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Performance of Africanized honeybee colonies settled by queens selected for different traits

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ABSTRACT. We evaluated varroa infestation and the performance of Africanized honeybee colonies with queens selected for honey and royal jelly production, and also unselected queens, correlating with climatic variables. In Campo Alegre, Santa Catarina State, Brazil, the experiment I used 10 Langstroth hives and in Mafra, Santa Catarina State, Brazil, the experiment II was performed with 15 Schenk hives. A mapping in areas of sealed and unsealed brood, honey and pollen was carried out on days zero, 45 and 90 days after the introduction of the queen. In the experiment I, there was interaction between the type of queen selection and the evaluation period for areas of sealed brood, honey, and total stored food. The group selected for royal jelly production presented larger sealed brood area and smaller honey area at 90 days. Varroa infestation was lower ($p < 0.05$) at 90 days. The type of queen selection and the evaluation period influenced the sealed brood area, the total brood and the total area occupied in the colony. The high relative humidity caused greater honey storage for the local group. The different groups of queens presented different behavior according to the environment in which they are settled.

Keywords: queen selection, honey production, royal jelly production, *Varroa destructor*.

Desenvolvimento de colônias de *Apis mellifera* formadas com rainhas selecionadas para diferentes características

RESUMO. Avaliaram-se a infestação por *Varroa destructor* e o desenvolvimento de colônias de *A. mellifera* com rainhas selecionadas para produção de mel, geleia real e rainhas não selecionadas, correlacionando-se com as variáveis climáticas. No experimento I, em Campo Alegre, Estado de Santa Catariana, utilizaram-se dez colmeias Langstroth e, no experimento II, em Mafra, Estado de Santa Catariana, utilizaram-se 15 colmeias Schenk. Procedeu-se ao mapeamento das áreas de cria aberta, operculada, mel e pólen, na data de introdução, aos 45 e 90 dias após a introdução das rainhas. No experimento I, houve interação entre o tipo de seleção da rainha e a época de avaliação para as áreas de cria operculada, mel e total de alimento. O grupo selecionado para geleia real apresentou maior área de cria operculada e menor área de mel aos 90 dias. A infestação por varroa foi menor ($p < 0,05$) aos 90 dias. No experimento II, não foi encontrado interação para as características avaliadas. As áreas de cria operculada, total de cria e total ocupada da colônia apresentaram efeito do tipo de seleção da rainha e época de avaliação. A alta umidade relativa do ar influenciou o maior armazenamento de mel para o grupo local. Os diferentes grupos de rainhas responderam diferentemente em função do ambiente onde estão inseridas.

Palavras-chave: seleção de rainhas, mel, geleia real, *Varroa destructor*.

Introduction

Beekeeping is a growing activity, with highly social, economic and ecological relevance. Besides being appropriate to the properties of family farming, it also stands out as one of the activities with largest development in agribusiness (Khan, Matos & Lima, 2009). However, the national average of honey yield is still considered low, reaching only 18 kg^{-1} $hive^{-1}$ year Instituto Brasileiro de Geografia e Estatística (IBGE, 2013).

The main determinants of low yield include the genetic quality of the queens. The development and productivity of a colony of *Apis mellifera* basically depend on the age and the characteristics of the queen (Bienefeld, Ehrhardt & Reinhardt., 2007). Desirably, queen should be young (Akyol, Yeninar, Karatepe, Karatepe & Özkök, 2009) and has good genetic traits (Costa-Maia et al., 2011).

The population of a colony is determined by the laying capacity of the queen and the adaptation capacity to different environmental conditions

(Bienefeld et al., 2007). The number of eggs and larvae will determine the colony population, and encourage workers to forage by releasing pheromones. The greater release of the pheromone by brood stimulates higher consumption of pollen by nursing workers honeybees to feed the larvae and queen, thus stimulating increased egg laying rate and, consequently, increased brood and population (Pankiw, Sagili & Metz, 2008).

The assessment of colony development indicates disturbances in the queen laying activity and the performance of workers in the collection of food for the supply of the colony. The development of the colony is also influenced by climate and food availability in the region and the availability of food resources determine the number of brood produced (Costa et al., 2007).

Colony working occurs in a feedback system involving the activity of the queen and the internal and external environmental conditions of the colony. When a colony has a good quality queen, we expect a high egg laying rate and viable brood, resulting in a vigorous population, which will determine the maintenance and success of the colony (Bienefeld et al., 2007), as well as greater resilience to environmental change.

The availability of queens selected for production traits as well as for hygienic behavior is crucial for increasing the productivity of colonies (Costa-Maia et al., 2011), contributing to the expansion and strengthening of beekeeping agribusiness in the country. Nevertheless, the genetic material selected in breeding programs should be assessed under field conditions to validate the effectiveness of selection and adaptation of the lineages (Baitala et al., 2010) in other regions. This analysis also allows a better understanding of the genotype-environment interactions, since there are only a few studies on these interactions with bees (Faquinello et al., 2011).

The present study evaluated the development of *A. mellifera* colonies with queens selected for honey and royal jelly production, in two regions, correlating with the level of infestation by *V. destructor*, honey yield and climatic variables.

Material and methods

Two experiments were conducted to evaluate the queens. In both, we evaluated two groups of queen honeybees from the selection program of the State University of Maringá, coming from Maringa, located in the State of Paraná Northwest, located at latitude 23°25'30" South and longitude 51°56'20" West, with an altitude of 550 m subtropical climate - Cfa of Köppen.

One group consisted of daughters of queens selected on the basis of genetic evaluation of mother queens for honey production and hygienic behavior. Selection for honey production was performed by evaluation of morphometric characteristics and emergence weight of queens and hygienic behavior by assessing the removal of dead brood within 24h by worker honeybees in colonies daughters of mother queens (Costa-Maia et al., 2011; Wielewski et al., 2012). The other group was composed of daughters of queens selected for royal jelly production using molecular markers for expressing the protein MRJP3 (Baitala et al., 2010) with a tendency to homozygosity for this locus (Parpinelli, Ruvolo-Takasusuki & Toledo, 2014). In the experiment II, besides these groups, we also evaluated a local group of queens, which were chosen at random in the apiary and were not subjected to any breeding process.

Queens of the breeding program were produced in the beekeeping sector of State University of Maringá using the method adapted from Doolittle (1889) from mother colonies selected for honey or royal jelly production. Queens were mated in the air in Maringá, Paraná State, using individual mating nucs.

The colonies were dequeening 24h before the introduction of queens, in both experiments. They were introduced in cages, JZ/BZ™ model, without worker honeybees, in a central comb of the nest, containing sealed or unsealed brood. The experimental design was completely randomized with five replicates for each group of queens. In the experiments I and II, the variables were: unsealed brood area (eggs+larvae), sealed brood (pupae), food (honey and pollen) and the percentage of infestation by *V. destructor* on adult honeybees.

The evaluation of food and brood areas was performed according to Al-Tikrity, Hillmann, Benton and Clarke (1971) with modifications. The percentage of colony occupation was calculated considering the total area occupied by brood and food depending on the total area available in the hive for occupation by colony. We also computed the equivalent of this area in number of occupied combs with each variable analyzed from the prior knowledge of the area in cm^2 of each comb of the hive. Varroa infestation was conducted using the method described by Stort, Gonçalves, Malaspina, and Duarte (1981). Evaluations were performed on the date of queen introduction (initial), on days 45

and 90 after the introduction; the evaluation period corresponded to the period from early winter to early spring. In the experiment II, we evaluated the honey yield, with honey collection in October 2012, due to the nectar flow in the region, considered the beginning of the beekeeping season. The production of honey per colony was measured as the difference between the weight of full and empty supers after centrifugation.

Climate information was provided by the Information Center of Environmental Resources and Hydrometeorology of Santa Catarina (Ciram), from the daily record of weather stations in the counties of Campo Alegre and Mafra, State of Santa Catarina, for the experiments I and II, respectively. Importantly, they are two independent experiments conducted in two production systems, in different regions and not under comparison, that is, we do not intend to say if one was better but rather to verify the behavior of different types of selection in both regions.

Experiment I

The experiment I was conducted in an apiary located in the county of Campo Alegre, State of Santa Catarina, located at 26°11'34" S and 49°15'57" W, and 870 m altitude predominance of temperate climate - Cfb of Köppen and Geiger (1928)(Figure 1).

The introduction of fertilized and marked queens occurred in May 2012. The colonies received approximately 300 g of protein-energetic food, composed of sugar, honey and pollen, provided on the combs. The flowering of the native species *Mimosa scabrella* (Bracatinga) started one week after the introduction of queens.

Ten colonies of *A. mellifera* were settled in nuclei with five frames, Langstroth standard, each frame side corresponded to 760 cm² in area. Once all the frames of the nucleus were occupied with brood and food, the colonies were transferred to standard Langstroth hives with ten nest frames.

Experiment II

The experiment II was performed in an apiary located in the county of Mafra, state of Santa Catarina, located at 26°06'39" S and 49°48'18" W, and 793m altitude and predominance of temperate climate - Cfb of Köppen and Geiger (1928) (Figure 1). Noteworthy, this experiment is different and independent from the previous experiment.

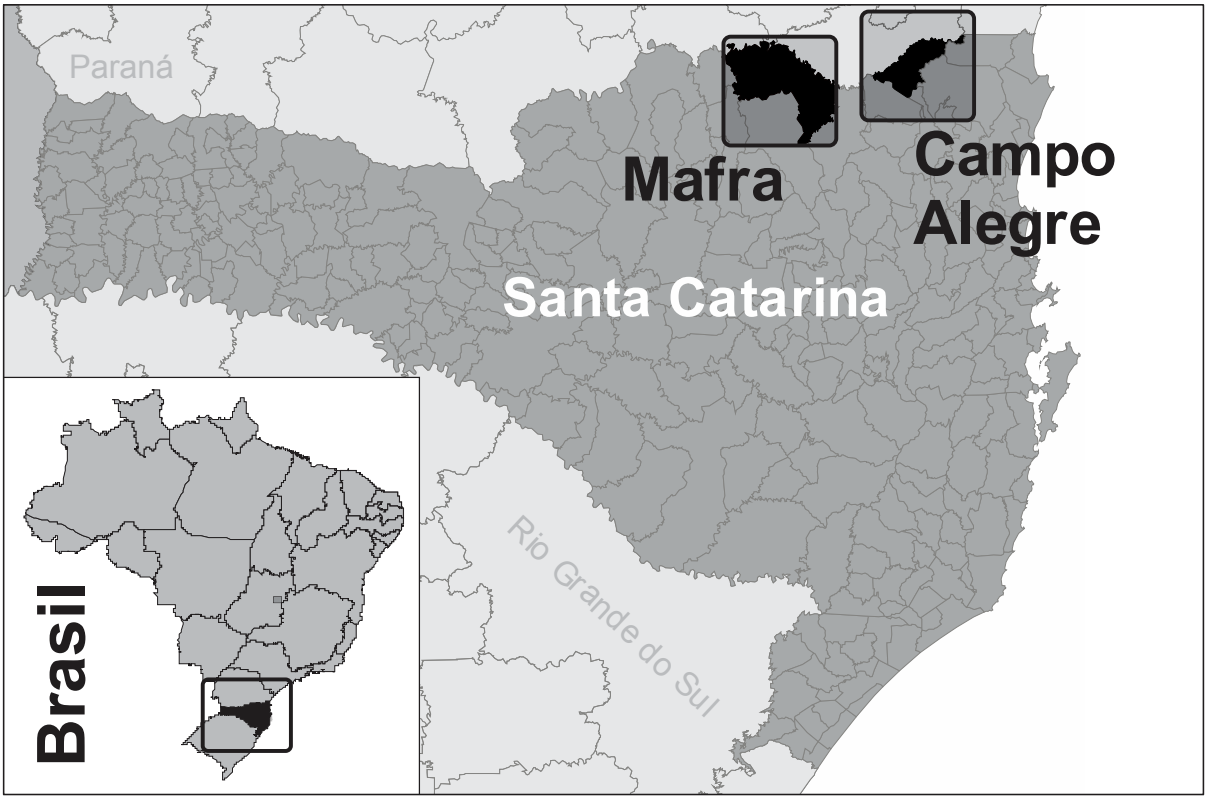

Figure 1. Location of the counties in which the apiaries of the experiments were installed, experiment I (Campo Alegre) and experiment II (Mafra), State of Santa Catarina, Brazil.

The introduction of fertilized queens took place in June 2012. The colonies were given 600 mL of energy food consisting of sugar and honey, in the proportion of 2: 1, supplied in Boardman feeders. The apiary was installed near a canola crop (*Brassica napus*), in early developmental stage, which reached full flowering 40 days after the introduction of queens. The flowering of the native species *M. scabrella* (Bracatinga) started approximately 15 days after the introduction of queens.

A. mellifera colonies (n = 15) were installed in standard Schenk hives, composed of 15 frames, and each frame side corresponded to 504 cm² in area. Supers were added according to the development of the colonies and nectar flow.

Statistical analysis

The software SAS was used for the statistical tests SAS Institute Inc., (SAS, 2004). Data were previously tested by the Shapiro-Wilk test to check for normality. All variables had normal distribution ($p < 0.05$). Thus, data were subjected to analysis of variance in PROC GLM, considering the completely randomized design with the treatments arranged in a factorial design. Means were compared by Tukey test at 5% significance level.To test the influence of climatic conditions on the variables, a multiple regression analysis was run with climate data and parameters of colony development and infestation by *V. destructor* using the stepwise method.

Results and discussion

Experiment I

The evaluation period affected the areas of sealed brood and pollen. The largest areas ($p < 0.05$) were observed 90 days after the introduction of queens (Table 1).

Table 1. Area occupied, in cm², by unsealed brood, sealed brood, honey, pollen, total brood area, total food area, total area occupied in the colony, and Varroa infestation, for colonies with queens selected for honey and royal jelly production, in the experiment I.

Evaluation★	Type of queen selection		Mean	CV (%)[1]
	Honey	Royal jelly		
	Unsealed brood (eggs+larvae)			
Initial	836.0 ± 304.0	1045.0 ± 285.0	940.5 ± 201.4 b[2]	42.6
45 days	1330.0 ± 190.0	2280.0 ± 0.0	1805.0 ± 217.1 b	
90 days	4560.0 ± 438.8	3800.0 ± 1160.9	4180.0 ± 580.5 a	
Mean	1931.7 ± 489.4 A	2242.0± 495.3 A		
	Sealed brood (pupae)			
Initial	380.0± 169.9	627.0± 337.6	503.5 ± 169.9	68.4
45 days	330.6 ± 162.8	633.3 ± 126.7	481.97 ± 116.6	
90 days	3040.0 ± 1520.0 B	7093.3 ± 1013.3 A	5066.7 ± 1220.2	
Mean	1028.5 ± 484.2	2568.8 ± 1029.3		
	Honey			
Initial	2736.0± 515.5	3990.0 ± 363.8	3363.0± 380.0	39.4
45 days	2850.0± 950.0	3293.3 ± 670.3	3071.7 ± 574.5	
90 days	4433.3 ± 705.3 A	1266.7 ± 253.3 B	2850.0± 783.4	
Mean	3198.3 ± 437.4	2964.0 ± 445.3		
	Pollen			
Initial	380.0 ± 169.9	0.0± 0.0	190.0 ± 111.7 b	112.4
45 days	45.6 ± 45.6	126.7 ± 126.7	86.1 ± 56.2 b	
90 days	760.0 ± 219.4	1013.3 ± 506.7	886.7 ± 253.3 a	
Mean	363.5 ± 115.8 A	342.0 ± 199.9 A		
	Total brood area (eggs+larvae+pupae)			
Initial	1216.0 ± 368.4	1672.0 ± 604.0	1444.0 ± 323.9 b	33.6
45 days	1660.6 ± 323.2	2913.3 ± 126.7	2286.9 ± 310.2 b	
90 days	7600.0 ± 1160.9	10893.3 ± 1266.7	9246.7 ± 1064.3 a	
Mean	2960.2 ± 864.3 B	4810.8 ± 1395.6 A		
	Total food area (honey+pollen)			
Initial	3116.0 ± 486.6	3990.0 ± 363.8	3553.0 ± 333.8	37.0
45 days	2895.6 ± 917.1	3420.0 ± 658.2	3157.8 ± 559.8	
90 days	5193.3 ± 770.5 A	2280.0 ± 760.0 B	3736.7 ± 811.6	
Mean	3561.9 ± 469.9	3306.0 ± 375.9		
	Total area occupied (brood+food)			
Initial	4332.0 ± 391.2	5662.0 ± 412.2	4997.0 ± 354.2 b	23.1
45 days	4556.2 ± 894.0	6333.3 ± 770.5	5444.8 ± 664.9 b	
90 days	12793.3 ± 552.1	13173.3 ± 2026.7	12983.3 ± 943.2 a	
Mean	6522.1 ± 1141.2 B	8116.8 ± 1249.9 A		
	Varroa infestation (%)			
45 days	11.0 ± 2.6	6.5 ± 1.3	8.8 ± 1.7 a	34.5
90 days	4.7 ± 0.9	2.5 ± 2.2	3.6 ± 1.1 b	
Mean	7.9 ± 1.9 A	4.5 ± 1.5 A		

Mean ± standard error; [1]CV(%) – coefficient of variation; [2]Means followed by different letters, lowercase in the same column and uppercase in the same row, are significantly different by Tukey's test ($p < 0.05$). ★There was a variation in the number of repetitions between the evaluation periods caused by loss of colonies due to death, disappearance or swarming, as follows: queens selected for honey: initial: n = 5; 45 days n = 4; 90 days: n = 3 and queens selected for royal jelly: initial: n = 4; 45 days: n = 3; 90 days: n = 3.

There was a significant interaction between the type of queen selection and evaluation period for the areas of sealed brood, honey and occupied with food (honey and pollen). The group of queens selected for royal jelly production exhibited a larger sealed brood area and smaller honey and total food areas (honey + pollen) 90 days after the introduction of the queens (Table 1).

The type of queen selection and the evaluation period affected the total brood area (eggs + larvae + pupae) and the total area occupied in the colony (brood+ food). Mean values for these variables were higher ($p < 0.05$) 90 days after the introduction of queens. The group selected for royal jelly production presented values significantly higher for area occupied with brood and total area occupied in the colony (Table 1).

Varroa infestation was lower ($p < 0.05$) 90 days after the introduction of queens and with no significant difference between the two groups (Table 1). The results corroborate those of Harris & Harbo (2000), which verified that varroa infestation caused no change in the first brood cycle after the introduction of selected queen, and differences were detected only from the fifth week.

The largest sealed brood area and smaller food area for the group selected for royal jelly production and the largest food area and smallest sealed brood area for the group selected for honey production indicates the greater prolificacy of the first group, possibly due to the greater capacity for royal jelly production, which also provided a greater availability of nutrients to larvae. The low temperatures recorded in this period (Table 2) contributed to reduce foraging activity of honeybees, leading to greater consumption of food stored by colonies with greater amount of brood. It is important the environment supports this prolificacy, both in food availability, and sufficient area for the egg laying.

The largest unsealed brood and pollen areas on day 90, regardless of queen selection, presented a response to egg laying stimuli available in the environment through the availability of food resources. One of the stimuli is due to the flowering of the native species *M. scabrella*, one week after the introduction of queens, increasing the availability of nectar and pollen. This result corroborates Jevtic et al. (2009), who found that pollen area in the colony increases along with brood area. The area of stored pollen is also influenced by the colony size (Jevtić, Mladenović & Nedić, 2005, Jevtic et al., 2009; Taha & A-Kahtani, 2013).

It is noteworthy that the method of Al-Tikrity et al. (1971) is based on counting the number of squares, and each square has 2 x 2 cm or 4 cm^2. From this count and respective multiplication by four and applying a rule of three (knowing that each standard hive had 10 combs in the nest), we obtained the percentages of occupation of brood and food areas. The colony occupation, for the evaluation period, was 42.3% (1.3 combs with unsealed brood, 0.7 with sealed brood, 2.1 with honey and 0.2 with pollen) for the group selected for honey production and 53.4% (1.5 combs with unsealed brood, 1.7 with sealed brood, 1.9 with honey and 0.2 with pollen) for the group selected for royal jelly production. The results obtained are superior to those observed by Costa et al. (2007) in Maringá, State of Paraná, within one year, where the average total occupation of the colony was four combs (38.18%), corresponding to one comb with unsealed brood, one with sealed brood, 1.5 with honey and 0.5 with pollen.

The results obtained, in the winter period, are lower than those determined by Winston (1991) of at least 59.2% to consider the colonies as productive. However, considering only the occupation 90 days after the introduction of queens, which corresponded to a period of greater availability of food in the region, allowing the colonies to start nectar storage and honey production, the average colony occupation was 85.4% (8.5 combs), higher than the values reported by Winston (1991) to consider the colonies as productive.

The relationship between the evaluated parameters and climate variables is presented in Table 3. The group selected for honey production was negatively affected by the maximum temperature in unsealed brood and total food. The minimum temperature presented a positive effect on areas of unsealed and sealed brood, pollen and total area occupied by brood, food and brood+ food and negative effect on varroa infestation. The relative humidity had a negative effect on areas of unsealed brood, pollen and total area occupied in the colony (Table 3).

The group selected for royal jelly production was negatively influenced by minimum temperature on the area of honey and varroa infestation and positively influenced on the areas of sealed brood and total area occupied in the colony. The average temperature had a positive effect on the areas of pollen and total food. The relative humidity presented a negative effect on the areas of sealed brood and total area occupied in the colony (Table 3).

Table 2. Mean values of climatic variables for the experiments I and II.

Period	T (°C)	T max (°C)	T min. (°C)	Rainfall (mm)	RH (%)
Experiment I (Campo Alegre)					
Initial	15.1	18.1	11.8	*	90
45 days	14.3	21.3	9.4	2.2	90
90 days	14.9	20.1	9.9	10.6	89
Experiment II (Mafra)					
Initial	13.0	14.4	8.4	3.8	92
45 days	15.9	22.7	15.9	89.0	90
90 days	17.2	25.2	11.7	70.6	84

T - average temperature; Tmax - maximum temperature; Tmin. - minimum temperature; RH – relative humidity. *Data not available for the period. Values correspond to the average of a 45 day-period prior to the evaluation date. Source: Epagri/Ciram (Weather Stations PL-F14MDA – Campo Alegre/Faxinal and PL-F14MDA – Mafra/Campo Novo, State of Santa Catarina).

Table 3. Multiple regression analysis using the stepwise method with the selected models for Africanized honeybee colonies with queens selected for honey and royal jelly production in the experiment I.

Final model	F	P	CV (%)	Adjusted R^2
Queens selected for honey (n = 5)				
CA = 8277.1021-69.3744(Tmax)+175.1256(Tmin.)-64.0319(UR)	7.67	0.0001	58.01	0.0637
CO = 622.8503+95.2163(Tmin.)	6.10	0.0141	126.15	0.0170
P = 78214998+28.0258(Tmin.)-7.7271(UR)	7.37	0.0008	109.84	0.0415
AT = 7784.1512+233.0013(Tmin.)-64.2417(UR)	8.82	0.0002	71.05	0.0505
AC = 3143.4457+80.7492(Tmin.)	5.41	0.0208	45.22	0.0148
AA = 22273-176.91184(Tmax)+446.6047(Tmin.)-163.2938(UR)	7.64	0.0001	49.97	0.0635
VAR = 10.3447-0.2216(Tmin.)	6.08	0.0142	56.75	0.0170
Queens selected for royal jelly (n = 5)				
CO = 7718.5198+243.1607(Tmin.)-68.6947(UR)	7.43	0.0007	82.30	0.0486
M = 2890.5231-66.0650(Tmin.)	6.90	0.0091	54.70	0.0229
P = 117.2926+31.9784(Tmd)	4.49	0.0351	116.13	0.0136
AT = 11751+298.1616(Tmin.)-85.6229(UR)	7.39	0.0008	57.67	0.0483
AA = 6352.4812+240.0673(Tmd)	7.43	0.0069	40.55	0.0249
VAR = 5.77-0.1346(Tmin.)	3.97	0.0474	74.05	0.0116

CA – unsealed brood (eggs+larvae); CO – sealed brood (pupae); M – honey; P – pollen; AC – total brood area (eggs+larvae+pupae); AA – total food area (honey+pollen); AT – total area occupied in the colony (brood+food); VAR – varroa infestation; Tmd –average temperature; Tmax - maximum temperature; Tmin. - minimum temperature; UR – relative humidity; F – F-value; P – probability; CV(%) – coefficient of variation.

The results differed from those of Costa et al. (2007), who found a positive correlation between maximum temperature and areas of honey, total area occupied in the colony and total food and negative correlation between minimum temperature and pollen area, total area and total food. The results were similar to that of these authors who also found negative correlation between minimum temperature and honey area.

The climatic variables presented different effects on the colony development parameters for the different groups of queens. The increase in relative humidity resulted in lower brood production and lower pollen storage in the group selected for honey, also decreasing the total food area and total area occupied in the colony. In the group selected for royal jelly, relative humidity caused no effect on food storage, but reduced the sealed brood area and the total area occupied in the colony.

Experiment II

There was no interaction between the type of queen selection and the evaluation period. Areas of unsealed brood (eggs + larvae) and pollen were significantly influenced by the evaluation period (Table 4). The area of sealed brood (pupae), the total brood area (eggs + larvae + pupae) and total area occupied in the colony (brood + food) presented effects from the evaluation period and type of queen selection. Mean values for these variables were significantly higher at 45 and 90 days compared to the initial period (Table 4).

No statistical difference was detected for varroa infestation between the different periods and between groups of queens (Table 4). The greater availability of pollen observed 45 days after the introduction of queens may have contributed to the maintenance of varroa infestation levels, because the mite females produce more offspring during pollen production than in other seasons (Mondragón Spivak & Vandame, 2005; Mondragón, Martin, & Vandame, 2006; Moretto, Gonçalves, & De Jong, 1997).

Larger pollen area 45 days after the introduction of queens coincided with the flowering of canola (*Brassica napus*), indicating a similar potential of the three groups for collecting pollen as well as similar honeybee population. These results are supported by Taha & A-Kahtani (2013), who found that the most populous colonies had stored highest amount of pollen than less populated colonies. The decrease of pollen area on the following date evidences a low availability of resources in the environment.

The occupation of the colony, for the study period, was 27.6% (1.1 combs with unsealed brood, 1.5 with sealed brood, 1.3 with honey and 0.3 with pollen) for the group with queens selected for honey; 27.8% (1.2 comb with unsealed brood, 1.1

with sealed brood, 1.6 with honey and 0.3 with pollen) for the group with queens selected for royal jelly and 36.3% (1.3 comb with unsealed brood, 2.1 with sealed brood, 1.7 with honey and 0.4 with pollen) for the local group of queens. Considering the percentage of occupation 90 days after the introduction of queens, when there was a greater availability of nectar due to the onset of the flowering of other species besides *M. scabrella* (bracatinga) and *Brassica napus* (canola), the percentages were 40.4, 36.9 and 40.6% for the colonies with queens selected for honey, royal jelly and local queen, respectively. The results obtained were lower than those verified by Winston (1991), in which colonies considered productive must have occupied at least 59.17% of the total area available. The results 90 days after the introduction of the queens were close to those reported by Costa et al. (2007), who evaluated colonies of Africanized honeybees over a year in Maringá, state of Paraná, and estimated the total area occupied in the colony at 38.2%.

The relationship between the evaluated parameters and climate variables is presented in Table 5. The average temperature had a positive effect on the total food area for groups selected for honey and royal jelly production; negative effect on varroa infestation for the group selected for honey, and on unsealed brood area, total brood area and total area occupied in the colony, for the local group of queens. The maximum temperature had a positive effect on the sealed brood area for the group selected for royal jelly and on areas of unsealed and sealed brood, pollen area, total colony area and total brood for the local group of queens. The minimum temperature presented a positive effect on unsealed brood area, honey area, total area occupied in the colony and total brood area for the three groups of queens; a positive effect on the sealed brood area only for the group selected for honey and on the total food area for the local group of queens. The rainfall had a negative effect on pollen area for the group selected for honey production (Table 5).

Table 4. Area occupied, in cm², by unsealed brood, sealed brood, honey, pollen, total brood area, total food area, total area occupied in the colony, and Varroa infestation, for colonies with queens selected for honey and royal jelly production, and selected in the local apiary, in the experiment II.

Evaluation*	Type of queen selection			Mean	CV(%)[1]
	Honey	Royal jelly	Local		
	Unsealed brood (eggs+larvae)				
Initial	277.2 ± 128.5	226.8 ± 140.3	277.2 ± 188.6	260.4 ± 82.9 b[2]	48.9
45 days	1461.6 ± 341.8	1638.0 ± 299.9	1915.2 ± 204.7	1671.6 ± 162.0 a	
90 days	1701.0 ± 215.2	1638.0 ± 577.4	1596.0 ± 366.1	1645.0 ± 193.8 a	
Mean	1146.6 ± 218.7 A	1167.6 ± 265.2 A	1262.8 ± 248.6 A		
	Sealed brood (pupae)				
Initial	252.0 ± 79.7	453.6 ± 94.3	1108.8 ± 188.6	604.8 ± 119.9 b	52.5
45 days	1612.8 ± 293.9	1417.5 ± 566.7	3074.4 ± 244.3	2034.9 ± 282.9 a	
90 days	2520.0 ± 534.6	1512 ± 1049.2	2100.0 ± 444.5	2044.0 ± 379.5 a	
Mean	1461.6 ± 306.7 AB	1127.7 ± 320.9 B	2094.4 ± 285.9 A		
	Honey				
Initial	1184.4 ± 497.0	1310.4 ± 332.4	2016.0 ± 0.0	1503.6 ± 208.8 a	62.7
45 days	907.2 ± 233.7	1323.0 ± 215.2	1159.2 ± 128.5	1129.8 ± 114.3 a	
90 days	1827.0 ± 799.4	2184.0 ± 1072.4	2100.0 ± 672.0	2037.0 ± 441.3 a	
Mean	1306.2 ± 293.1 A	1605.8 ± 293.4 A	1758.4 ± 187.4 A		
	Pollen				
Initial	25.2 ± 25.2	25.2 ± 25.2	75.6 ± 50.4	42.0 ± 20.1 b	86.1
45 days	680.4 ± 135.7	630.0 ± 162.7	705.6 ± 201.6	672.0 ± 91.1 a	
90 days	63.0 ± 63.0	252.0 ± 192.5	336.0 ± 222.2	217.0 ± 88.5 b	
Mean	256.2 ± 97.8 A	302.4 ± 101.9 A	372.4 ± 117.6 A		
	Total brood area (eggs+larvae+pupae)				
Initial	529.2 ± 100.8	680.4 ± 209.3	1386.0 ± 318.8	865.2 ± 157.4 b	36.8
45 days	3074.4 ± 614.2	3055.5 ± 773.9	4989.6 ± 256.9	3706.5 ± 391.9 a	
90 days	4221.0 ± 416.3	3150.0 ± 962.3	3696.0 ± 510.9	3689.0 ± 352.4 a	
Mean	2608.2 ± 484.6 AB	2295.3 ± 481.9 B	3357.2 ± 493.4 A		
	Total food area (honey+pollen)				
Initial	1209.6 ± 487.3	1335.6 ± 332.4	2091.6 ± 50.4	1545.6 ± 210.3 a	58.0
45 days	1587.6 ± 346.4	1953.0 ± 297.7	1864.8 ± 204.7	1801.8 ± 159.7 a	
90 days	1890.0 ± 851.5	2436.0 ± 1264.9	2436.0 ± 746.6	2254.0 ± 498.4 a	
Mean	1562.4 ± 304.7 A	1908.2 ± 339.9 A	2130.8 ± 176.2 A		
	Total area occupied in the colony (brood+food)				
Initial	1738.8 ± 399.2	2016.0 ± 347.4	3477.6 ± 353.2	2410.8 ± 283.0 b	25.2
45 days	4662.0 ± 949.6	5008.5 ± 626.6	6854.4 ± 244.3	5508.3 ± 454.3 a	
90 days	6111.0 ± 486.6	5586.0 ± 302.9	6132.0 ± 588.0	5943.0 ± 259.6 a	
Mean	4170.6 ± 618.5 B	4203.5 ± 542.6 B	5488.0 ± 483.2 A		
	Varroa infestation (%)				
45 days	4.6 ± 1.5	3.2 ± 0.9	1.5 ± 1.2	3.1 ± 0.8 a	35.9
90 days	4.4 ± 1.4	7.6 ± 2.5	3.7 ± 1.9	5.2 ± 1.1 a	
Mean	4.5 ± 0.9 A	5.4 ± 1.4 A	2.6 ± 1.1 A		

Mean ± standard error; [1]CV (%) – coefficient of variation; [2] Means followed by different letters, lowercase in the same column and uppercase in the same row, are significantly different by Tukey's test ($p < 0.05$). *There was a variation in the number of repetitions between the evaluation periods caused by loss of colonies due to death, disappearance or swarming, as follows: queens selected for honey production: initial: n = 5; 45 days; n = 4; 90 days: n = 3 and queens selected for royal jelly: initial: n = 4; 45 days: n = 5; 90 days: n = 3.

Table 5. Multiple regression analysis using the stepwise method with the selected models for Africanized honeybee colonies with queens selected for honey and royal jelly production and the local group of queens in the experiment II.

Final model	F	Prob.	CV (%)	Adjusted R^2
Queens selected for honey (n = 5)				
CA = 2933.9177+73.4799(Tmin.)-28.8507(UR)	73.81	0.0001	52.67	0.2038
CO = 3436.6667+125.3075(Tmin.)-37.7956(UR)	102.05	0.0001	56.40	0.2621
M = 730.8380+45.8529(Tmin.)	16.81	0.0001	81.69	0.0270
P = 1090.3594-5.7186(pct)-8.4655(UR)	16.43	0.0001	106.11	0.0515
AT = 8475.3596+236.8546(Tmin.)- 77.5157(UR)	89.13	0.0001	41.63	0.2365
AC = 6370.5815+198.7874(Tmin.)-66.6463(UR)	116.02	0.0001	47.97	0.2879
AA = 930.4757+40.5243(Tmd)	10.69	0.0011	67.33	0.0168
VAR = 7.4841-0.1477(Tmd)	16.63	0.0001	62.43	0.0267
Queens selected for royal jelly (n = 5)				
CA = 3507.0283+75.0481(Tmin.)-35.5068(UR)	60.11	0.0001	60.09	0.2006
CO = 131.1738+45.4052(Tmax)	23.43	0.0001	92.62	0.0455
M = 967.4039+53.0309(Tmin.)	21.0	0.0001	62.31	0.0408
P = 793.7314+10.0258(Tmax)- 7.6495(UR)	20.77	0.0001	93.33	0.0775
AT = 9121.2558+194.7844(Tmin.)- 79.7350(UR)	108.11	0.0001	31.60	0.3126
AC = 6225.5909+132.1410(Tmin.)-60.4860(UR)	50.02	0.0001	58.89	0.1723
AA = 832.4408+67.2233(Tmd)	23.22	0.0001	58.28	0.0451
VAR = -0.4911+0.0692(UR)	8.10	0.0046	71.73	0.0149
Local queens (n = 5)				
CA = 4191.5646+102.9140 (Tmax)+174.5986(Tmin.)-231.6126(Tmd)-38.5543(UR)	43.79	0.0001	47.78	0.2462
CO = 3407.9190+40.3411(Tmax)-23.0048(UR)	39.42	0.0001	37.72	0.1279
M = 269.5683+15.3878(UR)	14.94	0.0001	42.15	0.0259
P = 891.8407+11.0258(Tmax)-7.9436(UR)	17.08	0.0001	89.37	0.0578
AT = 11519+212.9472(Tmax)+356.5099(Tmin.)-478.5887(Tmd)-78.9079(UR)	52.61	0.0001	21.86	0.2826
AC = 9788.9680+233.4363(Tmax)+353.5068(Tmin.)-520.5730(Tmd)-80.0240(UR)	44.91	0.0001	35.83	0.2510
AA = 1816.2864+22.1670(Tmin.)	8.88	0.0030	31.52	0.0148
VAR = -0.17324+0.03042(UR)	3.87	0.0498	106.58	0.0054

CA – unsealed brood (eggs+larvae); CO – sealed brood (pupae); M – honey; P – pollen; AC – total brood area (eggs+larvae+pupae); AA – total food area (honey+pollen); AT – total area occupied in the colony (brood+food); VAR – varroa infestation; Tmd –average temperature; Tmax - maximum temperature; Tmin - minimum temperature; UR – relative humidity; F – F-value; P – probability; CV(%) – coefficient of variation.

The results confirm those obtained by Costa et al. (2007), which also pointed out a positive correlation between maximum temperature and total food area in the colony. Nevertheless, contrary to that observed in this study, these same authors verified a negative correlation between minimum temperature and the honey area, total area and total food area and a positive correlation between rainfall and pollen area.

The relative humidity had a negative effect on the unsealed brood area, pollen area, total area occupied in the colony and total brood area for the three groups of queens and on the sealed brood area in groups selected for honey and local queen. In turn, this variable presented a positive effect on varroa infestation for groups selected for royal jelly and local queen and on honey area for the group of local queens.

Moreover, the three groups exhibited a reduced brood production and pollen storage with high relative humidity, which also caused a smaller total area occupied in the colony. The local group of queens stored larger amount of honey with high relative humidity. Groups selected for honey and royal jelly production stored greater amount of honey under higher temperatures and humidity, which provides a stimulus for greater availability of nectar due to the greater abundance of flowers.

Honey yield per hive was 14.7 kg for the local group of queens, 6.7 kg for the group selected for royal jelly and 4 kg for the group selected for honey. The increased production of honey for the local group of queens is because this group, in addition to the positive effect of relative humidity, as seen in analysis associating the colony development parameters with climate variables, also presented a larger brood area, which resulted in a higher population of bees in this group. This is supported by Jevtic et al. (2009) that found a strong correlation between colony size and honey production.

Conclusion

Climate variables presented distinct effects on the parameters of colony development in the different groups of queens analyzed. The high relative humidity favored a larger storage of honey by the local group.

Colonies formed by queens most adapted to local conditions presented advantages as for honey storage in relation to queens introduced from another region, while honeybees selected for royal jelly production presented larger area of sealed brood.

Varroa destructor infestation remained low, approximately 5%, thus requiring no miticide application.

The use of queens selected by molecular markers, originating from regions with different climatic conditions to which they were subjected to, led to a better development of the colony compared to the use of queens selected considering their genetic value.

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