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Evaluation of *Apis mellifera* Carniolan and Africanized Honey Bees in Royal Jelly Production

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ABSTRACT

Investigations were carried out to evaluate Africanized and Carniolan hybrid Apis mellifera honeybees in royal jelly production. Ten colonies were randomly collected in nature and housed in Langstroth beehives. Each hive was having two nuclei with five frames. In superior nucleus frame was containing 28 acrylic cups in two bars. The five colonies with the best production remained in the experiment and their queens replaced by the respective daughters. Carniolan queens were placed in the five Africanized honeybees colonies with lower production. The hybrid Carniolan showed a higher production (P=0.0001) of royal jelly per colony, in three-day collect (3.95 ± 2.92 g) when compared to the Africanized honeybees (2.23 ± 2.13 g). The Carniolan also had a higher percentage of larvae acceptance (55.45 ± 36.7%) compared to the Africanized honeybees (35.8 ± 28.3%).

Key words: *Apis mellifera*, royal jelly, Africanized honeybees, Carniolan, hybrid honeybees

INTRODUCTION

Honeybees have been used in royal jelly, honey, wax and propolis production, besides being very important as pollinator agents. Among the apicultural products, the royal jelly is the result of hypopharyngeal gland (watery) and mandibular gland (milky) secretion. Such glands are located in the workers heads (Haydak, 1970). It is one of the most valued products of beehives, and is produced by 5-to-12-days old workers, called nurse bees. The commercial production of royal jelly is directly related to the production of queens and the workers do not stock royal jelly, being immediately utilized in larval feeding. The royal jelly has three uses: feeding worker honeybee larvae, up to 90 hour of larval life; feeding of the queen during all its larval phase and adult life (Wang, 1965); and feeding of drone larvae during all its larval phase (Haydak, 1970).

An alternative to diversify and increment the apiculture in huge sugarcane areas is to direct the colonies to the production of royal jelly. In areas of predominant sugarcane culture, the honey production is impaired by the organoleptic characteristics of the sugarcane-based honey, which has low commercial acceptance (Nogueira-Couto, 1991).

Descendants from highly royal jelly productive colonies had a higher acceptance of transferred larvae and also deposited more royal jelly per cup (Azevedo, 1996). The quantity of royal jelly per cup varies depending on the time it remains in the colony. Chang (1977) obtained more royal jelly when collecting 72 hours after 12-14 hour-old larvae transference than in collects done 48 hours after larvae transference.

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Toledo (1997) observed that Carniolan had higher larvae acceptance and higher royal jelly production than Africanized queens descending from: queens inseminated by Italian males; Africanized queens with Italian males; Italian queens inseminated by Italian males and Italian queens with Africanized males.

Different factors may interfere in royal jelly production: genetics; colonies internal population conditions; food flow; queen's egg-laying; and external environment related to weather conditions and food availability (Nogueira-Couto, 1992 and 1996, Toledo, 1997; Azevedo-Benitez et al., 1998). Thus, it is important to study the influence of these factors on royal jelly production, and honeybee's regional adaptability.

The aim of this experiment was to evaluate comparatively the royal jelly production in Africanized and Carniolan hybrid *Apis mellifera* colonies in Maringá, Paraná, Brazil.

MATERIAL AND METHODS

The experiments were carried out in the Apiculture Sector of Fazenda Experimental de Iguatemi (FEI), Universidade Estadual de Maringá (UEM), from August 1996 to July 1997. The method for royal jelly production was the mini-hives system, similar to the one developed by Santos and Message (1984). Mini-hives were composed by two superposed nuclei, separated by a queen excluder. The lower nucleus had five frames and the upper, four plus the cup-bar frame. The colonies received supplementary food (water + sugar) every time the transference was made.

Aiming to keep the highest quantity of young workers in contact with transferred larvae, every ten days, or any time it was necessary, a shift of combs was carried out in the mini-hives. In the shifts, two combs with sealed brood of lower part were placed in the upper part; and two empty combs were placed in the lower part. In this handling routine, assessments were also performed in the colonies to detect any abnormality, such as the queen on the upper nucleus, absence or irregular egg laying of the queen, lack of food or a virgin queen emerged from a queen cell hiding on a piece of comb that could be affect the larvae acceptance, consequently the royal jelly production (Free, 1985).

Two-cup bars with 28 acrylic cups, 14 in the upper picket and 14 in the lower, were utilized in each frame. In each mini-hive were evaluated: the acceptance %, quantity of royal jelly produced/cup (mg), and the quantity of royal jelly produced (g)/colony/collect during the assay. The royal jelly was collected between 68 and 72 hours after the transferences.

Experiment I

The colonies of Africanized honeybees were collected from nature in August 1996, and submitted to royal jelly production. From September to November 1996, they were evaluated in their transferred larvae acceptance % and royal jelly production/colony/collecting day. After statistically analyzing the data, the five worst Africanized queens according to the colony production were replaced by Carniolan queens and their respective daughters replaced the five best Africanized queens.

Experiment II

Ten mini-hives were used, five with hybrid Carniolan honeybees and five Africanized honeybees, mated in the air, daughters of the five best queens from the last experiment. These hybrid Carniolan honeybees were obtained by crossing pure Carniolan honeybees mated in the air with Africanized honeybees' males. This was done because in Maringá region there are no European honeybees' males.

The experiment began in January 1997, when all the population was changed, sixty days after the replacing of the queens (November, 1996). The Africanized honeybees were produced in the FEI apiary; following Doolittle's method (1899) and the Carniolan were acquired from a queen breeder in the city of Rio Claro, state of São Paulo. Quantifying the areas with food, honey and pollen, and sealed brood and unsealed brood four mappings were made, adapting Al-Tikrity et al. (1971) method's, which consists of placing the combs inside a stand with laterals made of horizontally and vertically stretched wire, thus forming a 2-cm square.

Data about maximum and minimum temperature (ºC), air relative humidity (%) and pluviometric precipitation (mm) were also obtained.

The data were analyzed in a totally randomized design. The transferred larvae acceptance % data were analyzed and transformed to the arc $\sin\sqrt{x+\alpha}$, which α=0.5, according to Pimentel Gomes (1990) and the production of royal

jelly/colony/collect was analyzed without transforming the data.

RESULTS

The data obtained with experiment I are shown in Table 1. In this phase the five best royal jelly-producer colonies were selected, number 1, 4, 6, 8 and 10 (based on the total royal jelly production), and were kept with Africanized honeybee queens. The other colonies (number 2, 3, 5, 7 and 9) the Carniolan queens replaced the old Africanized queens, beginning thus experiment II. From the ten colonies observed, only two (5 and 9) differed statistically from the highest producer (1), as shown in Table 1. Thus, the Africanized queens from these two colonies, plus the three ones from the lowest producers were changed by Carniolan queens for the rest of the experiment.

The data obtained in experiment II are in Table 2. It was observed that the hybrid Carniolan showed higher transferred larvae acceptance and also produced more royal jelly/colony.

Table 3 shows the variance analysis summary for the parameters egg-larva, pupa, honey, pollen, total brood and total food, total occupied area and total available area, and also the means of each subspecies and mappings. It was observed that there was no significant effect among the subspecies ($P>0.05$) and also for the interaction subspecies*month (S*M). This means, that the Africanized and hybrid Carniolan had the same number of occupied combs, and also that none of the hybrids showed better or worse during a determinate time of the year. However, among the mapping months there was a significant ($P<0.05$) difference for some parameters: combs occupied by egg/larva, honey and total brood (egg-larva and pupae). The number of combs occupied with honey was significantly higher ($P= 0.0216$) in the mappings carried out in February and March 1997, and the total food areas (honey + pollen) were higher ($P= 0.0041$) in March.

In Figs. 1 and 2, the variation occurred in the total brood comb occupation and food occupation % during each month of the experiment may be seen. It was noticeable that the occupation total brood and also with food were very similar for Africanized and hybrid Carniolan. It would be important to emphasize that during March, even though the occupation with food increased to more than 100%, the occupation with total brood decreased.

According to the regression analysis, the royal jelly production/colony/collect (g) and the royal jelly produced/cup (mg) did not suffer influence from environmental factors: maximum and minimum temperatures, relative humidity and pluviometric precipitation. However, the transferred larva acceptance percentage (TLAP) suffered influence from the maximum external temperature ($P=0.0395$) and the regression equation obtained was $TLAP = -0.5129 +0.0330MET$ (MET= maximum external temperature).

DISCUSSION

The results obtained showed that there was a high variation between Africanized honeybees, opening a perspective to select these honeybees for any production, be it honey, wax, pollen, propolis or royal jelly, as occurred with European honeybees selected to pollinate alfalfa cultures in USA (Nye and Mackensen, 1970). The criteria to be adopted for the selection should be very carefully chosen not to cause the selected honeybees to loose adaptive characteristics, such as resistance to diseases.

The results obtained about European honeybees producing more royal jelly than the Africanized honeybees, agreed with Durán (1991), Garcia (1992), Nogueira-Couto (1996) and Toledo (1997). The Africanized honeybees have high genetic variability towards royal jelly production and therefore, if not submitted to a selection before comparative analysis, not just of royal jelly production but other products as well, these honeybees will be in disadvantage.

The utilization of selected Africanized honeybees to increase apicultural production has been reported by Ferreira (1998) who mentioned that, using Africanized honeybees, the beekeepers surpassed many production difficulties, such as weather changes, lack of feeding flora, tolerance to *Varroa destructor* and resistance to disease. Hence, the abundance of Africanized honeybees in nature and its facility to be collected and acquired would allow a lower-cost, more accessible production of royal jelly to beekeepers. The beekeeper could use the Carniolan immediately to obtain a higher production, but he would have to depend on the production centrals

or queen producers to renew his colonies, increasing production costs.

The results obtained here agreed with Toledo (1997), who did not find statistical difference in the parameters egg-larva, pupa, honey, pollen, total brood, total food and total occupied area among the *Apis mellifera* subspecies studied (Africanized, Carniolan and Italian honeybees hybrids). Durán (1991) also observed that there were no significant differences (P<0.05) among the Carniolan, Italian and Africanized honeybees subspecies in quantity of brood (egg-larva, pupa and total brood) of drones, workers (separately) and quantity of food (honey, pollen and total food) from July 1989 to February 1991. Nogueira-Couto (1991) observed from March 1983 to October 1983 that there was no significant difference (P>0.05) in total brood and pollen production between *Apis mellifera* subspecies, Africanized, Carniolan, Caucasian and Italian honeybees.

Table 1 - F Values with respective probability, variation coefficient (VC%), means and standard deviation of royal jelly production/colony/collect (g) form September/1996 to February/1997 and transferred larvae acceptance %, in Africanized honeybees, at Fazenda Experimental de Iguatemi, Universidade Estadual de Maringá. The data of transferred larva acceptance % were analyzed transforming to the arc sin $\sqrt{x+\alpha}$, which α= 0.5 and the royal jelly production/colony/collect without transforming the data.

Variation sources	**Total royal jelly production/colony/ collect (g)**	**% of transferred larva acceptance/ colony/collect (g)**
Treatments (Colony)	**2.82 P=0.0041**	**3.16 P=0.0014**
VC (%)	**126.25**	**68.48**
Beehive 1 (n=19)	2.145 ± 1.697 a	0.695 a[1] (42.48 ± 20.51)[2]
Beehive 2 (n=19)	0.929 ± 1.003 ab	0.470 ab (24.31 ± 26.90)
Beehive 3 (n=19)	0.800 ± 1.067 ab	0.375 b (18.61 ± 26.85)
Beehive 4 (n=19)	1.040 ± 1.142 ab	0.409 ab (18.42 ± 13.64)
Beehive 5 (n=19)	0.371 ± 0.490 b	0.253 b (8.27 ± 11.04)
Beehive 6 (n=19)	1.359 ± 1.605 ab	0.563 ab (31.58 ± 24.49)
Beehive 7 (n=19)	0.941 ± 1.375 ab	0.430 ab (21.80 ± 23.87)
Beehive 8 (n=19)	1.063 ± 1.681 ab	0.458 ab (24.06 ± 30.02)
Beehive 9 (n=19)	0.410 ± 0.610 b	0.316 b (13.72 ± 18.91)
Beehive 10 (n=19)	1.597 ± 1.962 ab	0.488 ab (25.75 ± 27.24)

[1] Means followed by equal letters, in the same column, do not differ statistically (P<0.05) by Tukey's test.
[2] Numbers between parentheses indicate mean without transformation and standard deviation.

Table 2 - F Values with respective probability, variation coefficient (VC%) and mean of royal jelly production/colony/collect (g), royal jelly produced/cup (mg) and transferred larva acceptance % in Africanized and hybrid Carniolan *Apis mellifera* honeybees, from January/1997 to March/1997, at Fazenda Experimental de Iguatemi, Universidade Estadual de Maringá. The data of transferred larva acceptance % were analyzed transforming to the arc sin $\sqrt{x+\alpha}$, which α= 0.5 and the royal jelly production/cup (mg) and /colony/collect (g) were analyzed without transforming the data.

Variation sources	**Royal jelly production/colony /collect (g)**	**Royal jelly produced/ cup (mg)**	**% of acceptance transferred larvae**
F Value	**19.25 P=0.0001**	**0.79 P=0.3735**	**16.27 P=0.0001**
VC (%)	**77.48**	**72.17**	**51.28**
Carniolan honeybees (n=90)	3.951 ± 2.921 a	207.79 ± 114.21 a	0.919 a[1] (55.4 ± 36.7)[2]
Africanized honeybees (n=90)	2.230 ± 2.132 b	188.32 ± 166.79 a	0.616 b (35.8 ± 28.3)

[1] Means followed by equal letters, in the same column, do not differ statistically (P < 0.05) by Tukey's test.
[2] Numbers between parentheses indicate mean without transformation and standard deviation

Table 3 - F Values with respective probability (P), variation coefficient (%) and mean number of combs occupied with egg-larva, pupa, honey, pollen, total brood, total food, total occupied area and total available area, data without transformation, from November 1996 to March 1997, at Fazenda Experimental de Iguatemi, Universidade Estadual de Maringá, state of Paraná, Brazil.

Variation source	Egg-larva	Pupa	Honey	Pollen
Subspecies	3.72 P=0.0632	0.02 P=0.8931	0.60 P=0.4445	0.80 P=0.9556
Month	6.06 P=0.0024	1.60 P=0.2091	3.73 P=0.0216	1.83 P=0.1632
Subspecies*month	0.11 P=0.9541	1.49 P=0.2364	1.48 P=0.2404	1.10 P=0.3639
VC (%)	55.65	54.74	42.81	53.51
Carniolan	0.578	1.183	2.639	0.333
Honeybees (n=5)	(± 0.467)[1]	(± 0.671)	(± 1.102)	(± 0.181)
Africanized	0.815	1.140	2.990	0.340
Honeybees (n=5)	(± 0.444)	(± 0.663)	(± 1.636)	(± 0.193)
November/96	0.330 b[2]	1.030	2.350 b	0.310
(n=10)	(± 0.424)	(± 0.917)	(± 0.963)	(± 0.202)
January/97	0.980 a	1.160	2.190 b	0.240
(n=10)	(± 0.278)	(± 0.510)	(± 0.810)	(± 0.107)
February/97	0.910 a	1.520	2.810 ab	0.410
(n=10)	(± 0.463)	(± 0.555)	(± 0.806)	(± 0.233)
March/97	0.563 ab	0.875	4.225 a	0.400
(n=10)	(± 0.378)	(± 0.420)	(± 2.091)	(± 0.131)

Variation source	Total brood	Total food	Total occupied area	Total available area
Subspecies	0.53 P=0.4727	0.33 P=0.5687	0.96 P=0.3341	0.03 P=0.8713
Month	3.07 P=0.0427	5.46 P=0.0041	4.69 P=0.0084	0.86 P=0.4732
Subspecies*month	0.54 P=0.6604	1.21 P=0.3222	0.68 P=0.5726	0.34 P=0.7995
VC (%)	49.52	36.36	25.10	7.95
Carniolan	1.783	3.033	4.833	8.211
Honeybees (n=5)	(± 0.934)	(± 1.054)	(± 1.269)	(± 0.553)
Africanized	1.975	3.330	5.295	8.165
Honeybees (n=5)	(± 1.052)	(± 1.674)	(± 1.623)	(± 0.691)
November/96	1.380	2.650 b	4.020 b	8.300
(n=10)	(± 1.183)	(± 1.089)	(± 0.907)	(± 0.506)
January/97	2.190	2.470 b	4.660 ab	7.900
(n=10)	(± 0.702)	(± 0.763)	(± 1.079)	(± 0.721)
February/97	2.440	3.200 b	5.650 a	8.300
(n=10)	(± 0.912)	(± 0.837)	(± 1.176)	(± 0.709)
March/97	1.438	4.750 a	6.200 a	8.263
(n=10)	(± 0.713)	(± 1.821)	(± 1.805)	(± 0.487)

[1] Numbers in parentheses indicate standard deviation from average.
[2] Means followed by different letters, in the same column, differ statistically (P<0.05) by Tukey's test.

The results differed from Garcia (1992), who observed that beehives with Africanized honeybees tended to produce more worker brood than the ones with European queens; and that the beehives with European queens tended to stock more honey and the Africanized honeybees, more pollen. Toledo (1991) observed that European honeybee colonies showed a higher young forms and food % when compared to the Africanized honeybees, and European hybrids showed more constant answers and the Africanized honeybee higher fluctuations along the experiment.

The number of combs occupied by egg-larva increased (P= 0.0024) during summer (January and February 1997), when probably there was more food available in nature. Durán (1991), Nogueira-Couto (1991), Toledo (1991), Garcia (1992), under the conditions of their respective experiments, agreed that there was a difference (P<0.05) between the mappings, the season or month, in which the mapping of combs was made.

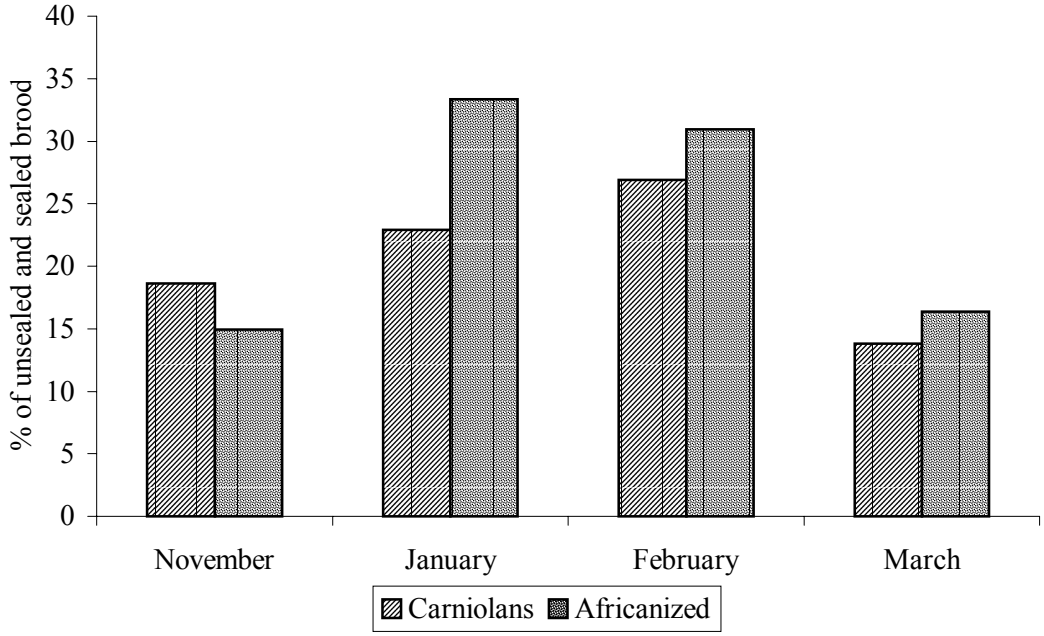

Figure 1 - Comparison of unsealed and sealed brood area (egg-larva and pupa) of workers, in Africanized and Carniolan *Apis mellifera* colonies at Fazenda Experimental de Iguatemi, Universidade Estadual de Maringá, from November 1996 to March 1997.

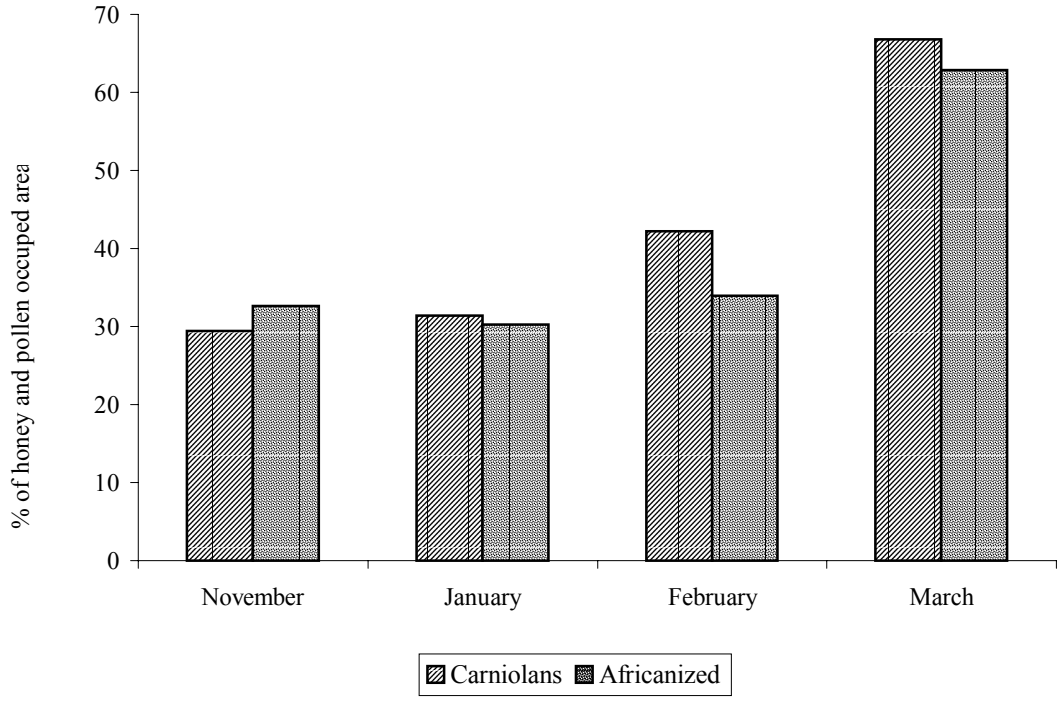

Figure 2 - Comparison of the total food area (pollen and honey) in Africanized and Carniolan *Apis mellifera* colonies at Fazenda Experimental de Iguatemi, Universidade Estadual de Maringá, from November 1996 to March 1997.

The determination coefficient adjusted for the equation of the transferred larva acceptance percentage (TLAP) was very low (3.4%). This indicated that there was a high variation between the obtained data and that there could be other factors influencing the results.

This may be explained because environmental variables determine the species flowering, and with this, there may be higher or lower food availability for honeybees, especially pollen, and the brood quantity may be proportional to food availability (Nogueira-Couto, 1991; Garcia, 1992). Many aspects must be considered when a colony is submitted to production. For instance, an increase of 21% to 25% in honey production may be obtained simply replacing old queens by young ones, besides, the management techniques in royal jelly production (Vantooor and Littlejohn, 1994). The increase in the productivity of colonies for honey production may reach 46 to 50% using young queens selected for this characteristic (Duay, 1996; Soares et al., 1996). Few researches have been carried out for the royal jelly selection. In this experiment, the mean royal jelly production of the ten colonies was 1.066 g. After the selection, the production increased to 2.230 g, which meant a 109.19% increase. Queiroz et al. (2001) reported the highest production of royal jelly in northeast of Brazil between 54 to 59 hours after the grafting, but in the present study, the royal jelly deposited by cup was 207 mg and 188 mg by Carniolan and Africanized honeybees, respectively, after 66-70 hours of grafting.

To achieve an increase in royal jelly production it is indispensable to select the queens. The hybrid Carniolan honeybees could be an option to the beekeepers to begin the royal jelly production during the time in which the Africanized honeybees selection has been carried out.

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RESUMO

O objetivo deste trabalho foi comparar abelhas *Apis mellifera* africanizadas e cárnicas híbridas quanto à produção de geléia real. Foram utilizadas 10 colônias de abelhas africanizadas coletadas ao acaso na natureza e alojadas em colméias do tipo Langstroth, mini-recrias, com quadro porta-cúpulas que possuíam 28 cúpulas de acrílico, sendo 14 em cima e 14 em baixo. As cinco colônias africanizadas com maior produção permaneceram no experimento e suas rainhas foram substituídas pelas respectivas filhas e nas cinco colônias de africanizadas com as menores produções foram introduzidas rainhas cárnicas. As cárnicas híbridas apresentaram uma maior (P=0,0001) produção de geléia real/colônia/coleta, 3,95 ± 2,92 g, em relação às africanizadas 2,23 ± 2,13 g. As cárnicas foram superiores (P=0,0001) quanto à porcentagem de aceitação de larvas, 55,4 ± 36,7%, em relação às africanizadas, 35,8 ± 28,3%.

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