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Pattern of the daily flight activity of *Nannotrigona testaceicornis* (Lepeletier) (Hymenoptera: Apidae) in the Brazilian semiarid region

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Abstract

The flight activities of Meliponini (stingless bees) are associated with a series of particular behaviors as collection of pollen, nectar, resin, and clay during the day, which can be influenced by extrinsic (e.g. abiotic factors) or intrinsic factors (e.g. internal conditions of the colony, morphology, physiology of the individuals, and others). This study aims to analyze the pattern of the daily flight activity of *Nannotrigona testaceicornis* (Lepeletier) and the influence of climatic factors (temperature, relative humidity, and light intensity) on these activities in Chapada Diamantina, Bahia, Brazil. The study was conducted every two months between October 2010 and October 2011 during three consecutive days in two colonies (one managed and the other unmanaged). The managed colony was more active than the unmanaged one in all the months. Nevertheless, both colonies showed regularity of times for their first activities and for the preferential time for the most part of the activities analyzed, which occurred generally in the morning, until noon. This daily pattern of activity of both colonies was mainly influenced by light intensity. In this sense, these activities began with the sunrise and become more intense with the increase of the light intensity in the environment.

Introduction

The daily activity patterns of eusocial stingless bees are mainly associated with activities as collection of pollen, nectar, resin, and clay. These activities occur during the day, but in higher intensity at specific moments, influenced by climatic factors (Hilário et al., 2001; Souza et al., 2006; Rodrigues et al., 2007; Ferreira-Junior et al., 2010). According to Corbet et al. (1993) there would be an ideal temperature range in which the eusocial bees are active, able to carry out activities that are external to the nest. The light intensity is another climatic factor that influences the flight activity of the bees (Kleinert-Giovannini, 1982; Corbet et al., 1993), acting as an indicator for the onset of the flight activities (Lutz, 1931). Heard and Hendrikz (1993) studying *Tetragonula carbonaria* (Smith) verified a diurnal pattern of activity with the influence of temperature and radiation. For these authors, there was no good correlation between activity and temperature, but it is not conclusive.

Factors such as rainfall, for *Melipona asilvai* Moure (Nascimento & Nascimento, 2012), and relative air humidity, for *Plebeia remota* (Holmberg) (Hilário et al., 2007), can also influence the daily activities of the stingless bees.

The time to supply with floral resources also exerts great influence on the foraging of eusocial bees. Since most plants show another dehiscence in the morning, the presence of a large quantity of bees in the flowers is common during this period for pollen collection (Roubik, 1989).

Many factors, such as those mentioned above are important for interpreting the patterns of daily activity, mainly related to the behavior in the collection of resources. However, the patterns of daily activity can also be manifestations of the circadian timing system, which can be related to many of the aspects of rhythmic daily activities of Meliponini (Bloch, 2008). Endogenous rhythmicity in the activity of Meliponini has already been observed by Bellusci and Marques (2001). According to Moore (2001), eusocial bees present a daily rhythm in the collection of floral resources such as nectar and pollen and these rhythms are generally synchronized with environmental (light/dark) or climatic (temperature) cycles. In this sense, there are very few studies in literature that analyze the daily activities based on the biological rhythm. Moreover, the pattern of daily activity can occur in a different way in the different months of the year, depending on the climatic

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characteristics of every month, as noted by Nunes-Silva et al. (2010) when they observed the flight activity of *P. remota*.

In order to verify the pattern of daily activity of Meliponini and the influence of environmental and climatic factors, an analysis was performed on two nests of *Nannotrigona testaceicornis* (Lepeletier), a Neotropical bee that occurs in several regions of Brazil (Moure et al., 2012).

Material and Methods

Study area

The work was developed in the Valley of Capão, municipality of Palmeiras, Chapada Diamantina (Bahia State, Brazil) (12º 31'S, 41º 33'W, at 1000 m of altitude). This area has low, fairly homogenous, and dense vegetation. The canopy is high, reaching 10 m height with trees emerging over 15 m mainly in the lower altitudes, which can be characterized as dry forest (semideciduous) (Queiroz et al., 2005). The climate of Palmeiras varies between sub-humid and dry with a yearly average temperature of 24.3 ºC (Köppen-Geiger: BSh). The rainy period occurs between October and July and the annual rainfall index is 1,361.7 mm (SEI, 2011).

The climatic data were collected at intervals of one hour, from 5:00 to 18:00 during the observations. The data of temperature and relative air humidity were collected with a digital thermo hygrometer fixed above the soil (ca. 1.5 m) in a shadowy area and the data of light intensity (illuminance) were measured with a digital luximeter (Lutron LX-107) at a distance of approximately 1 m from the soil. Sunrise and sunset times were obtained from the almanac of the National Observatory (http://euler.on.br/ephemeris/index.php).

Flight activity

For this study were used two colonies of *N. testaceicornis*, one managed and one unmanaged. The managed colony of *N. testaceicornis* was captured in the Valley of Capão (Chapada Diamantina), and had been maintained in a wooden box, under a roof, for eight years. The unmanaged colony was located in the trunk of a tree which was transferred from Caatinga environment (semiarid region, located around Palmeiras, Bahia, Brazil) to Valley of Capão, approximately four years ago and had been kept in an open area.

Individuals of *N. testaceicornis* were collected and deposited in the Entomological Collection "Johann Becker" at the Museum of Zoology of the State University of Feira de Santana.

The observations of the managed colony were made every two months from October 2010 to October 2011 while the unmanaged colony was observed in February, June, and October 2011. The observations in each colony were conducted between 5:00 and 19:00 (intervals of 15 min/hour) over three consecutive days. Quantification of the number of bees was made by counting the bees that entered and left the nest (observation of the individuals at the entrance of the colonies). The bees were separated in categories: a) did not carry apparent material; b) carrying pollen, and c) carrying resin on the corbiculae.

Statistical analyses of data

The analyses of the daily activities of *N. testaceicornis* colonies were performed using the Rayleigh test of the Circular Statistics Method (Batschelet, 1980; Zar, 2010). Preferential times (or acrophases) of activities were considered for values with the significant vector (r) above of 0.7, which can range from 0 to 1, according to dispersion of data ($p<0.05$). The analyses were only applied when the number of each activity observed reached 10 or more in the month. The daily activities of both colonies were compared using the Watson-Williams test ($p<0.05$) (Batschelet, 1980; Zar, 2010).

Pearson's correlation coefficient (r) was applied for verifying the correlation between the abiotic variables (temperature, relative humidity, and light intensity in open area and light intensity in hive entrance) and biotic variables (entrance, entrance with pollen, entrance with resin and exit). Correlations were considered statistically significant when $p<0.01$, by Student's t test. Correlations were made using the program PAST version 1.85 (Hammer et al., 2001). They were considered moderate correlations when the values of r (Pearson's correlation coefficient) were between 0.39 and 0.69 and strong correlations when the r values were above 0.7 (Dancey & Reidy, 2005 in Figueiredo-Filho et al., 2009).

Results

During the observations of the daily flight activities of *N. testaceicornis* in the study area, the temperature varied from 17 ºC to 28 ºC with an average temperature variation of 25 ºC (October 2010 and February 2011) to 21ºC (June 2011) (Table 1). The lowest values of temperature and light luminosity were registered close to sunrise and sunset and the highest values were registered in the afternoon between 12:00 and 17:00, generally higher than 25 ºC. The relative humidity varied from 41% to 90% with the average varying from 55% (August) to 83% (December) (Table 1). Sunrise times oscillated between 5:10 (October) and 6:00 (June) and sunset occurred between 17:27 (June) and 18:19 (February).

Table 1. Time of the first activities of the unmanaged and managed colonies of *Nannotrigona testaceicornis* and the meteorological factors in these times (light intensity at the entrance of the colony, LI, sunrise, temperature, Temp. at the onset of the activity and monthly average, relative humidity, RH at the onset and monthly average) from October 2010 to October 2011 in Valley of Capão (Chapada Diamantina, Bahia, Brazil).

Months	Hour	LI (lux)	Sunrise	Temp. (°C)	Temp. Average	RH (%)	RH Average
Managed Colony							
Oct/10	7:00	504 – 1110	5:15	19	25	82	59
Dec/10	6:00	28 – 126	5:11	20	24	90	83
Feb/11	6:00	1 – 95	5:39	20	25	77	61
Abr/11	6:00	78 – 85	5:50	20	23	76	70
Jun/11	8:00	410 – 786	6:03	18	21	80	69
Aug/11	8:00	12500 – 19400	6:00	19	23	70	55
Out/11	6:00	125 – 157	5:14	20	24	78	59
Unmanaged Colony							
Feb/11	6:00	3 – 230	5:39	20	25	77	61
Jun/11	8:00	2100 – 3850	6:03	18	21	80	69
Oct/11	6:00	798 – 1333	5:14	20	24	78	59

Monthly activities

The managed colony of *N. testaceicornis* showed daily activity in all the seven months of observation. The numbers of total daily flight activities (entrance plus exit), entrance with pollen, and entrance with resin were always higher for the managed colony than for the unmanaged colony in all the months when both colonies were observed (Figs 1 and 2). In October 2011 (Temperature average: 24°C), a higher number for total activity and entrance with pollen in both colonies could be observed and also in August 2011 (Temperature average: 23°C) for the managed colony (Fig 1).

Daily Activities

The opening and closing of the cerumen tube at the nest entrance by the bees were observed daily and these movements marked respectively the starting and the ending of the activities for both colonies. The first activities of both colonies of *N. testaceicornis* occurred at the same time of the day. However, these times varied in different months of the year (Table 1; Fig 1). The first total activities and the first entrance activities with pollen occurred earlier (interval between 6:00 and 7:00) in December 2010, February 2011, April 2011, and October 2011 for the managed colony and in February 2011 and October 2011 for the unmanaged one. The first activities of the bees occurred later (between 8:00 and 9:00) in June 2011 (managed and unmanaged colonies) and August 2011 (managed colonies). During these activities, the temperature usually ranged from 18°C (June 2011) to 20°C (in the other months), the relative humidity ranged from 76% to 90% and the values of light intensity were always higher than 1 lux.

The last total activities and collection of pollen by the bees of the two colonies occurred between 17:00 and 18:00, generally a little before sunset while the light intensity still above 1 lux, with the exception of February 2011 in which the activities ended later (between 18:00 and 19:00), month that values of temperature were higher (media 24.5°C) (Fig 1) and sunset occurred later (18:19).

Total activities (Fig 1) and the entrance with pollen (Fig 2) in the managed and unmanaged colonies occurred at a higher intensity in the morning until 12:00, declining after this time. Through the analysis of Circular Statistics was observed the presence of preferential times (or acrophases) for most of the flight activities of the bees for both colonies (Table 2).

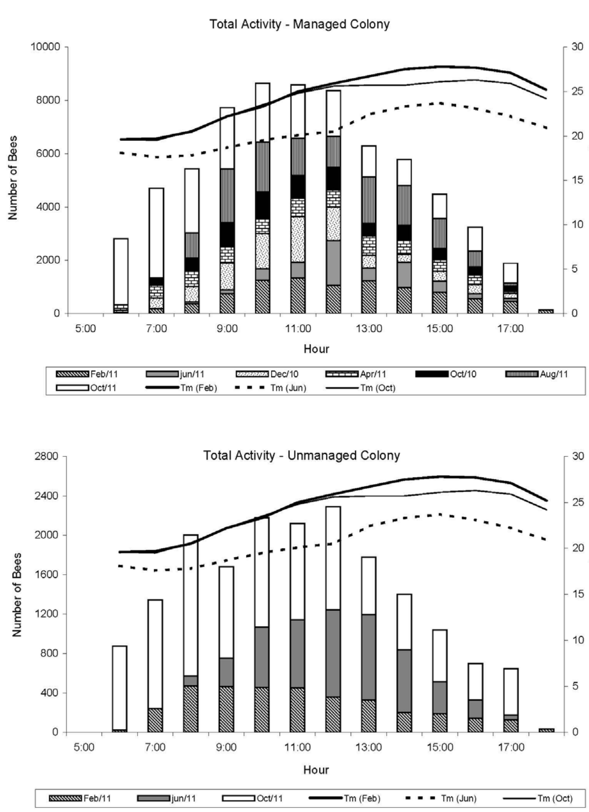

Fig 1. Total activity (entrance plus exit, without materials apparent) of *Nannotrigona testaceicornis* and temperature values in colony unmanaged (in February, June and October/11) and managed (from October 2010 to October 2011) in the Valley of Capão (Chapada Diamantina), Bahia, Brazil. Tm: Temperature.

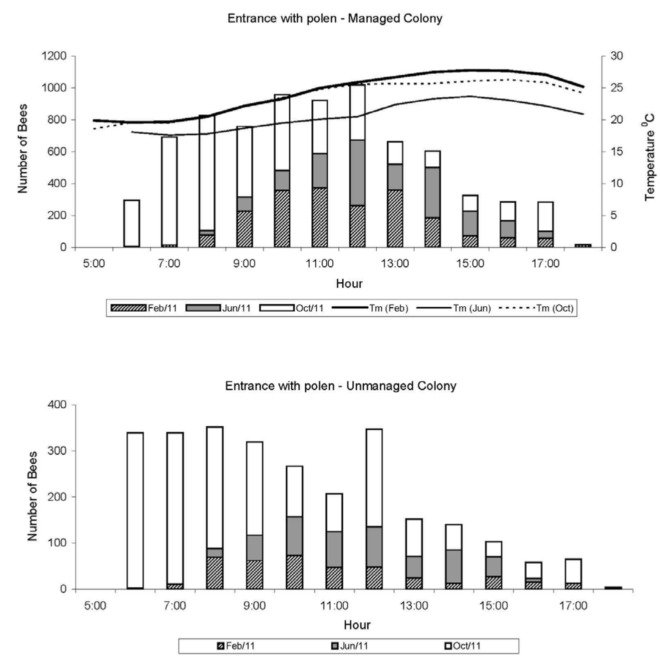

Fig 2. Entrance activity with pollen of *Nannotrigona testaceicornis* and temperature values in colony unmanaged and managed, in February, June and October/11 in the Valley of Capão (Chapada Diamantina) Bahia, Brazil. Tm: Temperature

Although no difference was found between the times of the first and last activities of the managed and unmanaged colonies, there was a difference in the acrophases of the colonies. In the unmanaged colony, located in an open area, the acrophases of all activities occurred generally earlier than in the managed colony (Table 2). The acrophases values for the unmanaged colony varied from 8:57 (October 2011) for the activity of entrance with pollen to 12:00 (June 2011) for the activity of exit. In the managed colony, the times of the acrophases varied from 9:31 (October 2011) for the entrance with pollen to 12:46 (June 2011) for the entrance into the nest (Table 2).

Furthermore, the Watson-Williams test showed significant difference between the acrophases of the two colonies in all

Table 2. Acrophases times of exit and entrance activity; entrance with pollen and entrance with resin for *Nannotrigona testaceicornis* from October 2010 to October 2011 in the Valley of Capão (Chapada Diamantina, Bahia, Brazil).

Bee/activity	Oct/10	Dec/10	Feb/11	Apr/11	Jun/11	Aug/11	Oct/11
Unmanaged colony							
Entrance			11:01		12:21		*
Exit			10:49		12:00		10:23
Pollen			10:27		11:42		08:57
Resin							*
Managed colony							
Entrance	11:25	11:03	12:18	11:12	12:46	11:43	09:45
Exit	11:20	10:48	12:02	11:06	12:22	11:32	09:51
Pollen	09:51	10:35	11:34	10:56	12:38	11:49	09:31
Resin	10:04	11:06	11:41	*	13:26	11:11	

*value non-significant (r < 0.7)

activities that were compared (entrance, exit, and entrance with pollen) in February 2011, June 2011, and October 2011 (Table 3).

All bees' acrophases were later in October 2010 than in October 2011. Although the value of mean temperature was the same in October 2010 and October 2011 (24°C), the light intensity was different. The mean of light intensity in open area was 17,160 lux in 2010 and 39,314 lux in 2011 and in the entrance of the managed colony, a light intensity reached 807 lux in 2010 and 2,446 lux in 2011.

The acrophases for the entrance with resin were detected only for the managed colony; this was generally later than for the other activities, varying from 10:04 (October 2010) to 13:26 (June 2011).

There were significant positive correlations (moderate) between the light intensity and entrance activities (r = 0.48, p<0.01) and exit (r = 0.50, p<0.01) in the managed colony at the open area. Also, there were significant positive correlations (moderate) between the light intensity at the entrance of the colony and the activities of entrance (r = 0.40, p<0.01) and exit (r = 0.44; p<0.01) in unmanaged colony. There were no significant correlations between the two colonies with temperature and also with the relative humidity (Table 4).

Table 3. Comparison of the daily flight activities of the managed and unmanaged colonies of *Nannotrigona testaceicornis* in February, June and October 2011 in the Valley of Capão (Chapada Diamantina, Bahia, Brazil). Significance determined by Watson-Williams test (F) (p < 0.05).

Month/Activity	F	Critical value of F	Statistical significance
February/11			
Entrance	316.93	0.05(1).1.7582 = 3.86	S
Exit	218.51	0.05(1).1.4968 = 3.86	S
Pollen	83.91	0.05(1).1.3719 = 3.86	S
June/11			S
Entrance	62.56	0.05(1).1.5517 = 3.86	S
Exit	41.71	0.05(1).1.4268 = 3.86	S
Pollen	78.06	0.05(1).1.2148 = 3.86	S
October/11			
Entrance	61.57	0.05(1).1.17794 = 3.86	S
Exit	90.95	0.05(1).1.13387 = 3.86	S
Pollen	45.45	0.05(1).1.5725 = 3.86	S

Table 4. Correlation analysis between abiotic variables (temperature, relative humidity, RH and light intensity in open area, LI–AO, and in hive entrance, LI-EC) and biotic variables (bees activities), of the managed (from October 2010 to October 2011) and the unmanaged colonies (in February, June and October 2011) in the Valley of Capão (Chapada Diamantina, Bahia).

Managed colony	Temperature	LI – EC	LI – AO	RH
Entrance	0.15	0.32	0.48*	-0.18
Exit	0.09	0.31	0.50*	-0.15
Polen	-0.02	0.26	0.39	-0.03
Resin	0.05	0.14	0.06	0.17
Unmanaged colony				
Entrance	0.08	0.40*	0.31	-0.05
Exit	-0.01	0.44*	0.32	0.05
Polen	-0.03	0.27	0.26	0.09
Resin	-0.01	0.19	0.05	-0.02

*moderate significance

Discussion

The highest values of the activities in the managed colony of *N. testaceicornis* observed in all months of the study may be an indication that this colony is stronger than the unmanaged one, since the number of flights of the colony characterized it as weak, medium, or strong (Hilário et al., 2000). In general, the two colonies were more active in October 2011 and the managed colony also in August 2011.

The onset of the daily flight activities of *N. testaceicornis* showed regularity in the months investigated for both colonies. The beginning of activities occurred generally in the early morning and the end of the activities in the late afternoon for both colonies. This regularity was also observed by the occurrence of a preferential time for most part of the flight activities of the bees, which was statistically significant and occurred generally in the morning. Based on the first and last activities and on the acrophases observed in *N. testaceicornis,* it can be suggested that the two colonies showed a daily biological rhythm for most of the activities that were observed in the study. In literature, there is a great number of studies showing that daily flight activities occur at specific times through the day (Iwama, 1977; Kleinert-Giovannini, 1982; Guibu & Imperatriz-Fonseca, 1984; Kleinert-Giovannini & Imperatriz-Fonseca, 1986; Souza et al., 2006). However, few studies relate these activities with rhythmic aspects of the foraging behavior of the bees. Heard and Hendrikz (1993), working with *T. carbonaria* in Australia, noted the presence of activity peaks of the workers, indicating the presence of a daily periodicity independently from the meteorological variables considered (temperature, radiation, and relative humidity). Some studies in Brazil consider the pattern of the daily activity of Meliponini as rhythmic manifestations that may have an endogenous origin. Hilário et al. (2003) studying *Melipona bicolor* Lepeletier in the Southeast, and Gouw and Gimenes (2013) studying *Melipona scutellaris,* Latreille and *Frieseomelitta doederleini* (Friese) in the Northeast of Brazil observed the occurrence of the daily activity rhythm in these species.

The daily activities rhythm observed for the two colonies of *N. testaceicornis* may be synchronized by environmental meteorological factors or even by the time that the floral resources are available to the flower visitors. The influence of light/dark and photoperiodic cycles as synchronizers of the activities of *N. testaceicornis* can be observed in the first and last flight activities of both colonies since these activities occur very close to the time of sunrise and sunset, respectively. Moreover, the acrophases occur earlier in October and December (end of Spring and beginning of Summer, when the sun rises earlier) compared to June (Winter, when the sun rises later) in the area of the study, mainly for the managed colony. Besides this, in February 2011, the activities finished later than in the other months in both colonies, and in this month the sunset occurred later. The entrainment of the daily activities by the light/dark cycle was observed by Bellusci and Marques (2001) for the workers of *Scaptotrigona* aff. *depilis* (Moure), suggesting an endogenous character of this synchronization.

The entrainment of the daily rhythm of insect activities by the daily light/dark cycles and the annual photoperiodic cycle has already been discussed in the chronobiological literature (Saunders, 2002; Dunlap et al., 2004). This entrainment would lead to the adjustment of the time activities of the insects (such as bees) with the most favorable moments of the day, when the abiotic factors are favorable for the flight, allowing the organisms to anticipate the favorable or unfavorable cyclical environmental conditions (Dunlap et al., 2004).

The synchronization of the bees with the flowers may also be related to the time of the presentation of resource, as pollen or nectar. In this study, the workers from both colonies of *N. testaceicornis* presented their acrophases for the entrance with pollen in the morning, perhaps related to the greater availability of pollen by the plants at this time of the day, as also considered by Roubik (1989). Moreover, the light/dark and photoperiodic cycles can act in the synchronization of daily rhythm of foraging bees with the flower opening, the pollen exposure, and nectar production as were observed by Gimenes et al. (1993; 1996) in the interaction between flowers of *Ludwigia elegans* (Cambess.) H.Hara (Onagraceae) and flower-visiting bees.

The daily flight activities of both colonies of *N. testaceicornis* were probably influenced by the light intensity because it showed positive correlations between the activities of entrance and exit in both colonies. Moreover, the bees were never active when the light intensity was under 1 lux. The effects of light intensity can be better observed when the activities of both colonies of *N. testaceicornis* are compared. The fact that the acrophases of the unmanaged colony had occurred earlier may be related to the positive influence of light intensity. This influence is due to the higher values of light intensity that acts on the entrance of the unmanaged colony whose nest was located in an open area favoring the earlier exit of the bees.

Another point to be considered is the fact that in October 2011 all acrophases of the managed colony occurred earlier than in the same month in 2010. In both months, the temperature average did not vary, but the intensity light was lowest in 2010. Also, the light intensity seems influencing the end of the flight activity of *N. testaceicornis* in both colonies. According to Corbet et al. (1993) the flight activities of social bees can be more related to radiation than to temperature, especially at the onset of daily activity, next to sunrise. Lutz (1931) also observed the influence of light intensity at the beginning of flight activity of one species of Meliponini, *Trigona mosquito* (Smith). Such effect of light intensity could be modulator of the daily flight activities of bees. In this regard, Bellusci and Marques (2001) observed the endogenous circadian rhythm having light intensity as a modulator factor, in what clearer days influenced positively the increase of the external activities of *S.* aff. *depilis.*

Statistical analysis showed no significant effect of temperature on the activities of *N. testaceicornis*. However, the onset of the activities always occurred when the temperature was over 18°C. In other studies in the literature, it was observed a correlation between temperature and flight activities of the Meliponini, mainly for *Melipona* spp. (Souza et al., 2006; Ferreira-Junior et al., 2010), *Plebeia pugnax* Moure (in litt.) (Hilário et al., 2001), and *Tetragona clavipes* (Fabricius) (Rodrigues et al., 2007). The effect of the temperature on the activities of the bees may be related to the body size; species considered small (such as *N. testaceicornis*) start their activity at a higher temperature than those that are of a larger size. According to Teixeira and Campos (2005) the onset of the external activity of bees of a small body size such as *Plebeia droryana* (Friese), *Frieseomelitta varia* (Lepeletier), *Friesella schrottkyi* (Friese), and *N. testaceicornis* occurred at temperature values ranging from 18°C to 21°C, while larger Meliponini such as *Melipona quadrifasciata anthidioides* Lepeletier and *M. bicolor* started their activities at lower temperatures (ca. 12°C).

The fact of no significant correlation between the daily flight activities of *N. testaceicornis* and the temperature may be related to the fact that bees living in tropical regions, where the average temperature ranges from 20°C to 30°C, so, there are not great thermal stresses (Heinrich, 1993). However, Heard and Hendrikz (1993) correlated daily activity with both climatic factors, temperature and light intensity in flight activities of *T. carbonaria*.

The results from this study showed the influence of light/dark cycle in different months of the year and also the light intensity in the daily flight activities of *N. testaceicornis* in an area of the Brazilian semiarid, where temperatures are relatively favorable to the flight of bees. Therefore, the synchronization of the flight activities with the times of day, and favorable climatic factors can ensure bee survival, especially for Meliponini of small size, as *N. testaceicornis*.

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