UNIVERSIDADE FEDERAL DE MINAS GERAIS
INSTITUTO DE CIÊNCIAS BIOLÓGICAS
Departamento de Botânica
Programa de Pós-Graduação em Biologia Vegetal

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FERNANDA FIGUEIREDO DE ARAUJO

A COMPETIÇÃO DE ABELHAS NOTURNAS, ABELHAS DO MEL E ABELHAS SEM FERRÃO PELO PÓLEN DE PLANTAS DE FLORAÇÃO MACIÇA

Tese apresentada ao Programa de Pós-Graduação em Biologia Vegetal do Departamento de Botânica do Instituto de Ciências Biológicas da Universidade Federal de Minas Gerais, como requisito parcial à obtenção do título de Doutor em Biologia Vegetal.

Área de Concentração: Fisiologia Vegetal e Ecologia

BELO HORIZONTE – MG

2021

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Área de Concentração: Fisiologia Vegetal e Ecologia

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Dedico.

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SUMÁRIO

RESUMO

Espécies de floração maciça, cujas flores se abrem em grande quantidade e ao mesmo tempo durante um curto período, são conhecidas como fontes excepcionais de pólen para as abelhas. Em espécies de floração maciça com flores que se abrem a noite, durante o crepúsculo ou pouco antes do nascer do sol, o número de espécies de abelhas que visitam suas flores em busca do pólen cresce à medida que o brilho aumenta após o amanhecer, aumentando também a competição entre elas. Várias espécies de abelhas, incluindo abelhas crepusculares/noturnas, abelhas do mel e abelhas sem ferrão exploram essas flores e competem pelo pólen. No entanto, a competição por esse recurso raramente foi quantificada. Usamos quatro espécies de Myrtaceae de floração maciça como modelo para investigar a competição entre abelhas nativas e a introduzida *Apis mellifera* por pólen. Em *Campomanesia pubescens*, *C. adamantium*, *Blepharocalyx salicifolius* e *Myrcia rufipes*, espécies que possuem típicas flores de pólen, quantificamos quanto pólen é coletado por diferentes grupos de abelhas que visitam suas flores. Para isso, realizamos um experimento de remoção de pólen em flores acessadas apenas por determinados grupos de abelhas. Mostramos que o pólen das flores de *C. pubescens* foi coletado por abelhas crepusculares, abelhas do mel e abelhas sem ferrão, enquanto que nas demais espécies o pólen foi removido por abelhas do mel e abelhas sem ferrão. Em todas as quatro espécies de plantas estudadas, operárias de *A. mellifera* foram, de longe, os visitantes florais mais abundantes e melhores competidoras, pois coletaram a maior parte do pólen das flores (Capítulos 1, 2 e 3). Além disso, medimos o impacto da presença massiva de *A. mellifera* na frequência de visitas e na coleta de pólen por abelhas nativas em flores de *C. pubescens*, *B. salicifolius* e *M. rufipes* (Capítulos 1 e 2). Em *C. pubescens,* a presença massiva de operárias da abelha do mel encurtou o tempo efetivo que as abelhas crepusculares, principais polinizadores dessa espécie, coletam pólen sem competidores ao amanhecer e diminuem fortemente o ganho de pólen por abelhas nativas diurnas. Contudo, a exclusão das abelhas do mel das flores resultou em um aumento drástico do fluxo de pólen para as espécies diurnas nativas, especialmente as abelhas sem ferrão. Em *B. salicifolius* e *M. rufipes*, após a exclusão de *A. mellifera* das flores, a frequência das abelhas sem ferrão triplicou e o ganho de pólen das abelhas sem ferrão aumentou consideravelmente nas duas espécies. Esses resultados demonstram que as abelhas nativas sofrem deslocamento pela abelha introduzida via competição por exploração. Apesar do impacto negativo para as abelhas nativas, a abelha do mel foi polinizador efetivo

das três espécies de planta. No capítulo 4, estudamos aspectos relacionados à interação da espécie de floração maciça *Caryocar brasiliense* com abelhas que visitam suas flores. A espécie apresenta flores quiropterófilas típicas que se abrem a noite e fornecem recursos até o amanhecer, atraindo abelhas noturnas/crepusculares e várias espécies de abelhas diurnas. Avaliamos a importância de *C. brasiliense* como recurso floral para abelhas noturnas e se essas abelhas atuam como polinizadores efetivos das flores de *C. brasiliense*. Nossos resultados mostraram que embora o pólen de *C. brasiliense* seja um importante recurso alimentar das abelhas noturnas, essas abelhas não contribuíram para a polinização dessa árvore típica do Cerrado.

Palavras-chave: Polinização, Myrtaceae, Caryocaraceae, Interações planta-polinizador

ABSTRACT

Mass flowering species, whose flowers open in large amount at the same time for a short period, are known as exceptional sources of pollen for bees. In the mass flowering species with flowers that open at night, during twilight or just before sunrise, the number of bee species that visit their flowers in search of pollen grows as the brightness increases after dawn, also increasing the competition among them. Several bee species, including crepuscular/nocturnal bees, honey bees and stingless bees exploit these flowers and compete for their pollen. However, competition for this resource was rarely quantified. We used four mass-flowering Myrtaceae species as a model, to investigate the competition among native bees and the introduced honey bees for pollen. In *Campomanesia pubescens, C. adamantium, Blepharocalyx salicifolius* and *Myrcia rufipes*, species with typical pollen flowers, we quantified how much pollen is collected by different bee groups. We conducted a pollen removal experiment in flowers accessed only by bee groups. We showed that the pollen of *C. pubescens* flowers was collected by crepuscular bees, honey bees and stingless bees, while in the other species the pollen was removed by honey bees and stingless bees. In all four plants species studied, honey bee workers were by far the most abundant floral visitors and the best competitors, collecting most of the flower pollen (Chapters 1, 2 and 3). In addition, we measured the impact of the massive presence of honey bees on the frequency of visits and pollen collection by native bees on *C. pubescens, B. salicifolius* and *M. rufipes* flowers (Chapters 1 and 2). In *C. pubescens*, the massive presence of honey bee workers shortened the effective time that crepuscular bees, the main pollinators of this species, collect pollen without competitors at dawn and strongly decrease pollen gain by diurnal native bees. However, the exclusion of honey bees from flowers resulted in a drastic increase in pollen flow for native diurnal species, especially stingless bees. In *B. salicifolius* and *M. rufipes*, after the exclusion of honey bees from the flowers, the frequency of stingless bees was threefold and the pollen gain of stingless bees increased considerably in both species. These results demonstrated that native bees are displaced by the introduced bee via exploitative competition. Despite the negative impact on native bees, the honey bees were effective pollinators of the three plant species. In chapter 4, we study aspects related to the interaction of the massive flowering species *Caryocar brasiliense* with bees that visit their flowers. The species has typical chiropterophilous flowers that open at night and provide resources until dawn, attracting nocturnal bees and several diurnal bee species. We evaluated the importance of *C. brasiliense* as a floral resource for nocturnal bees and whether, these bees act as

effective pollinators of *C. brasiliense* flowers. Our results showed that although *C. brasiliense* pollen are an important food resource for nocturnal bees, these bees did not contribute to the pollination of this typical Cerrado tree.

Keywords: Pollination, Myrtaceae, Caryocaraceae, Plant-pollinator interaction

INTRODUÇÃO GERAL

Espécies de floração maciça se caracterizam pela abertura de uma enorme quantidade de flores ao mesmo tempo durante um curto período (Gentry, 1974), e podem ser consideradas como uma estratégia que favorece a atração de polinizadores (Bawa 1983; Kang & Bawa 2003). Muitas espécies de floração maciça apresentam numerosas flores generalistas pequenas, com pólen e néctar de fácil acesso aos visitantes (Bawa, 1980). Apesar do limitado período de plena floração, essas espécies constituem consistente fontes de recursos florais que suprem as demandas de diversos grupos de visitantes, principalmente as abelhas (Wilms et al., 1996, Wilms & Wiechers ,1997, Ramalho, 2004).

Diversas espécies de floração maciça são conhecidas como fontes excepcionais de pólen para as abelhas e, em função disso, suas flores são exploradas por vários grupos de abelhas, incluindo abelhas solitárias que utilizam o pólen para a alimentação das suas crias (Linsley; 1958; Wcislo & Cane, 1996) e abelhas eussociais, que coletam massivamente o pólen dessas espécies e estocam este alimento em suas colônias perenes para serem utilizados nos períodos de escassez de recursos (Michener, 1974).

Como o pólen é um recurso finito e sua disponibilidade é o principal fator que regula as populações de abelhas, em espécies de plantas que são exploradas por muitas espécies de abelhas pode haver competição por este recurso (Roulston & Goodell, 2010; Cane & Tepedino, 2017). Por exemplo, em espécies cujas flores se abrem a noite, durante o crepúsculo ou pouco antes do nascer do sol, o número de espécies de abelhas que visitam suas flores em busca do pólen cresce à medida que a luminosidade aumenta após o amanhecer, aumentando também a competição entre elas. Nessas espécies, o pólen é explorado principalmente por abelhas crepusculares/noturnas, abelhas do mel e abelhas sem ferrão (Carneiro & Martins, 2012; Cordeiro et al., 2017; Siqueira et al., 2018; Araujo et al., 2020; Araújo et al., no prelo). Apesar da grande importância do pólen para as abelhas, a competição por esse recurso raramente foi quantificada.

Nestes sistemas, a abelha *Apis mellifera* Linnaeus, 1758, espécie introduzida e extraordinariamente adaptada às condições tropicais, pode apresentar vantagens na exploração do pólen devido ao seu refinado sistema de comunicação, através do qual recruta um elevado número de operárias para a eficiente exploração das flores (von Frisch, 1967; Roubik, 1980; Dyer, 2002; Seeley, 2012). Contudo, a eficiente capacidade de forrageamento de *A. mellifera* e sua alta abundância nas flores pode ocasionar impactos negativos para espécies de abelhas nativas, sobretudo no esgotamento de recursos compartilhados por elas

(Wilms & Wiechers 1997; Roubik et al., 1986; Roubik & Villanueva- Gutiérrez 2009; Cane & Tepedino, 2017).

Aqui, investigamos a competição entre espécies de abelhas nativas sociais e solitárias com a introduzida *A.mellifera* por pólen usando como modelo quatro espécies de Myrtaceae de floração maciça: *Campomanesia pubescens* (DC.) Berg, *C. adamantium* (Cambess.) O.Berg, *Blepharocalyx salicifolius* (Kunth) O.Berg e *Myrcia rufipes* DC. Essas espécies possuem típicas flores de pólen (pollen-flowers, *sensu* Vogel, 1978), pois oferecem apenas pólen como recurso floral para as abelhas. Nas quatro espécies quantificamos quanto pólen é coletado por diferentes grupos de abelhas que visitam suas flores, incluindo abelhas crepusculares, abelhas sem ferrão e *A. mellifera* (Capítulos 1, 2 e 3). Em *C. pubescens*, *B. salicifolius* e *M. rufipes* medimos o impacto de *A. mellifera* na frequência de visitação e ganho de pólen por abelhas nativas. Para essas espécies também definimos seus polinizadores efetivos (Capítulos 1 e 2). No capítulo 4, estudamos aspectos relacionados à interação da espécie de floração maciça *Caryocar brasiliense*, árvore típica do Cerrado brasileiro e popularmente conhecido como pequi, com abelhas que visitam suas flores. A espécie apresenta flores quiropterófilas típicas que se abrem a noite e fornecem recursos até o amanhecer, atraindo abelhas noturnas/crepusculares e várias espécies abelhas diurnas. Avaliamos a importância de *C. brasiliense* como recurso floral para abelhas noturnas e se essas abelhas atuam como polinizadores efetivos das flores de *C. brasiliense*.

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CAPÍTULO 1

Honey bees displace native bees from a mass-flowering fruit crop and gain the major pollen amount

Honey bees displace native bees from a mass-flowering fruit crop and gain the major pollen amount

Abstract

Melittophilous species whose flowers open before daybreak can be exploited by crepuscular and diurnal bees. When the brightness enhances after dawn, the number of flower visiting bee species increases, thus increasing also the competition among them. The fruit crop *Campomanesia pubescens* (Myrtaceae) is a mass-flowering shrub with melittophilous flowers that open before dawn and attract crepuscular bees, invasive honey bees and native diurnal bees. In this system, the impact of honey bees on native bees in terms of pollen gain and visiting frequencies remain unknown. Here, we determined the effective pollinators of *C. pubescens*, the frequency of flower visits, and the pollen competition among bee groups. We conducted an experiment of pollen removal in flowers access only by groups of bees and other of the net-covered flowers that avoid visits of honey bees. Honey bees were by far the most abundant flower visitors and collected >80% of the pollen of *C. pubescens*. The massive presence of the introduced honey bees causes negative impacts on (a) crepuscular bees, because the foraging time overlaps with that of crepuscular bees and this shortens the effective time crepuscular bees collect pollen without competitors at dawn and (b) especially on native diurnal bees, for dominating on flowers and due early mass foraging. Exclusion of honey bees, resulted in a drastic increase of the pollen flow to native diurnal species, especially stingless bees, which collected ~20 times more pollen than under honeybee presence. Therefore, invasive honeybees cause displacement of native diurnal bees through exploitative competition.

Keyword: Brazil, *Campomanesia*, Cerrado, Crepuscular bees, Myrtaceae, Pollen competition, Pollen depletion,

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1. INTRODUCTION

Many melittophilous plant species open their flowers before daybreak – still in the night or at dawn. In several of these species nocturnal/crepuscular bees (hereafter referred to as "crepuscular bees") were recorded as the first flower visitors and, in general, as efficient pollinators (Linsley & Cazier, 1970; Shelly et al., 1993; Faria & Stehmann, 2010; Cane et al., 2011; Franco & Gimenes, 2011; Carneiro & Martins 2012; Krug et al., 2015; Cordeiro et al., 2017, 2021; Siqueira et al., 2018). The crepuscular bees exhibit a brief flight activity restricted to the short matinal and vespertine dim light periods. It is assumed that the foraging activity under low light evolved to escape competition with other floral visitors (Wcislo et al., 2004; Smith et al., 2017). The ability to visit the flowers before the diurnal bees arrive, therefore, makes them highly efficient in removing pollen during the periods of twilight (Araújo et al., in press).

With increasing brightness at and after dawn, diurnal bees start to visit the flowers, and the sequence in which bees of the different diurnal species appear at the flowers in the early morning, seems to be related to their ability to fly and forage under still restricted light intensities. The increment in flower visiting species rapidly enhances competition, especially in pollen flowers (Carneiro & Martins, 2012; Cane & Tepedino, 2017; Araújo et al., in press). The most efficient competitors may deplete the pollen resources, cause temporal displacement of bee species with less adaptated foraging ability or displace these species to other host plants (Roubik et al., 1986; Roubik & Villanueva-Gutierrez, 2009, Carneiro & Martins, 2012). Thus, the bee species must be able to develop different foraging strategies to optimize efficiency in pollen harvesting, such as the ability to distinguish rewarding and non-rewarding flowers (Goulson, 1999, 2003; Russell et al., 2017).

Among the matinal diurnal bees that fly early in the morning are especially highly social bees like honey bees (*Apis mellifera* Linnaeus, 1758) and stingless bees (Meliponini), but also carpenter bees (*Xylocopa*) and bumble bees (*Bombus*) among others (Cordeiro et al., 2017; Siqueira et al., 2018, Soares & Morellato, 2018; Araujo et al., 2020, Araújo et al., in press). Honey bees are repeatedly cited to be among the first diurnal bee species after the crepuscular bees that appear at the flowers, overlapping with them and also the subsequent flower visiting diurnal bee species (Linsley & Cazier, 1970; Carneiro & Martins, 2012; Cordeiro et al., 2017, Araujo et al., 2020, Araújo et al., in press). As invasive species, honey bees thus may exert strong impact on the removal of pollen, which should diminish pollen flow to the native diurnal bees. However, the quantity of pollen removed by honey bees in

such systems to know the dimension on their impact on native bees has rarely been addressed. Honey bees have a highly efficient social recruitment that enables them to rapidly locate and efficiently exploit rentable mass-flowering species, which makes them strong competitors in pollen harvesting (von Frisch, 1967; Seeley, 1995; Dyer, 2002; Dornhaus & Chittka, 2004; Rader et al., 2009; González-Varo & Vilà, 2017).

Many species of woody Myrtaceae are among mass-flowering plants used as a pollen source for highly social bee (Pedro & Camargo, 1991; Wilms et al., 1996; Ramalho et al., 2007). The fruit crop *Campomanesia pubescens* (DC.) Berg is one of the numerous mass-flowering Myrtaceae species with melittophilous flowers in the neotropics that open before dawn, offer a high amount of pollen, which is explored by a variety of bee species, including honey bees (Torezan-Silingardi & Del-Claro, 1998; Rodrigues et al., 2017).

We studied the pollination of *C. pubescens* in the natural environment and analyzed pollen competition between native bees and honey bees and asked: (a) What species are effective pollinators of *C. pubescens*? (b) What is the pollen removal dynamics along the anthesis, and how many pollen grains are removed from the different bee groups from flowers of *C. pubescens*? (c) Does the abundance of honey bees alter the pollen flow and amount of pollen collected by native bees? (d) Does the abundance of flower visits of native bees change when honey bees are absent? To answer these questions, we described the floral traits and determined the breeding system of *C. pubescens*. We determined the frequency of flower visits and conducted an exclusion experiment allowing only restricted access to different groups of bees to the flowers. In addition, we conducted an experiment that prevented the access of honey bees and larger bees to flowers and measured the abundance of flower visits of native bees under experimental conditions and in flowers free accessible to all visitors. Finally, we assessed the pollen removal by quantifying the pollen decrease per flower throughout anthesis in open assess flowers and net covered flowers.

2. METHODS

2.1 Study Site

The fieldwork was conducted in the *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), during two flowering periods (2019-2020), from August to October. The study site is located in the Cadeia do Espinhaço (Espinhaço Mountain range) and is covered with Cerrado (Brazilian savannah) and Campo Rupestre (rupestrian fields) vegetation in an

area of 10,755 ha. The climate of the region is characterized by a hot and rainy summer (October to March) and a well-defined dry season (April to September). The average annual temperature is 19.9ºC and average annual rainfall is 1,550 mm (IEF, 2004; Neves et al., 2005).

2.2 Study Species

Campomanesia pubescens (DC.) Berg, popularly known as "gabiroba, guabiroba or guavira", is a native Brazilian deciduous shrub up to 3 m high that occurs widely in the Cerrado. Shortly after the appearance of new leaves, from August to October the plants exhibit mass-flowering (Landrum, 1986; Rodrigues et al., 2017). The hermaphrodite flowers have a white pentamerous corolla (Proença & Gibbs 1994; Rodrigues et al., 2017) and are typical pollen-flowers without nectaries (*sensu* Vogel, 1978), which is characteristic of many neotropical Myrtaceae. The flowers are visited by a variety of bee species, including several native species and the introduced honey bee (Torezan-Silingardi & Del Claro, 1998; Rodrigues et al., 2017). The fruits can be consumed *in natura* and are used for production of frozen pulps, juices, sweets, liqueurs and wine (Duarte et al., 2009; Rocha, 2011), thus presenting economic value and sociocultural importance in Brazil. The population studied was native in the study area. A voucher specimen was deposited at BHCB herbarium – Universidade Federal de Minas Gerais Belo Horizonte, Brazil.

2.3 Floral morphology, biology and anthesis

We described the floral morphology of *C. pubescens* in 20 flowers from ten individual plants (N=2 flowers/individual), measured the diameter of the open corolla and counted the number of stamens. We monitored the anthesis from opening to senescence, considering its beginning when the petals separated and style and anthers became visible. We determined the time of anther dehiscence with a hand-magnifying lens. Stigma receptivity was tested from flower opening to senescence by adding a drop of H_2O_2 to the stigma and the formation of bubbles indicated viability (Dafni et al., 2005).

The number of pollen grains per flower was determined by macerating the anthers of pre-anthesis flower buds in Eppendorf tubes containing a solution of 0.5 mL lactic acid and glycerin at 3:1 (Lloyd, 1972). We extracted an aliquot from this solution for counting the pollen grains using a Neubauer chamber (Maêda, 1985). The mean number of grains per flower was calculated from 10 flower buds from ten different plant individuals. In the same

flowers, we counted the number of ovules under a stereomicroscope and determined the pollen to ovule ratio.

2.4 Breeding system

We determined the breeding system of *C. pubescens* using the following controlled pollination treatments: hand self-pollination, spontaneous self-pollination, hand cross-pollination and pollination under natural conditions (open pollination control). For each of the first three treatments, we bagged 100 flower buds in pre-anthesis on ten different individuals. On the same plants, we marked 200 flower buds to measure fruit set under natural conditions. The number of flowers used in each treatment per plant individual varied slightly, especially the open pollination control flowers due to flower availability. In all treatments, fruit and seed set were determined.

2.5 Flower visitors

We determined the spectrum of the flower-visitors in 10 non-consecutive days in the 2019 flowering season. We collected the bees with entomological nets during the visits on flowers from 0430 to 1200 h and recorded their behaviour when collecting pollen, for example, if there was vibration of the anthers. The collected specimens were prepared, identified, labeled and deposited in the entomological collection of the Centro de Coleções Taxonômicas, Federal University of Minas Gerais (CCT-UFMG).

To determine the frequency of flower visits we counted all visitors that occurred in a group of ~100 flowers/individual for 20 seconds from 0430 to 1200 h every 5 minutes. We opted for the short scanning for flower visitors to determine visitation frequency because of the temporarily high number of honeybee visitors that frustrated the determination of the number of bees without multiple counting of the same bee individuals. We stopped counting after ~100 visits per 20 seconds, thus considering 100 as the maximum value. During the counts, we recorded also the names of the flower visiting species or morphospecies of bees. The scans of the number of flower visitors were repeated on 15 plant individuals during 10 days, which was possible because in some days more than one observer performed counts on different shrubs simultaneously. The data were grouped to 30-minute intervals (six countings) and the flower visitors were grouped to (1) crepuscular bees (species of *Megalopta* and *Ptiloglossa*), (2) honey bees (*Apis mellifera*) and (3) native diurnal bees (all other bees).

2.6 Pollinator effectiveness

In the 2020 flowering season, we measured the contribution of each group of bee to the fruit and seed set of *C. pubescens*. For this, three treatments were established in which the flowers were exclusively available to flower visitors during daytimes, that corresponded to their period of flower visitation: 1- Crepuscular bees - bags from bagged flowers were removed between 0430 and 0500 h; 2- honey bees – bags from bagged flowers were removed between 0630 and 0730 h; and 3- native diurnal bees - bags from bagged flowers were removed between 0830 and 1200 hr. After the period of access n which there were several visits, the flowers were bagged again. During the period, the flowers were available, we checked whether the flowers were visited only by bees of the respective group. For each treatment, we bagged 50 flower buds in pre-anthesis in ten plant individuals. The flowers were monitored until senescence, when fruit and seed set was determined for each group. The fruit set was compared to that of non-bagged flowers freely accessible to flower visitors (flowers accessible to pollinators in Reproductive system; $N = 200$).

2.7 Experimental exclusion of bees

To evaluate the influence of honey bees mass foraging on the abundance and resource collection on native diurnal bees, we conducted a honey bees exclusion experiment on *C. pubescens.* In the 2019 flowering season, we marked 12 shrubs and, in each of them, we randomly selected two branches with abundant new flowers for the honey bees exclusion experiment. One of the branches of each shrub was not manipulated, and flower visitors had free access to the flowers; we denominated this treatment "Open access flowers". The second flowering branch was bagged with a green nylon net of 4 mm long and 6 mm wide which allowed only the passage of small bees (such as Meliponini and Halictidae) (Figure 1). Honey bees and other bees of similar or bigger size (e.g., crepuscular bees) were not able to access these net-covered flowers. We denominated this treatment "Net-covered flowers". The flowers used in this treatment were bagged one day before the experiment. The "open access flowers" were visited by bees of all species. The honey bees exclusion experiment was conducted in 10 non-consecutive days. The number of flowers used in the paired treatments varied slightly, because it was not possible to determine exactly how many flowers would open on the subsequent day of the experiment. Overall, a total of 1200 flowers were monitored in the open access flowers *and* 1197 flowers in the net-covered flowers. In both treatments, we determined the abundance and richness of bees using observational

scans (see details above in “Flower visitors”). Here, the counts of flower visits were carried out between 0630 and 1200 h, which is equivalent to the foraging period of the native diurnal bees in flowers of *C. pubescens*.

FIGURE 1 Nylon net used in the treatment “Net-covered flowers”, which allowed only the passage of Meliponini and Halictidae bees and prevented access to flowers by honey bees and other bees of similar or bigger size, as crepuscular bees.

2.8 Pollen removal

We determined the amount of pollen grains in the flowers at different times of anthesis: (0) Pre-anthesis - flower buds; (1) 0630 h - after visits by crepuscular bees and massive visits by *A. mellifera*; (2) 0830 h - after the end of massive honey bee visits and native bee visits; (3) 1200 h - after the end of bee visits in general. First, we calculated the number of pollen grains of flowers using flower buds (stage 0) (N=10) (see above in “Floral Biology”). Then, we counted the pollen grains collected by bees after (i) period of crepuscular bee and honey bee overlap (flowers removed at 0630 h) and (ii) period of diurnal native bee and honey bees overlap (flowers removed at 0830 and 1200 h).

In each time of anthesis, 10 individual flowers were removed and transferred to an Eppendorf tube containing 70 % ethanol. Subsequently, the anthers were removed and macerated, while the pollen adhering to the corolla was washed out with ethanol. The samples were homogenized in a vortex stirrer and centrifuged at 6000 r.p.m for 5 min. The alcohol was decanted and 0.5 mL lactic acid and glycerin at 3:1 was added (Lloyd, 1972).

We extracted an aliquot from this solution for counting pollen grains in a Neubauer chamber (Maêda, 1985).

Amount of pollen grains removed by different groups of bees when these have exclusive access to a flower

We calculated the number of pollen grains removed by different groups of bees: (1) crepuscular bees, (2) honey bees and (3) native diurnal bees. To calculate the average number of pollen grains collected by each bee group, we counted the number of residual pollen grains in the flowers and the % of pollen collected per flower by each bee group, relative to the mean total number of pollen grains per flower bud. The quantity of pollen collected by crepuscular bees was counted in flowers visited exclusively by these bees between 0430 and 0500 h (N=10), by honey bees in flowers visited exclusively by these bees between 0630 and 0800 h (N=10) and by native diurnal bees in flowers visited exclusively by these bees between 0830 and 1200 h (N=10). These intervals were chosen because there was little or no foraging overlap of the bee groups, which allowed us to guarantee only group visits at the respective intervals. Here, we use the same pollen counting methodology used previously (see details above in "Pollen Flow")

Pollen removal of native diurnal bees in the presence and absence of honey bees

We predicted that the exclusion of *A. mellifera* (and due to the size, crepuscular bees too) increases the amount of pollen available and removed by native diurnal bees. Therefore, to test this, we calculated the number of pollen grains collected by native diurnal bees using net-covered flowers from the exclusion experiment (N= 10) compared with the number of pollen grains collected by these bees in open-access flowers (N= 10), that were visited also by honey bees and crepuscular bees. We used pollen counting methodology described above (see "Pollen Flow").

2.9 Statistical analysis

We used a Generalized Linear Mixed Model (GLMM) with Poisson distribution (logarithmic link function) to compare the number of fruits produced in each treatment of the breeding system, with treatment as a fixed factor, number of flowers as a random factor. To compare the contribution of each group of bees on the fruit and seed set of *C. pubescens*, we used a

GLMM with Poisson distribution and number of flowers as a random factor. To compare the frequency of visits by native bees in the presence and absence of honey bees, we used a GLMM with Poisson distribution, considering treatment (Open access flowers or Net-covered flowers) and time intervals as fixed factors and number of flowers as a random factor. To compare the number of pollen grains remaining within flowers at different times of anthesis and to compare the number of pollen grains collected by each bee group, we used the Generalized Linear Model (GLM), with Gaussian distribution. We utilized likelihood ratio tests to select the appropriate model (Zuur et al., 2009) and post-hoc Tukey tests to analyze the response variable in relation to the fixed effects. The data were analysed in R using the packages "lme4" (Bates et al., 2015) and "emmeans" (Lenth, 2019).

3. RESULTS

3.1 Floral morphology and anthesis

The studied population of *C. pubescens* bloomed in August and September. Individual shrubs exhibited mass flowering, which lasted about five to eight days once a year, but flowering was not synchronized among individuals. The number of opening flowers on a blooming day varied and was higher in the first two days of flowering of a plant individual. The nectarless flowers had a mean diameter of 26.2 ± 1.4 mm when fully open and contained on average 152.8 (± 27.3; N=20) stamens, that produced on average 918,200 (± 157,970; N=10) pollen grains per flower. The inferior ovaries contained on average 59.6 (± 5.3; N=10) ovules and the pollen to ovule ratio was 15,406:1. The flowers opened before sunrise, between 0400 and 0430 h (Figure 2a). Anthers dehisced at the time of floral opening (Figure 2b) and the stigmas were already receptive and remained so until floral senescence. The floral senescence occurred approximately five days after opening, when the corolla and stamens detached and fell (Figure 2c). The yellow berries matured in October and November and measured 1 to 3 cm in diameter (Figure 2d).

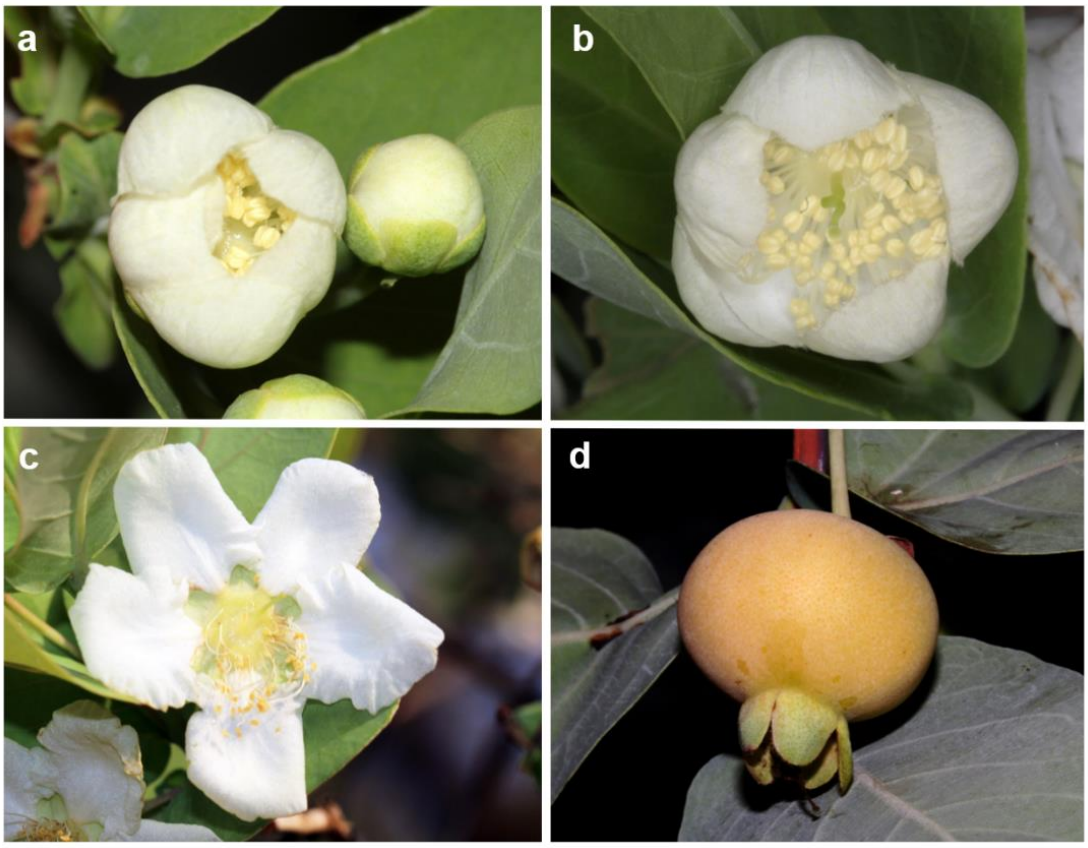

FIGURE 2 Floral anthesis stages and fruit of *Campomanesia pubescens.* **(a)**, Floral bud and flower starting the opening; **(b)**, Freshly opened flower, around 0430 h; **(c)**, Senescent flower with stamens standing out; **(d)**, Gabiroba fruit with permanent sepals.

3.2 Breeding system

The pollination experiment revealed that *C. pubescens* was predominantly xenogamous. The fruit set after spontaneous self-pollination was low and did not differ from hand self-pollination (P=0.82; Tables 1, S1). Fruit set after hand cross-pollinated flowers was conspicuously higher than those after self-pollination (P<0.05; Tables 1, S1) and did not differ from that in flowers accessible to pollinators (P=0.55; Tables 1, S1). The same was true for seed set, which was similar in flowers accessible to floral visitors and in hand cross-pollinated flowers and much higher than that after spontaneous self-pollination (Table 1).

TABLE 1 Breeding system of *Campomanesia pubescens*: fruit and seed set after spontaneous self-pollination, hand self-pollination, hand cross-pollination and open pollination (flowers accessible to pollinators; control).

Treatment	Number of flowers	Fruit set [N (%)]	Seed set (mean ± SD)
Spontaneous self-pollination	100	2 (2%) a	6 ± 4.2
Hand self-pollination	100	4 (4%) a	8.3 ± 1
Hand Cross-pollination	100	41 (41%) b	9.2 ± 0.9
Open pollination	200	76 (36%) b	8.9 ± 1.2

Different letters in each line of fruit set refer to $P<0.05$.

3.3 Flower visitors

The flowers were visited by bees of 23 species (Table 2), of which eight were stingless bees, 11 diurnal solitary bees, three crepuscular bees and the introduced honey bees *Apis mellifera*.

TABLE 2 Floral visitors of *Campomanesia pubescens* in Parque Estadual do Rio Preto, Minas Gerais, Brazil, including sex and daily foraging period.

Bee species		Daily foraging period
Apidae		
Apini	*Apis mellifera* Linnaeus, 1758	diurnal
Bombini	*Bombus* sp.	diurnal
Centridini	*Centris* sp.	diurnal
Meliponini	*Melipona* sp. 1	diurnal
	Melipona sp. 2	diurnal
	Oxytrigona tataira (Smith, 1863)	diurnal
	Plebeia sp.	diurnal
	Scaptotrigona postica (Latreille, 1807)	diurnal
	Tetragonisca angustula (Latreille, 1811)	diurnal
	Trigona spinipes (Fabricius 1793)	diurnal
	Trigona sp.	diurnal
Xycolopini	*Xylocopa grisescens* Lepeletier, 1841	diurnal
	Xylocopa sp.	diurnal
Colletidae		
Caupolicanini	*Ptiloglossa latecalcarata* Moure, 1945	crepuscular
Halictidae		
Augochlorini	*Augochlora* sp. 1	diurnal
	Augochlora sp. 2	diurnal
	Augochloropsis sp. 1	diurnal
	Augochloropsis sp. 2	diurnal
	Augochloropsis sp. 3	diurnal
	Megalopta aegis (Vachal, 1904)	crepuscular
	Megalopta amoena (Spinola, 1853)	crepuscular
	Thectochlora alaris (Vachal, 1904)	diurnal
Halictini	*Dialictus* sp.	diurnal

Honey bee workers (Figure 3a) were by far the most frequent floral visitors (74%),

followed by the crepuscular bees *Megalopta aegis* (Vachal, 1904), *M. amoena* (Spinola, 1853) (14%; these species could not be distinguished in the field) (Figure 3b) and *Ptiloglossa latecalcarata* Moure, 1945 (3%) (Figure 3c). Other flower visitors (9%) were mainly stingless bees (Figure 3d), carpenter bees and halictid bees.

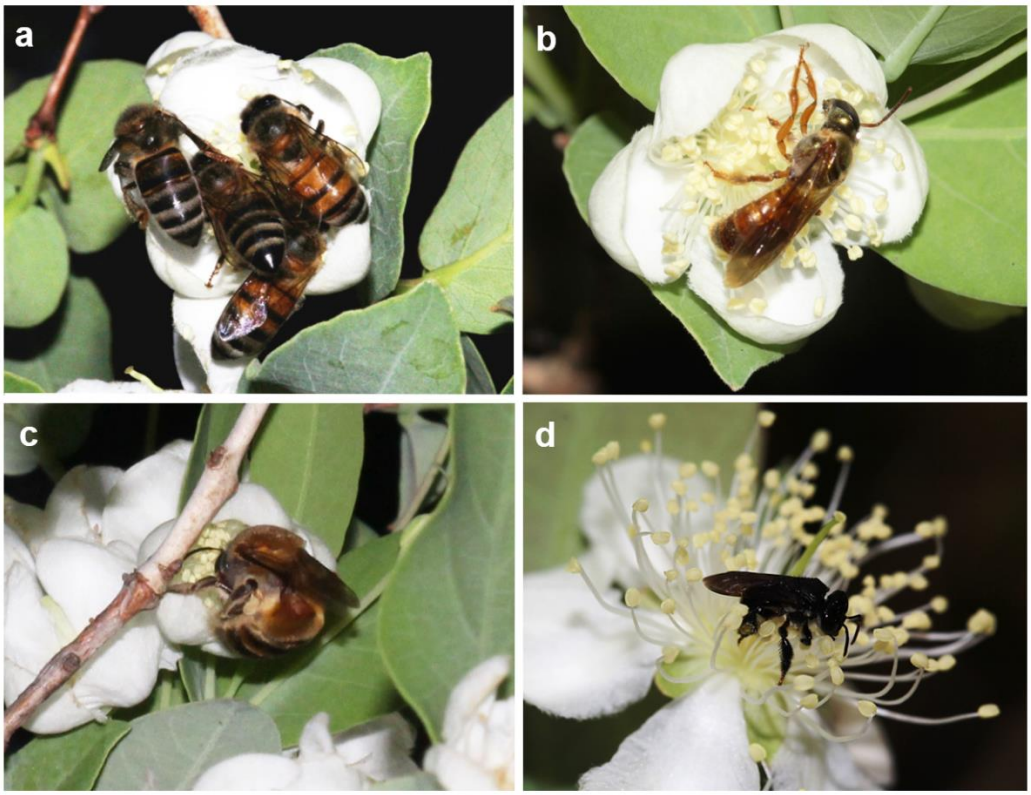

FIGURE 3 Females of bee species collecting pollen in flowers of *Campomanesia pubescens*. **(a)**, Honeybee workers (*Apis mellifera).* **(b)**, Female of crepuscular *Megalopta* sp. **(c)**, Female of crepuscular *Ptiloglossa latecalcarata*. **(d)**, Worker of the diurnal Meliponini species.

During the 10 days of floral monitoring along anthesis, we recorded always the same daily visitation patterns: the first floral visitors were crepuscular bees ***(****Megalopta aegis*, *M. amoena* and *Ptiloglossa latecalcarata*) soon after the beginning of anthesis (0435 h) before sunrise visiting the flowers until 0630 h (Figure 4). Bees of *Megalopta* were more abundant than *Ptiloglossa* and accounted for 75 % of the crepuscular bee visits. All crepuscular bees grabbed a set of stamens and vibrated the anthers during the visits. *Apis mellifera* (Figure 4) started visiting shortly before sunrise, ~0500 h, overlapping the visiting period with crepuscular bees. Between 0530 and 0630 h honey bees visited the flowers of *C. pubescens* intensely. Around 0700 h, their abundance decreased and ~0800 h, their flower visits

virtually ceased. Carpenter bees of *Xylocopa grisescens* visited the flowers already before 0630 h, but with only low abundance (1.5% of all visits). The stingless and halictid bees (Figure 4), started flower visits ~ 0630 h, and after 0800 h their visits increased and outnumbered the number of honey bee visits. Overall flower visits of native diurnal bees summed 7.5%. From ~1200 h onward, no more flower visits were recorded (Figure 4). During visits, bees of all species touched the stigmas with exception of the small bees of *Plebeia* and *Dialictus*.

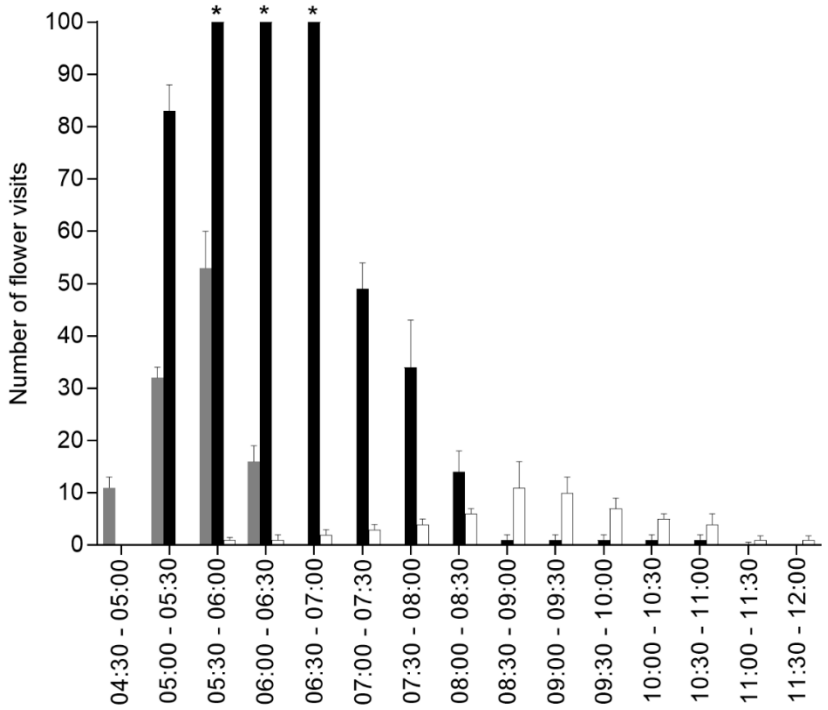

FIGURE 4 Number of visits to flowers of *Campomanesia pubescens* in Parque Estadual do Rio Preto. Observations were made on 10 non-consecutive days and data were grouped into 30-min intervals. Intervals marked with an asterisk mean that exact counting of the number of bees (honey bees) was not possible due to the huge abundance. Therefore, we stopped counting after ~100 visits and considered 100 as the maximum estimated value. Thus, honeybee frequencies might be somewhat underrepresented in the three intervals. Gray bars = crepuscular bees; black bars = honey bees; white bars = native diurnal bees.

3.4 Pollinator effectiveness

The fruit set of flowers accessible exclusively to crepuscular bees (16 fruits; 32%) was bigger than in flowers accessible only honey bees (15 fruits; 32%) and only to native bees (11 fruits; 22%) (Table 2). However, there was no significant difference between the three treatments

(Table S2). When we compared the set fruit of each group of bees with flowers accessible to pollinators, we also found no significant difference (Table S2). There was no difference in the seed set after visits of the three bee groups (P>0.05) (Tables 3, S3).

TABLE 3 Fruit and seed set of *Campomanesia pubescens* after visits of the nocturnal, native and honey bees. For each bee group we used 50 flowers.

Bee group	Fruit set [N (%)]	Seed set (mean ± SD)
Crepuscular bees	16 (32%)	8.5 ± 1.3
Honey bees	15 (30%)	9.3 ± 0.7
Native diurnal bees	11 (22%)	8.5 ± 0.6

4.5 Experimental exclusion of bees

The exclusion of honey bee visits in net-covered flowers caused 100.3% increase in native bee abundance throughout the activity period of these bees. In total, we recorded 1290 native diurnal bees in net-covered flowers and 643 of these bees in open access flowers, of which 98 % were stingless bees and 2 % halictid bees. Native diurnal bees were the more abundant flower visitors when honey bees were absent until 1000 h (P <0.05; Figure 5A; Table S4). After this time there was no significant difference in the abundance of native bees in net-covered flowers when compared to the open access flowers that were intensely visited by honey bees (P> 0.05; Figure 4A; Table S4). The difference between native diurnal bee visits was especially high until 0800 h, when their abundance was 74 to 82 % higher than in open access flowers (Figure 5B).

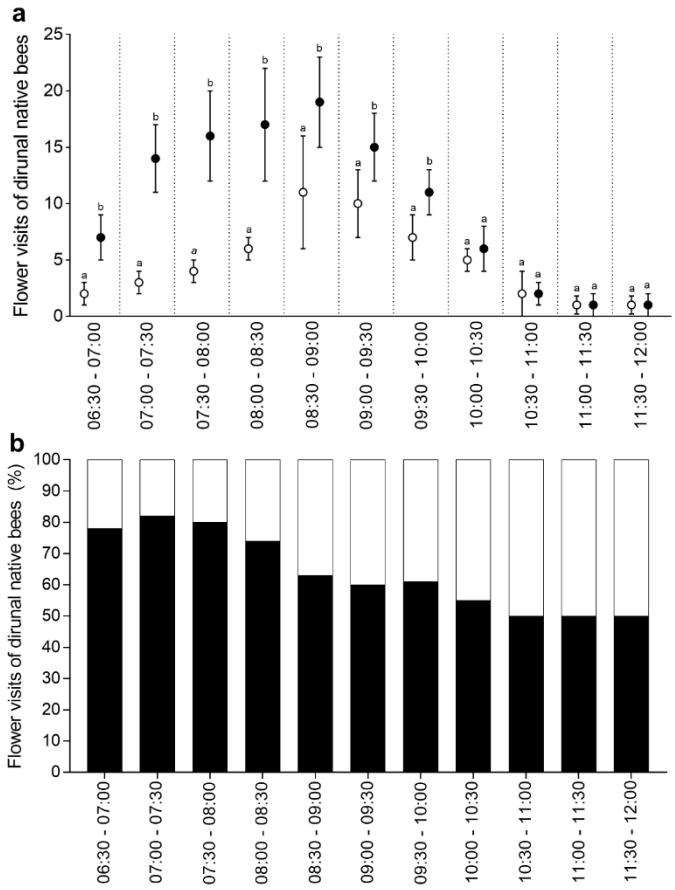

FIGURE 5 Abundance of native diurnal bees on flowers of *Campomanesia pubescens*. **(a),** Number of native diurnal bee visits in open access flowers (flowers also visited by honey bees) and net-covered flowers that hindered access to the flowers for honey bees. Clear circles = open access flowers; filled circles = net-covered flowers. Different letters mean significant differences between treatments ($P<0.05$). **(b),** Relative frequency of native diurnal bees in open access flowers and net-covered flowers. White bars = open access flowers; black bars = net-covered flowers.

3.6 Pollen Flow

We measured the decrease in the total amount of pollen grains in the flowers of *C. pubescens* during the activity of bees, from 0430 to 1200 h. In the first two hours of anthesis there was a drastic reduction in the pollen content per flower, and on average only 13.4% (112,600 ± 39,328) of the pollen grains remained in the flowers at 0630 h. At 0830 h on average 6.4% (58,550 ± 17,930) and at 1200 h (end of the bee visits) only 3% (27,800 ± 9,340) (residual pollen) (Figure 6a, b). From the time of flower opening (~0430 h) until 0630 h, pollen grains were collected by crepuscular bees and honey bees. These bees together collected 86.6% (795,600) of the total number of pollen grains per flower. From 0630 to 1200 h, the pollen grains were collected by both honey bees and native diurnal bees, and in this period these

bees together collected 10.4% (94,800) of the grains (Figure 6b). There was significant difference in the number of remaining pollen grains per flower when comparing the 0630 and 1200 h intervals (P=0.05), but there was no significant difference between the intervals 0630 and 0830 h (P>0,05) (Figure 5; Table S4) (Figure 5; Table S5).

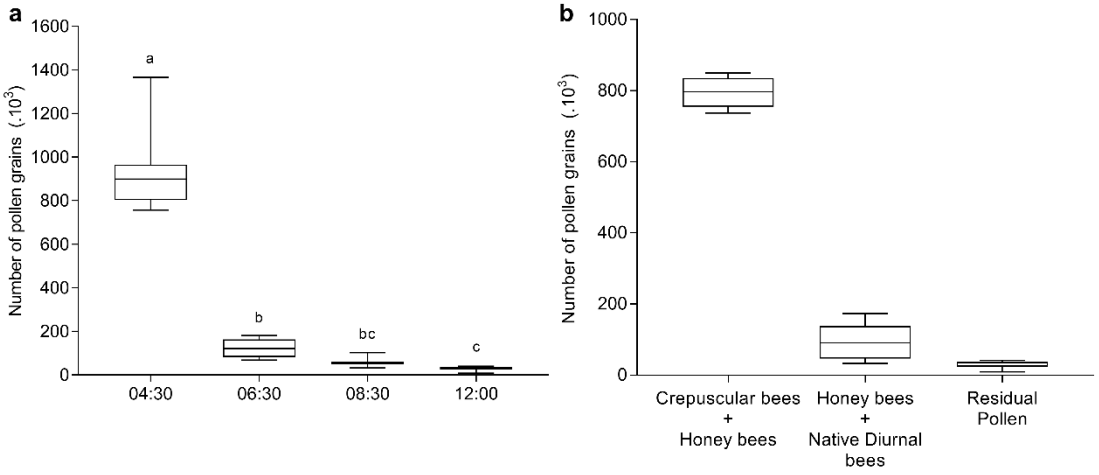

FIGURE 6 The amount of pollen grains in the *Campomanesia pubescens* flowers at different times of anthesis. **(a)**, Number of pollen grains per flower until 1200 h when flower visitors had disappeared. 0430h before visits; N=10) and during the activity of bees (N=10 for each interval). Different letters mean significant differences between treatments (P<0.05). **(b)**, Pollen grains collected by bees in foraging overlap periods.

3.7 Amount of pollen grains removed by different groups of bees when these have exclusive access to a flower

When only crepuscular bees visited a flower of *C. pubescens*, they collected on average 23% of the pollen grains, honey bees alone on average 87%, and when exclusively native diurnal bees are allowed to visit a flower they removed on average 76% of the pollen grains (Figure 7). There was no significant difference in the number of pollen grains collected by honey bees and native diurnal bees (P< 0.001) (Table S6).

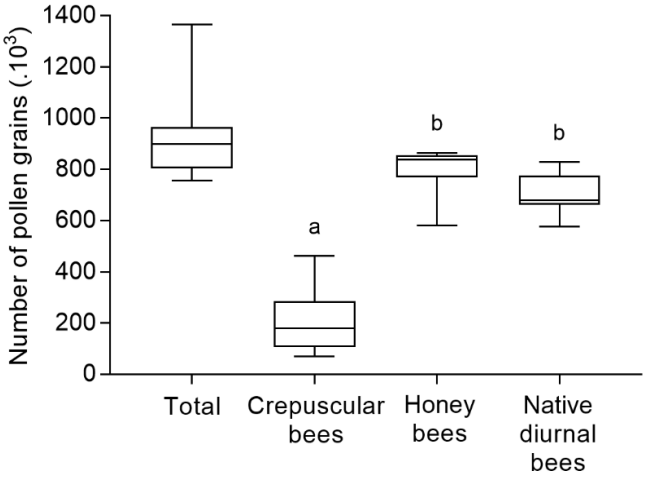

FIGURE 7 Pollen removal from bagged flowers of *Campomanesia pubescens* available to groups of flower visitors exclusively during their visiting intervals. Pollen grains destined to different bee groups: Crepuscular bees, Honey bees and Native diurnal bees (N=10 for each group) in relation to the total grains of the flower buds (Total). Bagged flowers were opened to crepuscular bees between 0430 and 0500 h, to honey bees between 0630 and 0800 h and to native diurnal bees between 0830 and 1200 h. After the visits, the flowers were removed to count the remaining pollen. Different letters mean significant differences between treatments (P<0.05).

3.8 Pollen removal of native diurnal bees in the presence and absence of honey bees

In open flowers accessible for all bees, native diurnal bees collected only 3.7% of the pollen grains from flowers of *C. pubescens.* In net-covered flowers that excluded crepuscular bees and honey bees, the native diurnal bees gained on average 76% of the pollen grains of a flower, which is ~20 times more pollen collected when compared to flowers available to honey bees and crepuscular bees (Figure 8).

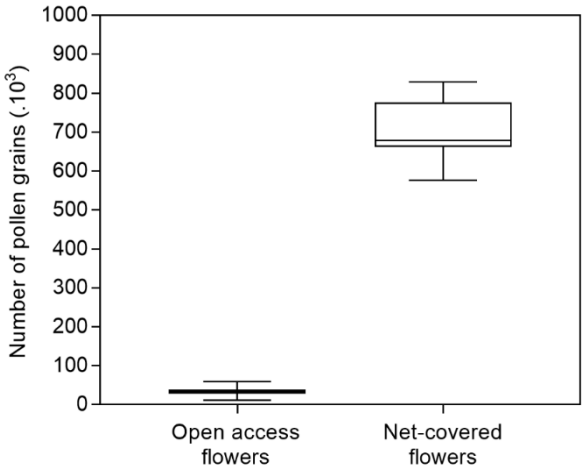

FIGURE 8 Pollen removal by native diurnal bees in the presence (Open access flowers) and absence (Net-covered flowers) of *Apis mellifera*.

4. DISCUSSION

Our results show that crepuscular bees are effective pollinators of *C. pubescens* and guarantee the maximum fruit set (32%) in only a few visits at dawn. Honey bees and native diurnal bees are also effective pollinators, but they do not increase the fruit set after the flower visits of the crepuscular bees. Honey bees are the most efficient competitors, as they gained by far the largest amount of pollen grains from the flowers of *C. pubescens*. The massive visitation by honey bees caused displacement of native diurnal bees through exploitative competition, as shown by the exclusion experiment, which resulted in a drastic increase of the pollen flow to native diurnal species, especially stingless bees.

Crepuscular bees - the most efficient pollinators of *Campomanesia pubescens*

Crepuscular bees were the most efficient pollinators of *C. pubescens* flowers. In on average only 1.3 visits per flower at dawn, these bees guarantee the maximum fruit set of *C. pubescens,* considering that the fruit set after exclusive visits of crepuscular bees did not differ from that of hand cross-pollinated flowers and open access flowers. The crepuscular bees of *Megalopta* and *Ptiloglossa* have been identified as effective pollinators of other species of Myrtaceae with pollen flowers, such as *Campomanesia phaea* (O.Berg) Landrum (Cordeiro et al., 2017), *Eugenia florida* DC. and *Myrciaria floribunda* (H.West ex Willd.) O.Berg (Souza, 1996). This reinforces the high potential of crepuscular bees as effective pollinators of crop species in Myrtaceae and other families (Cordeiro et al., 2019; 2021). Species of Myrtaceae were repeatedly shown as important pollen source for these bees, as demonstrated in analyzes of their pollen loads and brood cells (Smith et al., 2012; Araujo et al., 2020). The crepuscular bees utilize vibration to collect pollen of *C. pubescens* flowers as reported also for other representatives of the family Myrtaceae (Gressler et al., 2006; Fidalgo & Kleinert, 2009).

Because the flowers of *C. pubescens* achieve maximum fruit production already after the first few flower visits of the crepuscular bees, these bees are prominent pollinators and should strongly shape the floral traits of the plant, especially flower opening time, anther dehiscence and stigma viability. Honey bees and the native diurnal bee species were also effective pollinators of *C. pubescens*, given that their visits alone lead also to high fruit set. However, their visitation frequency to maximum fruit production was four to five times higher (on average 6.1 and 5.1 visits, respectively) than that of the crepuscular bees. To affirm that these bees are in fact less efficient pollinators would be necessary to perform

single-visit experiments, for example. In other population of *C. pubescens* from a Cerrado area of the state of São Paulo in southeastern Brazil, honey bees were abundant flower visitors but inefficient pollinators (Torezan-Silingardi & Del-Claro, 1998; Rodrigues et al., 2017). They collected most pollen before effective pollinators were active or by gleaning residual pollen dispersed in the flowers without depositing on stigmas, thus were classified as pollen thief (Hargreaves et al., 2009). In *C. phaea*, honey bees were also inefficient pollinators, because they transferred large amounts of pollen grains especially between flowers of the same individual and did not contribute to fruit set of this self-incompatible species (Cordeiro et al., 2017).

Competition for pollen between honey bees and crepuscular bees

In flowers of *C. pubescens*, pollen collecting bees remove the pollen grains extraordinarily fast and the pollen pool per flower diminish by over 80% in the first two hours of anthesis. This is similar to other associations of species with synchronously opening flowers and pollen collecting bees (Carvalho & Schlindwein, 2011; Siqueira et al., 2018; Cerceau et al., 2019). This pollen flows to crepuscular bees and honey bees, with vast pollen amount to honey bees. Our data about flowers with restricted access to crepuscular bees point that about 20% of the pollen grains to these bees, while the rest flows to the honey bees.

The honey bees arrived at the flowers only few minutes (~25 min) after the crepuscular bees. The sudden visits of honey bees in mass, as in flowers of *Pseudobombax longiflorum* (Mart.) A. Robyns (Malvaceae) (Araújo et al., in press), overlaps with the foraging period still under low light of the crepuscular bees and thus, these bees directly shorten the effective time that crepuscular bees collect pollen without competitors in the early morning. Because they are extraordinarily abundant, honey bees often competitively displace native bee species from their preferred pollen hosts or reduce their foraging benefit causing temporal displacement of the native bee species (Roubik et al., 1986; Roubik & Villanueva-Gutierrez 2009; Cane & Tepedino, 2017; Carneiro & Martins, 2012).

We do not know how strong honey bees negatively impact the pollen gain of the crepuscular bees in the period of foraging overlap, because it is not possible in an exclusion experiment with different mesh sizes to allow crepuscular bees and exclude honey bees because of their similar body size to honey bees (*Megalopta*) or because they are larger (*Ptiloglossa*). However, considering that the direct competition in the overlap time with honey bees was ~90 min and, thus, much larger than the period that crepuscular bees in fact forage without competitors and there was no overlap with the native diurnal bees that started

foraging ~ 0630 h, the crepuscular bees could remove much more pollen grains per visit in in this overlap-period when there were no honey bees. In other plant species not sought by honey bees in the same study site and flowering period, the daily exclusive foraging time of crepuscular bees is longer than in *C. pubescens*, by ~120 min in *Machaerium opacum* Vogel (Fabaceae) (Siqueira et al., 2018) and by ~40 min in *Caryocar brasiliense* Cambess. (Caryocaraceae) (Araujo et al., 2020). Therefore, the presence of invasive honey bees has a strong impact on crepuscular bees by shortening their foraging time and also pollen gain on lucrative pollen resources due to the ability of the honey bees to forage earlier than native diurnal bee competitors.

Competition for pollen between honey bees and native diurnal bees

Our study shows, that the early depletion of pollen by crepuscular bees and especially honey bees before the native diurnal bees foraging, was the determining factor for the abundance and pollen gain of native diurnal bees, especially stingless bees. When these bees start foraging, the flowers of *C. pubescens* contained only less than a fifth of the pollen grains and the stingless bees still had to compete for this small pollen rest with the yet foraging honey bees. Only when the flowers were almost empty, at around 0630 h, the native stingless and halictid bees were among themselves. Several studies demonstrated that honey bees deplete floral resources before native pollinator species began to forage on flowers (Wills et al., 1990; Horskins & Turner, 1999; Carmo et al., 2004; Carneiro & Martins, 2012).

However, when the honey bee access to the flowers was hindered by a fine meshed net, the major pollen fate of *C. pubescens* are the native bees. The abundance of these bees dramatically increased, especially in the first hours of anthesis, and depleted the pollen of flowers until ~1000 h. This shows that the native diurnal bees suffer vigorous competitive displacement by the invasive honey bees. This happened even with honey bees intensely foraging from surrounding accessible flowers on neighboring branches at the same time. Interestingly, the net-covered flowers exerted no or almost no influence on the access of the native diurnal bees to the flowers, despite strongly changing the visual aspect of the flowers.

The number of workers of stingless bees allocated to pollen collecting depends on the resource availability (Eltz et al., 2002; Biesmeijer & Slaa, 2004; Slaa et al., 2006), similar to honey bees, and changes in resource accessibility and amount alter the forager recruitment. As food generalists, these species may switch to more lucrative resource plants in the surroundings and reduce overlap in resource usage, which can reduce the impact of competitive displacement (Roubik et al., 1986; Biesmeijer et al., 1999). In the absence of

the introduced honey bees, the crepuscular bees, most likely, would be the only competitors of the polylectic stingless and halictid bees.

We conclude that the introduced honey bees gain much more pollen of this mass flowering myrtacean shrub than all native bees combined, because they shorten the effective time that crepuscular bees could collect pollen without competitors and, in special, they drastically reduce the amount of pollen available to native diurnal bees through effective mass exploitation of this profitable pollen resource. The massive presence of honey bees at the study site was surprising as it is a full-protected nature reserve. The study emphasizes that honey bees exert a strong negative impact on the interactions of native bee pollinators to their host plants and make the management of invasive honey bee colonies desirable especially in natural areas under protection.

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SUPPLEMENTARY INFORMATION

Tables

Table S1. Breeding system of *Campomanesia pubescens*: fruit and seed set after spontaneous self-pollination (SS), hand self-pollination (HS), hand cross-pollination (HC) and open pollination (OP - flowers accessible to pollinators; control). We tested whether there was difference in the number of fruits formed between treatments using generalized mixed-effect linear models (GLMM). Non-significant differences are in bold (*P*-value). N flowers = number of flowers; N fruits = number of fruits.; SE = Standard error.

Treatment	N flowers	N fruits	Comparison	Estimate	SE	*z*-value	*P*-value
SS	100	2	SS - HS	-0.759	0.873	-0.87	**0.82**
HS	100	4	SS - OP	-3.389	0.736	-4.60	<0.05
HC	100	41	SS - HC	-3.036	0.729	-4.16	<0.05
OP	200	72	HS - OP	-2.630	0.551	-4.77	<0.05
			HS - HC	-2.277	0.531	-4.29	<0.05
			OP - HC	0.353	0.266	1.33	**0.55**

Table S2. Pollinator effectiveness in flowers of *Campomanesia pubescens*: Fruit set after visits of the Crepuscular Bees (CB), Honey Bees (HB) and Native diurnal bees (NDB). We tested whether there was difference in the number of fruits formed between treatments using a generalized linear model (GLM), with Gaussian distribution. Non-significant differences are in bold (*P*-value). N flowers = number of flowers; N fruits = number of fruits.; SE = Standard error.

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Treatment	N flowers	N fruits	Comparison	Estimate	SE	*z*-value	*P*-value
CB	50	16	CB - HB	0.100	0.402	0.249	**0.97**
HB	50	15	CB - NDB	0.500	0.402	1.244	**0.43**
NDB	50	11	HB - NDB	0.400	0.402	0.995	**0.57**

Table S3. Pollinator effectiveness in flowers of *Campomanesia pubescens*: Fruit set after visits of the Crepuscular Bees (CB), Honey Bees (HB) and Native diurnal bees (NDB). We tested whether there was difference in the number of fruits formed between treatments using a generalized linear model (GLM), with Gaussian distribution. Non-significant differences are in bold (*P*-value). N flowers = number of flowers; N fruits = number of fruits.; SE = Standard error.

Treatment	N flowers	N fruits	Comparison	Estimate	SE	*z*-value	*P*-value
CB	50	16	CB - HB	-0.75	0.320	-2.34	**0.06**
HB	50	15	CB - NDB	-0.00	0.320	-1.71	**1.00**
NDB	50	11	HB - NDB	0.75	0.320	2.34	**0.06**

Table S4. The abundance of native bees on *Campomanesia pubescens* flowers in relation to the visitor exclusion experiment treatments (Open access flowers - OA and Net-covered flowers - NC). We tested whether there was difference in the frequency of visits by native bees in presence and absent of *Apis mellifera*, we used a GLMM with treatment (OA or NC) and time intervals as fixed factors, number of flowers as a random factor and Poisson distribution. Model summaries containing model comparisons, Akaike information criterion (AIC) and likelihood ratio test on marginal effects of fixed factors. Non-significant differences are in bold (*P*-value). Full model = Visits ~ treatment + intervals + treatment * intervals + (1 | Flowers). N = 1200 flowers in OA; N = 1197 flowers in NC. N visits = number of visits. SD = Standard deviation; SE = Standard error

			Interval Effect						Treatment effect				
Intervals	N visits Mean ± SD		Comparision	Treatment	Estimate*	SE	*z*-value	*P*-value	Comparision	Estimate*	SE	*z*-value	*P*-value
	OA	NC											
06:30	2±1	7±2	06:30 - 07:00	NC	-0,69	0,13	-5,20	< 0.05	OA – NC	12763	0.231	5529	< 0.05
07:00	3±1	14±3	06:30 - 07:30	NC	-0,82	0,13	-6,32	< 0.05	OA – NC	17.405	0.198	8.793	< 0.05
07:30	4±1	16±4	06:30 - 08:00	NC	-0,84	0,13	-6,50	< 0.05	OA – NC	14.888	0.167	8.920	< 0.05
08:00	6±1	17±5	06:30 - 08:30	NC	-0,95	0,13	-7,47	< 0.05	OA – NC	10.886	0.141	7.707	< 0.05
08:30	11±5	19±4	06:30 - 09:00	NC	-0,72	0,13	-5,49	< 0.05	OA – NC	0.5506	0.111	4.962	< 0.05
09:00	10±3	15±3	06:30 - 09:30	NC	-0,39	0,14	-2,79	**0,16**	OA – NC	0.4140	0.119	3.474	< 0.05
09:30	7±2	11±2	06:30 - 10:00	NC	0,26	0,16	1,62	**0,88**	OA – NC	0.3556	0.138	2.572	< 0.05
10:00	5±1	6±2	06:30 - 10:30	NC	1,12	0,22	5,16	< 0.05	OA – NC	0.1466	0.181	0.811	**0.42**
10:30	2±2	2±1	06:30 - 11:00	NC	2,26	0,35	6,44	< 0.05	OA – NC	-0.1335	0.259	-0.516	**0.61**
11:00	1±0.8	1±1	06:30 - 11:30	NC	2,15	0,33	6,44	< 0.05	OA – NC	-10.609	0.387	-2.743	**0.60**
11:30	1±0.8	1±1	07:00 - 07:30	NC	-0,13	0,10	-1,25	**0,98**	OA – NC	-0.5878	0.394	-1.490	**0.14**
			07:00 - 08:00	NC	-0,15	0,10	-1,45	**0,93**					
			07:00 - 08:30	NC	-0,26	0,10	-2,57	**0,27**					
			07:00 - 09:00	NC	-0,03	0,11	-0,32	**1,00**					
			07:00 - 09:30	NC	0,30	0,12	2,54	**0,28**					
			07:00 - 10:00	NC	0,95	0,14	6,57	< 0.05					
			07:00 - 10:30	NC	1,81	0,20	8,88	< 0.05					
			07:00 - 11:00	NC	2,94	0,34	8,61	< 0.05					
			07:00 - 11:30	NC	2,84	0,33	8,73	< 0.05					
			07:30 - 08:00	NC	-0,02	0,10	-0,20	**1,00**					

			Interval Effect						Treatment effect				
Intervals	N visits Mean ± SD		Comparision	Treatment	Estimate*	SE	*z*-value	*P*-value	Comparision	Estimate*	SE	*z*-value	*P*-value
	OA	NC											
			07:30 - 08:30	NC	-0,13	0,10	-1,32	**0,97**					
			07:30 - 09:00	NC	0,10	0,10	0,93	**1,00**					
			07:30 - 09:30	NC	0,43	0,11	3,76	< 0.05					
			07:30 - 10:00	NC	1,08	0,14	7,61	< 0.05					
			07:30 - 10:30	NC	1,94	0,20	9,60	< 0.05					
			07:30 - 11:00	NC	3,08	0,34	9,02	< 0.05					
			07:30 - 11:30	NC	2,97	0,32	9,16	< 0.05					
			08:00 - 08:30	NC	-0,11	0,10	-1,12	< 0.05					
			08:00 - 09:00	NC	0,12	0,10	1,13	< 0.05					
			08:00 - 09:30	NC	0,45	0,11	3,95	< 0.05					
			08:00 - 10:00	NC	1,10	0,14	7,77	< 0.05					
			08:00 - 10:30	NC	1,96	0,20	9,72	< 0.05					
			08:00 - 11:00	NC	3,10	0,34	9,09	< 0.05					
			08:00 - 11:30	NC	2,99	0,32	9,23	< 0.05					
			08:30 - 09:00	NC	0,23	0,10	2,25	**0,47**					
			08:30 - 09:30	NC	0,56	0,11	5,02	< 0.05					
			08:30 - 10:00	NC	1,21	0,14	8,65	< 0.05					
			08:30 - 10:30	NC	2,07	0,20	10,32	< 0.05					
			08:30 - 11:00	NC	3,21	0,34	9,43	< 0.05					
			08:30 - 11:30	NC	3,10	0,32	9,59	< 0.05					
			09:00 - 09:30	NC	0,33	0,12	2,85	**0,14**					
			09:00 - 10:00	NC	0,99	0,14	6,84	< 0.05					
			09:00 - 10:30	NC	1,84	0,20	9,07	< 0.05					
			09:00 - 11:00	NC	2,98	0,34	8,72	< 0.05					
			09:00 - 11:30	NC	2,87	0,33	8,84	< 0.05					
			09:30 - 10:00	NC	0,65	0,15	4,31	< 0.05					
			09:30 - 10:30	NC	1,51	0,21	7,24	< 0.05					
			09:30 - 11:00	NC	2,65	0,34	7,67	< 0.05					
			09:30 - 11:30	NC	2,54	0,33	7,74	< 0.05					
			10:00 - 10:30	NC	0,86	0,23	3,80	< 0.05					

			Interval Effect						Treatment effect				
Intervals	N visits Mean ± SD		Comparision	Treatment	Estimate*	SE	*z*-value	*P*-value	Comparision	Estimate*	SE	*z*-value	*P*-value
	OA	NC											
			10:00 - 11:00	NC	1,99	0,36	5,61	< 0.05					
			10:00 - 11:30	NC	1,89	0,34	5,56	< 0.05					
			10:30 - 11:00	NC	1,13	0,38	2,96	**0,10**					
			10:30 - 11:30	NC	1,03	0,37	2,79	**0,16**					
			11:00 - 11:30	NC	-0,11	0,46	-0,23	**1,00**					
			06:30 - 07:00	OA	-0,22	0,27	-0,81	**1,00**					
			06:30 - 07:30	OA	-0,61	0,25	-2,39	**0,37**					
			06:30 - 08:00	OA	-1,03	0,24	-4,32	< 0.05					
			06:30 - 08:30	OA	-1,67	0,22	-7,53	< 0.05					
			06:30 - 09:00	OA	-1,58	0,22	-7,07	< 0.05					
			06:30 - 09:30	OA	-1,31	0,23	-5,70	< 0.05					
			06:30 - 10:00	OA	-0,86	0,24	-3,55	< 0.05					
			06:30 - 10:30	OA	-0,29	0,27	-1,07	**0,99**					
			06:30 - 11:00	OA	-0,08	0,28	-0,28	**1,00**					
			06:30 - 11:30	OA	0,29	0,31	0,92	**1,00**					
			07:00 - 07:30	OA	-0,38	0,24	-1,62	**0,88**					
			07:00 - 08:00	OA	-0,80	0,22	-3,66	< 0.05					
			07:00 - 08:30	OA	-1,45	0,20	-7,15	< 0.05					
			07:00 - 09:00	OA	-1,36	0,20	-6,65	< 0.05					
			07:00 - 09:30	OA	-1,09	0,21	-5,15	< 0.05					
			07:00 - 10:00	OA	-0,64	0,23	-2,85	**0,14**					
			07:00 - 10:30	OA	-0,06	0,25	-0,25	**1,00**					
			07:00 - 11:00	OA	0,14	0,27	0,53	**1,00**					
			07:00 - 11:30	OA	0,51	0,30	1,71	**0,83**					
			07:30 - 08:00	OA	-0,42	0,19	-2,17	**0,53**					
			07:30 - 08:30	OA	-1,07	0,17	-6,11	< 0.05					
			07:30 - 09:00	OA	-0,98	0,18	-5,53	< 0.05					
			07:30 - 09:30	OA	-0,70	0,18	-3,82	< 0.05					
			07:30 - 10:00	OA	-0,26	0,20	-1,29	**0,97**					
			07:30 - 10:30	OA	0,32	0,23	1,37	**0,96**					

		Interval Effect						Treatment effect				
Intervals	N visits Mean ± SD	Comparision	Treatment	Estimate*	SE	*z*-value	*P*-value	Comparision	Estimate*	SE	*z*-value	*P*-value
	OA NC											
		07:30 - 11:00	OA	0,53	0,25	2,13	**0,56**					
		07:30 - 11:30	OA	0,89	0,28	3,19	**0,05**					
		08:00 - 08:30	OA	-0,65	0,15	-4,29	< 0.05					
		08:00 - 09:00	OA	-0,56	0,15	-3,64	< 0.05					
		08:00 - 09:30	OA	-0,28	0,16	-1,76	**0,81**					
		08:00 - 10:00	OA	0,16	0,18	0,90	**1,00**					
		08:00 - 10:30	OA	0,74	0,21	3,44	< 0.05					
		08:00 - 11:00	OA	0,95	0,23	4,10	< 0.05					
		08:00 - 11:30	OA	1,31	0,27	4,95	< 0.05					
		08:30 - 09:00	OA	0,09	0,13	0,70	**1,00**					
		08:30 - 09:30	OA	0,36	0,14	2,63	**0,23**					
		08:30 - 10:00	OA	0,81	0,16	5,08	< 0.05					
		08:30 - 10:30	OA	1,39	0,20	7,01	< 0.05					
		08:30 - 11:00	OA	1,59	0,22	7,41	< 0.05					
		08:30 - 11:30	OA	1,96	0,25	7,79	< 0.05					
		09:00 - 09:30	OA	0,27	0,14	1,94	**0,69**					
		09:00 - 10:00	OA	0,72	0,16	4,45	< 0.05					
		09:00 - 10:30	OA	1,30	0,20	6,50	< 0.05					
		09:00 - 11:00	OA	1,50	0,22	6,94	< 0.05					
		09:00 - 11:30	OA	1,87	0,25	7,39	< 0.05					
		09:30 - 10:00	OA	0,45	0,17	2,63	**0,23**					
		09:30 - 10:30	OA	1,02	0,21	4,96	< 0.05					
		09:30 - 11:00	OA	1,23	0,22	5,52	< 0.05					
		09:30 - 11:30	OA	1,60	0,26	6,18	< 0.05					
		10:00 - 10:30	OA	0,58	0,22	2,61	**0,24**					
		10:00 - 11:00	OA	0,78	0,24	3,32	< 0.05					
		10:00 - 11:30	OA	1,15	0,27	4,26	< 0.05					
		10:30 - 11:00	OA	0,21	0,26	0,79	**1,00**					
		10:30 - 11:30	OA	0,58	0,29	1,95	**0,68**					
		11:00 - 11:30	OA	0,37	0,31	1,20	**0,98**					

994 **Table S5**. The number of pollen grains inside the flower before anthesis (N=10) and during
995 the activity of bees (N=10 for each interval). We tested whether there was difference the
996 number of pollen grains remaining within flowers at different times of anthesis using a
997 generalized linear model (GLM), with Gaussian distribution. Non-significant differences are
998 in bold (*P*-value). T0 = 04:30h, bud flowers; T1 = 06:30h; T2 = 08:30h; T3 = 12:00h. N
999 flowers = number of flowers SD = Standard deviation; SE = Standard error.

1000

Treatment	N flowers	Mean ± SD Pollen grains	Comparison	Estimate	SE	*z*-value	*P*-value
T0	10	918200 ±157970	T0 – T1	795600	36681.01	21.69	<0.05
T1	10	122600 ±39328	T0 – T2	859650	36681.01	23.44	<0.05
T2	10	58550 ±17930	T0 – T3	890400	36681.01	24.27	<0.05
T3	10	27800 ±9340	T1 – T2	64050	36681.01	1.75	**0.30**
			T1 – T3	94800	36681.01	2.58	0.05
			T2 – T3	30750	36681.01	0.84	**0.84**

1001

1002 **Table S6.** Pollen grains of *Campomanesia pubescens* destined for each group of floral
1003 visitors: Nocturnal bees, Honey bees and Native diurnal bees. We tested whether there was
1004 difference in the number of pollen grains collected by each bee group, we used the
1005 generalized linear model (GLM), with Gaussian distribution. Non-significant differences are
1006 in bold (*P*-value). HB = Honey Bees; CB = Crepuscular Bees; NB = Diurnal Native Bees.
1007 N flowers = number of flowers SD = Standard deviation; SE = Standard error.
1008

Treatment	N flowers	Mean ± SD Pollen grains	Comparison	Estimate	SE	*z*-value	*P*-value
HB	10	796330 ± 91730	HB – NDB	97780	44787.2	2.18	< **0.07**
CB	10	206400 ± 126261	HB – CB	589930	44787.2	13.17	<0.05
NDB	10	34000 ± 12193	NDB – CB	492150	44787.2	10.99	<0.05

1009

CAPÍTULO 2

A presença *de Apis mellifera* diminui a remoção de pólen e a abundância de abelhas nativas sem ferrão em espécies de floração maciça de Myrtaceae

A PRESENÇA *DE APIS MELLIFERA* DIMINUI A REMOÇÃO DE PÓLEN E A

ABUNDÂNCIA DE ABELHAS NATIVAS SEM FERRÃO EM ESPÉCIES DE FLORAÇÃO MACIÇA DE MYRTACEAE

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ABSTRACT

PREMISSA DO ESTUDO: *Apis mellifera* é uma das espécies mais amplamente distribuída e abundante no mundo. Nos Neotrópicos, essa abelha introduzida pode impactar fortemente a apifauna nativa, porque compartilha os recursos florais com espécies nativas, especialmente espécies de abelhas sem ferrão. No entanto, há poucas abordagens experimentais que quantificam o impacto dessa espécie invasiva no ganho de recursos florais de abelhas nativas. Usamos duas espécies de Myrtaceae com flores de pólen generalistas e floração maciça como modelos para quantificar o impacto da presença de *A. mellifera* na frequência de visitas e na coleta de pólen por abelhas nativas.

MÉTODOS: Nas duas espécies, determinamos o espetro dos visitantes florais e a formação de frutos por visitas de *Apis mellifera* e de abelhas nativas. Num experimento de exclusão de *A. mellifera* com um tecido de malha fina, determinamos a frequência de visitas florais, a remoção de pólen ao longo da antese e quantificamos o ganho de pólen de ambos os grupos de abelhas.

RESULTADOS CHAVE: Em ambas as espécies de plantas, *A. mellifera* removeu a maior porção do pólen das flores. Na exclusão de *A. mellifera,* a frequência das visitas florais das abelhas sem ferrão triplicou nas duas espécies e o ganho de pólen aumentou 3,1 vezes em *Blepharocalyx salicifolius* e 6,2 vezes em *Myrcia rufipes*. Ambos os grupos de abelhas foram polinizadoras efetivos.

CONCLUSÕES: O estudo revela *Apis mellifera* como espécie altamente competitiva que diminui drasticamente o ganho de pólen de abelhas nativas, especialmente das abelhas sem ferrão. A presença das abelhas do mel introduzidas, desta maneira, tem como consequência que as abelhas nativas coletam menos pólen ou são deslocadas para outras espécies de plantas que fornecem menos pólen.

PALAVRAS-CHAVE: Competição, esgotamento de pólen, polinização, *Blepharocalyx*, *Myrcia*

INTRODUÇÃO

Apis mellifera Linnaeus, 1758, originária da África, Ásia Ocidental e Europa (Michener, 1973; De Jong, 1996; Moritz et al., 2005), é uma das espécies mais amplamente distribuídas no mundo (Goulson, 2003). Nos Neotópicos a abelha africanizada *A. mellifera* mostrou surpreendente capacidade de adaptação à diferentes ambientes e às condições tropicais, o que lhe permitiu uma rápida expansão (Kerr, 1967, Moritz et al., 2005). A espécie possui enorme capacidade em coletar recursos florais em flores com diversas morfologias e explora pólen de numerosas famílias sendo, portanto, considerada amplamente polilética (Roubik, 1978; Huryn 1997; Cane e Sipes, 2006; Köppler et al., 2007; Kleinert e Giannini, 2012). Além disso, essa abelha foi identificada como o principal visitante floral em estudos de comunidade de abelhas e plantas no Brasil (Viana et al., 1997; Schlindwein, 1998; Aguiar, 2003; Milet-Pinheiro e Schlindwein, 2008; de Mendonça Santos et al., 2010; Carneiro e Martins, 2012; Cordeiro et al., 2017).

A alta abundância de *A. mellifera* pode impactar negativamente as interações planta-polinizador nativos, e vários estudos demonstram diferentes efeitos da introdução dessa abelha nas últimas décadas em ecossistemas neotropicais. A maioria destes estudos foca na medição indireta dos impactos, abordando a sobreposição de nicho (Pedro & Camargo 1991; Aizen & Feinsinger 1994; Wilms et al. 1996; Wilms & Wiechers, 1997; Ramalho et al. 2007), mudanças no forrageamento e na busca por fontes de recursos florais (Roubik, 1980, 2009), além de redução nas taxas de visitação e depleção de recursos via competição por exploração (Roubik, 1978, 1996; Roubik et al., 1986; Roubik & Villanueva & Gutiérrez, 2009; Carneiro & Martins 2012). No entanto, impactos diretos que as abelhas do mel exercem às abelhas nativas em forma de ganho de recursos de plantas hospedeiras, raramente foram mensurados e quantificados.

A disponibilidade de pólen influencia fortemente a dinâmica de forrageamento das abelhas, e como pólen é um recurso limitado, o aumento de coleta por uma espécie frequentemente leva à redução para outra (Roubik, 1978; Cane e Tepedino, 2017). Há evidências que competição pelo pólen é comum entre *Apis mellifera* e abelhas sem ferrão (Roubik 1989; Pedro e Camargo, 1991; Aizen e Feinsinger, 1994; Wilms et al., 1996; Biesmeijer e Slaa 2006; Ramalho et al., 2007; Cane e Tepedino, 2017).

Dentre as fontes de pólen intensamente exploradas por estes dois grupos de abelhas sociais são espécies de Myrtaceae com floração maciça (Pedro e Camargo 1991; Wilms et al. 1996; Ramalho et al. 2007). No Brasil, muitas Myrtaceae melitófilas com floração maciça

possuem típicas flores de pólen (*sensu* Vogel, 1978, flores com pólen como único recurso), incluindo, por exemplo, espécies de *Blepharocalyx*, *Campomanesia*, *Eugenia* e *Myrcia* (Proença e Gibbs, 1994; Torezan-Sllingardi e Del-Claro, 2004; Silva e Pinheiro, 2007; Diniz e Buschini, 2016; Rodrigues et al., 2017). Isto vale também para *Blepharocalyx salicifolius* (Kunth) O.Berg e *Myrcia rufipes* DC, espécies que florescem no mesmo período e ocorrem no mesmo local em uma área de Cerrado em Minas Gerais.

Estudamos a polinização de *B. salicifolius* e *M. rufipes* e analisamos a competição por pólen entre *Apis mellifera* e abelhas nativas sem ferrão. O objetivo desse estudo foi quantificar o impacto da presença de *A. mellifera* na coleta de pólen por abelhas nativas em espécies nativas de Myrtaceae com "flores de pólen" e floração maciça. Para responder esta questão inicialmente determinamos quais espécies visitam e polinizam as flores dessas espécies, qual a frequência dos grupos de abelhas nas flores e qual a quantidade de pólen coletado por elas. Para saber se a frequência de visitas de abelhas nativas nas flores das duas espécies de Myrtaceae e se a quantidade de pólen removidos por elas aumenta na ausência de *A. mellifera* conduzimos um experimento cuja flores foram cobertas por rede para impedir visitas da abelha introduzida.

MATERIAIS E MÉTODOS

Local de estudo

O trabalho de campo foi realizado no Parque Estadual do Rio Preto, localizado no município de São Gonçalo do Rio Preto, Minas Gerais, sudeste do Brasil (18 ° 07'04 "S; 43 ° 20'42" O), durante os períodos de floração em 2019 e 2020, de agosto a outubro. O local de estudo está localizado no setor meridional do Complexo da Cadeia do Espinhaço e a vegetação é composta, principalmente, por fitofisionomias do Cerrado, Campos rupestres, capões de matas e matas de galeria. O clima da região é sazonal, caracterizado por um período quente e chuvoso (outubro a março) e uma estação seca bem definida (abril a setembro). A temperatura média anual é de 19,9ºC e a precipitação média anual de 1.550 mm (IEF 2004; Neves et al., 2005).

Espécies estudadas

Blepharocalyx salicifolius ocorre na Argentina, Bolívia, Brasil, Equador, Paraguai e Uruguai (Landrum ,1986). No Brasil, a espécie ocorre no cerrado, em floresta estacional decidual e semidecidual, restinga, floresta ombrófila e em campos (Carvalho, 2013; Vasconcelos, 2020). A espécie mostra grande variedade de hábito e pode ser encontrada na forma de

arbusto até árvores de até 30m de altura (Vasconcelos, 2020).

Myrcia rufipes é uma espécie arbustiva de até 2 m de altura, endêmica do Brasil e frequentemente encontrada no cerrado, campo rupestre e na mata Atlântica (Morais e Lombardi, 2006; Rosa e Romero, 2012; Santos et al., 2020).

Biologia floral

Monitoramos 20 flores de cada espécie, desde a sua abertura até a senescência. Testamos a receptividade do estigma com uma solução de 10% de peróxido de hidrogênio (Dafni et al., 2005) em diferentes momentos da antese. Para determinar o número de grãos de pólen por flor, maceramos as anteras indeiscentes de botões florais em pré-antese em tubos contendo 0,5 mL de ácido lático glicerinado 3:1 (Lloyd, 1972). Em seguida a solução com as anteras foi homogeneizada durante 2 min em agitador vórtex. Extraímos uma alíquota dessa solução para contar os grãos de pólen em câmara Neubauer (Maêda, 1985). O número médio de grãos de pólen por flor foi calculado a partir de 10 botões florais coletados em indivíduos diferentes de cada espécie.

Sistema reprodutivo

Determinamos o sistema reprodutivo de *B. salicifolius* e *M. rufipes* usando os seguintes tratamentos de polinização: autopolinização manual, autopolinização espontânea, polinização cruzada manual e polinização em condições naturais (polinização aberta) (Sage et al., 2015). Em 10 indivíduos marcados por espécie utilizamos inflorescências inteiras por tratamento (veja o tamanho amostral na Tabela 1), uma vez que não foi possível ensacar as flores individualmente por causa do pequeno tamanho e para evitar o aborto de flores devido à manipulação. Dessa forma, fizemos os testes à medida que as flores abriam. As inflorescências permaneceram ensacadas até a abertura da última flor, exceto no tratamento de polinização aberta.

Visitantes florais

Para cada espécie, determinamos o espectro dos visitantes florais em seis dias não consecutivos na estação de floração de 2019. Coletamos as abelhas com redes entomológicas desde a abertura das flores (05:00 h) até 12:00 h do primeiro dia da antese. Após esse horário não houve mais visitas florais. Os espécimes coletados foram montados, identificados e tombados na coleção do Centro de Coleções Taxonômicas, Universidade Federal de Minas Gerais (CCT-UFMG).

Determinamos a frequência das visitas florais, contando todas os visitantes que ocorreram em um grupo de ~100 flores/indivíduo (N = 12 plantas) por 20 segundos a cada 5 minutos, das 05:00 às 12:00 h. Optamos por esta contagem em varreduras curtas, para evitar contagem múltipla dos mesmos indivíduos e por causa do alto número de visitas de abelhas em determinados horários. Seis contagens, correspondendo um intervalo de 30 minutos foram agrupados. Os visitantes florais foram agrupados em (i) *Apis mellifera* e (ii) abelhas nativas (todas as outras espécies de abelhas).

Eficiência de polinização das abelhas

Na estação de floração de 2020, medimos a contribuição de cada grupo de abelhas para a frutificação de *B. salicifolius* e *M. rufipes*. Para isso, ensacamos 11 inflorescências com botões florais em pré-antese para cada tratamento, em três indivíduos por espécie e estabelecemos dois tratamentos nos quais as flores estavam exclusivamente disponíveis aos visitantes florais durante o intervalo correspondente ao período de visitação às flores e para evitar a disponibilização das mesmas entre horários de sobreposição de visitas dos grupos de abelhas: (1) Os sacos foram removidos desde a abertura das flores até 07:00 h; nesse período as flores foram visitadas exclusivamente por *A. mellifera* (N = 142 flores em *B. salicifolius* e 127 em *M. rufipes*). (2) Os sacos foram removidos entre 09:00 e 12:00 h; nesse período, as flores das duas espécies foram visitadas exclusivamente por abelhas sem ferrão (N = 140 flores em *B. salicifolius* e 135 em *M. rufipes*). Após o período de exposição, as flores foram novamente ensacadas. A taxa de frutificação foi determinada para cada tratamento.

Remoção de pólen das flores

Para conhecer a dinâmica de remoção de pólen nas flores de *B. salicifolius* e *M. rufipes*, contamos os grãos de pólen presentes nas flores em diferentes horários da antese: (0) pré-antese (botões florais removidos às 05:00 h); (1) 07: 00 h – flores removidas após visitas massivas de *A. mellifera*; (2) 09: 00 h – flores visitadas exclusivamente por *A. mellifera*, removidas antes das visitas das abelhas sem ferrão; (3) 12:00 h – flores removidas após o término geral das visitas das abelhas. Em cada intervalo, armazenamos 10 flores por espécie individualmente em tubos contendo etanol 70%. Para o número de pólen por flor, utilizamos as contagens no item “Biologia floral”. Para os demais intervalos, maceramos as anterase lavamos o pólen aderido à corola com etanol. A solução de etanol com os grãos de pólen foi centrifugada durante 5 min a 6.000 r.p.m. Em seguida, a solução suspensa foi retirada e

adicionado 0,5 mL de ácido lático glicerinado 3:1, para homogeneização em agitador vórtex durante 2 minutos (Lloyd, 1972). Extraímos uma alíquota dessa solução para contar os grãos de pólen em câmara Neubauer (Maêda, 1985). Extrapolamos o valor encontrado para a amostra inicial de cada intervalo analisado.

Para calcular a quantidade de pólen removida pelos visitantes florais ao longo da antese, subtraímos o número de grãos de pólen dos diferentes intervalos ($pólen_{T1}$ = T1 - T0, e assim sucessivamente). Posteriormente, calculamos a quantidade de grãos de pólen removida por diferentes grupos de abelhas: (1) *A. mellifera* e (2) abelhas nativas. Para isso, utilizamos a porcentagem de visitas às flores realizadas durante três intervalos (05:00 - 07:00 h, 07:00 – 09:00 h e 09:00 – 12:00 h). A partir dos valores de porcentagem estimamos a quantidade de grãos de pólen coletada por grupo de abelha nos intervalos e no geral, considerando a soma do que foi coletado por grupo em todos os intervalos.

Experimento de exclusão de *Apis mellifera*

Para avaliar a influência do forrageamento em massa de *A. mellifera* sobre a abundância e coleta de pólen de abelhas nativas, conduzimos um experimento de exclusão de abelhas melíferas em *B. salicifolius* e *M. rufipes*. Marcamos 12 indivíduos de cada espécie e selecionamos dois ramos por planta com abundantes flores novas para este experimento. Um dos ramos não foi manipulado (tratamento "flores de acesso aberto"; N = 1.515 flores em *B. salicifolius* e 1.209 em *M. rufipes*). O segundo ramo foi ensacado com tecido de náilon verde de malha de 4 mm de comprimento e 6 mm de largura (tratamento "flores cobertas por rede"; N = 1.504 flores em *B. salicifolius* e 1.198 em *M. rufipes*) que permitia a passagem de pequenas abelhas (Meliponini) e inibiu o acesso de *Apis mellifera* e abelhas maiores. Os ramos utilizados nos tratamentos foram ensacados um dia antes do experimento. Coletamos os dados em seis dias não consecutivos e determinamos a abundância e a riqueza das abelhas usando varreduras de observação (veja detalhes em "Visitantes florais").

Calculamos a quantidade de grãos de pólen coletada por abelhas nativas em "flores de acesso aberto" e "flores cobertas com rede" (veja método em "Remoção de pólen das flores"), de 10 plantas (uma flor por indivíduo e tratamento) e após o fim do período de visitação para contagem de grãos de pólen restantes.

Análise de dados

Utilizamos modelo linear generalizado misto (GLMM) com distribuição de Poisson (função de ligação logarítmica) para comparar o número de frutos produzidos em cada tratamento

do sistema reprodutivo, considerando o número de frutos como variável resposta, os tratamentos de polinização como efeito fixo e o número de flores como efeito aleatório. Para comparar a contribuição de cada grupo de abelhas na frutificação de *B. salicifolius* e *M. rufipes*, foi utilizado modelo linear generalizado (GLM) com distribuição Gaussiana. Para comparar a frequência de visitas de abelhas nativas na presença e na ausência de *Apis mellifera*, utilizamos GLMM com distribuição de Poisson, considerando o número de visitas como variável resposta, os tratamentos ("flores de acesso aberto" ou "flores cobertas por rede") e intervalos de tempo como efeitos fixos e o número de flores como efeito aleatório. Para comparar o número de grãos de pólen remanescentes nas flores em diferentes intervalos da antese e para comparar o número de grãos de pólen coletados por cada grupo de abelhas, foi utilizado GLM com distribuição Gaussiana. Selecionamos o melhor modelo usando testes de razão de verossimilhança (Zuur et al., 2009) e analisamos a variável resposta em relação aos efeitos fixos por meio de testes Tukey post-hoc. Realizamos as análises dos dados no "R" usando os pacotes "lme4" (Bates et al., 2015) e "emmeans" (Lenth, 2019).

RESULTADOS

Biologia floral

As flores de *B. salicifolius* e *M. rufipes* abriram sincronizadamente às ~ 05:00 h, cerca de 15 minutos antes do nascer do sol. Primeiro, as pétalas se abriram e os estames ficaram por ~5 min ainda curvados para o centro da flor (Fig. 1A). Quando os estames se estendiam as anteras deisceram. Os estigmas já estavam receptivos no início da antese tanto em *B. salicifolius* (Fig. 1B) quanto em *M. rufipes* (Fig. 1C). As flores de *B. salicifolius* produziram em média 96.200 ± 21.064 e as de *M. rufipes*, 99.800 ± 25.955 grãos de pólen por flor. No final do primeiro dia de antese os estames enrolam-se no centro das flores em ambas as espécies (Fig. 1D). Os estigmas permaneceram receptivos até a senescência floral em ambas as espécies, que ocorreu aproximadamente três dias após a abertura, quando a corola e os estames se destacaram e caíram.

Figura 1. Estágios da antese floral de *Blepharocalyx salicifolius* e *Myrcia rufipes*. **A**, Flores de *B. salicifolius* no imediatamente após a abertura, com as pétalas totalmente estendidas e os estames curvados para o centro da flor; **B**, Flores de *B. salicifolius* com inúmeros estames estendidos; **C**, Flores de *M. rufipes* com os estames estendidos; **D**, Flor de *M. rufipes* no final do primeiro dia de antese.

Sistema reprodutivo

Blepharocalyx salicifolius é auto-incompatível, uma vez que não houve formação de frutos em ambos os tratamentos de autopolinização (Tabela 1). Observamos diferenças não-significativa de aumento da frutificação após polinização cruzada manual em relação à polinização aberta ($p = 0,93$; Tabelas 1, S1).

O sistema reprodutivo de *M. rufipes* também é predominantemente xenogâmico. A frutificação após polinização cruzada manual foi maior do que nos tratamentos de autopolinização ($p < 0,05$; Tabelas 1, S1) e não diferiu da polinização aberta ($p = 0,83$; Tabelas 1, S1).

Tabela 1. Resultados dos testes de polinização realizados em *Blepharocalyx salicifolius* e *Myrcia rufipes* no Parque Estadual do Rio Preto, Minas Gerais, Brasil.

Tratamento	***Blepharocalyx salicifolius***		***Myrcia rufipes***	
	Número de inflorescências (flores)	Frutos desenvolvidos [N (%)]	Número de inflorescências (flores)	Frutos desenvolvidos [N (%)]
Autopolinização espontânea	45 (328)	0 (0%) a	19 (217)	12 (7%) a
Autopolinização manual	40 (284)	0 (0%) a	13 (143)	23 (16%) a
Polinização cruzada manual	42 (296)	101 (34%) b	13 (152)	55 (36%) b
Polinização aberta	52 (364)	106 (29%) b	20 (324)	63 (28%) b

Valores seguidos pela mesma letra na coluna "Frutos desenvolvidos" não diferiram estatisticamente pelo teste de Tukey a 5% de probabilidade

Visitantes florais

As flores de *B. salicifolius* foram visitadas por abelhas de 15 espécies, das quais 11 eram abelhas sem ferrão, três espécies de Halictidae, além de *Apis mellifera* (Fig. 2A). As flores de *M. rufipes* foram visitadas por abelhas de 17 espécies, sendo 12 espécies de abelhas sem ferrão (Fig. 2B), quatro espécies de Halictidae, além de *A. mellifera* (Tabela 2).

Tabela 2. Visitantes florais de *Blepharocalyx salicifolius* e *Myrcia rufipes* no Parque Estadual do Rio Preto, Minas Gerais, Brazil.

Espécies		***Blepharocalyx salicifolius***	***Myrcia rufipes***
Apidae			
Apini	*Apis mellifera* Linnaeus, 1758	x	x
Meliponini	*Frieseomelitta* sp.1	x	x
	Frieseomelitta sp.2	x	x
	Melipona sp.		x
	Paratrigona sp.	x	x
	Plebeia sp.	x	x
	Scaptotrigona postica (Latreille, 1807)	x	x
	Tetragonisca angustula (Latreille, 1811)	x	x
	Trigona fulviventris Guérin, 1844	x	x
	Trigona hyalinata (Lepeletier, 1836)	x	x
	Trigona spinipes (Fabricius, 1793)	x	x
	Trigona sp. 2	x	x
	Trigona sp. 2	x	x
Halictidae			
Augochlorini	*Augochloropsis* sp. 1	x	x
	Augochloropsis sp. 2		x
	Pseudaugochlora pandora (Smith, 1853)	x	x
Halictini	*Dialictus* sp.	x	x

Em ambas as espécies de planta, operárias de *A. mellifera* foram os visitantes florais os mais frequentes, seguidas por espécies de abelhas nativas sem ferrão e espécies de

Halictidae, que foram visitantes raros. Em *B. salicifolius* operárias de *A. mellifera* somaram 81,3% (1.445 visitas) e as abelhas sem ferrão 18,1% (322) das visitas florais. Em *M. rufipes*, *A. mellifera* somou 83,8% (2056) das visitas, as abelhas sem ferrão 15,8% (387). Nas duas espécies de plantas, as três espécies de Halictidae registradas nas flores somente visitaram as flores raramente, somando apenas 0,6 % (11) das visitas em *B. salicifolius* e 0,4% (10) em *M. rufipes*. Portanto, daqui em diante nos referiremos às abelhas nativas como abelhas sem ferrão.

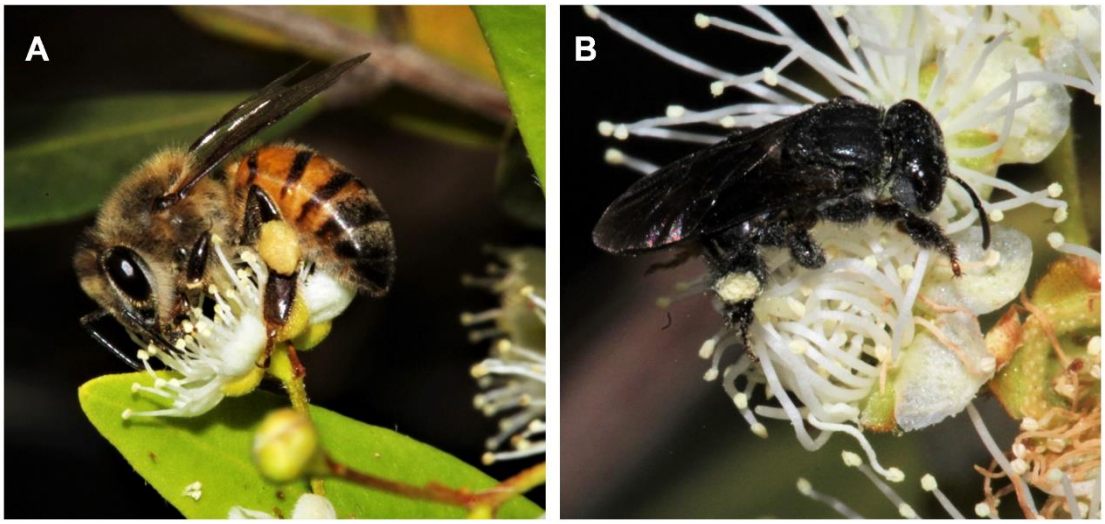

Figura 2. Operárias de abelhas sociais nas flores de duas espécies de Myrtaceae, no Parque Estadual do Rio Preto, Minas Gerais, Brasil. **A**, *Apis mellifera* durante visita a flor de *Myrcia rufipes*; **B**, Meliponini durante visita a flor de *Blepharocalyx salicifolius*.

Registramos o mesmo padrão de visitação diária em ambas as espécies. Os primeiros visitantes florais foram operárias de *A. mellifera*, que começaram a forragear nas flores pouco antes do nascer do sol (~ 05: 00h) e a partir das ~08:00 h sua abundância diminuiu e cessou às ~11:00 h. As abelhas sem ferrão e abelhas de Halictidae visitaram as flores a partir de ~06: 30h até -as 12:00 h (Fig. 3A, B). Em ambas as espécies, o período de forrageio das abelhas nativas se sobrepôs parcialmente ao das abelhas melíferas (Fig. 3).

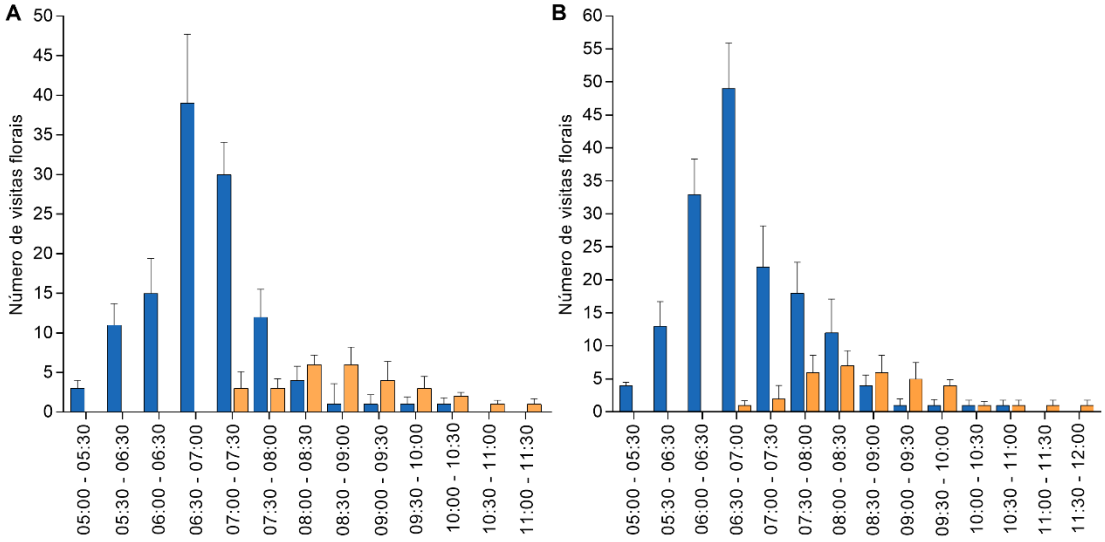

Figura 3. Número de visitas às flores de duas espécies de Myrtaceae no Parque Estadual do Rio Preto, Minas Gerais, Brasil: **A**, *Blepharocalyx salicifolius*; **B**, *Myrcia rufipes*. Em ambas as espécies, as observações foram feitas em seis dias não consecutivos e os dados foram agrupados em intervalos de 30 minutos. Barras azuis = *Apis mellifera*; Barras laranjadas = abelhas nativas.

Eficiência dos polinizadores

Em ambas as espécies, não houve diferença significativa na taxa de frutificação entre flores visitadas apenas por operárias de *A. mellifera* e flores acessíveis apenas para abelhas sem ferrão. No entanto, houve diferenças significativas entre os dois tratamentos para flores acessíveis à todas as espécies de abelhas (Tabela S2).

Remoção de pólen das flores

De 96.200 ± (21.064) grãos de pólen de *B. salicifolius*, em média 61% sobrou às 07:00 h, 23,1% às 09:00 h, e 6,5% no final da atividade das abelhas (12:00 h) (Fig. 4A; Tabela S3). De 99.800 (± 25.955) grãos de pólen de *M. rufipes*, 42,5% dos grãos de pólen restavam nas flores às 07:00 h, 20%, às 09:00 h e (20.100 ± 3.992) apenas 4,8% às 12h00, (Fig. 4B). Não houve diferença significativa entre os intervalos 09:00 e 12:00 h (P> 0,05) (Fig. 4B; Tabela S3).

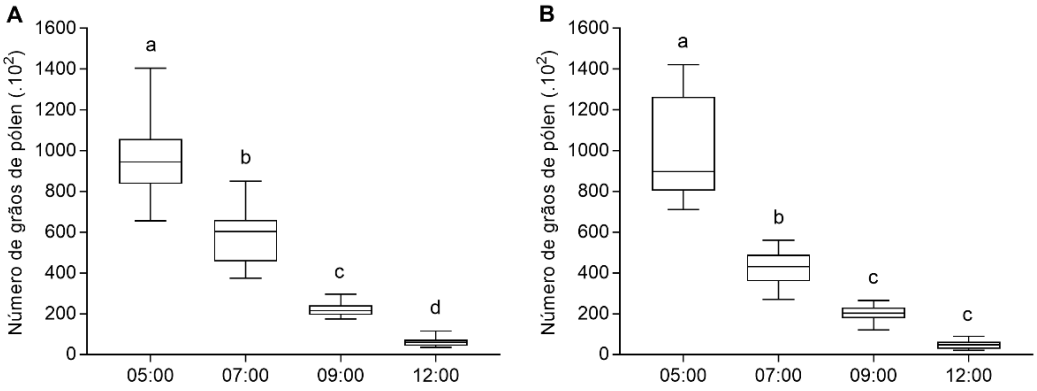

Figura 4. Quantidade de pólen em flores de duas espécies de Myrtaceae ao longo da antese. **A**, *Blepharocalyx salicifolius*; **B**, *Myrcia rufipes*. 05:00 h representa o número de grãos de pólen antes das visitas (botões florais, N = 10) e os demais intervalos, o período de atividade das abelhas (N = 10 flores para cada intervalo). Médias seguidas pela mesma letra não diferiram estatisticamente pelo teste Tukey a 5% de probabilidade.

Do total de grãos de pólen de *B. salicifolius*, 39% foi coletado até às 07:00 h, somente por abelhas do mel. Entre 07:00 e 09:00 h, abelhas do mel e abelhas sem ferrão removeram 38%, e entre 09:00 e 12:00 h 17% dos grãos de pólen foram removidos pelos dois grupos de abelhas (Fig. 5A). Considerando a soma dos grãos de pólen de todos os intervalos e a frequência das abelhas, as abelhas do mel coletaram em média 71,6%, enquanto que as abelhas sem ferrão coletaram 21,9% (Fig. 5B).

Em *M. rufipes*, em todos os intervalos o pólen foi coletado por abelhas do mel e abelhas nativas. Do total de grãos de pólen, essas abelhas removeram 58% até às 07:00 h, 22% entre 07:00 e 09:00 h e 15% entre 09:00 e 12:00 h (Fig. 5C). No total, considerando a soma dos grãos de pólen de todos os intervalos e a frequência das abelhas, *A. mellifera* coletou 81,9% do total de grãos pólen de flores de *M. rufipes*, enquanto as abelhas nativas coletaram 13,3% (Fig. 5D).

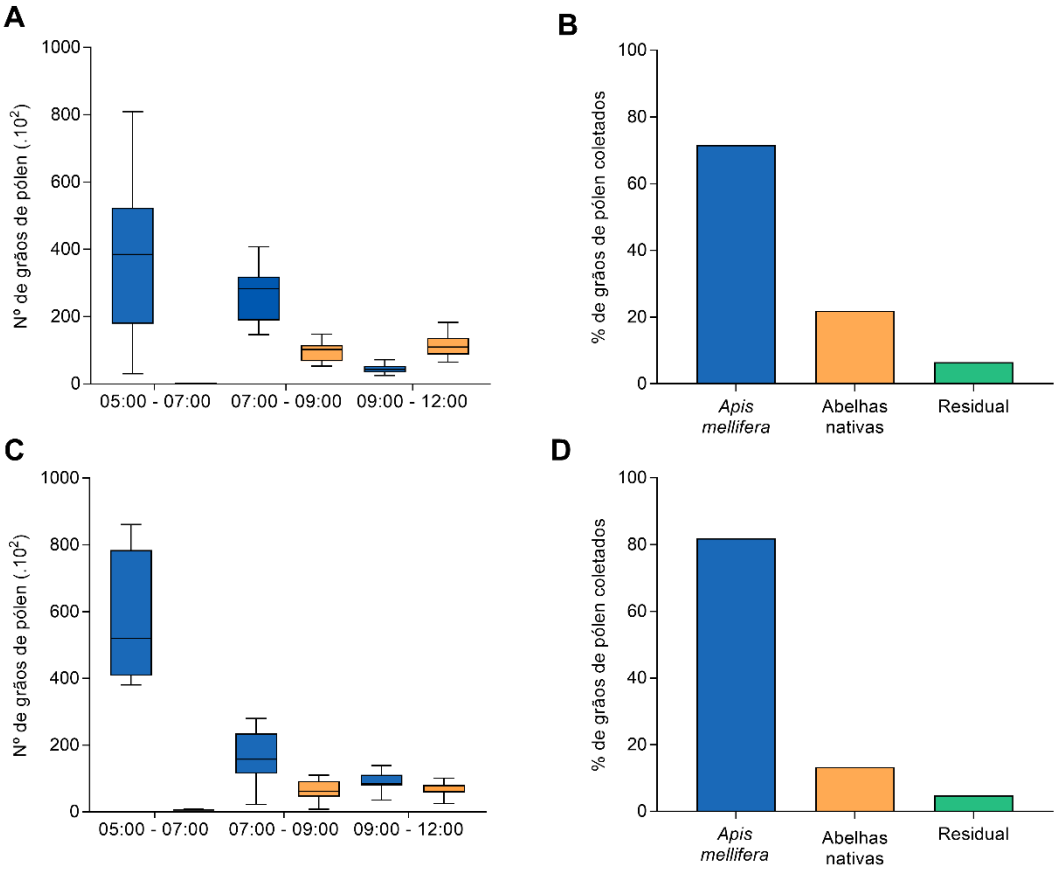

Figura 5. Remoção de pólen por abelhas em flores de duas espécies de Myrtaceae ao longo das primeiras sete horas de antese. **A**, Número de grãos de pólen removidos por grupo de abelhas em flores de *Blepharocalyx salicifolius*; **B**, Proporção de grãos de pólen coletados por grupo de abelhas em flores de *B. salicifolius;* **C**, Número de grãos de pólen removidos por grupo de abelhas em flores de *Myrcia rufipes*; **D**, Proporção de grãos de pólen coletados por grupo de abelhas em flores de *M. rufipes.* Barras azuis = *Apis mellifera*; Barras laranjadas = abelhas nativas; Barras verdes = pólen residual, ou seja, grãos de pólen não coletados que permanecem nas flores após o término das visitas das abelhas.

Experimento de exclusão de *Apis mellifera*

Registramos 982 visitas de abelhas sem ferrão nas flores de *B. salicifolius* cobertas por rede e 334 nas flores de acesso aberto. Picos significativos de diferenças no número de visitas de abelhas nativas ocorreu de 08:30 às 10:00 h na ausência de *A. mellifera* (ver "Efeito do intervalo" na Tabelas S4, S5). As abelhas sem ferrão foram significativamente mais frequentes na ausência de *A. mellifera* de 07:00 às 11:00 h (ver "Efeito do tratamento" na Tabela S5) (P> 0,05; Fig. 6A; Tabela S5).

Em flores de *M. rufipes*, registramos 1228 visitas de abelhas sem ferrão em flores cobertas por rede e 399 em flores de acesso aberto. Picos significativos diferenças na visitação de abelhas nativas ocorreu de 08:30 às 10:00 h na ausência de *A. mellifera* (ver

"Efeito do intervalo" na Tabelas S4, S6). As abelhas sem ferrão foram significativamente mais frequentes na ausência de *A. mellifera* de 06:00 até 11: 00h (P <0,05; Fig. 6B; "Efeito do tratamento" na Tabela S6).

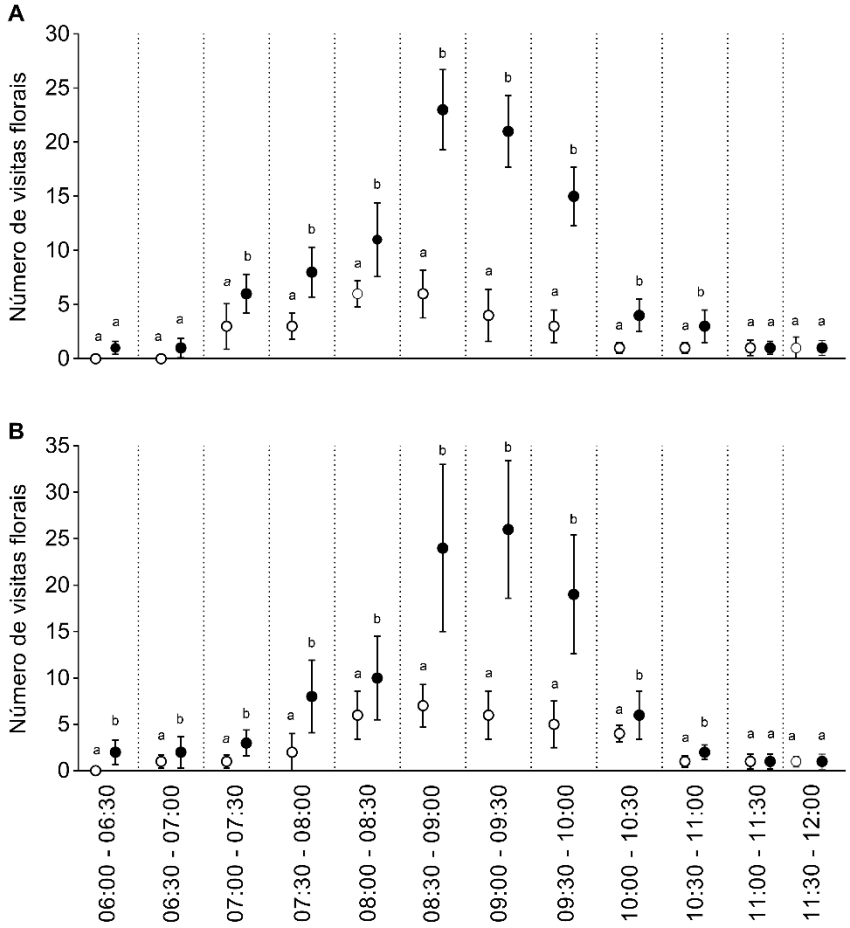

Figura 6. Número de visitas (média ± dp) de abelhas sem ferrão nas flores de duas espécies de Myrtaceae. **A**, *Blepharocalyx salicifolius;* **B**, *Myrcia rufipes*. Abundância de abelhas nativas nas flores quando *Apis mellifera* estava presente (flores de acesso aberto) e em flores ensacadas que impediam o acesso de *A. mellifera*, mas não das abelhas nativas (flores cobertas por rede). Círculos claros = flores de acesso aberto; Círculos preenchidos = flores cobertas por rede. Letras diferentes significam diferenças significativas entre os tratamentos (P <0,05).

Em *B. salicifolius*, as abelhas sem ferrão coletaram em média 22% dos grãos de pólen nas flores de acesso aberto, enquanto que em flores cobertas por rede, essas abelhas coletaram em média 72% (69.250 ± 23.574) dos grãos de pólen (Fig. 7A). Em *M. rufipes*, em flores de acesso aberto, as abelhas sem ferrão coletaram apenas 13% do total de grãos de pólen das flores, enquanto que em flores cobertas por rede, essas abelhas coletam 81% (80.700 ± 24.886) dos grãos de pólen de uma flor de *M. rufipes* (Fig. 7B).

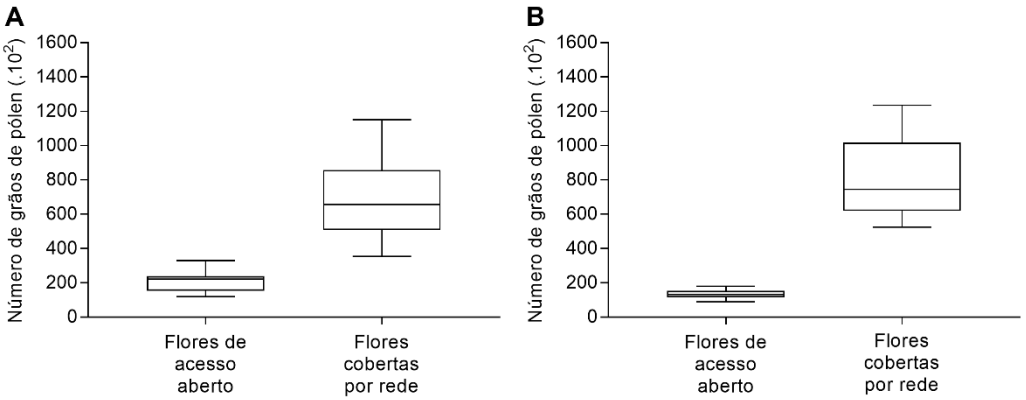

Figura 7. Remoção de pólen por abelhas sem ferrão em flores de acesso aberto e em flores cobertas por rede. **A**, *Blepharocalyx salicifolius*; **B**, *Myrcia rufipes.*

DISCUSSÃO

Nosso estudo demostrou que operárias da abelha introduzida *A. mellifera* coletaram a maior porção do pólen das flores de *B. salicifolius* e *M. rufipes*, portanto foram os competidores mais eficientes. A alta frequência de *A. mellifera* nas flores das duas espécies de Myrtaceae diminuiu a disponibilidade de pólen para as abelhas sem ferrão, resultando em uma baixa frequência destas abelhas nas flores. No entanto, quando *A. mellifera* foi excluída de flores de *B. salicifolius* e *M. rufipes*, a frequência de visitas e a remoção de pólen por abelhas sem ferrão aumentou de forma consistente. Isto demonstra o impacto da competição por exploração de *A. mellifera* ao forrageamento de abelhas sem ferrão, que foram deslocadas das flores ricas em pólen. Apesar da competição entre elas, *A. mellifera* e abelhas sem ferrão foram polinizadoras efetivos de ambas as espécies de Myrtaceae.

O domínio de *Apis mellifera* nas flores de *Blepharocalyx salicifolius* e *Myrcia rufipes*

Apis mellifera foi a melhor competidora por pólen das flores de *B. salicifolius* e *M. rufipes.* As operárias chegaram nas flores de ambas as espécies logo após a abertura das flores e em 30 minutos eram extraordinariamente abundantes. Elas dominaram as flores durante as três primeiras horas de antese, quando a quantidade de pólen caiu drasticamente em ~80%, e, então, abandonaram as flores massivamente. A alta eficiência de coleta de pólen de *A. mellifera* não é apenas uma consequência de sua capacidade de voar mais cedo que abelhas sem ferrão, mas principalmente devido ao seu refinado sistema de comunicação e recrutamento (von Frisch, 1967; Schaffer et al., 1979, Dyer, 2002; Seeley, 2012). Elas recrutam um grande número de operárias das colônias e utilizam a excelente memória espacial e olfativa para a localização de flores ricas em recursos, tornando-as forrageadoras

altamente eficientes e assim, fortes competidoras (von Frisch, 1967; Roubik, 1980; Reinhard et al., 2004; Beekman, 2005; Menzel et al., 2006). As visitas massivas de *A. mellifera* causou redução drástica do pólen disponível para abelhas sem ferrão. Casos semelhantes foram relatados para outras espécies de Myrtaceae (Silva e Pinheiro, 2007; Diniz e Buschini, 2016; Capítulos 1 e 2) e Anacardiaceae (Carneiro e Martins, 2012; Calaça et al., no prelo).

Apis mellifera desloca abelhas sem ferrão de recursos florais rentáveis

Devido ao forragemanto massivo e a alta eficiência de forrageio de *A. mellifera* nas flores de *B. salicifolius* e *M. rufipes.* menos de 1/4 do pólen foi de fato removido por abelhas sem ferrão. Em flores de *Eucalyptus costata* F. Muell, uma espécie de Myrtaceae que ocorre na Austrália onde *A. mellifera* também é introduzida, a disponibilidade dos recursos florais para a espécies de abelhas nativas foi significativamente reduzido após visitas matinais massivas da abelha do mel (Horskins e Turner 1999).

A baixa frequência de visitação quando *A. mellifera* estavam presentes nas flores, demostra que abelhas sem ferrão foram capazes de diferenciar rapidamente flores vazias de flores lucrativas, por meio da visualização do pólen ou pelo odor emitido por esse recurso. Em flores de *B. salicifolius* e *M. rufipes,* o odor adocicado característico das flores era perceptível somente após a deiscência das anteras e até quando havia pólen disponível (obs. pes.). Flores de *B. salicifolius* e *M. rufipes* cujo o conteúdo polínico já haviam sido esvaziados principalmente por *A. mellifera* e flores a partir do segundo de dia de antese não foram visitadas por abelhas sem ferrão. Isso ocorre também pois, tanto a alocação de forrageadoras para a coleta de pólen quanto o comportamento das forrageadoras de pólen são ajustados para a escolha de fontes ricas de recursos (Biesmeijer et al., 1999; Biesmeijer e Slaa 2004).

Quando *A. mellifera* foi excluída das flores de *B. salicifolius* e *M. rufipes*, houve o aumento consistente de visitas de abelhas sem ferrão nas flores, além de aumentar o ganho de pólen dessas abelhas em 3,1 e 6,2 vezes em *B. salicifolius* e *M. rufipes*, respectivamente. É interessante que isso ocorreu mesmo em flores cobertas por rede, que podem ter traços florais visuais fortemente alterados e com intenso forrageio de *A. mellifera* nas flores acessíveis circundantes. Portanto, nossos resultados mostram que a disponibilidade de pólen nas flores foi o fator regulador da visitação de abelhas sem ferrão nas flores de *B. salicifolius* e *M. rufipes.* Além do aumento na visitação, operárias de abelhas sem ferrão iniciaram visitas nas flores cobertas por rede ~06:00 h, cerca de uma hora antes comparado quando operárias de *A. mellifera* estão presente nas flores, demonstrando que as abelhas sem ferrão sofrem

deslocamento competitivo pelas abelhas introduzidas. Por serem forrageadoras extraordinariamente abundantes, *A. mellifera* frequentemente deslocam as espécies de abelhas nativas de seus hospedeiros de pólen preferidos ou reduzem suas visitas às flores dessas plantas (Roubik et al., 1986; Roubik & Villanueva-Gutierrez 2009; Cane & Tepedino, 2017; Carneiro & Martins, 2012).

Abelhas eussociais, como abelhas sem ferrão, necessitam fortemente de amplas quantidades de recursos florais para manutenção do grande número de indivíduos e de suas colônias perenes (Eltz et al., 2002; Biesmeijer e Slaa 2004; Slaa et al., 2006). Espécies de floração maciça, que produzam numerosas flores que se abrem a cada dia fornecendo abundantes quantidades de pólen, são intensamente exploradas por abelhas sem ferrão e também por *A. mellifera* (Ramalho, 2004; Almeida et al., 2011; Carneiro e Martins, 2012; Calaça et al., 2018; Siqueira et al., 2018), incluindo diversas espécies de Myrtaceae (Wilms et al., 1996; Ramalho, 2004; Torezan-Silingardi e Oliveira, 2004; Fidalgo e Kleinert, 2009).

Polinização de *Blepharocalyx salicifolius* e *Myrcia rufipes*

Apis mellifera e abelhas sem ferrão foram polinizadores eficientes de *B.salicifolius* e *M. rufipes*. Isso ocorreu devido ao ajuste entre o tamanho das flores das duas espécies e o tamanho corporal dessas abelhas, o que faz com que o pólen seja facilmente depositado nos estigmas. Além disso, devido à floração maciça e sincronizada entre os indivíduos, é provável que essas abelhas eussociais fazem voos abundantes entre diferentes indivíduos da mesma espécie de planta para suprir suas altas demandas por pólen. Como em *B.salicifolius* e *M. rufipes,* várias espécies de Myrtaceae do Brasil são polinizadas por abelhas sem ferrão e nessas espécies, *A. mellifera* também exerce um importante papel na polinização (Proença e Gibbs, 1994; Oliveira e Gibbs, 2000; Torezan-Silingardi e Oliveira, 2004).

Ausência de abelhas crepusculares em flores de *B. salicifolius* e *M. rufipes*

É curioso que não foram registradas espécies de abelhas noturnas nas flores das duas espécies estudadas. Abelhas noturnas são polinizadores efetivos de várias espécies de Myrtaceae (Cordeiro et al., 2017; Cordeiro et al., 2021; Capítulo 1). Essas abelhas são atraídas pelo forte perfume emitido por flores desta família (Cordeiro et al., 2017; Cordeiro et al., 2019). Um forte odor adocicado era perceptível logo após a aberturas das flores de *B. salicifolius* e *M. rufipes* (obs. pes.). No local de estudos, abelhas noturnas, principalmente espécies de *Ptiloglossa* e *Megalopta*, são comuns e visitam flores de diferentes espécies de plantas com recursos disponíveis no crepúsculo (Siqueira et al., 2018; Araujo et al., 2020; Capítulo 1).

Uma possível explicação para ausência de visitas de abelhas noturnas nas flores de *B. salicifolius* e *M. rufipes*, é a abertura das flores que ocorre apenas poucos minutos antes do amanhecer, sendo relativamente tarde para as abelhas noturnas, que apresentam curto período de forrageamento no crepúsculo. Flores melitófilas visitadas por abelhas noturnas se abrem ainda durante a noite ou durante o crepúsculo matutino (Martins e Carneiro, 2012; Krug et al., 2015; Cordeiro et al., 2017; Siqueira et al., 2018).

CONCLUSÕES

Atualmente, *Apis mellifera* já se tornou parte da apifauna dos Neotrópicos devido sua excelente adaptação às condições ambientais. Assim, é difícil de imaginar como seriam caracterizadas, nos diferentes ambientes e comunidades, as interações abelhas-plantas na ausência desta espécie. Nossos resultados mostraram que, apesar de serem polinizadoras eficientes de *B. salicifolius* e *M. rufipes*, o impacto da espécie introduzida *A. mellifera* para abelhas nativas sem ferrão em relação à remoção de pólen é de fato fortemente negativo. E, especialmente em unidades de conservação, como o local desse estudo, seria desejável o manejo desta espécie introduzida para conservação de espécies de abelhas nativas e consequentemente, a manutenção das interações planta-polinizador.

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MATERIAL SUPLEMENTAR

Tabelas

Tabela S1. Sistema reprodutivo de *Blepharocalyx salicifolius* e *Myrcia rufipes*: frutos formados após autopolinização espontânea (AE), autopolinização manual (AM), polinização cruzada manual (PC) e polinização aberta - controle (PA). Testamos se houve diferença no número de frutos formados entre os tratamentos usando modelos lineares generalizados mistos com distribuição de Poisson. Diferenças não significativas estão destacadas em negrito (Valor *p*). N flores = número de flores; N frutos = número de frutos; EP = erro padrão.

Tratamento	N flores	N frutos	Comparação	Estimativa*	EP	Valor *z*	Valor *p*
Blepharocalyx salicifolius							
AE	328	0	AE - AM	8,51	0,69	1,2	**1,0**
AM	284	0	AE - PA	-27,67	0,98	-2,80	< 0,05
PC	296	101	AE - PC	-27,62	0,99	-2,79	< 0,05
PA	364	106	AM - PA	-36,18	0,69	-5,19	< 0,05
			AM - PC	-36,13	0,69	-5,19	< 0,05
			PA - PC	0,05	0,01	3,47	**0,98**
Myrcia rufipes							
AE	167	12	AE - AM	-0,42	0,35	-1,19	**0,64**
AM	143	23	AE - PA	-1,76	0,30	-5,80	< 0,05
PC	152	55	AE - PC	-1,26	0,32	-3,90	< 0,05
PA	224	63	AM - PA	-1,34	0,27	-4,96	< 0,05
			AM - PC	-0,84	0,26	-3,25	< 0,05
			PA - PC	0,50	0,23	2,21	**0,12**

*Os coeficientes estão apresentados na escala do modelo.

Tabela S2. Eficácia de polinizadores em flores de *Blepharocalyx salicifolius* e *Myrcia rufipes*: Frutos formados após visitas das *Apis mellifera* (AM), abelhas nativas (AN) e polinização aberta (PA). Testamos se houve diferença no número de frutos formados entre os tratamentos usando modelos lineares generalizados mistos com distribuição de Poisson. Diferenças não significativas estão destacadas em negrito (Valor *p*). N flores = número de flores; N frutos = número de frutos; EP = erro padrão.

Tratamentos	N flores	N frutos	Comparação	Estimativa*	EP	*z*-value	*P*-value
Blepharocalyx salicifolius							
AM	142	38	AM - AN	0,14	0,27	0,53	**0,85**
AN	140	32	AM-PA	-1,26	0,21	-6,10	< 0,05
PA	364	106	AN-PA	1,41	0,22	6,42	< 0,05
Myrcia rufipes							
AM	135	32	AM - AN	0,17	0,26	0,65	**0,79**
AN	127	27	AM-PA	-0,68	0,22	-3,12	< 0,05
PA	224	63	AN-PA	0,85	0,23	3,68	< 0,05

*Os coeficientes estão apresentados na escala do modelo.

Tabela S3. Número médio de grãos de pólen nas flores de *Blepharocalyx salicifolius Myrcia rufipes* antes da antese e durante o período de atividade das abelhas (N = 10 para cada intervalo). Testamos se havia diferença no número de grãos de pólen nas flores em diferentes horários da antese usando modelo linear generalizado com distribuição Gaussiana. Diferenças não significativas estão destacadas em negrito (Valor *p*). T0 = 05:00 h (botões florais); T1 = 07:00 h; T2 = 09:00 h; T3 = 12:00 h. N flores = número de flores; DP = desvio padrão; EP = erro padrão.

Tratamento	Grãos de pólen (Média ± DP)	Comparação	Estimativa*	EP	Valor *z*	Valor *p*
Blepharocalyx salicifolius						
T0	96,200 ± 21,064	T0 – T1	37,500	5,687	6,59	< 0,05
T1	58,700 ± 13,612	T0 – T2	74,150	5,687	13,04	< 0,05
T2	22,050 ± 3,531	T0 – T3	89,950	5,687	15,82	< 0,05
T3	6,250 ± 2,312	T1 – T2	36,650	5,687	6,44	< 0,05
		T1 – T3	52,450	5,687	9,22	< 0,05
		T2 – T3	15,800	5,687	2,78	< 0,05
Myrcia rufipes						
T0	99800 ± 25955	T0 – T1	57350	6263	9,16	< 0,05
T1	42450 ± 9482	T0 – T2	79700	6263	12,73	< 0,05
T2	20100 ± 3992	T0 – T3	95050	6263	15,18	< 0,05
T3	4750 ± 2214	T1 – T2	22350	6263	3,57	< 0,05
		T1 – T3	37700	6263	6,02	< 0,05
		T2 – T3	15350	6263	2,45	**0,07**

*Os coeficientes estão apresentados na escala do modelo.

Tabela S4. Resumos dos modelos contendo comparações dos modelos, critério de informação de Akaike (AIC) e testes de razão de verossimilhança sobre os efeitos marginais de fatores fixos. Modelo completo: visitas ~ tratamento + intervalo + tratamento * intervalo + (1 | Flores).

Modelos e comparações	Termos descartados	AIC	Teste de verossimilhança
Blepharocalyx salicifolius			
1	Nenhum	261,9	
2 vs. 1	tratamento*intervalo	226, 8	$X^2 = 57,1$; df = 11; $p < 0,05$
Myrcia rufipes			
1	Nenhum	371,5	
2 vs. 1	tratamento*intervalo	345,7	$X^2 = 47,8$; df = 11; $p < 0,05$

Tabela S5. A abundância de abelhas nativas nas flores de *Blepharocalyx salicifolius* em relação aos tratamentos do experimento de exclusão de visitantes (flores de acesso aberto - AA e flores cobertas por rede - CR). Testamos se havia diferença na frequência de visitas de abelhas nativas na presença e ausência de *Apis mellifera*, usamos modelo linear generalizado misto com distribuição de Poisson, considerando os tratamentos (AA ou CR) e os intervalos de tempo como efeitos fixos e número de flores como efeito aleatório. Resumos de modelos contendo comparações de modelos, critério de informação de Akaike (AIC) e teste de razão de verossimilhança nos efeitos marginais de fatores fixos. Diferenças não significativas estão destacadas em negrito (Valor *p*). Modelo completo: visitas ~ tratamento + intervalo + tratamento * intervalo + (1 | Flores). N = 1200 flores em AA; N = 1197 flores em CR. N visitas = número de visitas. DP = desvio padrão; EP = erro padrão.

			Efeito do intervalo						Efeito do tratamento				
Intervalos	N visitas Média ± DP		Comparação	Tratamento	Estimativa*	EP	Valor *z*	*Valor P*	Comparação	Estimativa*	EP	Valor *z*	*Valor P*
	AA	CR											
06:00	0	1±0,6	06:00 - 06:30	CR	-0,10	0,44	-0,22	**1,00**	AA – CR	1,28	0,23	5,53	0,91
06:30	1±0,7	1±0,9	06:00 - 07:00	CR	-1,89	0,34	-5,56	< 0,05	AA – CR	1,74	0,2	8,79	**0,93**
07:00	3±2,1	6±1,8	06:00 - 07:30	CR	-2,22	0,33	-6,66	< 0,05	AA – CR	1,49	0,17	8,92	< 0,05
07:30	3±1,2	8±2,3	06:00 - 08:00	CR	-2,53	0,33	-7,71	< 0,05	AA – CR	1,09	0,14	7,71	< 0,05
08:00	6±1,2	11±3,4	06:00 - 08:30	CR	-3,31	0,32	-10,29	< 0,05	AA – CR	0,55	0,11	4,96	< 0,05
08:30	6±2,2	23±3,7	06:00 - 09:00	CR	-3,20	0,32	-9,93	< 0,05	AA – CR	0,41	0,12	3,47	< 0,05
09:00	4±2,4	21±3,3	06:00 - 09:30	CR	-2,88	0,33	-8,88	< 0,05	AA – CR	0,36	0,14	2,57	< 0,05
09:30	3±1,4	15±2,7	06:00 - 10:00	CR	-1,46	0,35	-4,15	< 0,05	AA – CR	0,15	0,18	0,81	< 0,05
10:00	2±0,5	4±1,5	06:00 - 10:30	CR	-1,13	0,36	-3,11	**0,08**	AA – CR	-0,13	0,26	-0,52	< 0,05
10:30	1±0,5	3±1,5	06:00 - 11:00	CR	-0,41	0,41	-0,99	**1,00**	AA – CR	-1,06	0,39	-2,74	< 0,05
11:00	1±0,7	1±0,6	06:00 - 11:30	CR	-0,34	0,41	-0,81	**1,00**	AA – CR	-0,59	0,39	-1,49	**0,34**
11:30	0	1±0,7	06:30 - 07:00	CR	-1,79	0,33	-5,50	< 0,05	AA – CR	1,28	0,23	5,53	**0,94**
			06:30 - 07:30	CR	-2,12	0,32	-6,66	< 0,05					
			06:30 - 08:00	CR	-2,44	0,31	-7,75	< 0,05					
			06:30 - 08:30	CR	-3,22	0,31	-10,47	< 0,05					
			06:30 - 09:00	CR	-3,11	0,31	-10,08	< 0,05					
			06:30 - 09:30	CR	-2,79	0,31	-8,98	< 0,05					
			06:30 - 10:00	CR	-1,36	0,34	-4,03	< 0,05					
			06:30 - 10:30	CR	-1,04	0,35	-2,95	**0,12**					
			06:30 - 11:00	CR	-0,31	0,40	-0,78	**1,00**					

			Efeito do intervalo						Efeito do tratamento				
Intervalos	N visitas Média ± DP		Comparação	Tratamento	Estimativa*	EP	Valor *z*	*Valor P*	Comparação	Estimativa*	EP	Valor *z*	*Valor P*
	AA	CR											
			06:30 - 11:30	CR	-0,24	0,40	-0,60	**1,00**					
			07:00 - 07:30	CR	-0,33	0,16	-2,06	**0,65**					
			07:00 - 08:00	CR	-0,65	0,15	-4,25	< 0,05					
			07:00 - 08:30	CR	-1,43	0,14	-10,41	< 0,05					
			07:00 - 09:00	CR	-1,32	0,14	-9,49	< 0,05					
			07:00 - 09:30	CR	-1,00	0,14	-6,93	< 0,05					
			07:00 - 10:00	CR	0,43	0,20	2,19	**0,56**					
			07:00 - 10:30	CR	0,76	0,22	3,47	< 0,05					
			07:00 - 11:00	CR	1,48	0,29	5,18	< 0,05					
			07:00 - 11:30	CR	1,55	0,29	5,27	< 0,05					
			07:30 - 08:00	CR	-0,31	0,14	-2,29	**0,48**					
			07:30 - 08:30	CR	-1,09	0,12	-9,09	< 0,05					
			07:30 - 09:00	CR	-0,98	0,12	-8,05	< 0,05					
			07:30 - 09:30	CR	-0,67	0,13	-5,19	< 0,05					
			07:30 - 10:00	CR	0,76	0,18	4,12	< 0,05					
			07:30 - 10:30	CR	1,09	0,21	5,24	< 0,05					
			07:30 - 11:00	CR	1,81	0,28	6,51	< 0,05					
			07:30 - 11:30	CR	1,88	0,29	6,56	< 0,05					
			08:00 - 08:30	CR	-0,78	0,11	-7,25	< 0,05					
			08:00 - 09:00	CR	-0,67	0,11	-6,11	< 0,05					
			08:00 - 09:30	CR	-0,35	0,12	-3,02	**0,10**					
			08:00 - 10:00	CR	1,08	0,18	6,09	< 0,05					
			08:00 - 10:30	CR	1,40	0,20	6,99	< 0,05					
			08:00 - 11:00	CR	2,13	0,27	7,79	< 0,05					
			08:00 - 11:30	CR	2,20	0,28	7,80	< 0,05					
			08:30 - 09:00	CR	0,11	0,09	1,27	**0,98**					
			08:30 - 09:30	CR	0,43	0,10	4,47	< 0,05					
			08:30 - 10:00	CR	1,86	0,16	11,31	< 0,05					
			08:30 - 10:30	CR	2,18	0,19	11,52	< 0,05					
			08:30 - 11:00	CR	2,91	0,27	10,97	< 0,05					

			Efeito do intervalo						Efeito do tratamento				
Intervalos	N visitas Média ± DP		Comparação	Tratamento	Estimativa*	EP	Valor *z*	*Valor P*	Comparação	Estimativa*	EP	Valor *z*	*Valor P*
	AA	CR											
			08:30 - 11:30	CR	2,98	0,27	10,87	< 0,05					
			09:00 - 09:30	CR	0,32	0,10	3,24	**0,06**					
			09:00 - 10:00	CR	1,74	0,17	10,55	< 0,05					
			09:00 - 10:30	CR	2,07	0,19	10,87	< 0,05					
			09:00 - 11:00	CR	2,80	0,27	10,52	< 0,05					
			09:00 - 11:30	CR	2,87	0,27	10,43	< 0,05					
			09:30 - 10:00	CR	1,43	0,17	8,40	< 0,05					
			09:30 - 10:30	CR	1,75	0,19	9,01	< 0,05					
			09:30 - 11:00	CR	2,48	0,27	9,22	< 0,05					
			09:30 - 11:30	CR	2,55	0,28	9,18	< 0,05					
			10:00 - 10:30	CR	0,33	0,24	1,39	**0,97**					
			10:00 - 11:00	CR	1,05	0,30	3,51	< 0,05					
			10:00 - 11:30	CR	1,12	0,31	3,65	< 0,05					
			10:30 - 11:00	CR	0,73	0,31	2,31	**0,47**					
			10:30 - 11:30	CR	0,79	0,32	2,47	**0,36**					
			11:00 - 11:30	CR	0,07	0,37	0,19	**1,00**					
			06:00 - 06:30	AA	-0,64	5497,51	0,00	**1,00**					
			06:00 - 07:00	AA	-20,49	4448,40	0,00	**1,00**					
			06:00 - 07:30	AA	-20,44	4448,40	0,00	**1,00**					
			06:00 - 08:00	AA	-20,99	4448,40	0,00	**1,00**					
			06:00 - 08:30	AA	-20,99	4448,40	0,00	**1,00**					
			06:00 - 09:00	AA	-20,77	4448,40	0,00	**1,00**					
			06:00 - 09:30	AA	-20,38	4448,40	0,00	**1,00**					
			06:00 - 10:00	AA	-19,69	4448,40	0,00	**1,00**					
			06:00 - 10:30	AA	-18,75	4448,40	0,00	**1,00**					
			06:00 - 11:00	AA	-19,10	4448,40	0,00	**1,00**					
			06:00 - 11:30	AA	-18,75	4448,40	0,00	**1,00**					
			06:30 - 07:00	AA	-19,85	3230,23	-0,01	**1,00**					
			06:30 - 07:30	AA	-19,80	3230,23	-0,01	**1,00**					
			06:30 - 08:00	AA	-20,35	3230,23	-0,01	**1,00**					

			Efeito do intervalo						Efeito do tratamento				
Intervalos	N visitas Média ± DP		Comparação	Tratamento	Estimativa*	EP	Valor *z*	*Valor P*	Comparação	Estimativa*	EP	Valor *z*	*Valor P*
	AA	CR											
			06:30 - 08:30	AA	-20,35	3230,23	-0,01	**1,00**					
			06:30 - 09:00	AA	-20,13	3230,23	-0,01	**1,00**					
			06:30 - 09:30	AA	-19,74	3230,23	-0,01	**1,00**					
			06:30 - 10:00	AA	-19,05	3230,23	-0,01	**1,00**					
			06:30 - 10:30	AA	-18,11	3230,23	-0,01	**1,00**					
			06:30 - 11:00	AA	-18,46	3230,23	-0,01	**1,00**					
			06:30 - 11:30	AA	-18,11	3230,23	-0,01	**1,00**					
			07:00 - 07:30	AA	0,05	0,23	0,23	**1,00**					
			07:00 - 08:00	AA	-0,50	0,20	-2,50	**0,34**					
			07:00 - 08:30	AA	-0,50	0,20	-2,50	**0,34**					
			07:00 - 09:00	AA	-0,28	0,21	-1,34	**0,97**					
			07:00 - 09:30	AA	0,11	0,23	0,46	**1,00**					
			07:00 - 10:00	AA	0,80	0,28	2,81	**0,17**					
			07:00 - 10:30	AA	1,74	0,41	4,25	< 0,05					
			07:00 - 11:00	AA	1,39	0,35	3,92	< 0,05					
			07:00 - 11:30	AA	1,74	0,41	4,25	< 0,05					
			07:30 - 08:00	AA	-0,55	0,20	-2,71	**0,22**					
			07:30 - 08:30	AA	-0,55	0,20	-2,71	**0,22**					
			07:30 - 09:00	AA	-0,33	0,21	-1,56	**0,92**					
			07:30 - 09:30	AA	0,05	0,23	0,23	**1,00**					
			07:30 - 10:00	AA	0,75	0,29	2,61	**0,27**					
			07:30 - 10:30	AA	1,69	0,41	4,11	< 0,05					
			07:30 - 11:00	AA	1,33	0,36	3,76	< 0,05					
			07:30 - 11:30	AA	1,69	0,41	4,11	< 0,05					
			08:00 - 08:30	AA	0,00	0,17	0,00	**1,00**					
			08:00 - 09:00	AA	0,22	0,18	1,19	**0,99**					
			08:00 - 09:30	AA	0,61	0,21	2,92	**0,13**					
			08:00 - 10:00	AA	1,30	0,27	4,88	< 0,05					
			08:00 - 10:30	AA	2,24	0,40	5,64	< 0,05					
			08:00 - 11:00	AA	1,89	0,34	5,56	< 0,05					

			Efeito do intervalo						Efeito do tratamento				
Intervalos	N visitas Média ± DP		Comparação	Tratamento	Estimativa*	EP	Valor *z*	*Valor P*	Comparação	Estimativa*	EP	Valor *z*	*Valor P*
	AA	CR											
			08:00 - 11:30	AA	2,24	0,40	5,64	< 0,05					
			08:30 - 09:00	AA	0,22	0,18	1,19	**0,99**					
			08:30 - 09:30	AA	0,61	0,21	2,92	**0,13**					
			08:30 - 10:00	AA	1,30	0,27	4,88	< 0,05					
			08:30 - 10:30	AA	2,24	0,40	5,64	< 0,05					
			08:30 - 11:00	AA	1,89	0,34	5,56	< 0,05					
			08:30 - 11:30	AA	2,24	0,40	5,64	< 0,05					
			09:00 - 09:30	AA	0,39	0,22	1,79	**0,82**					
			09:00 - 10:00	AA	1,08	0,27	3,96	< 0,05					
			09:00 - 10:30	AA	2,02	0,40	5,03	< 0,05					
			09:00 - 11:00	AA	1,67	0,34	4,84	< 0,05					
			09:00 - 11:30	AA	2,02	0,40	5,03	< 0,05					
			09:30 - 10:00	AA	0,69	0,29	2,40	**0,41**					
			09:30 - 10:30	AA	1,64	0,41	3,96	< 0,05					
			09:30 - 11:00	AA	1,28	0,36	3,58	< 0,05					
			09:30 - 11:30	AA	1,64	0,41	3,96	< 0,05					
			10:00 - 10:30	AA	0,94	0,45	2,12	**0,61**					
			10:00 - 11:00	AA	0,59	0,39	1,49	**0,94**					
			10:00 - 11:30	AA	0,94	0,45	2,12	**0,61**					
			10:30 - 11:00	AA	-0,36	0,49	-0,72	**1,00**					
			10:30 - 11:30	AA	0,00	0,53	0,00	**1,00**					
			11:00 - 11:30	AA	0,36	0,49	0,72	**1,00**					

*Os coeficientes estão apresentados na escala do modelo.

Tabela S6. A abundância de abelhas nativas nas flores de *Myrcia rufipes* em relação aos tratamentos do experimento de exclusão de visitantes (flores de acesso aberto - AA e flores cobertas por rede - CR). Testamos se havia diferença na frequência de visitas de abelhas nativas na presença e ausência de *Apis mellifera*, usamos modelo linear generalizado misto com distribuição de Poisson, considerando os tratamentos (AA ou CR) e os intervalos de tempo como efeitos fixos e número de flores como efeito aleatório. Resumos de modelos contendo comparações de modelos, critério de informação de Akaike (AIC) e teste de razão de verossimilhança nos efeitos marginais de fatores fixos. Diferenças não significativas estão destacadas em negrito (Valor *p*). Modelo completo: visitas ~ tratamento + intervalo + tratamento * intervalo + (1 | Flores). N = 1200 flores em AA; N = 1197 flores em CR. N visitas = número de visitas. DP = desvio padrão; EP = erro padrão.

			Efeito do intervalo						Efeito do tratamento				
Intervalos	N visitas Média ± DP		Comparação	Tretamento	Estimativa*	EP	Valor *z*	Valor *P*	Comparação	Estimativa*	EP	Valor *z*	Valor *P*
	AO	NC											
06:00	0	2±1,7	06:00 - 06:30	CR	2,25	0,32	7,01	<0,05	AA – CR	1,08	0,20	5,30	<0,05
06:30	1±0,7	3±1,4	06:00 - 07:00	CR	0,46	0,17	2,77	**0,19**	AA – CR	2,38	1,05	2,27	<0,05
07:00	6±2	8±3,9	06:00 - 07:30	CR	0,13	0,15	0,84	**1,00**	AA – CR	0,48	0,21	2,33	<0,05
07:30	7±2,6	10±4,5	06:00 - 08:00	CR	-0,19	0,14	-1,31	**0,98**	AA – CR	0,86	0,20	4,35	<0,05
08:00	6±2,3	24±9	06:00 - 08:30	CR	-0,97	0,13	-7,64	<0,05	AA – CR	0,63	0,16	3,93	<0,05
08:30	5±2,6	26±7,4	06:00 - 09:00	CR	-0,86	0,13	-6,67	<0,05	AA – CR	1,41	0,15	9,68	<0,05
09:00	4±2,5	19±6,5	06:00 - 09:30	CR	-0,54	0,13	-4,01	<0,05	AA – CR	1,52	0,16	9,53	<0,05
09:30	1±0,9	6±2,6	06:00 - 10:00	CR	0,89	0,19	4,71	<0,05	AA – CR	1,58	0,19	8,38	<0,05
10:00	1±0,6	2±0,8	06:00 - 10:30	CR	1,22	0,21	5,75	<0,05	AA – CR	0,85	0,28	2,99	<0,05
10:30	1±0,8	1±0,8	06:00 - 11:00	CR	1,94	0,28	6,91	<0,05	AA – CR	1,47	0,42	3,49	<0,05
11:00	1±0,8	1±0,8	06:00 - 11:30	CR	2,01	0,29	6,95	<0,05	AA – CR	0,39	0,41	0,94	**0,35**
11:30	0	1±1	06:30 - 07:00	CR	-1,79	0,33	-5,50	<0,05	AA – CR	0,67	0,47	1,45	**0,15**
			06:30 - 07:30	CR	-2,12	0,32	-6,66	<0,05					
			06:30 - 08:00	CR	-2,44	0,31	-7,76	<0,05					
			06:30 - 08:30	CR	-3,22	0,31	-10,47	<0,05					
			06:30 - 09:00	CR	-3,11	0,31	-10,08	<0,05					
			06:30 - 09:30	CR	-2,79	0,31	-8,98	<0,05					
			06:30 - 10:00	CR	-1,36	0,34	-4,04	<0,05					

			Efeito do intervalo						Efeito do tratamento				
Intervalos	N visitas Média ± DP		Comparação	Tretamento	Estimativa*	EP	Valor *z*	Valor *P*	Comparação	Estimativa*	EP	Valor *z*	Valor *P*
	AO	NC											
			06:30 - 10:30	CR	-1,04	0,35	-2,95	**0,12**					
			06:30 - 11:00	CR	-0,31	0,40	-0,78	**1,00**					
			06:30 - 11:30	CR	-0,24	0,40	-0,60	**1,00**					
			07:00 - 07:30	CR	-0,33	0,16	-2,06	**0,65**					
			07:00 - 08:00	CR	-0,65	0,15	-4,26	<0,05					
			07:00 - 08:30	CR	-1,43	0,14	-10,41	<0,05					
			07:00 - 09:00	CR	-1,32	0,14	-9,49	<0,05					
			07:00 - 09:30	CR	-1,00	0,14	-6,93	<0,05					
			07:00 - 10:00	CR	0,43	0,20	2,19	<0,05					
			07:00 - 10:30	CR	0,76	0,22	3,47	<0,05					
			07:00 - 11:00	CR	1,48	0,29	5,18	<0,05					
			07:00 - 11:30	CR	1,55	0,29	5,27	<0,05					
			07:30 - 08:00	CR	-0,31	0,14	-2,29	**0,48**					
			07:30 - 08:30	CR	-1,09	0,12	-9,09	<0,05					
			07:30 - 09:00	CR	-0,98	0,12	-8,05	<0,05					
			07:30 - 09:30	CR	-0,67	0,13	-5,19	<0,05					
			07:30 - 10:00	CR	0,76	0,18	4,12	<0,05					
			07:30 - 10:30	CR	1,09	0,21	5,24	<0,05					
			07:30 - 11:00	CR	1,81	0,28	6,51	<0,05					
			07:30 - 11:30	CR	1,88	0,29	6,56	<0,05					
			08:00 - 08:30	CR	-0,78	0,11	-7,26	<0,05					
			08:00 - 09:00	CR	-0,67	0,11	-6,11	<0,05					
			08:00 - 09:30	CR	-0,35	0,12	-3,02	**0,10**					
			08:00 - 10:00	CR	1,07	0,18	6,09	<0,05					
			08:00 - 10:30	CR	1,40	0,20	6,99	<0,05					

			Efeito do intervalo						Efeito do tratamento				
Intervalos	N visitas Média ± DP		Comparação	Tretamento	Estimativa*	EP	Valor *z*	Valor *P*	Comparação	Estimativa*	EP	Valor *z*	Valor *P*
	AO	NC											
			08:00 - 11:00	CR	2,13	0,27	7,79	<0,05					
			08:00 - 11:30	CR	2,20	0,28	7,80	<0,05					
			08:30 - 09:00	CR	0,11	0,09	1,27	**0,98**					
			08:30 - 09:30	CR	0,43	0,10	4,47	<0,05					
			08:30 - 10:00	CR	1,86	0,16	11,31	<0,05					
			08:30 - 10:30	CR	2,18	0,19	11,52	<0,05					
			08:30 - 11:00	CR	2,91	0,27	10,97	<0,05					
			08:30 - 11:30	CR	2,98	0,27	10,87	<0,05					
			09:00 - 09:30	CR	0,32	0,10	3,24	**0,06**					
			09:00 - 10:00	CR	1,74	0,17	10,55	<0,05					
			09:00 - 10:30	CR	2,07	0,19	10,87	<0,05					
			09:00 - 11:00	CR	2,80	0,27	10,52	<0,05					
			09:00 - 11:30	CR	2,87	0,27	10,43	<0,05					
			09:30 - 10:00	CR	1,43	0,17	8,40	<0,05					
			09:30 - 10:30	CR	1,75	0,19	9,01	<0,05					
			09:30 - 11:00	CR	2,48	0,27	9,22	<0,05					
			09:30 - 11:30	CR	2,55	0,28	9,18	<0,05					
			10:00 - 10:30	CR	0,33	0,24	1,39	**0,97**					
			10:00 - 11:00	CR	1,05	0,30	3,51	<0,05					
			10:00 - 11:30	CR	1,12	0,31	3,65	<0,05					
			10:30 - 11:00	CR	0,73	0,31	2,31	**0,47**					
			10:30 - 11:30	CR	0,79	0,32	2,47	**0,36**					
			11:00 - 11:30	CR	0,07	0,37	0,19	**1,00**					
			06:00 - 06:30	AA	3,55	1,02	3,49	<0,05					
			06:00 - 07:00	AA	-0,14	0,24	-0,59	**1,00**					

			Efeito do intervalo						Efeito do tratamento				
Intervalos	N visitas Média ± DP		Comparação	Tretamento	Estimativa*	EP	Valor *z*	Valor *P*	Comparação	Estimativa*	EP	Valor *z*	Valor *P*
	AO	NC											
			06:00 - 07:30	AA	-0,09	0,24	-0,37	**1,00**					
			06:00 - 08:00	AA	-0,64	0,22	-2,95	**0,12**					
			06:00 - 08:30	AA	-0,64	0,22	-2,95	**0,12**					
			06:00 - 09:00	AA	-0,42	0,23	-1,87	**0,78**					
			06:00 - 09:30	AA	-0,04	0,24	-0,15	**1,00**					
			06:00 - 10:00	AA	0,66	0,30	2,22	**0,54**					
			06:00 - 10:30	AA	1,60	0,42	3,83	<0,05					
			06:00 - 11:00	AA	1,24	0,36	3,42	<0,05					
			06:00 - 11:30	AA	1,60	0,42	3,83	<0,05					
			06:30 - 07:00	AA	-3,69	1,01	-3,64	<0,05					
			06:30 - 07:30	AA	-3,64	1,01	-3,59	<0,05					
			06:30 - 08:00	AA	-4,19	1,01	-4,16	<0,05					
			06:30 - 08:30	AA	-4,19	1,01	-4,16	<0,05					
			06:30 - 09:00	AA	-3,97	1,01	-3,93	<0,05					
			06:30 - 09:30	AA	-3,58	1,01	-3,54	<0,05					
			06:30 - 10:00	AA	-2,89	1,03	-2,81	**0,17**					
			06:30 - 10:30	AA	-1,95	1,07	-1,82	**0,81**					
			06:30 - 11:00	AA	-2,30	1,05	-2,20	**0,55**					
			06:30 - 11:30	AA	-1,95	1,07	-1,82	**0,81**					
			07:00 - 07:30	AA	0,05	0,23	0,23	**1,00**					
			07:00 - 08:00	AA	-0,50	0,20	-2,50	**0,34**					
			07:00 - 08:30	AA	-0,50	0,20	-2,50	**0,34**					
			07:00 - 09:00	AA	-0,28	0,21	-1,34	**0,97**					
			07:00 - 09:30	AA	0,11	0,23	0,46	**1,00**					
			07:00 - 10:00	AA	0,80	0,28	2,81	**0,17**					

			Efeito do intervalo						Efeito do tratamento				
Intervalos	N visitas Média ± DP		Comparação	Tretamento	Estimativa*	EP	Valor *z*	Valor *P*	Comparação	Estimativa*	EP	Valor *z*	Valor *P*
	AO	NC											
			07:00 - 10:30	AA	1,74	0,41	4,25	<0,05					
			07:00 - 11:00	AA	1,39	0,35	3,92	<0,05					
			07:00 - 11:30	AA	1,74	0,41	4,25	<0,05					
			07:30 - 08:00	AA	-0,55	0,20	-2,71	**0,22**					
			07:30 - 08:30	AA	-0,55	0,20	-2,71	**0,22**					
			07:30 - 09:00	AA	-0,33	0,21	-1,57	**0,92**					
			07:30 - 09:30	AA	0,05	0,23	0,23	**1,00**					
			07:30 - 10:00	AA	0,75	0,29	2,61	**0,27**					
			07:30 - 10:30	AA	1,69	0,41	4,11	<0,05					
			07:30 - 11:00	AA	1,33	0,36	3,76	<0,05					
			07:30 - 11:30	AA	1,69	0,41	4,11	<0,05					
			08:00 - 08:30	AA	0,00	0,17	0,00	**1,00**					
			08:00 - 09:00	AA	0,22	0,18	1,19	**0,99**					
			08:00 - 09:30	AA	0,61	0,21	2,92	**0,13**					
			08:00 - 10:00	AA	1,30	0,27	4,89	<0,05					
			08:00 - 10:30	AA	2,24	0,40	5,64	<0,05					
			08:00 - 11:00	AA	1,89	0,34	5,56	<0,05					
			08:00 - 11:30	AA	2,24	0,40	5,64	<0,05					
			08:30 - 09:00	AA	0,22	0,18	1,19	**0,99**					
			08:30 - 09:30	AA	0,61	0,21	2,93	**0,13**					
			08:30 - 10:00	AA	1,30	0,27	4,89	<0,05					
			08:30 - 10:30	AA	2,24	0,40	5,64	<0,05					
			08:30 - 11:00	AA	1,89	0,34	5,56	<0,05					
			08:30 - 11:30	AA	2,24	0,40	5,64	<0,05					
			09:00 - 09:30	AA	0,39	0,22	1,79	**0,82**					

			Efeito do intervalo						Efeito do tratamento				
Intervalos	N visitas Média ± DP		Comparação	Tretamento	Estimativa*	EP	Valor *z*	Valor *P*	Comparação	Estimativa*	EP	Valor *z*	Valor *P*
	AO	NC											
			09:00 - 10:00	AA	1,08	0,27	3,96	<0,05					
			09:00 - 10:30	AA	2,02	0,40	5,03	<0,05					
			09:00 - 11:00	AA	1,67	0,34	4,84	<0,05					
			09:00 - 11:30	AA	2,02	0,40	5,03	<0,05					
			09:30 - 10:00	AA	0,69	0,29	2,40	**0,40**					
			09:30 - 10:30	AA	1,64	0,41	3,96	<0,05					
			09:30 - 11:00	AA	1,28	0,36	3,58	<0,05					
			09:30 - 11:30	AA	1,64	0,41	3,96	<0,05					
			10:00 - 10:30	AA	0,94	0,45	2,12	**0,61**					
			10:00 - 11:00	AA	0,59	0,39	1,49	**0,94**					
			10:00 - 11:30	AA	0,94	0,45	2,12	**0,61**					
			10:30 - 11:00	AA	-0,36	0,49	-0,72	**1,00**					
			10:30 - 11:30	AA	0,00	0,53	0,00	**1,00**					
			11:00 - 11:30	AA	0,36	0,49	0,72	**1,00**					

*Os coeficientes estão apresentados na escala do modelo.

CAPÍTULO 3

Competition of invasive honeybees and native stingless bees for pollen of a mass-flowering shrub of Myrtaceae

Competition of invasive honeybees and native stingless bees for pollen of a mass-flowering shrub of Myrtaceae

Abstract

Eusocial bees preferentially exploit and compete for aggregated floral resources such as pollen rich flowers of mass-flowering species. The honeybees, invasive in the New World, frequently dominate over native stingless bees on these plants, but information is scarce on the magnitude honeybees usurp floral resources. The Myrtaceae *Campomanesia adamantium* from the Cerrado of Brazil is such a mass-flowering pollen source. Our main objective was to quantify how much pollen are collected by honeybees and how much by stingless bees from flowers of this plant under natural condition. The honeybees dominated the flowers of *C. adamantium* and removed pollen extraordinarily fast. Within the first three hours of anthesis they removed on average 84% of the pollen grains and then abandoned the flowers rapidly. Native stingless bees gained only 11%. Thus, the massive abundance of honeybees drastically decreased the pollen availability for stingless bees and dislodge these bees from their natural resource.

Keywords: *Apis mellifera*, *Campomanesia*, pollen harvest, eusocial bees

*Manuscrito formatado de acordo com as normas de "**Journal of Apicultural Research**"

Scientific Note

Plant-pollinator interactions are strongly shaped by the availability of floral resources provided by plants to attract their pollinators. The highly social honeybees (*Apis mellifera*) and stingless bees (Meliponini), in general, are intensely attracted to resource-rich plant species, which include mass-flowering species with pollen-only flowers that have only short flowering periods per year (Proença & Gibbs, 1994; Carneiro & Martins, 2012).

In the Neotropics, invasive honeybees and native stingless bees, demonstrate overlap of trophic niches (Pedro & Camargo, 1991; Wilms et al., 1996; Ramalho et al., 2007). Nowadays, the Africanized honeybees, introduced in the mid 1900's, dominate bee-plant communities in most ecosystems (Schlindwein, 1998; Pacheco Filho et al., 2015), affect pollination, cause pollen depletion and changes in the foraging behavior of native bees (Wilms & Wiechers, 1997; Roubik et al., 1986; Roubik & Villanueva-Gutiérrez, 2009; Cane & Tepedino, 2017).

Campomanesia adamantium (Cambess.) O.Berg (gabiroba-do-campo; Myrtaceae) is a mass-flowering native crop species, that occurs in the Cerrado and Atlantic Forest of Brazil, with generalist easy accessible pollen flowers, typically explored by the polylectic social bees. Using this species as model, we asked how much pollen grains are removed by invasive honeybees and how much pollen flows to native stingless bees?

The study was conducted in the Nature Reserve *Parque Estadual do Rio Preto* located in the Cadeia de Espinhaço, Minas Gerais, Brazil (18°07'04" S; 43°20'42" W) in the 2018 flowering season. To answer the question, we first determined the spectrum of the flower-visiting bees of *C. adamantium.* During eight non-consecutive days, we counted all flower visiting bees that occurred in flowering twigs with ~20 flowers/individual for 20 seconds from 04:30 to 12:00h, every 5 minutes. Every six countings were grouped to 30-minute intervals and the flower visitors were grouped to (i) honeybees and (ii) stingless bees (and rare native bees) (see Supplementary Information – SI1). We determined the mean number of pollen grains per flower: (0) Pre-anthesis (flower buds); (1) 06:30h (2) 08:30h and (3) 12:00h (see Supplementary Information – SI2). We then calculated the amount and percentage of pollen grains collected by honeybees and stingless bees. To compare the number of pollen grains present in the flowers at different times of anthesis, we used the generalized linear model (GLM). The data were analyzed in R (R Core Team, 2021).

The *C. adamantium* flowers were visited by bees of nine species (see Supplementary Information – Table S1). Honeybees (Figure 1) were the most frequent floral visitor (85%

of visits), followed by native stingless bees (14%) and halictid bees (1%). Honeybees were the sole flower visitors from 05:00 to 08:00h. The stingless bees, mainly species of Trigona started the visits ~08:00h (Figure 1). Honeybees are known for their ability to fly earlier than stingless bees, and thus, explore full pollen rewarding flowers (Schaffer et al., 1979; Horskins & Turner, 1999).

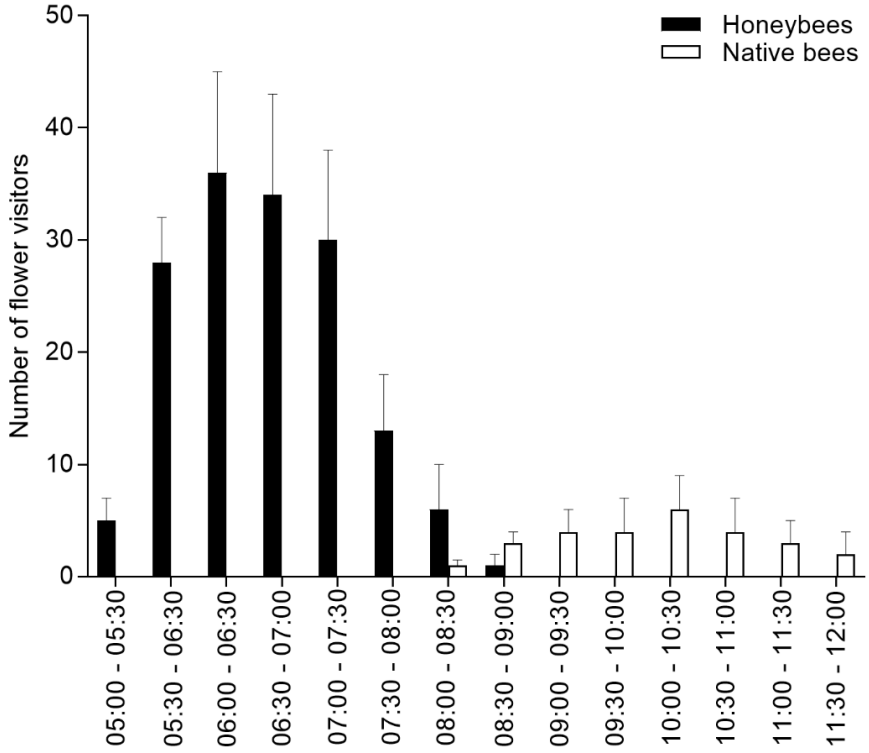

Figure 1. Number of visits of honeybees and stingless bees to flowers of *Campomanesia adamantium*. Observations were made on 10 non-consecutive days and data were grouped into 30-min intervals; mean and sd.

The pollen-only flowers of *Campomanesia adamantium* opened synchronously with already dehisced anthers. A flower produced on average 258,083 (±27,547) pollen grains. Honeybees demonstrate high efficiency in the exploration of the fresh pollen-rich flowers. They had exclusive flower access during the first three hours of anthesis and removed the pollen grains extraordinarily fast (Figure 2A; Table S2). In this time interval, honeybees collected 84% (216,300 ± 13,344) pollen grains of the *C. adamantium* flowers (Figure 2B). When the stingless bees arrived in the flowers, at ~08:30h, about 16% (41,800 ±6,484) of the pollen grains remained in the flowers (Figure 2A), of which on average 11% (28,600 ± 3,173) were collected by them (Figure 2B).

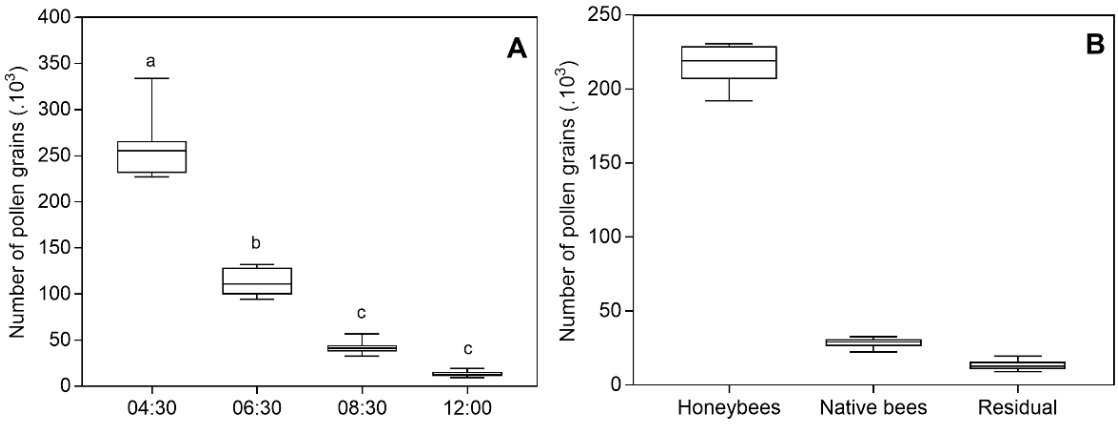

Figure 2. Pollen removal in flowers of *Campomanesia adamantium;* **A**, Number of pollen grains per flower until 12:00h, when flower visitors had disappeared. 04:30h before visits; N=10) and during the activity of bees (N=10 for each interval). Different letters mean significant differences between treatments ($P<0.05$). **B**, Pollen grains collected by different bee groups and residual pollen.

Honeybees were six times more abundant than native bees and gained most of the pollen of *C. adamantium* flowers. Their refined communication system allows mass-recruitment of nest mates to the rich food sources (von Fisch, 1967; Dyer, 2002; Seeley, 2012) making them strong competitors for stingless bees (Wilms et al., 1996). Therefore, the pollen gain of stingless bees was strongly influenced by the abundant honeybees that decreased the pollen availability. Similar cases were reported from flowers of other plant species dominated by honeybees, including further Myrtaceae (see chapter 1, 3) and other families (Roubik et al., 1986; Horsinks & Turner, 1999; Carneiro & Martins, 2012).

Future studies with possible exclusion of honeybees of the flowers would be interesting to know the possible effects of the massive presence of honeybees on stingless bees.

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SUPPLEMENTARY INFORMATION

MS1. Methodology – Frequency of flower visits

To determine the frequency of flower visits we counted all visitors that occurred in a group of ~20 flowers/individual for 20 seconds from 04:30 to 12:00h every 5 minutes. We opted for the short scanning for flower visitors to determine visitation frequency to avoid multiple counting of the same bee individuals. During the counts, we recorded also the names of the flower visiting species or morphospecies of bees. The scans of the number of flower visitors were repeated on 38 individuals on eight different days, in a total of 570 flowers, which was possible because in some days more than one observer performed counts on different shrubs simultaneously. The scans of the number of flower visitors were repeated on 38 individuals on eight non-consecutive days, in a total of 570 flowers, which was possible because in some days more than one observer performed counts on different shrubs simultaneously. The data were grouped to 30-minute intervals (six countings) and the flower visitors were grouped to (i) honey bees (*Apis mellifera*) and (ii) stingless bees (and other bee species).

MS2. Methodology – Pollen Count

We determined the number of pollen grains per flower by macerating the anthers of pre-anthesis flower buds in Eppendorf tubes containing a solution of 0.5 mL lactic acid and glycerin at 3:1 (Lloyd 1972). We extracted an aliquot from this solution for counting the pollen grains using a Neubauer chamber (Maêda 1985). The mean number of grains per flower was calculated from 10 flower buds from ten different plant individuals.

To determine the pollen removal dynamics along the anthesis by the flower visitors, we count the amount of pollen grains within the flowers at different times of anthesis: (0) Pre-anthesis - flower buds; (1) 06: 30h - after massive visits of *A. mellifera*; (2) 08: 30h - after the end of visits of *A. mellifera* and the start visits of native bees; (3) 12: 00h - after the end of the visit period of all groups of bees. First, we calculated the amount of pollen from flower buds (N=10). In each time of anthesis, 10 individual flowers were removed and transferred to an Eppendorf tube containing 70% ethanol. Subsequently, the anthers were removed and macerated, while the pollen adhering to the flower was washed out with ethanol. The samples were homogenized in a vortex stirrer and centrifuged at 6000 r.p.m for 5 min. The alcohol was decanted and 0.5 mL lactic acid and glycerin at 3:1 was added (Lloyd

1972). We extracted an aliquot from this solution for counting pollen grains in a Neubauer chamber (Maêda 1985).

TABLES

Table S1. Floral visiting bees of *Campomanesia adamantium* in Parque Estadual do Rio Preto, Minas Gerais, Brazil, including sex and foraging period.

Bee species		Sex
Apidae		
Apinae		
Apini	*Apis mellifera* Linnaeus, 1758	♀
Meliponini	*Melipona* sp.	♀
	Plebeia sp.	♀
	Scaptotrigona postica (Latreille, 1807)	♀
	Tetragonisca angustula (Latreille, 1811)	♀
	Trigona spinipes (Fabricius, 1793)	♀
	Trigona sp.	♀
Halictidae		
Halictinae		
Augochlorini	*Augochora* sp.	
Halictini	*Dialictus* sp.	♀

Table S2. Pollen removal in flowers of *Campomanesia adamantium*. The number of pollen grains inside the flower before anthesis (N=10) and during the activity of bees (N=10 for each interval). We tested whetherthere was difference the number of pollen grains remaining within flowers at different times of anthesis using a generalized linear model (GLM), with Gaussian distribution. Non-significant differences are in bold (*P*-value). T0 = 04:30h, buds; T1 = 06:30h; T2 = 08:30h; T3 = 12:00h. N flowers = number of flowers SD = Standard deviation; SE = Standard error.

Treatment	N flowers	Mean ± SD Pollen grains	Comparison	Estimate	SE	*z*-value	*P*-value
T0	10	918200 ±157970	T0 – T1	145750	7721,768	18,88	<0.05
T1	10	122600 ±39328	T0 – T2	216300	7721,768	28,01	<0.05
T2	10	58550 ±17930	T0 – T3	244900	7721,768	31,72	<0.05
T3	10	27800 ±9340	T1 – T2	70550	7721,768	9,14	<0.05
			T1 – T3	99150	7721,768	12,84	<0.05
			T2 – T3	28600	7721,768	3,70	**0,10**

CAPÍTULO 4

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

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

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

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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; Cordeiroet 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). Commontraits 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 spe- cies 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; Fae- gri 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 flow- ers 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,b). 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).

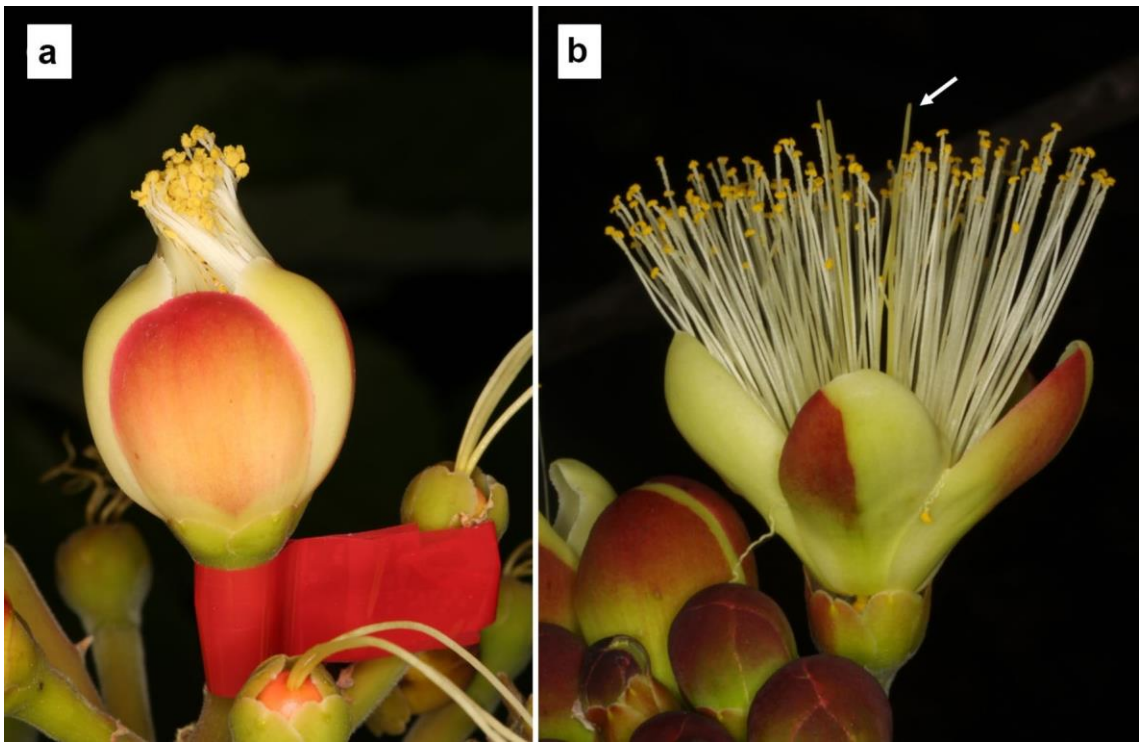

Fig. 1 Flowers of *Caryocar brasiliense*. **a** Beginning of anthesis; petals begin to separate and stigmas and anthers become visible. **b** Open flower with exposed stamens and stigmas. The arrow indicates the height of the stigmas.

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.

Flowers 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 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).

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

Females of *Ptiloglossa latecalcarata* accounted for 15% of all bee visits (Fig. 2a) while females 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.

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

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).

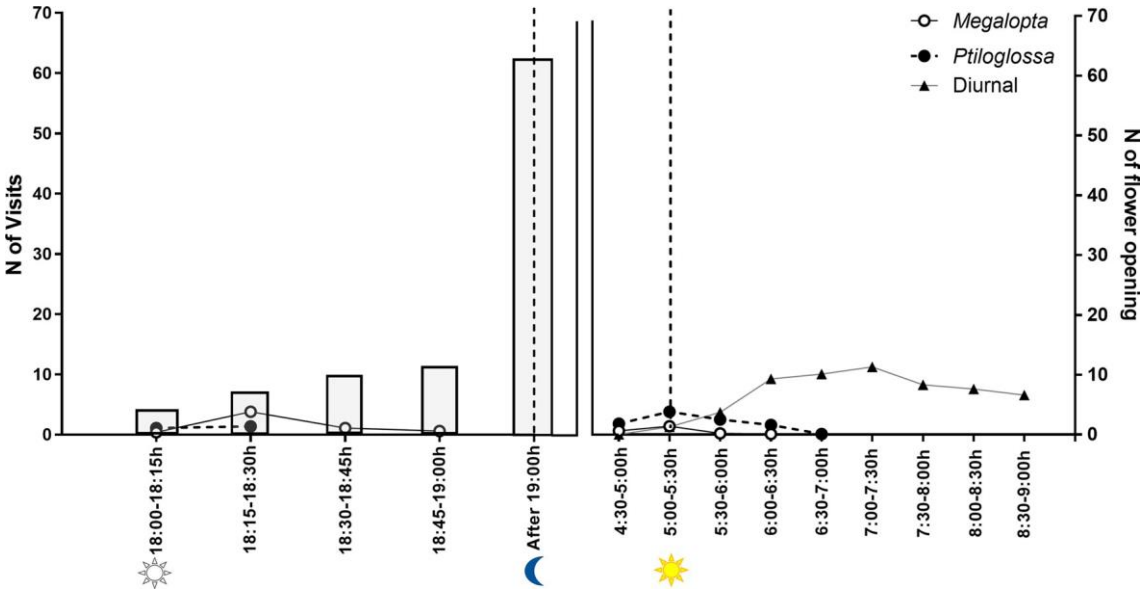

Fig. 3 Number of bee visits (lines) and opening hours of marked flowers of *Caryocar brasiliense* (bars), at half-hour intervals during dawn and 15-min intervals during dusk (N = 95 flowers from five trees). Dashed line indicates astronomical twilight end (19:09 h; 04:42 h); white sun: indication of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

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) ($X2 = 49.1$; $df = 1$; $p < 0.001$). 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).

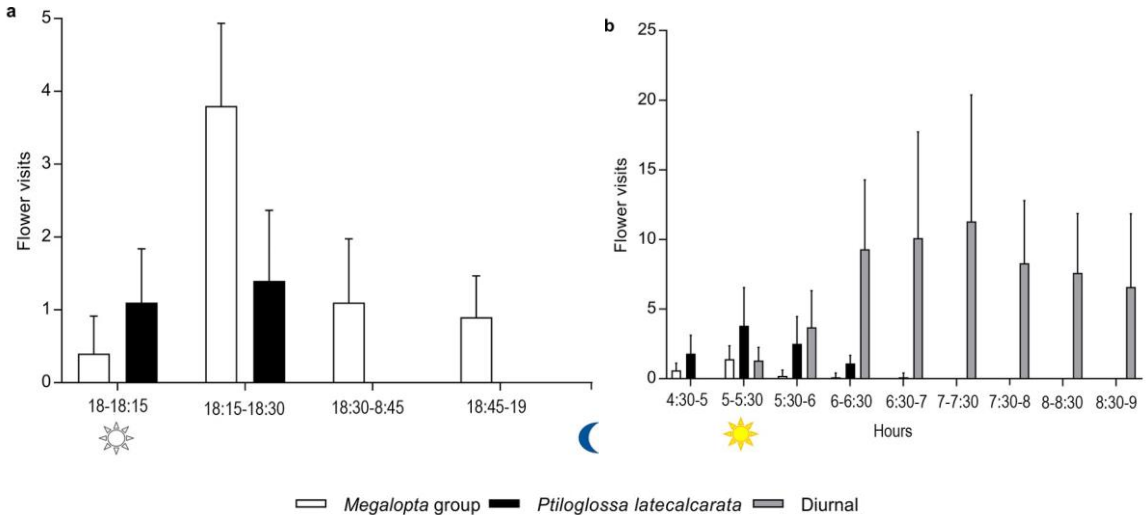

Fig. 4 Number of floral visits to flowers of *Caryocar brasiliense*. **a** Floral visits during evening twilight (mean ± SD). No diurnal bees visited the flowers of *C. brasiliense* at dusk. **b** Floral visits during dawn and the morning. Observations were made on 10 non-consecutive days. White sun: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

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) ($X2 = 346.9$; $df = 1$; $p < 0.001$) (N = 562).

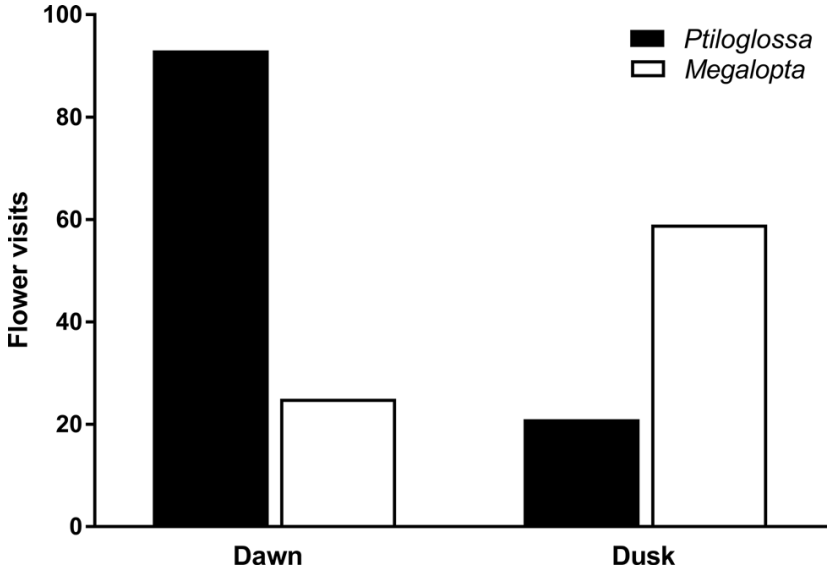

Fig. 5 Frequency of floral visits by *Megalopta* and *Ptiloglossa* at dawn and dusk

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.

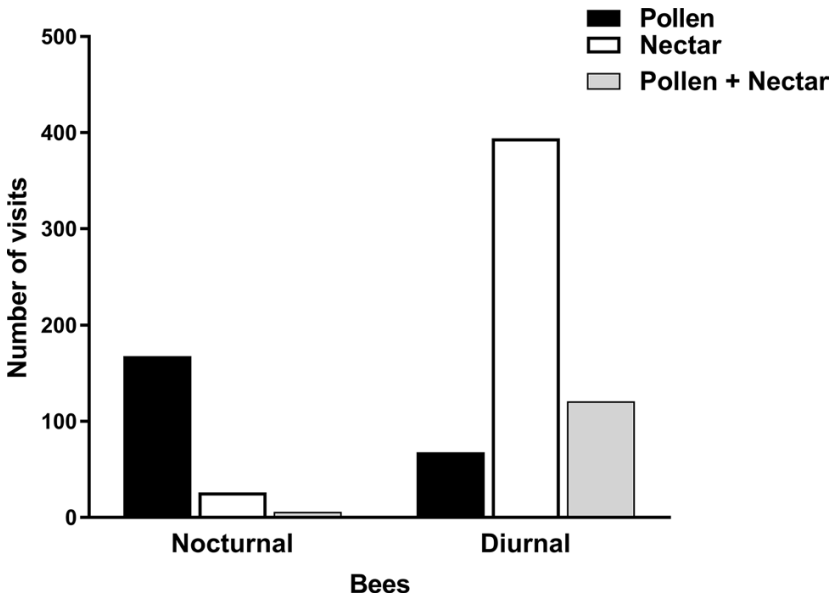

Fig. 6 Resources collected by diurnal and nocturnal bees during visits to flowers of *Caryocar brasiliense*

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 morpho- types 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 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.

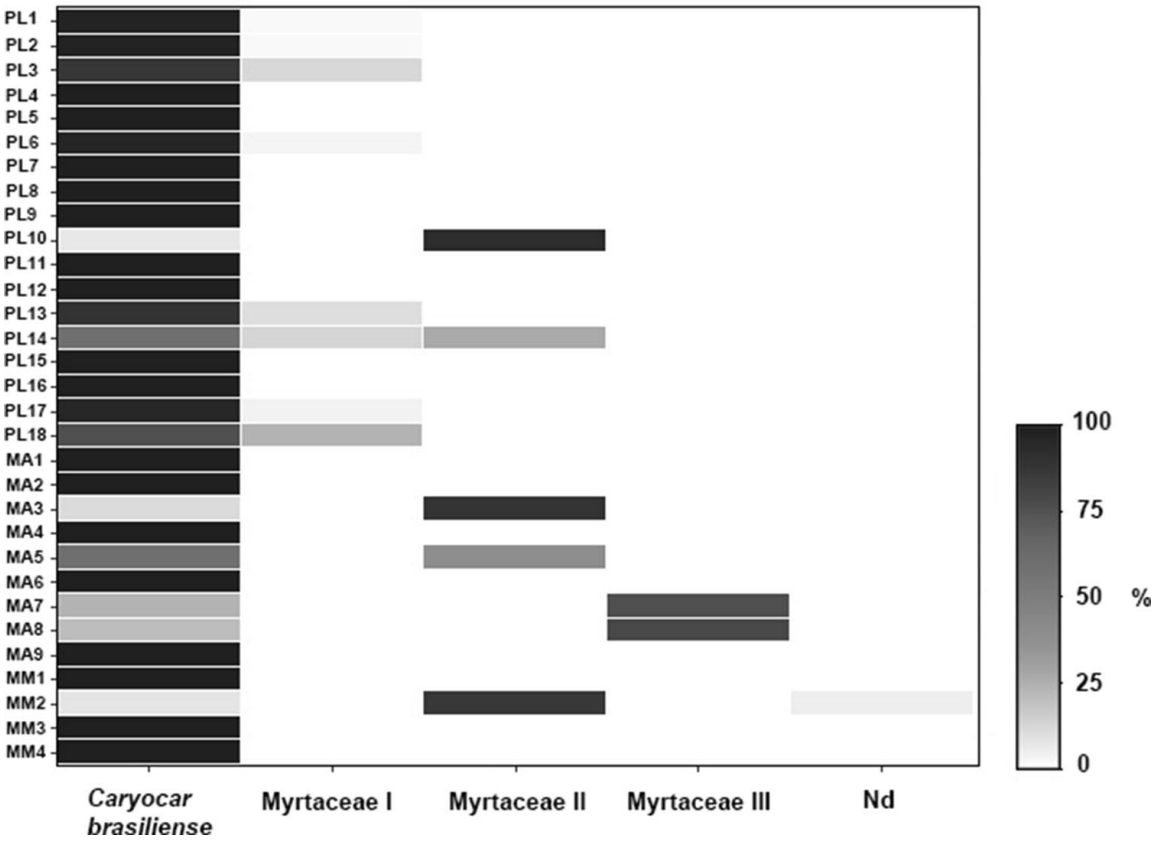

Fig. 7 Female scopa contents, shown as relative frequency of pollen types, for *Ptiloglossa latecalcarata*, *Megalopta aegis* and *M. amoena* collected in flowers of *Caryocar brasiliense*. The heatmap representation shows the percentage of each pollen type (columns) on each female (rows). PL = *Ptiloglossa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

Pollen 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).

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

Compounds	Relative contribution (%)
Aliphatic compounds	
(*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.

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 avail- able 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 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 com- pounds, 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 flow- ers. 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 con- tent, 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 them- selves. 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 fermenta-tion 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.

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ELECTRONIC SUPPLEMENTARY MATERIAL

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

Arthropod-Plant Interactions

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Nest of *Ptiloglossa latecalcarata* Moure, 1945

The nest of *Ptiloglossa latecalcarata* (Colletidae) was found next to a path in an area with sandy soil covered by characteristic Cerrado vegetation. The nest was not part of a nesting aggregation, and the nest entrance was not surrounded by a tumulus. The main tunnel had a diameter of 0.6 cm and was 110 cm long, descending vertically through the soil (Fig. 1a, 1b). The nest contained four closed brood cells and one brood cell under construction. The closed brood cells were at a depth of 90–110 cm and were positioned at the end of lateral side-tunnels, 10–18 cm from the main tunnel (Fig. 1b). The brood cell under construction was at the end of the main tunnel. The brood cells were vertical oriented, urn-shaped and with an average length of 3.1 cm and an average maximum diameter of 2.1 cm. The entrance of each cell had a curved neck, which averaged 0.7 cm in diameter. The walls of the cells were clearly delimited and smooth, with the inner surface and the neck being covered with a thin, semi-transparent, whitish cellophane-like membrane (Fig. 1c). Cell provisions consisted of a watery transparent-yellow fluid containing yellow pollen grains. The larvae measured 0.1 – 0.9 cm. The first-instar larvae floated on the surface of the fluid (Fig. 1d).

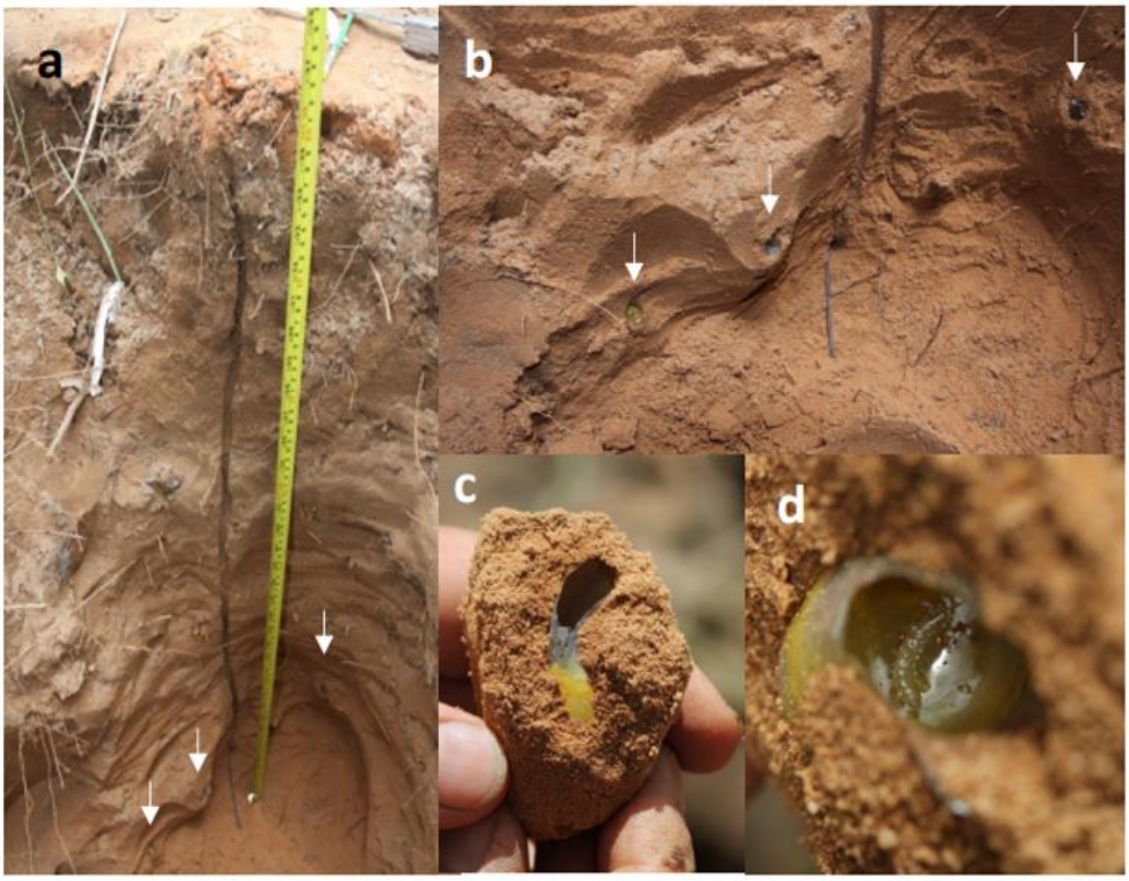

Fig. 1 Nest of *Ptiloglossa latecalcarata*. **a, b**- Main tunnel of the nest showing the position of three brood cells (arrows). **c**- Lateral view of a brood cell with part of the provisions and cellophane-like membrane. **d**- The larvae floating of the larval storage.

CONSIDERAÇÕES FINAIS

Nosso estudo mostrou, por meio de quantificações, que a presença massiva da espécie introduzida *Apis mellifera* nas espécies de Myrtaceae de floração maciça, têm impactos negativos no forrageamento de abelhas nativas, ao diminuir a frequência com que essas abelhas visitam suas plantas hospedeiras, além de diminuir drasticamente o ganho de pólen por elas. Vimos que em flores de *Campomanesia pubescens*, *A. mellifera* foi o visitante mais frequente e encurtou o tempo de forrageamento que as abelhas crepusculares, as principais polinizadoras de suas flores, sem presença de competidores. É muito provável que, na ausência das abelhas do mel, as abelhas crepusculares conseguiriam coletar uma maior quantidade de pólen. Além disso, a abelha introduzida diminui a frequência de visitas e a quantidade de pólen coletados por abelhas nativas diurnas, principalmente abelhas sem ferrão. Demonstramos também o domínio de *A. mellifera* para outras espécies de Myrtaceae. Em *Blepharocalyx salicifolius* e *Myrcia rufipes*, essa abelha introduzida também reduziu a visitação e reduziu drasticamente o ganho de pólen por abelhas sem ferrão. Seria interessante medir competição por pólen em *C. adamantium* em estudos futuros, uma vez que mais de 80% dos grãos de pólen das suas flores foram coletados por *A. mellifera.*

Nossos resultados mostram que, embora *A. mellifera* já faça parte da apifauna brasileira, há uma necessidade urgente de um maior entendimento das suas relações com a flora nativa e impactos para apifauna local, bem como o manejo desta espécie introduzida, especialmente em unidades de conservação de proteção integral, como o local desse estudo. Para isso, a condução de estudos de longa duração e quantificação dos possíveis impactos dessa espécie para abelhas nativas que visitam outras espécies de plantas seria interessante, a fim de conservar as interações planta-polinizador.

Nos capítulos 1 e 4, observamos diferentes relações de espécies de abelhas crepusculares/noturnas com flores. Essas abelhas foram polinizadores eficientes de *C. pubescens*, espécie com flores melitófilas que se abrem antes do amanhecer e oferecem uma grande quantidade de pólen. No entanto, elas não atuaram na frutificação de *Caryocar brasiliense*, embora visitaram intensamente suas flores tipicamente quiropterófilas para coleta de pólen. Com isso, estudos sobre percepção de sinais florais olfativos de plantas quiropterófilas e melitófilas de antese noturna usados pelas abelhas crepusculares são necessários para entender melhor as interações entre essas abelhas e suas plantas associadas.