Pollination Biology of *Jacaranda oxyphylla* with an Emphasis on Staminode Function

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**Background and Aims** Bignoniaceae is a Neotropical family with >100 genera, only two of which, *Jacaranda* and *Digomphia*, have a developed staminode. *Jacaranda oxyphylla*, whose flowers possess a conspicuous glandular staminode, is a zoophilous cerrado species. Here, the composition of the secretion of the glandular trichome and the influence of the staminode on the pollination biology and reproductive success of *J. oxyphylla* were studied.

**Methods** The floral morphology, pollen viability, stigma receptivity, nectar volume and nectar concentration were studied. Compatibility system experiments were performed and floral visitors were observed and identified. Experiments comparing the effect of staminode presence and absence on pollen removal and pollen deposition efficiency were conducted in open-pollinated flowers. Histochemistry, thin-layer chromatography (TLC) and gas chromatography coupled to flame ionization detection (GC–FID) analyses were performed to determine the main chemical components of the staminode’s glandular trichome secretion.

**Key Results** Flower anthesis lasted 2 d and, despite the low frequency of flower visitation, pollination seemed to be effected mainly by medium-sized *Eulaema nigrita* and *Bombus morio* bees, by the small bee *Exomalopsis fulvofasciata* and occasionally by hummingbirds. Small bees belonging to the genera *Ceratina*, *Augochlora* and *Trigona* were frequent visitors, collecting pollen. *Jacaranda oxyphylla* is predominantly allogamous. Staminode removal resulted in fewer pollen grains deposited on stigmas but did not affect total pollen removal. The secretion of capitule glandular trichome occurs continually; the main chemical compounds detected histochemically were phenolic and terpenoid (essential oils and resins). Monoterpene cineole, pentacyclic triterpenes and steroids were identified by TLC and GC–FID.

**Conclusions** The staminode of *J. oxyphylla* is multifunctional and its importance for female reproductive success was attributed mainly to the secretion produced by capitule glandular trichomes. This secretion is involved in complex chemical interactions with pollinating bees, including the solitary bees Euglossini. These bees are common pollinators of various species of *Jacaranda*.

**Key words:** Bignoniaceae, *Jacaranda oxyphylla*, pollination, bee, staminode, glandular trichomes, reproductive success, terpenes, steroids, phenolics.

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**INTRODUCTION**

The plant family Bignoniaceae is predominantly Neotropical and plays an important ecological role in the forests of these regions (Lohmann, 2006), especially due to the zoophilous nature of its flowers (Gentry, 1974, 1978, 1990). There are approx. 50 genera of Bignoniaceae in Brazil (Souza and Nascimento, 2005), most of them presenting flowers with rudimentary staminodes. However, in *Jacaranda* and *Digomphia*, the staminode is well developed, conspicuous, larger than the stamens, and seems to play an important role in the pollination ecology of species belonging to these genera (Gentry, 1992; Endress, 1994).

The role of staminodes in pollination has been described in various families of Angiosperms (Armstrong and Irvine, 1990; Endress, 1994; Walker-Larsen and Harder, 2000; Decraene and Smets, 2001). Experimental studies aimed at ascertaining the influence of this structure on components of reproductive success have indicated its importance, especially for female reproductive success. In those studies, lower pollen deposition on the stigma and a lower seed set were found in flowers where the staminode had been removed (Dierenger and Cabrera, 2001, 2002; Walker-Larsen and Harder, 2001).

Many species of *Jacaranda* grow in the Brazilian cerrado, a savannah-like vegetation that is predominant in Central Brazil (Mendonça et al., 1998). All species of *Jacaranda* are characterized by the abundant glandular trichomes that are present throughout the staminode (Martius et al., 1897; Gentry and Morawetz, 1992; Lohmann et al., 2008). These secretory structures might lead to specialized interactions with anthropophilous animals. However, their exact role in the pollination biology of those species is yet to be determined. In studies concerning the pollination biology of some species of *Jacaranda*, several roles have been attributed to the staminode. Among those are a secondary pollen presentation (Yanagizawa and Maimoni-Rodella, 2007), a mechanism for increased bee contact with the reproductive organs through a reduction of the space inside the floral tube (Vieira et al., 1992; Bittencourt and Semir, 2006; Yanagizawa and Maimoni-Rodella, 2007), visual orientation through contrast with the corolla (Vieira et al., 1992; Sérsic and Rando, 2004), guidance through scent emission (Vieira et al., 1992; Sérsic...
and Rando, 2004; Bittencourt and Semir, 2006) and a physical barrier against pollen robbers (Sérsic and Rando, 2004).

Despite the ecological importance of this structure in species of Jacaranda, experimental studies testing how the staminode might influence components of reproductive success are yet to be carried out. The sole study that addressed this question to any extent in Jacaranda was conducted with Jacaranda mimosifolia (Sérsic and Rando, 2004).

In the present study, the floral biology of Jacaranda oxyphylla Cham. was investigated and the role of the staminode and exudates of its glandular trichomes in the interactions with floral visitors was examined. In addition, the effect of staminode removal on the reproductive success of J. oxyphylla was evaluated.

**MATERIALS AND METHODS**

**Study site and study organism**

Fieldwork was conducted from 2004 to 2006 in two fragmented areas of cerrado located in Pratânia (22°48′52″S, 48°44′35″W) and Botucatu (22°57′38″S, 48°31′22″W), in the state of São Paulo, South-eastern Brazil.

Jacaranda oxyphylla Cham. is widely distributed in cerrado areas of South-eastern Brazil. This species is very common in open ‘campo limpo’ grasslands and the state of São Paulo, South-eastern Brazil.

In the present study, the floral biology of Jacaranda oxyphylla Cham. was investigated and the role of the staminode and exudates of its glandular trichomes in the interactions with floral visitors was examined. In addition, the effect of staminode removal on the reproductive success of J. oxyphylla was evaluated.

**Staminode morphology and composition of the secretion of staminode glandular trichomes**

Staminode samples were fixed with 2.5 % glutaraldehyde in 0.1 M phosphate buffer, pH 7.3, for 6–12 h at 4 °C. In addition, staminode samples were post-fixed with Karnovsky solution (Karnovsky, 1965), dehydrated in a graded series of ethanol solutions and embedded in histo resin (Gerrits, 1991). Sections of 8 mm were stained with 0.05 % toluidine blue (O’Brien et al., 1964). The slides were sealed with Entellan resin and examined under an Olympus BX 41 light microscope (Japan) equipped with a Olympus C7070 digital camera (Olympus, Japan).

Complementary analyses were carried out with an Olympus SZ 61 (Japan) stereoscopic microscope, also equipped with an Olympus C7070 digital camera (Olympus, Japan).

For morphological analyses by scanning electron microscopy (SEM), staminode samples from five newly opened flowers were fixed for 24 h in 2.5 % glutaraldehyde with 0.1 M phosphate buffer, pH 7.3. In addition, staminode samples were post-fixed with 1 % osmium tetroxide for 2 h, dehydrated in a graded alcohol series, critical point dried, coated with gold and examined under a Fei-Quanta 200 scanning electron microscope (Phillips, Czechoslovakia).

Fresh hand-cut sections were subjected to eight different histochemical tests: (a) periodic acid–Schiff (PAS) reaction to detect water-insoluble polysaccharides (Jensen, 1962); (b) 0.02 % ruthenium red aqueous solution to detect mucilage/pectin (Johansen, 1940); (c) Sudan IV to detect total lipids (Johansen, 1940); (d) naphthol + dimethyl-paraphenylene-diamine (NADI) reagent to detect terpenes (David and Carde, 1964); (e) 10 % ferric trichloride aqueous solution to label phenolic compounds (Johansen, 1940); (f) mercuric bromophenol blue to detect total proteins (Mazia et al., 1953); (g) Dragendorff reagent to detect alkaloids (Svendsen and Verpoorte, 1983); and (h) Fehling’s solution to detect reducing sugars (Purvis et al., 1964). Standard control procedures were carried out simultaneously, following the indicated protocols.

Temporary slides were mounted in glycerine and analysed under an Olympus BX 41 light microscope (Japan) equipped with an Olympus C7070 digital camera (Olympus, Japan). Complementary analyses were carried out with an Olympus SZ 61 (Japan) stereoscopic microscope, also equipped with an Olympus C7070 digital camera (Olympus, Japan).

Thin-layer chromatography (TLC) was used in order to investigate the presence of terpenes in staminodes. For those analyses, samples from staminodes were taken from 102 fresh flowers and immersed in chloroform for 30 min, following Siebert (2004).

Using a glass capillary tube, samples were spotted onto Silica gel 60 F354 (Merck) TLC plates, using toluene–ethyl acetate (93 : 7) as eluant. Terpenes were visualized by spraying the plates with AS (anisaldehyde–sulfuric acid) reagent, heating the plates at 100 °C for 10 min, and then evaluating the terpenes in visible light (Wagner and Bladt, 1996).

For the gas chromatography (GC) analysis, chloroform extracts of J. oxyphylla staminodes that had been ultrasonicated at room temperature for 20 min were used. The chromatograph used was a VARIAN CP-3380 coupled to an ADCB (1 V) flame ionization detector (FID), and equipped with an LM-5 capillary tube (phenyl 95 % methylpolysiloxane with a length of 15 m, internal diameter of 0.33 m and film thickness of 0.5 mm). Results were recorded on a computer equipped with VARIAN GW-V509NO Workstation software. Operating conditions were as follows, detector = 250 °C; detector = 290 °C; heating ramp-up = 150–280 °C (rate of 10 °C/min) and 28 °C for 18 min, total time of 31 min; gas flow = air at 480 mL min⁻¹, N2 at 43 mL min⁻¹ and H2 at 2 mL min⁻¹; and gas ratios = N2/H2/air 1 : 10 : 20. Authentic samples of thymol, terpineol, progesterol, tingenone, α-tocopherol, stigmasterone, campesterol, stigmasterol, α-espinasterol, β-sitosterol, α-amin, α-amin acetate, β-amin acetate, lupeol, lupeol acetate, friedelanol and friedelin were injected under identical GC conditions.

**Pollination ecology of Jacaranda oxyphylla**

Flowers were monitored to check for visitors at different times of the day, from early in the morning at 0500 h to
Staminodes from half of the flowers were removed; the and the bags were removed on the morning of anthesis. Visible surfaces were counted using an Olympus SZ 61 acetic carmine solution. Pollen grains deposited on stamens and stigmas were carefully removed and fixed in acetic carmine solution. Samples (20 μL) were mounted on slides and then pollen grains were counted using an Olympus BX 41 microscope (Japan). Data from these experiments were tested for normal distribution (Kolmogorov–Smirnov) and compared using a two-tailed t-test and Mann–Whitney U test, using GraphPad Instat v.3.01 software (San Diego, CA, USA).

RESULTS

Staminode morphology and composition of the secretion of staminode glandular trichomes

The staminode is composed of a cylindrical filament (2.8–4.3 mm long) with a broader, slightly bifid tip, and a thin base, attached to the bottom of the corolla tube. It emerges obliquely and its distal end rests upon the entrance of the corolla tube (Fig. 1B). The filament of the staminode is densely covered with capitate glandular trichomes over its entire length except the basal 10 mm (Fig. 1C). Its abaxial portion shows only capitate glandular trichomes (Fig. 1G), while the adaxial apical portion contains numerous hyaline, simple, uniseriate, un- to pluricellular long trichomes (Fig. 1H). The capitate glandular trichomes are constituted by an approximately spherical multicellular head, and a stalk varying in length, number of cells and degree of ramifications. These trichomes can be divided into three basic types according to their size. The short trichomes (Fig. 2A) are distributed over the entire abaxial surface of the staminode (Fig. 1C, G). The intermediary trichomes (Fig. 2C) may present ramifications, and are concentrated at the sides of the median portion of the staminode, forming a kind of channel situated 10–35 mm above the base (Fig. 1C). The third long-stalked ramificated trichomes (Fig. 2D) are predominantly located on the top of the abaxial apical portion of the staminode, forming a small tuft together with the simple trichomes (Fig. 1C, G). Trichome heads present 17–24 cells (n = 10) arranged concentrically (Fig. 2B) around a central cell (Fig. 2A, B). Occasionally, trichomes with two concentric layers of cells forming the glandular head are also present. In SEM analyses, capitate glandular heads present a marked surface, indicating the close attachment of the cuticle to the secretory upper cell walls and making the cell outlines evident (Fig. 2E). Alternatively, a small sub-cuticular space is formed by the detachment of the cuticle (Fig. 2F).

In pre-anthesis, droplets were found in the style and corolla. These droplets were seen in the median glandular portion of the staminode, indicating secretory activity of this structure prior to flower opening. Under the stereomicroscope, large droplets were observed on the head surface of the capitate glandular trichomes of newly opened flowers.

During the flower’s functional period, capitate glandular trichomes were found with different degrees of cuticle distension. Some of these trichomes did not present the formation of a sub-cuticular space (Fig. 2E), while others presented a slightly distended cuticle forming a small sub-cuticular...
space filled with hyaline secretion (Fig. 2C, F), and others presented a wrinkled cuticle indicating previous release of the secretion (Fig. 2G). This variation suggested that the release of secretion from the capitate glandular trichomes of the staminode of *J. oxyphylla* is continuous.

The secretion of the capitate glandular trichomes of the staminode is composed of predominantly lipophilic material. This material stained positively with Sudan IV and differentially with NADI reagent, indicating the presence of terpenes and resinic acids. The intensity and colours of the positive reaction to NADI reagent varied among trichomes of the same morphology located side by side. A strongly positive reaction occurred with phenolic compounds. Treatments with ruthenium red for mucilage/pectin and with Dragendorff solution for alkaloids proved negative. The assays for detecting proteins, sugars, neutral polysaccharides and starch showed weakly positive reactions (Table 1) and were highly variable among neighbouring trichomes.

TLC revealed the presence of several terpenoid compounds in the chloroform extract of the staminode of *J. oxyphylla*. With AS reagent, terpenes stained pinkish-purple zones; one of them, of *R*ₚ 0.33, was identified as cineole. This compound was confirmed by comparing its retention time with that of an authentic sample. The other terpenoid compounds could only be identified using...
GC–FID. The gas chromatogram of *J. oxyphylla* stamnodes presented peaks that were characteristic of pentacyclic triterpene and steroid compounds (Table 2). The identity of these compounds was confirmed by comparing their relative retention time with those of authentic samples.

**Pollination ecology of Jacaranda oxyphylla**

Flowering of *J. oxyphylla* occurred between August and October. Flowers opened predominantly at around 0700 h, and anthesis lasted about 2 d. Each inflorescence (Fig. 1A) presented one to two flowers in anthesis per day, and each plant had between one and 16 inflorescences.
TABLE 1. Histochemistry of mature capitate trichomes from the staminode of Jacaranda oxyphylla

<table>
<thead>
<tr>
<th>Staining procedure</th>
<th>Target compounds</th>
<th>Colour observed</th>
<th>Reactivity of head cells*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sudan IV</td>
<td>Total lipids</td>
<td>Red</td>
<td>+</td>
</tr>
<tr>
<td>NADI</td>
<td>Terpenes</td>
<td>Blue and dark red</td>
<td>++</td>
</tr>
<tr>
<td>Ruthenium red</td>
<td>Phenolic compounds</td>
<td>Green to black</td>
<td>++</td>
</tr>
<tr>
<td>Ferric trichloride</td>
<td>Phenol glycosides</td>
<td>Pink</td>
<td>+</td>
</tr>
<tr>
<td>Schiff (PAS)</td>
<td>Neutral polysaccharides</td>
<td>Black</td>
<td>–</td>
</tr>
<tr>
<td>Dragendorff</td>
<td>Alkaloids</td>
<td>–</td>
<td>+</td>
</tr>
<tr>
<td>Mercuric</td>
<td>Proteins</td>
<td>Dark blue</td>
<td>+</td>
</tr>
<tr>
<td>Bromophenol blue</td>
<td>Starch grains</td>
<td>Purple</td>
<td>+</td>
</tr>
<tr>
<td>Fehling’s solution</td>
<td>Sugars</td>
<td>Reddish</td>
<td>+</td>
</tr>
</tbody>
</table>

*– negative, + slightly positive, ++ strongly positive.

TABLE 2. Terpenoid composition of chloroform extract from the staminode of Jacaranda oxyphylla by GC–FID

<table>
<thead>
<tr>
<th>Peak</th>
<th>Retention time</th>
<th>Compounds</th>
<th>Phytochemical classes</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>12.7</td>
<td>Campesterol</td>
<td>Steroids</td>
</tr>
<tr>
<td>2</td>
<td>12.9</td>
<td>Stigmasterol</td>
<td>Steroids</td>
</tr>
<tr>
<td>3</td>
<td>16-4</td>
<td>β-Sitosterol</td>
<td>Steroids</td>
</tr>
<tr>
<td>4</td>
<td>16-8</td>
<td>α-Amyrin</td>
<td>Pentacyclic triterpenes ursane type</td>
</tr>
<tr>
<td>5</td>
<td>17-7</td>
<td>β-Amyrin</td>
<td>Pentacyclic triterpenes oleane type</td>
</tr>
<tr>
<td>6</td>
<td>18-8</td>
<td>Lupeol</td>
<td>Pentacyclic triterpenes lupane type</td>
</tr>
<tr>
<td>7</td>
<td>20-5</td>
<td>Lupeol acetate</td>
<td>Pentacyclic triterpenes lupane type</td>
</tr>
<tr>
<td>8</td>
<td>20-9</td>
<td>Friedelanol</td>
<td>Pentacyclic triterpenes friedelane type</td>
</tr>
<tr>
<td>9</td>
<td>21-5</td>
<td>Friedelin</td>
<td>Pentacyclic triterpenes friedelane type</td>
</tr>
</tbody>
</table>

presenting 7.5 ± 5.2 first-day flowers per day (mean ± s.d.). Three- to 6-d-old flowers remained in the inflorescences, even though these were no longer receptive, perhaps acting as a visual attractor for visitors. After the third day, the corolla presented faded colouring, and a darkened anther and staminode, with the stigma being the last structure to display signs of senescence. The floral tube was 32–50 mm longer (mean ± s.d.), and the pollen grains remained clustered inside the anthers. Pollen grains were only released when they were lightly squeezed against the corolla tube roof by visitors. At the start of anthesis, the corolla lobes were completely distended and an elongated platform was formed by the lower lobe, upon which the bright tip of the staminode contrasted (Fig. 1B). The stigma was sensitive, and closed in a matter of seconds when touched. When pollen deposition did not occur, the stigma remained closed for about 30 min, after which it gradually opened again, completing the process after 2 h.

Pollinators

The populations studied here were preferentially xenogamous, with the formation of fruits occurring in only 3.85% of the selfed flowers. Natural fruit set was low (7.25%) and no case of spontaneous self-pollination or autonomous agamospermy was found, indicating the need for a pollen transfer vector. The hand-cross pollination test produced greater fruit set (Table 3).

Small bees of the genera Ceratina, Trigona, Augochlora and Exomalopsis were observed visiting flowers of J. oxyphylla (Table 4). Among these small bees, only Exomalopsis fulvofasciata presented a legitimate visiting behaviour. This bee entered deep into the floral tube, passing over the staminode, seeking the nectar chamber and gathering nectar by extending its 4 mm long proboscis. Whenever it left the flower, its head and the dorsal portion of its thorax were covered with pollen. The narrowing of

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the tube diameter caused by dorsoventral compression and the arrangement of the staminode in relation to the reproductive structures favoured the contact of *E. fulvofasciata* with those structures. Despite its small size, this bee is quite heavy and can lower the staminode upon entering the floral tube and treading on it. *Ceratina, Augochlora* and *Trigona*, on the other hand, normally entered the staminode sideways and did not present legitimate visiting behaviour. These bees visited flowers from sunrise to dusk. At first, they investigated each flower visually; if there was no other visitor, they entered the floral tube laterally (Fig. 3A). Upon reaching the anthers, bees turned their ventral side toward the corolla tube roof and collected pollen actively with their first pair of legs, while simultaneously cleaning their bodies and transferring pollen to the subsequent pair of legs (Fig. 3B). This process lasted from 1 to 3 min. During these movements various parts of the bee’s body, covered with large quantities of pollen from this flower, established contact with the stigmatic surface, favouring a potential self-pollination. However, stigma closure was never observed during this contact. After pollen collection, the bees turned their abdomen downwards and actively scraped the glandular portion of the staminode. Then, the bees flew to other flowers of the same plant or to neighbouring plants. These bees were never observed gathering nectar from flowers of *J. oxyphylla*.

Besides *E. fulvofasciata*, medium-sized bees also visited *J. oxyphylla* flowers legitimately (Table 4). *Eulaema nigrita* (Euglossini) was observed on two occasions, visiting about ten flowers of neighbouring plants (Fig. 3E). After approaching an inflorescence, the bee landed on the anterior lobe of the corolla and then walked along the corolla tube floor, passing over the staminode, towards the nectar chamber. The bee touched the stigma and the anthers with its back. After 10–20 s, the female bee reversed out of the corolla tube, hovering for a few seconds in front of the visited flower, apparently cleaning its body. The anthers were arranged 15–30 mm above the nectar chamber, and the extended proboscis of *E. nigrita* was about 25 mm long. In order to reach the nectar, individuals of *E. nigrita* need to touch the reproductive structures with their head and dorsal portion of their thorax, while the ventral portion touches the entire median glandular region of the staminode.

Visits by a species of bumble-bee, *Bombus morio*, were also observed on two occasions (Fig. 3D). Before entering the flowers, the bee hovered for a few seconds in front of a flower and then landed on the anterior lobe of the corolla, walking on the tube floor towards the flower base. When *B. morio* left the flower, it displayed a large quantity of white pollen deposited on its head and the dorsal region of its thorax. Individuals of *B. morio* were subsequently observed visiting another flower on the same plant or flowers on some neighbouring plants.

Both medium-sized bees, *E. nigrita* and *B. morio*, were legitimate pollinators carrying pollen and touching the receptive surface of stigmatic lobes upon entering flowers of *J. oxyphylla*. Whenever these species presented pollen deposited on their dorsal region, only the sterile portions of the stigmatic lobes were subsequently touched, when leaving the flower, restricting the possibility of self-pollination.

Visits by an unidentified species of hummingbird (Trochilidae) were also observed. These hummingbirds visited 5–15 receptive flowers per flight, between 0800 h and 0900 h. During flower visits, the hummingbirds thrust their heads into the floral tube for 1 or 2 s, usually remaining in hovering flight. In some instances, the hummingbirds also rested their feet briefly on the inflorescence. Given that the hummingbird’s size (beak length approx. 20 mm) fit the reproductive structures and nectar chamber of *J. oxyphylla* very well, this species may be considered a legitimate pollinator. However, corolla abscission frequently occurred

### Table 3. Experimental pollination and fruit set of *Jacaranda oxyphylla*, in a cerrado patch, Botucatu, SP, Brazil *(n* = 30 plants)*

<table>
<thead>
<tr>
<th></th>
<th>Spontaneous self-pollination</th>
<th>Autonomous agamospermy</th>
<th>Hand self-pollination</th>
<th>Hand cross-pollination</th>
<th>Natural pollination (control)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Flowers</td>
<td>478</td>
<td>182</td>
<td>39</td>
<td>78</td>
<td>41</td>
</tr>
<tr>
<td>Fruits</td>
<td>32</td>
<td>0</td>
<td>0</td>
<td>3 (3.85 %)</td>
<td>19 (46.34 %)</td>
</tr>
</tbody>
</table>

### Table 4. Visitors to flowers of *Jacaranda oxyphylla* in patches of cerrado vegetation, in Botucatu and Pratânia, SP, Brazil

<table>
<thead>
<tr>
<th>Species</th>
<th>Visiting behaviour</th>
<th>Foraged resource</th>
<th>Frequency*</th>
</tr>
</thead>
<tbody>
<tr>
<td>ANDRENIDAE</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>Oxaea flavescens</em></td>
<td>Non-legitimate</td>
<td>Nectar</td>
<td>High</td>
</tr>
<tr>
<td><em>Bombus</em> (Fervidobombus) morio</td>
<td>Legitimate</td>
<td>Nectar</td>
<td>Low</td>
</tr>
<tr>
<td><em>Ceratina</em> (Crewella) maculifrons</td>
<td>Non-legitimate</td>
<td>Pollen</td>
<td>High</td>
</tr>
<tr>
<td><em>Ceratina</em> (Crewella) gossypii</td>
<td>Non-legitimate</td>
<td>Pollen</td>
<td>High</td>
</tr>
<tr>
<td><em>Ceratina</em> (Crewella) asuncionis</td>
<td>Non-legitimate</td>
<td>Pollen</td>
<td>High</td>
</tr>
<tr>
<td><em>Eulaema</em> (Apelaema) nigrita</td>
<td>Legitimate</td>
<td>Nectar</td>
<td>Intermediate</td>
</tr>
<tr>
<td>Exomalopsis</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>fulvofasciata</em></td>
<td>Legitimate</td>
<td>Nectar and pollen</td>
<td>Low</td>
</tr>
<tr>
<td><em>Trigona spinispe</em></td>
<td>Non-legitimate</td>
<td>Pollen</td>
<td>High</td>
</tr>
<tr>
<td><em>Xylocopa</em> sp.</td>
<td>Non-legitimate</td>
<td>Nectar</td>
<td>Low</td>
</tr>
<tr>
<td>HALICTIDAE</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>Augochlora</em> (Augochlora) sp.</td>
<td>Non-legitimate</td>
<td>Pollen</td>
<td>High</td>
</tr>
<tr>
<td>TROCHILIDAE</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>Trochilidae</em></td>
<td>Legitimate</td>
<td>Nectar</td>
<td>Intermediate</td>
</tr>
</tbody>
</table>

*Frequency: high (30 visits d<sup>-1</sup>), intermediate (1–5 visits d<sup>-1</sup>), low (<1 visit d<sup>-1</sup>).
a few seconds after the hummingbird left the flower, leaving behind only the calyx and the gynoecium and precluding further visits.

Lastly, *Oxaea flavescens* and *Xylocopa* sp. were observed stealing nectar (Table 4). Both presented similar behaviour, landing externally on the floral tube, introducing their mouth apparatus between the calyx and the corolla and actively stealing nectar through longitudinal slits produced at the base of the corolla tube (Fig. 3C).

**Staminode removal experiments and reproductive success**

Even though flowers without a staminode differed visually from intact flowers, no significant differences were found between treatments considering the presence and absence of staminode. No significant difference was found for pollen deposition on stigmas (n = 32) (Mann–Whitney U test, P = 0.89) and for pollen removal from anthers (n = 32) (t-test, P = 0.49) when comparing flowers with and without a staminode. Flowers with staminodes received pollen deposition on stigmas varying from 0 to 144 pollen grains (Fig. 4). On the other hand, flowers without staminodes only presented deposition of small quantities of pollen in all samples evaluated, with 0 to 15 pollen grains per stigma (Fig. 4). Small Halictidae and Anthophoridae bees were observed visiting flowers with and without a staminode, displaying the same behaviour (Fig. 3A, B) in both treatments. Although flowers whose staminodes had been removed were visited more frequently (15 : 1) by these bees, the total pollen grain removal was similar in flowers with and without staminodes (Fig. 5).

The percentage of flowers with staminodes that received a large amount of pollen grains on the stigma (6-25 %) was close to the natural fructification, i.e. 7-25%. This deposition (>140 pollen grains per stigma, Fig. 4) was compatible with the number of ovules per flower (113.28 ± 21.14,
mean ± s.e.). On the other hand, the number of pollen grains (<20 pollen grains, Fig. 4) deposited on stigmas of flowers without staminodes was much lower than the mean number of ovules per flower.

**DISCUSSION**

Staminode morphology and composition of the secretion of staminode glandular trichomes

In some species the secretion produced by glandular trichomes remains accumulated inside the sub-cuticular space, and is exclusively released through mechanical contact. This contact is usually provided by herbivores that break the cuticle and cause the release of substances that are normally associated with chemical defence (Ascensão et al., 1995, 1997; Sacchetti et al., 1999; Pichersky and Gershenzon, 2002; Machado et al., 2006). However, in the staminode of *J. oxyphylla* it was found that the secretion was already available at flower opening and could be scattered in the air, collected and easily transferred to the body of the pollinator during a visit. Sérsic and Rando (2004) detected the presence of secretion components produced by the glandular trichomes of the staminode of *J. mimosifolia* in the body of *B. morio*, indicating transfer of secretion components during visits.

Chemical analyses of the secretion produced by capitate glandular trichomes of the staminode of *J. oxyphylla* allowed for a better understanding of the role of this structure in the interactions with floral visitors. Phenolic and terpenic compounds constitute the secondary compounds most widely distributed among plants (Harborne, 1997). The phenolic compounds comprise approx. 8000 molecules, including flavonoids and tannins that contribute to the flavour, odour and colour of a variety of plants (Harborne, 1985, 1997; Nishida, 2002). Phenolic compounds in the capitate trichomes of the staminode of *J. oxyphylla* can be of different types and may play different roles. For example, trichomes may play a role in protection against ultraviolet radiation (Harborne, 1997), an important aspect in open vegetation formations such as the cerrado.

On the other hand, terpenoids constitute a wide and complex class of secondary compounds that play essential roles in plant–animal and plant–plant interactions, acting as feeding deterrents, pheromones, defence agents and allelochemicals (Harborne, 1997). The presence of monoterpene sesquerpenes and sesquiterpenes in flowers has been related to the attraction of pollinators, especially bees, moths and butterflies, that may, in some cases, be species specific (Harborne, 1997; Pichersky and Gershenzon, 2002). The monoterpene cineole, identified in the capitate glandular trichomes of the staminode of *J. oxyphylla*, comprises highly volatile fragrances associated with the localization of the flowers of these plants by ‘trapliners’ and opportunistic pollinators, and are commonly found in Orchidaceae (Cancine and Damon, 2007).

Triterpenes make up a more diversified sub-group of terpenes and can perform ecological defence-related functions.
(Harborne, 1997; Cheng et al., 2007). In this study, pentacyclic triterpenes and steroidal triterpenes produced by the staminode of *J. oxyphylla* may also perform these functions. Pentacyclic triterpenes can be found in combinations containing variable quantities of terpenes mixed with other classes of substances (Wagner and Bladt, 1996), such as the oil–resin mixture identified in the capitate glandular trichomes of the *J. oxyphylla* staminode. Although these compounds are widely distributed throughout higher plants, their presence in flowers has been described for only a few species. In particular, they have been found in *Dalechampia* (Armbuster, 1984) and *Tipuana tipu* (Pereira and Aquino Neto, 2003). Floral resins, normally composed of triterpenes, are collected by various genera of Neotropical Euglossini bees (Armbuster and Webster, 1979).

According to Roubik and Hanson (2004), both male and female Euglossini bees depend greatly on non-food materials in their environment. Males need to collect chemicals and females must find nesting material. Unlike bumble-bees, stingless bees and honey bees, Euglossini bees use no wax, depending heavily on resins for nest construction. The resin from flowers remains soft and pliable for a long time, unlike the resin or resinous sap that exudes from plant wounds (Roubik and Hanson, 2004). In addition to nesting material, some triterpenes, the primary compound of plant resins, provide antibiotics. Oliveira et al. (1996) tested the effectiveness of resin from *Clusia grandiflora* flowers and found that those resins are highly effective against Gram-positive bacteria, a deadly microbial enemy of bees.

On the other hand, steroidal triterpenes play an important role in plant–insect interactions, since many phytophagous and omnivorous insects are unable to biosynthesize the steroid nucleus (Svoboda and Feldlaufer, 1991). Steroids such as sitosterol, campesterol and stigmasterol are essential in structural and hormonal functions (Roitberg and Isman, 1992). Rasmont et al. (2005) found the presence of β-sitosterol in the pollen of a legume pollinated by *Bombus terrestris*. The authors state that this compound has a feeding deterrent effect on *Apis mellifera*, which fed on the nectar but not on the pollen of this species. This may be one of the reasons why no visits by *A. mellifera* to the flowers of *J. oxyphylla* were recorded.

Females of *E. nigrita* act as legitimate pollinators of *J. oxyphylla*. Even though male visits were also observed, it is unclear whether they also act as legitimate pollinators. It should be noted that males of this species collect volatile substances (fragrances) from floral and non-floral sources and store those substances in cavities located in the posterior tibia, where they accumulate complex, species-specific blends of fragrances (Schiestl and Roubik, 2003; Eltz et al., 2006). Eltz et al. (2003) analysed the content of the tibia of Euglossini males and detected mixtures of terpenoids and aromatic compounds totalling 70 substances, including cineole. Euglossini males feed on nectar of plant species that are not necessarily producers of fragrances; hence, cineole may act in the attraction of *E. nigrita* males to flowers of *J. oxyphylla*, which offers nectar as a reward, in addition to the secretions of the staminode.

In other species of this genus, *J. caroba* and *J. decurrens*, males of *Euglossa* were observed collecting fragrance from the staminode (Gottsberger and Silberbauer-Gottsberger, 2006). The authors reported that males, when exiting flowers, hover in front of those flowers and make typical movements with their legs that are associated with the transfer of liquid odour (fragrance) to the tibial capsule, similar to the behaviour performed by a male of *E. nigrita* in flowers of *J. oxyphylla*. Visits from Euglossini males were also described in other Bignoniaceae (Roubik and Hanson, 2004; Silva et al., 2007).

It is also noteworthy that Euglossini bees are the main pollinators of most species of *Jacaranda* studied so far, including *J. copaia*, *J. ulei*, *J. simplicifolia*, *J. rufa*, *J. racemosa*, *J. paucifoliolata*, *J. rugosa* and *J. decurrens* (Vieira et al., 1992; Stevens, 1994; Maués et al., 2004; Bittencourt and Semir, 2006; Sampaio et al., 2007; Yanagizawa and Maimoni-Rodella, 2007). According to Gottsberger and Silberbauer-Gottsberger (2006), small bees feed on pollen, while male Euglossini bees collect fragrances from the staminode of *Jacaranda*. Additionally, females of Euglossini and other species of large bees feed mainly on nectar in *Jacaranda*, leading to what Gottsberger and Silberbauer-Gottsberger (2006) call a superimposed pollination system. The behaviour of the females of *E. nigrita*, hovering and cleaning their bodies in front of the visited flower of *J. oxyphylla*, suggests that this bee could be collecting the secretion of the staminode capitate glandular trichomes transferred to its body during the visit.

The presence of substances related to nest building and chemical defences (i.e. resins and pentacyclic triterpenes), to the structural and hormonal development of bees (i.e. sterols) and to the attraction of Euglossini males (cineole) suggests that the secretions of the capitate glandular trichomes of the staminode are involved in complex chemical interactions. In particular, those trichomes seem to provide a variety of substances that are essential to the biology of bee pollinators of *J. oxyphylla*.

**Pollination ecology of Jacaranda oxyphylla**

Several structural and functional features of *J. oxyphylla* flowers indicate pollination by bees (Faegri and Pijl, 1979; Proctor and Yeo, 1979). Flowers of *J. oxyphylla* are of the *Anemopaegma* type described by Gentry (1974), which presents nototribic pollination carried out by medium-sized and large bees, normally Apidae (Euglossini tribe), and are visited by nectar robbers such as *Xylocopa* and hummingbirds. Nevertheless, only legitimate hummingbird visits were recorded to *J. oxyphylla* flowers. This species blooms during the driest season of the year, when water and energetic resources for visitors are scarce. Considering that hummingbird visits were not observed in all studied populations, nor in previous studies of *J. oxyphylla* conducted by Yanagizawa and Maimoni-Rodella (2007), it is possible that hummingbird visits may simply be opportunistic. In cerrado woody plants opportunistic visits by hummingbirds were recorded in >30% of the species studied by Oliveira and Gibbs (2002).

The intensive activity recorded for *O. flavescens*, a nectar robber, could be related to the considerable increase in its population size in winter, when *J. oxyphylla* is in...
blossom. *Oxaea flavescens* is one of the most regular and abundant nectar robbers in the Brazilian cerrado, and is commonly observed robbing nectar in species of Bignoniaceae that occur in this biome (Gottsberger and Silberbauer-Gottsberger, 2006).

Nectar is the main caloric resource available to pollinators of *J. oxyphylla*. Therefore, it is possible that low nectar production allied to intensive pillage by *O. flavescens*, a low percentage of nectar-producing flowers (43\% of flowers lacked nectar) and flower sparseness at anthesis may lead to insufficient nectar available to pollinators. This low availability of nectar may be incompatible with the energetic needs of pollinators and may be responsible for the low visit rate and low rate of natural fructification observed in the studied populations.

Considering the flexible reproductive system of *J. oxyphylla*, selfing could represent an alternative to propagation via seeds, especially in cerrado populations where the frequency of medium-sized and large visitors was low. However, it was found that although *Ceratina*, *Trigona* and *Augochlora* removed pollen intensively from flowers, their visits resulted in a reduced deposition of pollen on stigmas. The findings suggest that these small bees do not participate substantially in the pollination of *J. oxyphylla*, acting predominantly as pollen robbers, different from the situation proposed by Vieira et al. (1992) for *J. caroba* and by Bittencourt and Semir (2006) for *J. racemosa*. The only small bee that behaved like a legitimate pollinator of *J. oxyphylla* was *E. fulvosaccata*, whose behaviour was similar to that observed by Silva et al. (2007) in *Tecoma stans*.

*Jacaranda oxyphylla* presents the ‘modified steady-state’ phenological pattern described by Gentry (1974). This pattern is characterized by a scanty flower production per day over a period of several weeks and is typical of plants pollinated by bees that establish fixed daily foraging routes (e.g. Euglossini bees; Janzen, 1971). Studies related to flight behaviour showed that these bees present strong orientation and odour perception abilities even on extremely large areas of continuous forest (Ackerman et al., 1982; Roubik, 1989; Roubik and Hanson, 2004). Even though Euglossini bees are exclusively from forest habitats, *E. nigrita* also occurs in fragmented areas (Wittmann et al., 1988; Tonhasca et al., 2003; Milet-Pinheiro and Schindwein, 2005). *Eulaema nigrita* is distributed from Costa Rica to northern Argentina (Roubik and Hanson, 2004), comprising the geographic distribution of *J. oxyphylla* (Gentry and Morawetz, 1992). This fact, associated with the foraging behaviour of *E. nigrita* (Roubik and Hanson, 2004), may lead to the dispersal of pollen of *J. oxyphylla* over large areas.

Unlike Euglossini, bees belonging to the genus *Bombus* depend on the concentration of floral supplies (Walther-Hellwig and Frankl, 2000) and present a behaviour that tends to generate small-sized neighbourhoods (Schmitt, 1980). Thus, in the population of *J. oxyphylla* studied by Yanagizawa and Maimoni-Rodella (2007), the high density of flowering individuals in a small area may have favoured the high frequency of visits of *Bombus atratus*. In the present study, the low frequency of *B. morio* bees may have been due to the paucity of resources available.

The low natural fruit set observed in the populations of *J. oxyphylla* analysed in this study could be the result of the low frequency of pollinator visits recorded. Moreover, the reduced number of plants flowering simultaneously in the studied populations could lead to a transfer of mostly incompatible pollen. Given that previous studies indicated selfing rates of 26\% in this species (Yanagizawa and Maimoni-Rodella, 2007), the possibility that the compatibility system of *J. oxyphylla* is flexible should not be disregarded. In the case of selfing, seed production could be incremented through geitonogamy, since *J. oxyphylla* pollinators visit several flowers on the same plant sequentially. There is evidence that a mixed mating system combining high levels of allogamy with extra flexibility of permitting some selfing occurs in Bignoniaceae (Bertin and Sullivan, 1988; Bianchi et al., 2005; Bittencourt and Semir, 2006).

The low P/O ratio observed (154.64 ± 41.38) suggests that facultative autogamy is occurring in *J. oxyphylla*. However, its nototribic flowers could represent a more precise pollination mechanism, producing a deviation of the P/O ratio, as pointed out by Dafni et al. (2005) in predominantly allogamous species.

**Staminode removal experiments and reproductive success**

Intact flowers of *J. oxyphylla* tended to have higher pollen deposition on the stigma, indicating the participation of the staminode in female reproductive success, as observed by Walker-Larsen and Harder (2001) and Dieringer and Cabrera (2002) in *Penstemon* species pollinated by bees.

Several functions have been attributed to the staminode of *Jacaranda*, such as avoidance of pollen robbing, visual orientation, visual signal of the ending of nectar production, secondary pollen presentation, nectar guidance by odour emission and reduction of floral tube inner space (Morawetz, 1982; Vieira et al., 1992; Sérsic and Rando, 2004; Bittencourt and Semir, 2006; Yanagizawa and Maimoni-Rodella, 2007).

Overall, the staminode of *J. oxyphylla* does not seem to play any mechanical role in restricting access to pollen, as suggested for *J. mimosifolia* by Sérsic and Rando (2004), given that the small bees *Ceratina*, *Augochlora* and *Trigona* removed similar quantities of pollen grains from the anthers in flowers with and without a staminode. It is also unlikely that the staminode of *J. oxyphylla* has an essential role in visual orientation as found in other species of *Jacaranda* (Vieira et al., 1992). This is due to intrapopulation variation in the colour pattern of capitulate glandular trichomes encountered in *J. oxyphylla*, resulting in very distinctive visual patterns among flowers.

In addition, the role of the staminode as a visual indicator of flower senescence and consequent ending of nectar production was discarded for *J. oxyphylla* since the visual changes between fresh and old flowers were very discreet. Similar results were found in *J. racemosa* (Bittencourt and Semir, 2006). The function of secondary pollen presentation was not observed for the *J. oxyphylla* staminode since the pollen grains remain clustered inside the anthers.
An additional function attributed to the staminodes is the role of guidance, through odour emission. This role has been attributed to the staminodes of other species of Jacaranda (Vieira et al., 1992; Sérsic and Rando, 2004; Bittencourt and Semir, 2006). However, it was shown here that the white spot of the corolla tube may also carry out this function, given that it produces a similar mild and sweetish aroma as well as the fact that it reacts positively to neutral red. This emission might also be complemented by the capitate glandular trichomes of the staminode, which, together, form a tunnel of aroma emission that converges towards the reproductive structures and nectar chamber.

From a structural viewpoint, the spatial arrangement of the staminode in the floral tube of _J. oxyphylla_ may cause the reduction of floral tube inner space, favouring the contact of some small bees with reproductive organs, as observed for _E. fulvofasciata_.

The staminode may be involved in complex chemical interactions considering the presence of cineole, resins, steroids, pentacyclic triterpenes and phenolic compounds in the secretions of the capitate glandular trichomes. Some of these substances, such as cineole and other terpenoids, may act as secondary attractants for pollinating bees. On the other hand, considering the behaviour of females of _E. nigrita_ in flowers of _J. oxyphylla_ and the functions that have been ascribed to other substances found in the secretion of the capitate glandular trichomes, the possibility that the staminode may act as the primary attractant should not be disregarded.

In conclusion, it is suggested that the staminode of _J. oxyphylla_ is multifunctional and positively influences female reproductive success, acting physically as a lever and chemically as an attractant for pollinating bees.

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