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Foraging Behaviour of *Apis mellifera* L. and *Scaptotrigona bipunctata* on *Dombeya wallichii* Flowers in Southern Brazil

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Abstract

This research was carried out to evaluate the foraging behaviour of *Apis mellifera* and *Scaptotrigona bipunctata* and its relationship with environmental variables in *Dombeya wallichii* considering the following aspects: 1) Type of resource harvested, 2) Visitation rate, 3) Length of visit, 4) sugar concentration in the stored nectar in the honey crop of *A. mellifera* and secreted by the flower and 5) floral constancy. Both species collected mainly nectar, with a visitation rate of 4.2 flowers for *A. mellifera* and 1.1 flowers for *S. bipunctata* and visit length of 9.2 and 34.2 seconds, respectively. The sugar concentration had a positive relationship with temperature and luminosity, with mean values of 12.3% in the nectar and 14.2% in the honey crop. Bees were classified by specialists because the pollen of *D. wallichii* was predominant in the pollen basket, but the pollen of *Emilia sonchifolia* and *Raphanus sativus* was also found. These results suggest that the species under study take advantage of the resources offered by *D. wallichii*, therefore, this species is an important source for the maintenance of the bees in the season of lack of food.

Keywords

Flower Constancy, Foraging Behaviour, Honeybee, Stingless Bee, Plant-Pollinator Interaction

1. Introduction

Pollinators provide an essential service to the food production and natural ecosystems, *Apis mellifera* is the main species used for pollination of agricultural and horticultural crops because their body parts are modified to effect pollination, visit a wide variety of flower types and are relatively abundant and manageable [1] [2]. The pollination services offered by this species in North American crops were estimated at US $11.68 billion in the year 2009, similarly, on crops dependent in Brazil, the economic contribution of all pollinators corresponded to US $12 billion or 30% of the total production [3] [4]. Interspecific interactions between *A. mellifera* and wild bees may modify the behaviour and increase the pollination value of individual species [5]. However, many studies have reported declines in bee populations around the world and have been the subject of intense research due to the ecological and economic damages resulting from the loss of pollination services [6] [7] [8] [9].

Bee pollination increases the quality and yield of many agricultural crops [10] [11] [12] [13], however, in Brazil, the beekeeping is mostly focused on the production of honey, propolis and pollen [14] [15]. One of the great barriers of beekeeping is the cold and dry season, when the supply of floral resources in the environment decreases, causing a decrease in the development of the colonies [16]. Some of the alternatives for this period are the supplementation of the colonies [17] and the planting of melliferous flora [18] [19]. Among the species used for flowering in the winter period is the *Dombeya wallichii*.

The genus *Dombeya* of the family Malvaceae has a paleotropical distribution with 206 species, of which 173 are endemic to the islands of Madagascar and Comoros [20]; one of them, *D. wallichii* was introduced and adapted in Brazil, due to similar tropical conditions. It presents a shrub tree size of three to nine meters, forming large dense canopies with perennial life cycle, flowering period in fall/winter and flowering peak between June and July, with the influence of location and climatic conditions [21].

The efficiency in angiosperm reproduction is related to strategies developed to attract pollinators [22]; *D. wallichii* has floral characteristics such as colour, odour and nutritional rewards (nectar and pollen) to attract several floral visitors and thus to succeed in pollination [23] [24]. Among these visitors, bees are the dominant group, mainly *A. mellifera* and *Trigona spinipes*, in addition to other insects of the Hymenoptera, Lepidoptera, Coleoptera, Hemiptera and Diptera order [25] [26] [27].

Due to the lack of information on the interaction between the flower of *D. wallichii* and floral visitors, the present study evaluated the pollination behaviour of *A. mellifera* and *Scaptotrigona bipunctata* on *D. wallichii*, as well as the sugar concentration in the nectar along the day, in flower and in the honey crop of Africanized honeybee.

2. Material and Methods

Experimental Area

The experiment was developed at the Experimental Farm of Iguatemi (**Figure 1**), State University of Maringá, Maringá-State of Paraná, in the South of Brazil,

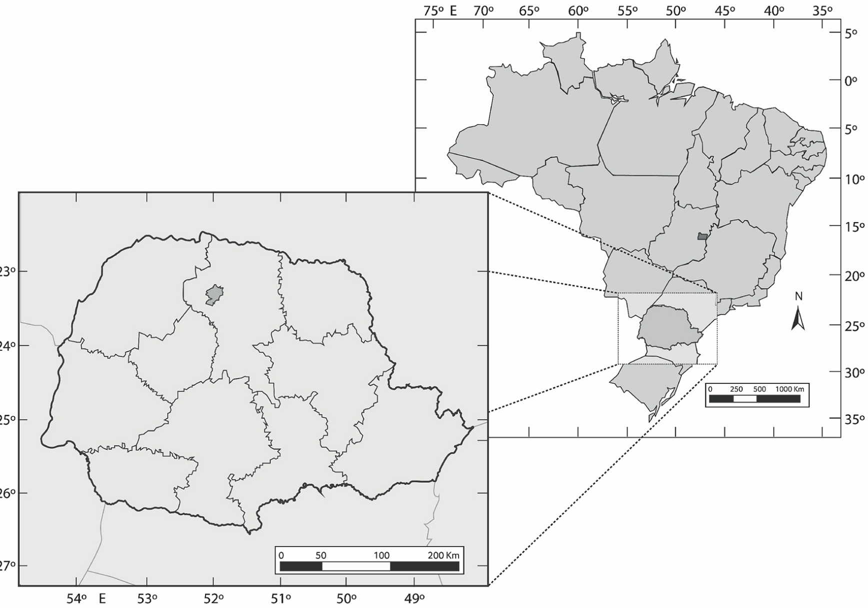

Figure 1. Location of the study area.

where the climate is subtropical, with warm, humid, mesothermal summer and the average temperature of the hottest month exceeding 22˚C, *i.e.*, Cfa according to the Köppen classification [28].

During the peak of flowering of *D. wallichii*, from 8 am to 5 pm, ten flowers and ten bees were collected to analyze the sugar concentration (˚Brix) in the nectar and in the honey crop, with a capillary tube (10 μL) and refractometer (Atago Refractometer). Also, the foraging behaviour of *Apis mellifera* and *Scaptotrigona bipunctata* was recorded, following the focal plant method (6.1.3.3) described by [29] in which were observed for 10 - 20 minutes per hour of pollinator activity his behaviour of a particular individual. For each visit, the following variables were observed: visitation rate; length of visit; type of floral resource collected (nectar or pollen) and if there was contact with anthers and stigma.

The floral constancy was evaluated in four times (8 and 11 am, 2 and 5 pm) by pollen analysis collected from the pollen basket [30], staining the pollen grains with 1% acetic carmine and comparing their external morphology with the pollen grains collected directly on the anthers of the flower buds. 500 grains were counted under an optical microscope, and the percentage of pollen of *D. wallichii* and other floral species was calculated. In addition, the climatic variables, temperature (˚C), humidity (%) and solar radiation (100X Lux) were measured on 28 June, 4 and 9 July 2018 using a thermo hygrometer and a digital lux meter.

3. Results

A. mellifera honeybees collecting nectar were more abundant (74.3%) than those collecting pollen (4.7%) and those collecting both resources (21.0%). At 4 pm, all

A. mellifera individuals were collecting nectar and at 1 pm, 50% were collecting nectar and 50%, nectar and pollen (**Figure 2(a)**). In *S. bipunctata*, a higher number of bees were observed collecting nectar (69.3%) compared to pollen foragers (17.0%) and collecting both resources (13.7%). At 11 am, there was the highest percentage of nectar foragers (83.3%) and at 5 pm, 66.7% of the bees were collecting pollen (**Figure 2(b)**).

The sugar concentration in the nectar ranged from 9.7 to 15.7 ˚Brix, while the content of the honey crop presented higher values, from 12.2 to 16.3 ˚Brix; at 12 pm, the highest sugar concentration was observed in both the plant and the honey crop (**Figure 2(c)**). There was a positive interaction between temperature and luminosity, in which, in the period from 12 to 1 pm the peaks in the two variables were found, around 24˚C and 550 (100X Lux), respectively. The humidity was inversely proportional to temperature and luminosity (**Figures 2(d)-(e)**).

A. mellifera visited on average 4.2 flowers per minute with a length of 9.2 seconds in each flower, and *S. bipunctata*, 1.1 flowers per minute, with a length of 34.2 seconds per visit. However, the dynamics of *S. bipunctata* specimens with the reproductive structures of the flower is less effective for plant fertilization, since 42.0% of the bees collected the nectar without touching the anther or the stigma. On the other hand, *A. mellifera* came into contact with the two reproductive structures in 80% of visits (**Table 1**).

The *A. mellifera* and *S. bipunctata* presented high constancy for the flower of *D. wallichii*, since the percentages of pollen grains of this species contained in the bees' pollen basket were 98.6% and 94.1%, respectively. In relation to the other plant species found in the pollen load, *A. mellifera* had a higher amount of pollen grains from *Emilia sonchifolia* and in *S. bipunctata*, pollen from *Raphanus sativus* L. After 10 am, we observed the highest constancy of *A. mellifera*, reaching 100%, while in *S. bipunctata*, it varied throughout the day from 50% to 100%.

4. Discussion

Pollination by bees depends on climatic conditions, as they influence both the crop and the bees [31], from 11 to 12 although there is an increase in sugar concentration in nectar, the data show a reduction in nectar collection, coinciding with temperature increase (22˚C - 23˚C), luminosity (632.5 - 662 100X lux) and relative humidity reduction (50% - 46%). However, the high temperature is not the only factor that interferes with the collection behaviour of the bees, so it is necessary to interact with the relative humidity and the luminosity. Given that at 3 to 4 pm, there is an increase in the nectar collected by bees, even with a decrease in sugar concentration in nectar, temperature, luminosity and increase in relative humidity (**Figure 2**). Temperature has a significant effect on foraging activity of pollinators, in which high temperatures favour the presence of floral visitors while high relative humidity suppresses activity [32]. Therefore, in colder periods there was less presence of floral visitors, this same behaviour was observed in two species of stingless bees, *Melipona beecheii* and *Melipona fasciata* [33].

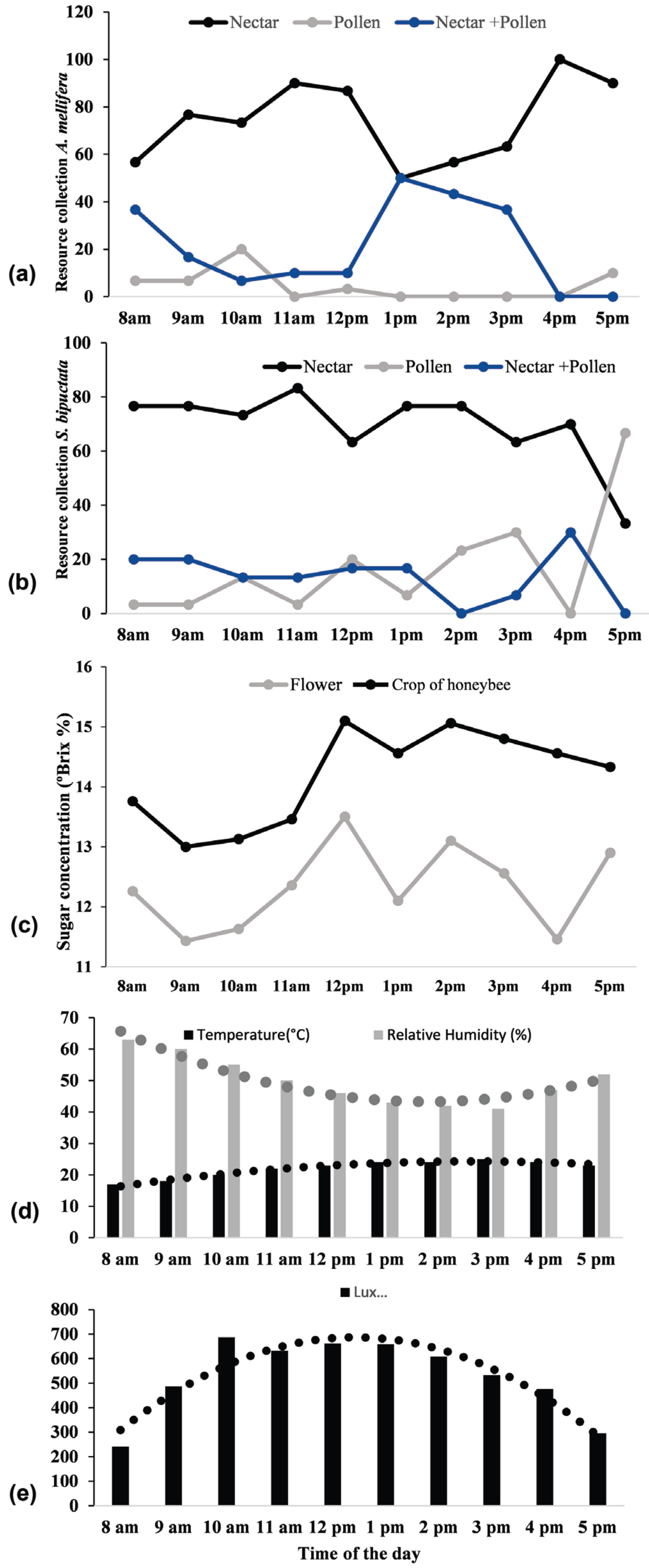

Figure 2. (a)—Foraging behaviour of *Apis mellifera*. (b)—Foraging behaviour of *Scaptotrigona bipunctata*. (c)—Sugar concentration in the nectar of the flower and in the honey crop of *Apis mellifera*. (d)-(e)—Climatic data of the evaluated period.

Table 1. Foraging behaviour of *Apis mellifera* and *Scaptotrigona bipunctata* on the flower of *Dombeya wallichii*.

	Apis mellifera	*Scaptotrigona bipunctata*
Visitation rate (flower/min)	4.2 ± 0.3	1.1 ± 0.2
Length of visit (s)	9.2 ± 1.8	34.2 ± 6.6
	Foraging behaviour	
Anther (%)	16.0	18.0
Stigma (%)	1.0	2.0
Anther and stigma (%)	80.0	38.0
None (%)	3.0	42.0

Regardless of the time, *A. mellifera* and *S. bipunctata* collected mainly nectar (74.3% and 69.3%, respectively), with a lower frequency of pollen harvesting (4.7% and 17.0%) or both resources (21.0% and 13.7%). Similar data were observed in canola pollination, in which *A. mellifera* collected mainly nectar (67.7% - 90.0%), followed by pollen (3.0% and 11.7%) and nectar and pollen (7.0% and 20.6%) [12] [34]. In *Tetragonisca angustula*, higher percentages were observed (74.3% nectar, 24.8 pollen and 0.4% of both resources), thus corroborating the results obtained in this study. These changes in the collection behaviour may be related to the supply of available pollen, the reduction of pollen and nectar hoarding and the consumption of the colony, occurring a greater demand and therefore a greater number of foragers when the storage is low or when the energy needs of the colony increase [35] [36].

During the evaluation period, the *A. mellifera* and *S. bipunctata* were constant on the flowers of *D. wallichii*, and only a small part of the pollen analyzed (1.4% and 6.0% respectively) does not belong to the species under study. Forager bees visit other flowers to follow the changes in rewards over time; their preference is altered in response to a sequence of low rewards or reduced availability of the preferred flower [37]. *A. mellifera* visited on average 4.2 flowers per minute, with a length of 9.2 seconds per flower while *S. bipunctata* visited 1.1 flowers with a length of 34.2 seconds per flower. An effective pollinator sequentially visits the flowers, transporting and transferring the pollen to the stigma during the visit [38] [39], *A. mellifera* was considered a pollinator because it visited a greater number of flowers per minute and in only 3.0% of the visits it had no contact with the reproductive structures of the plant.

Chambo *et al.* [12] analyzed pollination of canola and observed a visitation rate of 12.9, with a length of 4.2 seconds in each flower for *A. mellifera*, also reporting, that *Trigona spinipes* bee spend more time visiting the flower (36.0 s). However, in *Momordi cacharantia* (Cucurbitaceae) the visitation rate of *A. mellifera* is 2.2 with a length of 5.2 seconds per flower and for the genus *Trigona* sp., the visitation rate is 5.4 with 23.2 seconds per flower [40]. Thus, the visitation rate and the time of permanence of the bees vary depending on the biology and floral morphology of each plant and the species of visiting bees, usually stingless bees stay longer visiting the flower.

The distribution of floral visitors on the plant will depend on the age of the flower, because it interferes with the nectar volume and the sugar concentration [41]. This concentration is influenced by several factors, including environmental conditions [42]. Thus, high temperatures contribute to high sugar concentrations in the nectar secreted by the plant [43]. However, when the sugar concentration in the nectar decreases, visitors continue to attend the flower, since the *D. wallichii* nectar contains lipid and phenolic substances, protein compounds and acidic and neutral polysaccharides that are nutritive for bees [21].

In this study, we observed that the sugar concentration in the nectar of *D. wallichii* was not constant throughout the day, with a positive relationship with temperature and luminosity, this characteristic is considered an evolutionary strategy of plants to attract the insect pollinator for visiting other flowers [44]. Likewise, the sugar concentration in the honey crop varied with environmental conditions, agreeing with [45] who reported a positive correlation of sugar concentration in the honey crop with temperature for *Melipona ferruginea* and *Hypotrigona gribodoi*. Besides, the concentration of sugars is higher in the honey crop (14.2%) when compared to the concentration of nectar produced by the flower (12.3%), which is related to the behaviour of the worker bee that initiates the process of physical evaporation of the nectar and adds enzymes for conversion of sucrose into glucose and fructose [46].

5. Conclusion

The foraging behaviour of the two bee species was influenced by temperature, relative humidity and luminosity. Both species had a preference for collecting nectar throughout the day, and in the hottest times of the day, the bees collected both resources (pollen and nectar). *A. mellifera* is a potential pollinator because it has visited more flowers per minute, touching the two reproductive structures of the flower. However, *S. bipunctata* was considered like floral visitor because, even staying more time in each flower, it collects the nectar without touching the reproductive structures. Finally, the sugar concentration was higher in the honey crop of the bee than in the flower, with higher concentration in times with the highest temperatures.

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Conflicts of Interest

The authors declare no conflicts of interest regarding the publication of this paper.

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