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Pollination biology of melittophilous legume tree species in the Atlantic Forest in Southeast Brazil

Mardiore Pinheiro[1*], Vinicius Lourenço Garcia de Brito[2] and Marlies Sazima[3]

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ABSTRACT

This study describes the floral phenology and morphology, reproductive biology and pollinators for eight legume tree species, *Schizolobium parahyba, Senna macranthera*, and *Senna multijuga* (Caesalpinioideae), as well as *Andira fraxinifolia, Lonchocarpus cultratus, Pterocarpus violaceus, Swartzia oblata*, and *S. simplex* (Papilionoideae), in the Atlantic Forest in Southeast Brazil. All the studied species showed an annual flowering pattern, and almost all are of the cornucopia-flowering type, with the only exception being *Swartzia oblata*, which was of the steady-state type. In general, the legume flowers studied are conspicuous, mostly medium-sized, and offer nectar and/or pollen as a resource. Self-incompatibility associated with the production of many flowers and consequent pollen discounting due to self-pollination may contribute to low fruit set of these species in natural conditions. Fifty bee species were recorded visiting the flowers, with medium to large-sized Apidae bees, such as *Bombus morio,* and species of *Xylocopa,* Centridini and Euglossina, which were among the most frequent visitors and major pollinators. These bees showed high floral constancy, thus they are significant to the reproductive success of these tree species. This study provides information regarding the interactions between bees and these eight legume species and evaluates the importance of pollinators for their sexual reproduction.

Keywords: bee pollination, *Bombus*, Caesalpinioideae, Centridini, Euglossina, Faboideae, floral biology, reproduction, self-incompatibility, *Xylocopa*

Introduction

In Neotropical forests, the majority of tree species studied for their breeding system have been found to be self-incompatible; they are also mostly dependent on animals for their pollination and reproduction which probably increases the level of specialization in such places (Ollerton *et al.* 2006; 2011). Plant-pollinator interactions are important for the maintenance of biodiversity and are one of the critical services for sustainable ecosystems (Bawa 1990; Ollerton 2017). However, studies point that landscape changes resulting from anthropogenic disturbance, as habitat loss, fragmentation and degradation, are one of the primary threats to pollination services (Potts *et al.* 2010; Thomann *et al.* 2013). In this sense, studies of the interactions between plants and their pollinators can provide useful information to understanding ecological and evolutionary relationships of species (Mitchell *et al.* 2009) as well as to conserving species in threatened habitats (Mayer *et al.* 2011).

Although the Brazilian Atlantic Forest is one of the most diverse forest in tropical wet climate regions, and is also one of the most endangered ecosystems in the world (Morellato & Haddad 2000; Myers *et al.* 2000), the pollination and reproductive biology of such tree species has been poorly

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1 Programa de Pós-Graduação em Ambiente e Tecnologias Sustentáveis, Universidade Federal da Fronteira Sul, 979000-000, Cerro Largo, RS, Brazil
2 Instituto de Biologia, Universidade Federal de Uberlândia, 38400-902, Uberlândia, MG, Brazil
3 Departamento de Biologia Vegetal, Instituto de Biologia, Universidade Estadual de Campinas, 13083-970, Campinas, SP, Brazil

* Corresponding author: mardiore.pinheiro@gmail.com

studied and not encompass the taxonomic and functional diversity of this ecosystem. The absence of this kind of study is mainly due to difficulties in reaching the flowers in the canopy (Prata-de-Assis-Pires & Freitas 2008; Rocca & Sazima 2008). To our knowledge, only a few studies to date have described the pollinators of some ornithophilous species in the canopy (Rocca *et al.* 2006; Rocca & Sazima 2008), as well as the pollinators and reproductive biology of some melittophilous legume tree species (Borges *et al.* 2008; Prata-de-Assis-Pires & Freitas 2008; Wolowski & Freitas 2010; Amorim *et al*. 2013, Ávila *et al.* 2015), and the bee visitors of some mass flowering tree species (Ramalho 2004; Brito & Sazima 2012).

The knowledge of the reproductive biology of Neotropical trees has been increasing in recent decades (Bawa *et al.* 1985b; Ward *et al.* 2005), but it is still incipient for most tree species in the Atlantic Forest. Ward *et al.* (2005) reported that some legume tree species exhibit high patterns of mixed mating systems, while others are predominant outcrossers with rates higher than 0.80. Furthermore, despite the fact that in Neotropical rainforests the majority of plant species are pollinated by bees (Bawa *et al.* 1985a; Bawa 1990; Ollerton 2017), detailed studies on pollination by bees of canopy trees in the Atlantic Forest in Southeast Brazil are virtually nonexistent. The legume family is one of the most important and highly diverse families in the Atlantic Forest (Oliveira-Filho & Fontes 2000) and since most legume species are bee-pollinated (Arroyo 1981), they represent an important food source for these insects that are the largest pollination taxon in tropical communities (Ollerton 2017).

This paper provides information on the pollination biology and breeding systems of eight sympatric tree species of melittophilous Leguminosae in the Atlantic Forest in Southeast Brazil. The major goals were: (I) establish flowering phenology and floral biology of these species; (II), elucidate the main pollinators; and (III), evaluate whether the plant species are dependent on pollinators for reproduction.

Materials and methods

Study site and species

This study was conducted in the Atlantic Forest area in the Serra do Mar State Park (SMSP), Núcleo Picinguaba (NP) (ca. 23°20'S 44°48'W), municipality of Ubatuba, São Paulo state, Southeast Brazil. The SMSP covers 309,000 ha and the NP comprises 7850 ha (0 to ca 1300 m altitude) that includes dune and mangrove vegetation, coastal plain forest, lower montane forest (Atlantic rainforest) and montane forest (SEMA 2006). Climate is tropical rainy (Af), according to the Köppen (1948) system, with annual rainfall average up to 2600 mm and a mean annual temperature of 22 °C (Morellato *et al*. 2000). Field observations were made during two flowering seasons, between September 2005 and May 2007 in plant species of the coastal plain forest. Eight canopy legume tree species were studied: three of the subfamily Caesalpinioideae, *Schizolobium parahyba* (Vell.) S. F. Blake, *Senna macranthera* (DC. ex Collad.) H. S. Irwin & Barneby, *Senna multijuga* (Rich.) H. S. Irwin & Barneby, and five from the subfamily Papilionoideae/Faboideae, *Andira fraxinifolia* Benth., *Lonchocarpus cultratus* (Vell.) A. M. G. Azevedo & H. C. Lima, *Pterocarpus violaceus* Vogel, *Swartzia oblata* R. S. Cowan and *Swartzia simplex* Spreng. The height of individuals studied ranged from 4 to 17 meters. For floral biology studies, scaffolding and ladders were utilized for reproductive experiments and for observations of bee behavior. Voucher specimens for all tree secies are deposited in the UEC herbarium of the Universidade Estadual de Campinas.

Floral phenology and morphology

Flowering phenology was monitored in 68 individuals of the tree species at monthly interval: S. *parahyba* (n= 20), *S. macranthera* (n= 12), *S. multijuga* (n= 14), *A. fraxinifolia* (n= 3), *L. cultratus* (n= 5), *P. violaceus* (n= 1), *S. oblata* (n= 8), and *S. simplex* (n= 5). A flowering event was considered to begin with the occurrence of open flowers. The flowering intensity was estimated in terms of percentage in five levels ranging from 0 (absence) to 4, with a 25 % interval between each presence class 1 to 4 (Fournier 1974). The percentage of flowering individuals of each plant species (i.e., flowering activity) was calculated for all studied individuals of each legume tree species at the study site. The anthesis period with the highest values of flowering intensity and activity was considered the flowering peak. The flowering frequency was classified following Newstrom *et al.* (1994a) and the flowering pattern in relation to pollination systems was classified according to Gentry (1974).

Flower dimensions were taken in the laboratory from 10 flowers fixed in 50 % ethanol. For species with heteranthery (*S. macranthera*, *S. multijuga*, *S. oblate*, *S. simplex*), only the stamens involved in pollination were measured. Flowers were classified in three size categories, according to the corolla length and diameter, respectively: small (10-18 mm x 10-17 mm diam.), medium (32-42 mm x 32-47 mm diam.) and large (> 65 for both measures).

Floral biology and breeding system

Floral feature data, such as color, symmetry, flower longevity, presence of resource, and flower odor were recorded *in situ* (Kearns & Inouye 1993). Investigation of the breeding systems of these species (with exception of *Pterocarpus violaceus*) were performed and included autonomous autogamy, hand self- and cross-pollinations, and emasculation that used flowers from previously bagged inflorescences (Radford *et al.* 1974). The hand pollinations were made when stigmas were receptive. Stigma receptivity was estimated with the H_2O_2 catalase activity method (Dafni *et al.* 2005) and checked with a hand lens. The number of individuals used in the treatments was: *S. parahyba* (n= 2), *S. macranthera* (n= 3), *S. multijuga* (n= 3), *A. fraxinifolia* (n= 2), *L. cultratus* (n= 3), *S. oblata* (n= 8), and *S. simplex* (n= 3). The number of flowers used in each treatment can be checked in Table 1. These flowers were monitored until fruit set. Another group of flowers of each species was marked to estimate fruit set in natural conditions (control). Pollen viability was estimated by cytoplasmic stainability with the acetocarmine technique (Radford *et al.* 1974), using six flowers from different individuals.

Floral visitors

The spectrum of bee visitors to each plant species was determined by records on flowers at different periods of the day. The frequency and behavior of visitors on the flowers was recorded by focal observations from early morning to late afternoon. The total number of observation hours for each species was: *S. parahyba* (n= 20), *S. macranthera* (n= 14), *S. multijuga* (n= 20), *A. fraxinifolia* (n= 10), *L. cultratus* (n= 10), *P. violaceus* (n= 10), *S. oblata* (n= 10), and *S. simplex* (n= 49). Visitors that contacted the stigmas and anthers were considered pollinators (Alves-dos-Santos *et al.* 2016). To better visualize this data, we constructed a quantitative pollination network considering the links as the frequency of interactions using the *bipartite* package in R software (Dormann *et al.* 2008). Pollinators were also classified as r = rare (< 5 %), ff = few to frequent (5 to 15 %), f = frequent (> 15 to 30 %), and vf = very frequent (> 30 %) according to visit frequency and they were grouped in four categories for body length measure: very small (≤ 5 mm), small (> 5 to 12 mm), medium-sized (> 12 to 20 mm) and large (> 20 mm). The number of flowers visited by an individual bee during a foraging bout was recorded for the main pollinators of *Senna macranthera*, *Schizolobium parahyba*, *Swartzia oblata* and *S. simplex*. Some bees visiting the flowers were captured using an insect net, identified and housed in the MCT-bee collection of Pontifícia Universidade Católica do Rio Grande do Sul.

Results

Floral phenology, morphology, and biology

All species exhibited only one flowering episode per year, concentrated in the wettest period (September to April), with the highest values of flower intensity and activity (flowering peak) mainly during one month for each species (Fig. 1). Almost all species were of the cornucopia type with a large number of new flowers opening each day over a short period of the year. However, *Swartzia oblata* presented the steady state type where the plant produces a few flowers each day over an extended period of time (up to three months). Floral attributes of the eight legume tree species are presented in Table 2.

The Caesalpinioideae representatives, *Schizolobium parahyba*, *Senna macranthera* and *S. multijuga* have zygomorphic and conspicuous flowers (Fig. 2A-D). *Schizolobium parahyba* flowers have 10 stamens, one upright and adnate with the standard petal, and the others almost perpendicular facing this petal (Fig 2A). These stamens and pistil compose the androecium and gynoecium set, with the stigma placed between or slightly above the anthers, which dehisce longitudinally to the outside of the flower. Nectar is the main resource, hidden in a chamber, whose access is blocked by the base of the stamens and by the claw of the standard petal. *Senna macranthera* and *S. multijuga* flowers have a similar floral construction (Fig. 2B-D). However, the

Table 1. Pollen viability and breeding system of seven melittophilous Leguminosae tree species in the Atlantic Forest in Southeast Brazil. Percentage of fruits (n= number of flowers). ^{1} = pollen of small stamens, ^{2} = pollen of medium-sized stamens, ^{3} = pollen of large stamens, ^{4} = pollen of superior stamens, ^{5} = pollen of inferior stamens, - = no treatments were made.

Species	Number of individuals	Pollination treatment					
		Pollen viability	Agamospermy	Spontaneous self	Self-pollination	Cross-pollination	Natural conditions
		%	% fruit (n)	% fruit (n)	% fruit (n)	% fruit (n)	% fruit (n)
Schizolobium parahyba	2	95.7	0 (15)	0 (240)	0 (20)	6.4 (109)	2.8 (519)
Senna macranthera	3	93.8^{1}	0 (46)	0 (75)	0 (10)^{1}	42.8 (7)^{3}	2.0 (442)
		92.7^{2}			0 (18)^{3}		
		91.6^{3}					
Senna multijuga	3	96.6^{1}	0 (36)	0 (167)	0 (5)^{1}	-	3.6 (2.143)
		94.2^{2}					
		95.8^{3}					
Andira fraxinifolia	2	80.0	-	0 (80)	-	-	-
Lonchocarpus cultratus	3	98.5	-	0 (238)	0 (10)	-	2.8 (393)
Swartzia oblata	8	98.5^{4}	0 (10)	0 (361)	0 (11)^{4}	20.5 (39)^{4}	0.1 (1.721)
		98.3^{5}			0 (8)^{5}	38.4 (26)^{5}	
Swartzia simplex	3	95.4^{4}	0 (9)	0 (100)	0 (10)^{4}	0 (17)^{4}	0.9 (202)
		95.6^{5}			0 (10)^{5}	50.0(28)^{5}	

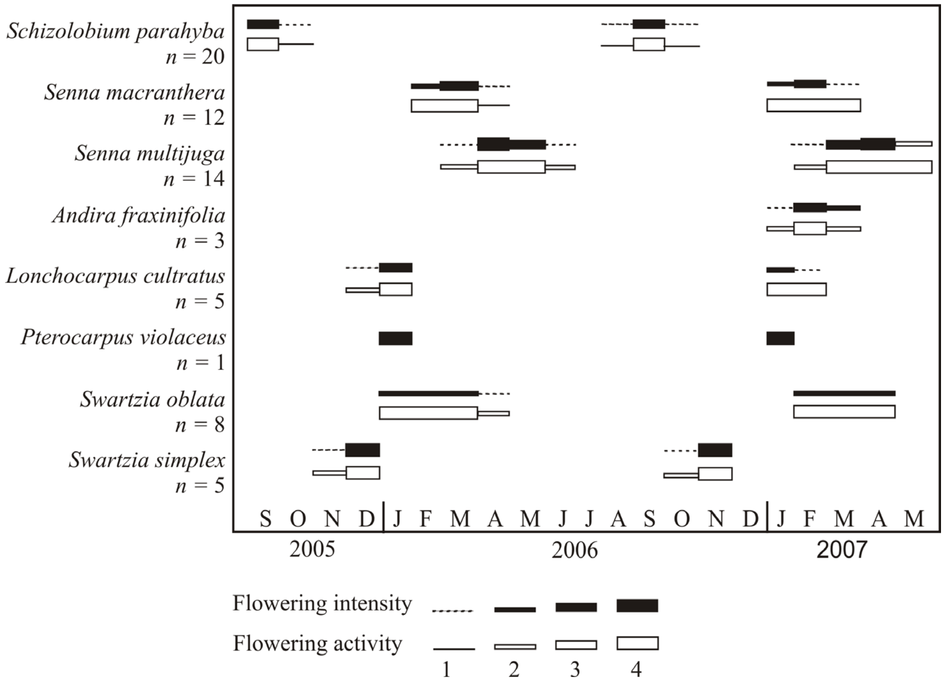

Figure 1. Flowering periods of eight melittophilous Leguminosae tree species in the Atlantic Forest in Southeast Brazil from September 2005 to May 2007. The number of individuals recorded (n) is given below each tree species. The percentages of flowering intensity and activity is scaled as 1= 1-25 %; 2= 26-50 %; 3= 51-75 %; 4= 76-100 %. Flowering intensity was measured as the percentage of branches in phenophase within the total canopy area of the tree. Flowering activity is the more comprehensive measure, calculated as the percentage of flowering individuals among all individuals of a tree species. The highest values of flowering intensity and activity were considered the flowering peak. The area of study was the Parque Estadual da Serra do Mar, Núcleo Picinguaba (an area of 7850 ha), São Paulo, Brazil.

Table 2. Floral characteristics of eight melittophilous Leguminosae tree species and their main pollinator size categories in the Atlantic Forest in Southeast Brazil. Flower size: small (s), medium-sized (ms), large (l). Odor: perceptible (+), not perceptible (-). Pollinating stamens (*).

Species	Flowers							Pollinator
	Symmetry	Color	Resource	Odor	Life span (h)	Corolla length / diam. (mm)	Gynoecium / Androecium length (mm)	size category
Schizolobium parahyba	bilateral	vivid yellow	nectar, pollen	(-)	36	32.85 / 34.9 (ms)	15.6 / 18.0	medium-sized, large
Senna macranthera	asymmetrical	deep yellow	pollen	(+)	12	69.02 / 65.9 (l)	28.9 / 14.6 (*)	large
Senna multijuga	asymmetrical	vivid yellow	pollen	(+)	12	41.74/ 32.3 (ms)	18.6 / 12.3 (*)	medium-sized, large
Andira fraxinifolia	bilateral	pink	nectar, pollen	(-)	36	15.33 / 12.6 (s)	13.8 / 14.2	small, medium-sized
Lonchocarpus cultratus	bilateral	pink	nectar, pollen	(-)	48	10.10 / 10.2 (s)	8.0 / 8.7	small, medium-sized
Pterocarpus violaceus	bilateral	vivid yellow	nectar, pollen	(-)	24	17.61 / 16.1 (ms)	13. 1 / 13.1	small
Swartzia oblata	bilateral	white	pollen	(+)	48	12.41 / 16.6 (ms)	9.5 / 9.0 (*)	medium-sized, large
Swartzia simplex	bilateral	deep yellow	pollen	(+)	24	36.75 / 46.8 (ms)	21.5 / 23.00 (*)	large

androecium is heterantherous and composed of three, small stamens uppermost with sterile anthers (Fig. 2B, arrow a) and three sets of fertile stamens of different filament lengths with the anthers dehiscing poricidally: (1) a central group of four stamens with short filaments and short anthers(Fig. 2B, arrow b), whose apertures are oriented toward the ventral side of the visitor, (2) one central-abaxial stamen with a short (*S. multijuga*) or long filament (*S. macranthera*) and a long anther oriented toward the gynoecium (Fig. 2B, arrow c), and (3) a group of two latero-abaxial stamens (Fig. 2B, arrow d) with long filaments and long anthers oriented toward the outermost adjacent somewhat curved petal (Fig. 2B, arrow f). Pollen is powdery and dry and is the only resource. The gynoecium is flexible, somewhat crooked and longer than the two latero-abaxial stamens (Fig. 2B, arrow e, Tab. 2). Flowers of both species show monomorphic enantiostyly (i.e., mirror-image flowers) (Fig. 2C, D).

The Papilionoideae, represented herein by *Andira fraxinifolia*, *Lonchocarpus cultratus* and *Pterocarpus violaceus*, also have zygomorphic flowers but less conspicuous (Fig. 2E-G). The gynoecium/androecium set is concealed within the keel petals. The staminal filaments are fused to form a tube, which ends with longitudinally dehiscing anthers. The style and the staminal tube follow the keel's curvature

Figure 2. Floral traits of eight melittophilous Leguminosae tree species, in the Atlantic Forest in Southeast Brazil. **A-D.** Caesalpinioid legume species. **A.** Flower of *Schizolobium parahyba*. **B-D.** Flowers of *Senna* species. **B.** *S. macranthera*; a= staminodes, b= medium-central stamens, c= central-abaxial stamen, d= latero-abaxial stamens, e= gynoecium, f= cucullate petal. **C-D.** Flowers of *S. multijuga* showing the pronounced asymmetry and enantiostily, the reproductive structures are positioned on opposite sides in relation to the center of the flower. **E-F.** Strongly zygomorphic Faboid flowers, with the connate petal standard and keeled petal asymmetry. **E.** *Andira fraxinifolia*. **F.** *Lonchocarpus cultrathus*, **G.** *Pterocarpus violaceus*. **H-I.** Faboid flowers with brush morphology and dimorphic androecium. **H.** *Swartzia oblata*, I. *S. simplex*.

upwards, and anthers and stigma are positioned near the keel apex. Nectar is the main resource, accumulating around the base of the gynoecium and confined within the chamber formed by the fusion of the filaments, and can be reached only by small openings on the base of the upper side of the staminal tube, whose access is blocked by the claw of the standard petal.

The remaining Papilionoideae, *Swartzia oblata* and *S. simplex,* also have zygomorphic flowers with a single upright conspicuous petal (Fig. 2H, I). The androecium is composed of two groups of dimorphic stamens with longitudinally dehiscing anthers: one group comprises a brush of ca. 100 short stamens oriented to the upper side of the flower, whilst the other group, oriented to the lower side of the flower, consists of four longer stamens in *S. oblata* and six in *S. simplex*. These long stamens accompany the curvature of the pistil and are rigid in *S. oblata* and flexible in *S. simplex* (Fig. 2H, I). Pollen is the only resource.

Reproductive system

Pollen viability of the seven species investigated was high (exceeding 80 %) and included pollen from the various stamens types of the four heterantherous species representing *Senna* and *Swartzia* (Tab. 1). The results of pollination experiments to examine the breeding system for the seven legume species are presented in Table 1. These species are most likely self-incompatible since no fruits developed in flowers bagged for autonomous selfing, hand self-pollinated and emasculated flowers. The fruit set in the four species with hand cross-pollinated flowers was higher than those obtained from natural conditions. Although a large number of flowers were randomly tagged to evaluate fruit set in natural conditions, the fruit set formed was low in plants of all studied species (Tab. 1).

Floral visitors

The flowers of these woody Leguminosae were visited by 50 bee species (Figs. 3-5). Among the four bee families recorded, Apidae (39 spp.) presented the highest number of species, followed by Halictidae (six spp.), Megachilidae (four spp.), and Andrenidae (one spp.) (Tab. 3).

Percentages of bee size categories were as follow: 8.0 % (n = 4) very small, 44.0 % (n = 22) small, 22.0 % (n = 11) medium-sized and 26.0 % (n = 13) large bees. Apidae represented the majority of bee visitors, constituting 78 % (n = 39) of all recorded species, and 56.4 % of them were medium to large-sized species. Among the medium to large-sized bees, the Centridini were the most represented group (12 species). The species acting as pollinators were mainly represented by medium to large-sized bees (Tab. 3).

Xylocopa frontalis visited more plant species (n = 7) than any other bee species, whereas 19 species visited two to three species, six visited four to five species and another 24 visited only one species (Fig. 5). Species of the genera *Bombus*, *Centris*, *Epicharis*, *Eufriesea*, *Eulaema*, *Xylocopa* and *Oxaea* were buzz-collectors on flowers of *Senna* species with poricidal anthers and were also observed to collect pollen by vibrating flowers with longitudinally dehiscing anthers in the two *Swartzia* species. These bees were the main visitors of most of the species with medium to large-sized flowers (Tab. 3). Smaller bee species of the genera *Thygater*, *Megachile*, *Ceratina, Exomalopsis,* as well as Meliponina and Halictidae visited mainly the flag-shaped flowers and were the principal visitors of *Andira fraxinifolia*, *Lonchocarpus cultratus* and *Pterocarpus violaceus* (Tab. 3). The bee visitors of the three former genera searched mostly for nectar, while Meliponina and Halictidae visited these flowers largely for pollen.

The highest number of bee species (n = 17) was recorded on *Swartzia* species with (33.3 % of the total observed), followed by *S. parahyba* (n = 15, 30.0 %), *S. multijuga* (n = 14, 25.5 %), *Andira fraxinifolia* (n = 12, 23.5 %), *L. cultratus* (n = 10, 19.6 %), and by *Pterocarpus violaceus* and *Senna macranthera*, both with eight species (Fig. 5). The mean number of flowers visited by an individual bee during a foraging bout to *Senna macranthera* was 8 ± 8.77 flowers (n = 648) by *Xylocopa frontalis,* 3.3 ± 4.3 flowers (n = 40) by *Centris decipiens* and 11.5 ± 12.2 flowers (n = 92) by *Eulaema cingulata*; to *Schizolobium parahyba* the mean number was 13.22 ± 14.64 flowers (n = 357) by *Xylocopa frontalis*, 8.42 ± 8.5 flowers (n = 539) by *Centris labrosa*, 10.21 ± 8.49 flowers (n = 245) by *C. varia* and 7.81 ± 7.16 flowers (n = 258) by *Megachile* sp. 1; to *Swartzia oblata* the mean number was 14.3 ± 13.10 flowers (n = 186) by *Epicharis flava*, 8.0 ± 5.32 flowers (n = 104) by *Eulaema seabrai* and 9.37 ± 5.57 flowers (n = 72) by *E. flava*; and to *Swartzia simplex* the mean number was 7.75 ± 10.09 flowers (n = 256) by *X. frontalis* and 6.36 ± 8.66 flowers (n = 140) by *Eulaema cingulata*. Most of the other species also made subsequent visits to several flowers of the same individual plant, which is related to the high number of open flowers available at the same time.

Pollination mechanisms

In *Schizolobium parahyba* bees landed on the gynoecium-androecium set and to access the nectar, bees pressed with their head at the claw of the standard petal and the base of the stamens, causing the pollen to adhere to the ventral portion of their body, where the stigma would also be touched (Fig. 3A-C). *Centris labrosa*, *Megachile* sp. indet. 1 and *Xylocopa frontalis* were the main pollinators of *S. parahyba* and showed similar behavior during all visits (Fig. 3A-C). Other medium and large-sized bees also acted as pollinators of *S. parahyba* flowers (Tab. 3), but their visits were very scarce. Smaller bees visited the flowers for pollen but worked the anthers one by one with their forelegs and rarely touched the stigma.

The flowers of *Senna macranthera* and *S. multijuga* have a similar morphology and pollination in these species was demonstrated as quite similar. Bee visitors landed on the stamen groups (Fig. 3D-I), gripped the filaments and gynoecial base and then vibrated it. Consequently, the anthers of the central group and of the central-abaxial stamens ejected pollen on the ventral surface of the bees and on the internal surfaces of all legs (pollen for bee collection), while pollen of the two latero-abaxial stamens, mostly for pollination, was ejected on the dorsal side of the thorax of *S. macranthera* visitors (Fig. 3D-F, this spot indicated by *), as well as in the groove between the thorax and abdomen (Fig. 3G, arrow), on the forewings (Fig. 3H, arrow) and on the dorsal side of the thorax (Fig. 3I, arrow) of visitors to *S. multijuga*.

In *Senna macranthera* the gynoecium is quite long, so that the stigma remains distant from the bee's body (Fig. 3D-F, arrows). During field observations no direct contact with

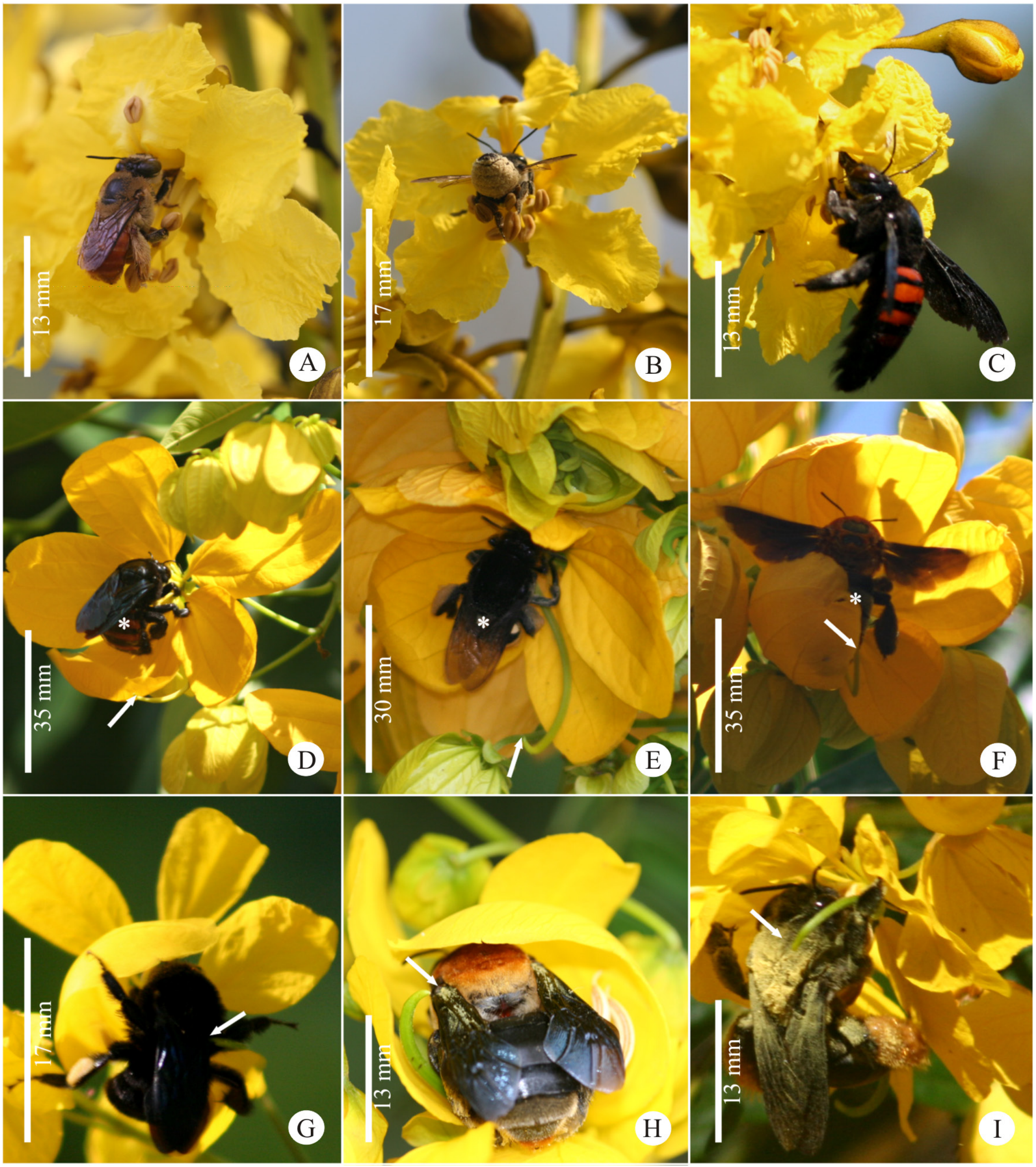

Figure 3. Pollinators and visitors of melittophilous Caesalpinioideae tree species in the Atlantic Forest in Southeast Brazil. **A-C.** Main pollinators of *Schizolobium parahyba*: to access the nectar the bees land on the androecium-gynoecium set contacting anthers and stigma simultaneously. **A.** *Centris labrosa*. **B.** *Megachile* sp. indet 3. **C.** *Xylocopa frontalis*. **D-F.** Pollinators of *Senna macranthera* collect pollen by vibration. **D.** *Xylocopa frontalis*. **E.** *Eulaema seabrai*. **F.** *Centris decipiens*. In flowers of *S. macranthera* the stigma (arrows) is distant from the anthers; thus, during visits contact with the stigma was rarely observed. * = Place where pollen is deposited. **G-I.** Pollinators of *Senna multijuga*. **G.** *Bombus morio*. **H.** *Centris* (*Melacentris*) sp. indet 2. **I.** *Epicharis flava*. The stigma touches the back or the sides of the bee's thorax, precisely at the places where the pollen is deposited (arrows).

Figure 4. Pollinators and visitors of melittophilous Faboideae tree species in the Atlantic Forest in Southeast Brazil. **A-C.** Bees visiting flowers of *Andira fraxinifolia*. **A.** *Xylocopa frontalis* is a pollinator; while it takes nectar, the stigma touches the ventral side of its thorax. **B.** *Melipona* sp. indet 2 is a nectar thief. **C.** *Plebeia* sp. indet 1 is a pollen thief. **D-F.** Bees visiting *Swartzia oblata* flowers. **D.** *Eulaema seabrai* is a pollinator and collects pollen by vibration. **E.** *Exomalopsis* sp. 3. **F.** *Augochloropsis* sp. indet 2. Both species are pollen thieves, vibrating only the upper anthers and not touching the stigma (arrows).

the receptive stigmatic surface was noticed, even when the largest bees visited. However, it is likely that the stigma does touch the dorsal side of the bee (Fig. 3D-F, place indicated by *), where pollen from the two latero-abaxial stamens is deposited. This can occur because the flexible gynoecium oscillates during the visitor's vibration and may touch the dorsal side of the bee or when the bee withdraws from the flower. The large and robust bee species of *Xylocopa*, *Eulaema* and *Centris* (see Tab. 3 for body length) are most likely to make contact with the stigma during the oscillation of the gynoecium, and it is assumed that these larger bees are the effective pollinators. Pollen of the latero-abaxial stamens of *S. multijuga* flowers is deposited only at the places of the bee's body that actually touch the stigma (Fig. 3G-I). The bees *Bombus morio*, *Xylocopa brasilianorum* and *Centris* sp. indet. 2 were the most frequent visitors and the main pollinators of this plant species (Tab. 3). Other medium and large-sized bees also acted as pollinators of *S. multijuga*, but their visits were much scarcer. Individuals of *Augochloropsis* sp. indet. 3 collected pollen by vibration on *Senna* species; however, they vibrated the anthers one by one and were not observed contacting the stigma.

On flowers of the Papilionoideae (with exception of *Swartzia* species), bees landed on the corolla wing-keel complex, and directed their heads between the claw of the flag and the basal portion of the staminal tube where they extended the proboscis through small openings to reach the nectar. Thus, the flower keel would move down, exposing anthers and stigma, which then touch the ventral side of the bee's abdomen. After the bee departed, the petals would return to their original orientation, characteristic features of the valvular mechanism of pollen presentation. The major pollinators of the Papilionoideae representatives visited the flowers only for nectar: *Andira fraxinifolia* was pollinated by medium-sized bee species of *Centris* and *Megachile* and large-sized *Xylocopa* (Fig. 4A); *Lonchocarpus cultratus* was

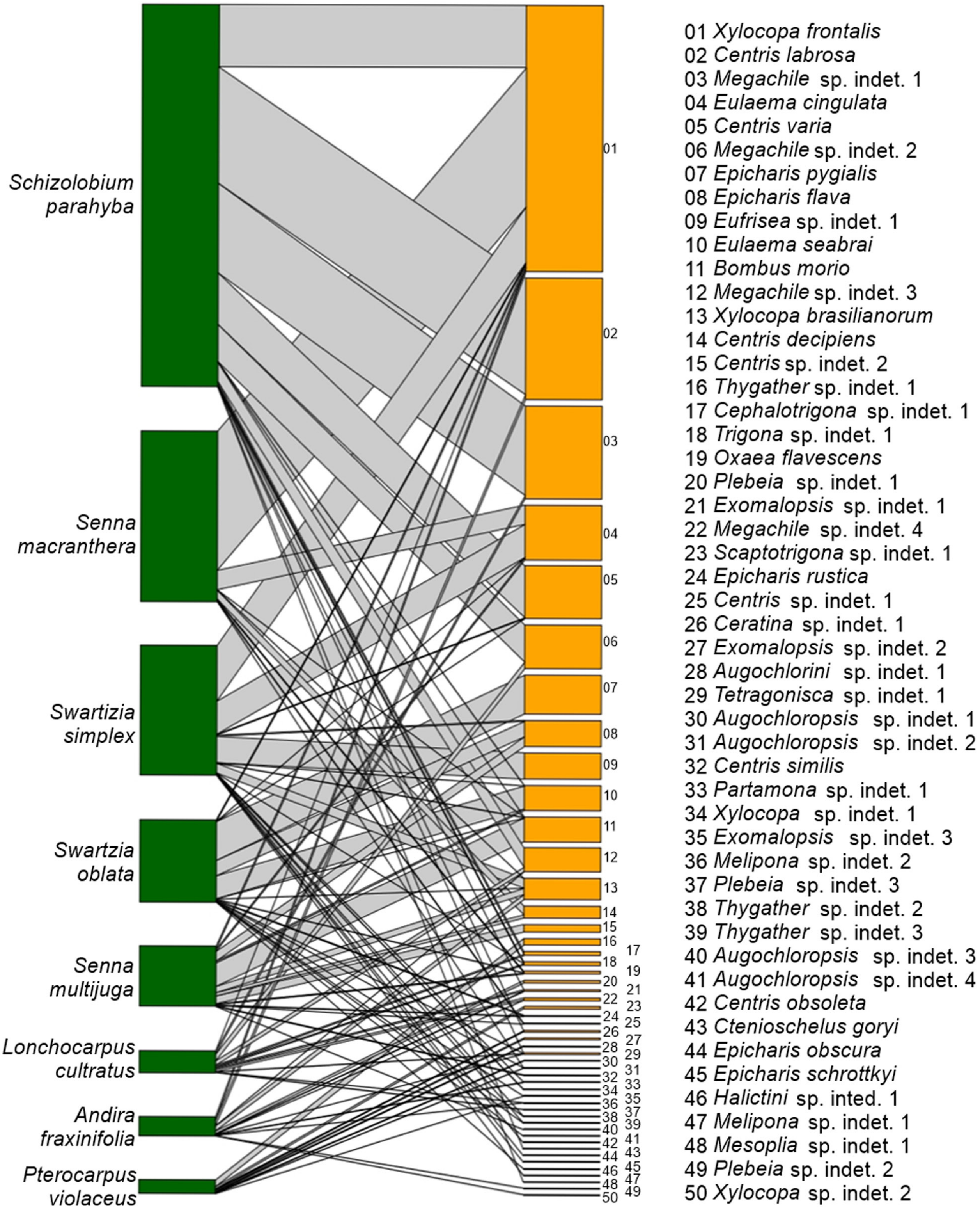

Figure 5. Melittophilous legume trees and their bee visitors' quantitative network in the Atlantic Forest in Southeast Brazil. Plants are represented in green and bees in orange. The links represent the frequency of interactions, while the size of the box represents the relative amount of visits received by the plants or made by the bees.

Table 3. Bee species recorded on eight melittophilous Leguminosae tree species in the Atlantic Forest in Southeast Brazil. Bees body size: very small bee (vs), small bee (sb), medium-sized bee (mb), large bee (lb). The letters in brackets after the plant names indicate its pollinators (p) and their frequency of visits: rare (r), few frequent (ff), frequent (f), very frequent (vf), frequency not registered (fn).

Bee species	Body size (mm)	Plant species
Andrenidae		
Oxaea flavescens Klug, 1807	17.4 mb	*Senna macranthera, Senna multijuga* (p, r), *Swartzia oblata, Swartzia simplex*
Apidae		
Bombus (Fervidobombus) morio (Swederus, 1787)	16.1 mb	*Schizolobium parahyba* (p, fn), *Senna macranthera, Senna multijuga* (p, vf), *Swartzia oblata* (p, fn), *Swartzia simplex*
Centris (Ptilotopus) decipiens Moure & Seabra, 1960	21.7 lb	*Senna macranthera* (p, ff), *Senna multijuga* (p, r)
Centris (Heterocentris) labrosa Friese, 1899	13.2 mb	*Andira fraxinifolia* (p, fn), *Schizolobium parahyba* (p, vf)
Centris (Trachina) similis (Fabricius, 1804)	16.6 mb	*Senna multijuga* (p, vf)
Centris (Melacentris) sp. indet 1	23.7 lb	*Senna macranthera* (p, r), *Senna multijuga* (p, r)
Centris (Melacentris) sp. indet 2	23.0 lb	*Senna multijuga* (p, fn)
Centris (Melacentris) obsoleta Lepeletier, 1841	20.8 lb	*Swartzia simplex*
Centris (Centris) varia (Erichson, 1848)	15.0 mb	*Schizolobium parahyba* (p, ff), *Swartzia oblata, Swartzia simplex*
Cephalotrigona sp. indet 1	9.0 sb	*Lonchocarpus cultratus*, *Schizolobium parahyba*
Ceratina sp. indet 1	9.0 sb	*Pterocarpus violaceus*, *Schizolobium parahyba*
Ctenioschelus goryi (Romand, 1840)	14,5 mb	*Schizolobium parahyba* (p, r)
Epicharis (Epicharana) flava (Friese, 1900)	22.3 lb	*Senna multijuga* (p, f), *Swartzia oblata* (p, f), *Swartzia simplex*
Epicharis (Triepicharis) schrottkyi (Friese, 1900)	21.1 lb	*Swartzia simplex*
Epicharis (Epicharitides) obscura (Friese, 1899)	13.3 mb	*Swartzia simplex*
Epicharis (Epicharana) rustica (Olivier,1789)	22.2 lb	*Senna multijuga* (p, f), *Swartzia oblata* (p, r)
Epicharis (Epicharana) pygialis (Friese, 1900)	20.6 lb	*Swartzia oblata* (p, vf), *Senna multijuga* (p, fn)
Eufriesea sp. indet 1	18.4 mb	*Swartzia simplex*
Eulaema (Apeulaema) cingulata (Fabricius, 1804)	22.4 lb	*Senna macranthera, Senna multijuga* (p, r), *Swartzia oblata* (p, r), *Swartzia simplex*
Eulaema (Eulaema) seabrai Moure, 1960	22.8 lb	*Schizolobium parahyba* (p, r), *Senna multijuga* (p, r), *Swartzia oblata* (p, f), *Swartzia simplex*
Exomalopsis sp. indet 1	6.0 sb	*Andira fraxinifolia, Lonchocarpus cultratus* (p, ff), *Schizolobium parahyba*
Exomalopsis sp. indet 2	6.1 sb	*Pterocarpus violaceus*
Exomalopsis sp. indet 3	6.1 sb	*Pterocarpus violaceus*, *Swartzia oblata*
Melipona sp. indet 1	7.0 sb	*Swartzia simplex*
Melipona sp. indet 2	9.6 sb	*Andira fraxinifolia, Lonchocarpus cultratus* (p, r)
Mesoplia sp. indet 1	12.7 mb	*Schizolobium parahyba* (p, r)
Partamona sp. indet 1	6.5 sb	*Andira fraxinifolia*, *Swartzia simplex*
Plebeia sp. indet 1	4.4 vs	*Andira fraxinifolia*
Plebeia sp. indet 2	4.3 vs	*Andira fraxinifolia*
Plebeia sp. indet 3	4.4 vs	*Lonchocarpus cultratus*
Scaptotrigona sp. indet 1	6.0 sb	*Pterocarpus violaceus*
Tetragonisca sp. indet 1	4.0 vs	*Andira fraxinifolia*, *Lonchocarpus cultratus*, *Schizolobium parahyba*, *Swartzia oblata*
Thygater sp. indet 1	9.3 sb	*Pterocarpus violaceus* (p, f)
Thygater sp. indet 2	12.0 sb	*Swartzia oblata*, *Swartzia simplex*
Thygater sp. indet 3	12.0 sb	*Swartzia oblata*, *Swartzia simplex*
Trigona sp. indet 1	6.0 sb	*Lonchocarpus cultratus*, *Schizolobium parahyba*, *Swartzia simplex*
Xylocopa (Neoxylocopa) brasilianorum (Linnaeus, 1767)	20.3 lb	*Andira fraxinifolia* (p, fn), *Senna macranthera, Senna multijuga* (p, f), *Swartzia oblata* (p, fn), *Swartzia simplex*
Xylocopa (Neoxylocopa) frontalis (Oliver, 1789)	26.1 lb	*Andira fraxinifolia* (p, fn), *Lonchocarpus cultratus* (p, r), *Schizolobium parahyba* (p, f), *Senna macranthera* (p, vf), *Senna multijuga* (p, ff), *Swartzia oblata* (p, r), *Swartzia simplex* (p, vf)
Xylocopa sp. indet 1	18.0 mb	*Swartzia simplex*
Xylocopa sp. indet 2	25.0 lb	*Andira fraxinifolia* (p, fn)
Halictidae		
Augochlorini sp. indet 1	7.2 sb	*Pterocarpus violaceus*
Augochloropsis sp. indet 1	8.6 sb	*Pterocarpus violaceus*
Augochloropsis sp. indet 2	7.4 sb	*Pterocarpus violaceus*, *Swartzia oblata*, *Senna macranthera*
Augochloropsis sp. indet 3	7.8 sb	*Senna multijuga*
Augochloropsis sp. indet 4	7.2 sb	*Swartzia oblata*

Table 3. *Cont.*

Bee species	Body size (mm)	Plant species
Halictini sp.	10.0 sb	*Swartzia oblata*
Megachilidae		
Megachile sp. indet 1	11.0 sb	*Lonchocarpus cultratus* (p, ff), *Schizolobium parahyba* (p, f)
Megachile sp. indet 2	14.3 mb	*Lonchocarpus cultratus* (p, ff), *Schizolobium parahyba* (p, ff)
Megachile sp. indet 3	10.0 sb	*Andira fraxinifolia* (p, fn), *Lonchocarpus cultratus* (p, f), *Schizolobium parahyba* (p, ff)
Megachile sp. indet 4	11.0 sb	*Andira fraxinifolia* (p, fn)

pollinated by small and medium-sized species of *Megachile*; and *Pterocarpus violaceus* was pollinated mainly by *Thygater* sp. indet. 1. Small bees are not heavy enough to depress the keel and exposing anthers and stigma; however, some of them will incidentally collect pollen (since the keel petals are loosely joined), and they sometimes touch the stigma. Social bees such as *Partamona*, *Plebeia*, *Scaptotrigona*, and *Tetragonisca* were mainly pollen and nectar thieves (Fig. 4B, C).

Flowers of *Swartzia oblata* and *S. simplex* are morphologically similar, and the pollination mechanism is also very similar. The bees land on the upper stamen group (Fig. 4D), grip and vibrate it. The liberated pollen was deposited on the ventral portion of the bee's body (the bees collected pollen actively only from these stamens), whilst from contact with the anthers of the lower stamens pollen was deposited at the end of the dorsal part of the bee's abdomen, precisely at the place where the stigma touched the bees. *Swartzia oblata* pollinators were medium and large-sized species (Fig. 4D), with *Epicharis pygialis* and *Eulaema seabrai* the most frequent visitors (Tab. 3, Fig. 5). Small bee species (Tab. 3) collected pollen only and made no contact with the stigma (Fig. 4E, F, arrows indicate stigmatic position). *Swartzia simplex* pollinators were most likely large bees, such as *Xylocopa frontalis*, since the gynoecium and the lower stamens are quite long, more than 20 mm (Tab. 2, Fig. 2I) the stigma and the anthers are distant from the upper stamen group where the bees land. Even the large individuals of *X. frontalis*, as long as 26.1 mm, were not observed making contact with the reproductive organs but contact may occur when the bees leave the flower or during vibration, as the gynoecium and the lower stamens are quite flexible as in of *Senna macranthera* flowers. Medium-sized and smaller bees primarily function as pollen thieves of *S. simplex*.

Discussion

The legumes trees studied here were of the cornucopia-flowering type, except for *Swartzia oblata*, and flowered only once a year, mainly during the wet season. The flowers offer nectar and/or pollen as resources to bee pollinators as well as to thieves; however through complex mechanisms at the flower level. All the legume species presented some incompatibility, which means that the interaction with pollinators is a necessary condition to their reproduction. Taking together, these results suggest that legume trees are important resources to bees and vice-versa, and that this interaction may benefit other plants at the Atlantic Forest. The specific discussion about each reproductive aspect of these species is presented below.

Floral phenology, morphology, and biology

The eight legume species presented a distinct annual and seasonal flowering phenophase, as described for a large number of Atlantic Forest trees (Morellato *et al.* 2000). High flowering synchrony at population level has been recorded for many other Atlantic Forest trees from the same locality (Talora & Morellato 2000; Bencke & Morellato 2002) and it is presumably an important strategy to attract pollinators and promote outcrossing (Rathcke & Lacey 1985; Kudo 2006). The apparent sequential flowering seasons, as recorded for the species in this study, are most likely important for the maintenance of autochthonous bees in the community, and this phenomenon has been previously documented in tropical communities (see Frankie *et al.* 1983; Rathcke & Lacey 1985; Newstrom *et al.* 1994b; Buzato *et al.* 2000; Freitas & Sazima 2006). Most species (*Swartzia oblata* was the only exception) showed the cornucopia flowering, a pattern common in other lowland tropical rainforests (Gentry 1974). By producing many flowers at once and flowering sequentially, these plants may indirectly facilitate each other over the year by maintaining their main pollinators. The same process has already been proposed for plants pollinated by hummingbirds in tropical landscapes (Stiles 1977; Buzato *et al.* 2000).

The floral attributes of the studied species show the distinctive traits associated with melittophily (Faegri & Pijl 1979), and, papilionoid flag-shaped flowers with hidden nectar or pollen resource are typical examples of bee-flowers (Endress 1994). The restricted access to the nectar of the flag-shaped flowers studied here is a general tendency in many Faboideae species pollinated by bees (Arroyo 1981; Westerkamp 1997; Etcheverry *et al.* 2003; Brito *et al.* 2010). Hidden nectar is also found in some Caesalpinioideae, such as *Schizolobium parahyba* and other bee-pollinated species in which access to nectar is prevented by floral structures (Lewis & Gibbs 1999; Cocucci *et al*. 1992). Floral bilateral symmetry combined with concealment of nectar requires the ability and appropriate mouthparts to reach the resource (Pellmyr 2002). This kind of floral morphology can thus limit the spectrum of visitors able to forage on flowers and may work as a strategy for effective pollination.

Pollen deposition on different areas of the pollinator's body in the studied *Senna* and *Swartzia* species is a result of heteranthery, an attribute already recorded for other *Swartzia* species (Lopes & Machado 1996; Moço & Pinheiro 1999), in various genera of the tribe Cassiinae (Dulberger 1981; Gottsberger & Silberbauer-Gottsberger 1988; Westerkamp 2004) and in at least 20 families (Endress 1994; Vallejo-Marín *et al*. 2010). Heteranthery, resulting in functional separation of pollen for feeding and pollen for pollination, has been considered an evolutionary response to assure pollination while feeding voracious foraging bees (Vogel 1978; Buchmann 1983; Luo *et al*. 2008; Vallejo-Marín *et al*. 2009; 2010; Papaj *et al*. 2017). In such flowers, pollen for pollination is deposited where the bee cannot groom it easily but is placed in the correct position where the stigma touches the bee (Buchmann 1983; Luo *et al*. 2008). Pollen release in the studied *Senna* species herein occurred in directed pollen jets; food pollen for bee collection is deposited on the ventral side of the bee, whereas pollen destined for pollination reached the upper parts of the bee's body, probably after being "ricocheted" from a petal, as described for other Cassiinae (Westerkamp 2004; Amorim *et al*. 2017).

The elongated gynoecium in *Senna macranthera* and *Swartzia simplex* keeps the stigma away from the flower center. This arrangement allows free access of the pollinator to the feeding anthers and may protect the style and stigma from possible damage during vibration by the heavy-bodied visitor (Dulberger 1981). Furthermore, in these species the gynoecium greatly exceeds the size of the largest bees; thus, the pollination may depend on incidental contact with the stigma and the bee's body. Such contact with the stigma was also rarely recorded for *Senna affinis* (Gottsberger & Silberbauer-Gottsberger 1988), a species with a floral morphology similar to *S. macranthera*.

The monomorphic enantiostyly in the studied *Senna* species follows the pattern already well documented in Cassiinae species (Gottsberger & Silberbauer-Gottsberger 1988; Westerkamp 2004). Such trait is directly associated with poricide anthers and heteranthery in pollen rewarding flowers trough the evolutionary history of angiosperms (Buchmann 1983; Vallejo-Marín *et al*. 2010). It has been shown that monomorphic enantiostyly reduces the amount of geitonogamy since the style deflection would allow pollination of half the flowers in the same individual plant (Jesson & Barrett 2002; 2005). However, the enantiostilic *Senna* species also presented a self-incompatible system (see below). In other words, geitonogamy is not a possibility in this species. The correlated evolution of monomorphic enantiostyly and a self-incompatible system in these plants points to the possibility of other evolutionary causes for style deflection in both directions in the same individual as the reduction of pollen interference and the reaching of pollen safety sites in the bee's body (Dulberger 1981; Westerkamp 2004; Koch *et al*. 2017).

Reproductive system

The high pollen viability in both the feeding anthers and the pollinating anthers in the *Senna* and *Swartzia* species has been recorded for other species of these genera (Lopes & Machado 1996; Carvalho & Oliveira 2003; Laporta 2005; Luo *et al*. 2009), but not in *Senna alata* and *S. bicapsularis* in which the pollinating anthers had higher proportions of viable pollen than the feeding anthers (Luo *et al*. 2009).

Since no fruits developed after spontaneous and hand self-pollination in the studied species, they probably are self-incompatible. Self-incompatibility has been suggested as prevalent in tropical trees (Bawa 1974; Bawa *et al*. 1985b). In fact, several tropical Leguminosae tree species, such as *Swartzia pickelli* (Lopes & Machado 1996) and *Swartzia apetala* (Moço & Pinheiro 1999) in the north-eastern Atlantic rainforest, *Pseudopiptadenia contorta* (Prata-de-Assis-Pires & Freitas 2008) and *S. multijuga* (Wolowski & Freitas 2010) in a montane Atlantic rainforest, *Senna silvestris* (Carvalho & Oliveira 2003) and *Copaifera langsdorffi* (Freitas & Oliveira 2002), both in the Brazilian savanna, and *Caesalpinia calycina* (Lewis & Gibbs 1999) in the caatinga, have been demonstrated as self-incompatible. However, since the number of treated flowers and individuals in this study was low, more experiments are necessary to detect the mating-system of these species. Furthermore, mixed mating was also reported for *S. multijuga* (Ribeiro & Lovato 2004).

The low fruit set in natural conditions of the studied species may be because of self-deposition due to high floral constancy and behavior of pollinators, which remain for long periods foraging on the same individual plant promoting self-pollination followed by stigma clogging and pollen discount (Wilcock & Neiland 2002). Low fruit set under natural conditions and/or following hand cross-pollinations has been reported in many trees of the legume and other families (Bawa & Webb 1984; Bawa & Bullock 1989; Lopes & Machado 1996; Jausoro & Galetto 2001; Freitas & Oliveira 2002; Carvalho & Oliveira 2003; Prata-de-Assis-Pires & Freitas 2008).

Floral visitors

The medium to large-sized bees observed in this study have been reported as pollinators of several other legume species with medium and large-sized flowers: Euglossina species are the main pollinators of *Swartzia pickelli* (Lopes & Machado 1996), and *Xylocopa* and Centridini bees of

Swartzia apetala (Moço & Pinheiro 1999) and of several *Senna* species (Carvalho & Oliveira 2003), as well as several Cassiinae species with pollen flowers (Dulberger 1981; Gottsberger & Silberbauer-Gottsberger 1988). These cohorts of buzz-collectors have also been reported as pollinators of several Caesalpinioideae with nectar flowers (Cocucci *et al.* 1992; Lewis & Gibbs 1999), and most of them are important pollinators of many canopy tree species of Neotropical forests (Frankie *et al.* 1983; Bawa 1985a; 1990).

The medium to large-sized bees that pollinate the studied species are known to forage over long distances (Frankie *et al*. 1976; Janzen 1971), a behavior that ensures pollen flow among conspecifics which is essential for fruit set in these self-incompatible legume species. On the other hand, subsequent visits to several flowers of the same individual plant of this study is a foraging behavior promoting self-pollination, and possibly related to low fruit-set on mass flowering self-incompatible species (Augspurger 1980; Baker *et al*. 1983).

Pollination of most of the studied legume species including those with small flowers by the large bee *Xylocopa frontalis* is a feature of the genus whose individuals have a physical morphology that allows a good positional fit between floral parts of many kinds of flowers. Due to this aspect and generalist behavior, these bees have frequently been reported among pollinators of many Neotropical species (Arroyo 1981 and reference therein; Roubik 1989), not only by those grouped in the *Xylocopa*-flower syndrome (Pijl 1954).

The bees recorded on *Senna* (poricidal anthers) and *Swartzia* (longitudinal anthers) species are known to vibrate flowers to collect pollen. Although the buzzing behavior on poricidal anthers is well understood (Buchmann 1983; Luca & Vallejo-Marín 2013), it is still not obvious why bees also buzz flowers with longitudinal anthers. However as buzzing bees can quickly extract a large proportion of pollen from longitudinal anthers the benefit of collecting more pollen per flower may have favored this behavior (see Buchmann 1985 for commentaries).

Pollination mechanisms

Schizolobium parahyba, one of the less specialized Caesalpinioideae studied here, is the only species of this family that produce nectar and the flowers show some degree of nectar protection. However, the nectar is easily available since the flowers do not require special manipulation. Therefore, these characteristics may favor the higher visitor richness in this species, which encompass specialized and non-specialized pollinators (Arroyo 1981; Brito *et al.* 2017), although restricted to large- and medium-sized bee species. On the other hand, since *S. parahyba* is one of the first species in flower after the winter months, the high number of pollinators may also be related to scarcity of other flowering species, with exception of the flowering events of some sympatric Malpighiaceae species in the vicinity whose flowers were visited by the same *Centris* bee species (Sazima *et al.* 2009).

The other Caesalpinioideae of this study, the pollen–flowers of *Senna macranthera* and *S. multijuga*, are species in which resource protection and pollinator selection is evident. Pollen is the only resource, and to obtain it from the different types of poricide anthers, the special extraction technique of vibrating - the buzz-pollination syndrome (Buchmann 1983; Vallejo-Marín *et al.* 2010) - is required. In these species, the pollination mechanism seems to require equivalence between the size of bees and the size of the flowers, as may be illustrated by *S. macranthera*, with large flowers that are pollinated by the largest bees, in contrast to the smaller flowers of *Senna multijuga*, which are pollinated by large- and medium-sized bees.

In the nectar-offering flowers of *Andira fraxinifolia*, *Lonchocarpus cultratus* and *Pterocarpus violaceus*, nectar and pollen are both concealed and are becoming available only after tripping, a mechanism that also requires specialized pollinators (Arroyo 1981). Among the various mechanisms of staminal column release, the studied species had the valvular mechanism (Delpino 1868; Westerkamp 1997) since the wing and keel petals are entirely free. Some large but mainly medium-sized bee species were the only pollinators capable of successfully working these papilionoid flowers.

Pollen harvesting with the buzz-pollination mechanism from anthers with longitudinal dehiscence as observed in the pollen-flowers of *Swartzia oblata* and *S. simplex* is already known for several other species with clumped brush-like stamens with non-poricidal anthers (Buchmann 1985; Lopes & Machado 1996 and references therein). Again, the pollination of these species shows a relationship between the size of the bees and the flowers; *S. oblata* flowers have both large- and medium-sized bees as pollinators, whereas the larger flowers of *S. simplex* are probably only effectively pollinated by the largest *Xylocopa* species. Bees of similar size categories were also recorded as the main pollinators of *S. pickelli* and *S. apetala* (Lopes & Machado 1996; Moço & Pinheiro 1999).

Populations that flower annually in a sequence during part of the year and bloom copiously are essential for the maintenance of autochthonous bees in the Atlantic Forest. Floral traits of the legume tree species studied were clearly melittophilous and require specialized medium to large-sized bees to access the resources. Furthermore, self-incompatibility in these legume species indicates the need for partners for reproduction; thus, the pollinators are of utmost importance. Although several bee visitors were recorded on the flowers of the studied species, the number of pollinator species was very low for the majority of the plant species. Despite their relative abundance, behavior and adequate morphology, few or only one pollinator species was prevalent. In addition, legume species represent an important food source for many Centridini and Euglossina bee species that are related to various specialized interactions of other plant species in the Atlantic Forest. Habitat disturbance has been responsible for significant decreases in bee diversity (Frankie *et al*. 2009), with direct impact on plant mating systems (Ward *et al*. 2005), and can be a cause for loss of biodiversity in several ecosystems. The Atlantic Forest, one of the world's most threatened biodiversity hotspots (Myers *et al*. 2000), has been reduced to less than eight percent of its original area, and the natural vegetation remaining (in scattered fragments) is heavily affected by anthropogenic activities (Morellato & Haddad 2000). This study has provided valuable information regarding the interactions of bee species and legume tree species in the Atlantic Forest that can be utilized toward the future conservation of both pollinators and plants.

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