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Floral, reproductive and pollinators biology of *Myrcianthes pungens* (Berg) Legrand, neglected species

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ABSTRACT. Studies related to floral biology are essential for the understanding of the ecological relations between different species, and the beginning of breeding programs. In this way, the aim of the study was to elucidate aspects of floral and reproductive biology and floral visitors of this species. Information about floral morphology and morphometry, anthesis, nectaries and pollinator attractive structures, characterization of floral visitors, receptivity of androcytic stigma and maturation, in vitro pollen storage and germination, and characterization of the reproductive system were obtained. The *guabiju* tree has hermaphrodite flowers, and the floral opening occurs mainly during the night, but also in the morning. Anthers are the main attractive structure to the pollinating insects, releasing fetid odor, attracting mainly flies and wasps characterized as occasional pollinators, and moths characterized as effective pollinators. For the germination of pollen, it is recommend using it without desiccation, collected in post-anthesis, and for the culture medium the use of 11% of sucrose and 7% of boric acid. Pollen presents recalcitrant behavior, so even when stored in refrigerator, freezer, liquid nitrogen and natural environment lose viability in less than 30 days. It presents high reproductive efficiency, and can be considered self-compatible; however, fertilization also occurs by cross-pollination.

Keywords: *guabiju* tree; Myrtaceae; floral visitors; pollination; germination of pollen.

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Introduction

The Myrtaceae family belongs to the Angiosperm classification, Myrtales order and comprises about 100 genera and 3,000 species of trees and shrubs, distributed by all continents, except Antarctica (Marchiori & Sobral, 1997).

It is one of the most representative families in the different vegetation formations of Brazil, possessing great wealth and phytosociological importance for the Brazilian forests (Leitão-Filho, 1993; Barroso & Peron, 1994). In addition, the native species occupy a prominent position in the physiognomy of the forests of Southern Brazil, being represent in the Mixed Ombrophylous Forest, Pluvial of the Atlantic Slope, and in the Seasonal forests of Alto Uruguai or Serra Geral (Marchiori & Sobral, 1997).

They are also among the most popular fruit trees because of their technological potential, being found in commercial and domestic orchards and occupying a prominent place in natural ecosystems (Simarelli, 2007; Fior, Rodrigues, Calil, Leonhardt, Souza, & Silva, 2010), which increases the interest for these species, besides the nutritional and quality characteristics of fruits, which drives the research and industrialization sector.

Among the species of Myrtaceae, the *guabiju* tree (*Myrcianthes pungens* (Berg) Legrand) stands out mainly for its ecological, ornamental and nutritional characteristics of the fruits. It has a large occurrence and was found from the State of São Paulo to Rio Grande do Sul in the semideciduous forests of altitude (Marchiori & Sobral, 1997).

The plant can reach 27 meters in height, trunk of smooth bark and little thick, evergreen and rounded crown. The leaves were present in a simple, bald, having three to seven centimeters in length and have a spiny apex, an important characteristic in the identification of the species (Marchiori & Sobral, 1997). The flowers were hermaphrodite, light in color, 1.5 to 2.0 centimeters in diameter, usually occurring in the

'branch of the year' (new sprout). The fruits are of the pubescent berry type with fleshy pulp and sweet taste, velvety texture and purple coloration, each fruit having one to two seeds (Marchiori & Sobral, 1997; Lorenzi, 2006).

There are few studies conducted with *guabiju* tree, such as those on chemical characterization and bioactive compounds of the fruits (Silveira, Lucena, Pereira, Garnés, Romagnolo, Takemura, & Laverde Junior, 2011; Nora, Danelli, Souza, Rios, Jong, & Flôres, 2014a; Nora, Jablonski, Rios, Hertz, Jong, & Flôres, 2014b; Nora et al., 2014c), nutritional composition (Reis, Bernardi, Silva, & Facco, 2016), properties of leaf essential oils (Zygadlo, Alicia, Rotman, Perez, & Negueruela, 2011), and seed quality (Fior et al., 2010; Souza, Fior, Souza, & Schwarz, 2011).

It is worth highlighting the potential of the species for industrialization and reforestation of degraded areas, mainly due to the attractiveness of the bird fauna. However, studies on the pollinators and floral, reproductive biology of the species were not found in the literature. These are essential for the understanding of the ecological relations between different species, the conservation of the germplasm, the elaboration of management protocols in cultivation and the beginning of breeding programs (Torres & Galetto, 2011; Danner, Citadin, Sasso, Sachet, & Malagi, 2011a; Françoso, Guaraldo, Prada, Paiva, Mota, & Pinto, 2014).

With this in mind, the present study sought to elucidate aspects related to floral and reproductive biology of *guabiju* tree, neglected specie.

Material and methods

The study was developed with matrices of different genotypes located in the *Universidade Tecnológica Federal do Paraná*, Dois Vizinhos Campus and also the urban area of the same city, and the analyzes conducted in the Laboratory of Plant Physiology of the same institution. The matrices were register in the Herbarium of the *Universidade Tecnológica Federal do Paraná*, Dois Vizinhos Campus (BRASIL. PARANÁ: Dois Vizinhos, Herbarium of the *Universidade Tecnológica Federal do Paraná*, 19. IV. 2018, fl., and fr., Guollo, K. 5829 (DVPR) – urban area; Brazil. Paraná: Dois Vizinhos, Herbarium of the *Universidade Tecnológica Federal do Paraná*, 19. IV. 2018, fl., and fr., Guollo, K. 5830 (DVPR) – rural area).

To identify the two matrices used as a single species, they were compare through synoptic frames (Vidal & Vidal, 2010; Gonçalves, 2011). Through these, it was also performed floral characterization (Vidal & Vidal, 2010), and morphometry was performed using 100 flowers in post-anthesis, with the use of digital caliper.

For identification of the anthesis moment, observations were made in five non-consecutive days (5:00 a.m. to 8:00 p.m.) in four matrices of *guabiju* tree. One hundred floral buds were marked for follow-up, identifying the moment of each phenophase (early floral opening, full floral opening, early senescence, and total senescence).

In the laboratory environment (± 25°C), the controlled anthesis of pre-anthesis flowers fixed to phenolic foam was observed, accompanied until the moment of the complete floral opening.

The identification of nectar presence in fresh flowers was accomplish through the arrangement of micro capillary tubes in the pedicel, base of the sepals, petals, stamens and carpels, being identified its presence or absence (Versieux, Acosta, Jordao, Zidko, & Maia, 2014). For visual identification of nectars, it was used a handheld digital microscope (Digital Microscope® Model HD Color CMOS Sensor U500X).

Through direct observation of fresh flowers (post-anthesis) in an adapted camera provided with ultraviolet light (luminescence), floral structures of ultraviolet light absorption and reflection were identified, because the flower visitors' recognition of the flower is due to the contrast difference of these areas.

In order to identify the areas of greatest metabolic activity at the time of anthesis, fresh flowers were maintained for 60 minutes in a solution of 1% neutral red, diluted in distilled water. Therefore, through observation in a stereoscope, the presence of metabolic activity and osmophores (gland producing volatile substances, odor), which is represented in colored fabrics (Dafni, Kevan, & Husband, 2005).

In order to identify a floral resource guide, a test with ammonium hydroxide was carried out in a capped container, cotton soaked with 10 mL of ammonium hydroxide, remaining for 10 minutes for atmosphere saturation. After this period fresh flowers were allocated into the same container, keeping it closed for an additional 10 minutes. The contrast difference observed is due to flavonoid-like pigments that absorb

ultraviolet light while the other parts of the flower reflect it, allowing floral visitors to identify and locate the resources (Storti, 2002).

To identify odor-releasing floral structures, flowers were dissected into sepals, petals, stamens and pistils, which were placed in test tubes sealed with film paper for five hours. After this period, ten volunteers were interview in order to identify the main odor releasing structure, and which type of exhaled odor (sweet, floral, citric, woody, among others) (Versieux et al., 2014).

Still, for the identification of the moment of odor release, olfactory bioassay was conducted. For this, completely post-anthesis flowers were placed in a test tube sealed with film paper, being olfactory evaluated by volunteers at hourly intervals for 10 hours.

For the characterization of pollinators and floral visitors, observations were made on non-consecutive days, from 5:00 am to 8:00 pm, obtaining the visiting times and identification of the most frequent visitors (Kiill & Simão-Bianchini, 2011; Versieux et al., 2014).

Pollinators were those who made frequent and legitimate visits (contact in all reproductive structures), occasional pollinators those who presented less frequent visits, even being a legitimate visitor and plunderers those who do not make contact with the reproductive structures (Matias & Consolaro, 2014).

Photographic registration was also carry out and some visitors were capture, using a network and entomological vacuum cleaner being keep in FAA, for later identification of family, gender and species when possible, with the aid f an identification guide (Fujihara, Forti, Baldin, & Almeida, 2011).

For the identification of the period of the stigma receptivity, 15 flowers for each floral stage (1) pre-anthesis; (2) beginning of the anthesis; (3) total anthesis; (4) early senescence), were kept in a phenolic sponge in a natural environment, throughout the evaluation period. For the observation, 3% hydrogen peroxide were use, which through the peroxidase activity promoted the bubbling on the stigma, thus confirming the receptivity of the same (Kiill, Martins, & Silva, 2014; Versieux et al., 2014; Matias & Consolaro, 2014).

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, the flowers were observed in stereoscope, and the presence of metabolic activity were represented in the stained tissues, indicating the receptivity (Kearns & Inouye 1993; Dafni et al., 2005).

The evaluation of the androceutical maturation was carry out through a stereoscope observation, identifying the release moment of the anthers pollen grains in pre and post-anthesis.

For the pollen storage and germination in vitro pre-anthesis floral balloons were collect, remaining in phenolic sponge until opening, so flower anthers in pre- and post-anthesis were detached and placed to dry in paper trays placed in a silica chamber at room temperature (± 25°C) for 8, 16 and 24 hours, causing anther dehiscence and release of the pollen grain. As control, pollen grains from fresh anthers were use, without desiccation.

The determination of the culture medium was carried out based on the concentration of the chemical components that provided the best germination of the pollen grains, in the previous experiment to carry out the following treatments, according to Figueiredo, Pio, Silva, and Silva (2013).

Initially, the design was completely randomized (CRD), in a bi-factorial scheme, with factor A corresponding to the time of the pollen grains collection (pre- and post-anthesis), and factor B corresponding to the desiccation time (0; 8, 16 and 24 hours). The culture medium used at first stage consisted of 1% agar for solidification and 10% sucrose. Then the sucrose concentration in the culture medium was evaluate with five levels (0, 10, 20, 30 and 40%).

After obtaining the results, using the ideal condition for collection and desiccation of the pollen grains, and the concentration of sucrose that gave the highest percentage of germination, the addition of boric acid (0, 5, 10, 15 and 20%) to the culture medium.

To solidify the culture medium 1% agar was added, dissolved in distilled water and heated in a microwave oven until complete dissolution. The medium poured into Petri® dishes was cut with the help of a spatula after cooling, forming small blocks that were arrange on slides on which the pollen was sprinkle with a brush n° 4.

The slides were place in Gerbox®-type boxes containing moistened paper and incubated in an oven B.O.D. (Biological Demand Oxygen) type at controlled temperature (25°C) for 24 hours. For each treatment, eight replicates were use, each block of culture medium in each of the slides representing one replicate.

Grains with pollen tube length equal to or greater than the diameter of the pollen grain were considered to be germinate.

After obtaining germination results above 80%, the pollen grains were stored in refrigerator (5°C), freezer (-17°C), liquid nitrogen (-147°C) and natural environment (± 25°C), evaluating the germination monthly, until total loss of viability.

The obtained data were submitted to the normality test (Lilliefors) and homogeneity of variance (Bartllet). The observed means resulting from the first experiment were transformed into an arc of $\sqrt{x/100}$. Given the assumptions of the model, they were submitted to analysis of variance (ANOVA) to verify the significance of the factors and their interactions. When significant, the Duncan averages test at 5% of probability for the qualitative factor and regression analysis for the quantitative factors was applied, using Genes software (Cruz, 2013).

For the characterization of the reproductive system, two plants were selected, due to the occurrence of greater flowering, using 25 flowers for each treatment, performing: (1) spontaneous self-pollination (flower buds bagged); (2) manual self-pollination or geitonogamy (flowers bagged the day before anthesis and, after the flowers were opened, manual pollination with flower pollen from the same plant); (3) control or open pollination (marked flower buds, making them accessible to flower visitors); (4) cross-pollination or xenogamy (pre-anthesis emasculation and pollination using pollen from another plant). For cross-pollination, pollens were used with 88% germinative percentage, previously tested.

For the execution of the treatments, the receptivity of the stigma was considered, and all the manipulated flowers were isolated (paper bags) the day before the procedures were performed, to avoid contamination with pollen from other species. The treatments were followed until fruit maturation or floral abscission.

The self-incompatibility index (SII) were calculate from the relation between the percentage of fruit from manual self-pollination and the percentage of fruit from cross-pollination. When the SII does not exceed 0.25, it means that the species is self-incompatible (Oliveira & Gibbs, 2000). The rate of spontaneous self-pollination (SSP) was calculate from the relation between the percentage of fruits formed by spontaneous self-pollination and the percentage of fruits formed by manual self-pollination. The closer to zero the SSP, the smaller is the possibility of this reproductive strategy (Polatto & Alves-Junior, 2009).

The reproductive efficiency (RE) was obtained through the ratio between the percentage of fruits formed by open pollination (control) and that of fruits formed by manual cross-pollination (Polatto & Alves-Junior, 2009).

Results and discussions

Through the observations made in this study (Figure 1), it is possible to infer about the characterization that *guabiju* tree has single leaves with a whole margin, with characteristic spinescent apex, and opposite disposition. It has hermaphrodite and diclamid flowers with four petals of white color and four sepals of green coloration. The flowers are actinomorphs, dialipetals, gamosepals, heterodyne and dialysemane stamens free from each other, with yellowish coloration and rhyming (longitudinal) dehiscence, and are solitary in groups of 5 to 6 flowers. The stylet is erect, simple fillet and exerts (protruding in the throat of the calyx / crown); infertile ovary, bilocular and pluriovular, possessing on average 30 eggs per lobe.

The floral opening of *guabiju* tree occurs mainly during the night, with approximately 75% starting the anthesis at approximately 10:00 p.m., completing it in approximately 2 hours. Approximately 25% of the flowers begin anthesis at 5:00 p.m., completing it in approximately 1 ½ hour after, which was also observed in *Myrciaria dubia* (HBK) McVaugh (Maués & Couturier, 2002), and may be related to the habit of visits of the pollinators of the species.

Approximately 30 hours after total anthesis occurs senescence, remaining only the chalice and the stylet until the beginning of the fruit formation.

Flowers of *guabiju* tree are devoid of nectaries, which is common in other species of the Myrtaceae family, and the main resource offered by these is the pollen (Maués & Couturier, 2002).

No ultraviolet light reflectance was observed in *guabiju* flowers through the luminescence test, which does not prove to be an efficient test for the species. However, in other tests, it was possible to detect the presence of osmophores in the anthers and pollen of flowers. These results show that the anthers presented significant color changes through the exposure to ammonia hydroxide, which shows the presence of

flavonoids that absorb ultraviolet light, thus contrasting with the ultraviolet light reflecting regions, characterizing in this case the floral resource guides (Storti, 2002).

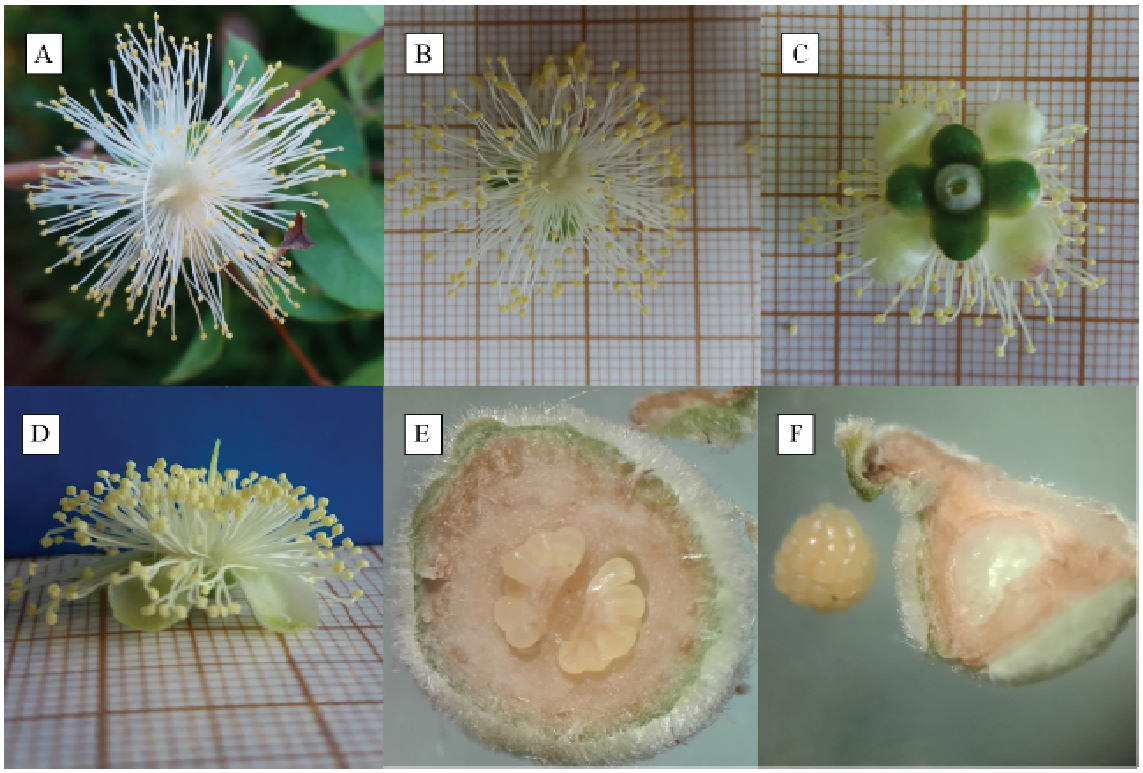

Figure 1. *Myrcianthes pungens* (Berg) Legrand. Flower: A) Complete anthesis. B) Front side. C) Back side. D) Stigma protruding the stamens. E) Transverse section of ovary (Small cavity with ovule). F) Longitudinal cut of the ovary with removed ovule.

The odor of *guabiju* tree flowers is concentrated in the male structures (anther / pollen), and it has been maintained since the beginning of the anthesis and lasts for an average period of 8 hours. The odor on pollen grains occurs due to the presence of volatile oils characteristic of the Myrtaceae family (Maués & Couturier, 2002; Silva & Pinheiro, 2007).

The fetid odor characteristic found in *guabiju* flowers is not common in species of Myrtaceae, which is generally characterize as fruity and sweet (Maués & Couturier, 2002; Pires & Souza, 2011), but can be characterized for the genus *Myrcianthes* O. Berg as slightly sour.

Regarding the observation of floral visitors, it was found that the Syrphidae family had the highest number of visits and visitors, mainly of the Diptera order (Figure 2D, E, F), with 48.96% of the visitors, followed by the Vespidae (Hymenoptera) families with 22.4%; Apidae (Hymenoptera) with 13% (Figure 2A), Pyralidae (Lepdoptera) with 5%; Formicidae (Hymenoptera) with 4.12%; Chrysomelidae (Coleoptera) with 3.52% (Figure 2B); and Coccinellidae (Coleoptera) with 2.94%.

The identified flies and wasps were observed moving between the flowers of the same plant, sometimes touching both reproductive structures, thus being able to be characterized as occasional *guabiju* pollinators (Matias & Consolaro, 2014). According to Faegri and Van der Pijl (1979) flies are not stimulated to visit special parts of the flowers such as anthers or stigma but rather due to the foul odor that becomes attractive for oviposition, ensuring food for the larvae.

Few bees were observed, however, being the first visitors in the morning, which showed preference for flowers at the beginning of the anthesis, remaining on the reproductive structures for approximately 5 seconds. Although they are legitimate visitors, the bees observed were characterize in this case as occasional pollinators due to less frequent visits (Matias & Consolaro, 2014). The main resource sought in this case are the pollen grains.

The moths identified in the nocturnal period (*Ettiela* sp., *Elasmupalpus* sp. (Pyralidae)) were observed moving between the flowers, always touching both reproductive structures, remaining in the flower for approximately 5 seconds, being characterized as effective pollinators of *guabiju* tree, also due to frequent visits in the observed period (Matias & Consolaro, 2014).

Visitors from other families (Chrysomelidae, Formicidae, Coccinellidae) were characterized as plunderers of the species (Matias & Consolaro, 2014), by looting fallen pollen grains on the perianth or feeding on part of the flower structures, and by the little movement, not allowing the efficiency transfer of the pollen grains.

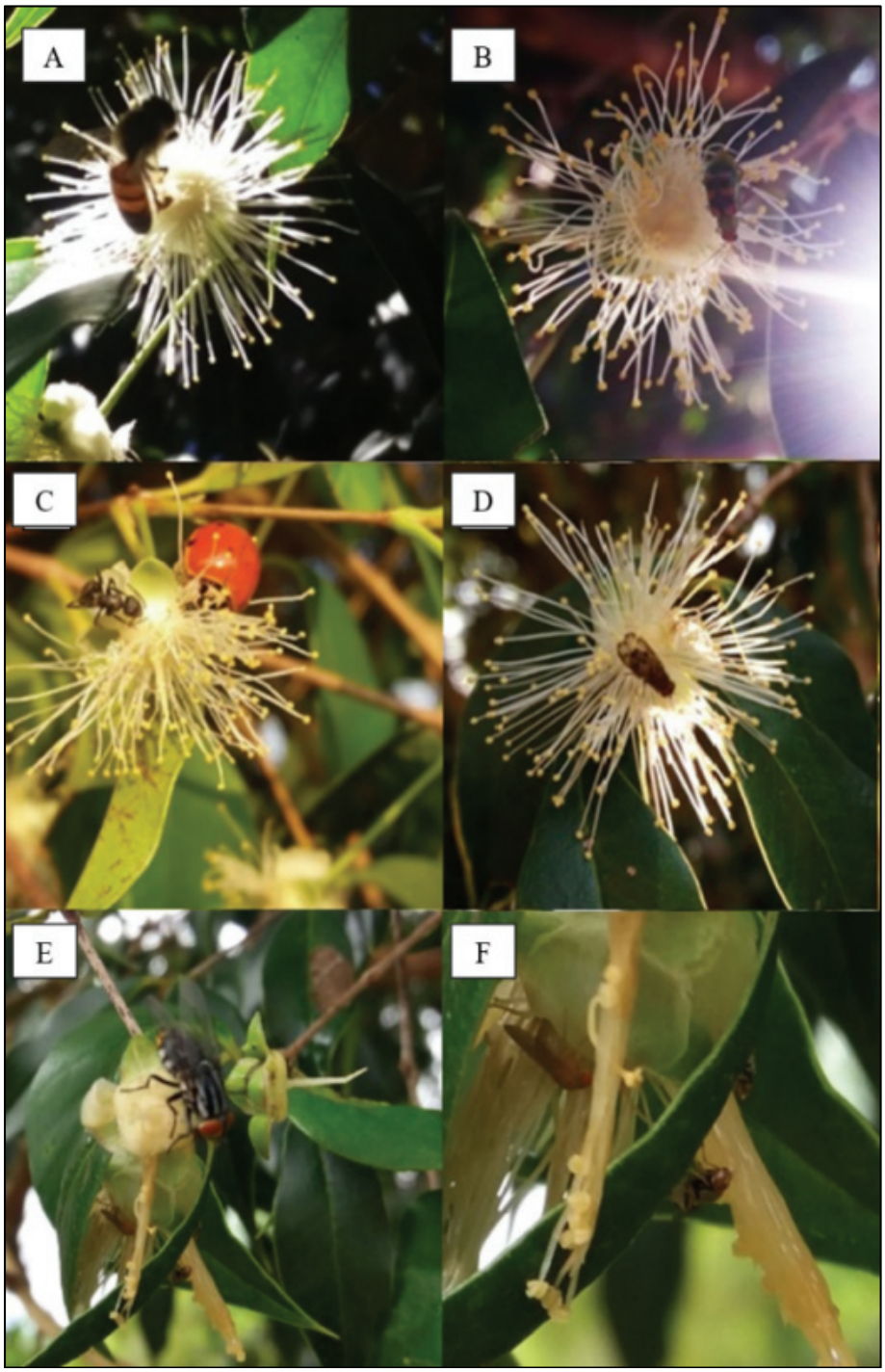

Figure 2. *Myrcianthes pungens* (Berg) Legrand. Identification of flowers visitors: A) *Apis mellifera (*Apidae) B) *Diabrotica speciosa* (Chrysomelidae) C) *Formica* sp. (Formicidae); *Coccinela septempunctata* (Coccinellidae). D) *Drosophila* sp. (Muscidae). E) *Cochliomyia* sp. (Muscidae). F) *Musca* sp. and *Drosophila* sp. (Muscidae).

In both tests, it was observed that 100% of the *guabiju* flowers are receptive from the time of pre-anthesis to the beginning of senescence. However, the enzymatic activity of peroxidase, visualized by bubbling on stigma was higher in post-anthesis flowers. As well as observed in *Myrcia guianensis* (Aubl.) DC. and *M. laruotteana* Cambess (Pires & Souza, 2011), and citrus (Ramos, Pasqual, Salles, Chagas, & Pio, 2008).

In addition, the anthers are dehiscent at the beginning of the floral balloon opening, thus releasing the pollen along with the peak receptivity of the stigma.

There was a significant interaction at 1% probability level of the error between floral development stage and desiccation time, rejecting the hypothesis of nullity H0 (Table 1).

Table 1. ANOVA: Coefficient of variation (CV), degrees of freedom (DF) and mean squares (MS) of the analysis of variance for the pollen germination variable, in an experiment conducted in CRD with eight replicates.

Sources of variation	DF	MS
Floral Development Stage (A)	1	637.56**
Desiccation time (B)	3	572.10**
A X B	3	573.43**
Treatment	7	582.02**
Error	56	1.50
CV (%)	21.69	

** Significant at the 1% probability level of error by F test (p <.01). * Significant at the 5% probability level of error by F test (0.01 = <p <0.05). ns Not significant (p> = 0.05).

The adjusted regression equations were significant at 1% probability level by the t-test. Regarding the stage of floral development, the best germination percentages were observed in post-anthesis (25%), and when obtained in pre-anthesis the germination of the pollen was only 2%, proving unsuitable for collection (Figure 3).

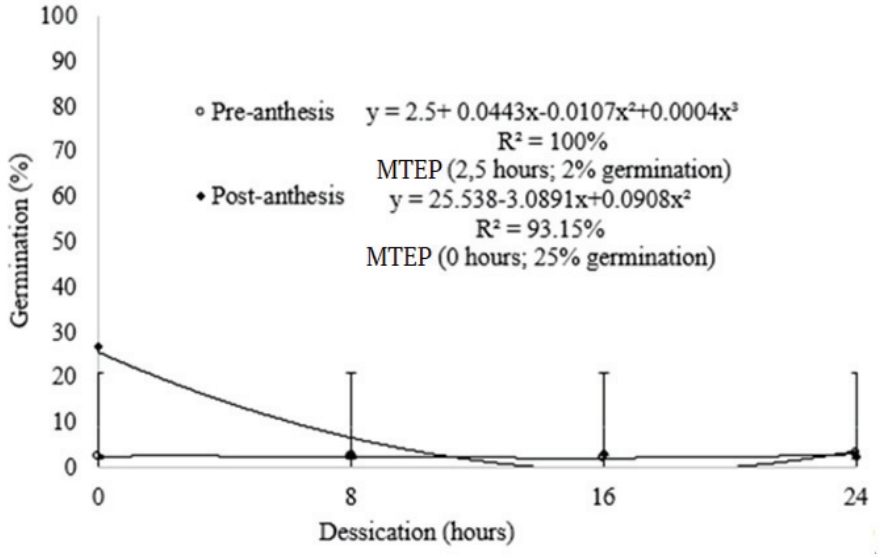

Figure 3. In vitro germination of *guabiju* pollen as a function of the collection moment of the flower buds and the time of desiccation of the pollen grains.

As for desiccation, the viability of the pollen grains decreases as this period increases, possibly showing recalcitrant behavior. It is also observed in Figure 2 that a higher percentage of germination can be obtained with fresh pollen grains (without desiccation), and 25% can be obtained when it is dried for a maximum of 2.5 hours.

The loss of water from the pollen grains can occur even before dispersion, and the time to reduce the water content is short when compared to the seeds, which can occur within a few hours after the anthesis or even before this (Franchi, Piotto, Nepi, Baskin, Baskin, & Pacini, 2011). Thus, the study of this behavior becomes paramount considering that these factors have a direct influence on the success of fertilization (Cabral, Rossi, Klein, Vieira, & Giustina, 2013).

Subsequently, using fresh pollen grains from post-anthesis flowers, the effect of adding sucrose to the culture medium was verify. The sucrose factor has been shown to be significant, however, the germination percentage obtained can still be considered low, and according to the MTEP (maximum technical efficiency point), and it is possible to obtain 25% germination by adding approximately 11% sucrose to the culture medium (Figure 4).

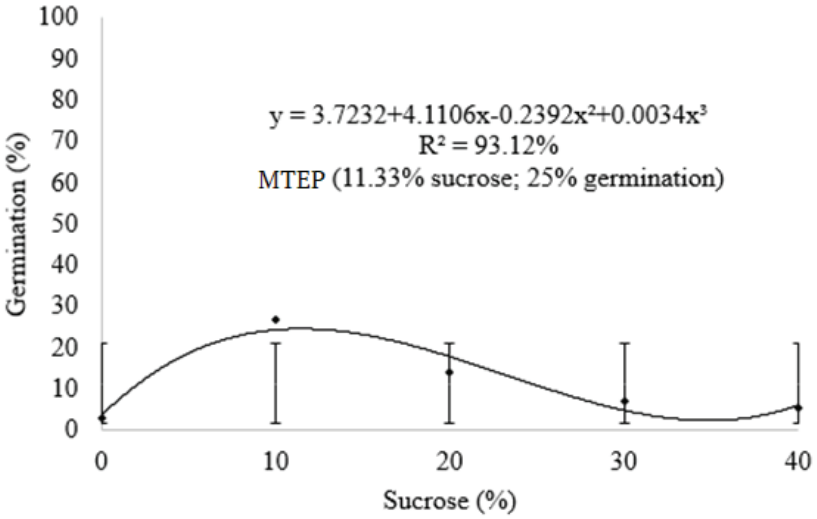

Figure 4. In vitro germination of *guabiju* tree pollen as a function of the sucrose concentration in the culture medium.

Results found in the literature demonstrate that sugars are the most important components of the culture medium for the in vitro germination of pollen grains (Lyra, Sampaio, Pereira, Silva, & Amaral, 2011; García, Guarnieri, & Pacini, 2012). However, for *guabiju*, the presence of other substances is necessary, since combinations of sugars and boric acid may be more efficient in the germination of the pollen grain.

In this way, as the germinative percentage obtained was show below value considered satisfactory (80%), the effect of adding boric acid to the culture medium was evaluated, considering the best results obtained previously.

The addition of boric acid to the culture medium showed to be efficient on the germination of *guabiju* tree pollen grains, reaching high germinative percentage. The MTEP shows that when 7% of boric acid is used the germination can reach 99% (Figure 5).

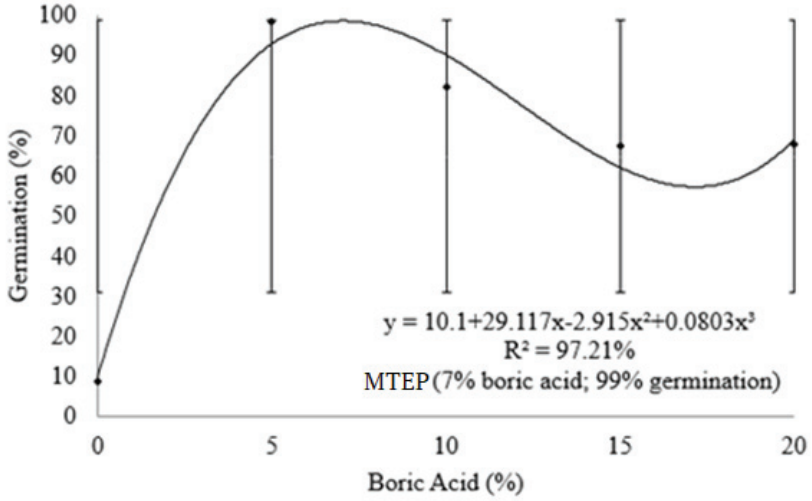

Figure 5. In vitro germination of *guabiju* tree pollen as a function of the concentration of boric acid in the culture medium.

Pollen germination can be stimulated by different chemical components, however, the boric acid according to Franzon and Raseira (2006), stimulates the growth of the pollen tube and decreases the probability of disruption of the pollen grains.

Possibly, due to this complex, the addition of boron was beneficial in the germination of the *guabiju* tree pollen grains. The addition of boron was also efficient on the germination of pear and mulberry pollen grains (Chagas, Pio, Chagas, Pasqual, & Bettiol Neto, 2010; Figueiredo et al., 2013).

Regarding the storage of pollen grains, for both conditions tested, there was a total loss of viability in the first evaluation (30 days). Contradictory result to that obtained with *Plinia cauliflora* (DC.) Kausel, *P.*

trunciflora (O. Berg) Kausel and *P. jaboticaba* (Vell.) Berg), (Danner, Citadin, Sasso, Scariot, & Benin, 2011b) also from Myrtaceae, in which the pollen viability was maintained for up to 90 days in a freezer.

The analysis of pollen viability is crucial before crossing, since the flowering period of the plants under study can be short and, when not feasible the crosses (Chagas et al., 2010).

In view of the above, it was recommended for future studies that the evaluation of the pollen viability of *guabiju* tree be carried out weekly, considering that the pollen grains have recalcitrant behavior, and in this way besides the impossibility of water loss, the loss of viability occurs more quickly.

It was verified the formation of fruits of *guabiju* tree in all forms of pollination tested, mainly through natural pollination (effective fruiting), however, the percentage of fertilized flowers and fruits formed was only 20% (Table 2).

Table 2. Pollination tests, number of fertilized flowers, number of fruits formed, auto incompatibility index, spontaneous self-pollination index and reproductive efficiency, in *guabiju* tree.

Pollination tests	Number of flowers user	Fertilized flowers (%)	Formed fruits (%)
Manual Sel-pollination	25	8	8
Natural pollination	25	20	20
Self-pollinating	25	8	8
Spontaneous			
Cross pollination	25	12	12
SII		0.66	
SSP		1.00	
ER		1.66	

According to studies carried out by Danner et al. (2011b) with *Plinia cauliflora*, *P. trunciflora* and *P. jaboticaba*, the effective fruiting can also vary between the periods of the crosses, species and evaluated genotypes.

For the other forms of crossing, there was also no decrease in the ratio of fecundated flowers and formed fruits (Table 2). According to Fidalgo and Kleinert (2009), the reproductive system of species of the Myrtaceae family can vary from complete self-sterility to apomixis and may be associated with the evolutionary processes of each species. Similar result was find in guava tree (*Psidium guajava* L.) which produced fruits in all forms of crossing, even when flowers were prevent from visiting insects (spontaneous self-pollination). The results show that the wind besides the insects, can exert influence on the pollination, as well as observed in guava tree (*P. guajava*) (Alves & Freitas, 2007).

As for the reproductive indexes obtained, *guabiju* tree can be considered self-compatible because the SII obtained is higher than 0.25 (Table 2), which according to Oliveira and Gibbs (2000) characterizes self-incompatible species. Self-compatibility may be associated with pollinator inefficiency (occasional pollinators or pollinators) and the low number of visits, which could be visualize in *guabiju* in the present study and in *Stigmaphyllon paralias* A. Juss. (Malpighiaceae) (Costa, Costa, & Ramalho, 2006).

Self-incompatibility may contribute to the prevention of inbreeding; however, it may be a limiting factor on self-fertilization in situations where it is not possible to cross (Goldberg, Kohn, Lande, Robertson, Smith, & Igic, 2010). Thus, self-compatibility is beneficial in some cases, ensuring the perpetuation of species where there is low population density and deficiency of pollinating agents and cross-fertilization is less frequent (Schoen & Busch, 2008).

In relation to SSP, the value obtained (1.00) shows that there is probably no need for pollinators to carry out the pollination in this species (Table 2), due to their self-polluting capacity (autogamous), attesting to the hypothesis that this occurs mainly through the wind. Contrary to what has seen in other species, such as *Bauhinia curvula* Benth (Fabaceae), in which there is no fruiting after spontaneous self-pollination, thus presenting pollinator dependence due to the position of the stigma in relation to the anthers (Munin, Teixeira, & Sigrist, 2008), which does not occur in *guabiju* tree.

As for ER, it was high (1.66) (Table 2), due to the high percentage of fruiting, and the absence of abortions in flowers submitted to cross-pollination and in natural conditions, indicating good efficiency in the transfer of viable pollen to the stigma of the flower, either through pollinators or also by the wind.

Despite the high reproductive efficiency, it is suggested that in order to carry out the management to conserve *guabiju* trees, further studies should be carried out on the populations of pollinators observed in

this study, since many of them have been identified as occasional pollinators or pollinators and pollinators with fewer visitors, which may be impacted by habitat degradation.

Conclusion

With the data obtained in this research, a great advance in the understanding regarding the floral and reproductive biology of *guabiju* tree, as well as interactions with pollinators were elucidate. This information will be useful and primordial for future studies, such as breeding projects, as well as dissemination of knowledge for the exploration and formation of commercial orchards, making possible the domestication of the species.

References

Alves, J. E., & Freitas, B. M. (2007). Requerimentos de polinização da goiabeira. *Ciência Rural, 37*, 1281-1286. doi: 10.1590/S0103-84782007000500010.

Barroso, G. M., & Perón, V. (1994). *Myrtaceae.* In M.P.M. Lima, & R.R. Guedes-Bruni (Eds.). *Reserva Ecológica de Macaé de Cima, Nova Friburgo, RJ. Aspectos florísticos das espécies vasculares* (Vol. 1, p. 261-302). Rio de Janeiro, RJ: Jardim Botânico do Rio de Janeiro.

Cabral, J. C., Rossi, A. A. B., Klein, M. B., Vieira, F. S., & Giustina, L. D. (2013). Estimativa da viabilidade polínica em acessos de *Theobroma cacao* L. baseada em testes colorimétricos. *Enciclopédia Biosfera, 9*(17), 2780-2788.

Chagas, E. A., Pio, R., Chagas, P. C., Pasqual, M., & Bettiol Neto, J. E. (2010). Composição de meio de cultura e condições ambientais para germinação de grãos de pólen de porta-enxertos de pereira. *Ciência Rural, 40*(2), 261-266. doi: 10.1590/S0103-84782010000200002

Costa, C. B. N., Costa, J. A. S., & Ramalho, M. (2006). Biologia reprodutiva de espécies simpátricas de Malpighiaceae em dunas costeiras da Bahia, Brasil. *Revista Brasileira de Botânica, 29*(1), 103-114. doi: 10.1590/S0100-84042006000100010

Cruz, C. D. (2013). GENES – A software package for analysis in experimental statistics and quantitative genetics. *Acta Scientiarum. Agronomy, 35*(3), 271-276. doi: 10.4025/actasciagron.v35i3.21251

Dafni, A., Kevan, P. G., & Husband, B. C. (2005). *Practical pollination biology.* Cambridge, UK: Cambridge University Press.

Danner, M. A., Citadin, I., Sasso, S. A. Z., Sachet, M. R., & Malagi, G. (2011a). Modo de reprodução e viabilidade de pólen de três espécies de jabuticabeira. *Revista Brasileira de Fruticultura, 33*(2), 345-352. doi: 10.1590/S0100-29452011005000077

Danner, M. A., Citadin, I., Sasso, S. A. Z., Scariot, S., & Benin, G. (2011b). Genetic dissimilarity among jabuticaba trees native to Southwestern Paraná, Brazil. *Revista Brasileira de Fruticultura, 33*(2), 517-525. doi: 10.1590/S0100-29452011005000078

Faegri, K., & Van der Pijl, L. (1979). *The principles of pollination ecology.* Oxford, UK: Pergamon Press.

Fidalgo, A. O., & Kleinert, A. M. P. (2009). Reproductive biology of six Brazilian Myrtaceae: Is there a syndrome associated with buzzpollination? *New Zealand Journal of Botany, 47*(4), 355-365. doi: 10.1080/0028825x.2009.9672712

Figueiredo, M. A., Pio, R., Silva, T. C., & Silva, K. N. (2013). Características florais e carpométricas e germinação in vitro de grãos de pólen de cultivares de amoreira-preta. *Pesquisa Agropecuária Brasileira, 48*(7), 731-740. doi: 10.1590/S0100-204X2013000700005

Fior, C. S., Rodrigues, L. R., Calil, A. C., Leonhardt, C., Souza, L. S., & Silva, V. S. (2010). Qualidade fisiológica de sementes de guabijuzeiro (*Myrcianthes pungens* (Berg) Legrand – Myrtaceae) em armazenamento. *Revista Árvore, 34*(3), 435-442. doi: 10.1590/S0100-67622010000300007

Franchi, G. G., Piotto, B. N., Nepi, M., Baskin, C. C., Baskin, J. M., & Pacini, E. (2011). Pollen and seed desiccation tolerance in relation to degree of developmental arrest, dispersal and survival. *Journal of Experimental Botany, 62*(15), 5267–5281. doi: 10.1093/jxb/err154

Françoso, R., Guaraldo, A. C., Prada, M., Paiva, A. O., Mota, E. H., & Pinto, J. R. R. (2014). Fenologia e produção de frutos de *Caryocar brasiliense* Cambess. e *Enterolobium gummiferum* (Mart.) J.F. Macbr. em diferentes regimes de queima. *Revista Árvore, 38*(4), 579-590. doi: 10.1590/S0100-67622014000400001

Franzon, R.C., & Raseira, M.do C.B. (2006). Germinação in vitro e armazenamento do pólen de *Eugenia involucrata* DC (Myrtaceae). *Revista Brasileira de Fruticultura, 28*(1), 18-20. doi: 10.1590/S0100-29452006000100008

Fujihara, R. T., Forti, L. C., Baldin, E. L. L., & Almeida, M. C. (2011). *Insetos de importância econômica: guia ilustrado para identificação de famílias.* Botucatu, SP: FEPAF.

García, C. C., GuarnierI, M., & Pacini, E. (2012). Tomato pollen tube development and carbohydrate fluctuations in the autotrophic phase of growth. *Acta Physiologiae Plantarum, 34*(6), 2341-2347. doi: 10.1007/s11738-012-1037-4

Goldberg, E. E., Kohn, J. R., Lande, R., Robertson, K. A., Smith, S. A., & Igic, B. (2010) Species selection maintains self-incompatibility. *Science, 330*(6003), 493-495. doi: 10.1126/science.1194513

Gonçalves, E. G. (2011). *Morfologia vegetal: organografia e dicionário ilustrado de morfologia de plantas vasculares* (2a ed.). Nova Odessa, SP: Instituto Plantarum de Estudos de Flora.

Kearns, C. A., & Inouye, D. (1993). *Techniques for pollinations biologists*. Niwot, CO: University press of Colorado.

Kiill, L. H. P., & Simão-Bianchini, R. (2011). Biologia reprodutiva e polinização de *Jacquemontia nodiflora* (Desr.) G. Don (Convolvulaceae) em Caatinga na região de Petrolina, PE, Brasil. *Hoehnea, 38*(1), 511-520. doi: 10.1590/S2236-89062011000400001.

Kiill, L. H. P., Martins, C. T. V. D., & Silva, P. P. (2014). Biologia reprodutiva de *Sideroxylon obtusifolium* (Roem. & Schult.) T.D. Penn. (Sapotaceae) na região semiárida da Bahia. *Revista Árvore, 38*(6), 1015-1025. doi: 10.1590/S0100-67622014000600006

Leitão Filho, H. F. (1993). *Ecologia da mata atlântica em Cubatão (SP)*. São Paulo, SP: Universidade Estadual Paulista; Campinas, SP: Universidade Estadual de Campinas.

Lorenzi, H. (2006). *Frutas brasileiras e exóticas cultivadas: de consumo in natura*. São Paulo, SP: Nova Odessa.

Lyra, D. H., Sampaio, L. S., Pereira, D. A., Silva, A. P., & Amaral, C. L. F. (2011). Pollen viability and germination in *Jatropha ribifolia* and *Jatropha mollissima* (Euphorbiaceae): Species with potential for biofuel production. *African Journal of Biotechnology, 10*(3), 368-374. doi: 10.5897/AJB10.808

Marchiori, J. N. C., & Sobral, M. (1997). *Dendrologia das angiospermas: Myrtales*. Santa Maria: Ed. UFSM.

Matias, R., & Consolaro, H. (2014). Pollination biology of *Geissomeria pubescens* Nees (Acanthaceae) in a forest remnant in central Brazil. *Botany, 92*(3), 215-222. doi: 10.1139/cjb-2013-0211

Maués, M. M., & Couturier, G. (2002). Biologia floral e fenologia reprodutiva do camu-camu (*Myrciaria dubia* (H.B.K.) McVaugh, Myrtaceae) no Estado Pará, Brasil. *Revista Brasileira Botânica, 25*(4), 441-448. doi: 10.1590/S0100-84042002012000008

Munin, R. L., Teixeira, R. C., & Sigrist, M. R. (2008). Esfingofilia e sistema de reprodução de *Bauhinia curvula* Benth. (Leguminosae: Caesalpinioideae) em cerrado no Centro-Oeste brasileiro. *Revista Brasileira de Botânica, 31*(1), 15-25. doi: 10.1590/S0100-84042008000100003

Nora, C. D., Danelli, D., Souza, L. F., Rios, A. O., Jong, E. V., & Flôres, S. (2014a). Protective effect of guabiju (*Myrcianthes pungens* (O. Berg) D. Legrand) and red guava (*Psidium cattleyanum* Sabine) against cisplatin-induced hypercholesterolemia in rats. *Brazilian Journal of Pharmaceutical Sciences, 50*(3), 483-491. doi: 10.1590/S1984-82502014000300006

Nora, C. D., Jablonski, A., Rios, A. O., Hertz, P. F., Jong, E. V., & Flôres, S. H. (2014b). The characterization and profile of bioactive compounds in red guava (*Psidium cattleyanum* Sabine) and guabiju (*Myrcianthes pungens* (O. Berg) D. Legrand). *International Journal of Food Science & Technology, 49*(8), 1842-1849. doi: 10.1111/ijfs.12493

Nora, C. D., Müller, C. D. R., Bona, G. S., Rios, A. O., Hertz, P. F., Jablonski, A., ... Flôres, S. H. (2014c). Effect of processing on the stability of bioactive compounds from red guava (*Psidium cattleyanum* Sabine) and guabiju (*Myrcianthes pungens* (O. Berg) D. Legrand). *Journal of Food Composition and Analysis, 34*(1), 18-25. doi: 10.1016/j.jfca.2014.01.006

Oliveira, P. E., & Gibbs, P. E. (2000). Reproductive biology of woody plants in a cerrado community of Central Brazil. *Flora, 195*(4), 311-329. doi: 10.1016/S0367-2530(17)30990-8

Pires, M. M. Y., & Souza, L. A. (2011). Morfoanatomia e aspectos da biologia floral de *Myrcia guianensis* (Aubletet) A. P. de Candolle e de *Myrcia laruotteana* Cambesse (Myrtaceae). *Acta Scientiarum, Biological Science, 33*(3), 325-331. doi: 10.4025/actascibiolsci.v33i3.6647

Polatto, L. P., & Alves-Junior, V. V. (2009). Sistema reprodutivo de *Sparattosperma leucanthum* (Vell.) K. Schum. (Bignoniaceae). *Revista Árvore, 33*(2), 289-296. doi: 10.1590/S0100-67622009000200010

Ramos, J. D. R., Pasqual, M., Salles, L. A., Chagas, E. A., & Pio, R. (2008). Receptividade do estigma e ajuste de protocolo para germinação in vitro de grãos de pólen de citros. *Interciência, 33*(1), 51-55.

Reis, L. C. R., Bernardi, J. R., Silva, A. C. P., & Facco, E. M. P. (2016). Análise da composição nutricional e estabilidade de compostos fenólicos e antocianinas totais do guabijú (*Myrcianthes punges*). *Brazilian Journal of Food Research, 7*(1), 89-104. doi: 10.3895/rebrapa.v7n1.3517

Schoen, D. J., & Busch, J. W. (2008). On the evolution of self-fertilization in a metapopulation. *International Journal of Plant Sciences, 169*(1), 119-127. doi: 10.1086/523356

Silva, A. L. G., & Pinheiro, M. C. B. (2007). Biologia floral e da polinização de quatro espécies de *Eugenia* L. (Myrtaceae). *Acta Botanica Brasilica, 21*(1), 235-247. doi: 10.1590/S0102-33062007000100022

Silveira, S., Lucena, E. V., Pereira, T. F., Garnés, F. L. S., Romagnolo, M. B., Takemura, O. S., & Laverde Junior, A. (2011). Atividade anticolinesterasica dos frutos de *Myrcianthes pungens* (o.Berg) d. Legrand (Myrtaceae). *Arquivo Ciência e Saúde, 15*(2), 127-133. doi: 10.25110/arqsaude.v15i2.2011.3707

Simarelli, M. (2007). Afinal o que é biodiversidade? *Revista Frutas e Derivados, 2*(8), 33-34.

Souza, L. S., Fior, C. S., Souza, P. V. D., & Schwarz, S. F. (2011). Desinfestação de sementes e multiplicação in vitro de guabijuzeiro a partir de segmentos apicais juvenis (*Myrcianthes pungens* O.Berg) D. Legrand. *Revista Brasileira de Fruticultura, 33*(3), 691-697. doi: 10.1590/S0100-29452011005000081

Storti, E. F. Biologia da polinização e sistema reprodutivo de *Passiflora coccinea* Aubl. em Manaus, Amazonas, Brasil. *Acta Amazônica, 32*(3), 421-429, 2002. doi: 10.1590/1809-43922002323429

Torres, C., & Galetto, L. (2011). Flowering phenology of co-occurring Asteraceae: a matter of climate, ecological interactions, plant attributes or of evolutionary relationships among species? *Organisms Diversity & Evolution, 11*(1), 9-19. doi: 10.1007/s13127-011-0038-2

Versieux, L. M., Acosta, A. L., Jordao, A. L., Zidko, A., & Maia, U. M. (2014). Floral biology, morphology and ecological niche modelling of *Caraipa grandifolia* (Calophyllaceae), an important Amazonian floodplain tree. *Ciências Naturais, 9*(3), 621-638.

Vidal, W. N., & Vidal, M. R. R. (2010). *Botânica – organografia: quadros sinóticos ilustrados de fanerógamos*. Viçosa, MG: Universidade Federal de Viçosa.

Zygadlo, J. A., Alicia, D., Rotman, M. J., Perez, A., & Negueruela, A. V. (1997). Leaf oils of two *Myrcianthes* species from Argentina: *M. pungens* (Berg.) Legrand and *M. cisplatensis* (Camb.) Berg. *Journal of Essential Oil Research, 9*(2), 237-239. doi: 10.1080/10412905.1997.9699470