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Regulation of Pollen Foraging Activity in *Apis mellifera* Africanized Honeybees Colonies

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Abstract

Efficient honey production requires knowledge about the behavior of the workers and the parameters that influence the strength of the colony. In this study, the objective was to analyze the interaction between the foraging behavior of worker honeybees and pollen storage levels in Africanized honeybees colonies. Colonies with low pollen storage increased pollen intake rates, but this value was 15% lower than colonies with high pollen storage, demonstrating a direct relationship between the pollen storage levels and foraging activity. The difference in pollen intake rates varied according to the number of foraging honeybees and pollen load collected by each individual. Under both high and low pollen storage, colonies returned pollen storage to initial level within 16 days, suggesting that honeybees regulate pollen storage levels around a homeostatic set point. Relationship between pollen storage levels and colony brood production was also found, indicating how alterations in the behavior of each individual can affect the strength of the colony.

Keywords

Comb Mapping, Honeybee Nutrition, Management in Beekeeping, Behavior

1. Introduction

Foraging behavior of *Apis mellifera* is extremely important to the colony development. Under normal colony

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conditions, the forager honeybees are workers with an age of over 21 days, at which time they shift to perform tasks outside of the hive, including water, nectar, pollen or resin collection [1]. Species of large honeybees require large amounts of pollen to feed their larvae and tend to leave areas with low abundance of food [2]. This is because the pollen foraging activity conducted by *Apis mellifera* is directly related to pollen storage and the amount of larvae in the colony [3].

Forager honeybees can be classified into two categories: scout honeybees which search for the best food resource and the reticent honeybees which wait in the beehive until the scout honeybees return and give them information about the food source by dancing [1] so that more forage honeybees are encouraged to go to the food source found. Forage honeybees have the ability to remember the time of day in which larger quantities of food sources are available [4]. Regarding the distances traveled by these honeybees [5] found that the foraging range of honeybees ranged from 45 m to 5983 m. It seems that the foraging distance for colonies in the same region is impacted by honeybee specie, colony strength, food resource, month and the time of the day [1].

Understanding the foraging behavior is important for understanding the biology of the honeybee and it is essential to improve management techniques. However, there are very few studies about foraging decisions when changes occur in the colony. Given these characteristics, this research was carried out to study the foraging behavior of *Apis mellifera* Africanized honeybees with changes in the amount of pollen stored in the colony.

2. Material and Methods

The study was conducted in December 2014, at Experimental Farm of Iguatemi, belonging to the State University of Maringa. Six colonies of *Apis mellifera* Africanized honeybees were used, each colony contained approximately 25.000 adult honeybees kept in Langstroth hives. The observations of foraging occurred during 16 days.

Initially colonies were mapped and standardized according to the method adapted from Al-Tikrity *et al*. (1971) [6]. This method consists of the introduction of each hive frame on a wooden support with wire subdivided into small squares with an area of four cm^2. After the introduction of the frame, photographs were taken with a digital camera on both sides of the comb, for subsequent counting of the food area (honey and pollen), and brood area (capped and uncapped) of drones and workers separately. After mapping, colonies were standardized to the same amount of food and brood, in which the average pollen area in the colonies was 488.67 cm^2.

To observe the relationship between the pollen storage level and foraging behavior, two manipulations were performed by removing or adding pollen combs in the colony. The first group consisted of three colonies with high amount of stored pollen (973 cm^2) and the second group three colonies with low amount of stored pollen (52 cm^2).

Regarding the amount of pollen stored in the colony, it was observed by mapping in the first, eighth and sixteenth days, being the first day for standardization, eighth and sixteenth days to observe the colony of pollen storage behavior. Rates of foragers collecting pollen and other foragers were observed in the entrance of the hive of both groups during five minutes, every other day, at 9.00 am.

To verify the forage for pollen load per forager a set of tem pollen foragers per colony was used, these foragers were collected in the entrance of the colony with the aid of an entomological aspirator in the four-day break. In the laboratory the pollen load was removed from the corbiculae of these forages to determine fresh and dry weight. The loads were weighed on an analytical balance, placed in an oven at 42˚C for three days and weighed again.

The pollen entrance rate was measured by inserting pollen collectors in the entrance in the third and eighth day of the experiment, for an hour. Pollens were removed from the drawers, placed in plastic bags, identified in the laboratory and analyzed the wet and dry weight as described above.

Climatic data of the maximum and minimum temperature (˚C), relative maximum and minimum humidity of air (%) and sum of precipitation (mm) were obtained through Climatological Station of the Experimental Farm of Iguatemi, State and Maringa University.

Data were statistically analyzed using the procedure generalized linear models-proc GLM (General Linear Models) of the statistical package SAS (2013) [7] using the Tukey's test for comparison of means at 5% significance level.

3. Results and Discussion

After the manipulation of the colonies there was a significant difference between the means of pollen areas (cm^2)

in hives with high (973 cm^2) and low (52 cm^2) initial amount of pollen (**Table 1**), during the 16-day trial. Redundant. This was described above.

The colonies with low pollen storage, eight days after the manipulation managed to increase the stored pollen area from 52 cm^2 to 160 cm and after 16 days presented an average of 85 cm^2. While colonies with high of pollen storage decreased the area of 973 cm^2 to 261 cm^2; and 16 days after the manipulation the average area stocked with pollen was 188 cm^2.

A higher number of pollen forager honeybees were observed at 9.00 am. There was no significant difference between the average number of foragers for pollen, other foragers or total of forager honeybees in colonies with high or low amount of initial pollen. Colonies with high storage pollen presented higher pollen load entering per hour (1.97 g/h), when compared to colonies with low storage pollen (1.25 g/h). There was no significant difference between means of fresh pollen load and dry pollen load weight taken from the pollen basket.

During the experimental period the average minimum external temperature was 20.0˚C and the maximum was 27.9˚C. Regarding precipitation, it rained rainfall was only one day and it was 2.4 mm.

The amount of stored pollen changed in both groups (**Figure 1**), in the colonies with high pollen reserve (973 cm^2) as the low reserve (52 cm^2). These results show that honeybees respond to manipulations of the quantity of pollen stored in their hive. Pernal and Currie (2001) [8] verified that the quality of pollen stored is also a parameter to honeybees increase or decrease the amount of pollen in the hive.

The decrease or stimulating of pollen foraging activity in response to addition or removal of it in the colony was also observed by [3]. These data indicate that in situations of protein food shortage within the colony, foragers honeybees are encouraged to collect and store greater amounts of pollen to feed the larvae. Rodríguez-Gironés and Bosch (2012) [2] observed that species of large honeybees, such as *Apis mellifera*, require large amounts of pollen to feed the larvae and tend to leave local with shortages of flowers. However, when there is plenty of pollen stored in the combs, the foraging activity decreases significantly. Amdam *et al*. (2009) [9] found that reduction in the queen oviposition delayed onset of honeybees foraging activity and mortality was associated with increased vitellogenin levels.

Table 1. Means of pollen areas (cm^2) in colonies with high and low initial amount of pollen.

Colony	8˚ day	16˚ day
High pollen storage	261a	188a
Low pollen storage	160a	85b

Means followed by different letters in the same column are significantly different ($P < 0.05$).

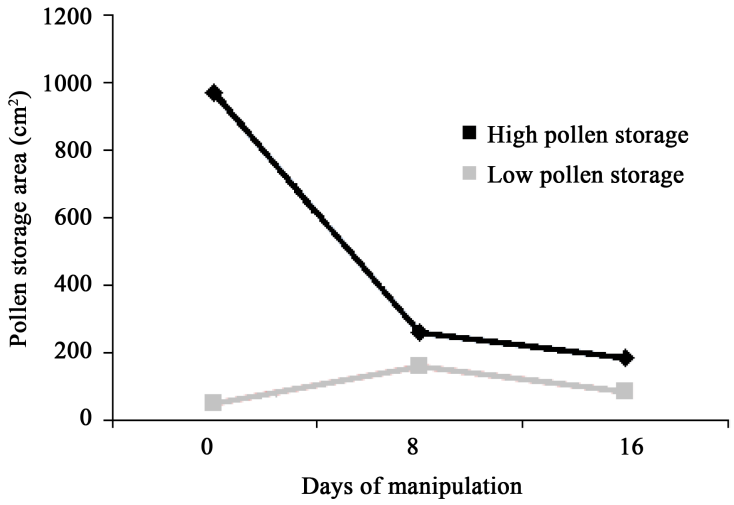

Figure 1. Changes in the amount of stored pollen in the colonies with high and low pollen reserve.

Initially colonies were standardized with average pollen area of 48,867 cm^2 and none returned to baseline, but were left with values close to each other at 16 days after manipulation. Possibly, colonies regulate pollen storage rate based on a set point. This point may be related to floral resources available at the time and the number of uncapped brood within the colony [3].

Figure 2 shows that the number of foragers honeybee (pollen, nectar, resin and water) returning to the colony in five minutes, there was a higher number of pollen foragers at 9.00 am, agreeing with [10] who found that honeybees prefer morning period, between 7.00 and 11.00 am for pollen collection.

The foraging period of *Apis mellifera* honeybees may vary due to numerous factors, may start early in the morning and finish in the evening. Joshi e Joshi (2010) [11] observed that honeybee workers started foraging activity at 6.17 am and 6.00 - 6.15 am [12]. Woyke *et al*. (2003) [13] observed high correlation between the temperature and the number of forager honeybees for temperatures above 16˚C. This indicates that *Apis mellifera* is well adapted to Brazilian climate conditions. Nowadays, this behavior is still observing in Africanized honeybees.

The foraging activity or floral visitation is analyzed using different parameters, such as foraging commencement or/and cessation time [12]; the number of honeybees returning to the beehive [8]; the peak and fluctuations of foraging over time [10]; the number of foragers per flower [14]; the number of visited flowers per forager [15]; the proportion of pollen or nectar foragers relative to total foragers; foraging type; the load of pollen and pollen type; concentration of crop nectar sucrose [16] and competition with other pollinators [17]. As a result of crossing between European races with the African honeybee (*Apis mellifera scutellata*), Africanized honeybees inherited the feature of start foraging early in the morning, as observed by Woyke, J. (1992) [18] with African honeybees.

Colonies with high storage pollen presented higher pollen load entering per hour (1.97 g/h), when compared to colonies with low storage pollen (1.25 g/h), which represents a difference of approximately 58% between groups. Colonies with high storage pollen had a mean fresh pollen load weight taken from the pollen basket of 36.43 mg, and dry weight 24.68 mg. While colonies with low pollen storage had a mean fresh weight of 39.40 mg and dry weight 26.81 mg. Pollen loads after removing the humidity was approximately 32% lighter than the fresh load in both groups.

Colonies with high pollen reserves presented higher pollen entry due to large amount of foragers honeybees, 15% more, but managed to reduce pollen in the colony area decreasing the size of pollen load by forager. While colonies with low pollen reserves increases the stored pollen area, increasing the size of load per trip, despite having lower quantity of forager honeybees collecting pollen. Free (1967) [19] found a decrease in foraging activity in response to the addition of pollen in the colonies. Forager honeybees from the colonies with low pollen reserves increased foraging effort, by 8% pollen load heavier than the load collected by forage honeybees with high reserves. This behavior was also found by [3] in European honeybees, in which the values for average of pollen load weight for colonies with high pollen storage was 14.0 mg, and low storage 16.7 mg, increasing the

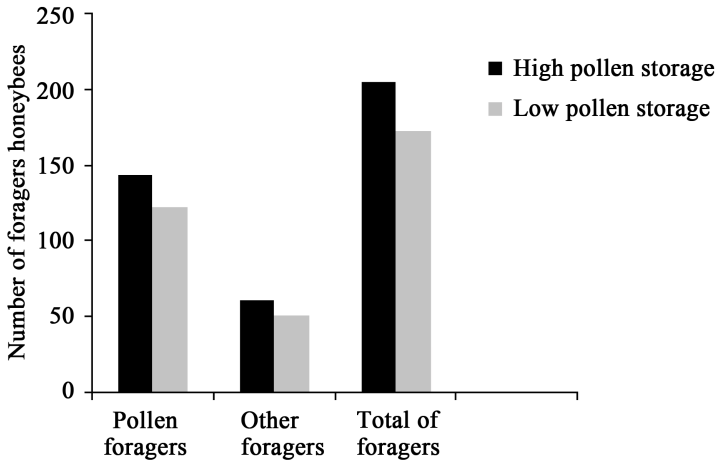

Figure 2. Differences in foraging activity rates of colonies on condition of high and low pollen reserve.

load weight in 19%.

In a study conducted in Africanized honeybees, Seeley (1995) [20] found that the average occupancy of ten combs available in the colony, less than half of a comb had stored pollen, indicating that pollen is collected according to the need of the colony in receiving protein food and its storage is lower compared the honey. Thus under favorable foraging conditions a colony will amass 50 or more kg of honey in its hive, but at the same time it will store up less than 1 kg of pollen [3]. It occurs because the colony needs a lot more of energy to keep itself and in contrast, a colony consumes pollen (protein food) mainly during brood rearing [21]. Woyke (1980) [22] found out that colonies maintained with enough storage pollen to keep the brood showed a higher survival rate of brood and [23] concluded that in periods of low pollen flow honeybees start cannibalism of the larvae.

During the experimental period the average minimum external temperature was 20.0˚C and the maximum was 27.9˚C. Joshi and Joshi (2010) [11] observed that *Apis mellifera* honeybees start foraging when the ambient temperature is at least 16.0˚C, and the highest activity was recorded at ambient temperature of about 20˚C [24], while at 43˚C the lowest foraging activity was found [25], as well as at or below 10˚C [11]. Moreover, significant negative correlation was found ($r = -0.09$) between foraging activity and temperature [26]. Regarding precipitation, there was no interference of this variable during the period of study because according to the climatic data, precipitation was only one day and it was 2.4 mm, not disturbing pollen collection activity by forager honeybees.

The experiment was conducted during the summer season when there is plenty of pollen in the assessed region, which possibly allowed the colonies with low pollen storage increase pollen entrance rate in a short time. The quality of pollen available in the area is also an important factor in foraging rate, Pernal and Currie (2001) [8] found that in periods of pollen shortage or poor quality pollen prevalence, colonies increase the proportion of forager honeybees without increasing foraging rate.

Moreover, if the beekeeper is looking for high pollen production, establishing a genetic breeding program is a key factor to improve the results. Page and Fondrk (1995) [27] reported that high pollen-hoarding strain honeybees maintain increased levels of pollen intake. Colony population also depends on the length of worker life and the amount of brood [28]. As the pollen is the main source of protein to the colony, it is an essential nutrient to keep brood-rearing and as a consequence, a high colony population of workers honeybees.

4. Conclusion

Honeybees stored the amount of pollen needed to maintain the colony brood by changing the foraging behavior according to the amount of pollen stocked. As the colonies were standardized in population and were located in the same area, there was no difference in forager honeybee number and amount of pollen collected between colonies with high and low pollen stocked initially. Colonies increased or decreased the number stored pollen area according to the colony conditions.

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