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Pollination biology in *Jacaranda copaia* (Aubl.) D. Don. (Bignoniaceae) at the "Floresta Nacional do Tapajós", Central Amazon, Brazil[1]

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ABSTRACT – (Pollination biology in *Jacaranda copaia* (Aubl.) D. Don (Bignoniaceae) at the "Floresta Nacional do Tapajós", Central Amazon, Brazil). *Jacaranda copaia* (Aubl.) D. Don is a pioneer tree widespread in the Brazilian Amazon, usually found colonizing forest gaps and altered areas, and the forest fragment edges. This study investigated aspects of the floral biology, breeding system and pollinators of *J. copaia* trees. Flowering lasts from August to November, during the low rainfall period extending up to four weeks per tree and 3-4 months for the population as a whole, characterizing a cornucopia flowering pattern. The fruit set ends in the beginning of the rainy season, with wind dispersed winged seeds. Fruit set from open pollination was 1.06% (n = 6,932). Hand pollination using self-pollen (n = 2,099) did not set fruits. Cross-pollination resulted in 6.54% fruit set (n = 2,524), representing six times more than the natural pollination rate (1.06%, n = 6,932). Flowers excluded from insect visitation (automatic self-pollination) did not set fruits (n = 5,372). Pollen tube growth down to ovary was detected under fluorescence microcoscopy in cross-pollinated and selfed pistils. The species is an obligate allogamous plant, with late-acting self-incompatibility system. Approximately 40 species of native bees visited the flowers, but the main pollinators were medium-sized solitary bees as *Euglossa* and *Centris* species due to the compatibility between their body sizes with the corolla tube, direct contact with the reproductive structures and high frequency of visits.

Key words - bees, floral biology, late-acting self-incompatibility (LSI), phenology, pollinators

RESUMO – (Biologia da polinização de *Jacaranda copaia* (Aubl.) D. Don (Bignoniaceae) na Floresta Nacional do Tapajós, Amazônia Ocidental, Brasil). *Jacaranda copaia* (Aubl.) D. Don é uma árvore pioneira distribuída por toda Amazônia brasileira, encontrada colonizando clareiras, áreas alteradas e bordas de fragmentos florestais. O presente estudo investigou aspectos da biologia floral, sistema reprodutivo e polinizadores de *J. copaia*. O florescimento ocorre de agosto a novembro, durante o período de menor precipitação pluviométrica, estendendo-se por até quatro semanas por indivíduo e três ou quatro meses para a população, caracterizando um padrão de floração cornucopia. A frutificação termina no início da estação chuvosa, com a dispersão anemocórica das sementes aladas. A taxa de frutificação natural foi de 1,06% (n = 6.932). As flores autopolinizadas manualmente (n = 2.099) não produziram frutos. A polinização cruzada (n = 2.524) resultou em 6,54% frutos, representando seis vezes mais do que a polinização natural (1,06%, n = 6.932). As flores protegidas da visita de polinizadores (autopolinização espontânea) não formaram frutos (n = 5.372). O crescimento dos tubos polínicos foi detectado sob microscopia de fluorescência tanto nos pistilos autopolinizados quanto nos submetidos à polinização cruzada. A espécie foi considerada alógama obrigatória, com mecanismo de auto-incompatibilidade de ação tardia. Aproximadamente 40 espécies de abelhas nativas visitaram as flores, entretanto os polinizadores legítimos foram principalmente abelhas solitárias de médio porte dos gêneros *Euglossa* e *Centris*, em função da compatibilidade entre o tamanho corporal com o tubo da corola, que facilitava o contato direto com as estruturas reprodutivas, e a elevada freqüência de visitas.

Palavras-chave - abelhas, auto-incompatibilidade de ação tardia, biologia floral, fenologia, polinizadores

Introduction

Large rainforest woody species are commonly self-incompatible (Bawa 1974, 1990) and dependent on long distance pollinators. But despite low density and sometimes asynchronous flowering, which have led to misconceptions about the ability of these plants to attract pollinators and have allogamous fruit set (Corner 1954, Fedorov 1966), they do attract efficient long distance pollinators as large solitary or subsocial bees (Bawa *et al.* 1985, Dick *et al.* 2004). For the tropical Bignoniaceae, a diverse assemblage of pollinators seems to have influenced flowering morphology and guaranteed fruit-set of these largely self-incompatible plants (Gentry 1974a, Gibbs & Bianchi 1999, Bittencourt Júnior & Semir 2004, Gottsberger & Silberbauer-Gottsberger 2006). Flowering phenology patterns, defined according to the duration and

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intensity, were also related with pollination biology (Gentry 1974b, Van Schaik *et al.* 1993, Morellato *et al.* 2000).

Despite numerous studies on pollination biology of the Bignoniaceae, few published studies have been focused so far on *Jacaranda* species, *e.g. J. macrantha* Cham. (Bittencourt 1981), *J. caroba* (Vell.) A. DC. (Vieira *et al.* 1992) and *J. racemosa* Cham. (Bittencourt Júnior & Semir 2006). These studies showed clear evidences of self-incompatibility (SI) (Bittencourt 1981) and ovarian or late-acting self-incompatibility (LSI) (Vieira *et al*. 1992, Bittencourt Júnior & Semir 2006). Gottsberger & Silberbauer-Gottsberger (2006) discuss about the superimposed pollination system of this genus, where three layered types of pollination coexist.

Jacaranda copaia (Aubl.) D. Don are medium to large trees, up to 30-35 m tall and 75 cm of DBH under natural conditions (Silva *et al.* 1985). It is a pioneer tree, usually found colonizing forest gaps, altered areas, and the edge of forest fragments (Guariguata *et al.* 1995). The species can also be established inside the forest, where adult trees can reach the canopy, despite being more frequent at the understory (Ribeiro *et al.* 1999). The species is distributed in the Neotropical region, widespread in lowland moist and wet forest from Belize to Bolivia, where two subspecies coexist (Gentry 1992), *J. copaia* subspecies *copaia* and *J. copaia* subspecies *spectabilis*, which are distinguished by features of the leaves and fruits. However, the acceptance of these subspecies is still controversial (Ribeiro *et al.* 1999, Lohmann *et al.* 2006, Lohmann & Ulloa-Ulloa 2006). *J. copaia* has been recommended for use in agroforestry, reforestation and degraded land recovery projects in South and Central America (Brienza Júnior *et al.* 1991), but basic information on its reproductive biology in order to subsidize its use was still lacking.

In this study, floral biology and breeding system of *J. copaia* were investigated in Pará State, Brazil, in order to provide information to future species use and management. The species is one of the target species of the Dendrogene project, coordinated by Embrapa Amazônia Oriental and several partners. In this project, some woody species with different ecological growing conditions and life history strategies are being studied for their genetic structure, reproductive process and regeneration (Kanashiro *et al.* 2002).

Material and methods

The main study area was located at the "Floresta Nacional doTapajós" (Flona Tapajós), in central Brazilian Amazon (2.89° S and 54.95° W). It comprises approximately 600,000 hectares of lowland native forest which has been submitted to controlled timber extraction and sustainable forest management studies (Silva *et al.* 1985, Kanashiro *et al.* 2002). The climate, according to Köppen classification, is AmW, characterized by annual dry period of 2-3 months and average rainfall of 2,000 mm (600 mm to 3,000 mm) (Espírito-Santo *et al.* 2005). The average annual air temperature is 25 °C, mean relative humidity is 86% (Carvalho *et al.* 2004). This forest may experience severe drought during El Niño events (Nepstad *et al.* 2002). From 1999 to 2004, a low impact selective logging project was conducted in 3,222 hectares under supervision of the "Instituto Brasileiro do Meio Ambiente e Recursos Naturais Renováveis" (Ibama) and the International Tropical Timber Organization (ITTO). The study plot was a 500 ha area within this larger area most of which is still untouched forest.

Complementary studies on floral biology and reproductive systems were also done with adult trees (> 20 years) planted at the experimental area of "Embrapa Amazônia Oriental" in Belém, Pará State (1°27' S and 48°29' W). The climate according to Köppen, is Afi, characterized by an average annual temperature of 25.9 °C (21 °C to 31.6 °C) and annual average rainfall of 2,900 mm.

Phenological observations were carried out every two weeks from October 2001 to July 2004 on 60 *Jacaranda copaia* trees, considering the occurrence, duration and frequency of the following events: (1) flowering (*e.g.*, floral buds and flowers); (2) fruit set (*e.g.*, immature fruit, mature fruit and seed dispersal) and (3) leaf changes (*e.g.*, juvenile and mature leaf; partial and total defoliation), in accordance with the methodology of Fournier & Charpantier (1975). The phenological records were associated with meteorological data (*e.g.*, precipitation, temperature, relative humidity and photoperiod) obtained in the same area from 2001 to 2003 by the LBA Project Team (2007), in accordance with Miller *et al.* (2004).

Inflorescence structure, flower morphology and aspects of the floral biology (anthesis, number of flowers opened/day, flower longevity, stigma receptivity, pollen viability, osmophores detection, sugar concentration and volume of the nectar, pollen/ovule ratio), were observed in five trees at Belém area, from August to October 2002. Peroxtesmo KO (Dafni & Maués 1998) was used to check stigma receptivity. The Peroxtesmo test indicates the main receptive area on the stigma surface, which turns dark blue or purple in contact with the solution. Pollen viability was tested with the DAB procedure (Sigma Fast™ 3.3' diaminobenzidine) and *in vitro* pollen germination on sucrose and agar medium (Dafni *et al.* 2005). The osmophores were detected with neutral red solution (1:10,000; Kearns & Inouye 1993) in fresh flowers. The flowers were immersed in the neutral red solution for 30 minutes, removed and washed in distilled water. Nectar was removed from previously bagged flowers and volume estimated with 1 µL glass microcapillary tubes. This procedure was carried out on fresh flowers (n = 30) (two hours after fully opening) and other 30 flowers one

day after opening. Total sugar concentration or "sucrose equivalents" on the nectar was scored after volume measurements with a Bellingham & Stanley pocket refractometer (Dafni *et al.* 2005). Different parts of the flower (corolla, calyx, staminode, stamens, pistil) where placed in covered glass vials for 5-10 minutes to organoleptic evaluation of scent (Dafni *et al.* 2005).

Fresh flowers were collected and fixed on FAA (acetic acid 5%, formaldehyde 5% and ethanol 90%) and 48 h later transferred to ethanol 70% for laboratory analysis. The floral morphology was described using stereomicroscope and scanning electron microscope (SEM). Flower structure, size, shape and color, as well as number of flowers per inflorescence, number of opened flowers per day and flower longevity were documented. Flower measurements were carried out for fixed flowers in five plants. The number of ovules and anthers were counted under stereomicroscope. The number of pollen grains was estimated in three flowers of each five plants, using all four anthers per flower, with a haematocytometer. To estimate the number of pollen grains per flower, each anther was gently squashed in 1 mL of 50% ethanol + 0.5%-1% of detergent, to facilitate pollen removal and homogeneous spread of pollen for counting under microscope. Six sub samples of 1 µL where dropped on the haemacytometer, counting all the pollen grains in a surface unit (Dafni *et al.* 2005).

Hand pollination experiments in previously bagged flowers were also performed to evaluate breeding system using five different trees. The subsequent treatments were carried out (following Radford *et al.* 1974): (1) cross-pollination; (2) manual self-pollination; (3) spontaneous (automatic) self-pollination; (4) control – tagged flowers left to natural pollination. Fruits set from each treatment were monitored until the complete fruit maturation, denoted by the beginning of capsules' dehiscence. In order to access the pollen germination and pollen tube growth by means of aniline blue staining and fluorescence microscopy (Martin 1959), 20 pistils of each treatment were collected 24 h and 48 h after manual pollination, fixed in FAA for 48 h and preserved in ethanol 70%. The index of self-incompatibility (ISI) was assessed by the ratio between fruit set from self- and cross-pollinated pistils (Bullock 1985). The reproductive efficacy (RE) was obtained by the ratio between fruit set of natural and cross-pollinated pistils (Ruiz & Arroyo 1978).

In order to have access to tree crown and observe flower visitors, a 34 m wood tower with a 2 m^2 platform on its top was built beside a target tree at the Tapajós forest site. Similar platforms with 10-18 m were also built beside *J. copaia* trees at the Embrapa site, to facilitate the handling of the flowers throughout the controlled pollination experiments. Observations of insect behavior on the flowers were accomplished, as well as capture with insect nets and photographic records, in order to identify legitimate pollinators. Frequency of visits was considered as follows: high (more than 20 visits per day), medium (at least 10 visits per day) and low or occasional (less than five visits per day). The observations were performed from 7:00 a.m. (anthesis initiation) to 18:00 p.m. during five days (approximately 50 h). Most insects were identified by comparison with previously identified specimens in the Entomological collection of Embrapa Amazônia Oriental. The following data about the insect visitors were registered: (1) species name; (2) if there was any contact between the visitor body and the reproductive structures of the flowers; (3) if pollen or nectar was collected and/or consumed. These observations were carried out during the main flowering season of 2001-2002, from September to October, corresponding to approximately 82 h of observations. Voucher specimens of the studied plants, insect visitors and pollinators, were deposited at the IAN Herbarium (numbers 178633, 176899, 176900, 17901, 17902) and the Entomological Museum of the Embrapa Eastern Amazon.

Results

Jacaranda copaia displays large erect panicles up to 37 cm long at the branches' tip, with an average of 3,596 flowers (± 613, n = 9) per inflorescence and 96.4 ± 58 (n = 16) opened flowers per inflorescence per day during the peak of the flowering phase. The total blooming per inflorescence lasted an average of 35 days (± 11, n = 5).

The most expressive flowering period occurred during the dry season, extending from September to December, when up to 97% of the individuals were flowering (figure 1). The same pattern was found during the whole study period, characterizing an annual flowering pattern (*sensu* Newstrom *et al.* 1994). Fruit set occurred from November to March, and the seed dispersal was concentrated during the peak of the raining season, from February to May. Leaf changes occurred just prior to the flowering phase, mainly from June to August.

The flowers are hermaphrodite, zygomorphic and nectariferous. The calyx is short (5-6 mm), cupular, brown, glabrous and gamosepalous. The corolla is tubular-infundibuliform, violet-blue (or lilac) in the outer surface and white inside the petal hood (throat), pubescent, gamopetalous, with five free lobes, 2.4-3.0 cm long. Androecium presents four didynamous stamens and one visually attractive staminode with glandular trichomes and bifurcated apex. Anthers are basifixed and monothecate (divaricate) with a mostly apical longitudinal opening, which remains partially closed after the dehiscence, without exposing totally the pollen grains (figure 2C and 5). The gynoecium presents a single filiform style, shorter than the staminode, with a bilobed tactile and humid stigma covered with clavate papillae of distinct lengths at the inner surface (figure 2A, 2E, 4, 5 and 6),

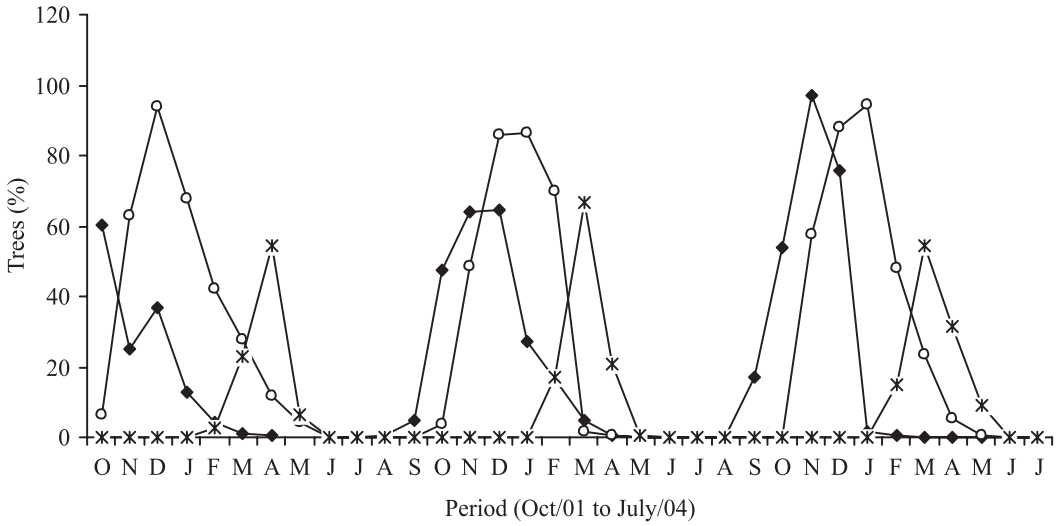


Figure 1. Percentage of trees in flowering (♦), fruiting (○) and seed dispersal (⚹) of *Jacaranda copaia* at the "Floresta Nacional do Tapajós", Central Amazon region, from October 2001 to July 2004.

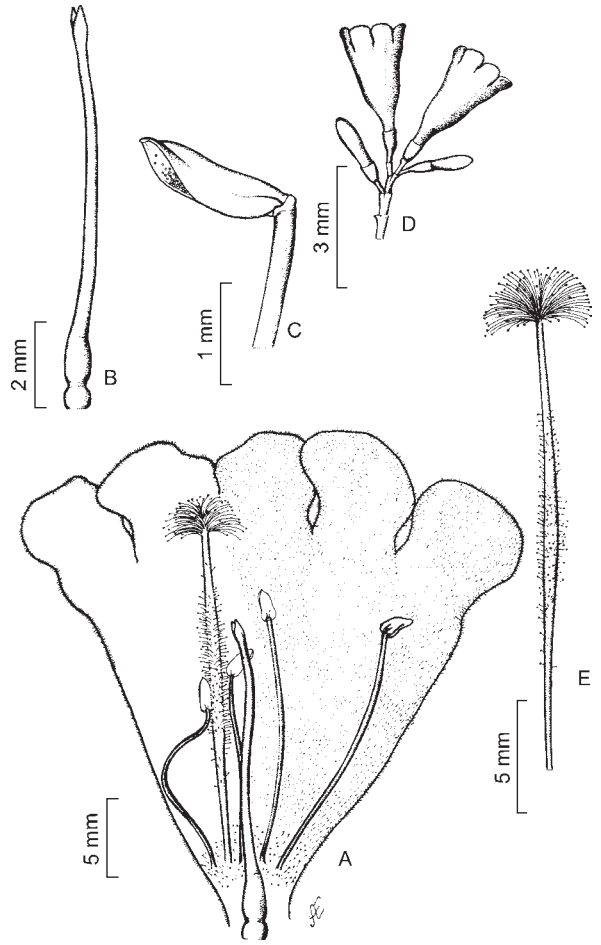


Figure 2. *Jacaranda copaia* flower. A. Opened flower showing the reproductive organs and the staminode position inside the corolla chamber. B. Pistil. C. Dehisced anther with pollen grains. D. Flowers and flower buds. E. Staminode with trichomes.

and a flattened and elongated ovary containing an average of 243 (± 33, *n* = 20) ovules.

During pre-anthesis phase flower buds were closed only by the petals edges, which opens with the simple touch of the first visitor. The anthesis started from 7:30 to 8:30 a.m, according to visitors' movement. The nectary is a disk located at the base of the ovary. The anthers dehisced soon after full anthesis phase, but the pollen was released only when the visitors squeezed the anthers, removing small amounts of pollen grains. The number of pollen grains estimated per flower was 30,425 (*n* = 4), and the pollen/ovule ratio was 125.2. The osmophores were mainly located in the corolla and staminode, which was consistent with results of the organoleptic test.

The best sucrose concentration for pollen germination was 25%, in which case 70.8% of the pollen spread on the agar media after 24 hours exhibited pollen tube growth. The DAB test showed highest pollen viability period among 8:00 to 9:00 a.m. (75.5%), decreasing gradually during the rest of the day (figure 3). The nectar was produced in small amounts during the flower life span. The average volume in first day flowers was 1.01 µL (± 0.2, *n* = 20; 0.5 to 1.5 µL) and for second day pistils the volume was 1.06 µL (± 0.3, *n* = 32; 0.5 to 2 µL). Sugar concentration varied from 23% to 41% (mean = 28.5% ± 4.4; *n* = 19) for first day flowers and from 20% to 53 % (mean = 34.7% ± 6.7; *n* = 31) for second day remaining pistils. Stigma receptivity is mainly located at the inner surface (papillate) region, lasting from the anthesis until 24 h of lifespan, as shown by the Peroxtesmo KO tests.

Flowers had a life span of approximately 24 h (intact flower – holding all the verticiles), after which the corolla

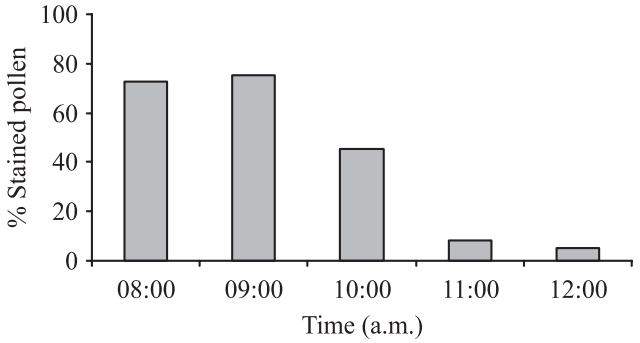


Figure 3. Pollen viability of *Jacaranda copaia* expressed in percentage of stained pollen grains with DAB (Sigma Fast™ 3.3' diaminobenzidine).

collapsed together with the stamens and staminode. The calyx, nectary disc and pistil lasted for another day, and nectar was still secreted, although very few visitors collected nectar during this second day. Total abscission of these flower structures occurred 48 h after flower opening. The dry dehiscent fruits took approximately four months to mature and comprised an average of 245 winged seeds (± 26, *n* = 25), which were wind dispersed.

There was a plethora of 61 different species of flower visitors, including medium to large-sized bees, butterflies, wasps and hummingbirds (table 1). Medium-sized bees belonging to the genus *Euglossa* and *Centris*, were the most frequent visitors in both study sites and the main pollinators due to their frequency, body size and behavior. These bees were usually the first visitors, assisting in the process of anthesis, as they touched the petal lobes triggering their final expansion and corolla opening. The visits of *Centris* were very fast, lasting from 3 to 6 s (*n* = 46). The euglossine bees were very frequent visitors and their visits lasted 8 to 12 s (*n* = 55). The *Centris* spp. entered the flower tube and collected both nectar and pollen, pollen collection indicated by body grooming after visits. As for *Centris* spp., *Euglossa* bees contacted reproductive structures at every visit while entering the corolla tube. When leaving the corolla chamber they squeezed the anthers, receiving pollen on the upper head and thorax. Their long glossa proved to be very useful in nectar collection. *Euglossa* males were also frequent visitors, but they were more restricted to the upper part of the corolla chamber, where they grasped the inner petal surface and, apparently the staminode trichomes glands. Halictidae (*Augochlora*, *Augochloropsis*, *Pseudoaugochloropsis*), *Exomalopsis* and Meliponina (*Paratetrapedia*) used the staminode as a bridge to access the nectary, and sometimes collected pollen adhered to its trichomes, behaving as occasional pollinators.

The flower visitation period extended from 7:30 a.m. to 17:00 p.m., with higher frequency of bees from 8:00 to

Table 1. Flower visitors and pollinator agents collected in *Jacaranda copaia* at the "Floresta Nacional do Tapajós" (Tap) and Belém (Bel), in the Brazilian Amazon. (O = occasional pollinator; L = legitimate pollinator; R = pollen/nectar robber; [+]Floral resources used by the visitors/pollinators: P = pollen; N = nectar).

FLOWER VISITORS	Locality	Category	Resource used[+]
INSECTA			
HYMENOPTERA			
Apidae			
Aparatrigona impunctata (Ducke, 1986)	Bel/Tap	O	P
Bombus brevivillus Franklin, 1913	Bel/Tap	L	N
Bombus transversalis (Olivier, 1789)	Bel/Tap	L	N
Centris (Heterocentris) analis (Fabricius, 1804)	Bel	L	N, P
Centris (Heterocentris) dichrootricha (Moure, 1945)	Tap	L	N, P
Centris (Centris) flavifrons (Fabricius, 1775)	Bel	O	N
Centris (Hemisiella) trigonoides Lepeletier, 1841	Bel/Tap	L	N, P
Centris sp. 1	Bel/Tap	L	N, P
Centris sp. 2	Bel/Tap	L	N, P
Epicharis (Epicharis) rustica (Olivier, 1789)	Bel	O	N
Epicharis (Hoplepicharis) affinis Smith, 1874	Bel	L	N
Epicharis (Parepicharis) zonata Smith, 1854	Bel	O	N
Epicharis sp. 1	Tap	O	N
Eufriesea mussitans (Fabricius, 1787)	Bel	O	N
Eufriesea surinamensis (Linnaeus, 1758)	Bel	O	N
Eufriesea sp.	Bel	O	N

continue

continuation

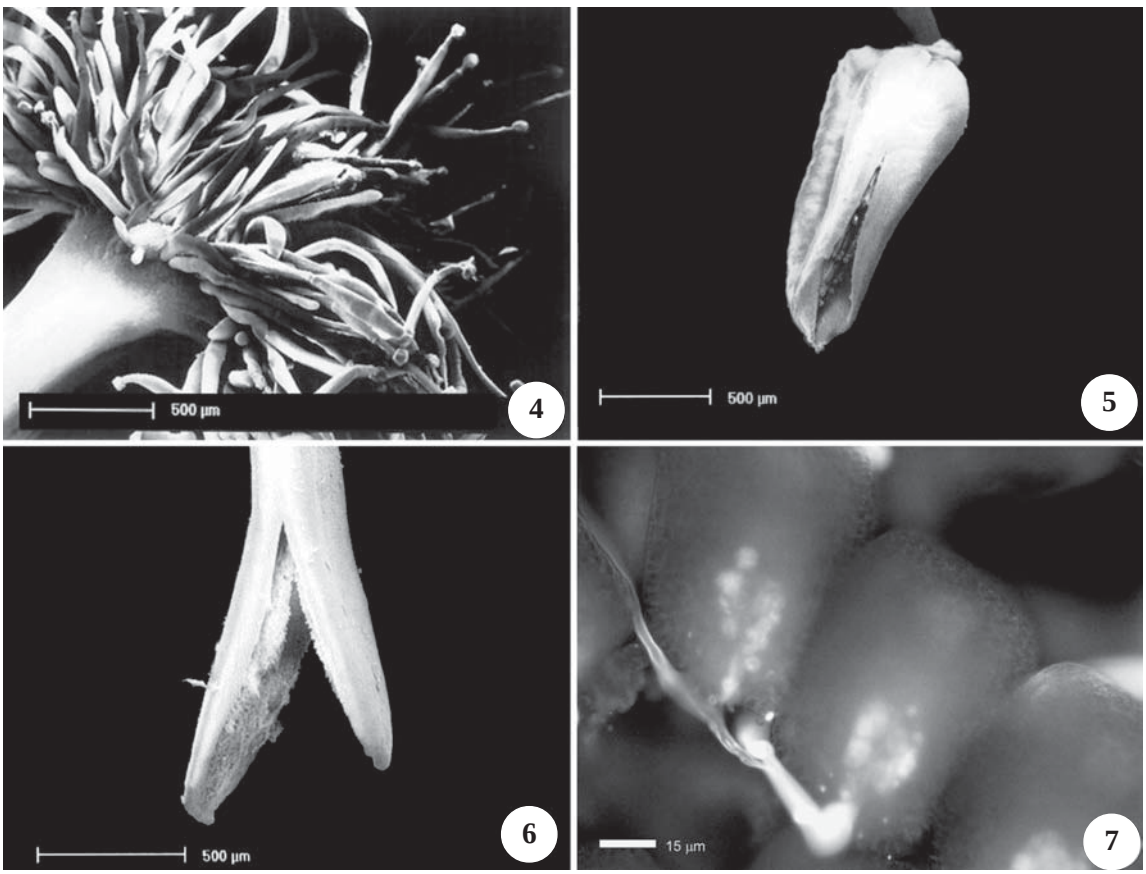
FLOWER VISITORS	Locality	Category	Resource used[+]
Euglossa chlorina (Dressler, 1982)	Bel	L	N, P
Euglossa sp. 1	Tap	L	N, P
Euglossa sp. 2	Tap	L	N, P
Euglossa sp. 3	Tap	L	N, P
Euglossa sp. 4	Tap	L	N, P
Eulaema meriana (Olivier, 1789)	Bel	O	N
Eulaema nigrita Lepeletier, 1841	Bel	L	N
Exomalopsis sp. 1	Bel	O	P
Exomalopsis sp. 2	Bel	O	P
Melipona compressipes (Fabricius, 1804)	Tap	O	N, P
Meliponina (6 species)	Tap	O	P
Paratetrapedia sp. 1	Bel	O	P
Paratetrapedia sp. 2	Bel	O	P
Xylocopa frontalis (Olivier, 1789)	Bel	R	N
Megachilidae			
Megachile (Chrysosaurus) ruficornis Smith, 1853	Tap	O	N
Andrenidae			
Oxaea sp.	Tap	O	N
Halictidae			
Augochlora (Augochlora) esox (Vachall, 1911)	Bel	O	N
Ceratina sp. 1	Tap	O	N
Pseudoaugochlora sp. 1	Bel	O	N, P
Pseudoaugochlora sp. 2	Tap	O	N, P
Vespidae			
Synoeca virginea (Fabricius, 1804)	Bel/Tap	O	N
DIPTERA			
Bibionidae			
1 species	Tap	O	P
Syrphidae			
Ornidia obesa Fabricius, 1775	Bel	O	P
COLEOPTERA			
Chrysomelidae			
4 species	Tap	O	P
Scarabaeidade			
Cnemida leprieuri Arrow 1899	Tap	O	P
Cnemida retusa (Fabricius, 1801)	Tap	O	P
Scarabaeinae (2 species)	Tap	O	P
LEPIDOPTERA			
Pieridae			
Phoebis statira (Cramer, 1777)	Bel/Tap	R	N
Phoebis trite (Linnaeus, 1758)	Tap	R	N
Lycaenidae			
1 species	Tap	O	N
Nymphalidae			
Philaetria dido (Linnaeus, 1763)	Tap	O	N
AVES			
Trochilidae			
Anthracothorax nigricollis (Vieillot, 1817)	Bel/Tap	O	N
Glaucis hirsuta Gmelin (1788)	Tap	O	N
Hylocharis sapphirina Gmelin (1788)	Tap	O	N
Thalurania furcata (Gmelin, 1788)	Bel	O	N
Topaza pella (Linnaeus, 1758)	Tap	O	N

10:30 a.m. *Xylocopa frontalis* visited the flowers at irregular intervals from the beginning of anthesis to the end of the day, perforating the base of the corolla tube to take the nectar in illegitimate visits which resulted in no pollination. Butterflies were late visitors, from 11:00 a.m. to 15:30 p.m., and nectar robbers, using the holes made by *X. frontalis* to access the nectar chamber. The legitimate and illegitimate pollinators visited the flowers together along the day, although the illegitimate visitors (*e.g. Xylocopa* and butterflies) were more frequent during the afternoon. No aggressive behavior was noticed among them.

Controlled pollination tests (table 2) resulted in no fruit set from manual and automaticself-pollination, although a single selfed fruit was initiated and aborted two weeks after manual pollination. Fruit set from open pollination was initially 4.99% but only 1.06% reached maturation (n = 6,932). Cross-pollination resulted in 21.7% of initiated fruits but only 6.54% reached maturation (n = 2,524). Pollen tube growth was detected both in self and cross-pollinated pistils after the first 24 hours, but only cross-pollen penetrated the ovules (figure 7).

Table 2. Percentage (%) of fruit set from hand-pollination treatments and open pollination flowers (control) in *Jacaranda copaia*. Data corresponds to initiated (three weeks) and mature fruits (number of fruits/number of flowers per treatment). (ISI = Self-incompatibility index; RE = Reproductive efficacy).

Treatments	Initiated fruits	Mature fruits
Cross-pollination	21.7% (469/2,524)	6.54% (173/2,524)
Manual self-pollination	0.06% (1/2,099)	0 (0/2,099)
Automatic self-pollination	0 (0/5,372)	0 (0/5,372)
Control (open pollination)	4.99% (414/6,932)	1.06% (91/6,932)
ISI	0	
RE	0.16	



Figures 4-7. Scanning electron micrography (SEM) of *Jacaranda copaia* flower. 4. Staminode apical region (38x). 5. Dehisced anther (33x). 6. Stigmatic lobes (48x). 7. Pollen tubes penetrating the ovules 48 hours after cross-pollination under epifluorescence pistil.

Discussion

Jacaranda copaia flowers attributes, such as diurnal anthesis, tubular violet zygomorphic corolla, presence of nectar in a protected chamber, hidden reproductive organs and sweet fragrance are compatible with the bee pollination syndrome (Faegri & van der Pijl 1979, Proctor *et al.* 1996). According to Gentry's (1974a) classification, *Jacaranda copaia* flowers belong to the *Anemopaegma* type, generally pollinated by medium to large bees, usually Euglossini and Anthophorinae, although it may be visited by illegitimate pollinators, nectar and pollen robbers (*e.g.* Trochlidae, Meliponina, Lepidoptera and *Xylocopa*). The anther's monothecate type is atypical within the genus *Jacaranda*, as most species presents dithecate anthers (Dr. Lúcia Lohmann, unpublished data).

The flowering phenology of *Jacaranda copaia* can be classified as cornucopia (Gentry 1974b) with a relatively long and massive flowering period between 3 to 10 weeks. The large and showy inflorescences at the canopy layer, with hundreds of flowers opening at the flowering peak, results in a flowering display which may attract visitors from long distances. This flowering pattern seems to be the most widespread and generalized among the Bignoniaceae (Gentry 1974a)

The corolla tube allowed visits of small to medium-sized bees and was a constraint to larger visitors. The position of the anthers and stigma inside the petal hood promoted the pollen deposition on the upper head and thorax of the pollinators. The basal constriction of the corolla tube was compatible with long-tongued bees, such as *Euglossa*, as reported also by Bittencourt Júnior & Semir (2006). The same foraging behavior of these agents was registered in the pollination biology of *Arrabidaea conjugata* (Vell.) Mart. (Correia *et al.* 2005) and *Jacaranda racemosa* Cham. (Bittencourt Júnior & Semir 2006).

Euglossini female bees were considered the most efficient melittophilous pollinators of Bignoniaceae species in Panama (Gentry 1974b). The characteristic long glossa of this tribe helps in the process of nectar foraging in tubular flowers (Pinheiro & Schlindwein 1998). *Centris* were also reported as main pollinators of *Tabebuia* flowers in Costa Rica (Frankie *et al.* 1983) and Central Brazil (Barros 2001). Besides, *Centris* spp. bees also demonstrated compatible pollinator behavior, once they penetrated up to the second third of the corolla tube (distal part), where the anthers and stigma are located, and thus were able to properly transfer pollen to the reproductive structures.

On the other hand, some of the visitors, as *Xylocopa frontalis*, which were not able to enter the corolla tube, collected nectar by perforating the soft corolla tissue at the nectary level. This behavior has been recognized in many species of the Bignoniaceae family (Gentry 1974a, b, Gobatto-Rodrigues & Stort 1992, Vieira *et al.* 1992, Galetto 1995, Correia *et al.* 2005) and is repeated by *Xylocopa* bees in other plants with tubular flowers (Barrows 1980).

The staminode "selected" the legitimate visits reducing the corolla chamber, therefore large-sized visitors were not able to properly enter the flower and contact the stigma and anthers, although some species such as *Centris flavifrons, C. similes, Eulaema meriana, Epicharis rustica, Bombus, Eufriesea* and *Oxaea* forced the entrance and acted as occasional pollinators, usually collecting nectar. It was also used as a platform by smaller bees such as Meliponina, Halictidae and *Exomalopsis*, which used the staminode as a path to reach the nectar chamber.

The staminode function has been comprehensively discussed (Bittencourt Júnior & Semir 2006, Gottsberger & Silberbauer-Gottsberger 2006). Vieira *et al.* (1992) suggested that this structure had three different functions in *Jacaranda caroba* (Vell.) DC. flowers: visual orientation, smell attraction guide and assistance in the contact of the pollinators with the reproductive structures. It has been also suggested that the staminode may be used as a bridge by halictid bees to reach the anthers, eventually touching the stigma (Gottsberger & Silberbauer-Gottsberger 2006). It also retains pollen grains on the glandular hairs (trichomes), which are collected by bees (Morawetz 1982 *apud* Gottsberger & Silberbauer-Gottsberger 2006), and diminishes the space inside the corolla chamber, thus promoting better contact of the pollinators with the reproductive organs (Bittencourt Júnior & Semir 2006). In *J. copaia*, all those functions can be assumed.

Finally, staminode's trichomes secret a scent (Bittencourt Júnior & Semir 2006) which may explain the visits of *Euglossa* males. Due to those different attractants, including nectar, pollen and scent, besides the multiple role of the staminode adapting the flower to different sizes of visitors, *Jacaranda* seems to have superimposed pollination systems (Gottsberger & Silberbauer-Gottsberger 2006).

Jacaranda copaia is self-incompatible with abscission of selfed pistils within one or two days after hand pollinations. ISI was null and the reproductive efficacy 0.16 was very low, indicating that natural pollination in the Belém plantation is much less efficient than hand cross pollination. Pollen germination and pollen tube growth was similar in both self and cross-pollinated pistils, but in selfed pistils the pollen tubes were not

observed penetrating the ovules. This type of late-acting self-incompatibility system (LSI) or ovarian self-incompatibility (Seavey & Bawa 1986) is common in many tropical Bignoniaceae, including *Tabebuia aurea* (Manso) Benth. & Hook. f. ex S. Moore (syn. *T. caraiba*) and *T. ochracea* (Cham.) Standl. (Gibbs & Bianchi 1993, Barros 2001), *Dolichandra cynanchoides* Cham. and *Tabebuia nodosa* (Griseb.) Griseb. (Gibbs & Bianchi, 1999), *Jacaranda macrantha* (Bittencourt 1981) and *Jacaranda racemosa* (Bittencourt Júnior & Semir 2006). Studies over the last two decades have shown that outbreeding and LSI are common features in many tropical trees (Bawa *et al.*, 1985) but seem to be such common features in the Bignoniaceae that a phylogenetic component has been argued to explain such familial clustering (Gibbs & Bianchi 1999). However, self-fertility and apomixis have been also described in some woody tropical Bignoniaceae (Costa *et al.* 2004). The extremely low fruit set from open pollination is consistent with other tropical trees (Bawa *et al.* 1985).

The pollen/ovule ration *sensu* Cruden (1977) indicated that the species would be self-compatible, but field tests on the reproductive system refuted that supposition. The pollen grains are not exposed, but released from the anthers in small amounts according to pollinators' movements in the flowers. This may result in a very efficient pollen dispensing and pollination strategy, which may explain the relatively low number of pollen grains produced per flower. In *Arrabidaea conjugata* the pollen was released by the same process, therefore even when visitors arrived at the end of the anthesis they still can be dusted with pollen grains (Correia *et al.* 2005).

The legitimate visitors observed confirmed the melittophilous pollination syndrome *sensu* Faegri & van der Pjil (1979) in *J. copaia*. Solitary bees tend to show higher oligolectic species-specific preferences (Cane 2001), but the superimposed pollination system (Gottsberger & Silberbauer-Gottsberger 2006), with nectar, pollen and perfume as rewards, would explain the great number of potential pollinators attracted. Hummingbird pollination is reported in *Zeyheria montana*. Mart. (Bittencourt Júnior & Semir 2004), but in *J. copaia* these agents were considered simply occasional pollinators, due to their low frequency and unspecialized bird syndrome flower traits. Cornucopia flowering phenology also contributes to long distance pollinators' attraction. Roubik & Degen (2004) modelling studies with *J. copaia* trees at the "Floresta Nacional do Tapajós", showed that this species was nearly completely out-crosser and the mean pollen dispersal range was 147.9 (± 42.0 m). Assuming that in this site the species is regularly distributed (1.8 trees ≥ 20 cm DBH per hectare), and considering its diversified pollination system, we expect the maintenance of the reproductive success of *J. copaia* under natural conditions.

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