Arthropod-Plant Interactions (2020) 14:785–797
https://doi.org/10.1007/s11829-020-09784-3

ORIGINAL PAPER

Nocturnal bees exploit but do not pollinate flowers of a common bat-pollinated tree

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Received: 7 May 2020 / Accepted: 12 September 2020 / Published online: 22 September 2020

Abstract

Some species of bees restrict foraging to the twilight period before sunrise or after sunset. Among the plants sought by these nocturnal bees are species described as chiropterophilous, such as *Caryocar brasiliense*. The flowers of this species open in the evening and provide resources until dawn. We determined the pattern of flower visitation by nocturnal bees and their role in pollination and fruit set of *C. brasiliense* and evaluated its importance as floral resource for nocturnal bees. We analyzed the pollen composition of cell provisions of nocturnal bees of *Ptiloglossa* (Colletidae) and compared its scent with floral scent compounds of *C. brasiliense*. Moreover, we conducted a pollinator exclusion experiment to determine the contribution of nocturnal bees to its fruit set. Disregarding bats, *Ptiloglossa latecalcarata* and two species of *Megalopta* (Halictidae) were consistent nectar and pollen gathering visitors, along with some social diurnal bees. The visitor exclusion experiment revealed that bee visits do not result in fruit set, which only occurs through visits by bats. The flowers supply a significant amount of pollen for nocturnal bees, as demonstrated through pollen analysis of brood cells and scopa loads. This interaction, therefore, is only beneficial to the commensalist bees. The scent collected from brood cells was dominated by hexanoic acid and 1-hexanol and differed strongly from the floral scent of *C. brasiliense*. These results substantiate that bat-pollinated flowers are an important part of the food niche of nocturnal bees, which implies that they are sensorially equipped to recognize floral traits shaped by bats.

Keywords *Caryocar brasiliense* · Caryocaraceae · Cerrado · Food niche · Dim-light foraging · Crepuscular bees · *Megalopta* · *Ptiloglossa* · Pollen loads

Handling Editor: Heikki Hokkanen.

Electronic supplementary material The online version of this article (https://doi.org/10.1007/s11829-020-09784-3) contains supplementary material, which is available to authorized users.

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Introduction

While most bees forage during the day, some species restrict floral resource exploitation to the twilight period before sunrise, after sunset or even into the night (Linsley 1958; Roulston 1997; Wcislo et al. 2004; Warrant 2007). With the ability to fly under low light, these crepuscular/nocturnal bees (hereafter referred to as just 'nocturnal bees') occupy a temporal niche distinct from that of diurnal bees—they collect floral resources before diurnal bees at dawn and after them at dusk (Wcislo et al. 2004; Kelber et al. 2006; Wcislo and Tierney 2009). Nocturnal bees possess visual adaptations (Warrant et al. 2004; Kelber et al. 2006; Warrant 2008) and use olfactory cues (Carvalho et al. 2012; Knoll and Santos 2012; Cordeiro et al. 2017; Krug et al. 2018) to detect flowers in low light conditions. At least 250 species of bees of the families Andrenidae, Apidae, Colletidae and Halictidae have nocturnal or crepuscular habits (Wcislo and Tierney 2009), with most of those in the Neotropics belonging to Halictinae (Halictidae) and Diphaglossinae (Colletidae) (Wcislo et al. 2004; Kelber et al. 2006; Warrant 2008; Wcislo and Tierney 2009).

Nocturnal bees use a wide array of plant species, especially those with melittophilous or chiropterophilous blossoms (Wcislo et al. 2004; Smith et al. 2012). Common traits of flowers that attract nocturnal bees include white petals, flower opening at night or during twilight and the emission of a strong nocturnal odor (Krug et al. 2015; Cordeiro et al. 2017; Siqueira et al. 2018). Associations involving nocturnal bees are still poorly studied, probably because of the difficulty in observing their behavior under the conditions of twilight. Some recent studies, however, have demonstrated that these bees are effective pollinators of species with melittophilous flowers, such as *Passiflora pohlii* Mast. (Passifloraceae) (Faria and Stehmann 2010), *Cambessedesia wurdackii* Martins (Melastomataceae) (Franco and Gimenes 2011), *Trembleya laniflora* Cong. (Melastomataceae) (Soares and Morellato 2018) and *Machaerium opacum* Vogel (Fabaceae) (Siqueira et al. 2018). Some of the melittophilous species pollinated by nocturnal bees are of economic importance, such as yellow mombin (*Spondias mombin* L., Anarcadiaceae) (Carneiro and Martins 2012), guarana (*Paullinia cupana* (Mart.) Ducke, Sapindaceae) (Krug et al. 2015, 2018), cambuci (*Campomanesia phaea* (O. Berg) Landrum) and other species of Myrtaceae (Cordeiro et al. 2017, 2019). Although there are several species with chiropterophilous flowers among the host plants of nocturnal bees (Roulston 1997; Wcislo et al. 2004; Piechowski et al. 2010; Smith et al. 2012), there is little information on whether these bees are effective pollinators of their flowers. *Caryocar brasiliense* Cambess. (Caryocaraceae), popularly known as Pequi and a common fruit crop of the Brazilian Cerrado, is one such species with chiropterophilous flowers (Vogel 1968; Faegri and van der Pijl 1979), which are intensely visited by nocturnal bees based on our preliminary observations. The large, yellowish-white brush flowers of the species possess numerous stamens, open early in the evening and produce a large volume of nectar. They are pollinated by bats of the species *Glossophaga soricina* and *Anoura geoffroyi* (Glossophaginae) (Gribel and Hay 1993), which visit the flowers at night after dusk and before dawn (Gribel and Hay 1993, personal observation). The flowers, as well as the androecium alone, emit a strong characteristic odor that is mainly composed of aliphatic hydrocarbons, such as a heptadecene and pentadecane, and, to a lesser extent, sulfur-bearing compounds, such as dimethyl sulfide and methanethiol (Paiva et al. 2019). In addition to bats, moths, hummingbirds and diurnal bees have also been recorded as visitors to the flowers of *C. brasiliense* (Gribel and Hay 1993; Melo 2001).

Studying the interaction between nocturnal bees and flowers of *C. brasiliense,* we asked: (a) What is pattern of flower visitation by nocturnal bees and their role in pollination and fruit set of *Caryocar brasiliense*? (b) What is the importance of *C. brasiliense* as a floral resource for nocturnal bees? (c) Do cell provisions of nocturnal bees of the genus *Ptiloglossa* that consist of pollen of *C. brasiliense* release compounds known to be released from the stamens/staminodes of this plant? To address these questions, we identified the species of nocturnal bees visiting the flowers of *C. brasiliense*, determined their flower-visiting frequency and analyzed their pollen loads. Moreover, we analyzed pollen composition and scent of the brood cells of a nest of a nocturnal species of *Ptiloglossa* found in the study area. Furthermore, we conducted an experiment that permitted restricted flower access to nocturnal bees to determine their contribution to fruit set of this chiropterophilous plant.

Material and methods

Study site

Fieldwork was carried out in *Parque Estadual do Rio Preto* (Rio Preto State Park), located in the municipality of São Gonçalo do Rio Preto, Minas Gerais, Brazil (18°07′04″S; 43°20′42″W) in October and November of 2013, 2015, and 2018. The park is located in the Espinhaço Mountain Range and encompasses an area of 10,755 ha covered mainly by vegetation of Cerrado (savannah) and Campo Rupestre (rupestrian fields). The climate is characterized by a hot and rainy summer (October–March) and a well-defined dry season

(April–September). The average annual temperature and precipitation are 19.9 °C and 1550 mm, respectively (IEF 2004; Neves et al. 2005).

Study species

Caryocar brasiliense Cambess. is a highly characteristic and abundant tree species of the Cerrado, a global conservation hotspot (Mittermeier et al. 1999). It reaches heights of up to 15 m and has tortuous branches and trunk. Inflorescences are terminal racemes containing 10–30 yellowish-white hermaphroditic flowers with numerous long stamens. The styles are slightly longer than the filaments (Fig. 1a). Staminodes with short filaments and rudimentary anthers are present in the innermost portion of the androecium. The distal region of the filaments and staminodes appear rough due to the presence of protruding foraminous cells, which act as an osmophore by release a strong odor consisting of compounds of various chemical classes, such as aliphatic (acetoin, various alkanes and alkenes) and sulfur-bearing compounds (methanethiol, dimethylsulfide, dimethyldisulfide) (Paiva et al. 2019). The fruits of *C. brasiliense* contain 1–4 seeds and are of great socioeconomic and nutritional value (Gribel and Hay 1993).

Anthesis and floral biology of *Caryocar brasilienses*

The number of flowers per inflorescence was determined by counting the flowers of 100 inflorescences from 10 individuals. Anthesis was observed for 95 flowers of five individuals (15–25 flowers per individual) from opening to senescence, considering the beginning of anthesis to be when petals separated and the style and anthers became visible (Fig. 1b). The beginning of anther-dehiscence was determined with a hand-magnifying lens, while stigmatic receptivity was determined by the formation of bubbles in a drop of hydrogen peroxide (H_2O_2) deposited onto stigmas (Dafni et al. 2005).

The number of pollen grains per flower was determined by macerating anthers of flower buds in Eppendorf tubes containing a solution of 0.5 ml lactic acid and glycerin at 3:1 (Lloyd 1972) and counting the pollen grains using a Neubauer chamber (Maêda 1985). The average number of grains per flower was calculated by counting the number of grains in two samples per flower for 15 buds of five different plants.

Flower visitors

Flower-visiting bees were collected with entomological nets for 10 non-consecutive days (60 h of observation). The frequency of floral visitors was determined by marking 95 flowers on five trees and counting the number of flower visits

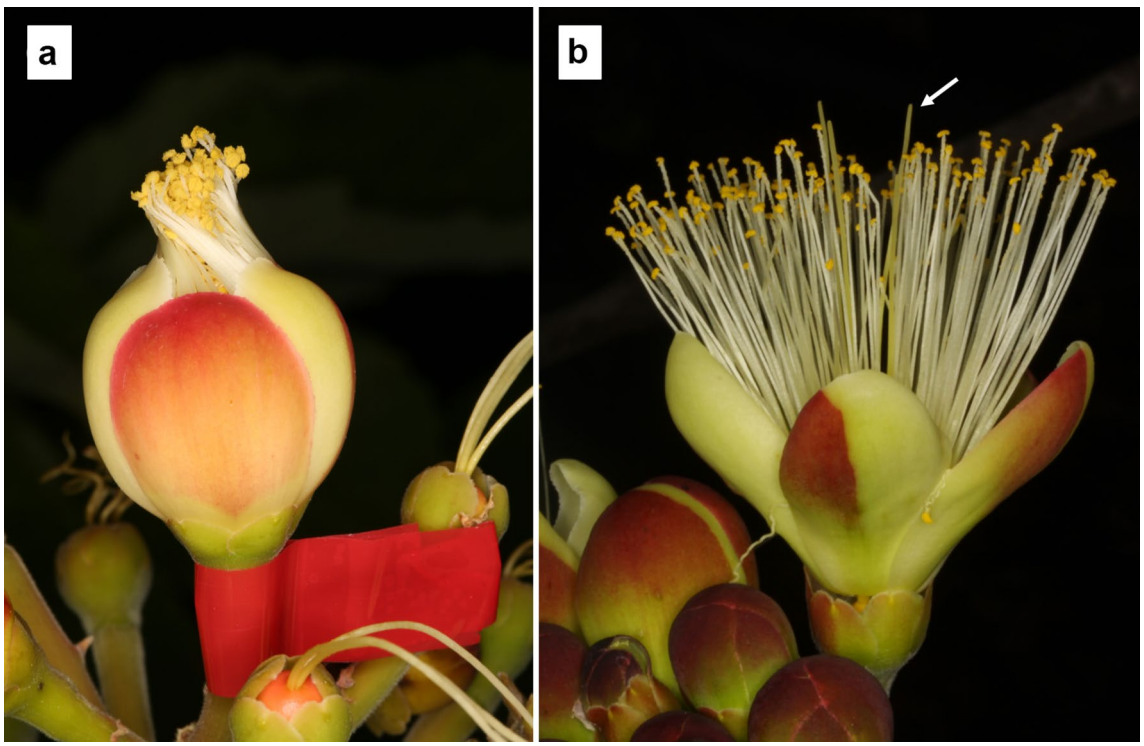

Fig. 1 Flowers of *Caryocar brasiliense*. **a** Beginning of anthesis; petals begin to separate and stigmas and anthers become visible. **b** Open flower with exposed stamens and stigmas. The arrow indicates the height of the stigmas

during 30-min intervals in the morning (4:30–9:00 h), and at 15-min intervals in the evening (dusk, 18:00–19:00 h). The time that nocturnal bees first appeared in the morning and left the evening had been previously determined. The time of sunset, sunrise and astronomical twilight were obtained for the study site from the online database "Date and time AS" (https://www.timeanddate.com). The following were noted during floral visits: (a) species/genus of the visitor (the two species of *Megalopta* could not be differentiated during frequency counts); (b) collected resource (pollen and/or nectar); and (c) visitor contact with stigmas. Visiting specimens were collected, prepared, labeled, identified, and deposited in *Centro de Coleções Taxonômicas*, Universidade Federal de Minas Gerais (CCT-UFMG).

Chi-square tests of independence (2 × 2 tables) were used determine if there were significant differences in: (a) the number of visits to flowers of *C. brasiliense* at dusk and dawn between bees of the genera *Megalopta* and *Ptiloglossa*; and (b) the resource collected (pollen versus nectar) between diurnal and nocturnal bees. Statistical tests were performed using the R environment (R Core Team 2013).

Visitor exclusion experiment

A visitor exclusion experiment was conducted over five consecutive days in October 2018 to determine whether nocturnal bees contribute to fruit set. A total of 50 flower buds were bagged in ten individual plants, which were then exposed to floral visitors exclusively during dusk (18:00–19:30 h) and dawn (4:00–5:30 h), when only bees visited the flowers. Whether nocturnal bees had visited the flowers of *C. brasiliense* was also checked. Bats that visited the flowers of *C. brasiliense* were common in tree crowns during nighttime hours. Another 50 individually marked flowers remained accessible to floral visitors throughout anthesis. The flowers were monitored until senescence, when fruit set was determined.

Analysis of scopa pollen loads

Pollen loads were removed from the scopa of nocturnal bees collected in flowers of *C. brasiliense* ($N = 18$ *Ptiloglossa latecalcarata*; 13 *Megalopta* spp.). The load of each bee was placed on a watch glass, to which 70% ethanol was added and mixed with an insect pin. After evaporation, a representative sample was removed with a small piece of glycerin gelatin, which was then transferred it to a microscope slide, covered with a cover glass and sealed with paraffin (Barth 1989; Darrault and Schlindwein 2002). Pollen grains were identified under a microscope and at least 500 pollen grains were counted per slide to determine the frequency of different pollen types in each scopa load.

Pollen analysis of brood cells

To access the brood cells of a nest of *Ptiloglossa* encountered at the study site, the nesting female was collected while leaving the nest entrance, and a hole was carefully dug about 40 cm to the side of nest entrance towards the central tunnel. The measured diameter and length of the main tunnel and the lateral tunnels were measured. The length and maximum and basal diameter of each brood cell were measured according to Rozen (1984).

The pollen content of brood cells was diluted in glycerinated lactic acid at a 3:1 solution and homogenized in a vortex stirrer. Three samples were obtained from each cell to prepare microscope slides and identify the pollen grains by comparison with pollen of the reference collection of *Fundação Ezequiel Dias* -FUNED, Belo Horizonte, Minas Gerais. The first 500 pollen grains per sample were counted and the relative frequencies of the different pollen types determined.

The total number of pollen grains was determined for two brood cells with larval supply—one with an egg and one with a first instar larva. The pollen content was stored in 70% ethanol and, after centrifuging and removing of ethanol, had 0.5 ml of the glycerinated lactic acid solution added. The solution was homogenized with a vortex stirrer and an aliquot was transferred to a Neubauer counting chamber to estimate the total number of pollen grains per brood cell (Maêda 1985; Schlindwein and Martins 2000; Schlindwein et al. 2009).

Scent analysis of brood cells

A dynamic headspace sample (Dötterl et al. 2005) was collected of the volatiles of four pooled brood cells. The cells were bagged with polyester oven bags (Toppits) for 30 min, after which the air enriched with volatiles was sucked through an adsorbent tube for ten minutes using a vacuum pump (G12/01EB; Rietschle Thomas, Puchheim, Germany) with a constant airflow of 200 ml/min. The adsorbent tube was made of quartz glass (25 mm long, internal diameter 2 mm) containing 1.5 mg Tenax-TA 60–80 and 1.5 mg Carbotrap B 20–40 (both Supelco, Bellefonte, US) fixed with glass wool. A negative control sample was collected from an empty oven bag.

The volatile sample was analyzed by TD-GC/MS (thermal desorption-gas chromatography/mass spectrometry, model QP2010 Ultra EI, Shimadzu, Japan), using the same method as described in Zito et al. (2019). Data from GC/MS were analyzed using GCM Solution package, Version 2.72 Shimadzu Corporation 2012. Compounds were identified using the mass spectral libraries ADAMS (2007), FFNSC 2, W9N11, and ESSENTIAL OILS (available in MassFinder 3), and the Kovats retention indices of the compounds (based

on n-alkane series). Mass spectra and retention times were also compared to standard components available in the reference collection of the Plant Ecology Lab of the Paris-Lodron-University of Salzburg.

Results

Anthesis and floral biology of *Caryocar brasiliense*

Inflorescences had an average of 19 (± 5; $N = 100$) flowers. The marked flowers opened between 18:00 and 21:00 ($N = 95$). Senescence occurred about 23 h after flower opening (15:00–17:00 h), when the corolla and stamens detached from the flower and fell. All anthers were already dehisced and stigmas were receptive when flowers opened. The flowers produced an average of 279,875 ± 72,832 ($N = 15$) pollen grains and still contained numerous pollen grains in the morning, as checked with a hand-magnifying lens.

Floral visitors

The flowers of *C. brasiliense* were visited by bees of 11 species (Table 1). Females of *Ptiloglossa latecalcarata* accounted for 15% of all bee visits (Fig. 2a) while females

Table 1 Floral visitors of *Caryocar brasiliense* in Parque Estadual do Rio Preto, Minas Gerais, Brazil, including sex, foraging period and resource collected

Family/species	Sex	Foraging period	Resource collected
Apidae			
Apis mellifera Linnaeus, 1758	♀	Diurnal	N
Bombus sp.	♀	Diurnal	P/N
Plebeia sp.	♀	Diurnal	P
Tetragonisca angustula (Latreille, 1811)	♀	Diurnal	P
Trigona spinipes (Fabicius, 1793)	♀	Diurnal	P/N
Trigona sp.	♀	Diurnal	P/N
Xylocopa (Neoxylocopa) grisescens Lepeletier, 1841	♀	Diurnal	P/N
Xylocopa sp.	♀	Diurnal	P/N
Colletidae			
Ptiloglossa latecalcarata Moure, 1945	♀	Nocturnal	P/N
Halictidade			
Megalopta aegis (Vachal, 1904)	♀/♂	Nocturnal	P/N
Megalopta amoena (Spinola, 1853)	♀/♂	Nocturnal	P/N

N Nectar, *P* pollen

Fig. 2 Females of nocturnal bee species collecting pollen in flowers of *Caryocar brasiliense*. **a** *Ptiloglossa latecalcarata*; **b** *Megalopta* sp

and males of *Megalopta aegis* and *M. amoena* (Fig. 2b) together accounted for 11%; it was not possible to differentiate the two species of *Megalopta* during flower visits. Bees of the genera *Ptiloglossa* and *Megalopta* visited flowers during dusk and dawn. The eusocial stingless bee species *Trigona spinipes* (56%) and the honeybee *Apis mellifera* (9%) were the most common diurnal flower visitors in the morning.

All marked flowers that opened prior to 19:00 h (34%) were visited by nocturnal bees, while the rest of the marked flowers (66%) opened after the flight activity of nocturnal bees (Fig. 3).

Bees of *P. latecalcarata* visited flowers from 18:00 to18:30 h and from 04:30 to 07:00 h (Fig. 4a, b), while bees of *Megalopta* visited from 18:15 to 19:00 h and from 04:30 to 06:30 h. While bees of the genus *Megalopta* visited more flowers at dusk ($N=59$ visits) than at dawn ($N=23$ visits), bees of *Ptiloglossa latecalcarata* visited flowers of *C. brasiliense* predominantly at dawn (93 of 118 visits) ($X^2=49.1$; df = 1; $p<0.001$). The first flower visits of diurnal bees in the morning (first visit of *Xylocopa grisescens* 05:10 h, of *Apis mellifera* 05:15 h and of *Trigona spinipes* 05:25 h) (Fig. 4b) partly overlapped with the flower-visiting period of the nocturnal bees; however, flower visits of nocturnal bees clearly dominated over the visits of diurnal bees until 05:30 h (Fig. 4b).

Nocturnal bees visited the flowers of *C. brasiliense* mainly to collect pollen (84% of the visits; $N=168$), while diurnal bees collected predominantly only nectar (79% of the visits; $N=394$) (Fig. 5) ($X^2=346.9$; df = 1; $p<0.001$) ($N=562$).

Prior to floral visits females of *P. latecalcarata* hovered in front of the flower, after which they grabbed a set of stamens that served as a landing pad and vibrated the anthers in single short buzzes soon after landing. The released pollen grains initially adhered to the ventral part of the body, from which they were transferred to the scopa. Despite their large body size, bees of *P. latecalcarata* touched the stigmas in only 6% of the flower visits. These bees exclusively collected pollen during 102 visits (86%), while females moved through the filaments to the nectar chamber and took up nectar during the remaining 16 (14%) visits, (Fig. 6); bees of this species were never observed to collected only nectar during a visit. No sonication sound was audible when females of *Megalopta* collected pollen. Bees of the genus *Megalopta* collected only pollen during 66 visits (81%), only nectar and during 6 (7%) visits, and took up nectar after gathering pollen by moving through the filaments to the nectar chamber during 10 (12%) visits (Fig. 6). Workers of the diurnal *Trigona spinipes* and *Apis mellifera* hovered in front of flowers, landed on the petals and went to the nectar chamber. These bees collected only pollen during 68 visits (12%), only nectar during 394 (67%) visits and both resources during 121 (21%) visits (Fig. 6). These bees were never observed to make contact with the stigmas.

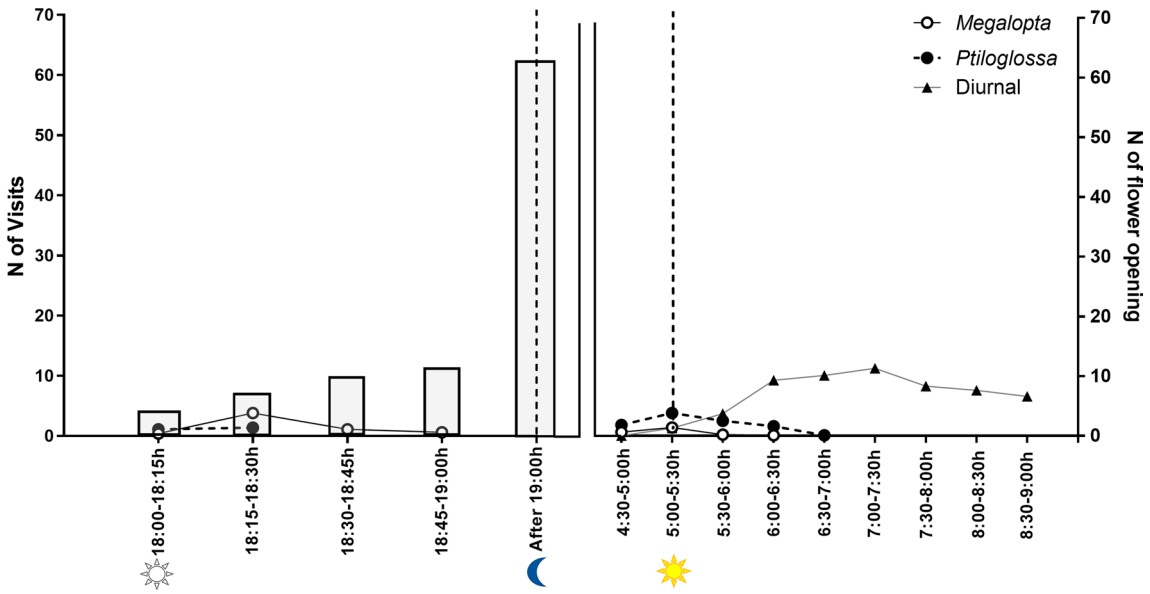

Fig. 3 Number of bee visits (lines) and opening hours of marked flowers of *Caryocar brasiliense* (bars), at half-hour intervals during dawn and 15-min intervals during dusk ($N=95$ flowers from five trees). Dashed line indicates astronomical twilight end (19:09 h; 04:42 h); white sun: indication of sunset (17:55 h); yellow solid sun: indication of sunrise (05:20 h); Moon: Indication of astronomical twilight start (19:10 h)

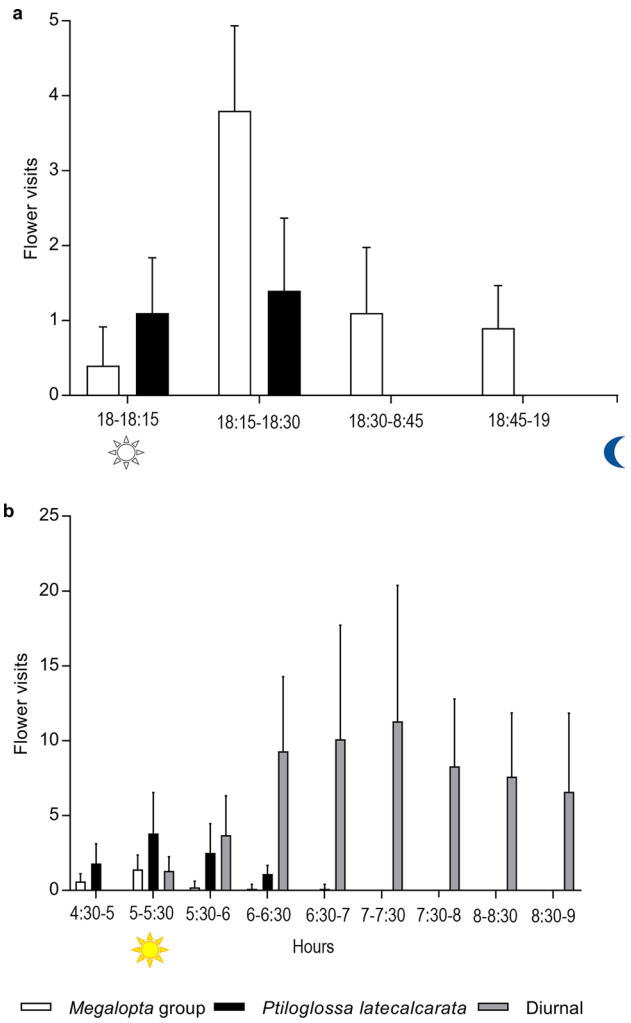

Fig. 4 Number of floral visits to flowers of *Caryocar brasiliense*. **a** Floral visits during evening twilight (mean ± SD). No diurnal bees visited the flowers of *C. brasiliense* at dusk. **b** Floral visits during dawn and the morning. Observations were made on 10 non-consecutive days. White sun: Indication of sunset; yellow solid sun: indication of sunrise; moon: Indication of astronomical twilight

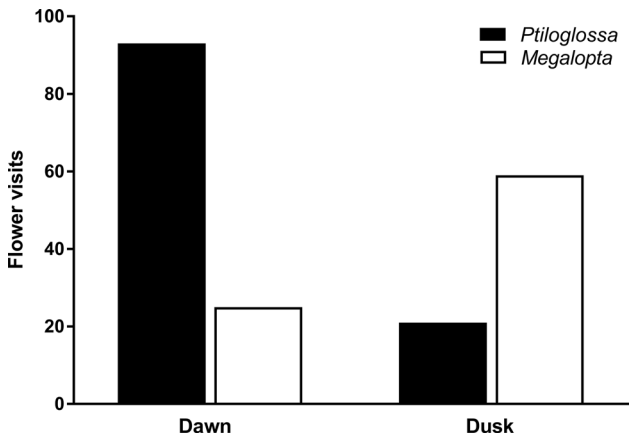

Fig. 5 Frequency of floral visits by *Megalopta* and *Ptiloglossa* at dawn and dusk

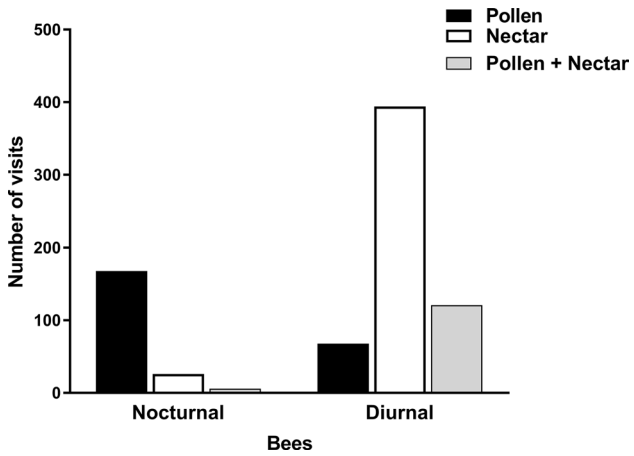

Fig. 6 Resources collected by diurnal and nocturnal bees during visits to flowers of *Caryocar brasiliense*

Visitor exclusion experiment

The visitor exclusion experiment, in which flowers were accessible to visitors only at dusk and dawn, revealed that flowers of *C. brasiliense* that were visited by nocturnal bees in the twilight did not produce any fruit. The fruit set for flowers accessible to floral visitors throughout anthesis was 22% (Table 2).

Table 2 Fruit set in flowers of *Caryocar brasiliense* left un-bagged and accessible to floral visitors throughout anthesis (open pollination) and bagged during the night

Treatments	No. of flowers	No. of fruits (% fruit set)
Open pollination (un-bagged flowers)	50	11 (22)
Crepuscular pollination (flowers bagged during the night)	50	0* (0)

The flowers were accessible only during dusk (18:00–19:30 h) and dawn (04:00–05:30 h)

*One fruit developed, but without seeds

Analysis of scopa pollen loads of nocturnal bees

Analyses of the pollen content of the scopa of 18 females of *P. latecalcarata* revealed that they all carried pollen from *C. brasiliense*. Half of these females had pure pollen loads of this species, whereas the pollen loads of the other individuals contained one or two additional pollen morphotypes of Myrtaceae. Only one female possessed a scopa that was not carrying predominantly pollen of *C. brasiliense* (Fig. 7). Eight of the 13 females of *Megalopta* had pure

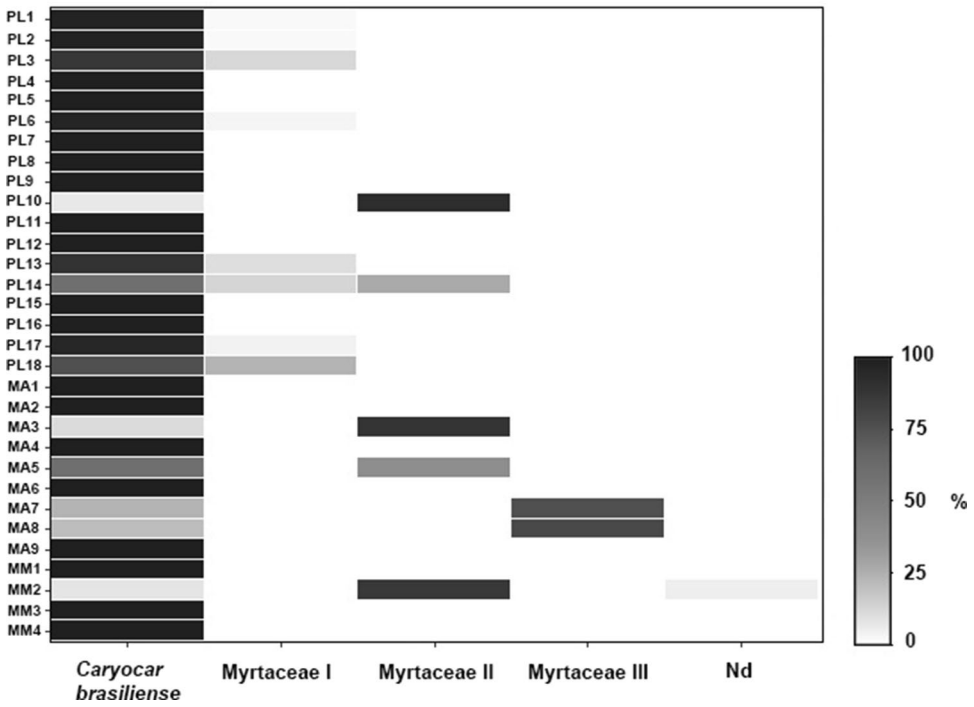

Fig. 7 Female scopa contents, shown as relative frequency of pollen types, for *Ptiloglossa latecalcarata, Megalopta aegis* and *M. amoena* collected in flowers of *Caryocar brasiliense*. The heatmap representation shows the percentage of each pollen type (columns) on each female (rows). PL = *Ptiloglossa latecalcarata*, MA = *Megalopta aegis*, MM = *Megalopta amoena*, Nd = unidentified pollen type

pollen loads of *C. brasiliense* while five also carried other pollen grains, also of one or two morphotypes of Myrtaceae and of a morphotype not identifiable to family. All bees with mixed scopa pollen loads (14) were collected at dawn.

Pollen analysis of brood cells of *Ptiloglossa latecalcarata*

The nest of *P. latecalcarata* found at the study site had four closed cells and one under construction. The brood cells had transparent-yellow liquid provisions containing a lot of nectar and a few amounts of pollen (see nest description in Supplementary Material). Analysis of the pollen content of the brood cells revealed that all four brood cells contained only pollen of *C. brasiliense*. The two brood cells with complete larval supply contained 62.5 and 55.2 million pollen grains of *C. brasiliense*, respectively, the total pollen content of about 223 and 197 flowers of *C. brasiliense*, respectively.

Scent of the larval supply of the nest of *Ptiloglossa latecalcarata*

The brood cells of *P. latecalcarata* were characterized by a strong sour smell. Analysis of the scent of the brood cells revealed the presence of 37 volatile compounds, among which were aliphatic and C5-branched-chain compounds, and terpenoids (Table 3). The aliphatics hexanoic acid (48%) and 1-hexanol (33%) were the most abundant compounds, followed by hexyl acetate, also an aliphatic compound (7%) (Table 3). Only four minor compounds out of the 37 volatiles were previously also described for the scent of the androecium of *C. brasiliense* (Table 3).

Discussion

The present study revealed that *Caryocar brasiliense* is, at least temporarily, an important floral resource, particularly its pollen, for nocturnal bees in the studied area. However, despite intense flower visitations, the relationship is beneficial only to the nocturnal bees because their visits do not contribute to fruit formation.

Bat-pollinated *Caryocar brasiliense* provides floral resources for nocturnal bees

Typical bat-pollinated plant species with brush blossoms, numerous stamens and large open corollas (Vogel 1968; 1969a, b; Faegri and van der Pijl 1979; Sazima et al. 1999; Tschapka and Dressler 2002) are likely the most common host plants of nocturnal bees among non-melittophilous flowers. They provide both abundant pollen and nectar resources in individual flowers, which are easily accessible, as is the case with *C. brasiliense* studied here. Flowers of bat-pollinated species often remain open and provide available resources until dawn, which makes them attractive to other groups of animals. Indeed, this has been shown for chiropterophilous flowers of Bromeliaceae (Sazima et al. 1999), Cactaceae (Rivera-Marchand and Ackerman 2006; Rego et al. 2012; Martins et al. 2016, 2020), Fabaceae (Baker

Table 3 Relative amounts (contribution of each single compound to total aroma) of the volatile organic compounds detected in a sample collected from four brood cells of *Ptiloglossa latecalcarata*

Compounds	Relative contribution (%)
Aliphatic compounds	
Acetoin*	0.08
1,2-Propanediol	0.16
1-Pentanol	0.31
Butanoic acid*	0.33
2,3-Butanediol*	0.76
Ethyl lactate	**1.34**
(*Z*)-3-Hexen-1-ol*	tr
1-Hexanol*	**32.88**
Pentanoic acid*	1.25
2-Heptanone	tr
1-Pentyl acetate	0.67
n-Hexyl formate	0.27
1-Heptanol*	0.33
Hexanoic acid	**47.67**
Ethyl hexanoate*	**1.67**
Hexyl acetate*	**6.53**
2-Ethylhexanoic acid	0.01
Methyl octanoate*	0.20
Octanoic acid*	0.50
Ethyl octanoate*	0.60
C5-branched chain compounds	
Isovaleric acid*	0.31
2-Methylbutanoic acid*	0.22
Terpenoids	
α-Thujene	tr
δ-3-Carene*	0.58
(*Z*)-linalooloxide furanoid*	tr
(*E*)-linalooloxide furanoid*	0.03
Epoxyoxoisophorone*	0.05
4-Oxoisophorone*	0.06
Nerol*	0.15
Neral*	0.62
Geraniol*	0.84
Geranial*	0.80
Geranyl acetate*	0.04
Unknowns	0.48

tr: < 0.05%. The relative contributions of the five most abundant compounds are in bold, as are the names of compounds that were also detected in scent released from the androecium of *C. brasiliense*, according Paiva et al. (2019). The identity of compounds marked with an asterisk was confirmed with synthetic standards

and Harris 1957; Hopkins 1984), Gesneriaceae (Sanmartin-Gajardo and Sazima 2005), Malvaceae (Eguiarte et al. 1987; Roulston 1997; Gribel et al. 1999; Wcislo et al. 2004) and Agavaceae (Cane and Rozen 2019). While for some of these species diurnal animals may play the role of complementary pollinators (Rivera-Marchand and Ackerman 2006; Martins et al. 2016), in most cases, such as *C. brasiliense*, they are poor pollinators because of morphological mismatch or inadequate flight routes that do not cause cross-pollen flow.

The flowers of most sphingophilous species, often with narrow floral tubes and hidden nectar and pollen (Vogel 1954; Silberbauer-Gottsberger and Gottsberger 1975; Faegri and van der Pijl 1979; Oliveira et al 2004; Darrault and Schlindwein 2005; Avila et al. 2012), as well as flowers pollinated by settling moths (Funamoto and Sugiura 2016), are less suitable for bees with their comparatively short mouth parts. This is also true for the nocturnal pollination systems of robust cyclocephaline scarabs with, for example, species of Araceae and Annonaceae that demand specialized flower handling (Gottsberger 1990; Gibernau et al. 2000; Maia et al 2013; Pereira et al. 2014).

Flowers of *C. brasiliense* deliver a significant supply of pollen for nocturnal bees during the flowering period, as demonstrated by the pure pollen content of the four studied brood cells of *Ptiloglossa latecalcarata* and the *Caryocar*-dominant scopa pollen loads of more than half of the females of the three nocturnal bee species recorded here. These bees must also be restricting their nectar collection to flowers of *C. brasiliense* during this period since there was no pollen from other floral nectar resources in the analyzed scopa pollen loads and brood cells. Major non-*Caryocar* pollen content in scopa pollen loads was of species of Myrtaceae, whose representatives at the study site are nectarless pollen-flowers (species of *Campomanesia, Myrcia, Eugenia*). Their typical melittophilous blossoms open before sunrise, for which nocturnal bees are effective pollinators, as demonstrated for *Campomanesia phaea* (Myrtaceae; Cordeiro et al. 2017, 2019). The same is true for some other non-Myrtaceae species with matinal flowering patterns, such as *Passiflora pohlii* (Passifloraceae; Faria and Stehmann 2010), *Spondias mombin* and *Paullinia cupana* (Sapindaceae; Carneiro and Martins 2012; Krug et al. 2015, 2018), and *Machaerium opacum* (Fabaceae; Siqueira et al. 2018).

Floral traits

Considering the abundance of bat-pollinated species among the host plants of nocturnal bees, these bees must have sensory capabilities that are able to perceive the floral signals shaped by, and for, bats, such as scents and/or colors. Bat-pollinated flowers typically release sulfur-containing compounds, terpenoids, and short chain aliphatic ketones (Knudsen and Tollsten 1995; Bestmann et al. 1997; Dobson 2006; Paiva et al. 2019). These compounds are generally absent from the scents of melittophilous flowers associated with nocturnal bees (see Carvalho et al. 2012; Krug et al. 2018; Siqueira et al. 2018; Cordeiro et al. 2017, 2019), but might

be used by these bees to recognize chiropterophilous flowers. Analysis of electrophysical antennal responses to such compounds of bat flowers would, therefore, help to better understand the compounds that these bees are sensitive to. In addition to these signals themselves, flowering time needs to fit the activity time of nocturnal bees. Chiropterophilous flowers frequently open in the early evening, sometimes before dusk (Faegri and van der Pijl, 1979; Eguiarte et al. 1987; Gribel et al. 1999; Sanmartin-Gajardo and Sazima 2005), and last, in general, for one or sometimes two nights (Sazima et al. 1999). When anthesis starts later, after the twilight period, it is too dark for nocturnal bees to forage (Kelber et al. 2006). Anthesis of bat-pollinated flowers, however, usually extends into the morning, and so nocturnal bees focus their visits to these flowers during dawn when the flowers still provide available resources.

More than two-thirds of the flowers of *C. brasiliense* open when it is already too dark for nocturnal bees to fly. In dawn, however, these bees are the first floral visitors, making them the most competitive bees at collecting residual pollen and nectar before the social stingless bees and honeybees start foraging activity.

The nest of *Ptiloglossa latecalcarata*: larval supply, odor, and characteristics

The nest of *P. latecalcarata* encountered at the study site shares architectural characteristics with nests of other species of *Ptiloglossa* and, in general, of Diphaglossinae (Roberts 1971; Rozen 1984; Sarzetti et al. 2013; Rozen et al. 2019). These shared characteristics include urn shaped brood cells and their general distribution and position, the curvature of the entrance of the brood cells, the liquid content, and the semitransparent cellophane cell lining, which is exclusive to Colletidae (Rozen 1984; Almeida 2008). The nest was isolated, as have been most recently described nests of Diphaglossinae (Sarzetti et al. 2013). Females of some species [*P. tarsata* (Friese, 1900), *P. matutina* (Schrottky, 1904), *P. arizonensis* Timberlake, 1946], deposit wooly plant material ("similar to cotton") at the brood cell entrance, which is thought to have the function of closing the cell (Rozen 1984; Sarzetti et al. 2013). The nest examined here, however, did not contain such wooly material.

It is surprising that all four brood cells of the single nest of *P. latecalcarata* contained pollen exclusively from *C. brasiliense* given that bees of this species are known to intensely collect pollen from flowers of other families such as Myrtaceae (Cordeiro et al. 2017), and that several individuals of *P. latecalcarata* also carried pollen from Myrtaceae in their scopa pollen loads. Several species of Myrtaceae occur at the study site, the flowering of which largely overlaps with that of *C. brasiliense*. Most species of *Campomanesia, Myrcia, Eugenia, Calyptranthes* and *Plinia* (Myrtaceae) in the surrounding Cerrado vegetation, however, exhibit short mass-flowering or flower in short peaks, which are interrupted by non-flowering phases (personal observation), as found in other studies (Proença and Gibbs 1994; Torezan-Silingardi and Oliveira 2004; Fidalgo and Kleinert 2009). Thus, bees cannot specialize on these plants because resources are available only for a very short period of time, and so they must collect pollen and nectar from other families and species, such as *C. brasiliense*. Unfortunately, there is little information about the content of larval supply for representatives of *Ptiloglossa*. Brood cells of *Ptiloglossa guinnae* in Costa Rica contained exclusively pollen from Melastomataceae (Roberts 1971).

The source of the unpleasant smell of the brood cells of *P. latecalcarata* may come from compounds such as hexanoic acid and isovaleric acid. Among the volatile compounds found in the brood cells of *P. latecalcarata,* only acetoin, butanoic acid, 2-heptanone and 2-methylbutanoic acid, all minor compounds, also occur in the floral and androecial bouquet of *C. brasiliense* (Paiva et al. 2019). This shows that floral scents make only a minor contribution to the scent of brood cells. Instead, it is very likely that various compounds emitted from brood cells originate from pollen and nectar fermentation. Roberts (1971) mentioned an unmistakable and strong fermentation odor in nests of *P. guinnae*. This author even noticed gas bubbles at the bottom of the liquid brood cells, and interpreted it as products from yeasts. Indeed, compounds such as the most abundant hexanoic acid and 1-hexanol, as well as ethyl lactate, strongly suggest that microorganisms, such as fungi and bacteria, are involved in the production of volatiles released from brood cells (Maicas et al. 1999; Clemente-Jimenez et al. 2005; Choi et al. 2013). Other compounds detected in the present study, such as the terpenoids geraniol, geranial and neral are components known to arise form mandibular secretions of different genera of colletid bees (Hefetz et al 1979a; Zheng Ming et al. 1990) and, thus, might originate from the bees themselves. The present study did not find macrocyclic lactones (or their corresponding fatty acids), compounds used by species of *Ptiloglossa* and other colletids to line their brood cells, in the scent of the brood cells (Cane 1983; Hefetz et al 1979b). This might have to do with the low vapor pressure of these compounds and the fact that they polymerize in brood cells to form a semitransparent cellophane cell lining (Hefetz et al. 1979b). Overall, it seems that the volatiles detected from the brood cells are a mixture of floral scents, bee secretions and compounds produced through fermentation of plant- and possibly bee-derived chemicals.

We conclude that the typical bat-pollinated flowers of *C. brasiliense* are important food resources for nocturnal bees. As commensalists, however, these bees do not contribute to the reproduction of *C. brasiliense*. Nocturnal bees are strong competitors for diurnal bees in collecting the floral

resources remaining after nocturnal visits of the effectively pollinating bats, but they seem to be negligible competitors for the bats. This relationship between nocturnal bees and *C. brasiliense* appears to be similar to other associations of such bees with chiropterophilous species. It would be interesting to determine whether nocturnal bees use the same floral scent compounds as bats do to locate these flowers, as it would contribute to a better understanding of the interactions between these bees and their associated plants.

Acknowledgements We thank José Neiva and Juliana Ordones for help with fieldwork; Paula Calaça, Pietro Maruyama, Sidnei Mateus and Adriano Valentin da Silva for suggestions to a previous version of the manuscript; James Cane for comments on the characterization of the brood cell volatiles of *Ptiloglossa*; and Rita Velez Ruiz for help with bee identification. We thank Fundação Ezequiel Dias (FUNED-MG) and its employees for support with pollen analysis, and the employees, particularly to Antônio Almeida Tonhão, of Rio Preto State Park for logistic support. We thank Instituto Estadual de Florestas (IEF/MG) and Instituto Chico Mendes de Conservação da Biodiversidade (ICMBio) for the license to work in the Parque Estadual do Rio Preto (license number 072/2017). We acknowledge financial support from Conselho Nacional de Desenvolvimento Científico e Tecnológico (Program Science without Borders PVE/2014-CNPq 401466/2014-0, and Universal 436095/2018-1), and FAPEMIG Universal APQ- 00713-17), and individual research grants from Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES) to F.F.A., P.A and R.O. (88882.316026/2019-01), and from National Council for Scientific and Technological Development (CNPq) to C.S. (PQ 311935/2018-4) and I.A.S (PQ 309216/2016-8).

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