited in the studies included, and for those without such coordinates, the official GPS location of the municipality was considered.

Varroa infestation levels were compared by sampling year, Brazilian geographic region, and ontogenetic phase (adult bees or sealed brood). All honey bee samples were collected from stationary (non-migratory) apiaries.

PERMANOVA analysis used square root transformation on data in a similarity matrix based on Euclidean distances to test the interactions between independent, qualitative, and quantitative predictors, examining their effects on the dependent variable, $VIL_{(%)}$. Anderson-Darling was used to test the normality of the response variable. Kruskal-Wallis was used to examine differences between regions, and the Wilcoxon test was used to compare the two components of the ontogenetic phase. All the analyses were performed using R software (R Core Team 2021) with a significance level set at $p < 0.05$.

3. RESULTS

Of all 361 samples analyzed (Table S.M. 1 – Supplementary Material), most were from apiaries located in the Northeast (26.6%), South (33.5%), and Southeast (32.7%) regions of Brazil; only a few studies were available from the Midwest (3.3%) and Northern (3.9%) regions (Figure 2).

As the primary objective of this research was to determine whether VIL in AHB in Brazil has changed over time, we treated it as the response variable. However, according to the Anderson-Darling test, VIL was not normally distributed, with gaps or insufficient samples in some years. There were significant effects on varroa

infestation levels of year and region of the country but not of ontogenetic bee phase (Table I).

Based on PERMANOVA analysis, one of the factors with a significant influence on VIL in honey bee colonies was year ($p < 0.0010$). Differences among years and lack of data in some years were observed (Figure 3).

The VIL on AHB in the various Brazilian geographic regions varied significantly ($p = 0.0060$; Table I). The Southeast presented the highest VIL (5.5%), though not significantly different from the VIL recorded for the Midwest (5.0%), based on the Kruskal-Wallis test (Figure 4).

When we evaluated the ontogenetic phase ($p = 0.3700$, Table I), 86.15% of the samples were from AHB adult workers (A_W), and 13.85% were from worker brood cells (W_B). There was no significant difference in VIL between these two phases ($p = 0.3459$) by the Wilcoxon test (Figure 5).

4. DISCUSSION

Significant variation in *V. destructor* infestation levels was found in AHB in Brazil, considering the factors year and Brazilian geographic region. However, no colony losses have been reported due to varroa infestations (Maggi et al. 2016; Requier et al. 2018; Castilhos et al. 2019; Peixoto et al. 2021). Also, considerable fluctuation in VIL was found; in a few years, VIL was greater than 10%, which would be considered dangerous in other regions of the world. However, damage at the colony level was not reported, despite speculation that varroa infestation levels could increase over the years due to the introduction and subsequent dominance of a more virulent mitotype of the mite (Garrido et al. 2003; Carneiro et al. 2007).

The factor year had a moderate influence on the response variable VIL ($R^2 = 0.194$; $p = 0.001$), but this may be due to variations in sample numbers and gaps in years reported, which led to limitations in data, even though missing data did not affect the validity of the PERMANOVA analysis (Figure 6).

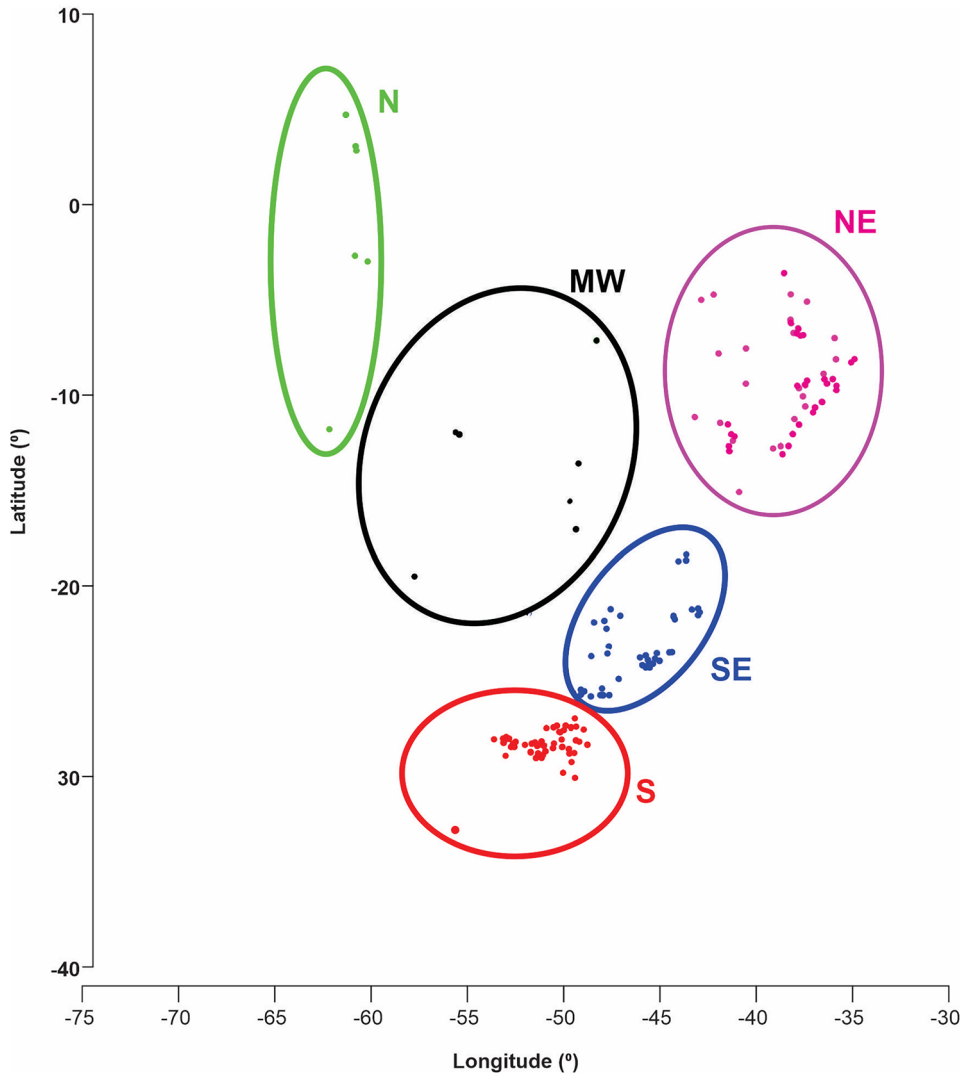


Figure 2. Distribution of studies of *Varroa destructor* infestation levels in Africanized honey bees grouped by Brazilian geographic regions based on latitude and longitude coordinates. N: North; NE: Northeast; MW: Midwest; S: South; and SE: Southeast.

The overall mean infestation level was 4.5% and median 3.8%, in this study, and *VIL* remained under 5% thanks to the confluence of many factors, according to published articles reviewed here, such as tropical and subtropical climate (Moretto et al. 1991a), honey bee Africanization (Moretto et al. 1991b; Guerra-Jr et al. 2000), grooming (Guzman-Novoa et al. 2012; Invernizzi et al. 2015) and hygienic

behaviors (Spivak and Danka 2021), mite haplotype (Calderón et al. 2010), capped cell period (Moretto et al. 1995; Rosenkranz 1999; Martin et al. 2020), brood cell dimensions and the low fertility of the mite in AHB worker brood cells (Maggi et al. 2010), AHB queen fertility and reproduction (Calderón et al. 2010), honey bee and mite genetic factors (Mendoza et al. 2020; Beaurepaire et al. 2022), low nutrition stress

Table 1 PERMANOVA analysis of effects of year, Brazilian geographic region, and ontogenetic phase (adult bee versus brood infestations) to determine their impact on *Varroa destructor* infestation levels in Africanized honey bee colonies in Brazil

Source	df	SS	MS	F	p	R ²	Significance	Perms
Year period	26	35.2010	1.3539	3.0445	0.0010	0.194	**	999
Brazilian region	4	7.2987	1.8247	4.1031	0.0060	0.048	*	999
Ontogenetic phase	1	0.34053	0.3405	0.7657	0.3700	0.002		998
Residuals	329	146.310	0.4447					
Total	360	191.730						

df degree of freedom, SS sum of squares, MS mean squares, F pseudo-F statistic, p p-value, R² size effect; significance: 0.001 “***” 0.01 “**”; Perms unique permutations

(DeGrandi-Hoffman and Chen 2015), low migratory stress (Simone-Finstrom et al. 2016), and local apiary management practices (De Jong 1996; Castilhos et al. 2021).

Nevertheless, we must consider that beekeeping management is inherently highly diverse; the large number of small-scale beekeepers limits uniform implementation of standardized best management practices (Kulhanek et al. 2021). However, there is relatively little migratory beekeeping in Brazil, which could be an advantage in facing the varroa problem.

Varroa mites survive and reproduce in AHB colonies with much less damage to the bees than in EHB colonies; however, this does not mean that

AHB are immune to the effects of the mite. Various studies have demonstrated that varroa significantly shortens the life span of AHB and reduces their size and weight (De Jong et al. 1982b; De Jong and De Jong 1983; Mattos and Chaud-Neto 2012; Koleoglu et al. 2018; Reyes-Quintana et al. 2019).

In Brazil, beekeepers do not normally use chemicals to treat varroa; instead, they rely on the ability of AHB to deal with this parasitic mite (De Jong 1996; Castilhos et al. 2021). With infestation levels remaining below 5 mites per 100 bees, beekeeping is conducted without chemically treating for varroa, which favors organic beekeeping throughout the country.

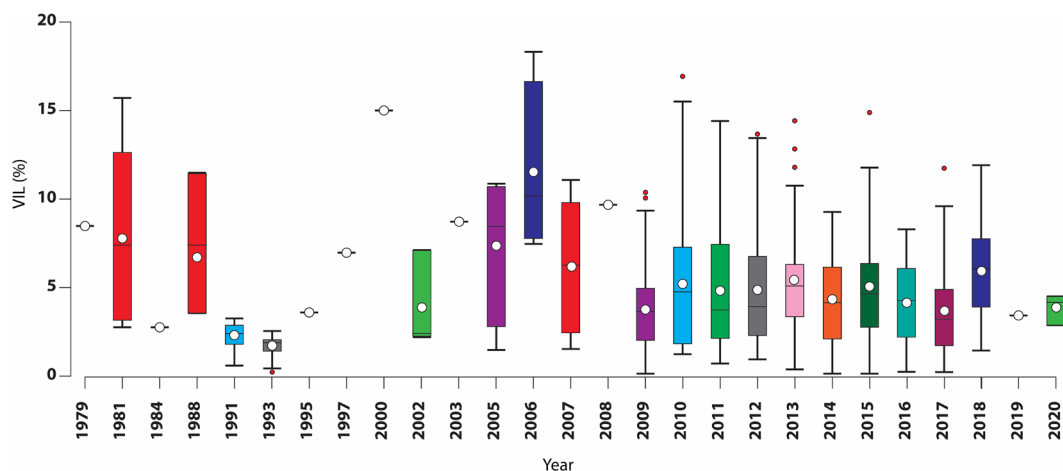


Figure 3. Varroa infestation levels in Africanized honey bee colonies in Brazil from the time varroa was first reported until 2020. Means are shown as white dots and medians as horizontal lines within the boxes.

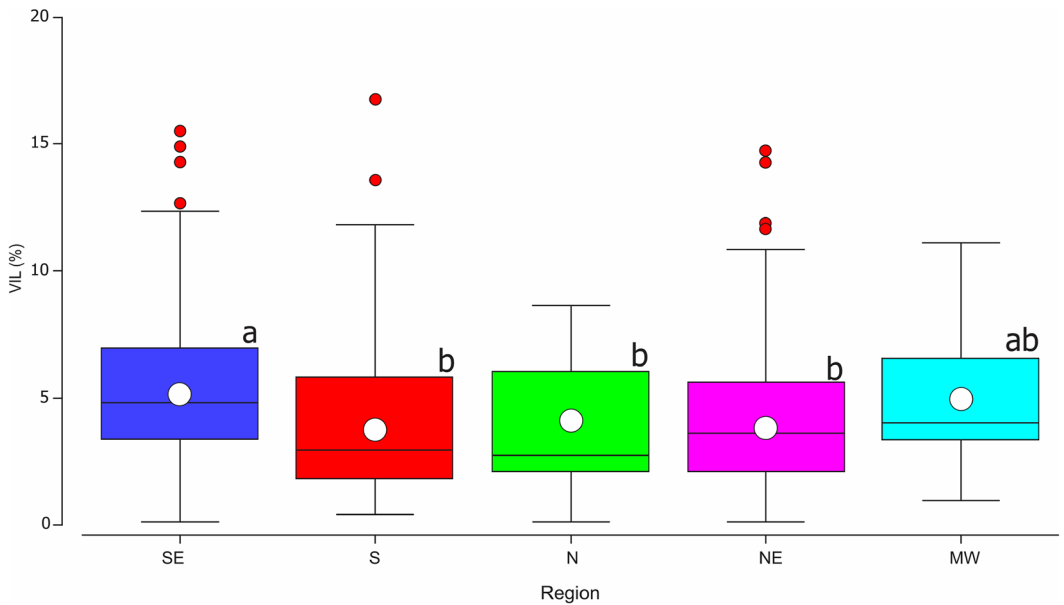


Figure 4. *Varroa* infestation levels in Africanized honey bee colonies plotted by *Geographic Region* of Brazil. Means indicated by white dots and medians by horizontal lines inside the box. Equal letters do not differ significantly according to the Kruskal-Wallis test; SE: Southeast; S: South; N: North; NE: Northeast; MW: Midwest.

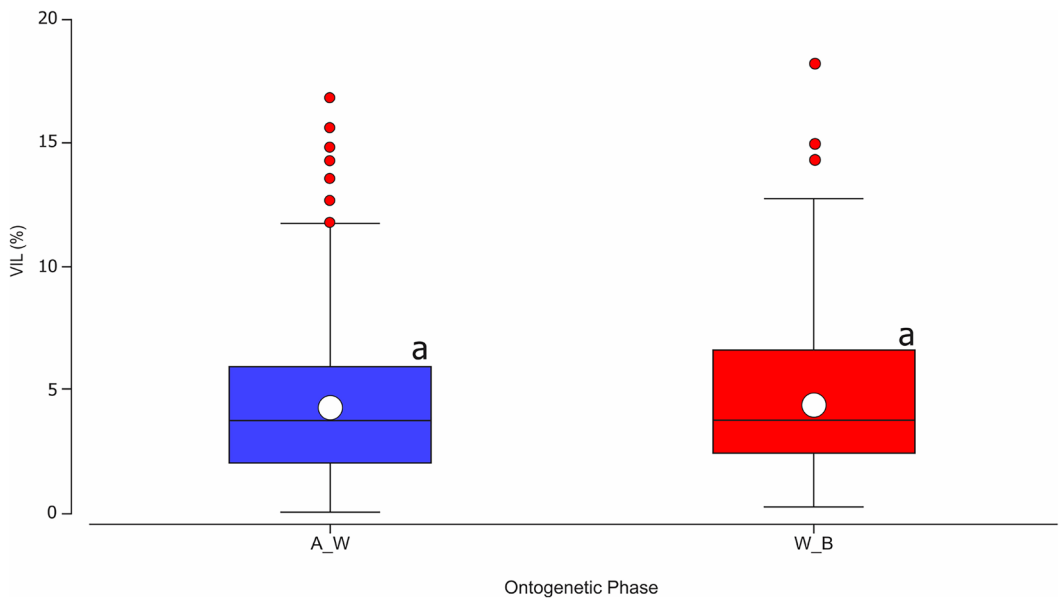


Figure 5. *Varroa* infestation levels (*VIL*) plotted by *Ontogenetic Phase* of the samples. Equal letters do not differ significantly according to the Kruskal-Wallis test; A_W, adult worker honey bee sample; W_B, worker brood bee sample.

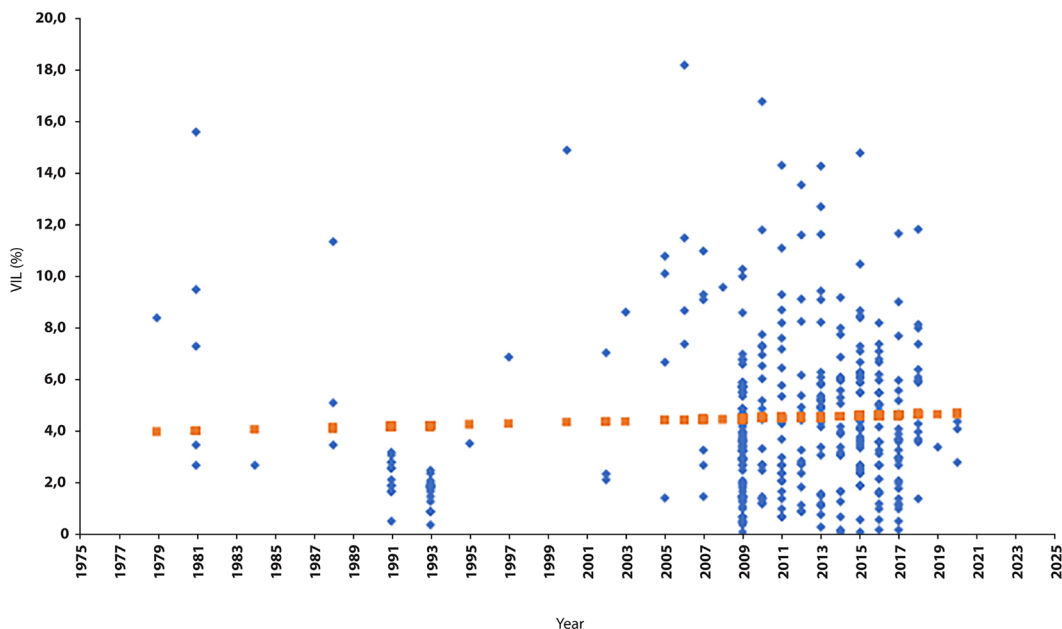


Figure 6. Year line fit plot with sample distribution of varroa infestation levels.

Nevertheless, though there is no evidence that varroa infestations cause colonies to collapse in Brazil, more research should be conducted to better assess the impact that the low varroa mite infestations have on AHB colony health and productivity.

SUPPLEMENTARY INFORMATION

The online version contains supplementary material available at <https://doi.org/10.1007/s13592-022-00984-9>.

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AUTHOR CONTRIBUTION

DC collected the data and structured the variables. GCB performed the R statistical analyses. DDJ and JK carefully edited the text. Other authors uploaded the databases. All

authors participated in writing and manuscript review. All authors read and approved the final manuscript.

AVAILABILITY OF DATA AND MATERIAL

Data collected are available at the Supplementary Material (Table S. M. 1).

CODE AVAILABILITY

Not applicable.

DECLARATIONS

Ethics approval This is an observational study. The Ethics Commission of UFERSA/RN, Brazil, has confirmed that no ethical approval is required.

Consent to participate Not applicable.

Consent for publication Not applicable.

Conflict of interest The authors declare no competing interests.

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