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External activity of colonies of *Melipona quinquefasciata* managed in different types of beehive[1]

Atividade externa de colônias de *Melipona quinquefasciata* manejada em diferentes tipos de colmeias

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ABSTRACT - This study evaluated the influence of the environment and of two models of beehive (wooden box and ceramic pot) on the flight activity of the ground-nesting bee, *Melipona quinquefasciata*. The experiment took place in the Chapada do Araripe, in the State of Ceará, Brazil, between July 2014 and June 2015. The study followed colonies in three ceramic pots and four wooden boxes. Observations of the flight activity of the bees and of the climate were made on five days every month, each colony being monitored for 5 min, at intervals of one hour, from 0500 to 1700. The internal temperature and relative humidity of the hives were also recorded every hour throughout the year. The temperature correlated positively with the bees' entering and leaving the hives, while air humidity correlated negatively. Light intensity positively influenced the two flows. Bee entry was higher during the dry period of the year (dry = 2.82 ± 0.22; wet = 1.19 ± 0.22). The mean internal temperature of the ceramic pots was higher ($p<0.01$) (dry = 23.34 ± 0.48 °C) (wet = 23.20 ± 0.25 °C) than of the wooden boxes (dry = 22.08 ± 0.31 °C and wet = 21.89 ± 0.29 °C), as was the internal humidity (dry = 87.41 ± 0.56% and wet = 89.35 ± 0.94%) ($p<0.01$) (dry = 82.24 ± 0.74% and wet = 86.47 ± 0.11%). It was concluded that the activity of *Melipona quinquefasciata* was related to the climate and the seasonality of the resources. The model of beehive used influences the homeostasis and foraging of the colonies.

Key words: Flight activity. Climate conditions. Types of beehive. Stingless bees.

RESUMO - Este trabalho avaliou a influência ambiental e dos modelos de colmeias (caixa de madeira e pote cerâmico) na atividade de voo da abelha de nidificação natural em solo, *Melipona quinquefasciata*. O experimento aconteceu na Chapada do Araripe, Ceará, entre julho de 2014 e junho de 2015. O estudo acompanhou colônias em três potes cerâmicos e quatro caixas de madeira. As observações do fluxo de voo das abelhas e do clima foram realizadas durante cinco dias em cada mês, sendo cada colônia monitorada por 5 min, em intervalos de uma hora, das 5h às 17 h. Ao longo do ano, foram ainda coletadas a temperatura e umidade relativa do ar internas das colmeias a cada hora. A temperatura correlacionou-se, positivamente, com entrada e saída de abelhas, enquanto a umidade do ar correlacionou-se negativamente. A intensidade de luz influenciou positivamente os dois fluxos. A entrada de abelhas foi maior no período seco do ano (seco = 2,82±0,22; úmido=1,19±0,22). As médias de temperatura interna dos potes cerâmicos foram maiores ($p<0,01$) (seco = 23,34±0,48 °C) (úmido = 23,20±0,25 °C) do que as das caixas de madeira (seco = 22,08±0,31 °C e úmido = 21,89±0,29 °C), bem como a umidade do ar interna (seco = 87,41±0,56% e úmido = 89,35±0,94%) foi maior ($p<0,01$) (seco = 82,24±0,74% e úmido = 86,47±0,11%). Concluiu-se que a atividade de *Melipona quinquefasciata* relacionou-se ao clima e à sazonalidade dos recursos. O modelo de colmeia usado influencia a homeostase das colônias e seu forrageio.

Palavras-chave: Atividade de voo. Condições climáticas. Tipos de colmeias. Abelhas sem ferrão.

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INTRODUCTION

The workers of Meliponini bees, also known as stingless bees, perform various activities inside and outside the nest, such the collection of resources for food and construction to ensure the development of the colonies. These activities are related to the biological characteristics of each species, the demand for resources and the environmental conditions (FIGUEIREDO-MECCA; BEGO; NASCIMENTO, 2013; SILVA; RAMALHO; ROSA, 2011).

Studies based on counting the workers that enter and leave the colonies and determining the type of material they transport have been carried out to understand the factors that influence foraging in stingless bees. Such studies can provide a better understanding of the biology of the bees, as well as an indication of the general state of the colonies (GOUW; GIMENES, 2013; HILÁRIO; IMPERATRIZ-FONSECA, 2009; NATES-PARRA; RODRÍGUEZ, 2011).

In Brazil, this type of evaluation has already been made with species of the genus *Melipona* (FIDALGO; KLEINERT, 2010; OLIVEIRA *et al*., 2012; OLIVEIRA-ABREU *et al*., 2014). However, no studies have been found on the influence of different beehive models used in the management of stingless bees. In addition, information on species that nest in the ground is scarce, such as *Melipona quinquefasciata*, which has an important potential for honey production (LIMA-VERDE; FREITAS, 2002), but for which there is still no consolidated production system. The aim of this study therefore, is to analyse the influence of environmental conditions and two models of beehive currently used in the management of *M. quinquefasciata*, on foraging behaviour. It is intended to gain information that may contribute to the construction of a rational management system for the species.

MATERIAL AND METHODS

The experiment was carried out over twelve months, from July 2014 to June 2015, in the Chapada do Araripe, on the border between the states of Ceará, Pernambuco and Piauí. The Chapada do Araripe has a tabular relief of sedimentary origin, and extends from east to west for about 180 km, at an elevation of between 800 and 950 m. The vegetation is characterised by a diversity of typologies, with areas covered by sedimentary caatinga (carrasco), cerrado, cerradão and sedimentary wet forest (MORO *et al*., 2015).

The management of the colonies of *M. quinquefasciata* was carried out in the Araripe National Forest (FLONA-ARARIPE), in the district of Barbalha, Ceará, in an area of sedimentary wet forest. The soil is a Red Yellow Latosol (MORO *et al*., 2015). The climate according to the Gaussen classification is type 4bTh - Tropical Hot Medium Dry, with a mean annual temperature varying between 24 ºC and 27 ºC, and a mean rainfall of 1,000 mm per year (GALVÃO, 1967; LIMA-VERDE; FREITAS, 2002).

The first model of beehive used was a ceramic pot. The pot is cylindrical, 25 cm in height and 25 cm in diameter, with no internal partitions and walls 8 mm thick. In the middle of the wall is a 16 mm hole through which was passed a flexible electrical conduit, 3/8” in diameter and 60 cm in length, used as the entrance tunnel. In the centre of the floor was a 6 mm hole to drain the excess moisture. The cover consisted of a 30 cm by 30 cm ceramic tile, below which was placed an inner cover of canvas. This model has been developed over almost a decade by experienced apiculturists in the Chapada do Araripe (Figure 1a-b).

The second model of beehive consists of a wooden box with no internal partitions, measuring 15 cm in height by 15 cm in width and 50 cm in length, the walls are 2.5 cm thick with a 16 mm hole in the side through which passed a similar conduit to the first model. The cover is of wood and is fixed with hinges; there is also a transparent acetate-plastic inner cover (Figure 1c-d).

The external activity of the colonies was evaluated as per Hilário and Imperatriz-Fonseca (2009). Initially, an energy food in a proportion of 1:1 (water to sugar) was provided to favour standardisation of the colonies regarding the number of combs and stocks of food. The experiment began when the colonies in each model of hive had 10 combs and 20 food pots. From then on, the average number of *M. quinquefasciata* workers entering and leaving the hives and the type of cargo they transported were evaluated (entry: nectar/water/nothing, pollen and building material; and exit: no cargo and waste). The observations were made on the first five days of each month, for three wooden boxes and four ceramic pots, which were monitored for 5 minutes every hour during the day (between 0500 and 1700). The following climate variables were also recorded every hour by means of a Digital Hygrometer-Anemometer (THAL-300): temperature, relative humidity, wind speed and luminosity.

Information on temperature and relative humidity inside the hives was obtained every hour throughout the period of the research, following a methodology adapted from Brasil *et al*. (2013), using Hobo model U12 data loggers. Care was taken to cover the data loggers with a 1-mm mesh wire screen to prevent the bees from depositing propolis on the sensor opening.

Figure 1 - Colonies of *Melipona quinquefasciata* bees: ceramic pot, external view (a) and internal view (b); and wooden box, external view (c) and internal view (d). Araripe National Forest (FLONA-ARARIPE), Chapada do Araripe, Barbalha, Ceará, Brazil, 2014

Data on the external activity of the workers and the meteorological variables were analysed for the dry (July to December 2014) and wet (January to June 2015) periods of the year, in order to verify any seasonal effect on the behaviour of *M. quinquefasciata*.

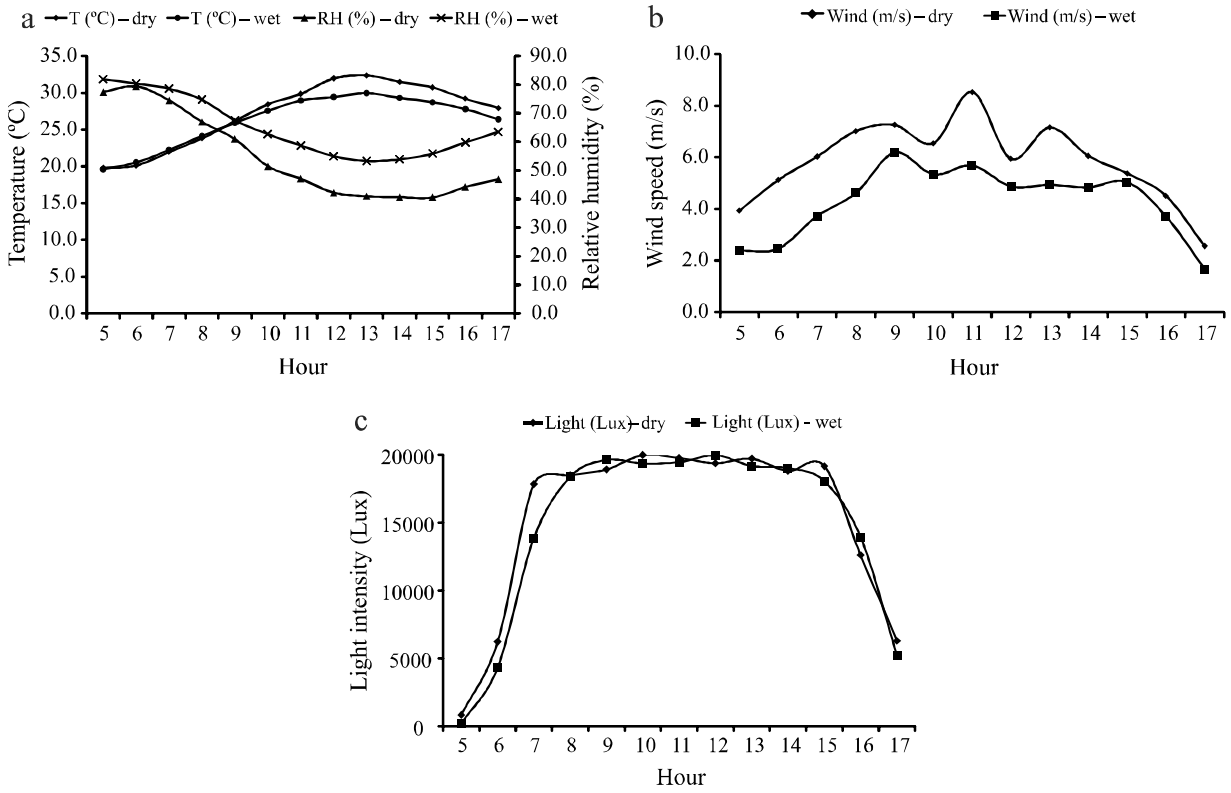
All the variables under analysis were initially submitted to the Kolmogorov-Smirnov test for normality (ZAR, 1984). In both cases, the data presented a nonparametric distribution ($p>0.05$). Spearman's correlation analysis was therefore applied between the external activities and the climate variables, considering the dry and wet periods. The Mann-Whitney test ($p<0.01$) was then applied in order to evaluate the seasonal effect (dry and wet periods) and the effect of the hive material (wooden box and ceramic pot) on the flow of workers. The Mann-Whitney test ($p<0.01$) was also applied to evaluate the environment in the colony from the temperature and relative humidity in each type of hive. The statistical analysis was carried out using the IBM® SPSS Statistics 20.0 software.

RESULTS AND DISCUSSION

The bees of the species *M. quinquefasciata* were active at temperatures of between 19.6 and 33.5 ℃, relative humidity between 38.0 and 80.4%, winds of from 2.5 to 8.5 m/s, and sunlight intensity from 210 to 20,000 Lux (Figure 2). The mean wind speed was higher during the dry period than during the wet period ($p<0.05$). Light intensity did not differ ($p>0.05$) between periods.

The entry of the *Melipona quinquefascita* workers showed a positive significant correlation (<0.01) with their leaving the hives during both periods of the year. The correlation between temperature and the external activity of the workers was positive and significant ($p<0.01$) for both entry and exit during the dry and wet periods of the year. The relative humidity correlated negatively and significantly ($p<0.01$) with both types of flow, irrespective of the period. The correlation between luminosity and the two types of flow was positive and significant ($p<0.01$) for both periods. In the case of wind speed, during the

Figure 2 - Daily mean temperature and relative humidity (a), wind speed (b) and light intensity (c), throughout the day during the wet (January-June 2015) and dry (July to December 2014) periods of the year. Chapada do Araripe, Barbalha,Ceará, Brazil



dry period there was a positive and significant correlation ($p<0.01$) with worker entry; however this was not significant during the wet period ($p>0.01$). While for exit, the correlation during the wet period was positive and significant ($p<0.01$), but not significant during the dry period ($p>0.01$) (Table 1).

The relationship between climate conditions and the flight of the *Melipona quinquefasciata* workers was mainly influenced by temperature, relative humidity and luminous intensity. Studies have shown that the climate variables of temperature and relative humidity are the main external factors influencing foraging in other species of *Melipona*, such as *M. quadrifasciata anthidioides* (OLIVEIRA-ABREU *et al*., 2014), *M. scutellaris* (SILVA; RAMALHO; ROSA, 2011), *M. eburnea* (NATES-PARRA; RODRÍGUEZ, 2011), *M. rufiventris* (FIDALGO; KLEINERT, 2010) and *M. subnitida* (OLIVEIRA *et al*., 2012), but the role of luminosity has been little investigated.

Sunlight intensity was fundamentally related to flight activity in *M. quinquefasciata*, and was positively correlated to both types of flow, serving as a trigger for the start and end of the activity, there being no flow without light. Gouw and Gimenes (2013) found similar behaviour in *M. scutellaris*.

Studies of *M. bicolor schencki* and *M. subnitida* have shown that wind speed does not have a strong relationship with flight activity, and may only have an influence together with temperature and relative humidity (FERREIRA JUNIOR; BLOCHTEIN; MORAES, 2010; OLIVEIRA *et al*., 2012). In the present study, variations in wind speed were not very significant and did not influence the flight activity of *M. quinquefasciata*, probably due to the barrier created by the surrounding forest where the colonies were located.

During the two seasons, the greater part of the collections is for nectar/water/nothing, followed by pollen and building material. The mean value for the transport of nectar/water/nothing and pollen for both models of hive was higher during the dry period, and for construction material during the wet period (Table 2). The greatest number of bees leaving the hives are cargo-free, irrespective of the type of hive or season (Table 3). For both models of beehive, the dry period showed greater mean values for bees exiting either with no material or with litter (Table 3).

Table 1 - Spearman correlation coefficients (r) between the external activity of *Melipona quinquefasciata* bees and climate factors (Barbalha, Ceará) during the dry (July to December 2014) and wet (January-June 2015) seasons. Chapada do Araripe, Brazil

Period	Parameter	Spearman correlation coefficients	
		Entry	Exit
Dry Period	Entry	-	*0.736*
	Exit	*0.736*	-
	Temperature (ºC)	*0.192*	*0.168*
	Relative humidity (%)	-0.191*	-0.160*
	Wind speed (m/s)	*0.040*	-0.028*
	Luminosity (Lux)	*0.180*	*0.180*
Wet Period	Entry	-	*0.554*
	Exit	*0.554*	-
	Temperature (ºC)	*0.302*	*0.233*
	Relative humidity (%)	-0.202*	-0.189*
	Wind speed (m/s)	*0.026*	*0.050*
	Luminosity (Lux)	*0.237*	*0.204*

*Correlation significant at 1%

Table 2 - Mean value (± standard deviation) of *Melipona quinquefasciata* workers entering hives managed away from the soil, during the dry (July to December 2014) and wet (January-June 2015) periods, in Chapada do Araripe, Barbalha, Ceará , Brazil

Model of hive	Season	Mean number of bees entering the hive transporting		
		Nectar/water/nothing	Pollen	Construction material
Ceramic pot	Dry	2.66±0.58 Aa	0.70±0.22 Ab	0.09±0.00 Bc
	Wet	1.32±0.31 Ca	0.12±0.06 Db	0.11±0.05 Ac
Wooden box	Dry	1.52±0.44 Ba	0.40±0.17 Bb	0.06±0.04 Bc
	Wet	1.02±0.32 Da	0.22±0.11 Cb	0.13±0.07 Ac

Mean values in the same column followed by the same uppercase letter and on the same line followed by the same lowercase letter do not differ by the Mann-Whitney test at 5% significance

Table 3 - Mean value (± standard deviation) of *Melipona quinquefasciata* workers leaving hives managed away from the soil, during the dry (July to December 2014) and wet (January-June 2015) periods, in Chapada do Araripe, Barbalha, Ceará , Brazil

Model of hive	Season	Mean number of bees leaving the hive	
		With no cargo	With waste
Ceramic pot	Dry	2.84 ± 0.64 Aa	0.23 ± 0.08 Ab
	Wet	1.11 ± 0.26 Ca	0.13 ± 0.06 BCb
Wooden box	Dry	1.56 ± 0.47 Ba	0.16 ± 0.10 Bb
	Wet	0.86 ± 0.28 Da	0.12 ± 0.06 Cb

Mean values in the same column followed by the same uppercase letter and on the same line followed by the same lowercase letter do not differ by the Mann-Whitney test at 5% significance

The total external activity of the *M. quinquefasciata* workers is greater during the dry period for both models of hive. The mean entry flow for the colonies of *M. quinquefasciata* managed in ceramic pots during the dry period was greater ($p<0.01$) than for the wooden boxes. However, during the wet period, entry did not differ

(p>0.01) for the type of hive (Table 4). In relation to the total leaving the hives, it was found that the flow was also greater during the dry period (p<0.01) for the two models, and that the ceramic pot saw a greater flow (p<0.01) during both seasons (Table 5).

In addition to climate factors, the availability of resources is also an important external influencer of flight activity in *Melipona* (GOUW; GIMENES, 2013). The flow of workers to a colony of *Melipona* is related to an optimal environmental window for foraging, conditioned by the existence of resources and a favourable climate (MAIA-SILVA *et al*., 2014). In the case of *M. quinquefasciata*, this optimal environmental window proved to be strongly related to the availability of resources, since the dry period was more expressive of the external activity of the workers. It is during this period that the greatest flowering occurs in the region (COSTA; ARAÚJO; LIMA-VERDE, 2004).

The only exception was the collection of construction material, which was greater during the wet period, possibly a consequence of the greater supply of moist, easy to handle mud. In the Amazon region, species of *Melipona* collect larger amounts of mud soon after the rains (CORTOPASSI-LAURINO; VELTHUIS; NOGUEIRA-NETO, 2007), which may be a response to the possibility of working the building material and the need to repair cracks in the hives to avoid low temperatures and humidity.

Data on internal temperature and relative humidity of the hives populated with colonies of *M. quinquefasciata* showed significantly higher mean values (p <0.01) than those recorded in the external environment. The highest mean values (p <0.01), both of temperature and of internal moisture, were for the colonies in ceramic pots (Tables 6).

In the case of places where the ambient temperature and relative humidity are lower than those required for the proper development of the brood, such as the Chapada do Araripe, the bees can be expected to develop ways of increasing these to an ideal level (JONES; OLDROYD, 2007). In fact, the mean data for temperature and relative humidity inside the colonies point to the production of heat and moisture by colonies of *M. quinquefasciata*, as well as indicating that maintaining these characteristics is more likely to happen in the ceramic pots.

During the colder hours, between 0500 and 0600 and around 1800, as well as when the temperatures are high, between 1200 and 1400, a noise could be heard of strong movement inside the hives, which could be an attempt to control the temperature by vibration of the thoracic muscles and/or ventilation, similar to behaviour seen in *Scaptotrigona depilis* (VOLLET-NETO; MENEZES; IMPERATRIZ-FONSECA, 2015) and *Melipona colimana* (MACÍAS-MACÍAS *et al*., 2011).

Table 4 - Mean values (± standard deviation) of the total entry flow of *Melipona quinquefasciata* bees for colonies managed in different models of hive (wooden box and ceramic pot) during the dry (July to December 2014) and wet (January-June 2015) seasons. Araripe National Forest, Barbalha, Ceará, Brazil

Model of hive	Mean number of bees entering the hive	
	Dry season	Wet season
Wooden box	1.96 ± 0.51 Ba	1.37 ± 0.34 Ab
Ceramic pot	3.45 ± 0.68 Aa	1.54 ± 0.32 Ab

Mean values in the same column followed by the same uppercase letter and on the same line followed by the same lowercase letter do not differ by the Mann-Whitney test at 5% significance

Table 5 - Mean values (± standard deviation) of the total exit flow of *Melipona quinquefasciata* bees for colonies managed in different models of hive (wooden box and ceramic pot) during the dry (July to December 2014) and wet (January-June 2015) seasons. Araripe National Forest, Barbalha, Ceará, Brazil

Model of hive	Mean number of bees leaving the hive	
	Dry season	Wet season
Wooden box	1.71 ± 0.49 Ba	0.98 ± 0.30 Bb
Ceramic pot	3.06 ± 0.65 Aa	1.24 ± 0.25 Ab

Mean values in the same column followed by the same uppercase letter and on the same line followed by the same lowercase letter do not differ by the Mann-Whitney test at 5% significance

Table 6 - Mean values (± standard deviation) of internal and external temperature and relative humidity for colonies of *Melipona quinquefasciata* bees managed in wooden boxes and ceramic pots during the dry (July to December 2014) and wet (January-June 2015) seasons. Araripe National Forest, Barbalha, Ceará, Brazil

Model of hive	Mean temperature (°C)		Mean relative humidity (%)	
	Dry period	Wet period	Dry period	Wet period
Ceramic pot	23.34±0.48 A	22.08±0.31 A	89.35±0.94 A	87.41±0.56 A
Wooden box	23.20±0.25 B	21.89±0.29 B	86.47±0.11 B	82.24±0.74 B
Control (external)	22.48±0.48 C	21.63±0.58 C	78.00±0.93 C	74.00±0.28 C

Mean values followed by the same letter in the columns do not differ at 1% probability by the Kruskal-Wallis test

Among stingless bees, high temperatures in the nests are common and indispensable for maintaining the brood (JONES; OLDROYD, 2007). In species of *Melipona*, a heat nucleus forms, generally in the centre of the colony, where the heat is mostly maintained by the presence of a casing of wax protecting the litter. This is an evolutionary characteristic providing homeostasis, especially to the brood, and thermal comfort to the adults at critical times (ROUBIK, 2006). Temperature and relative humidity also played an important role in the nests of *M. quinquefasciata*.

In the case of *Melipona quinquefasciata*, at no time throughout data collection were workers of the species seen collecting water from the containers that were made available. In this case, the behaviour reported for the genus prevailed, in which the highest internal levels of relative humidity would be the result of the process of removing moisture from the stored food. Water collection by species of stingless bees seems not to be widespread, probably due to the high levels of moisture present in nectar and to dehydration of the honey (ROUBIK, 2006). However, there are reports of the use of water for the active control of temperature in *Scaptotrigona depilis* (VOLLET-NETO; MENEZES; IMPERATRIZ-FONSECA, 2015) and some evidence that this may occur in species of *Melipona* (MACÍAS-MACÍAS *et al.*, 2011).

There are, therefore, two important points to be evaluated regarding homeostasis in the colonies of *M. quinquefasciata*. The first is that considering the importance of the casing used for temperature control in other species of *Melipona* (ROUBIK, 2006; SOUZA; CARVALHO; ALVES, 2008), it also seems to play a basic role for *M. quinquefasciata*, contributing to the higher temperatures in nests of this species when compared to the external environment. The second point is that movement within the colonies of *M. quinquefasciata* at certain times of high or low temperature could be an attempt to achieve homeostasis by beating their wings and vibrating their muscles, as seen in other stingless bees (MACÍAS-MACÍAS *et al.*, 2011; VOLLET-NETO; MENEZES; IMPERATRIZ-FONSECA, 2015). However, specific research is needed to clarify the existence of any action used for temperature control by this species, as well as the influence of material used in constructing the hives.

Ceramic materials tend to be better thermal insulators compared to wood (ASKELAND; FULAY; WRIGHT, 2010). However, no specific evaluations have yet been made of how much or in what way the physical characteristics of this material might interfere in the homeostasis of colonies of bees managed in hives constructed from it. Another important characteristic of the ceramic pot is that it is constructed of continuous material, with no joints or fissures, making it difficult for moisture and the external temperature to enter. This could favour the homeostasis of the colonies, unlike the wooden box that has a number of joints and cracks between its various parts.

The flight activity of stingless bees is influenced by the population situation of the colonies and by a series of external factors, such as climate and the availability of resources (MAIA-SILVA *et al.*, 2014; NASCIMENTO; NASCIMENTO, 2012; OLIVEIRA-ABREU *et al.*, 2014). Therefore, as the colonies were standardised for food reserves and brood combs, it is possible that, due to the better conditions for homeostasis, the ceramic pots reached better population levels and, because of this, a greater entry and exit flow of workers when compared to the wooden boxes. However, this difference does not seem to impair management of the species in either type of hive, and further evaluation of the productivity achieved under each of the management systems is necessary.

CONCLUSIONS

1. The external activity of *Melipona quinquefasciata* was influenced by climate factors, especially the temperature, relative humidity and luminosity, and by the seasonality of the resources offered by the vegetation in each area;

2. *Melipona quinquefasciata* is able to raise and maintain the internal temperature and relative humidity of the nests above the ambient temperature and relative humidity. These parameters appear to be important for the survival and development of the colonies;

3. The type of material used in constructing the models of hive used in the management of *Melipona quinquefasciata*, influences the homeostasis and foraging of the colonies, as the ceramic pots provided better ambient conditions for the colonies than did the wooden boxes. However, studies should be developed to test beehive models using this material.

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