Can the Environment Influence Varroosis Infestation in Africanized Honey Bees in a Neotropical Region?

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Source: Florida Entomologist, 101(3) : 464-469

Published By: Florida Entomological Society

URL: https://doi.org/10.1653/024.101.0304

Can the environment influence varroosis infestation in Africanized honey bees in a Neotropical region?

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Abstract

The African-derived honey bee, *Apis mellifera scutellata* L. (Hymenoptera: Apidae), is reported to be somewhat resistant to the parasitic mite *Varroa destructor* Anderson & Trueman (Mesostigmata: Varroidae). However, this parasitic mite is considered a danger to honey bee colonies around the world and is reported to have negative effects in Brazil as well. Climate and altitude can affect the infestation levels of this mite. However, the impact of climate and altitude on Varroa infestation rates in Africanized honey bee is not well known; therefore, the goal of this study was to assess environmental effects on Varroa and honey bees in Brazil. We sampled 193 colonies across Bahia state during the Brazilian winter period, also collecting climatic information (rainfall and temperature) and altitude data for each apiary location. We used several statistical tests, such as Kruskal-Wallis, Pearson's correlation, and canonical discriminant analysis to evaluate the possible influence of the environmental variables on Varroa infestation levels. *Varroa destructor* was present in 94% of the samples, with the colonies showing infestation levels (proportion of infested bees per sample) ranging from 0 to > 20%. We found that weather and altitude in Neotropical areas have moderate degrees of influence on *V. destructor* infestation rates in Africanized honey bees. Colonies of Africanized honey bees located in areas with greater rainfall and lower altitude can have greater mite presence and infestation levels than in warmer, higher areas. Beekeepers in the lower, wetter areas should monitor their colonies regularly to detect the presence, and avoid increase, in mite infestation rates.

Key Words: *Apis mellifera*; *Varroa destructor*; honey bee ectoparasite; climate factors

Resumo

A abelha africanizada é utilizada na apicultura brasileira e é relatada como resistente ao ácaro *Varroa destructor* Anderson & Trueman 2000 (Mesostigmata: Varroidae). Este ácaro parasita é considerado perigoso para essas abelhas em todo o mundo e é apontado como responsável por perdas de colônias. Os parâmetros climáticos e altitudes podem ter um efeito importante nos níveis de infestação deste ácaro, devido ao impacto que esses fatores podem causar sobre as abelhas. No entanto, em abelhas africanizadas, o impacto dos parâmetros climáticos e altitude sobre o nível de infestação pelo varroa ainda não é bem compreendido ou estudado, e este foi o objetivo desse estudo. Foram avaliadas 193 colônias na Bahia durante o inverno brasileiro, sendo ainda coletados dados sobre parâmetros climáticos e altitude dos locais onde os apiários estavam localizados. Os parâmetros climáticos e altitude em cinco diferentes mesorregiões foram avaliados pelos testes de Kruskal-Wallis, correlação de Pearson e análise de discriminantes canônicas. O *Varroa destructor* foi encontrado em 94% das amostras avaliadas e as colônias apresentaram níveis de infestação (proporção de abelhas infestadas por amostra) variando de ausência a acima de 20%. Os resultaram apontaram que em áreas neotropicais, os parâmetros climáticos e altitude influenciam moderadamente o nível de infestação pelo *V. destructor* em abelhas africanizadas. Onde colônias localizadas em áreas com maior pluviosidade e baixa altitude podem ter maior presença e nível de infestação por esse ácaro quando comparadas com as colônias de abelhas localizadas em áreas mais altas e quentes. Os apicultores das áreas com baixa altitude, maior precipitação pluviométrica e menor temperatura devem monitorar suas colônias maior frequência para detectar e evitar o aumento no nível de infestação por esse ácaro.

Palavras Chave: *Apis mellifera*; *Varroa destructor*; ectoparasita de abelhas; fatores climáticos

Brazilian honey is produced by the African-derived honey bee, *Apis mellifera* (Hymenoptera: Apidae); this honey bee is a polyhybrid mix of European and African subspecies that was generated by accident in São Paulo State and disseminated across South America reaching as far north as the United States (Clarke et al. 2002). Today this polyhybrid is called the Africanized honey bee and is used by beekeepers across the country.

The Africanized honey bee is not free from the effects of parasites such as *Varroa destructor* Anderson & Trueman 2000 (Mesostigmata: Varroidae). The Varroa mite was introduced to South America from Japanese honey bee colonies around 1971, and first detected in Paraguay and Brazil in 1978 (De Jong et al. 1984). This mite causes varroosis, a disease that is considered the most dangerous disease for honey bees. The disease's symptoms do not have a uniform pattern and are determined by infestation level and secondary infections (Boecking & Genersch 2008). This disease is one of the main causes of colony loss around the globe (Boecking & Genersch 2008; vanEngelsdorp et al. 2009; Nazzi & Le Conte 2016; Wilfert et al. 2016). Female *V. destructor* can parasitize the honey bee during its adult and pupal stages. The mite interferes in the development and longevity of honey bees, as well as acting as a vector for pathogenic viruses (Rosenkranz et al. 2010; Martin et al. 2012; Wilfert et al. 2016).

The Varroa mite is considered to be cosmopolitan (Wilfert et al. 2016), and infestation level on honey bees around the world varies from 2.0 to 31.4% (Akyol et al. 2006; Akyol & Yeninar 2009; Mumbi et al. 2014; Muli et al. 2014; Chemurot et al. 2016; Giaco-

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bino et al. 2016; Gracia et al. 2017). The infestation levels of this mite are higher in *A. mellifera* colonies from temperate climates than colonies from subtropical climates (Giacobino et al. 2016), suggesting that climate can have an important role in the host-parasite interaction (Muli et al. 2014) and may influence the mite infestation rates, but the influence of climate parameters on this mite are unclear (Rosenkranz et al. 2010). Changes in these parameters may be a stressor for the honey bees, leading to increased vulnerability to parasites (Goulson et al. 2015). Another environmental variable that may influence Varroa infestation levels is the altitude of the apiary. This factor can influence the size of organisms (Klok & Harrison 2009), possibly affecting the development of the mite by affecting the honey bee. Also, despite that fact the Africanized honey bees in Brazil are reported to be tolerant of Varroa (De Jong & Gonçalves 1998; Rosenkranz et al. 2010), beekeepers around the country have started to report colony loss due to unknown causes. There is a lack of information about Varroa infestation levels and whether temperature, rainfall, and altitude can influence the mite presence and infestation rates in Neotropical areas. Therefore, we investigated the relationships between temperature, rainfall, and altitude with Varroa infestation rates.

Materials and Methods

The samples were collected in Bahia State, located in the northeastern region of Brazil (Fig. 1). To classify the apiary locations, we used the Bahia geographic classification (IBGE, 2017a), which divides the state into 7 mesoregions (statistical subdivision of the state). We codified the mesoregions in which apiaries were sampled as BA1 (Sao Francisco Valley, n = 24 colonies), BA2 (Middle East, n = 54 colonies), BA3 (Metropolitan of Salvador, n = 50 colonies), BA4 (South Center, n = 36 colonies), and BA5 (South, n = 29 colonies).

Each sample was composed of approximately 300 adult honey bees that were collected from each of 193 colonies by beekeepers from 71 apiaries throughout Bahia (Fig. 1). The samples were collected between Jun and Aug of 2016 (Brazilian winter) and sent to the Federal University of Reconcavo of Bahia. The honey bees were preserved in absolute alcohol (99.6%) until the honey bees and mites were separated (if mites were found). The infestation level per colony was determined by dividing the number of mites by the number of honey bees then multiplied by 100 (Dietemann et al. 2013).

Averages of 10 years of temperature and rainfall were obtained from The Brazilian Institute of Geography and Statistics (IBGE 2017a). Altitudes were obtained from the same source.

Statistical analysis

The Kruskal-Wallis test was performed to establish the differences among variables (Varroa infestation, temperature, rainfall, and altitude) in the 5 mesoregions studied. To investigate the importance and influence of each variable for the geographical mesoregions studied, we used the canonical discriminant analysis, based on the distances of Mahalanobis (1948). Canonical discriminant analysis

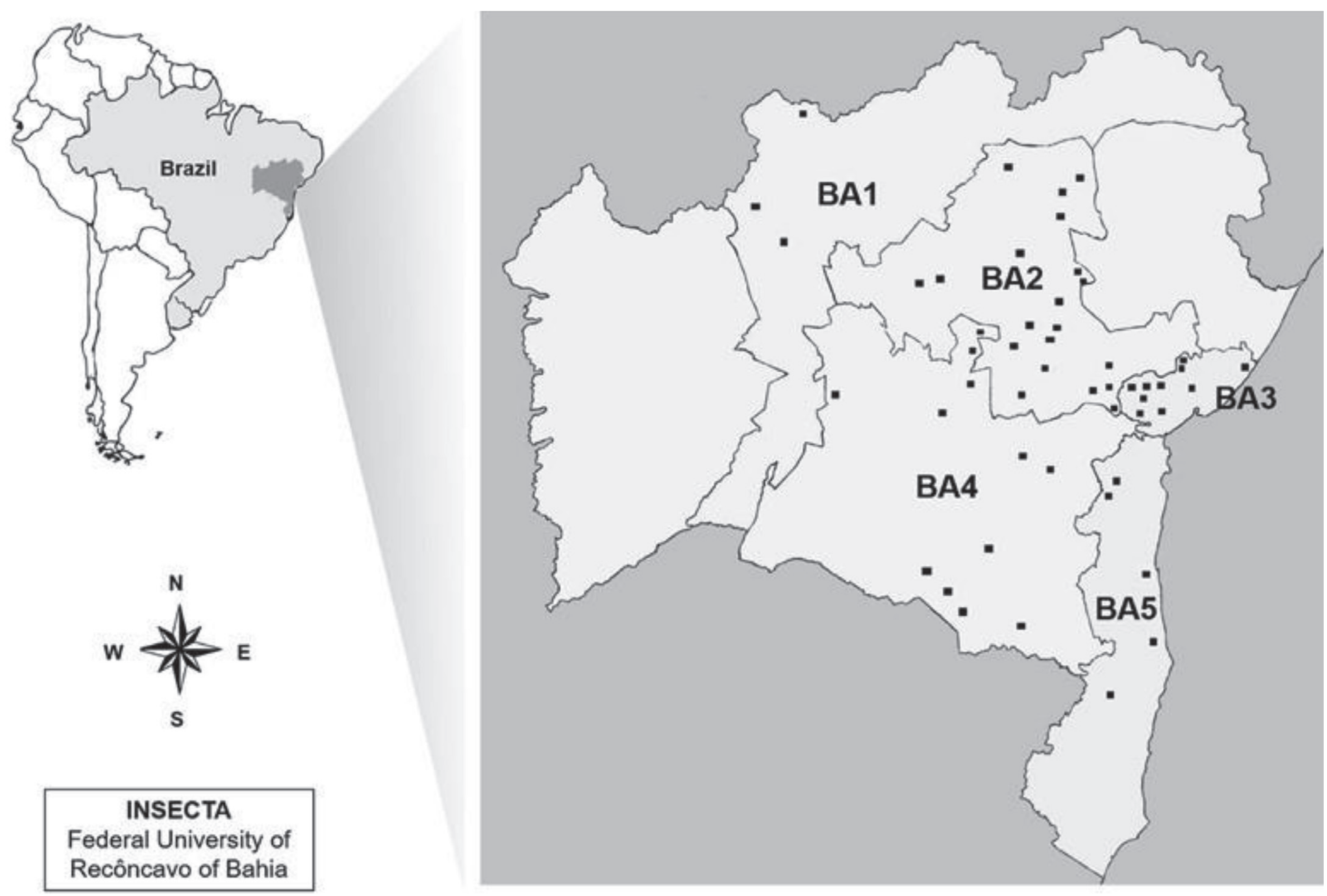

Fig. 1. Cities (black squares) where the apiaries are located from 5 different mesoregions in Bahia State, Brazil. BA1: São Francisco Valley; BA2: Middle East; BA3: Metropolitan of Salvador; BA4: South Center; BA5: South.

Table 1. Mean (± SD) of *Varroa destructor* mite infestation, temperature, rainfall, and altitude from 5 mesoregions in Bahia, Brazil.

	Mesoregion					
Treatment[a]	BA1	BA2	BA3	BA4	BA5	F
Infestation level (%)	3.0 ± 2.8b	5.18 ± 4.5a	4.65 ± 4.6ab	4.71 ± 3.5ab	6.37 ± 5.6a	0.0907
Temperature (°C)	25.3 ± 0.5a	23.16 ± 0.5c	23.96 ± 0.9b	22.42 ± 0.6c	23.93 ± 0.6b	<0.0001
Rainfall (mm)	774.4 ± 15cd	716.52 ± 11d	1369.84 ± 334b	806.40 ± 16c	1667.52 ± 26a	<0.0001
Altitude (m)	447.8 ± 40b	407.55 ± 16b	159.84 ± 162c	628.04 ± 18a	95.22 ± 16c	<0.0001

[a]Means in a row followed by different lowercase letters are significantly different by Kruskal-Wallis test. BA1: São Francisco Valley; BA2: Middle East; BA3: Metropolitan of Salvador; BA4: South Center; BA5: South.

establishes the significance of each variable across the mesoregions studied. Mahalanobis distance is based on the correlations between variables; with this, different patterns can be identified and analyzed, which is useful in determining the similarity among the different variables studied.

Before implementing the canonical discriminant analysis, Pearson's correlation among the variables was conducted to identify the variables which were not important for study. After the confirmation that all variables showed some correlation between each other from each of the geographical areas (Table 2), the data for each variable were standardized according to the following equation: , where is the standardized value of , is the average of the characteristic, and is the standard deviation. Standardization was necessary because of the presence of different units of measurement between the variables studied. The canonical discriminant analysis multicollinearity between the characteristics was studied to evaluate the linear dependence between the variables, which can result in singular or poorly conditioned matrices. The significance of the canonical discriminant analysis was evaluated by Wilk's Lambda test.

The analyses were performed using the functions *candisc*, *FactoMineR*, *vegan*, *maptools*, *maps*, *lattice*, *rgdal*, *spdep* from the R Language Development Core Team (2016).

Results

The Varroa mite was found in all 5 mesoregions studied and 94% of the 193 *A. mellifera* colonies contained this mite. The infestation level per mesoregion was > 10% and there were significant differences among regions ($P \leq 0.1$) (Table 1). The infestation rates per colony varied considerably, from 0 (6% of the colonies), 0.1 to 2.5% (31%), 2.6 to 5% (29%), 5.1 to 10% (21%), 10.1 to 15% (8%), 15.1 to 20% (4%), and > 20% (1% of the colonies).

The correlation between the variables and 5 mesoregions studied is shown in Table 2, which showed low correlation among temperature, rainfall, and altitude, and Varroa infestation in general, but each area presented different correlation levels when the rainfall, temperature, and altitude were compared independently with the infestation levels from each area studied.

Table 2. Pearson's correlation between rainfall, temperature, altitude, and *Varroa destructor* infestation level (Varroa I.) from mesoregions in Bahia State, Brazil, and total correlation between variables studied. BA1: São Francisco Valley; BA2: Middle East; BA3: Metropolitan of Salvador; BA4: South Center; BA5: South.

Mesoregions	Variables	Rainfall (mm)	Temperature (°C)	Altitude (m)	Varroa I. (%)
BA 1	Rainfall (mm)	—	−0.97	0.90	−0.50
	Temperature (°C)	−0.97	—	−0.77	0.47
	Altitude (m)	0.90	−0.77	—	−0.48
	Varroa I.L. (%)	−0.50	0.47	−0.48	—
BA 2	Rainfall (mm)	—	−0.12	0.10	0.10
	Temperature (°C)	−0.12	—	−1.22	0.31
	Altitude (m)	0.10	−1.22	—	−0.02
	Varroa I.L. (%)	0.10	0.31	−0.02	—
BA 3	Rainfall (mm)	—	0.91	0.12	−0.24
	Temperature (°C)	0.91	—	−0.02	−0.01
	Altitude (m)	0.12	−0.02	—	−0.30
	Varroa I.L. (%)	−0.24	−0.01	−0.30	—
BA 4	Rainfall (mm)	—	0.24	−0.26	0.17
	Temperature (°C)	0.24	—	−0.90	0.39
	Altitude (m)	−0.26	−0.90	—	−0.37
	Varroa I.L. (%)	0.17	0.39	−0.37	—
BA 5	Rainfall (mm)	—	0.77	−0.72	0.40
	Temperature (°C)	0.77	—	−0.75	0.40
	Altitude (m)	−0.72	−0.75	—	−0.41
	Varroa I.L. (%)	0.40	0.40	−0.41	—
Total Correlation	Rainfall (mm)	—	0.33	−0.62	0.08
	Temperature (°C)	0.33	—	−0.46	0.10
	Altitude (m)	−0.62	−0.46	—	−0.22
	Varroa I. (%)	0.08	0.10	−0.22	—

Table 3. Result of canonical discriminant analysis on temperature, rainfall, and altitude on *Varroa destructor* infestation rates from 5 mesoregions in Bahia, Brazil.

Function	CR[1]	DF[2]	F[3]	Eigenvalue	Percent	C%[4]	*P* value
1	0.89	16	47.82	4.72	80.30	80.30	< 0.001
2	0.48	9	21.61	0.91	15.54	95.84	< 0.001
3	0.19	04	10.90	0.23	03.97	99.82	< 0.001
4	0.01	01	01.98	0.01	00.17	100.00	> 0.001

[1]Canonical correlation; [2]Degrees of Freedom; [3]Approximate F; [4]Cumulative percent.

Three statistically significant discriminant functions were formed (Table 3) with the variables. The first canonical discriminant function explained 80.3% of the total data variance, while the second canonical discriminant function explained 15.5% of the total variance, and the third explained 3.9%. The first 2 canonical functions were enough to explain 95.8% of total variance (Figs. 2, 3).

The Mahalanobis quadratic distances matrix and probabilities of significant effects by the F test ($P < 0.001$) showed that the BA1 and BA5 mesoregions were the most dissimilar regions, while the most similar were the BA2 and BA4 mesoregions (Table 4, Fig. 2). The BA3 and BA5 mesoregions were discriminated from the other mesoregions by the discriminant function 1, influenced by rainfall and *V. destructor* infestation rates with high positive values (Figs. 2, 3). The BA2 and BA4 mesoregions were not separated by the canonical discriminant function 1, presenting high values for altitude and low values to temperature, rainfall, and *V. destructor* infestation (Figs. 2, 3). The BA3 (1.889) and BA5 (3.436) mesoregions were highlighted in the canonical discriminant function 1, while the BA1 (1.198) mesoregion was the region that contributed positively to the discriminant function 2 (Fig. 3).

Discussion

The Varroa infestation level can be considered low, because the highest average infestation level was 6.4% (Table 1). However, the high incidence of occurrence (94%) of this parasite in the Africanized honey bee colonies studied can be important because honey bee colonies with colony collapse disorder symptoms also can present low infestation rates (vanEngelsdorp et al. 2009). Mite infestation per mesoregion was variable, ranging from 0 to > 20%, showing that some colonies can be less tolerant. According to Medina-Flores et al. (2014), the varroa mites should benefit from tropical areas because honey bees can produce brood all year, which should allow the mite to reproduce constantly, so the mite level could be higher. Despite this, the Africanized honey bees in the areas studied are under different climate and altitude conditions (as shown in Table 1), and the variation in those variables can affect morphophysiological and behavioral characteristics in the honey bees, which can develop regional ecotypes (Meixner et al. 2010). These honey bee ecotypes may be the explanation for the variation of Varroa infestation rates in the areas studied and in other

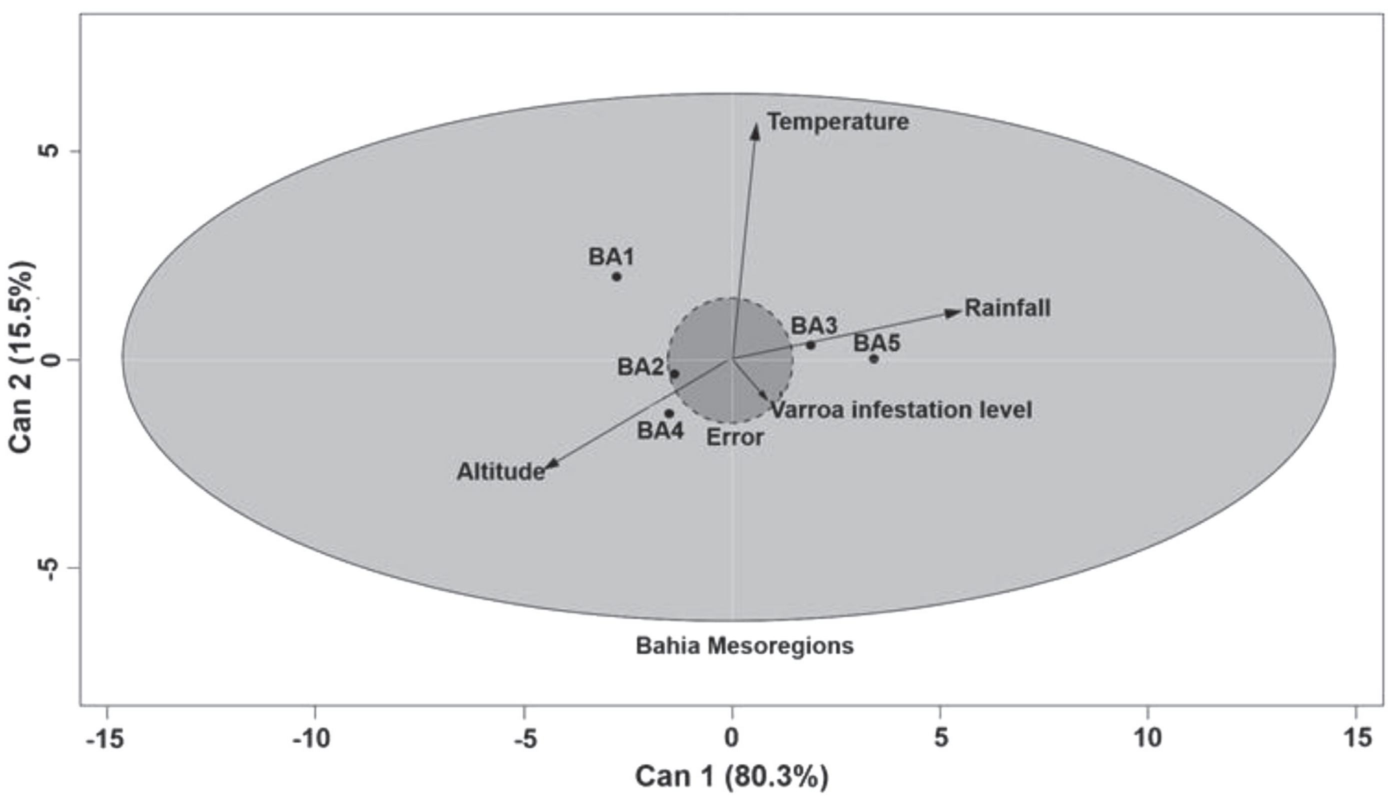

Fig. 2. Biplot of canonical discriminator of temperature (°C), rainfall (mm), altitude (m), and *Varroa destructor* infestation level (%) from Bahia State mesoregions, Brazil. BA1: São Francisco Valley; BA2: Middle East; BA3: Metropolitan of Salvador; BA4: South Center; BA5: South.

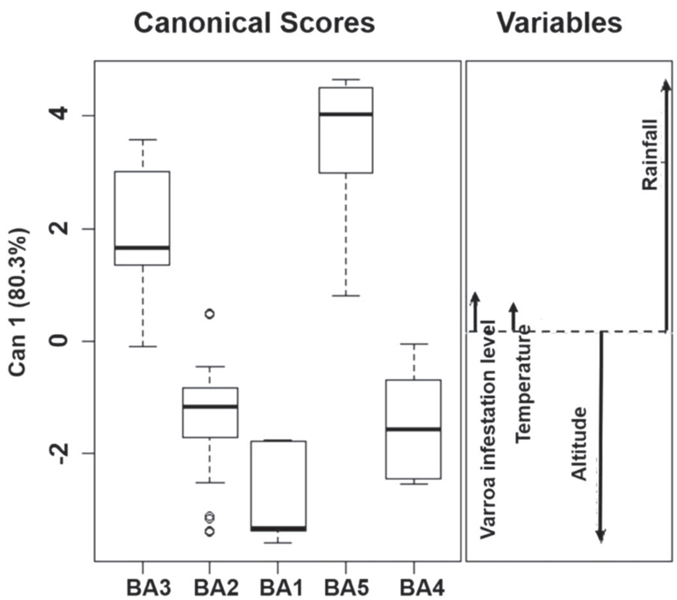

Fig. 3. Contribution of the variables in the first canonical discriminant function from temperature (°C), rainfall (mm), altitude (m), and *Varroa destructor* infestation level (%) from 5 different mesoregions in Bahia State, Brazil. BA1: São Francisco Valley; BA2: Middle East; BA3: Metropolitan of Salvador; BA4: South Center; BA5: South.

countries, such as Mexico and Costa Rica, where Africanized honey bees are apparently less tolerant to this mite when compared to the Africanized honey bees in Brazil (Calderón et al. 2010). Also, elements of climate, such as rainfall, can influence the hygienic behavior in Africanized honey bees (Sousa et al. 2015) including grooming activity, thereby increasing mite mortality (Junkes et al. 2007) and consequently reducing the infestation level.

The low degree of overall correlation observed between temperature, rainfall, altitude, and Varroa infestation may be explained by the great variation of the environmental parameters and altitude between the 5 areas studied. Bahia State covers a total area of 564,733.177 km² (a larger area than France), providing different biomes and altitudes (IBGE 2017b) in which 9 different climates can be found (tropical rainforest – Af; tropical monsoon – Am; tropical wet – Aw and As; hot desert – BWh; hot semi-arid – BSh; sub-humid, dry savanna to semi-arid – BSwh; monsoon-influenced humid subtropical – Cwa; subtropical highland – Cwb) according to Köppen climate classification (SEI 2017). However, some areas showed a moderate degree of correlation between the infestation rates and the environmental parameters and altitude studied (Table 2) showing that those variables likely influence the Varroa infestation rates in the colonies in some way.

Table 4. Pairwise distances between the 5 mesoregions calculated by the Mahalanobis distance.

Area	BA3	BA2	BA1	BA5	BA4
BA3	0	*11.25443*	*23.58176*	*2.73224*	*14.63204*
BA2	< 0.001	0	*7.90161*	*23.87691*	*2.57183*
BA1	< 0.001	< 0.001	0	*40.83151*	*11.94227*
BA5	< 0.001	< 0.001	< 0.001	0	*26.06312*
BA4	< 0.001	< 0.001	< 0.001	< 0.001	0

The squared Mahalanobis distances are in italics and the probability values for the contrasts (by F-tests) are not italicized. BA1: São Francisco Valley; BA2: Middle East; BA3: Metropolitan of Salvador; BA4: South Center; BA5: South.

The canonical discriminant analysis also confirmed the influence of temperature, rainfall, and altitude on Varroa infestation rates (Table 3). When the first discriminant function separated the BA3 and BA5 mesoregions, it formed a group with high rainfall and high *V. destructor* infestation rates with low altitude. On the other hand, the BA1, BA2, and BA4 mesoregions formed another group with high attitude and temperature with low rainfall and low *V. destructor* infestation rates (Figs. 2, 3). The temperature, rainfall, and altitude can affect the honey bee in different ways depending on the geographical area. In Turkey, a study found that honey bees in subtropical conditions have more problems with parasites in lowlands than in highlands during the winter (Ucak-Koc 2014), which agrees with the findings of this study where in the BA5 mesoregion, the infestation levels were highest and altitudes were lowest (Table 1). The temperature was the variable which contributed most in the second discriminant function, where the BA1 mesoregion was separated from the other mesoregions (Table 4, Figs. 2, 3). The brood rearing in honey bee colonies depends on sources of pollen and nectar in the environment, and food source availability is directly influenced by climatic conditions. This is important to the varroa infestation levels (Medina-Flores et al. 2014) because the mite needs the honey bee brood to reproduce (Rosenkranz et al. 2010).

Therefore, this study showed that in Neotropical areas, temperature, rainfall, and altitude have moderate degrees of influence on *V. destructor* infestation rates in Africanized honey bee colonies. Colonies located in areas with greater rainfall and low altitude may have higher mite incidence and infestation levels than in hotter, higher areas. Beekeepers in the lower, wetter areas should monitor their colonies regularly to detect and avoid an increase in mite infestation rates.

Acknowledgments

We thank the “Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq)” for funding via Special Visiting Researcher - PVE (number 400425/2014-9), Universal Call MCTI/CNPq number 01/2016, PDJ fellowship to M. E. C. O. (number 150731/2017-5), Junior Scientific Initiation scholarship for R. B. M., and PQ fellowship for C. A. L. C. (number 305228/2013-7); The Fundação de Amparo à Pesquisa do Estado da Bahia (FAPESB), for C. C. M. Master degree scholarship, and Junior Scientific Initiation scholarship for V. L. S. N; and Superintendência de Agricultura Familiar - Secretária de Desenvolvimento Rural do Estado da Bahia. Also, we thank the beekeepers for sending the honey bees to this study. The Africanized honey bees were collected under Sistema de Autorização e Informação em Biodiversidade (SISBIO) license number 50467 and 55056.

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