



Floral and reproductive biology and pollinators of *Campomanesia guazumifolia* (Cambess.) O. Berg., neglected species

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ABSTRACT. Studies on the Myrtaceae family are mainly distributed in floristic surveys, reproductive studies of plant communities or related to family taxonomy. Based on this, the objective of the present study was to elucidate aspects of floral and reproductive biology and, of floral visitors from *Campomanesia guazumifolia* (Cambess.) O. Berg., a neglected species. Information was obtained on floral morphology and morphometry, anthesis, nectary, and structures attractive to pollinators, characterization of pollinators, receptiveness of stigma and maturing of the androecium components, and characterization of the reproductive system. Sete-capote tree has hermaphrodite flowers, and the floral opening occurs mainly during the daytime. Pollen grains was the main resource offered to pollinators. The flowers had mellow sweet odor, attracting mainly native bees and *Apis mellifera*, which was characterized as effective pollinators. The species presents high reproductive efficiency and could be considered self-compatible; however, fertilization also occurs by cross-pollination.

Keyword: sete-capote tree; Myrtaceae; floral visitors; vibrating pollination.

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Introduction

The Myrtaceae family was considered to be of the best representation in the Brazilian biomes. It has approximately 5,500 species, which are distributed in 142 genus. Considering that there are approximately 400 families representing Angiosperm flora, this family represents about 1.32% of the total, being considerably representative (Wilson, 2011).

The species Myrtaceae are of great ecological importance in the structure of the forests, mainly in the Atlantic Forest, wet forests and in the bushy of the Brazilian east region (Lorenço & Barbosa, 2012; Morais, Conceição, & Nascimento, 2014; Flora do Brasil, 2020). These are essential for the preservation of the natural state of these areas, besides providing subsidies for insertion in the consumer market.

Within the Myrtaceae family stands out the species known as sete-capote tree (*Campomanesia guazumifolia* (Cambess.) O. Berg.), which has as differential characteristics the calyx completely closed in the floral bud, and big fruits of yellowish-green color, with characteristic flavor and high vitamin content. It also stands out for presenting medicinal characteristics (Sanquetta, Fernandes, Miranda, & Mognon, 2010). The fruit was also consumed by the fauna, which makes it commonly recommended in areas of reforestation (Carvalho, 2011).

Despite the importance it represents, many species of the Myrtaceae family was still neglected, lacking morphological and anatomical studies. These studies are primordial, since the knowledge of these aspects are essential for understanding ecological relations, in the elaboration of management protocols in cultivation, mainly regarding the necessity or not of the use of pollinator genotypes (Danner, Citadin, Sasso, Scariot, & Benin, 2011; Torres & Galetto, 2011; Pessoa, Mann, Santos, & Ribeiro, 2012; Françoso et al., 2014).

In view of the above and the scarcity of research on native species of the Myrtaceae family, the objective of this study was to elucidate aspects of floral and reproductive biology of sete-capote tree and its floral visitors.

Material and methods

The study was developed in the Laboratory of Plant Physiology and Orchard of Native Fruit Plants of the UTFPR Experimental Station, Campus Dois Vizinhos - PR, from October to November 2016 and 2017.

In order to identify the matrices used as a single species, this was compared through illustrated synoptic frames (Vidal & Vidal, 2010).

The species used were registered in the *Universidade Tecnológica Federal do Paraná*, Campus Dois Vizinhos (Estevan & Ferrari, 2018).

Floral morphology

The floral characterization was obtained through a illustrated synoptic frames (Vidal & Vidal, 2010; Gonçalves, 2011).

Determination of the anthesis

The floral opening was follow in five non-consecutive days (5:00 a.m. to 8:00 p.m.), of five matrix plants containing 10 flower balloons each, identifying the moment of each phenol-phases (early floral opening, full floral opening, early senescence, total senescence). Further, the controlled anthesis was observed in an environment of approximately 25°C, of pre-anthesis flowers fixed in phenolic foam, accompanied by the complete floral opening.

Identification of nectaries and attractive structures for flower visitors

Through the arrangement of micro capillary tubes in the pedicel, base of the sepals, petals, stamens and carpels of fresh flowers, the presence or absence of nectar had been identifying (Versieux, Acosta, Jordao, Zidko, & Maia, 2014). For the visual identification of nectars, a handheld digital microscope (Digital Microscope® Model HD Color CMOS Sensor U500X).

The test with ammonium hydroxide (NH₄OH) was realized for the identification of floral resource guides. The contrast difference when observed occurs due to the absorption of ultraviolet light by flavonoid type pigments, while the other parts of the flower reflect it, allowing floral visitors to identify and locate the resources (Storti, 2002). As a complementary test, the camera was fitted with ultraviolet light (luminescence), for direct observation of fresh flowers (post-anthesis), identifying the structures reflecting or absorbing ultraviolet light.

To identify areas of higher metabolic activity at the time of anthesis, fresh flowers were maintained for 60 minutes in 1% neutral red solution. After this period, it was possible to identify the presence of metabolic activity and osmophores (gland producing volatile substances, odor), through the stain tissues (Dafni, Kevan, & Husband, 2005).

In order to identify the odor-releasing floral structures, flowers were dissected in sepals, petals, stamens and pistils, by placing them in test tubes sealed with plastic film for five hours. Therefore, ten volunteers were interviewed in an attempt to identify the main odor-releasing from the floral piece and which kind of exhaled odor (sweet, floral, citric and woody, among others) (Versieux et al., 2014). For the identification of the moment of the odor release, 10 volunteers conducted an olfactory bioassay in which post-anthesis whole flowers were allotted in plastic-film-sealed containers and evaluated olfactory at hourly intervals for 10 hours.

Characterization of Pollinators and Floral Visitors

Observations of at least five matrices of sete-capote tree were carried out, in nonconsecutive days, from 5:00 a.m. to 8:00 p.m., obtaining the visiting schedules of the visitors (Kiill & Simão-Bianchini, 2011; Versieux et al., 2014).

The observed individuals were classified as pollinators (frequent and legitimate visits (contact in all reproductive structures); occasional pollinators (less frequent visits, even if they are legitimate visitors) and pillagers (without contact with reproductive structures)) (Matias & Consolaro, 2014). To identify them, when not possible in the field, these were captured, kept in FAA (formaldehyde - alcohol - acetic acid), for later identification of family, genus and species when possible, with the help of an identification guide (Fujihara, Forti, Almeida, & Baldin, 2014).

Receptivity of androecium stigma and maturity

To identify the moment the stigma became receptive 15 pre-anthesis flowers, beginning of the anthesis, total anthesis, and beginning of senescence were evaluated. For the observation, 3% hydrogen peroxide was used, which through peroxidase activity promotes bubbling on the stigma, confirming the receptivity (Matias & Consolaro, 2014; Versieux et al., 2014).

As a complementary test, 1% neutral red solution was used in which the flowers were maintained for 60 minutes. Therefore, the receptivity was confirmed by the presence of metabolic activity that is represented in the stain tissues (Dafni et al., 2005).

Reproductive system characterization

To characterize the reproductive system, 12 plants and 100 flowers were used for each treatment: (1) apomixis (emasculated and bagged flower buds); (2) spontaneous self-pollination (bagged flower buds, without manipulation of pollen); (3) manual self-pollination or geitonogamy (flower buds bagged the day before anthesis, manual pollination carried out after pollen of the same flower); (4) open pollination or control (flower buds without insulation with paper bag, allowing access for floral visitors); (5) cross-pollination or xenogamy (emasculature in pre-anthesis and pollination using pollen from another plant).

The treatments were applied based on the stigma receptivity test and, using fresh pollen, from flowers also bagged before anthesis. All manipulated flowers were isolated using paper bags, which were removed after four days. The treatments were followed until fruit maturation or floral abscission.

The self-incompatibility index (SII) was calculated based on the relationship between the percentage of fruits obtained from manual self-pollination and the percentage of fruiting from cross-pollination. When the SII does not exceed 0.25, it means that the species is self-incompatible (Oliveira & Gibbs, 2000). The spontaneous self-pollination index (SSPI) was calculated from the relation between the percentage of fruiting formed by spontaneous self-pollination and the percentage of fruit formed by manual self-pollination (Sobrevila & Arroyo, 1982). The closer to SSPI, the less likely is the reproductive strategy (Polatto & Alves-Junior, 2009).

The reproductive efficiency (RE) was obtaining through the ratio between the percentage of fruits formed by open pollination (control) and those by manual cross-pollination (Zapata & Arroyo, 1978). When there is evidence of low pollination efficiency, the RE will be close to zero (Polatto & Alves-Junior, 2009).

Results and discussion

Morphology and floral morphometric

Sete-capote tree has simple leaves, with opposite arrangement and hermaphrodite flowers, which are distribute evenly in all the quadrants of the tree. The flowers have an actinomorphic symmetry, 4 to 7 cm in diameter, a calyx with two to four green color gamosepalous (applies to the flower and to the calyx that has welded sepals), and a corolla generally containing seven white polypetalous (corolla with free petals between them), 4.5 cm wide and 5.5 cm long average (Figure 1).

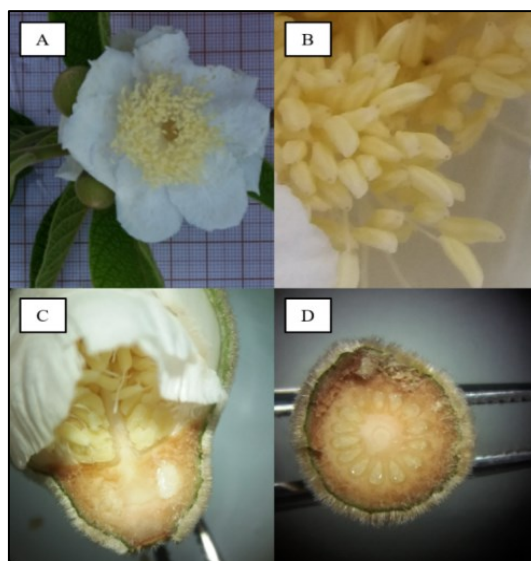


Figure 1. Flowers of sete-capote tree: A) Floral balloon in pre-anthesis and beginning of anthesis. B) Full flower (front). C) Longitudinal section of the ovary. D) Transverse section of the ovary.

Regarding the size of the stamens, these were classifying as heterodynamous (different stamens), and as welding were classifying as apostemonous (separated stamens). Each flower has approximately 900 anthers, of yellowish color with poricidal dehiscence (Figure 1B). The stylet is erect, the fillets being simple and exerts

(stand out in the neck of the calyx/corolla), and the stigma overhangs the length of the stamens. The inferior ovary (Figure 1C) has central placentae (Figure 1D) (loci attached to a central column), with approximately 12 loci (plurilocular) and 14 ovules per loci (pluriovular).

The observed characteristics were similar for other species of the genus *Campomanesia* (Lima, Goldenberg, & Sobral, 2011; Oliveira, Funch, & Landrum, 2012), being the number of anthers and the size of the flower superior and discrepant when compared to other species of the Myrtaceae family.

Determination of the anthesis and maturity of androecium

The floral opening occurred mainly during the day (80%). However, also during the night (20%), at different times, the maturity of the androecium/anther opening coinciding with the beginning of the floral opening, which is complete in approximately 12 hours. Approximately 30 hours after total anthesis, senescence occurred, maintaining only the calyx and stylet (the stigma to the ovary) until the beginning of the fruit formation, which was also observed in other species of the family Myrtaceae (Almeida, Naves, & Ximenes, 2000).

Identification of nectaries and attractive structures for flower visitors

It was not possible to investigate ultraviolet light reflecting regions, visible to the insects, or the presence of osmophores, through the observation by hand digital microscopy, development of the luminescence test and application of neutral red solution. However, through the exposure of floral pieces to ammonia hydroxide, we identified small contrast in the apical region of the stigma and in longitudinal lines in the anthers. This result coincides with the olfactory tests carried out, in which it was possible to identify the presence of mild sweet odor emitted by the anthers and possibly by the pollen grains and has been maintained since the anthesis and lasts for at least 10 hours.

The odor could be exhaled by the pollen grains due to the presence of volatile oils. Thus, pollen grains are possibly the main resource offered to sete-capote tree pollinators, as is the case for other species of the same family (Proença & Gibbs, 1994).

Characterization of pollinators and floral visitors

The Apidae family showed greater relevance in the number of visits and visitors, mainly in the Hymenoptera order, with 86.5% of the visitors (Figure 2A, B, C, D). Bees of the genus *Apis* and native bees of the genera *Trigona* and *Melipona* (Figure 2B, C) were observed, the last having a higher frequency of visits when compared to the first. In smaller number also the presence of bees of the genus *Bombus* (Figure 2D), also known as Bumblebee was observed.

The highest intensity of floral visitors was observed from 7:00 to 10:00 a.m., decreasing during the day, with the Apidae family being the first visitors with abundant presence. The pollen collected by all bees of the Apidae family was observed through the vibration method. Because the anthers are of the poricidal type, the pollen is removed more easily, and these cling to a group of stamens and vibrate. A fact also observed in *Campomanesia pubescens* (DC.) O. Berg. (Proença & Gibbs, 1994), and other species of the Myrtaceae family (*Campomanesia velutina* (Cambess) O. Berg.; *Eugenia speciosa* Camb.; *Eugenia desenterica* DC.; *Myrcia splendens* (SW.) DC.; *Myrcia multiflora* DC.; *Myrcia racemosa* Berg (Kiaersk); *Myrcia linearifolia* Cambess.; *Myrcia rhodosepala* Kiaeski; *Psidium cattleianum* Sabine; *Psidium firmum* Berg.) (Proença & Gibbs, 1994; Fidalgo & Kleinert, 2009).

The family Apidae is the most common among floral visitors of the family Myrtaceae, specifically for species of the genus *Campomanesia* (Nucci & Alves-Junior, 2017). Visitors of the Melyridae family (Coleoptera) with 12.5% (Figure 2E), and the presence (1%) of a belonging arachnid species of the Thomisidae family, known as crab-spider (*Misumena* sp.), which was observed preying on other floral visitors of the species, using mimicry (Figure 2F).

The bees showed preference for flowers at the beginning of the anthesis, which were moving between the flowers of the same plant and between different plants. The *Bombus* sp. is the only species that touches both reproductive structures at all visits. However, even though it does not always touch both reproductive structures, the other bees through the vibrating pollination perform self-pollination, which makes it possible to characterize them as effective sete-capote tree pollinators.

The greater number of anthers generates large amounts of pollen grains that possibly act as primary floral attraction for floral visitors. Due to the abundant availability of this resource on the petals, visitors of other families were observed piling the fallen pollen grains, and also feeding on part of the floral structures, or other visitors, thus not being characterized as pollinators, but as pillagers (without contact with reproductive structures) of this species (Figure 2E, F).

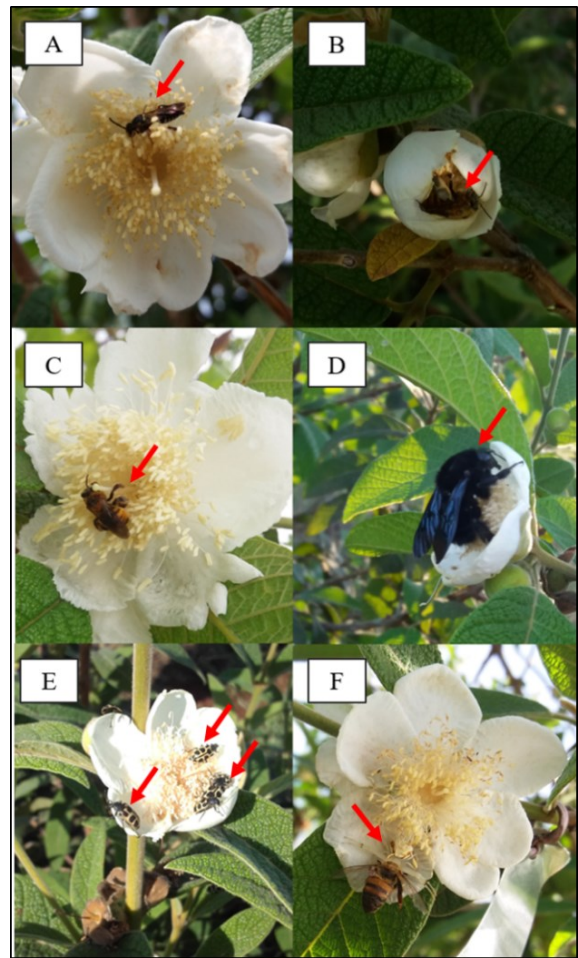


Figure 2. Floral visitors of sete-capote tree: A) Apidae (*Trigona* sp.). B, C) *Melipona* sp. (Apidae) performing vibrating pollination. D) Apidae (*Bombus* sp.). E) Melyridae (*Astylus variegatus*). F) Thomisidae (*Misumena* sp.).

Receptivity of the stigma

With the use of hydrogen peroxide, bubbling in the stigma cavity, we observed in 100% of the flowers from the pre-anthesis to the beginning of senescence, indicating peroxidase activity and consequently stigma receptivity throughout the period. An analogous result was obtained using the neutral red solution, in which 100% of the stigmas were stained during the same evaluation period.

Reproductive system characterization

Fruit formation was verified in 100% of the fertilized flowers mainly through manual self-pollination, followed by natural pollination (effective fruiting) and cross-pollination as shown in Table 1.

Table 1. Pollination tests, self-incompatibility index, spontaneous self-pollination index and reproductive efficiency, in sete-capote tree.

Testes de polinização	Number of flowers used	Fertilized flowers (%)	Fruits formed (%)
Manual self-pollination	100	88	88
Natural Pollination	100	56	56
Spontaneous self-pollination	100	0	0
Cross Pollination	100	46	46
Apomixis	100	0	0
Self-incompatibility Index (SII)		1.91	
Spontaneous Self-Pollination Index (SSPI)		0	
Reproductive Effectiveness (RE)		1.22	

There was no fertilization through spontaneous pollination and apomixis, which may be variable for each species of the Myrtaceae family, ranging from complete self-sterility to apomixis (Fidalgo & Kleinert, 2009), possibly associated with the evolution of each species.

In a study carried out with *Campomanesia adamantium* O. Berg., Nucci & Alves Junior (2017) obtained similar results, with 45% fruits formed through cross-pollination, and 60% for natural pollination, showing great similarity among the species.

As for the reproductive indexes obtained, sete-capote tree could be considered self-compatible because the SII obtained was higher than 0.25 (Table 1) (Oliveira & Gibbs, 2000). Self-compatibility may contribute to endogamy; however, it ensures the perpetuation of the species in situations where it is not possible to cross (Goldberg et al., 2010), such as where there is low population density and cross-fertilization is less frequent (Schoen & Busch, 2008). Furthermore, self-fertilization systems may favor local adaptation due to lower gene flow (Hereford, 2010).

In relation to SSPI, the result shows the total dependence of pollinating agents (Table 2), possibly due to the position of the stigma in relation to the anthers, as observed in *Bauhinia curvula* Benth. (Fabaceae) (Munin, Teixeira, & Sigrist, 2008).

As for RE, it was high (1.22), due to the high percentage of fruiting and the absence of abortions in flowers submitted to cross-pollination and under natural conditions. This result indicates good efficiency in the transfer of viable pollen to the flower stigma by pollinators or also by the wind, as observed in *C. adamantium*, which presented high reproductive efficacy (1.33), with the visit of pollinating agents (Nucci & Alves-Junior, 2017).

Conclusion

Sete-capote tree has hermaphrodite flowers, and the floral opening occurs mainly during the daytime period. Pollen grains are the main resource offered to pollinators. The flowers have mellow sweet odor, attracting mainly native bees and *Apis mellifera*, which was characterizing as effective pollinators.

Sete-capote tree it presents high reproductive efficiency, and can be considered self-compatible; however, fertilization also occurs through cross-pollination.

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