



RESEARCH ARTICLE - BEES

Colony performance of *Melipona quadrifasciata* (Hymenoptera, Meliponina) in a Greenhouse of *Lycopersicon esculentum* (Solanaceae)

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Abstract

The use of stingless bees in greenhouses has provided tremendous benefits to diverse crops in terms of productivity and fruit quality. However, knowledge about management techniques in these environments is still scarce. The present study aimed to evaluate colony performance of *Melipona quadrifasciata* Lepeletier, 1836 in a greenhouse of *Lycopersicon esculentum* Mill. and its potential use in pollinating this crop. Six nests of *M. quadrifasciata* were introduced in a greenhouse in Araguari, Minas Gerais state, Brazil. The development of colonies inside the greenhouse was investigated and the foraging behavior of the workers was assessed before introduction, into the greenhouse and after the nests had been removed from the greenhouse. The vital activities of colony maintenance were performed unevenly throughout the day inside and outside the greenhouse, but with confinement the daily period of foraging decreased and bees started collecting pollen from the flowers after approximately six months. The difficulty in orienting to and identifying flowers by the workers was attributed to sunlight diffusion and blockage of ultraviolet radiation caused by the cover on the greenhouse. Structural changes in the greenhouses, as well as improvements in management techniques, are required for better utilization of stingless bees for pollination of plant species grown in greenhouses.

Introduction

Worldwide, there are approximately 20,000 described bee species (Michener, 2007). Among them, the stingless bees are highly social organisms that constitute an important group due to the ecological and economic roles they play. These bees belong to the subtribe Meliponina, which consists of approximately 400 species grouped in about 50 genera, distributed across tropical and subtropical regions, in America, Southeast Asia, Africa, Madagascar and Australia (Silveira et al., 2002).

Stingless bees are responsible for the pollination of many native (Michener, 2007) and cultivated (Heard, 1999) plant species. As a result, since the 1990s, the number of studies involving the introduction of these bees in greenhouses to evaluate their pollination efficiency on different crops has grown steadily. Particularly noteworthy among these are: eggplant pollination, using *Melipona quadrifasciata* Lepeletier, 1836 (Bispo dos Santos, 2008); basil pollination, tested with *Nannotrigona testaceicornis* Lepeletier,

1836 (Bispo dos Santos, 2008); strawberry pollination, tested with *Tetragonisca angustula* Latreille, 1811 (Malagodi-Braga, 2002), *Scaptotrigona* aff. *depilis* Moure, 1942 (Roselino et al., 2009) and *N. testaceicornis* (Roselino et al., 2009); sweet pepper pollination, using *M. subnitida* Ducke, 1910 (Cruz et al., 2005), *M. quadrifasciata anthidioides* Lepeletier, 1836 (Roselino et al., 2010) and *M. scutellaris* Latreille, 1811 (Roselino et al., 2010); and tomato pollination, tested with *N. perilampoides* Cresson, 1878 (Cauich et al., 2004; Palma et al., 2008b) and *M. quadrifasciata* (Del Sarto et al., 2005; Bispo dos Santos et al., 2009).

Tomato, *Lycopersicon esculentum* Mill. (Solanaceae), is one of the most widespread vegetable crops in the world, being cultivated in almost all parts of the world under different cropping systems and various levels of management. It is a self-fertilizing plant and its flowers are bisexual, do not produce nectar and present poricidal anthers. Therefore, in order to release the pollen, vibration of the anthers with consequent opening thereof is necessary (Buchmann, 1983). Pollination can be performed by the shaking of the anthers



by the wind, but cross-pollination is ensured through visits of bees that exhibit vibratory behavior or buzz-pollination (Buchmann & Hurley, 1978; Heard, 1999; Nunes-Silva et al., 2010).

Tomato can be grown in open areas or in greenhouses. When grown in open areas, the released pollen is carried by the wind (McGregor, 1976; Free, 1993) and/or natural pollinators, especially bees, which have free access to the flowers. As for cultivation in greenhouses, pollination is normally performed by the mechanical method of vibrating the flowers, to compensate for the absence of wind and natural pollinators. However, studies involving the management of pollinators in greenhouses have indicated improvements in productivity and fruit quality (Cruz & Campos, 2009). Among the pollinators of tomato in greenhouses, *M. quadrifasciata* proved to be efficient in pollinating the long-lived variety (Del Sarto et al., 2005), but studies testing colony performance and pollination efficiency of this bee on the grape variety, grown mostly in greenhouses, are yet to be performed.

Stingless bees are considered particularly promising for use as commercial pollinators (Cruz & Campos, 2009), given that they do not present a functional sting, are easy to handle (usually low aggressive-nonstinging), have populous and perennial nests, present a marked worker recruitment behavior and stock a large amount of food (Heard, 1999; Malagodi-Braga et al., 2004). Nevertheless, the lack of techniques for management and multiplication of nests has hindered the availability and use of these bees in agriculture on a large-scale (Imperatriz-Fonseca et al., 2006).

The present study sought to advance knowledge about management of stingless bees in greenhouses for use as commercial pollinators. We have specifically evaluated colony performance of *M. quadrifasciata* in a greenhouse of grape tomato, *L. esculentum*.

Material and Methods

Study area

The study was performed at the “Meliponário da Universidade Federal de Uberlândia (UFU)”, located at the “Fazenda Experimental do Glória” (FEG) (18°56'57”S - 48°12'14”W), Uberlândia, Minas Gerais state, Brazil, and in the “Chácara Paraíso” (CP) (18°39'3.55”S - 48°11'7.51”W), located in the municipality of Araguari, Minas Gerais state, Brazil. At FEG, agricultural and cattle raising activities are developed, but preserved fragments of cerrado and semideciduous forest can be found. CP consists of areas of cultivation and pasture, fragments of cerrado and 12 greenhouses where grape tomatoes are grown. One of these was used for the experiments.

The climate of the regions of Uberlândia and Araguari is marked by two distinct seasons, the rainy season that extends from October to March and a dry season from April to

September. Annual rainfall varies between 1,160 and 1,460 mm/year and the average annual temperature is between 23 and 25°C, being uniform throughout the year (Alves & Rosa, 2008).

The greenhouse used in the experiment comprised approximately 1,344 m² (48 m x 28 m), being covered at the top with an Extra Long Life (ELL) Diffuser Antivirus plastic diffuser and fully enclosed on the sides with anti-aphid screens (Fig 1A). The greenhouse presented 24 planting rows and each of these had an average of 112 tomato plants, adding up 2,688 plants.

Introduction of nests of *M. quadrifasciata*

Six nests of *M. quadrifasciata* with similar population sizes were introduced at the onset of flowering, in March 2012. The nests were kept in wooden boxes with an approximate size of 40x25x25cm (Fig 1B). Before introduction, due to the difficulty in bee orientation inside the greenhouse (Bartelli, personal communication; Del Sarto, 2005), the population of old foragers was removed to avoid their loss in the enclosed space, following the methodology used by Cauich et al. (2004). Moreover, for the same reason, the nests were placed in the greenhouse after dark (Cuyppers, 1968).

In order to allow a homogeneous distribution of the bees on the flowers, nests were arranged in the central region of the greenhouse (Free, 1993) and supported by plastic boxes installed in eucalyptus logs located within the planting rows. To increase the number of reference points for the bees, since the uniformity of greenhouses may hinder orientation (Dyer, 1994), the entrance to each nest was painted with different color patterns.

Containers with water, mud and cerumen (alternative source for plant resin) of *T. angustula* were placed on the plastic boxes (Fig 1C). Nests were sporadically fed with *A. mellifera* pollen macerated with sugar and water until the onset of flower visitation, and fed weekly with syrup (a mixture of honey of *A. mellifera*, sugar and water in the ratio 1:1:1) over the entire period of confinement. For pesticide application, a common management practice, the entrances of all nests were sealed with paper, the nests themselves were protected with plastic bags and the containers covered with cardboard boxes. These protections were removed only after the pesticide had dried; then, the entrances of the nests were unobstructed after dark. Furthermore, to increase luminosity inside the greenhouse, another common management practice, the ceiling and sides of the greenhouse were washed with soap and water in late August.

In order to facilitate bee orientation, one mercury vapour lamp and two mixed lamps (alternative sources of light and ultraviolet radiation) were installed inside the greenhouse in one of the corridors where some of the nests were placed (Fig 1D). The lamps were connected to a digital timer, which was programmed to switch them on at 6 am and switch them



Fig 1. A) Greenhouse used in the experiment at Chácara Paraíso in Araguari; B) Nest of *Melipona quadrifasciata* installed inside the greenhouse; C) Containers with water, mud and cerumen available inside the greenhouse; D) Lamps installed inside the greenhouse.

off at 6 pm. They remained in the greenhouse from early June to mid-July. The lamps' efficiency, as well as the influence of the diffuser film on solar radiation focused on the greenhouse, was evaluated by analysing the pattern of reflectance of the flowers. 10 flowers were photographed between 10 and 11 am in a white background with a Sony Cyber-shot DSC-H20 digital camera. Each flower had its picture taken in three different circumstances: inside the greenhouse and next to the lamps; inside the greenhouse and 12m far from the lamps; and outside the greenhouse. Photoshop software CS3 10.0 was used to quantify patterns of chromatic (RGB - red, green and blue in the light spectrum) and achromatic (luminosity) saturation of flower corollas.

Colony performance of *M. quadrifasciata*

To assess colony performance, the internal conditions of nests, behavior of workers in flight and on flowers, foraging activities and temperature inside and outside the greenhouse were analyzed. Figure 2 outlines the arrangement of nests inside the greenhouse. Nests D and F remained in the greenhouse for three months and then they were relocated to the outside area.

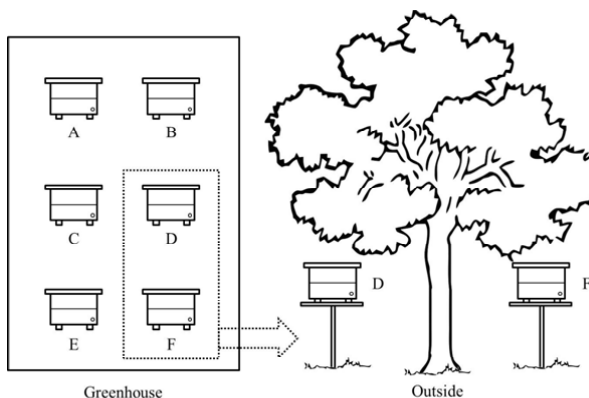


Fig 2. Diagram representing the arrangement of nests inside and outside the greenhouse.

Internal conditions

Biweekly, from March to October 2012, we assessed qualitative features of nests, such as the presence of cells in construction (yes / no), number of workers (very low / low / medium / high) and presence of guards (yes / no).

Behavior of workers

Biweekly, from May to October 2012, the flight behavior of bees and the behavior of workers visiting tomato flowers were observed inside the greenhouse. These behaviors were assessed every 15 minutes during each hour (from 6 am to 6 pm).

Foraging activities

Foraging activities of bees were assessed through direct observations of the flow of workers from nests. The quantity and quality of resources (pollen, nectar/water or water, resin, mud and garbage) that entered and/or left the nests were recorded every 10 minutes during each hour (from 6 am to 6 pm). In order to compare the daily pattern of resource collection of workers inside and outside the greenhouse, the procedures described above were performed: before introduction in three nests (B, D and F, Fig 2), over three non-consecutive days in late February 2012 at FEG; inside the greenhouse in two nests (C and E, Fig 2), biweekly from May to October 2012; and outside the greenhouse in one (nest F, Fig 2) of the nests removed from the greenhouse at CP, biweekly from June to October 2012.

Temperature

Through the use of a digital thermohygrometer, the temperature inside and outside the greenhouse was measured every day at 8 am, 11 am and 3 pm from April to October 2012.

Statistical analyses

To verify whether the patterns of reflectance of tomato flowers depended on the presence of the lamps and diffuser film or not, we conducted an analysis of variance (ANOVA) followed by Tukey's test, since there was no difference between blocks (flowers) for RGB ($F = 1.118$; $df = 9$; $P = 0.399$) and luminosity ($F = 1.037$; $df = 9$; $P = 0.449$) (Zar, 2010). In order to evaluate the daily pattern of resource collection of bees inside and outside the greenhouse, circular analyses were performed using Oriana 4.1 software (Kovach, 2011), using the Rayleigh test for the calculation of probabilities of distribution of bees throughout the day. To investigate whether the temperature differed inside and outside the greenhouse during the months the nests were confined, we conducted a paired t test (Zar, 2010).

Results

Internal conditions

Two nests (A and B, Fig 2) did not survive the conditions of confinement and died after about two months. To avoid their loss, after three months of confinement, two other nests (D and F, Fig 2) were removed from the greenhouse and placed outside due to the absence of cells in construction, the low number of workers and the absence of guard workers. One of these nests (F, Fig 2) survived and increased the number of workers to “high” after two months, but the other died (D, Fig 2), attacked by phorids (Diptera). Nests C and E (Fig 2) survived the conditions of confinement and remained in the greenhouse during the entire experiment, from March to October 2012, with cells in construction, guard workers and a high number of workers.

Behavior of workers

Inside the greenhouse, flight activities of bees began after 22 days of confinement. However, workers were limited only to the removal of garbage from the colonies and many of them clashed against the ceiling or sides of the greenhouse, where they remained and ended up dying. This flight behavior toward the ceiling and sides of the greenhouse decreased over time during confinement but did not cease, even after the beginning of collection activities in the flowers.

The beginning of pollen collection from the tomato flowers occurred in late July, but there was only one record during that period and none in the following month. Intensive visits of workers to flowers (six records of pollen collection per nest) occurred in early September, after nearly six months of confinement, and the ceiling and sides of the greenhouse had been washed with soap and water. Bees landed on the anthers of a flower and bowed around or at the apex of the anthers cone to grab it (Fig 3A). Thus, they transmitted vibrations to the anthers through their thorax and legs to release the pollen. For the transfer of pollen from the body to the corbicula, some bees remained stuck to the base of the anthers cone by the legs and/or jaws, while others performed the transfer during flight or on other parts of the plant, such as the leaves and fruits (Fig 3B).



Fig 3. A) *Melipona quadrifasciata* worker collecting pollen in tomato flower; B) *M. quadrifasciata* worker transferring pollen from the body to corbicula on a fruit.

Pattern of reflectance of the flowers

The lamps installed inside the greenhouse did not alter the flight behavior of the bees and did not influence the RGB and luminosity of tomato flower corollas. However, the patterns of chromatic ($F = 10.51$; $df = 2$; $P < 0.001$) and achromatic ($F = 8.44$; $df = 2$; $P = 0.001$) saturation of the flowers were significantly different inside and outside the greenhouse (Fig 4).

Foraging activities

Before introduction, at Fazenda Experimental do Glória (FEG), the bees began to forage at around 5:40 am. The number of forager workers coming in and out of nests was not uniform throughout the day and the period of highest activity occurred at 6-8 am. The relative frequency of collection per resource varied considerably between the nests. Of the workers observed, 7.7-18.2% transported pollen, 17.0-81.4% nectar/water, 3.6-55.7% resin and 0.2-11.1% mud. The resources were not obtained uniformly throughout the day. Pollen collection occurred only in the morning, with a peak at 6-8 am. The highest mean frequency observed for nectar/water occurred between 6 and 7 am and the collection of this resource gradually decreased throughout the day. The peaks of collection of resin and mud were, respectively, at 8-10 am and at 8-11 am (Fig 5, Table 1).

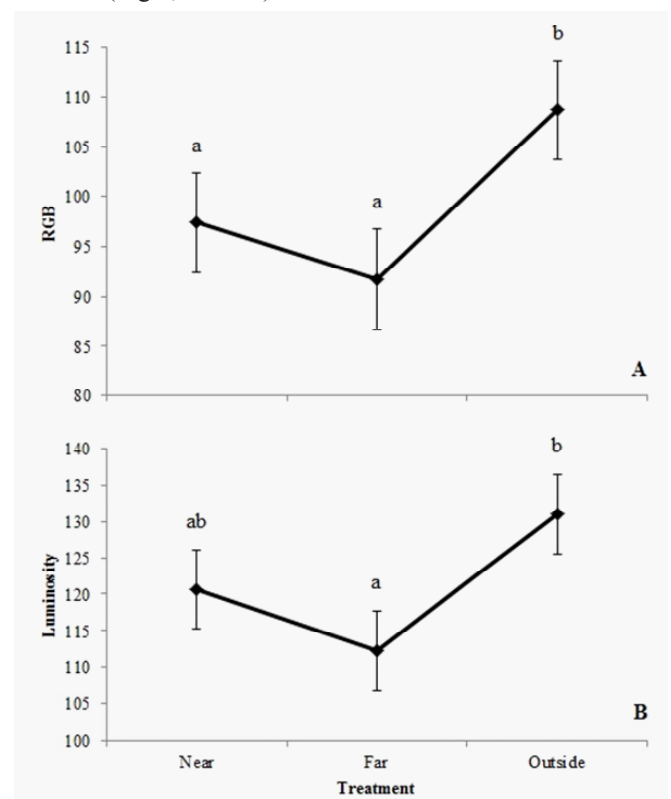


Fig 4. Mean values (\pm standard error) of patterns of chromatic (or RGB - A) and achromatic (or luminosity - B) saturation of tomato flowers in the different treatments (Near: inside the greenhouse and next to the lamps; Far: inside the greenhouse and far from the lamps; and Outside: outside the greenhouse) at Chácara Paraíso in Araguari. Distinct letters indicate significant differences between treatments.

Table 1. Mean time vector (with the number of observations (n) and probability values (p), according to Rayleigh test) for resources collection and total external activity throughout the day by *Melipona quadrifasciata* at Fazenda Experimental do Glória (FEG), in Uberlândia, and inside and outside the greenhouse at Chácara Paraíso, in Araguari.

Resource	FEG			Inside		Outside
	Nest B	Nest D	Nest F	Nest C	Nest E	Nest F
Pollen	7:56 am (n = 31) ($P < 0.001$)	6:51 am (n = 60) ($P < 0.001$)	6:44 am (n = 67) ($P < 0.001$)	7:40 am (n = 3) ($P = 0.036$)	8:10 am (n = 6) ($P < 0.001$)	7:29 am (n = 10) ($P < 0.001$)
Nectar/water or Water	7:18 am (n = 329) ($P < 0.001$)	7:44 am (n = 79) ($P < 0.001$)	7:52 am (n = 117) ($P < 0.001$)	4:00 pm (n = 3) ($P = 0.042$)	2:45 pm (n = 6) ($P = 0.011$)	7:33 am (n = 19) ($P < 0.001$)
Resin	10:41 am (n = 15) ($P < 0.001$)	9:50 am (n = 259) ($P < 0.001$)	9:39 am (n = 136) ($P < 0.001$)	-----	11:30 am (n = 2) ($P = 0.144$)	6:30 am (n = 2) ($P = 0.144$)
Mud	9:00 am (n = 1) ($P = 0.512$)	9:42 am (n = 50) ($P < 0.001$)	10:38 am (n = 23) ($P < 0.001$)	-----	-----	-----
Total external activity	7:32 am (n = 801) ($P < 0.001$)	9:00 am (n = 910) ($P < 0.001$)	8:36 am (n = 719) ($P < 0.001$)	11:43 am (n = 17) ($P < 0.001$)	11:28 am (n = 39) ($P < 0.001$)	7:45 am (n = 62) ($P < 0.001$)

(-----) Insufficient data for analysis or resource not collected

Inside the greenhouse, the activity of bees began approximately at 7 am and was not uniform throughout the day, with the greatest movement of workers occurring between 5 and 6 pm for nest C, and between 12 and 1 pm for nest E. The relative frequency of collection per resource varied between the nests. From the workers observed over the entire period of confinement, 14.8-21.4% transported pollen, 37.5-60.7% water and 7.8-23.3% resin (cerumen). There was no collection of mud. Workers obtained pollen and water in a heterogeneous way throughout the day and there was no defined peak of resin collection. Pollen collection was limited to the morning, with a peak between 8 and 9 am. The highest mean frequency observed for water occurred between 5 and 6 pm (Fig 5, Table 1).

After the nests were removed from the greenhouse and placed in the outer area, it was possible to observe workers carrying pollen in their corbiculas on the following day. The movement of bees was not uniform throughout the day and peak activity occurred between 6 and 7 am. Of workers observed over the months, 31.4% carried pollen, 57.5% nectar/water and 7.0% resin. The resources were obtained in a heterogeneous way throughout the day, except resin. Pollen collection, limited again to the morning, and nectar/water collection had peaks between 7 and 8 am, and between 6 and 7 am, respectively. There was no collection of mud (Fig 5, Table 1).

Temperature

The temperature inside the greenhouse was significantly higher over the entire period of confinement of the nests of *M. quadrifasciata* ($t = 6.99$; $df = 6$; $P < 0.001$; Fig 6).

Discussion

The results of our experiments showed that acclimation of *Melipona quadrifasciata* to conditions inside greenhouses are colony-dependent and, besides foraging activities varied a little inside and outside the greenhouse, the daily period of foraging into the greenhouse decreased and bees took a long time to visit flowers consistently for pollen collection.

The foraging behavior of stingless bees is related both to factors intrinsic to the nest, including the ability to communicate and population size, and extrinsic factors, such as the abundance and distribution of resources in the environment and susceptibility to abiotic factors (Fidalgo & Kleinert, 2007). However, despite the methodological differences used in the introduction of the nests and the different conditions of confinement (size and structure of the greenhouses, crop type, etc), our results about the foraging behavior of *M. quadrifasciata* were similar to results found in other studies that evaluated the introduction of stingless bees in greenhouses (Del Sarto et al., 2005; Nunes-Silva et al., 2013). The workers concentrated pollen collection in the morning.

Inside the greenhouse, the foraging behavior of *M. quadrifasciata* showed little variation compared to the outside. However, stronger patterns (represented by smaller probability values, Table 1) in the external activities of bees were observed at FEG, before confinement. Additionally, the daily period of foraging in the greenhouse decreased, as well as for *N. perilampoides* (Cauch et al., 2004; Palma et al., 2008a), and bees visited flowers consistently for pollen collection only after approximately six months of confinement. The time required for acclimation to protected environments

varies both between species and between colonies of the same species (Malagodi-Braga, 2002). However, this timing was probably determined by the presence of the diffuser film on the coverage of the greenhouse. As evidence of this, pollen collection observed in nests placed in the outer area started immediately after their removal from the greenhouse.

Due to the dispersant effect of plastic films used in greenhouses coverage, solar radiation is one of many environmental factors that can be changed by using protected crops (Schwengber et al., 1996). This could be evidenced via the different reflectance patterns found on tomato flowers inside and outside the studied greenhouse. This dispersant effect is favorable to plants, since the fraction of diffuse solar radiation is more effective for photosynthesis (Farias et al., 1993), but may have hindered bee orientation and identification of flowers by worker bees, affecting foraging activities inside the greenhouse and thereby delaying the beginning of pollen collection.

In relation to the external environment, global solar radiation (measured by luminous flux density) and diffuse solar radiation (multidirectional) are respectively lower and higher inside the greenhouse as a result of reflection and absorption

by the material of the plastic coverage. In turn, this reflection and absorption are determined, for example, by the conditions of coverage at the time of use and dust deposition (Farias et al., 1993). This explains why visits to flowers by *M. quadrifasciata* workers intensified when the ceiling and sides of the greenhouse were washed with soap and water as part of the management of the protected tomato crop.

For orientation, bees use elements like the sun, polarized light, visual cues present in the environment and ultraviolet radiation (Kerr, 1973; Dyer, 1994; Briscoe & Chittka, 2001). The presence of clouds and fog cause dispersion of light rays and reduces the polarization signal (Shashar & Cronin, 1998). Thus, greenhouse cover can produce a similar effect to that promoted by clouds and restrict bee activity to a period in which light rays are less dispersed by the cover (Malagodi-Braga, 2002).

Besides the dispersant effect, the film ELL Diffuser Antivirus eliminates the entry of ultraviolet (UV) to hinder the vision of tomato pest insects (Electro Plastic, 2013). This could also have been an aggravating factor hindering the orientation of *M. quadrifasciata* forager workers inside the greenhouse. Although this species present a wide distribution throughout Brazil (Camargo & Pedro, 2012), foraging in places relatively poor in UV, such as under the canopies of dense tropical forests (Briscoe & Chittka, 2001), the nests were in an open environment at FEG and were accustomed to high UV exposure before introduction to the greenhouse.

In temperate areas, where bumble bees are largely used for greenhouse tomato pollination, showing high efficiency (Banda & Paxton, 1991; Morandin et al., 2001), the structure of the greenhouses are similar to those used in this study. However, in the case of bumblebees, it seems that the reduction in UV light can be compensated and as a result their visit to flowers is not affected by the type of greenhouse coverage (Dyer & Chittka, 2004).

In the present study, as in Del Sarto et al. (2005), the performance of *M. quadrifasciata* to conditions of confine-

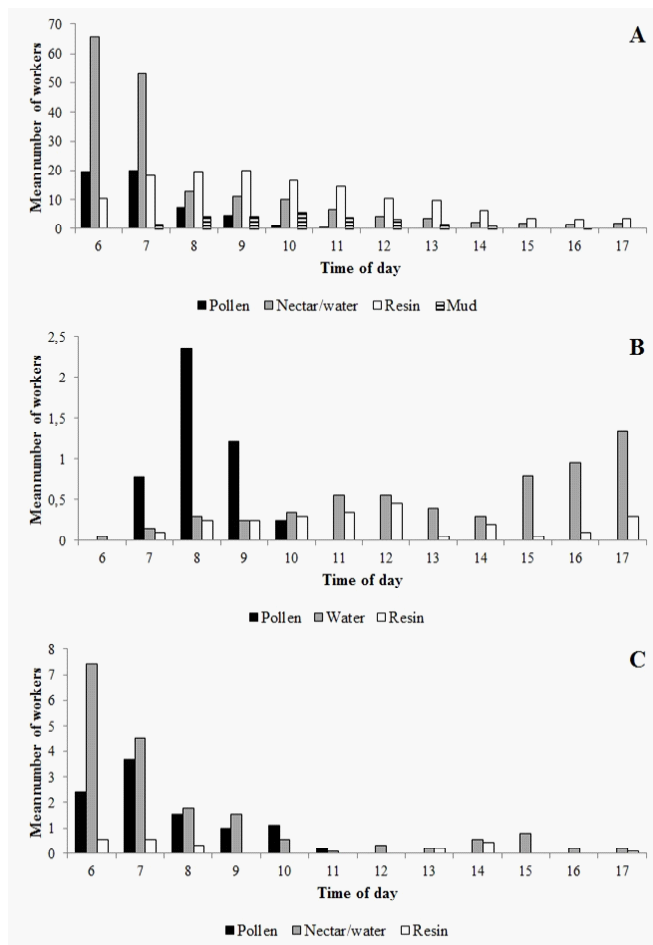


Fig 5. Mean number of workers for each resource collected throughout the day by *Melipona quadrifasciata* before introduction (A), at Fazenda Experimental do Glória, in Uberlândia, and inside (B) and outside (C) the greenhouse at Chácara Paraíso, in Araguari.

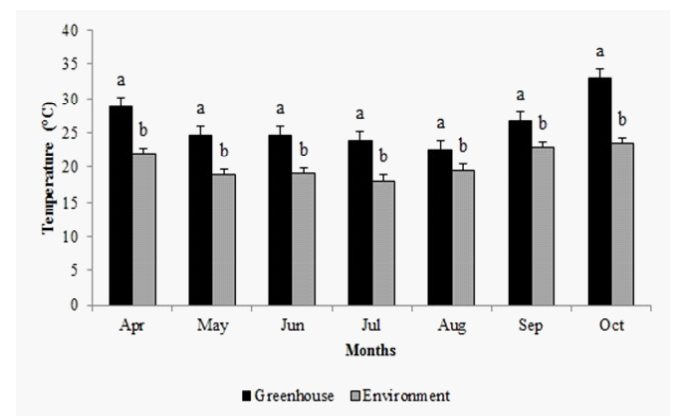


Fig 6. Mean values (\pm standard error) of temperature ($^{\circ}$ C) inside and outside (environment) the greenhouse during the period of confinement of nests of *Melipona quadrifasciata* at Chácara Paraíso in Araguari. Distinct letters indicate significant differences for each month.

ment proved to be colony-dependent. Studies have shown that stingless are tolerant to high temperatures and capable of cooling the inside of their colony by ventilation generated by beating their wings in the nest entrance. However, we believe that the high temperatures inside the greenhouse may have been an aggravating factor for colony development, since the intense heat decreases the density of larval food, causing the eggs to sink and death of larvae by drowning (Amano et al., 2000).

From results obtained in this study and considering the benefits that the use of stingless bees has provided to diverse crops grown in greenhouses (Cruz & Campos, 2009), the control of temperature and humidity inside greenhouses would be an important step. Another idea would be to exchange the plastic greenhouse coverings for materials that interfere less significantly in solar radiation, or, alternatively, a less drastic alternative would be to intercalate the plastic cover with such materials, allowing at least partial diffusion of solar radiation to occur in the greenhouse. For the farmer, taking into account the duration of the cycle of grape tomato in greenhouses, which is eight months, using stingless bees is not practical if these bees require a great deal of time to acclimate and initiate foraging activities. However, studies assessing the cost-benefit relationship of such structural changes in greenhouses must still be made and more information that will permit improvements in management techniques for stingless bees in greenhouses is needed.

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