Acta Botanica Brasilica, 2022, 36: e2021abb0121
doi: 10.1590/0102-33062021abb0121

Original article

Phenology, abundance and efficiency of pollinators drive the reproductive success of *Sarcoglottis acaulis* (Orchidaceae) at the Atlantic Forest

Nayara S.L. Albuquerque[1]*, Paulo Milet-Pinheiro[2], Denise D. Cruz[3], Georgiana M.S. Pimentel[3], Aércia C. Sousa[3], Adriennius M. Carneiro[3] and Isabel C. Machado[1]

Received: July 5, 2021
Accepted: January 27, 2022

ABSTRACT

Male euglossine bees (Euglossine: Apidae) pollinate flowers of as many as 1,000 Neotropical plant species and there is evidence that blooming in these plants is synchronized with the seasonality of euglossine pollinators. The present study aimed to monitor for two years the phenology of *Sarcoglottis acaulis* (Orchidaceae - Spiranthinae subtribe) and the seasonality of its pollinators at three Atlantic Forest fragments, two of which surrounded by urban anthropized areas. Phenologies in the three areas were related to abiotic factors, suggesting that precipitation on months prior to the beginning of the flowering period influenced the fructification rates of *S. acaulis*. Additionally, water shortage during the driest months resulted in vegetative dormancy. *Sarcoglottis acaulis* sets only a low number of fruits by self-pollination, and the plant's reproductive success depended on the frequency of its main pollinator, *i.e.*, the male euglossine bees *Eulaema atleticana*. Lower rates of pollinia removal were observed in areas where this bee is less frequent and, consequently, less fruits were formed, suggesting a high dependency on this pollinator.

Keywords: inflorescence development, insect-plant interaction, orchid bees, urban forest remnants/fragments, urban ecology

Introduction

Biotic and abiotic processes are central players in plant phenology (Lieth 1974; Morellato & Leitão Filho 1990; Wolf *et al*. 2017). In seasonal Rain Forests, phenology is limited by physiological stresses caused by variation in temperature and humidity. However, where climatic fluctuations are less pronounced, selective biotic pressures such as herbivory, predation, competition, pollination and dispersal, may influence phenological responses in plants (Aide 1988). It is known that global climate change interferes with the flowering patterns of plant species (Lesica & Kittelson 2010), and consequently the relationship with several pollinator

---

1 Programa de Pós-graduação em Biologia Vegetal, Departamento de Botânica, Universidade Federal de Pernambuco, 50372-970, Recife, PE, Brazil
2 Laboratório de Ciências Biológicas, Universidade de Pernambuco, 56328-900, Petrolina, PE, Brazil
3 Departamento de Sistemática e Ecologia, Universidade Federal da Paraíba, 58051-900, João Pessoa, PB, Brazil

* Corresponding author: nayarasla@hotmail.com

groups might also be affected (Kharouba & Vellend 2015; Hutchings *et al*. 2018).

The Atlantic Forest biome is one of the thirty-six world hotspots due to its high biodiversity, high endemism rate, and advanced degree of deforestation, with current vegetation cover of 28 % (Rezende *et al*. 2018). In Atlantic Rain Forests of southeastern Brazil, most Orchidaceae species are known to flower during rainy months (Pansarin & Pansarin 2008). However, there is also evidence that flowering of many orchids is related to seasonality of pollinators such as euglossine bees (Ackerman 1983). Therefore, biotic and abiotic factors may contribute to the population and community dynamics of orchids.

With approximately 26,460 described species (Christenhusz *et al*. 2017), the family Orchidaceae is one of the most species-diverse among angiosperms, with a high rate of endemism (Govaerts 2003). In Brazil, 2,760 species from 250 genera are currently known (Flora do Brasil 2020 2021), mostly from ombrophilous rainforests, including those from the Atlantic Forest biome, which harbors about half of all Brazilian orchid diversity (Barros *et al*. 2009). Orchidaceae presents a vast array of pollination strategy and may attract pollinators by offering different floral resources, such as nectar, oil, resin and fragrances, or by deceit (van der Pijl & Dodson 1969; Ackerman 1986; Nilsson 1992; Jersákova *et al*. 2006).

The terrestrial orchid *Sarcoglottis acaulis* is widespread across all Brazilian regions, including the Atlantic Forest (Barros *et al*. 2014). This species is pollinated by bees of the tribe Euglossini (Apidae) (Ackerman 1985; Singer & Sazima 1999; Albuquerque *et al.* 2021), which are key pollinators of many Neotropical plant families, particularly orchids (Dressler 1981; Roubik & Ackerman 1987; Carvalho & Machado 2002; Nemésio 2009; Milet-Pinheiro & Gerlach 2017).

The present study aimed to record the reproductive and vegetative phenology of *Sarcoglottis acaulis* and to monitor the seasonality of its pollinators during the flowering period in three fragments of the Atlantic Forest in the Northeast Brazil, two of them inserted in an urban and very anthropized area. Specifically, we investigated if there is difference in the pollinator frequency, in the natural fruit set and in the pollinia removal among fragments.

Materials and methods

Study plant species

Sarcoglottis acaulis (Spiranthinae subtribe, synonyms: *Sarcoglottis grandiflora* (Lindl) Klotzsch and *Sarcoglottis fasciculate* (Vell) Schltr) is a terrestrial orchid that occurs throughout Latin America (Tropicos.org 2015), and it is distributed in all regions of Brazil (Barros *et al.* 2014). Populations of *S. acaulis* occur scattered through the forest. Its pale-green flowers are arranged in racemose inflorescences (Pessoa & Alves 2012), produce small amount of nectar and are pollinated by euglossine bees (Ackerman 1985; Singer & Sazima 1999; Albuquerque *et al.* 2021). Flowers of *S. acaulis* produce a strong perfume and are pollinated, at the same study sites, only by males of two Euglossine bees, *Eulaema atleticana* Nemésio, 2009 and *E. niveofasciata* Friese, 1899, which collect nectar (Albuquerque *et al.* 2021).

Study sites

The study was conducted in three fragments of Atlantic Forest, in the Paraíba State, NE-Brazil: 1) Jardim Botânico Benjamim Maranhão (JBBM); 2) Mata do Departamento de Sistemática e Ecologia (DSE) of the Universidade Federal da Paraíba (UFPB) and 3) Reserva Biológica Guaribas (Rebio Guaribas).

The first two fragments are located within the urban perimeter of João Pessoa, and have typical seasonal semideciduous forest vegetation. These fragments are 1.5 km distant from each other and located southwest of João Pessoa's urban area (7º 6' Lat. Sul/ 34º 52' Long. Oeste), Paraíba state coast, at an average altitude of 45 m a.s.l (Barbosa 1996). Climate is typically hot and wet with mean annual temperature near 25 ºC and precipitation rates varying between 1500 to 1700 mm. Peak rainfall occurs between March and August and the dry season between October and December (Lima & Heckendorff 1985). The JBBM fragment has about 417 hectares. The DSE fragment is an Atlantic Forest remnant with 7 hectares, located at the Centro de Ciências Exatas e da Natureza of the UFPB, Campus I, João Pessoa, Paraíba (7º 8' S/ 34º 50' O).

The third fragment, Rebio Guaribas, is located at Mamanguape city, northern coastline of Paraíba state, 70 km from the state capital João Pessoa. The reserve has about 3,378 hectares, with vegetation consisting of a mosaic of Cerrado and Atlantic Forest remnants. Mean annual temperature varies between 24 and 26 ºC and total annual precipitation rates vary between 1,750 and 2,000 mm, with the rainiest months between April and June, and the driest months between October and December (Brasil 2003).

Phenology

The phenology of *S. acaulis* was monitored at the DSE and JBBM using two different approaches, one for observation of phenology phases (flowering longevity) of the individuals, and the other for recording the phenology in population level. In the DSE fragment, we marked 49 individuals, and monitored them in intervals of 2 - 4 days during one flowering season in order to record the stages ranging from the emission of the scape, during the first flower bud stages, until seed release by ripe fruits (Fig. 1).

In the JBBM fragment, we delimited a 0.8 hectare area and subdivided it into smaller 10 x 20 m sections in order to monitor phenophases in population level and to evaluate

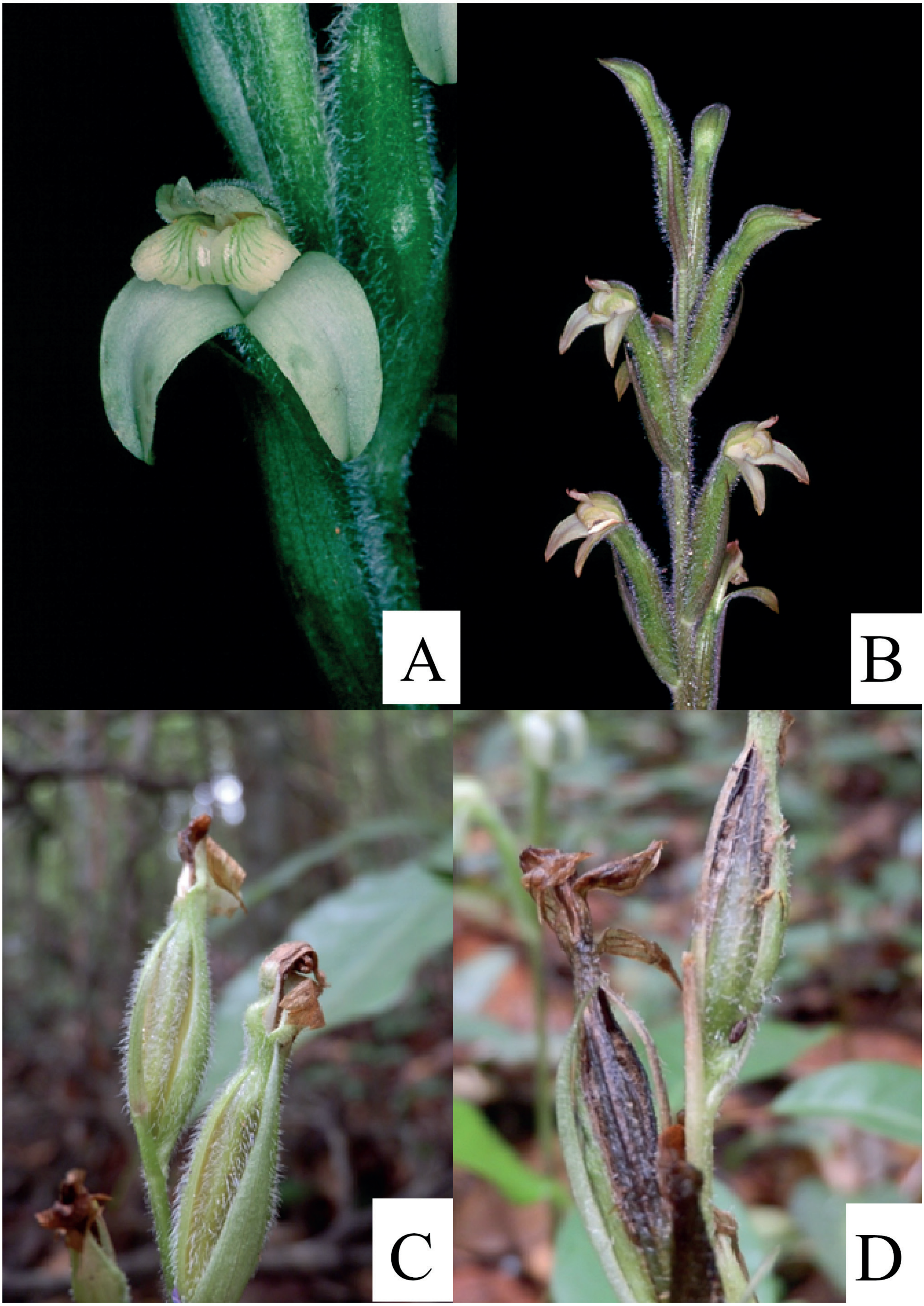

Figure 1. Flower and fruit longevity in *Sarcoglottis acaulis*. **A.** Open flowers, **B.** Buds and flowers, **C.** Unripe fruits and **D.** Ripe fruits.

possible variation in phenology in different years. All individuals observed within the area were tagged, totaling 132 plants of *S. acaulis*. To avoid tagging clones and to reduce the chance of pseudo-repetitions, a minimum radius of 3 m between individuals was delimited. The 132 individuals were then monitored monthly for two years (2011 and 2012). Phenology monitoring included the assessment of the following reproductive phenophases: floral bud, opened flower, unripe and ripe fruits. The number of individuals in each phenophase was counted. Onset of leaf dormancy was determined by the disappearance of the vegetative structure, whereas the end was determined by its regrowth. In some cases, the presence of a pseudobulb was detected on plants superficially connected to the substrate. Therefore, vegetative dormancy and presence/absence of pseudobulbs were also evaluated. Individuals that did not grow for four months or more were categorized as dead.

Phenology data were correlated to precipitation and the mean temperature values recorded for the study period, and to historical data available for the study areas. Data follow non-normal distribution according to the Shapiro-Wilk test (Zar 1999), and the nonparametric Spearman's rank correlations were performed using R software version 3.3.1 (R Development Core Team 2013). Circular statistics were used to estimate the intensity, frequency and seasonality of each phenophase by converting months into angles (*i.e.* January: 0° and December: 330°, with 30° intervals between months). This analysis generates the mean angles and standard deviations for the frequency of individuals on different phenophases, the mean months, the *r* vector value, and the significance level using Rayleigh's test. Tests were performed with the R software version 3.3.1. Precipitation data were provided by the Agência Executiva de Gestão das Águas do Estado da Paraíba (Paraíba 2020).

Comparison of the natural fruit set and pollinia removal among fragments

At the three study sites, flowers (N=333, 111 in Rebio, 69 in JBBM and 153 in DSE) of *S. acaulis* were marked and exposed to pollinators to assess natural fruit set from the different populations. Pollen removal was also quantified. To quantify pollinia removal in different periods of the day, 24 individuals per area were monitored for three days. On each day, observations were conducted early in the morning (05:30 am) and late in the afternoon (5 pm). Following the exposure period, total numbers of removed and non-removed pollinia were compared among areas.

To test whether 1) natural fruit-set and 2) pollinia removal differed among the fragments, nonparametric Kruskal-Wallis tests were performed, since data were not normally distributed. The Anderson-Darling normality test was applied for residuals and a post hoc test of Dunn for comparison by groups. The tests were performed using the R software version 3.6.1.

Flower availability and pollinator frequency

To evaluate an eventual relationship between flowering intensity of *S. acaulis* and pollinator abundance, the frequency of bees and available plants were monitored at the three fragments. The Euglossine species, *Eulaema atleticana* and *E. niveofasciata*, were monitored. These bees had been chosen because they were previously registered as pollinators of *S. acaulis* (see Albuquerque *et al*. 2021). In the DSE and JBBM, phenology of plants and bees were observed throughout the year (from July 2016 to June 2017). In the Rebio Guaribas, observations were restricted to the plant reproductive period (from July to October 2016). During the initial and final flowering months, June and October, respectively, frequency of bees was assessed monthly, and during peak flowering months, August and September, biweekly evaluations were made.

To monitor phenology, 50 plants per fragment were marked and the number of open flowers was recorded on the same days in which bees were collected. To attract bees, we used four synthetic compounds, *i.e.*, geraniol (97 %, Sigma-Aldrich), eucalyptol (99 %, Sigma-Aldrich), benzyl acetate (99 %, Sigma-Aldrich) and methyl salicylate (99 %, Sigma-Aldrich), which are known as potent attractants of euglossine bees (Bezerra & Martins 2001; Martins & Souza 2005). Filter papers were impregnated with the synthetic compounds individually and ranged at 1.5 m above the ground on different trees that were 3 m apart from each other. Bees were collected using an entomological net, cooled within an ice box, marked with non-toxic pens of colors corresponding to site of origin (Edding) and subsequently released. The bees with pollinia attached to the body were captured. Bees were collected on three consecutive days at the three areas, between 07:00 am and 2:00 pm.

Pollinator frequency and flower abundance were compared between sites using the Kruskal-Wallis test, as the data did not show normal distribution. The Anderson-Darling normality test was used for residuals and a post hoc test of Dunn for comparisons by groups. These tests were performed using the R software version 3.6.1 (http://www.r-project.org/). To test the correlation between the frequency of bees and the flowering frequency throughout the year, a Spearman Correlation was performed (no normal data) in the software R version 3.5.1 (http://www.r-project.org/).

Results

Phenology

During population monitoring (N=49 individuals) at the DSE fragment in 2011 we recorded a total of 49 inflorescences and 189 flowers in an area of 11,000 m^2. Mean number of open flowers per day was 3.05 ± 1.17 per inflorescence. The mean number of flowers and fruit set per inflorescence was 3.78 ± 1.3 and 3.18 ± 1.39, respectively. Flowers lasted on average 5.95 ± 2.34 days (N=189) (Fig. 2). The interval ranging from the beginning of fruit development to fruit maturation and seed dispersion lasted 11.76 ± 3.36 days (Fig. 2).

Sarcoglottis acaulis flowered between August and October, and flowering peaks differed between the two study years at JBBM. In 2011, flowering in the populations peaked in September (31 individuals; 23.48 %), whereas in 2012 it peaked in July and August (23 individuals; 17.4 %). Although a well-defined reproductive period was observed, phenophases were not concentrated on a particular period of the year at the population level (The Rayleigh test was not significant; Tab. 1).

Table 1. Circular statistics results for reproductive and vegetative phenophases and mean month for *Sarcoglottis acaulis* (Orchidaceae) in an Atlantic Forest area from northeastern Brazil. n.s. = not significant (p > 0.05).

	Reproductive phenophases			
	Buds	Flowers	Unripe fruits	Ripe fruits
Average degree	124.63°	154.63°	174.90°	170.10°
SD	47.64°	47.64°	51.44°	47.52°
Mean month	August	September	September	September
R	0.31	0.30	0.19	0.31
Rayleigh (p)	ns	ns	ns	ns
	Vegetative phenofases			
	Deaths	Pseudobulbs / dormancy		Re-budding
Average degree	90°	214.04°		306.74°
SD	51.46°	53.43°		47.90°
Mean month	June	November		February
R	0.19	0.13		0.30
Rayleigh (p)	ns	ns		ns

Of the 132 individuals monitored at the JBBM, 70 (53 %) flowered in 2011 and 27 (20.4 %) in 2012. There was variation in precipitation observed during the two years, with higher rates in 2011 (about 2,355 mm) than in 2012 (1,651 mm). Reproductive period of *S. acaulis* correlated positively with local precipitation, and specific responses to water availability were observed for each reproductive phenophase: floral bud emission was positively correlated with precipitation with a lag time of one and two months (r = 0.61, p = 0.001 and r = 0.59, p = 0.001, respectively). Flowering, on the other hand, was correlated with precipitation with a lag time of two and three months (r = 0.55, p = 0.003 and r = 0.55, p = 0.003, respectively). During the first year of monitoring, both flowering and fruiting peaked in September.

Fruiting frequency of *S. acaulis* varied slightly between years. In 2011, 14 (10.6 %) individuals set fruits, whereas 16 (12.1 %) individuals set fruits in the second year (2012). However, the period of fruit development varied between years. In 2011, fruiting began and ended in September, whereas in 2012, fruiting began in September but extended until October. Ripe fruits were correlated with precipitation of three months (r = 0.41, p = 0.03), whereas ripe fruit production correlated with precipitation of two and three months (r = 0.44, p = 0.02 and r = 0.40, p = 0.04, respectively).

Vegetative dormancy (leaf fall) was observed for 31 (23.5 %) of the individuals of *S. acaulis* monitored in 2011, peaking in December when 29 leafless individuals (22 %) were recorded. In 2012, the frequency was 69.7 %, and, again, December showed the highest proportion of leafless individuals (64.4 %). January 2012 and 2013 showed the highest rates of vegetative budding. Dormancy negatively

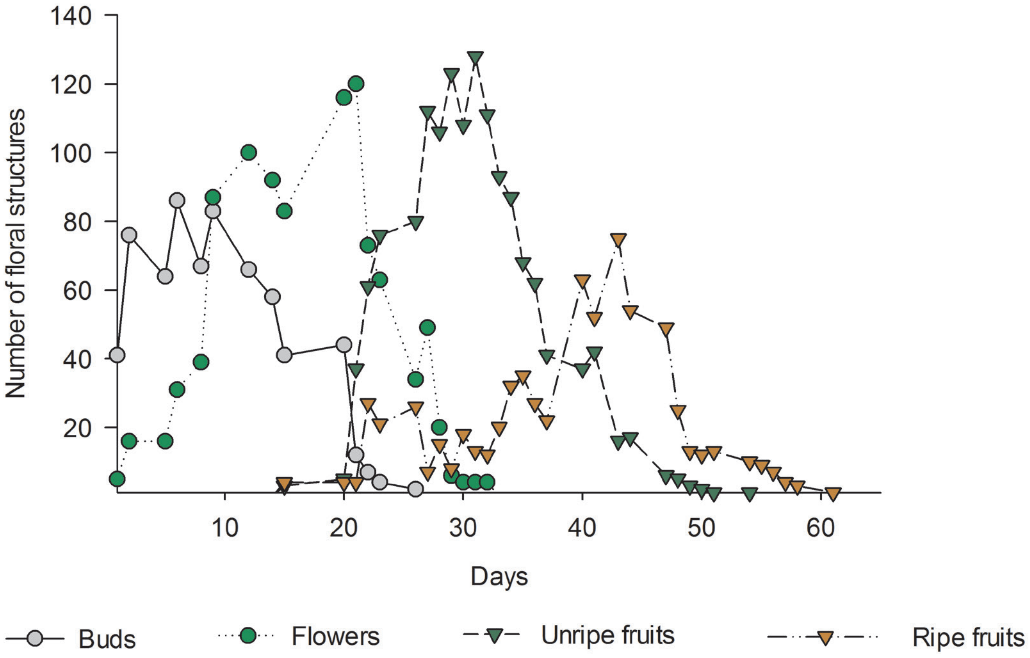

Figure 2. Number of buds, flowers, unripe and ripe fruits produced during the longevity of 49 inflorescences in the fragment of Departamento de Sistemática e Ecologia, Atlantic Forest, Paraíba, NE-Brazil.

correlated with precipitation (r = -0.47, p = 0.016; r = -0.73, p < 0.0001, *lag time*: 1 month; r = -0.68, p = 0.0001, *lag time*: 2). Re-budding negatively correlated with precipitation and a lag time of two and three months was observed (r = -0.47, p = 0.001 and r = -0.55, p = 0.003, respectively).

Comparison of the natural fruit set and pollinia removal among fragments

Natural fruit-set varied significantly among the three fragments (H = 10; df = 2; p = 0.006). Post hoc tests showed that the difference was due to higher fruit set observed at the DSE fragment in relation to the Guaribas Rebio (H = 9.27; df = 1; p = 0.002). Fruit set at JBBM was similar to the two other study sites (Tab. 2).

Table 2. Fruit set of three populations of *Sarcoglottis acaulis* at Paraiba State, Northeastern Brazil. (Rebio = Guaribas Biological Reserve; JBBM = Jardim Botânico Benjamim Maranhão; DSE = Departamento de Sistemática e Ecologia). Different letters are attributed to significant differences between areas (Chi-Square = 9.27; df = 1; p = 0.002).

Area	Flowers (N)	Fruits (N)	Fruits (%)
Rebio	111	76	68.47 ^{a}
JBBM	69	52	73.91 ^{ab}
DSE	153	130	84.98 ^{b}

Pollinia were always removed during the day. Removal rate differed significantly among the study sites (H = 6.41; df = 2; p = 0.04). Pollinia removal at the Rebio Guaribas was lower than those of both the JBBM (H= 2.021; p = 0.02) and the DSE fragments (H= 2.255; p = 0.01). Removal in the last two fragments was similar to each other (Tab. 3).

Table 3. Rates of pollinia removal in three populations of *Sarcoglottis acaulis* in fragments of Atlantic Forest, at Paraiba, NE-Brazil. Abbreviations: Rebio = Guaribas Biological Reserve; JBBM = Jardim Botânico Benjamim Maranhão; DSE = Departamento de Sistemática e Ecologia. Different letters are attributed to significant differences between areas (H = 6.41, df = 2, p = 0.04; Rebio x JBBM – H = 2.02, p = 0.02; Rebio x DSE – H = 2.25, p = 0.01).

Area	Flowers (N)	Pollinia removal (N)	Pollinia removal (%)
Rebio	99	5	5 % ^{a}
JBBM	100	26	26 % ^{b}
DSE	95	27	28 % ^{b}
Total	294	58	19.7%

Comparison of flower availability and pollinator frequency among fragments

Flowering intensity was similar at the three study sites (DSE = 14.3 ± 11.6; JBBM = 15 ± 12.6; Rebio Guaribas = 19.3 ± 13.7, mean number of flowering events, N = 6 days of observation for each study site, H=1.0; df=2; p=0.6) (Fig. 3). Frequency of *Eulaema niveofasciata* was also similar at the study sites (DSE = 3 ± 2.19; JBBM = 2 ± 0.63; Rebio Guaribas = 1.5 ± 1.04, mean number of individuals, N = 6; (H=2.15;

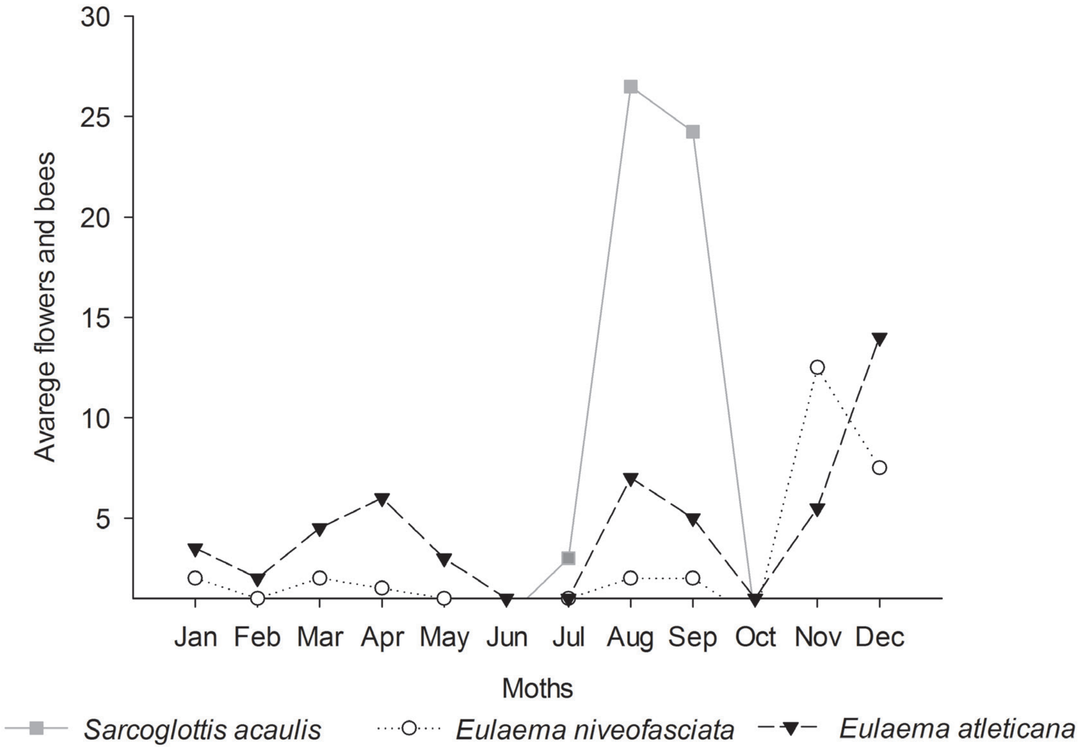

Figure 3. Annual flower availability of *Sarcoglottis acaulis* and seasonality of euglossine bees (*E. niveofasciata* and *E. atleticana*) in the fragments of Departamento de Sistemática e Ecologia, Jardim Botânico and Guaribas Reserve, Paraiba, NE - Brazil.

df = 2; p=0.34). However, the frequency of *E. atleticana* differed significantly among the fragments (DSE = 6.1 ± 4.7; JBBM = 5.6 ± 4.1; Rebio Guaribas = 0.8 ± 0.7, mean number of individuals, N = 6; H = 8.17; df = 2; p= 0.02). Post hoc analyses showed that *E. atleticana* is less frequent in the Rebio Guaribas than in both the DSE (H = 2.55; p= 0.005) and the JBBM fragments (H = 2.38; p= 0.008) (Fig. 3).

In the scent baits, we collected a total of 115 individuals of two species of *Eulaema*, of which 14 were sampled at Guaribas (5 *E. atleticana* and 9 *E. niveofasciata*), 55 at DSE (37 *E. atleticana* and 18 *E. niveofasciata*), and 46 in JBBM (34 *E. atleticana* and 12 *E. niveofasciata*). One bee captured and marked at the DSE was later re-captured at the JBBM (Fig. 4).

We did not detect a correlation between flowering intensity of *S. acaulis* and frequency of both pollinator species, *i.e.*, *E. niveofasciata* (r = 0,128; p < 0.05) and *E. atleticana* (r = 0,166; p < 0.05).

At the three fragments, *E. atleticana* (14) and *E. niveofasciata* (2) were observed carrying pollinia of *S. acaulis*, which were attached to the ventral portion of their labrum. At the Rebio Guaribas, an *E. niveofasciata* individual carrying pollinia of an unidentified orchid species was also observed at the same flowering time as *Sarcoglottis acaulis* (Fig. 4).

Discussion

The development from buds to fruit dispersion of *Sarcoglottis acaulis* is completed in about one month. The short interval observed is very similar to *Cyclopogon diversifolius,* another species belonging to the Pelexia alliance (Singer & Cocucci 1999). Our data show a steady state flowering pattern to *S. acaulis*, according to the classification of Gentry (1974), as the plants produce a few flowers a day over an extended period of time. This pattern is common in flowers pollinated by trap-liners euglossine bees (Janzen 1971). According to the classification of Newstrom *et al.* (1994), the flowering pattern is annual, similar to those of several orchids occurring at the Atlantic Forest, which also exhibits one major flowering cycle per year (Pansarin & Pansarin 2008). The peak flowering month in the present study differed from other populations of *S. acaulis* recorded in other geographic regions, such as in southeastern Brazil, where flowering peaked in June (Cunha & Forzza 2007), corresponding to the dry season in the southeastern region, while in the northeastern occurred in September. This divergence may be related to the difference in the dry month periods in the regions of Brazil. This suggests that the precipitation of the previous months triggers the flowering of *S. acaulis*, since they bloom in the first dry month of each region.

High rain volumes during the flowering period result in high flower production, but not necessarily in high pollination rates, since euglossine bees are more abundant during the driest periods of the year (Farias *et al*. 2008). This further explains why similar fruiting rates were observed between years despite different precipitation regimes. Also, fruiting during the rainy months, due to *S. acaulis*'s fast fruit maturation feature, seems to be disadvantageous, since seeds are dispersed by the wind. On the other hand, prolonged dry periods induce plants to undergo dormancy. Several terrestrial orchids have been previously observed to flower during the rainy period (Sahagun-Godinez 1996; Pansarin & Pansarin 2008), probably to increase energetic efficiency and to avoid the harsher and limited conditions of the dry period (Sahagun-Godinez 1996). *Sarcoglottis acaulis*, therefore, needs water to flower, to fruit and to maintain its leaves, but flowering needs to be extended to the less rainy months when pollinator frequency is higher to guarantee outbreeding.

The fragmentation of the Atlantic Forest reduces area and quality of habitat for euglossine bees (Nemésio 2013), which, although able to fly long distances in continuous forest areas (Wikelski *et al*. 2010), are negatively affected by habitat fragmentation (Nemésio & Silveira 2006). How

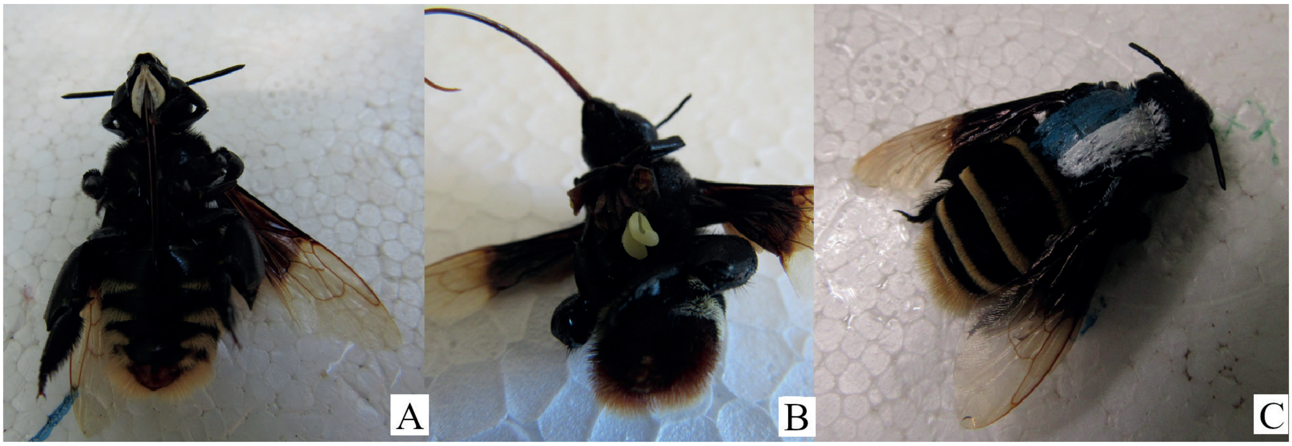

Figure 4. Floral visitors: **A.** *Eulaema atleticana* carrying a pollinia of *Sarcoglottis acaulis*; **B.** *Eulaema niveofasciata* carrying a pollinia of an unidentified species and **C.** *Eulaema atleticana* captured and marked at the Departamento de Sistemática e Ecologia and recaptured at the Jardim Botânico fragment, Paraiba, NE - Brazil.

vulnerable an organism is to fragmentation depends on its life history and ecological properties (Davies *et al.* 2004). For example, some euglossine species, such as *Eulaema niveofasciata* and *E. atleticana*, remain confined to forest fragments and, therefore, their foraging areas are limited to the frontiers of fragments (Milet-Pinheiro & Schlindwein 2005). This further increases reproductive isolation between plants located on different fragments due to a high dependency on bees for pollination. Alternatively, species such as *E. nigrita* Lepeletier, 1841 are more abundant in fragmented areas and, in fact, are frequently used as indicators of disturbance (Peruquetti *et al*. 1999; Nemésio & Silveira 2006). Therefore, fragment size and minimum distance between adjacent fragments need to be evaluated in order to guarantee the survival of bees and the plants that depend on them for pollination (Powell & Powell 1987).

Concerning fragmentation and pollinator dynamics, a lowest frequency of the main pollinator, *E. atleticana*, was observed at the largest fragment, *i.e.* the Rebio Guaribas. This might explain the lower rates of both pollinia removal and fruit set that were observed at this area. At the Rebio Guaribas, richness of orchids and other nectar-producing plants is higher than those of the other two areas (Araújo *et al*. 2009; Barbosa *et al*. 2011), suggesting the existence of a higher competition for pollinators. This idea may be supported by the fact that bees carrying pollinia from other species were also collected in this fragment in the same time the flowering of *S. acaulis*.

In the DSE fragment, although it is the smallest one, we observed at the same period, a high frequency of visits of *Eulaema atleticana* to *S. acaulis* flowers (Albuquerque *et al*. 2021), high fruit set and high pollinia removal rate. Bee abundance at this fragment may be a consequence of emigration from the Jardim Botânico, the Mata do DSE and other forest fragments within the Campus, which disperse to fulfill biological needs such as nidification sites and additional food sources on larger areas. Euglossine bee species fly over long distances (Wikelski *et al*. 2010) and similar species composition between forest fragments that are close (distance of 2 km) have been previously recorded (Ramalho *et al.* 2009).

In summary, our study highlights the importance of precipitation to flowering seasonality of *S. acaulis* and the dependency of this orchid on euglossine bees for pollination, considering that areas with higher abundance of bees showed higher pollinia removal and fruiting rates. The low rate of self-pollination of *S. acaulis,* as shown by our own data from the reproductive system of the same studied population (Albuquerque *et al.* 2021), reinforces the importance of pollinators for its reproduction. Furthermore, our data suggest that proximity of fragments is an important factor for conservation since even at small fragments, pollinators were abundant perhaps due to emigration from the larger, adjacent fragments. The lower frequency of bees from the scent baits in the largest fragment, that is, the Rebio Guaribas, which may have resulted in less pollinia removal and fruiting rates, is noteworthy and seems somewhat conflicting. Whether low abundance of bees was specific for the monitored period or, in fact, describe a particular feature of the conservation unit, is unknown. Future investigations should evaluate the causes of the low frequency of bees in this area.

Acknowledgements

Thanks are due to the staff of the Biologia Floral e Reprodutiva - Polinizar and Ecologia Química laboratories of the Universidade Federal de Pernambuco, the Ecologia Terrestre laboratory of the Universidade Federal da Paraíba, of the Reserva Biológica Guaribas and of the Jardim Botânico Benjamim Maranhão for logistic support. We also thank Dr. Celso Martins (UFPB) for his help in the methodology of collecting bees using odor baits. To CNPq (Conselho Nacional de Desenvolvimento Científico e Tecnológico for a research and productivity grants to ICM (311021/2014-0 and 310508/2019-3). Financial support was provided by the Coordenação de Aperfeiçoamento de Pessoal de Nível Superior - Brasil (CAPES) - Financing Code 001.

References

Ackerman JD. 1983. Specificity and mutual dependency of the orchid-euglossine bee interaction. Biological Journal of the Linnean Society 20: 301-314.

Ackerman JD. 1986. Mechanisms and evolution of food deceptive pollination systems in orchids. Lindleyana 1: 108-113.

Ackerman JD. 1985. Euglossine bees and their nectar hosts. In: D'Arcy WG, Correa MD. (eds). The botany and natural history of Panama. St. Louis: Missouri Botanical Garden 225-233.

Aide TM. 1988. Herbivory as a selective agent on the timing of leaf production in a tropical understory community. Nature 336: 574-575.

Albuquerque NSL, Milet-Pinheiro P, Cruz DD, Navarro DMAF, Machado IC. 2021. Pollination of *Sarcoglottis acaulis* (Orchidaceae) by male orchid bees: nectar as resource in spite of a strong floral scent. Plant Biology 23: 719-727.

Araújo AC, Quirino ZGM, Gadelha Neto PC, Araújo AC. 2009. Síndromes de polinização ocorrentes em uma área de Mata Atlântica, Paraíba, Brasil. Biotemas 4: 83-94.

Barbosa MRV. 1996. Estudo florístico e fitossociológico da Mata do Buraquinho, João Pessoa, Paraíba. PhD Thesis, Universidade Estadual de Campinas, Campinas.

Barbosa MRV, Thomas W, Zárate ELP, *et al*. 2011. Checklist of the vascular plants of the Guaribas Biological Reserve, Paraíba, Brazil. Revista Nordestina de Biologia 20: 79-106.

Barros F, Rodrigues VT, Batista JAN. 2009. Orchidaceae. In: JR Stehmann, RC Forzza, A Salino, M Sobral, DP Costa, LHY Kamino. (eds.). Plantas da Floresta Atlântica. Instituto de Pesquisa Jardim Botânico do Rio de Janeiro, Rio de Janeiro. p. 372-403.

Barros F, Vinhos F, Rodrigues VT, *et al*. 2014. Orchidaceae. In: Lista de Espécies da Flora do Brasil. Jardim Botânico do Rio de Janeiro. http://floradobrasil.jbrj.gov.br/jabot/floradobrasil/FB12194. 06 Oct. 2014.

Brasil. Ministério do Meio Ambiente/Instituto Brasileiro do Meio Ambiente e dos Recursos Naturais Renováveis. 2003. Plano de Manejo, Reserva Biológica Guaribas. http://www.icmbio.gov.br/portal/images/stories/imgs-unidades-coservacao/rebio_guaribas.pdf. 25 Feb. 2020.

Bezerra CP, Martins CF. 2001. Diversidade de Euglossinae (Hymenoptera, Apidae) em dois fragmentos de Mata Atlântica localizados na região urbana de João Pessoa, Paraíba, Brasil. Revista Brasileira de Zoologia 18: 823-835.

Carvalho R, Machado IC. 2002. Pollination of *Catasetum macrocarpum* (Orchidaceae) by *Eulaema bombiformis* (Euglossini). Lindleyana 17: 85-90.

Christenhusz, MJ, Fay MF, Chase MW. 2017. Plants of the World: an illustrated encyclopedia of vascular plants. Chicago, University of Chicago Press.

Cunha MFB, Forzza RC. 2007. Orchidaceae no Parque Natural Municipal da Prainha, RJ, Brasil. Acta Botanica Brasilica 21: 383-400.

Davies KF, Margules CR, Lawrence JF. 2004. A synergistic effect puts rare, specialized species at greater risk of extinction. Ecology 85: 265-271.

Dressler RL. 1981. The Orchids: Natural history and classification. Cambridge, Harvard University Press.

Farias RCAP, Madeira-da-Silva MC, Pereira-Peixoto MHE, Martins CF. 2008. Composição e sazonalidade de espécies de Euglossina (Hymenoptera: Apidae) em mata e duna na área de Proteção Ambiental da Barra do Rio Mamanguape, Rio Tinto, PB. Neotropical Entomology 37: 253-258.

Flora do Brasil 2020. 2021. Orchidaceae. Jardim Botânico do Rio de Janeiro. https://floradobrasil.jbrj.gov.br/FB179. 25 Feb. 2021.

Gentry AH. 1974. Flowering phenology and diversity in tropical Bignoniaceae. Biotropica 6: 64-68.

Govaerts R. 2003. Computer printout of the monocot checklist. Kew, Royal Botanic Gardens

Hutchings MJ, Robbirt KM, Roberts DL, Davy AJ. 2018. Vulnerability of a specialized pollination mechanism to climate change revealed by a 356-year analysis. Botanical Journal of the Linnean Society 186: 498-509.

Janzen DH. 1971. Euglossine bees as long-distance pollinators of tropical plants. Science 171: 203-205.

Jersákova J, Johnson SD, Kindlmann P. 2006. Mechanisms and evolution of deceptive pollination in orchids. Biological Reviews of the Cambridge Philosophical Society Journal 81: 219-235.

Lesica P, Kittelson PM. 2010. Precipitation and temperature are associated with advanced flowering phenology in a semi-arid grassland. Journal of Arid Environments 74: 1013-1017.

Lieth H. 1974. Purpose of a phenology book. In: Lieth H. (eds.). Phenology and seasonality modeling. Berlin, Springer. p. 3-19.

Lima PJ, Heckendorff WD. 1985. Climatologia. In: Governo do Estado da Paraíba. (eds.). Atlas Geográfico do Estado da Paraíba. João Pessoa, Universidade Federal da Paraíba.

Kharouba HM, Vellend M. 2015. Flowering time of butterfly nectar food plants is more sensitive to temperature than the timing of butterfly adult flight. Journal of Animal Ecology 84: 1311-1321.

Martins CF, Souza AKP. 2005. Estratificação vertical de abelhas Euglossina (Hymenoptera, Apidae) em uma área de Mata Atlântica, Paraíba, Brasil. Revista Brasileira de Zoologia 22: 91-913.

Milet-Pinheiro P, Gerlach G. 2017. Biology of the Neotropical orchid genus *Catasetum*: A historical review on floral scent chemistry and pollinators. Perspectives in Plant Ecology Evolution and Systematics 27: 23-34.

Milet-Pinheiro P, Schlindwein C. 2005. Do euglossine males (Apidae, Euglossini) leave tropical rainforest to collect fragrances in sugarcane monocultures? Revista Brasileira de Zoologia 22: 853-858.

Rezende CL, Scarano FR, Assad ED, et al. 2018. From hotspot to hopespot: An opportunity for the Brazilian Atlantic Forest. Perspectives in Ecology and Conservation 16: 208-214.

Morellato LPC, Leitão Filho HF. 1990. Estratégias fenológicas de espécies arbóreas em floresta semidecídua na Serra do Japí, Jundiaí, São Paulo. Revista Brasileira de Biologia 50: 163-173.

Nemésio A. 2009. Orchid bees (Hymenoptera: Apidae) of the Brazilian Atlantic Forest. Zootaxa. New Zealand: Magnolia Press 2041: 241.

Nemésio A. 2013. Are orchid bees at risk? First comparative survey suggests declining populations of forest-dependent species. Brazilian Journal of Biology 73: 367-374.

Nemésio A, Silveira FA. 2006. Edge effects on the orchid-bee fauna (Hymenoptera: Apidae) at a large remnant of Atlantic Rain Forest in southeastern Brazil. Neotropical Entomology 35: 313-323.

Newstrom LE, Frankie GW, Baker HG. 1994. A new classification for plant phenology based on flowering patterns in lowland tropical rain forest trees at La Selva, Costa Rica. Biotropica 26: 141-159.

Nilsson LA. 1992. Orchid pollination biology. Trends in Ecology and Evolution 8: 255-259.

Pansarin ER, Pansarin LM. 2008. A Família Orchidaceae na Serra do Japi, São Paulo, Brasil. Rodriguésia 59: 99-111.

Paraíba. 2020. Agência Executiva de Gestão das Águas do Estado da Paraíba. http://www.aesa.pb.gov.br/aesa-website/meteorologia-chuvas. 25 Feb. 2020.

Pessoa E, Alves M. 2012. Flora da Usina São José, Igarassu, Pernambuco: Orchidaceae. Rodriguésia 62: 341-356.

Peruquetti RC, Campos LAO, Coelho CDP, Abrantes CVM, Lisboa LCO. 1999. Abelhas Euglossini (Apidae) de áreas de Mata Atlântica: abundância, riqueza e aspectos biológicos. Revista Brasileira de Zoologia 16: 101-118.

Powell AH, Powell GVN. 1987. Population dynamics of male euglossine bees Amazonian forest fragments. Biotropica 19: 176-179.

Ramalho AV, Gaglionone MC, Oliveira ML. 2009. Comunidades de abelhas Euglossina (Hymenoptera, Apidae) em fragmentos de Mata Atlântica no Sudeste do Brasil. Revista Brasileira de Entomologia 53: 95-101.

R Development Core Team. 2013. R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. http://www.r-project.org/.

Roubik DW, Ackerman JD. 1987. Long-term ecology of euglossine orchid-bees in Panama. Oecologia 73: 321-333.

Sahagun-Godinez E. 1996. Trends in the Phenology of Flowering in the Orchidaceae of Western Mexico. Biotropica 28: 130-136.

Singer RB, Sazima M. 1999. The pollination mechanism in the 'Pelexia alliance' (Orchidaceae: Spiranthinae). Botanical Journal of the Linnean Society 131: 249-262.

Singer RB, Cocucci AA. 1999. Pollination mechanism in southern Brazilian orchids which are exclusively or mainly pollinated by halictid bees. Plant Systematics and Evolution 217:101-117.

Tropicos.org. 2015. Missouri Botanical Garden. www.tropicos.org/Name/40029773. 10 Jun. 2015.

van der Pijl L, Dodson CH. 1969. Orchid flowers: their pollination and evolution. Coral Gables, University of Miami Press.

Wikelski M, Moxley J, Eaton-Mordas A, *et al*. 2010. Large-range Movements of Neotropical Orchid Bees Observed via Radio Telemetry. PLOS ONE 5: 1-6.

Wolf AA, Zavaleta ES, Selmants PC. 2017. Flowering phenology shifts in response to biodiversity loss. PNAS 114: 3463-3468.

Zar JH. 1999. Biostatistical analysis. 4th edn. New Jersey, Prentice-Hall, Inc.