Acta Scientiarum

http://www.uem.br/acta
ISSN printed: 1806-2636
ISSN on-line: 1807-8672
Doi: 10.4025/actascianimsci.v37i3.26585

Varroa destructor mite in Africanized honeybee colonies *Apis mellifera* L. under royal jelly or honey production

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ABSTRACT. This study evaluated the level of invasion of *Varroa* mite into worker brood cells, the infestation rate on adult worker honeybees, total and effective reproduction rates of the mite in Africanized honeybee colonies under royal jelly or honey production. Invasion and infestation rates were not statistically different between honeybee colonies producing honey or royal jelly and the averages for these parameters were 5.79 and 8.54%, respectively. Colonies producing honey presented a higher ($p < 0.05$) total and effective reproduction of *Varroa* than colonies producing royal jelly. There was a negative correlation between levels of invasion and infestation with minimum external temperature, relative humidity and rainfall. The variables month and season influenced the development of the mite, but rates were low and within the range normally found in Brazil for Africanized honeybee colonies, which confirm the greater resistance of these honeybees to *Varroa destructor* than European honeybees.

Keywords: *Varroa* infestation, mite invasion rate in brood cells, mite total reproduction, mite effective reproduction, varroasis, honeybee queen selection.

Varroa destructor em colônias de abelhas africanizadas *Apis mellifera* submetidas à produção de geleia real ou mel

RESUMO. O objetivo foi analisar o nível de invasão do ácaro nas pupas, a taxa de infestação do ácaro nas abelhas adultas, as taxas de reprodução total e efetiva do ácaro em colônias de abelhas africanizadas e se esses níveis são influenciados pela produção de geleia real ou mel. As taxas de invasão nas pupas e infestação nas abelhas adultas não apresentaram diferença estatística entre os tratamentos, as médias para esses parâmetros foram 5,79 e 8,54%, respectivamente. As colônias submetidas à produção de mel apresentaram maior nível de reprodução total e efetiva ($p < 0,05$) do ácaro em relação às colônias produtoras de geleia real. Houve correlação negativa dos níveis de invasão nas pupas e infestação nas abelhas adultas com a temperatura externa mínima, umidade relativa do ar e precipitação. As variáveis mês e o período do ano influenciaram o desenvolvimento do parasita, entretanto, as taxas obtidas foram baixas e dentro dos valores normalmente encontrados no Brasil para abelhas africanizadas.

Palavras-chave: infestação de *varroa*, invasão do ácaro nas crias, reprodução total do ácaro, reprodução efetiva do ácaro, varroatose, seleção de rainhas.

Introduction

The mite *Varroa destructor* represents a huge concern in beekeeping and has been investigated as a major contributor to the mortality of colonies in much of the world (Genersch et al., 2010). The population dynamics of this parasite varies according to the region (Calderón et al., 2010). The recent introduction of a new haplotype K of the *Varroa* mite in Brazil may be related to increased reproductive capacity of this parasite, as this haplotype is found in regions with records of great damage caused by varroasis (Carneiro et al., 2007). Nevertheless, invasion rates in worker pupae cells at 1.67% found by Toledo and Nogueira-Couto (1996) and 1.57% observed by Wielewski et al. (2012) confirm that the Africanized honeybees have great resistance to this parasite.

The severity of varroasis may vary according to the subspecies of honeybees, climate conditions, the nectar flow, the development period of the brood and the ability to detect and remove the mite. In addition to direct damage, there is still the risk of virus transmission during feeding, causing a devastating effect on the colony health (Johnson et al., 2009). Among the major damage caused by *Varroa*, stands out the reduction in body weight and individual protein content of honeybees, which results in decreased longevity (Amdam et al., 2004).

The homogeneous genetic structure, the close physical contact and the high social interaction among individuals, make honeybees more vulnerable to pathogen infections (Chen & Siede, 2007). Besides, many beekeepers choose to use miticides in colonies, which can lead to parasite resistance to the active ingredient of the products used (Mullin et al., 2010).

The hygienic behavior is a natural resistance mechanism of honeybees to brood diseases and parasites (Rothenbuhler, 1964), characterized by removing dead, damaged or diseased brood. Furthermore, Oxley et al. (2010) observed that hygienic behavior is influenced by three pairs of genes and Harris (2007) found a genetic association between hygienic behavior and the level of infestation of adult honeybees by *Varroa destructor* mite.

In this sense, Goode et al. (2006) found that the behavioral profile of hygienic honeybees is driven by several factors including genetic, neural, social and environmental factors. The level of hygienic performance of individuals is related to genotypes and the selection of hygienic colonies has the potential to provide commercially significant increase in disease resistance (Oxley et al., 2010).

The understanding the factors and forms of biological interaction between parasite and host can determine which are the colonies most resistant to the mite, thus allowing to select colonies with more hygienic behavior, maintaining yield and quality of honeybee products without the use of miticides. Based on this, this study assessed the level of invasion of the mite into worker brood cells, the infestation level on adult honeybees, the total and effective reproduction rates of the mite in Africanized honeybee colonies of *Apis mellifera* and if these parameters are influenced by the production of royal jelly or honey.

Material and methods

The study was developed at the beekeeping sector of the Experimental Farm of Iguatemi, Universidade Estadual de Maringa. Colonies (n = 90) of the Africanized honeybee *Apis mellifera*, 40 colonies producing honey and 50 producing royal jelly, were subjected to monthly tests of infestation and invasion from February to December 2009. Royal jelly producing colonies were housed in vertical mini-hives with queen excluder, while the honey producing colonies were housed in standard Langstroth hives.

To carry out the test of invasion of mite into worker brood cells, we used the method cited by Toledo and Nogueira-Couto (1996). For the analysis, opercula were opened with anatomical forceps and 100 brown eyed pupae with body in early pigmentation were taken, 50 pupae from each side of the brood comb. The presence of female mite and descendants on the pupae and inside alveoli was analyzed using a light and a magnifying lens. After checking the presence or absence of the mite, pupae were discarded and the data were noted.

The invasion rate of pupae into worker pupae cells was calculated by the formula:

Invasion rate in pupae cells (%) = (number of mites found / number of pupae examined) x 100

The total reproductive rate of the mite was determined by the formula:

TRR (%) = (total number of descendants / number of adult females) x 100.

The effective reproductive rate of the mite was determined by the formula:

ERR (%) = (number of deutonymphs and young adults / number of adult females) x 100.

The infestation of the mite on adult honeybees was analyzed according to Stort et al. (1981). This method consists of removing approximately 100 adult honeybees from a brood comb at the center of the colony and placing them in 70% ethanol and shaking. Alcohol was collected by filtering through a screen which retained the honeybees and allowed the passage of the mite along with alcohol. The total number of honeybees and mites was counted to establish the percentage of infestation of each colony.

The mite infestation rate on adult honeybees was calculated by the formula:

Infestation rate on adult honeybees (%) = (number of mites found / number of worker honeybees examined) x 100.

Data of average maximum and minimum temperatures were obtained (°C), maximum and minimum relative humidity (%) and total rainfall (mm) of the Climatological Station of the Experimental Farm of Iguatemi, Universidade Estadual de Maringá.

Data were statistically analyzed using the procedure generalized linear models - proc GLM (General Linear Models) of the statistical package SAS (2004) using the Tukey's test for comparison of means at 5% significance level.

Results and discussion

The summary of analysis of variance and the mean value for each parameter are listed in table 1.

Table 1. F-values and respective probabilities (P), coefficients of variation (CV%), means and respective standard errors of invasion of the mite in worker pupae cells, infestation of the mite on adult honeybees, total and effective reproductive rate of the mite *Varroa destructor* in Africanized honeybee colonies under royal jelly or honey production.

Source of variation	Invasion of the mite in worker pupae cells	Infestation of the mite on adult honeybees	Total reproductive rate of the mite[2]	Effective reproductive rate of the mite[2]
Type of production	0.35 P =0.56	2.63 P = 0.11	5.12 P = 0.03	5.12 P = 0.03
CV%	45.64	37.62	116.52	120.90
Honey [n=101]**	0.22 ± 0.01 a[1] (5.55 ± 0.48)*	0.27 ± 0.09 a (7.67 ± 0.56)*	106.54 ± 11.01 a	64.58 ± 6.89 a
Royal jelly [n=28]	0.23 ± 0.02 a (6.03 ± 0.91)*	0.27 ± 0.01 a (9.41 ± 0.88)*	53.07 ± 20.90 b	31.05 ± 13.09 b

[1] Means followed by different letters in the same column are significantly different ($P<0.05$); [2] For the variables total and effective reproductive rate, data were not transformed; * For the variables invasion in worker pupae cells and infestation on adult honeybees, the numbers in parentheses indicate mean values not transformed and the standard error of the mean, the number outside parentheses were arcsine transformed $\sqrt{(x + \alpha)}$, with $\alpha = 0.5$; ** Values in square brackets indicate the number of observations.

There was no difference ($p > 0.05$) in rates of mite invasion in pupae cells and mite infestation on adult honeybees between colonies producing royal jelly or honey. Mean values of mite invasion rate in pupae cells and mite infestation rate on adults in colonies of Africanized honeybees *Apis mellifera* were 5.79 and 8.54% respectively.

Significant differences ($p < 0.05$) were found in total and effective reproductive rates of the mite in both systems, and the colonies producing honey presented higher total and effective reproduction rates of *Varroa*, with values 100.75 and 107.99% higher than the colonies producing royal jelly, respectively. Honey-producing colonies have greater population of worker honeybees than colonies that produce royal jelly, consequently, there are greater amounts of brood cells in these colonies, which could explain the higher values of total and effective reproduction of the mite observed in this production system. Removal of brood cells invaded by mites interrupts the reproductive cycle of *Varroa destructor*, prolongs its phoretic phase or kills the parasite (Zakar et al., 2014).

The mite infestation rate on adult honeybees is illustrated in Figure 1. Figure 2 presents the total and effective reproductive rates of the mite in honey-producing colonies.

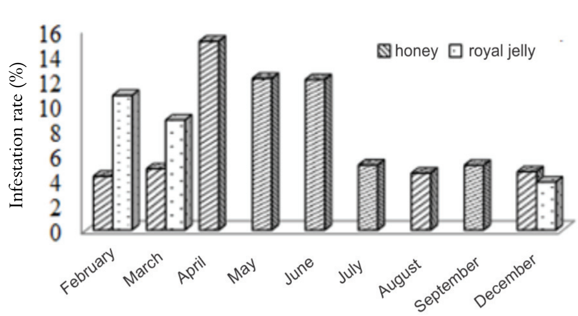


Figure 1. Infestation rate of the mite *Varroa destructor* in colonies under royal jelly or honey production, from February to December 2009.

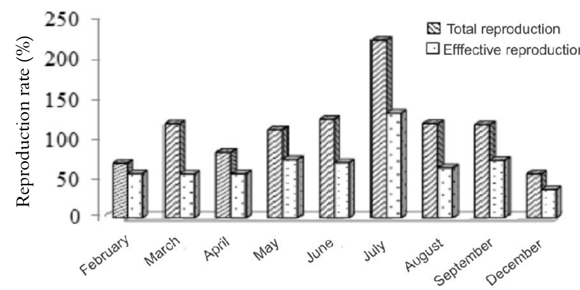
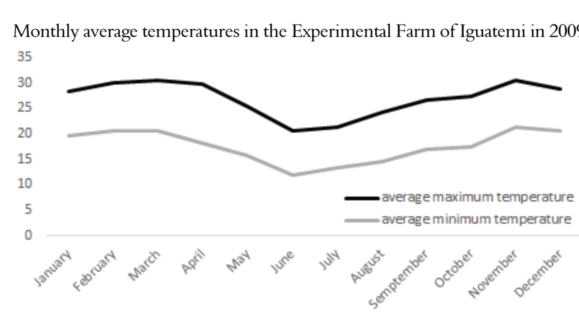


Figure 2. Total and effective reproductive rate of the mite *Varroa destructor* in honey-producing colonies, from February to December 2009.

Pernal et al. (2005) observed that the mites prefer nursing honeybees, which tend to stay in brood combs. The increased rates of invasion and infestation of colonies under honey production may be due to the greater presence of drone cells in these colonies in the period with greater abundance of food. Jay (1963) found that the different mite reproductive rates are related to the operculation time, generally greater in drones. In drone cells of European *A. mellifera* honeybees, this time is 10 days and in worker cells, it is eight days (Fernandez & Coineuu, 2006). The preference of the mite for drone cells has also been observed in Africanized honeybees (Calderone & Kuenen, 2001).

Temperature records during the experimental period are presented in Figure 3.



Source: Climatological Station of the Experimental Farm of Iguatemi, Universidade Estadual de Maringá.

Figure 3. Average monthly temperatures in the Experimental Farm of Iguatemi (EFI) – Universidade Estadual de Maringá, 2009.

Wielewski et al. (2013), in 2007, worked in the same experimental area and reported invasion rate of pupae of 1.69% in royal jelly-producing colonies and 1.35% for colonies producing queens. Regarding the infestation rate in adult honeybees, these authors observed average of 5.62% in colonies producing royal jelly and 3.55% for colonies producing queens.

Mustard et al. (2010) observed that the use of dopamine in worker honeybees leads to decrease in time for the flight and walking, but at the same time causes an increase in grooming behavior, grooming is the ability of the worker honeybee to identify and remove the mite from itself or other individual. Toledo et al. (2012) and Wielewski et al. (2013) found influence of season on the mite invasion rate in pupae cells, with lower levels verified in cooler months, along with a reduction of total and effective reproductive rates of the parasite.

Table 2 presents the Pearson correlation coefficients (r^2) between the parameters analyzed and climatic variables: maximum and minimum external temperature (°C); maximum and minimum relative humidity (%) and rainfall (mm), always pairwise, i.e., a dependent variable (parameter analyzed) and an independent variable (climatic variable).

The maximum external temperature was negatively correlated with the total and effective reproductive rates of the mite in colonies producing honey and royal jelly, while the minimum external temperature was the only one negatively correlated with all parameters analyzed (Table 2). This may be because in colder days the honeybees decrease the activities of foraging, remaining grouped in the hive, which can contribute positively to the hygienic behavior, lowering the mite infestation rate in adults and invasion in pupae cells.

Also, maximum and minimum values of relative humidity and rainfall presented a negative correlation with the mite invasion rate in brood cells and the mite infestation rate on adults (Table 2). On very hot days, the forager worker honeybees need to get water in the field to decrease the internal temperature of the colony, and in consequence, there is an increase in relative humidity inside the hive and decrease in rates of mite invasion in brood cells and mite infestation on adults, as they were negatively correlated, that is, when the humidity increases, rates of invasion in pupae cells and infestations of adults drop. The low fecundity of *Varroa* in winter is related to the smaller number of young worker honeybees (Fernandez & Coineuu, 2006), which serve as hosts to the parasite. Le Conte and Navajas (2008) reported that the reduced brood restricts the population of workers, decreasing the brood area, which confirms the data obtained in this research.

Table 3 presents the prediction equations obtained by multiple regression analysis using the stepwise method of SAS (2004) for the colonies under honey and royal jelly production. This method provides an equation that explains the variations in the parameter assessed according to independent variables, in this case all the environmental variables together, not just one at a time, as in the case of Pearson's correlation.

Table 2. Pearson's correlation coefficient (r^2) and its respective probability (P), number of samples (N) between the independent variables: maximum temperature (TMAX), minimum temperature (TMIN), maximum relative humidity (UMAX) and minimum relative humidity (UMIN), rainfall (PREC) and dependent variables: mite invasion in pupae cells, infestation of the mite on adult honeybees, total and effective reproduction of the mite.

	TMAX	TMIN	UMAX	UMIN	PREC
Invasion of the mite in pupae cells	r^2 = -0.0095 P = 0.5051 N = 3838	r^2 = -0.0667 P = 0.0001 N = 4976	r^2 = -0.0321 P = 0.0234 N = 4976	r^2 = -0.0857 P = 0.0001 N = 4976	r^2 = -0.0511 P = 0.0003 N = 4976
Infestation of the mite on adult honeybees	r^2 = 0.0018 P = 0.9123 N = 3838	r^2 = -0.0460 P = 0.0043 N = 3838	r^2 = -0.0907 P = 0.0001 N = 3838	r^2 = -0.0969 P = 0.0001 N = 3838	r^2 = -0.0497 P = 0.0021 N = 3838
Total reproduction of the mite	r^2 = -.0.1833 P = 0.0001 N = 3838	r^2 = -0.1874 P = 0.0001 N = 3838	r^2 = -0.0101 P = 0.5323 N = 3838	r^2 = 0.0279 P = 0.0835 N = 3838	r^2 = -0.0279 P = 0.1224 N = 3838
Effective reproduction of the mite	r^2 = -0.1818 P = 0.0001 N = 3838	r^2 = -0.1859 P = 0.0001 N = 3838	r^2 = 0.0090 P = 0.5777 N = 3838	r^2 = 0.0288 P = 0.0747 N = 3838	r^2 = -0.0164 P = 0.3086 N = 3838

Table 3. Multiple regression analysis using the stepwise method, with the selected models, in Maringá, state of Paraná, from February to December 2009, for Africanized honeybees *Apis mellifera* under royal jelly or honey production, independent of the production system.

Final model selected	N	F-value	P	adjusted R^2	CV (%)
Inv = 8.80 -0.22Umax – 0.02Umin	3656	20.07	0.0001	0.0103	84.50
Inf = 8.95 + 0.14Tmax – 0.27Tmin + 0.03Umax – 0.03Umin	3656	16.12	0.0001	0.0163	74.89
TRR = 249.23 -2.66Tmax – 2.87Tmin -0.55Umax + 0.29Umin	3656	37.95	0.0001	0.0389	115.44
ERR = 134.46 -1.49Tmax -1.99Tmin	3656	72.34	0.0001	0.0376	120.38

Inf = infestation of the mite on adult honeybees; Inv = mite invasion in pupae cells; TRR = total reproductive rate of the mite; ERR = effective reproductive rate of the mite.

Maximum and minimum values of relative humidity were negatively correlated with the invasion in brood cells. For the variable infestation of adult honeybees, the external maximum temperature and maximum relative humidity were positively correlated, while the external minimum temperature and the minimum relative humidity were negatively correlated.

The total reproductive rate of the mite was negatively correlated with maximum and minimum external temperature and with maximum relative humidity, and was positively correlated with minimum relative humidity. Both the maximum and minimum external temperatures were negatively correlated with the effective reproductive rate.

Table 4 lists the prediction equations obtained by multiple regression analysis using the stepwise method of SAS (2004) for the system of colonies producing honey.

The invasion rate in brood cells was negatively correlated with the minimum external temperature and the maximum and minimum relative humidity. The maximum external temperature was positively correlated with the infestation rate on adult honeybees, while the minimum external temperature and the minimum relative humidity were negatively correlated. The total and effective reproductive rates of the mite were negatively correlated with the maximum external temperature.

Van Dooremalen et al. (2012) reported high levels of mite infestation on adult honeybees during the transition from late summer to early winter may be the cause of the loss of colonies due to decreased longevity of winter honeybees infested with *Varroa*.

The longevity of worker honeybees is essential for colony survival, and to feed the first brood (Le Conte et al., 2010). Although there has been greater infestation by the mite in winter, the average found during the study period was 8.54%, considered low compared to the European honeybees. A similar result was registered by Teixeira et al. (2008), who found infestation of 10.68% for the mite *V. destructor* in adult honeybees working with mortality of honeybees associated with high rate of *Varroa* in colonies in the region of State of São Paulo. These values are close to the economic damage thresholds suggested by Currie and Gatien (2006).

Table 5 presents the prediction equations obtained by multiple regression analysis using the stepwise method of SAS (2004) for the system of colonies producing royal jelly.

The minimum relative humidity was negatively correlated with the mite invasion rate in pupae cells and the total reproductive rate of the mite. In turn, the maximum relative humidity was positively correlated with the effective reproductive rate of the mite.

Although the mite infestation rates found in Africanized honeybees are still low compared to European honeybees, the percentage of fertile female of the mite found herein was 85.56%, similar to that obtained by Carneiro et al. (2007), which found that the rate increased from 56% in 1980 to 86% in 2005-2006 and that 72% of females that invaded worker brood cells have left at least one viable descendant, compared with 35% in 1986-1987. The high fertility of *V. destructor* in Brazil is the result of a change in haplotype K of the mite (Strapazzon et al., 2009).

In recent years, losses in winter colonies reached 20% or more in many areas (Van Engelsdorp et al., 2007), whereas losses of 5 to 10% colonies were common during the winter 30 years ago (Le Conte et al., 2010). The weakening or even the loss of colonies is because parasite infestation leads to failure of the immune system of honeybees, suppressing the expression of immunity-related genes (Yang & Cox-Foster, 2007). When the infestation reaches excessive levels, the colony can be severely affected, enter into collapse or even die (Fernandez & Coineau, 2006), which also has a negative effect on the population of mites as well.

Thus, the infestation rate found close to the economic damage threshold did not cause significant loss of colonies, which probably occurred due to the parasite - host balance from the low reproductive capacity of the mite in worker brood cells of Africanized honeybees (Calderón et al., 2010) and the high mortality of the offspring (Mondragón et al., 2006). In France, Le Conte et al. (2007) observed that local honeybee colonies under honey production can survive the mite. Besides that, the increase in infestation rate during the colder months can be explained by decline of honeybee populations in the colonies, because the larger the size of the colony the smaller the infestation is (Lee et al., 2010).

Table 4. Multiple regression analysis using the stepwise method, with the selected models, in Maringá, State of Paraná, considering only the honey-producing colonies.

Final model selected	N	F-value	P	adjusted R^2	CV (%)
Inv = 10.53 -0.06Tmin -0.04Umax -0.02Umin	2848	17.58	0.0001	0.0172	85.84
Inf = 11.99 + 0.11Tmax -0.35Tmin -0.02 Umin -0.04					
	2848	35.95	0.0001	0.0468	70.00
TRR = 199.06 -3.36Tmax	2848	50.02	0.0001	0.0169	109.55
ERR = 124.09 -2.18Tmax	2848	52.40	0.0001	0.0177	114.67

Inf = infestation of the mite on adult honeybees; Inv = invasion of the mite in pupae cells; TRR = total reproductive rate of the mite; ERR = effective reproductive rate of the mite.

Table 5. Multiple regression analysis using the stepwise method, with the selected models, in Maringá, State of Paraná, considering only the royal jelly-producing colonies.

Final model selected	N	F-value	P	adjusted R^2	CV (%)
Inv = 7.62 -0.03Umin	808	3.86	0.0499	0.0035	79.19
Inf = -----------	808	---	---	---	82.28
TRR = 2.95 -0.78Umin	808	17.28	0.0001	0.0198	130.11
ERR = -18.63 + 0.56Umax	808	11.97	0.0006	0.0134	125.63

Inf = infestation of the mite on adult honeybees; Inv = invasion of the mite in pupae cells; TRR = total reproductive rate of the mite; ERR = effective reproductive rate of the mite.

In Tables 3, 4 and 5, it can be seen that the mite invasion in pupae cells was negatively correlated with minimum relative humidity in all evaluated models. Still, in all prediction equations, the adjusted R^2 was very low, probably because the other variables exerted a greater influence. The humidity and the adaptability of the parasite to the host are factors that influence the parasite egg viability (Fernandez & Coineau, 2006).

The population of adult honeybees exhibited a decrease in the amount of sealed brood at the end of the season with greater availability of nectar, also decreasing the mite population throughout the year, in agreement with Wielewski et al. (2013). The highest level of infestation of adult honeybees occurred at the end of the period with greater availability of food, when the infestation was 10.68% of the mite *V. destructor* in adult honeybees. The sharp increase in this rate can be explained by the decrease of hygienic behavior in the period, caused by a reduced availability of nectar, as noted by Somerville (2005).

Additionally, Wielewski et al. (2012) concluded that the selection of Africanized honeybees for the hygienic behavior decreases the total reproductive rate of the mite and, consequently, the infestation rate on adult worker honeybees. As the colony characteristics are determined by the queen genotype (Bienefeld et al., 2007), the availability of selected queens for production traits, as well as for hygienic behavior, contributes to increase the beekeeping productivity (Costa-Maia et al., 2011) and to the lowest infestation rate of *Varroa destructor* on adult worker honeybees.

Akyol et al. (2009) verified that colonies with young queens presented a greater production of brood cells and lower infestation rate on adult worker honeybees by *Varroa*. Therefore, the scheduled exchange of queens can be a viable alternative in parasite control, since the maintenance of heterozygosity and high levels of genetic diversity are important to ensure the success in controlling this parasite (Bourgeois & Rinderer, 2009).

The number of dead brood removed within 24 hours is an efficient selection criterion to improve hygienic behavior (Costa-Maia et al., 2011) and indicates the possibility of selecting queens based on this trait. It is still little known if the severity of the effects caused by *Varroa destructor* depends on the genotype of honeybees, the genotype of the mite or the interaction of both (Calderón et al., 2010). Based on this, we reinforce the idea that further tests are necessary to check the mite infestation levels, assemble production records and use hygienic honeybees; thus avoiding the (indiscriminate) use of chemicals.

Results for mite infestation on adult honeybees and mite invasion in brood cells - averages of 8.54 and 5.79%, respectively, indicate that Africanized honeybees have a high genetic variability, confirming Wielewski et al. (2013); the genetic diversity increases the health of the colony (Oldroyd & Fewell, 2007). Although the values were at levels below 10%, the reproductive capacity of the parasite has grown substantially since its arrival in Brazil, indicating that it is necessary to select more hygienic honeybees and breeding of queens for the maintenance of low levels of invasion and infestation, without the use of chemicals that could compromise the health of the colony and the honey quality.

Conclusion

The invasion levels of the mite in pupae and infestation on adult honeybees were not influenced by the production system, while total and effective reproductive rates of mite in colonies producing honey were higher compared to those producing royal jelly. Further studies are necessary, focusing on the effect of breeding on infestation levels and reproductive rates of the mite.

Acknowledgements

To CNPq processes 479329/2009-5; 308283/2011-2 and 311663/2014-1, to Araucária Foundation protocol 15095 – 422/2009.

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Received on February 10, 2015.

Accepted on May 8, 2015.