

RESEARCH PAPER

Pollination in *Jacaranda rugosa* (Bignoniaceae): euglossine pollinators, nectar robbers and low fruit set

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ABSTRACT

Nectar robbers access floral nectar in illegitimate flower visits without, in general, performing a pollination service. Nevertheless, their effect on fruit set can be indirectly positive if the nectar removal causes an incremental increase in the frequency of legitimate flower visits of effective pollinators, especially in obligate outcrossers. We studied pollination and the effect of nectar robbers on the reproductive fitness of *Jacaranda rugosa*, an endemic shrub of the National Park of Catimbau, in the *Caatinga* of Pernambuco, Brazil. Xenogamous *J. rugosa* flowers continuously produced nectar during the day at a rate of $1 \mu\text{l}\cdot\text{h}^{-1}$. Female and male *Euglossa melanotricha* were the main pollinators. Early morning flower visits substantially contributed to fruit set because stigmas with open lobes were almost absent in the afternoon. Ninety-nine per cent of the flowers showed damage caused by nectar robbers. Artificial addition of sugar water prolonged the duration of flower visits of legitimate flower visitors. Removal of nectar, simulating the impact of nectar robbers, resulted in shorter flower visits of euglossine bees. While flower visits of nectar-robbing carpenter bees (*Xylocopa frontalis*, *X. griseocens*, *X. ordinaria*) produced only a longitudinal slit in the corolla tube in the region of the nectar chamber, worker bees of *Trigona spinipes* damaged the gynoecium in 92% of the flowers. This explains the outstandingly low fruit set (1.5%) of *J. rugosa* in the National Park of Catimbau.

INTRODUCTION

Nectar robbing is a common phenomenon in species with narrow flower tubes and nectar chambers (Gentry 1980; Inouye 1980; Higashi *et al.* 1988; Morris 1996; Traveset *et al.* 1998; Irwin & Brody 1999; Maloof & Inouye 2000; Lara & Ornelas 2001, 2002; Maloof 2001; Irwin & Maloof 2002). Nectar robbers access floral nectar in illegitimate flower visits by means that do not use the entrance of the flower. In general, nectar robbers do not perform a pollination service and are found among birds, bees, ants, wasps and butterflies (Faegri & van der Pijl 1979; Inouye 1980). Nevertheless, in a few cases, nectar robbers may also be pollinators (Higashi *et al.* 1988; Navarro 2000). Primary nectar robbers gnaw a hole, perforate or cut the perianth from outside to reach the floral resources, while secondary nectar robbers use the perforations made by primary nectar robbers to suck nectar (Inouye 1980).

The effect of nectar robbing on the reproductive success of associated plants has interested many researchers. Depending on the species of nectar robber and on the morphology of the flower/inflorescence, the effect on the plant's reproductive success can be positive (Higashi *et al.* 1988; Navarro 2000; Richardson 2004a), neutral (Zimmerman & Cook 1985; Arizmendi *et al.* 1996; Morris 1996) or negative (Roubik 1982, 1989; Traveset *et al.* 1998; Irwin & Brody 1999).

Nectar robbing has been documented in several species of Bignoniaceae (Borrero 1972; Barrows 1977; Stephenson & Thomas 1977; Gentry 1980; Silberbauer-Gottsberger & Gottsberger 1988; Vieira *et al.* 1992; Barros 2001; Maués *et al.* 2004; Richardson 2004a; Souza *et al.* 2004). In this family, the fusion of stamens with the corolla forms a nectar chamber that prevents flower visitors with short mouthparts from reaching the nectar disk at the base of the flower. In illegitimate flower visits, insects of some

species with such short mouthparts perforate the flower tube at the base of the corolla to access nectar (Silberbauer-Gottsberger & Gottsberger 1988; Barros 2001; Maués *et al.* 2004; Souza *et al.* 2004).

In Bignoniaceae, several pollination syndromes are reported, melittophily being the most common (Gentry 1974a, 1980). *Jacaranda* is a Neotropical genus that comprises about 50 species (Sandwith & Hunt 1974; Morawetz 1982; Gentry 1992), pollinated exclusively by medium to large bees (Gentry 1990). *Jacaranda rugosa* Gentry is a shrub endemic to the National Park of Catimbu and its surroundings in Buíque, Pernambuco State, Brazil (Gentry 1992). Here, we present the pollination ecology of this species, pointing out the effect of nectar robbing on its reproductive success, and ask the following questions: (i) which species are effective pollinators of *J. rugosa*; (ii) which species are primary and secondary nectar robbers and (iii) how does nectar robbing influence the behaviour of effective pollinators and the reproductive success of the plant?

MATERIALS AND METHODS

Study site

The study was carried out in the National Park of Catimbu, a nature reserve of 62,000 ha, in the municipalities of Buíque, Tupanatinga and Ibimirim (Andrade *et al.* 2004). From March to December 2005, individuals of *Jacaranda rugosa* were monitored at Serra Branca (8°23'53.8"S, 37°14'51.3"W; 898 m above sea level), an area with dense shrubby vegetation intermingled with scattered trees up to 10-m high.

The meteorological station at Buíque has an annual average temperature of 25 °C and annual precipitation of 1095.9 mm. The rainy season is from April to June (SUDENE 1990).

Studied species

Jacaranda rugosa shrubs grow on rocky and sandy soils and reach up to 4-m high. The leaflets are hard and strongly wrinkled and the purple flowers, which are disposed in axillary racemes, are hermaphroditic and tubular (Gentry 1992). The species has two blooming peaks, March–April and October–December, with only a few flowers opening in other months. Flowers of *J. rugosa* are 44.1 ± 6.9 mm long (mean \pm SD) and 32.9 ± 4.4 mm in diameter. Their fused petals form a flower tube 43.4 ± 11.8 mm long with an entrance height/width of, respectively, 5.9 ± 1.2 mm and 15 ± 1.1 mm. In the middle part of the corolla, the flower has a dorsiventral constriction where the stigma and

anthers are located. Styles with a bilobate stigma measure 31.9 ± 2.7 mm and the two pairs of stamens are 25.1 ± 2.0 mm and 28.8 ± 2.5 mm long. The flowers have a long and well developed staminodes (42.2 ± 5.5 mm), which are densely covered by long, yellow glandular trichomes that cross the corolla tube longitudinally and are visible at the entrance of the flower (Fig. 1a). The filaments are fused with the corolla tube, forming a nectar chamber of 10.7 ± 0.7 mm in length. The opening of the nectar chamber is densely covered with hairs that hinder access to the nectar for insects with short mouthparts and for minute animals.

Flower biology and breeding system

Fifty flower buds were bagged from their opening to abscission in order to examine anthesis. We recorded the time of flower opening, anther dehiscence, stigma receptivity, pollen viability, nectar availability, corolla and nectar guide colouration and blooming patterns. Stigma receptivity was determined using H₂O₂ (10%). To determine pollen viability, grains were removed from anthers, fixed in FAA (formaldehyde (37%), glacial acetic acid and ethanol (50%), 1:1:2) and transferred to microscope slides with acetocarmine (Dafni *et al.* 2005). Three hundred pollen grains from each flower ($n = 10$) were counted under the microscope and the proportion of stained grains was determined. Flowers of five individuals of *J. rugosa* were examined.

Volume and nectar concentrations were measured with 20- μ l graduated microcapillaries (Brand) and a pocket refractometer (Atago), respectively. Measurements were made in 10 individuals at 07:00, 12:00 and 17:00 h, using 10 previously bagged flowers at each time point.

To verify the breeding system of *J. rugosa*, the following treatments were performed on each of 10 individuals: (i) spontaneous self-pollination, unmanipulated bagged flowers, (ii) hand self-pollination, bagged flowers were pollinated with self-pollen, (iii) geitonogamous pollination, bagged flowers were pollinated with pollen from the same individual, (iv) hand cross-pollination, flowers were pollinated with several pollen donors from at least 1 km distant and (v) pollination of open flowers by visitors in natural conditions (control). For each treatment, 40 previously bagged flowers were used, in addition to the 260 marked flowers exposed to natural pollination. After manipulation, the position of the stigma was recorded and the flower bagged again. Fruit set in each treatment was recorded.

The pollen–ovule ratio was determined from 10 flower buds collected randomly in the field. The pollen grains per flower were counted using a Neubauer chamber. The

Fig. 1. *Jacaranda rugosa*, flower, flower visitors and nectar robbers. (a) Staminode visible in the flower entrance; (b) flower with staminode manually removed; the left flower shows a longitudinal slit at the base of the flower tube in the region of the nectar chamber made by *Xylocopa* bees; (c) female of *Euglossa melanotricha* hovering in front of the flower before the flower visit; (d) flowers during and (e) after nectar robbing by worker bees of *Trigona spinipes*, calyx and corolla have circular holes.



anthers were softened in Eppendorf tubes containing 0.2 ml of glycerinate lactic acid 3/1. After homogenisation with a vortex stirrer, a part of the sample (0.0008 ml) was transferred to the chamber and grains were counted under a microscope. Ovaries were dissected and the ovules counted under a stereomicroscope. Average and standard deviation of the P/O ratio of *J. rugosa* was determined for ten flowers (Dafni *et al.* 2005).

Flower visitors and effective pollinators

The flower-visiting insects were captured with entomological nets, mounted and stored in the Entomological Collection of the Federal University of Pernambuco (UFPE). Collection data were included in the database of the working group. Vouchers of the plants were stored in the Herbarium Geraldo Mariz UPE, Recife.

The frequency of flower visitors was followed in three plant individuals for three non-consecutive days. To test whether the staminode is important in visual orientation of flower visitors, 20 flowers were monitored per individual and day (10 controls and 10 in which the staminode was removed) from 06:00–17:00 h for 30 min·h⁻¹, corresponding to a total of 18 observation hours. We recorded if the bees made legitimate or illegitimate visits, whether they contacted the anthers and stigma and which floral resources were collected. During three nights, flowers were monitored to verify possible nocturnal visitors.

To determine the effective pollinators, we considered the behaviour of bees during the flower visits, their relative frequency, if they contacted the reproductive organs and if they performed flights among conspecific plant individuals. The position of the stigma lobes was verified after each flower visit. Moreover, the stigma position (open or closed) was monitored in 30 flowers accessible to flower visitors at 05:00, 08:00, 12:00, and 17:00 h. Any mechanical stimulation triggers stigma closure and stigmas remain closed after deposition of conspecific self- or cross-pollen (Milet-Pinheiro *et al.* 2008).

Nectar robbers and their effects

The injuries to flowers, made by the illegitimate visitors, were classified according to their form (circular holes or longitudinal slits) and position (calyx, corolla, other flower parts) in the flower.

The rate of nectar robbing was determined in 100 flowers of 10 individuals, considering the type of damage to the perianth. To determine the relation between volume of nectar and duration of flower visits, we removed the nectar from the nectar chamber with microcapillaries in 62 flowers of 10 plant individuals. These empty flowers were exposed to flower visitors. In another 62 flowers of the same individuals, we added 10 µl of sugar water at 25% sugar concentration (similar to natural volume and concentration). The duration of the flower visits of legitimate visitors in these treatments was measured. Furthermore, buds of 62 flowers of the same 10 plants were

bagged and, on the next day, these flowers with accumulated nectar were exposed to flower visitors. In this treatment, we measured the duration of the first three flower visits of each legitimate visitor. The bee that arrived first in such a non-visited flower always made two consecutive visits. The third visit, in general, was made by another individual. The experiment was carried out between 07:00 and 9:00 h.

Statistical analysis

Nectar volumes recorded at different times of the day were compared with an ANOVA test. A chi-squared test was performed to verify differences in the frequency of fruit set among the breeding system treatments. To determine if there was a significant difference among the number of visitors in flowers with staminodes and flowers without staminodes, an ANOVA test was used. The duration of the flower visits for each treatment was compared with the Kruskal–Wallis test (Zar 1999). The normality of the data was verified using the Kolmogorov–Smirnov test. When necessary, the Tukey test was used for *a posteriori* comparisons. All the tests mentioned were accomplished with the software STATISTICA version 6.0 (Statsoft, Inc. 2001).

RESULTS

Flower biology and breeding system

The flowers of *Jacaranda rugosa* open at night, between 23:00 and 03:00 h, according to the individual. Soon after opening of the flower, the stigma was already receptive, the anthers dehisced and nectar was present. Thus, when the bees began their activity at sunrise, all flowers were in the same state, offering their resources synchronously. At 15:00 h the corollas of *J. rugosa* began to fall.

Nectar was produced continuously throughout the day, increasing from 5.1 µl on average at 07:00 h to 21.5 µl at 17:00 h. This means that a flower produced about 1.6 µl of nectar per hour (Fig. 2a). Nectar concentration varied, on average, between 25.6% and 28.8% throughout the day (Fig. 2b).

The flowers produced 21,775 ± 1734 pollen grains and 43.6 ± 6.3 ovules on average, corresponding to a P/O ratio of 499.4 ± 19.0. Pollen viability rate was 94.4 ± 3.0% (n = 10).

Controlled pollination showed that *J. rugosa* is a xenogamous species, since only hand cross-pollinated flowers and natural pollinated flowers set fruits (Table 1). Fruit set in open-pollinated flowers was low (1.5%). After self-, cross- and geitonogamous pollination the stigmas closed and did not open again.

Flower visitors and effective pollinators

Bees of 15 species were recorded in the flowers of *J. rugosa* (Table 2). The flower visitors were classified

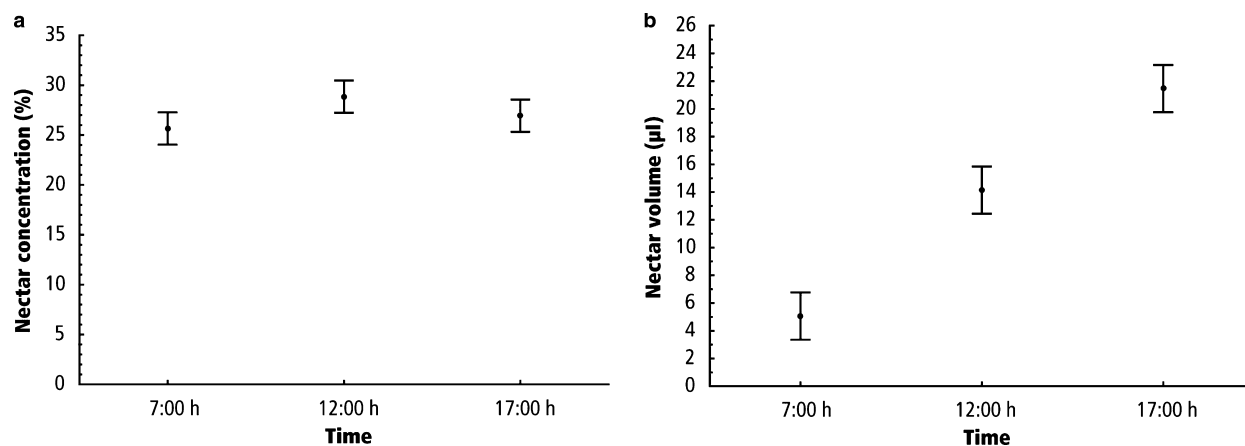


Fig. 2. Volume (a) and sugar concentration (b) of accumulated nectar throughout the day in bagged flowers of *Jacaranda rugosa* ($n = 10$, average and confidence interval).

Table 1. Controlled pollination in *Jacaranda rugosa*.

treatments	n	fruit set	fruit set (%)
hand self-pollination	40	0	0
spontaneous self-pollination	40	0	0
geitonogamous pollination	40	0	0
hand cross-pollination	40	19	47.5
open-pollination	260	4	1.5

In the treatments, hand self-pollination, spontaneous self-pollination, geitonogamous pollination and hand cross-pollination flower buds were bagged before anthesis. Open-pollinated flowers were marked and received legitimate and illegitimate flower visitors. The difference among the fruit set of the treatments was significant ($\chi^2 = 84.19$; $df = 1$; $P < 0.00001$).

in categories according to their behaviour: category I – legitimate flower visitors, nectar collectors, flowers visited at the regular corolla entrance; category II – legitimate visitors, pollen collectors; category III – illegitimate visitors, primary nectar robbers; category IV – illegitimate visitors, secondary nectar robbers (Table 2). Bees of 11 species were legitimate flower visitors. The medium to large species (*Bombus*, *Centris*, Euglossini) foraged only for nectar, while the small to tiny stingless bees, on the other hand, collected pollen in legitimate visits and robbed nectar from outside.

The legitimate visitors of *J. rugosa* distinguished flowers with staminodes (Fig. 1a) from flowers with staminodes experimentally removed (Fig. 1e). Unmanipulated flowers were visited at a significantly higher frequency ($F = 7.3119$; $g.l = 1$; $P < 0.01$; Fig. 3). Primary and secondary nectar robbers did not differentiate between flowers with or without staminodes.

Although the flowers opened around midnight, no nocturnal visitors were recorded and the first flower visits occurred only at 07:00 h. Visits to flowers of *J. rugosa* were most frequent between 09:00 and 12:00 h, mainly by

primary nectar robbers. Legitimate flower visits were more evenly distributed during anthesis (Fig. 4). The behaviour of *Euglossa melanotricha*, *E. cordata* and *Centris analis* was characteristic: before the flower visits, the bees hovered in front of the flower entrance, alighted in the flower opening, entered the flower and contacted first the stigma and then the anthers. After nectar collection, they left the flowers in the same way (Fig. 1b). In this category, males and females of *E. melanotricha* were the most abundant visitors (Table 3). After any flower visit by these bees, the stigmas closed and did not open again. At sunrise, before the first flower visitors arrived, all stigmas were open. At 08:00 h, 60% of the stigmas had already closed, reaching 93% at sunset (Fig. 5).

Workers of the stingless bee species, *Plebeia flavocincta*, *Plebeia* sp., *Paratrigona lineata* and *Trigona spinipes* and bees of two species of *Ceratina* (category II) were most abundant between 09:00 and 12:00 h. When collecting pollen grains, directly from the anthers, these bees also contacted the stigma.

The primary nectar robbers, *Trigona spinipes*, *Oxaea austera*, *Pseudaugochlora pandora* and the three species of *Xylocopa* bees, showed high flower visiting activity between 10:00 and 12:00 h. This group was responsible for 56.4% of all visits to flowers of *J. rugosa*, including 39% by workers of *Trigona spinipes*. The secondary nectar robbers were most frequent around noon, when most flowers already showed damage to the calyx and/or corolla tube. The secondary nectar robbers were also pollen collectors (category II).

Nectar robbers and their effects

In *Jacaranda rugosa*, 99% of the flowers showed damage from nectar robbers. Workers of *Trigona spinipes* were most abundant, chewing large (5 mm) circular to oval holes in the base of the corolla tube and sepals of 92% of the flowers. To access the nectar disk, worker bees stayed up to 40 min in the flowers (Fig. 1c and d). Moreover,

flower visitors	category	sex	resource collected
Andrenidae			
<i>Oxaea austera</i> Gerstaecker, 1867	III	♀/♂	N
Apidae			
Bombini			
<i>Bombus (Fervidobombus) brevivillus</i> Franklin, 1913	I	♀	N
Centridini			
<i>Centris (Trachina) fuscata</i> Lepeletier, 1841	I	♀/♂	N
Euglossini			
<i>Euglossa (Euglossa) cordata</i> (Linnaeus, 1758)	I	♀/♂	N
<i>Euglossa (Euglossa) melanotricha</i> Moure, 1967	I	♀/♂	N
<i>Eulaema (Apeulaema) nigrita</i> Lepeletier, 1841	I	♀/♂	N
Meliponini			
<i>Paratrigona lineate</i> (Lepeletier, 1836)	II, IV	♀	N/P
<i>Plebeia flavocincta</i> (Cockrell, 1912)	II, IV	♀	N/P
<i>Plebeia</i> sp.	II, IV	♀	N/P
<i>Trigona spinipes</i> (Fabricius, 1793)	II, III	♀	N/P
Xylocopini			
<i>Ceratina (Crewella) maculifrons</i> Smith, 1854	I, II	♀	N/P
<i>Ceratina (Crewella)</i> sp.	I, II	♀	N/P
<i>Xylocopa (Megaxylocopa) frontalis</i> (Olivier, 1789)	III	♀	N
<i>Xylocopa (Neoxylocopa) griseescens</i> Lepeletier, 1841	III	♀	N
<i>Xylocopa (Neoxylocopa) ordinaria</i> Smith, 1874	III	♀	N
Halictidae			
Augochlorini			
<i>Pseudaugochlora pandora</i> Smith, 1853	III, IV	♀/♂	N

Table 2. Flower visitors of *Jacaranda rugosa* in the National Park of Catimbau, Buíque Pernambuco, their visitor category, sex and type of resource collected.

Categories: I – legitimate visitors, nectar collectors; II – legitimate visitors, pollen collectors; III – illegitimate visitors, primary nectar robbers; IV – illegitimate visitors, secondary nectar robbers; N, nectar; P, pollen.

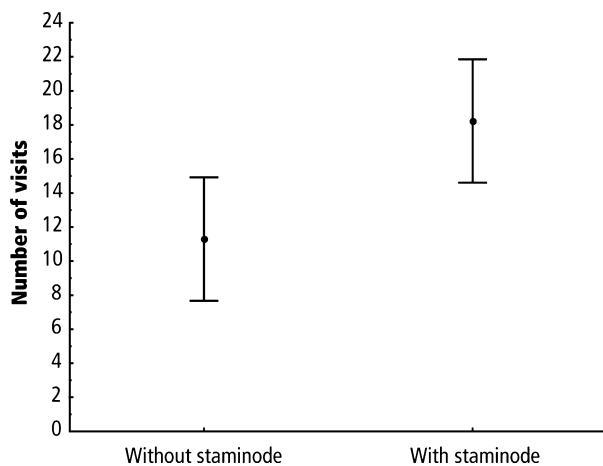


Fig. 3. Number of visits to non-manipulated flowers of *Jacaranda rugosa* and to flowers with the staminode removed ($n = 60$; 18 h of observation over 3 days; average and confidence interval). The differences among the treatments are significant ($P < 0.01$).

these bees were also pollen robbers. Using their mandibles, they often also cut other flower parts, such as styles, anthers and filaments and also attacked flower buds. While in a flower, workers were aggressive to legitimate

flower visitors and defended flowers even against the large carpenter bees. Bees of *Xylocopa* were also common nectar robbers and damaged 64% of the flowers. All species observed (*Xylocopa griseescens*, *X. frontalis* and *X. ordinaria*) showed the same behavioural pattern: they alighted on the upper side of the flower and perforated the tube of the corolla with their stiff galeae in the region of the nectar chamber, leaving a narrow longitudinal slit up to 8 mm long in the corolla (Fig. 1e). Flower visits by the *Xylocopa* bees were short and lasted only 1–3 s. *Pseudaugochlora pandora* bees inserted their tongue in the region between the sepals and petals at the base of the flower tube for a few seconds without damaging the flower. Injuries to flowers made by bees of both genera, *Trigona* and *Xylocopa*, were found in 57% of the flowers.

The duration of a visit of a pollinator to a flower with manipulated nectar availability and to control flowers was measured to verify whether the natural depletion of nectar by robbers would affect their behaviour. Visits of *Euglossa melanotricha* bees to flowers in which nectar was removed lasted 4.5 ± 2.1 s (mean \pm SD, $n = 62$), while those to flowers where 10 μ l of sugar water (25%) was added lasted 10.9 ± 6.6 s. In control flowers, which were previously bagged to accumulate nectar, the durations of the three first visits were monitored. The first visit to a flower was very brief, only 2.9 ± 1.2 s ($n = 62$). After this visit, the *Euglossa*

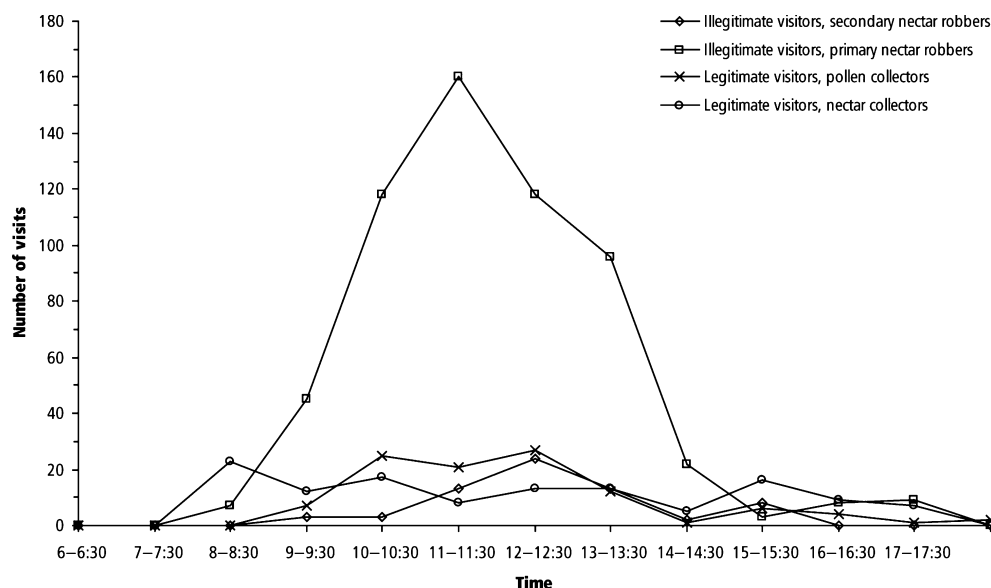


Fig. 4. Frequency of flower visitors per category to *Jacaranda rugosa* flowers during the day (n = 60; 18 h of observation over 3 days).

bees hovered in front of the flower and removed the pollen that had stuck to their facial area. Then, after 2–4 s, the same bees always made a second visit to the same flower, staying inside it for 9.8 ± 2.9 s (n = 62). The third, and not sequential, visit occurred after irregular intervals and lasted only 4.5 ± 1.1 s (n = 62). The third visitors, in general, were different bee individuals. The variation in the duration of the flower visits among the treatments was significantly different ($H = 202.24$; $g.l = 4$; $P < 0.001$). A *posteriori* tests showed that the duration of a visit in a similar pair to flowers with removed nectar, and the third visit to control flowers was significantly different from another similar pair (flowers with added sugar water and second visit to control flowers). Thus, the first visit to a flower on a given day differed from other visits and did not serve to collect nectar (Fig. 6).

DISCUSSION

Euglossine bees, especially those of *E. melanotricha*, are the main effective pollinators of *Jacaranda rugosa*. Euglossini are typical bees of Neotropical rain forests (Dressler 1982; Roubik & Ackerman 1987), and in the Atlantic rain forest of northeastern Brazil more than 20 species were recorded (Bezerra & Martins 2001; Neves & Viana 2003; Darrault *et al.* 2006). However, in the region of the highly seasonal *Caatinga*, which is dominated by spiny shrubs, small trees and succulent plants, and where *J. rugosa* is a local endemic species, species richness of Euglossini is low but not their abundance in *Jacaranda* flowers. Bees of all three euglossine species that occur at the study site, verified by collection of bees on scent baits, visited the flowers of *J. rugosa*. While *Eulaema nigrita* and *Euglossa cordata* are ubiquitous species and known to leave closed rain forest (Darrault *et al.* 2003, 2006; Milet-Pinheiro &

Schlindwein 2005), *E. melanotricha* predominantly occurs in open habitats like those of the *Caatinga* and *Cerrado* (Neves & Viana 2003; Nemésio & Faria 2004).

The importance of Euglossini in the pollination of melittophilous Bignoniaceae has frequently been demonstrated (Borrero 1972; Barrows 1977; Frankie *et al.* 1983; Barros 2001; Dutra & Machado 2001). According to Gentry (1974a,b), a great part of the 76 Bignoniaceae species studied in Costa Rica and Panama were pollinated mainly by euglossine bees, which demonstrate outstanding flight capacity and act as long-distance pollinators (Janzen 1971). *Jacaranda caroba* and *J. copaia* of the *Cerrado* and Amazon, respectively, are also pollinated mainly by male and female Euglossini (Vieira *et al.* 1992; Maués *et al.* 2004). Worker bees of several generalist species of Meliponini frequently collected pollen in flowers of *J. rugosa*. As these bees, in general, do not fly among different plant individuals, they do not, or only slightly, contribute to cross-pollination and fruit set.

Surprisingly, *Centris* bees were rare flower visitors and less important pollinators of *J. rugosa*, but were very common flower visitors of other species of Bignoniaceae in the National Park of Catimbau, such as *Tabebuia impetiginosa*, *Arrabidaea limae* and *Anemopaegma laeve* (P. Milet-Pinheiro & C. Schlindwein unpublished results; Carvalho *et al.* 2007). *Jacaranda rugosa* has nectariferous flowers and its effective pollinators do not actively collect pollen. The length of the nectar chamber of *J. rugosa* excludes bees with tongues shorter than 11 mm, because these do not reach the nectar at the base of the flower tube. *Centris* bees avoid the flowers of *J. rugosa* due to their shorter tongue length. The nectar volume in *J. rugosa* is relatively large when compared to other species of Bignoniaceae pollinated by bees (Frankie *et al.* 1983; Stevens 1994; Rivera 2000; Barros 2001; Souza *et al.* 2004)

Table 3. Frequency of flower visits to *Jacaranda rugosa* flowers per species of visitor over 30-min intervals throughout the day (n = 60 flowers, 3 days of observation).

flower visitors	interval															Total
	06:00–06:30 h	07:00–07:30 h	08:00–08:30 h	09:00–09:30 h	10:00–10:30 h	11:00–11:30 h	12:00–12:30 h	13:00–13:30 h	14:00–14:30 h	15:00–15:30 h	16:00–16:30 h	17:00–17:30 h				
<i>Centris fuscata</i>	–	–	–	2	–	–	–	–	–	–	–	–	–	–	–	2
<i>Euglossa cordata</i>	–	–	–	–	–	–	–	1	–	–	–	–	–	–	–	10
<i>Euglossa melanotricha</i>	–	–	10	10	3	–	–	–	12	–	–	–	–	–	–	61
<i>Ceratina</i> group	–	–	2	5	5	13	13	4	4	–	–	–	–	–	–	50
<i>Plebeia</i> group	–	–	9	27	34	51	24	3	13	–	–	–	–	–	–	164
<i>Pseudaugochlora pandora</i>	–	–	8	10	44	38	50	17	1	–	–	–	–	–	–	183
<i>Trigona spinipes</i>	–	–	7	109	116	79	47	5	3	–	–	–	–	–	–	410
<i>Xylocopa ordinalaria</i>	–	–	–	–	–	1	–	–	–	–	–	–	–	–	–	1
Total	0	30	67	163	202	182	134	30	33	21	17	2	–	–	–	881

Bees which could not be identified to species level in the field were grouped: *Ceratina* group, *Ceratina maculifrons* and *Ceratina* sp.; *Plebeia* group, *Paratrigona lineata*, *Plebeia flavocincta* and *Plebeia* sp.

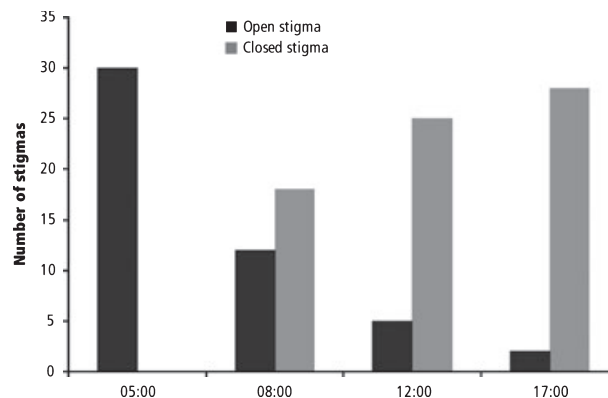


Fig. 5. Position of the stigma lobes of flowers of *Jacaranda rugosa* during the day (n = 30). The flowers were not bagged and were visited by flower visitors.

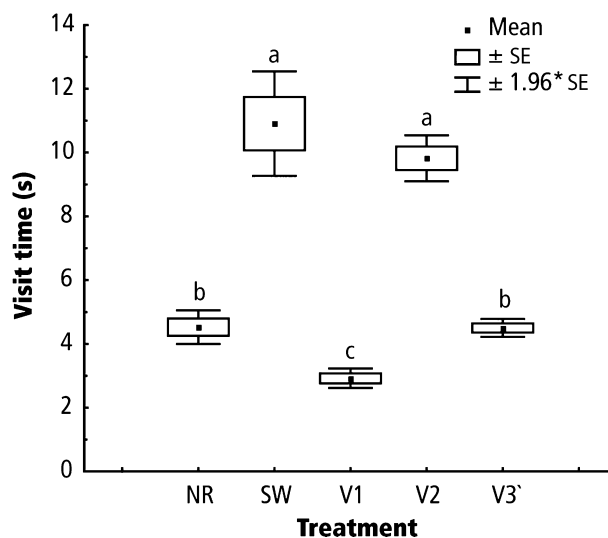


Fig. 6. Duration of flower visits of bees of *Euglossa melanotricha* to flowers of *Jacaranda rugosa* without nectar (NR), with sugar water added (SW), and first (V1), second (V2) and third visit (V3) to previously bagged control flowers. The treatments accompanied by different letters are significantly different ($P < 0.0001$); the difference between a similar pair NR/V3 and V2 is marginally significant ($P = 0.07$).

and the continuous production of nectar maintains the flowers attractive for visitors throughout the day. According to Gottsberger & Silberbauer-Gottsberger (2006), in some species of *Jacaranda* male euglossine bees collect the volatiles produced by the glandular trichomes of the staminode. We did not make such observations in *J. rugosa*.

Only the legitimate flower visitors of *J. rugosa* discriminated flowers with staminodes from those whose staminodes were experimentally removed. According to Vieira *et al.* (1992), the staminode has a triple function in the pollination of *J. caroba*: (i) visual orientation, due to its

contrasting colour to the opening of the corolla tube, (ii) olfactory orientation, through the scent liberated by the glandular trichomes on the staminode and (iii) aid in contact of the pollinator with the anthers and stigma of the flower, diminishing the diameter of the corolla. According to Maués *et al.* (2004), the staminode in *J. copaia* reduces the space of the perianth chamber of the corolla and consequently hinders access of larger bees to the nectar. These alternatives deserve further testing.

The attractiveness of *J. rugosa* flowers as a nectar source is also shown through the high rate of robbed flowers. Although we have no field data on the direct reproductive consequences of nectar robbers, indirect effects permit conclusions on its impact on fruit set. The high abundance of nectar robbers throughout the day maintains a low nectar level in the *Jacaranda* plants. Experimental removal of nectar and addition of sugar water revealed that the amount of nectar in the nectar chamber is indeed related to the duration of a flower visit by *Euglossa melanotricha*. When the nectar was removed manually, the visits of pollinators were very short, similar to the visits in flowers whose nectar was removed by other bees. After artificial nectar addition, the duration of visits was about two times longer and corresponded to visits to flowers with accumulated nectar, in other words, without the presence of robbers. This indicates that continuous nectar removal by robbers may reduce the permanence of pollinators in the flower by about 50%. Thus, the males and females of, for instance, the effective pollinating *Euglossa melanotricha* must at least duplicate the number of flower visits to collect the same amount of nectar as they would without the presence of nectar robbers. In this context, the reduction in the duration of a visit would increase flights among conspecific individuals of *J. rugosa* and, consequently, pollen flow and the rate of cross-pollination, a condition of fruit set in this xenogamous species.

Only medium to large bees that make legitimate visits to flowers of *J. rugosa* promote stigma closure and are effective pollinators. This corroborates studies in other Bignoniaceae, such as *Catalpa speciosa*, *Campsis radicans*, *Chilopsis linearis* and *Tecoma stans* (Stephenson & Thomas 1977; Bertin 1982; Singh & Chauhan 1996; Richardson 2004b; Yang *et al.* 2004). At sunset, more than 90% of the stigmas of *J. rugosa* were closed, showing that the flowers were intensely visited and that fruit set was not limited by the low frequency of pollinators, as demonstrated for other Bignoniaceae (Bertin 1982; Vieira *et al.* 1992). Soon after the beginning of the flight activity of the bees, early in the morning, the number of closed stigmas was already very high. This shows that only the first flower visits of a day were responsible for pollination and fruit set. The probability that a flower visit results in pollen deposition on the stigma decreases during the day. Therefore, bees that visited flowers in the afternoon did not, or only insignificantly, contribute to fruit set. The high fruit set after hand cross-pollination (about 45%)

shows that fruit set is not resource-limited, as demonstrated for *Tabebuia aurea* and *T. ochracea* (Barros 2001).

The reproductive success of *J. rugosa* in our study was extremely low (less than 2%) when compared to hand cross-pollinated flowers (above 45%), which were protected by pollination bags against destructive flower visits of *Trigona spinipes*. These bees were recorded in almost all flowers of *J. rugosa*. In places where this bee does not occur, the presence of nectar robbers like carpenter bees should have a positive effect on the fruit set, as proposed in other studies (Roubik 1982, 1989; Laroca & Almeida 1985; Silberbauer-Gottsberger & Gottsberger 1988; Vieira *et al.* 1992; Maloof & Inouye 2000; Barros 2001). The low fruit set of *J. rugosa* in the National Park of Catimbau was caused by the high rate of geitonogamy (in this self-incompatible species), combined with the definitive stigma closure after any conspecific pollen deposition and, especially, by the destructive flower visits of *Trigona spinipes*.

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