



## Original Papers

# Myrtaceae flowering strategies in a gallery forest, Chapada Diamantina, Brazil

Rosineide Braz Santos Fonseca<sup>1</sup>, Elaine Maiara Bonfim Nunes<sup>2,5</sup>, Jamerson Souza da Costa<sup>2,6,10</sup>,  
Isiara Silva Menezes<sup>3,7</sup>, Lia d'Afonseca Pedreira de Miranda<sup>3,8</sup>, Eduardo Leite Borba<sup>4</sup> & Ligia Silveira Funch<sup>2,3,9</sup>

### Abstract

Tropical plants exhibit virtually all known phenological responses, which can range from near-continuous to brief events, and from complete synchrony to complete asynchrony. This wide variety of phenological patterns is related to high tropical biodiversity and complex interactions with diverse abiotic and biotic factors, such as plant-pollinator interactions. Changes in these factors will therefore influence plant phenology, and differences in those phenological responses can impact reproductive success and plant fitness. We report here interspecific variations in the reproductive ecology (flowering phenology, floral biology, reproductive systems, and pollination) of 10 Myrtaceae species in a gallery forest in northeastern, Brazil. We evaluated flowering patterns and strategies, considering the frequency and duration of each phenophase from monthly observations, using the semi-quantitative Fournier scale. We also analyzed floral biology, recorded floral visitors, conducted pollination experiments (autonomous self-pollination, hand self-pollination, hand cross-pollination, and natural/open pollination), and calculated self-incompatibility (SII) and autogamy (AI) indices. Most species exhibited synchronous and annual flowering lasting between 1 and 5 months, with a “pulsed bang” strategy (*i.e.*, mass flowering concentrated within just a few days, with intervals). These patterns were repeated among congeneric species, except in *Myrcia*, in which half of the species accompanied were classified as having a “pulsed bang” and the other half a “big bang” strategy. The flowering strategies appeared to be associated with distinct patterns of inflorescence development, bud production, flowering duration, and the timing of flowering in individual plants. Bee species were considered pollinators. Most species studied were self-incompatible and allogamous. In general, the species produced large quantities of flowers during short temporal intervals, favoring crosses between individuals having precise synchrony.

**Key words:** flowering phenology, interspecific variation, montane forest, reproductive biology.

### Resumo

As plantas tropicais exibem quase todos os tipos de respostas fenológicas conhecidas, variando de eventos quase contínuos aos explosivos breves, e de total sincronia à completa assincronia. Essa ampla variedade de padrões fenológicos está relacionada à alta biodiversidade e às diferentes interações com fatores abióticos e bióticos, como as interações planta-polinizador. Portanto, mudanças nesses fatores influenciam a fenologia das plantas e diferenças nas respostas fenológicas podem impactar o sucesso reprodutivo da espécie a aptidão das plantas. Analisou-se a variação interespecífica na ecologia reprodutiva (fenologia da floração, briologia floral, sistemas reprodutivos e polinização) de 10 espécies de Myrtaceae em floresta ciliar, na Chapada

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<sup>1</sup> Instituto Federal de Educação, Ciência e Tecnologia Baiano, Campus Santa Inês, Zona Rural, Santa Inês, BA, Brazil. ORCID: <<https://orcid.org/0000-0002-6020-1467>>.

<sup>2</sup> Universidade Estadual de Feira de Santana - UEFS, Prog. Pós-graduação em Botânica, Feira de Santana, BA, Brazil.

<sup>3</sup> Universidade Estadual de Feira de Santana - UEFS, Depto. Ciências Biológicas, Lab. Flora e Vegetação, Feira de Santana, BA, Brazil.

<sup>4</sup> Universidade Federal de Minas Gerais - UFMG, Instituto de Ciências Biológicas, Depto. Botânica, Belo Horizonte, MG, Brazil. ORCID: <<https://orcid.org/0000-0002-9881-8630>>.

<sup>5</sup> ORCID: <<https://orcid.org/0000-0001-7064-4328>>. <sup>6</sup> ORCID: <<https://orcid.org/0000-0001-5318-2213>>. <sup>7</sup> ORCID: <<https://orcid.org/0000-0003-0745-7743>>.

<sup>8</sup> ORCID: <<https://orcid.org/0000-0002-1841-1663>>. <sup>9</sup> ORCID: <<https://orcid.org/0000-0001-7096-0187>>.

<sup>10</sup> Author for correspondence: [jamersonjse@yahoo.com.br](mailto:jamersonjse@yahoo.com.br)

Diamantina, Brasil. Avaliaram-se os padrões e estratégias de floração, considerando a frequência e duração da fenofase a partir de observações mensais e de acordo com a escala semiquantitativa de Fournier; também analisaram-se a biologia floral, visitantes florais e conduziram-se experimentos de polinização (autopolinização autônoma, autopolinização manual, polinização cruzada manual e polinização natural/aberta) e calcularam-se os índices de autoincompatibilidade (SII) e autogamia (AI). A maioria das espécies apresentou floração anual, com duração de 1 a 5 meses, alta sincronia e estratégia do tipo *pulsed-bang* (i.e., floração massiva concentrada em poucos dias intercalados por um intervalo de tempo). Os padrões se repetiram entre espécies congêneres, exceto em *Myrcia* spp., em que metade das espécies foi classificada como “pulsed bang” e as demais apresentaram estratégia “big bang”. A estratégia de floração parece estar relacionada com o padrão de desenvolvimento das inflorescências, produção dos botões, duração e sincronia da floração dos indivíduos. Abelhas foram consideradas os polinizadores. A maioria das espécies foram auto-incompatíveis e alogâmicas. Em geral, as espécies ofertaram grandes quantidades de flores em pequenos intervalos temporais, favorecendo cruzamentos entre indivíduos com sincronia precisa.

**Palavras-chave:** fenologia da floração, variação interespecífica, floresta montana, biologia reprodutiva.

## Introduction

Phenological patterns can be defined at different organizational levels - from communities to individuals - and are closely linked to biotic (e.g., plant-pollinator interactions) and abiotic (e.g., climatic variables) factors (Newstrom *et al.* 1994; Williams-Linera & Meave 2002; Stevenson *et al.* 2008). In tropical regions, precipitation, and photoperiod have demonstrated high relevance to phenology (Borchert *et al.* 2015; Mendoza *et al.* 2017; Souza & Funch 2017; Costa *et al.* 2021), affecting the duration, seasonality, and, most notably, the synchronicity of reproductive seasons (Morellato *et al.* 2016). The complexity of these relationships highlights the need for detailed research on the phenological triggers in tropical regions (Mendoza *et al.* 2017).

Considering that plant phenology is driven, in part, by biotic factors, temporal and spatial variations in reproductive phenology will directly affect the availability of resources for pollinators and seed dispersers, and thus, the reproductive success of tropical plant populations (Newstrom *et al.* 1994; Cortés-Flores *et al.* 2017) because of their greater dependence on animals for pollination and seed dispersal services (Ollerton *et al.* 2011) and the importance of the synchronization of those reproductive phenophases with favorable abiotic and biotic conditions (Elzinga *et al.* 2007; Carstensen *et al.* 2014; Rosas-Guerrero *et al.* 2014). As such, interspecific variations in flowering patterns can affect the stability of communities and the productivity of plant systems (Morellato *et al.* 2016). Flowering phenology is therefore highly relevant to the organization and structure of plant communities, the conservation of mutualists

and their interactions, and the maintenance of essential ecosystem services. The analyses of the reproductive ecology of plant species can aid our understanding of phenological differences (or similarities) and identify other selective forces (such as competition or facilitation), particularly among “phenospecies” - sympatric species that share the same phenological triggers and strategies (Proença *et al.* 2012; Morellato *et al.* 2016).

Many tropical trees exhibit brief flowering strategies (< 30 days) (Augsburger 1981; van Schaik *et al.* 1993; Newstrom *et al.* 1994; Bendix *et al.* 2006), resulting in mass and concentrated flowering (“big bang” or “pulsed bang”) with high synchrony between conspecific individuals - a strategy that favors cross-pollination and gene flow within natural plant populations (Proença & Gibbs 1994). Similarly, Staggemeier *et al.* (2010; 2015) demonstrated that flowering synchrony tends to increase with phylogenetic proximity in monophyletic groups, reflecting the evolutionary inheritance of their reproductive phenological niche.

Pollination systems can be affected by co-flowering species, aspects of the breeding system, and the diversity and availability of pollinators (Ramirez 2005). Plants with different pollination systems may exhibit flowering strategies linked to the needs of specific pollinators. Albor *et al.* (2020), Bergamo *et al.* (2020b), and Genini *et al.* (2021), however, noted a lack of studies addressing floral similarity among co-flowering species that share the same pollination systems and pollinators, as well as the potential influence of flower abundance on the synchronicity and temporal organization of resources in tropical forests. Such knowledge

would allow us to better understand the roles of phenodynamics and breeding systems on plant success.

Myrtaceae comprises approximately 6,000 species, distributed across 140 genera, with South America being one of its primary diversity hotspots (Wilson 2011; Lucas *et al.* 2019) with the identification of 1,195 species in Brazil (Proença *et al.* 2022). Despite the importance of this botanical family to the floristic composition of several Brazilian biomes (such as the Atlantic Forest, Cerrado, and Caatinga) (Forzza *et al.* 2012; Sobral *et al.* 2015; BFG 2018), little is known about its biology, interspecific phenological aspects, and reproductive ecology - especially when considering the wide variation in the breeding systems of its component taxa (*e.g.*, self-incompatibility, self-compatibility, spontaneous self-pollination, and apomixes) (Proença & Gibbs 1994; Nic Lughadha & Proença 1996; Nic Lughadha 1998; Torezan-Silingardi & Oliveira 2002; Vilela *et al.* 2012). Most of the studies on reproductive systems in this family evaluated a single or just a few species, with rare studies focusing on several species of the same group (*e.g.*, congeneric species; Nic Lughadha 1998; Silva & Pinheiro 2009) or an entire plant community (*e.g.*, Proença & Gibbs 1994; Fidalgo & Kleinert 2009). Such studies have demonstrated the occurrence of variations among the reproductive system of congeneric species, between phytophysiognomies of the same biome (Proença & Gibbs 1994), and even between congeneric species of the same phytophysiognomy (Silva & Pinheiro 2009).

Neotropical phenological studies associated with pollination and seed dispersal also tend to focus on one or just a few species, making it difficult to test hypotheses regarding the causes of certain phenological patterns and to analyze the variability of interspecific reproductive strategies (Williams-Linera & Meave 2002; Goulart *et al.* 2005; Elzinga *et al.* 2007). We therefore analyzed the flowering phenology of 10 Myrtaceae species in a gallery forest in the Chapada Diamantina Mountains in northeastern Brazil, and discuss here their phenological variations and diversity. Additionally, we evaluated the floral biology, pollination, and breeding systems of five Myrtaceae species to address the following questions: 1) Are their flowering patterns seasonal and associated with abiotic factors? 2) Do flowering strategies differ among the species? 3) What are the floral visitors and potential pollinators? 4) What are the

reproductive systems of the species? We expected to identify links between flowering patterns, rainfall, and photoperiod (Borchert *et al.* 2015; Mendoza *et al.* 2017; Souza & Funch 2017; Costa *et al.* 2021), and to observe similar flowering strategies among the species evaluated and highly overlapping phenophases (Morellato *et al.* 2016). We also expected bees to act as effective pollinators and be responsible for ensuring the reproductive success of self-incompatible species (Proença & Gibbs 1994).

## Materials and Methods

### Study sites and sampled species

The present study was conducted along a trail approximately 3 km long that passed through a narrow strip of gallery forest (15 to 25 m wide) following the Lençóis River and growing on shallow, rocky, dystrophic, and litholic neosols, at 400–500 m a.s.l., in the municipality of Lençóis, in the Chapada Diamantina Mountains (12°33'S–41°24'W and 12°32.8'S–41°25.5'W) in Bahia state, which is part of the larger Espinhaço Range in northeastern Brazil. The region shows a high floristic diversity associated with various vegetation types, including evergreen forests growing along riverbanks and on mountain slopes (Funch *et al.* 2008, 2009). The upper canopy of this gallery forest is formed by trees up to 10 m tall, with some emergent individuals up to 20 m tall; the discontinuous sub-canopy, ranging in height from 3.5 m to 8.0 m, includes several species of Myrtaceae (Funch *et al.* 2008). The region has a relatively humid tropical climate (type Aw by the Köppen system), with a rainy season concentrated in the Austral summer (from December to April) and a dry winter season (from July to August). The mean annual precipitation varies between 700 and 1,300 mm (Alvares *et al.* 2013), with mean monthly precipitation rates generally varying from 35 mm (July and August) to 184 mm (December). The mean monthly temperature varies from 18 °C (April to September) to 22 °C (October to February) (Alvares *et al.* 2013). During the study period (2005–2006), rainfall during January and February/2006 was considerably lower than in 2005, but considerably higher in October/2006 than in 2005 (Fig. 1). A total of 102 tree species belonging to 39 families have been identified in the gallery forest along the Lençóis River, with Myrtaceae contributing 15 species to the sub-canopy (Funch *et al.* 2008). This study focused on

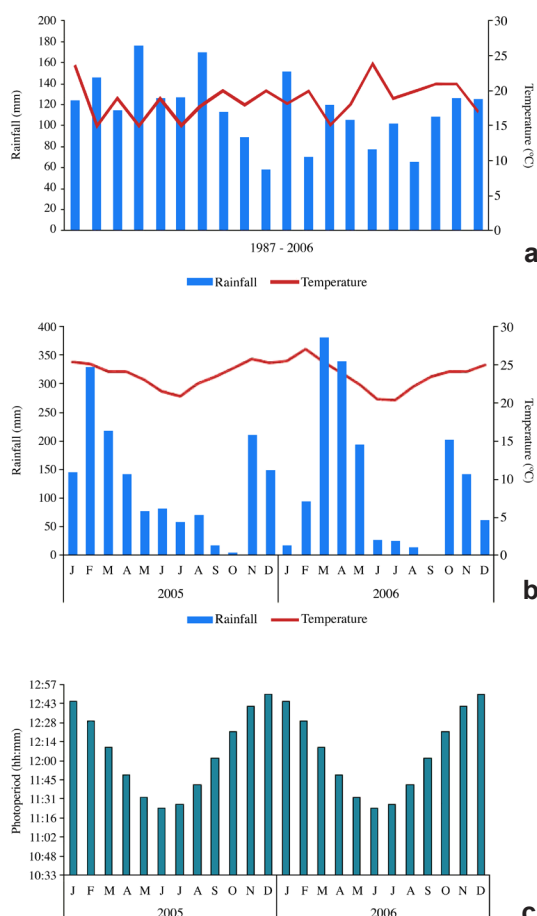
10 Myrtaceae shrub-tree species (Appendix S1, available on supplementary material <10.6084/m9.figshare.25923958>) that occur with high abundances in the study area (Funch *et al.* 2008): *Blepharocalyx salicifolius* (Kunth) O.Berg, *Eugenia gracillima* Kiaersk., *Eugenia puniceifolia* (Kunth) DC., *Myrcia amazonica* DC., *Myrcia blanchetiana* (O.Berg) Mattos, *Myrcia neoregeliana* E.Lucas & C.E.Wilson, *Myrcia sylvatica* (G.Mey.) DC., *Myrciaria floribunda* (H.West ex Willd.) O.Berg, *Myrciaria glanduliflora* (Kiaersk.) Mattos & D.Legrand, and *Psidium brownianum* Mart. ex DC. A voucher specimen for each species was herborized and deposited in the HUEFS herbarium at the Universidade Estadual de Feira de Santana.

## Environmental data

Environmental data for the study area (rainfall, temperature, and photoperiod) used to relate the phenological responses of the species with local climatic conditions were obtained from publically available datasets. Rainfall and temperature data were obtained from the National Institute of Meteorology (INMET - <<https://portal.inmet.gov.br/>>) and were based on the Lençóis Meteorological Station because of its proximity to the research site (1.41 km). Photoperiod data were obtained from the Astronomical Applications Department of the U.S. Naval Observatory (<[http://aa.usno.navy.mil/data/docs/RS\\_OneYear.php](http://aa.usno.navy.mil/data/docs/RS_OneYear.php)>).

## Flowering phenology

Ten adult reproductive individuals of each of the 10 focal Myrtaceae species (trees 5–15 m tall) were marked along a trail, varying from 15 to 25 m wide and approximately 3 km long, through the gallery forest. Phenological observations were made of those marked individuals every month for 24 months from January/2005 to December/2006, and daily during their respective reproductive periods. As proposed by Fournier (1974), the intensities of the phenophases (flower buds and flowers) were estimated using a semi-quantitative scale with five categories, at 25% intervals. We considered: a zero value of intensity (0), when a phenophase was absent; a value of one (1), when the phenophase were present at 1–25% of the observed total; two (2), when it was present at 26–50%; three (3), when it was present at 51–75%; and four (4), when the phenophases was present at 76–100% (Fournier 1974). The intensity of each phenophase is expressed as a percentage, according the five categories (San Martín-Gajardo & Morellato 2003). We evaluated the flowering patterns (flowering phenology) of each species, considering their frequency (the number of cycles per unit time), and duration (the period that an individual plant remains in a given phenophases) (Newstrom *et al.* 1994). The flowering strategies were classified according to Proença & Gibbs (1994). We considered that (i) the big-bang strategy occurs among species with synchronized mass flowering, with a duration of approximately one week; (ii) pulsed-bang flowering is discontinuous, and several days may pass when no flowers are open; and (iii) the steady-state flowering strategy involves relatively few flowers produced each day over a long period.



**Figure 1** – a-c. Climate data for the municipality of Lençóis, Chapada Diamantina, Brazil – a. historical annual total rainfall and temperature averages from 1987 to 2006 (INMET); b. monthly rainfall and temperature averages (Jan/2005 to Dec/2006); c. monthly average photoperiods (Jan/2005 to Dec/2006).



The seasonalities and synchronies of flowering during the observation period of the Myrtaceae species were tested using circular statistics (Morellato *et al.* 2010). For each year of observation, the frequency of occurrence of the phenological event of a given species in each month was calculated based on the total number of individuals showing that phenophase. The months were then converted to angles, where  $15^\circ = \text{January}$ , and the successive months were calculated at interval of  $30^\circ$ . We calculated basic parameters for circular distribution data (Morellato *et al.* 2010): i) the mean angle, which is similar to the linear mean, and represents the date mean associated with the phenological event; ii) concentration, which indicates how much of the observed data are concentrated around the mean; iii) angular standard deviation, which calculates the circular deviation around the mean angle; and iv) length of the  $r$  vector, which measures the concentration of frequencies around the estimated mean angle. Finally, we applied the Rayleigh test ( $z$  and  $p$ ), which is used to test the uniform distribution of circular data. A uniform distribution of the observed dates over time (year) indicates the absence of seasonality; the concentration of dates (concentration of frequencies) around the mean date implies seasonality. Flowering was considered seasonal and synchronic if the vector length ( $r$ ) was significantly greater or equal to 0.5 ( $r > 0.5$  and  $p \leq 0.05$ ) (Zar 2010). Flowering with a significant mean angle ( $p < 0.05$ ) was converted to a mean date, or the most probable date of the year that the species would be found in that phenophase. The Watson-Williams test was used to compare the mean dates ( $p < 0.05$ ) of the study years and among the different Myrtaceae species (Zar 2010). The normality of phenological data was examined using the Shapiro & Wilk test (Zar 2010). Generalized linear models, based on Gaussian distributions, and identity link functions, were used to test the effects of precipitation and photoperiod (predictor variables) on flowering (monthly activity index - response variable) for each Myrtaceae species during each month of the year. Temperature data were excluded from subsequent analyses because of collinearity between temperature and photoperiod (Spearman correlation test  $r = 0.86$ ). These analyses were performed using the “circular” package of R software (Agostinelli & Lund 2017).

## Morphology and floral biology

Floral biology, pollination, and breeding system observations were conducted for *B. salicifolius*, *E. puniceifolia*, *M. neoregeliana*, *M. sylvatica*, and *P. brownianum*, in addition to aspects of their inflorescence and floral morphology, such as the type and position of the inflorescence, flower length, and calyx and corolla size (Proença & Gibbs 1994). Due to the high synchronism of their flowering periods, we restricted morphological and floral biology measurements to only five of the 10 species studied. We closely observed the timing, sequence, and duration of anthesis of 20 pre-anthesis flower buds of each species (2–3 plants), from 00:00 to 17:00, for three consecutive days. We analyzed anthesis based on the classification used by Proença & Gibbs (1994), considering two types of floral anthesis: (i) the “Psidium” type, in which the stamens and style expand as the sepals and petals open; and (ii) the “Myrcia” type, in which the stamens remain completely curved while the style expands together with the sepals and petals. Stigma receptivity was evaluated by dipping the stigmas of 10 opened flowers per species into hydrogen peroxide and subsequently evaluating peroxidase activity (Dafni *et al.* 2005). Pollen availability (*i.e.*, the number of open anthers with the presence of pollen) was assessed by observing five unbagged floral buds per species (2–3 plants) throughout the floral cycle. The presence and location of pigments that reflect ultraviolet rays and osmophores (scent glands) were tested on a total of 20 flowers (10 flowers/experiment) from 2–3 plants of each species. The presence of UV-absorbing pigments was investigated by placing flowers in an ammonium hydroxide atmosphere for less than 5 minutes (Wilson & Brown 1957, adapted by Bandeira *et al.* 2011). Osmophores were verified by immersing the flowers in neutral red (1%) for 10 minutes and then washing them with a solution of glacial acetic acid (5%) (modified from Vogel 1990). Additionally, the scents of the flowers were determined via the olfactory perceptions of the researchers throughout the day, during the period of anthesis.

## Breeding systems

We performed pollination experiments for *B. salicifolius*, *E. puniceifolia*, *M. neoregeliana*, *M. sylvatica*, and *P. brownianum* using the following treatments: 1) autonomous self-pollination ( $n = 10$  individuals/species), in which autonomous pollen transfer was tested in 20–85 flowers per species,

whose flower buds were isolated in pre-anthesis with voile bags; 2) hand self-pollination ( $n = 12$  individuals/species), in which pollen grains from 20–65 flowers in pre-anthesis per species were manually transferred from the anthers to the stigmas of the same flowers, with the flowers then isolated with voile bags to prevent the access by floral visitors; 3) hand cross-pollination or xenogamy ( $n = 12$  individuals/species), where pollen grains from 20–53 pre-anthesis flowers per species were manually transferred to the stigmas of the same number of other emasculated flowers on different individuals, which were then bagged to prevent the access by floral visitors; 4) natural pollination ( $n = 12$  individuals/species), in which 30–64 pre-anthesis flowers per species were marked and left under natural conditions to test for natural pollen transfer by pollinators. The self-incompatibility (SII) and autogamy (AI) indices were then calculated (Ruiz-Zapata & Arroio 1978). The SII corresponds to the fruit/flower ratio produced by hand self-pollination divided by the fruit/flower ratio produced by hand cross-pollination. The AI was calculated by dividing the fruit/flower ratio produced by autonomous self-pollination by the fruit/flower ratio produced by hand cross-pollination. SII and AI assume values between 0 and 1, with values greater than 0.2 indicating self-compatibility and autogamy respectively (Ruiz-Zapata & Arroio 1978).

Approximately 10 hours after experimental pollinations, 3–5 flowers per treatment per species were fixed in 50% FAA (37% formaldehyde, acetic acid, 50% ethyl alcohol, 1:1:18 v/v) to test pollen germination. The pistils were rinsed in distilled water, treated with NaOH (10 N) at 60 °C for 10 minutes, transferred to distilled water overnight, cleared in 2% sodium hypochlorite for 1 h, rinsed in distilled water, and stained with 0.2% aniline blue (Kearns & Inouye 1993). Half of the pistil was placed on a microscope slide and compressed with a coverslip. Epifluorescence microscopy was used to verify of pollen grain germination (through the morphology and growth of the pollen tube bundles) and fertilization (Souza *et al.* 2015).

### Floral visitors

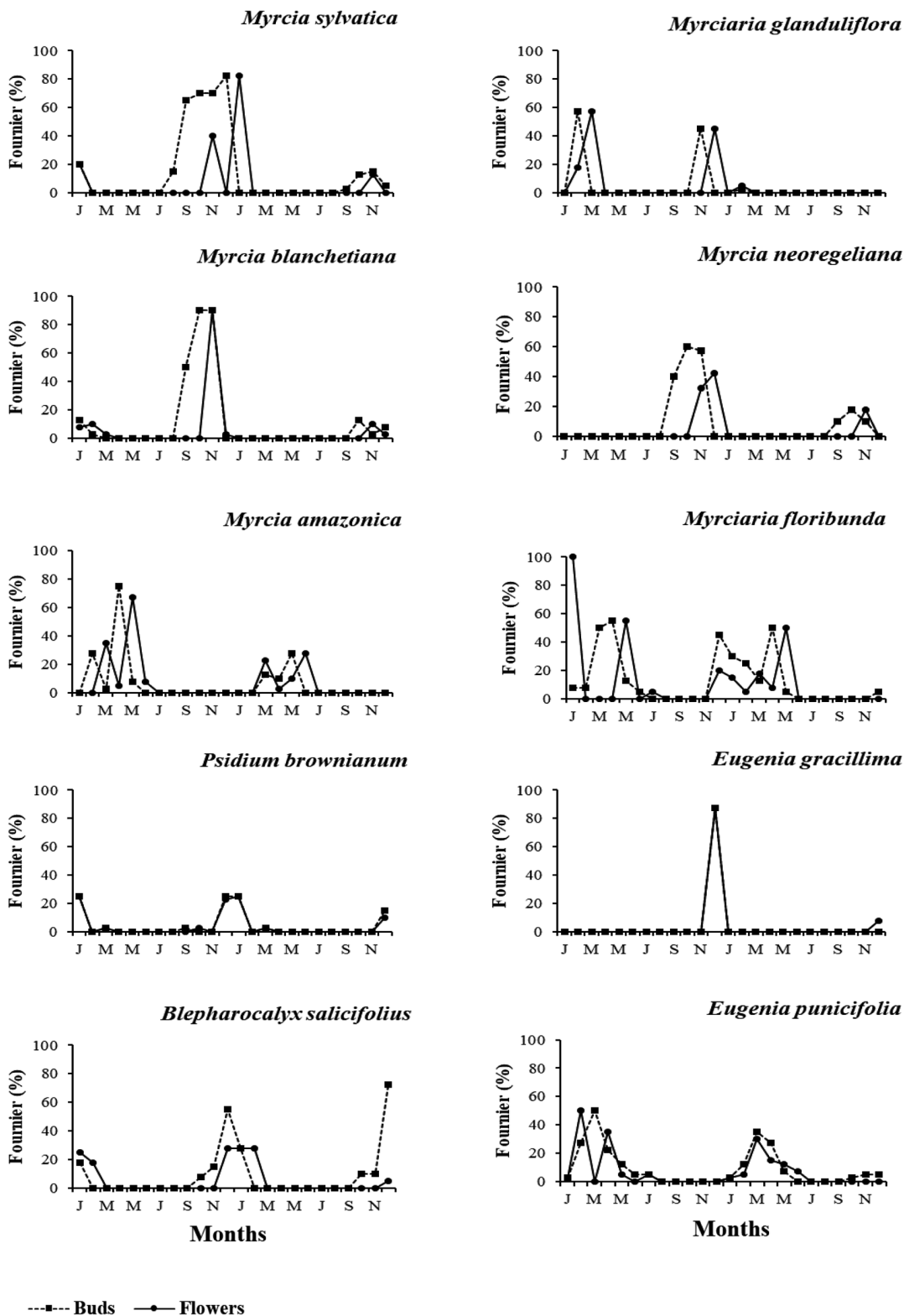
Focal observations of floral visitors to *B. salicifolius*, *E. puniceifolia*, *M. neoregeliana*, *M. sylvatica*, and *P. brownianum* were carried out from 5:00 to 17:00 h for 2–3 days, totaling 10–30 h per species (2–3 individuals/species) of observation on the duration and frequency of visits and foraging behavior (Rands & Whitney 2010). We considered

floral visitors to be any animal that visited the flowers, without necessarily acting as a pollinator (Inouye 1980). Floral visitors were classified as effective pollinators or occasional pollinators based on their body size, visitation time, frequency and duration of visits, and foraging behaviors on the flowers. Criteria used to evaluate effective pollinators considered the following: (i) visitation during periods of stigmatic receptivity and pollen availability; (ii) high frequency of visits; and (iii) body size large enough to carry pollen grains and contact the stigma of flowers during visits. Occasional pollinators were visitors with body size and behaviors suitable for pollen transfer but with low visitation frequencies and/or durations (Inouye 1980). The frequency of visits was calculated by dividing the total number of visits for each visitor species by the total number of hours the visitors were observed. Floral visitors were filmed and photographed to record and aid in the descriptions of their behaviors. On one of the observation days, viewing areas of approximately of 0.5 m<sup>2</sup> (*Myrcia neoregeliana*) and 1 m<sup>2</sup> (*Eugenia puniceifolia* and *Myrcia sylvatica*) were used because of the large number of concurrent visitors. Due to the difficulty in distinguishing *Partamona* sp. and *Trigona spinipes* in the field, the visitation numbers of these species were pooled. The insects were captured with an entomological net, mounted, and dried before being added as voucher specimens to the MZUEFS - Museu de Zoologia of the Universidade Estadual de Feira de Santana.

## Results

### Flowering patterns and strategies: interspecific variations

The Myrtaceae species in the tree community studied exhibited annual flowering patterns (Fig. 2; Tab. 1). Flowering duration ranged from brief to intermediate (40% for each class). Only *Eugenia gracillima* and *Myrciaria floribunda* (20%) exhibited extended flowering. In terms of flowering strategies, 70% of the Myrtaceae (*Blepharocalix salicifolius*, *Eugenia gracillima*, *E. puniceifolia*, *Myrcia amazonica*, *M. sylvatica*, *Myrciaria floribunda*, and *M. glanduliflora*) displayed “pulsed bang” flowering (mass, discontinuous, and concentrated in approximately seven days with intervals); another 20% (*Myrcia blanchetiana* and *M. neoregeliana*) displayed “big bang” flowering (mass and concentrated in approximately seven days); and only 10% (*Psidium brownianum*)



**Figure 2** – Flowering intensity (flower buds and flowers) of ten Myrtaceae species in a Gallery Forest, Chapada Diamantina, Brazil, recorded from Jan/2005 to Dec/2006.

**Table 1** – Phenological behavior of flowering in Myrtaceae species in terms of their frequency, duration, and flowering strategy, in the years 2005-2006, in Chapada Diamantina, Brazil. Frequency: A = annual or SA = supra-annual. Duration: B = brief, BI = brief to intermediate (interannual variation), I = intermediate, IE = intermediate to extended (interannual variation), and E = extended (according to Newstrom *et al.* 1994). Flowering strategies: B = “big bang”, BP = “big bang” to “pulsed bang” (interannual variation), P = “pulsed bang”, and S = “steady state” (according to Proença & Gibbs 1994, adapted from Gentry 1974).

Species	Population level			Individual level (%)		
	Frequency	Duration	Strategy	Frequency	Duration	Strategy
<i>Blepharocalyx salicifolius</i>	A	I	P	90A, 10SA	70B, 20I	100P
<i>Eugenia gracillima</i>	A	E	P	90A, 10SA	50BI, 30I, 10IE, 10E	40BP, 60P
<i>Eugenia puniceifolia</i>	A	I	P	70A, 30SA	30B, 30BI, 40I	60B, 20BP, 10P
<i>Myrcia amazonica</i>	A	B	P	40A, 60SA	100B	100B
<i>Myrcia blanchetiana</i>	A	B	B	90A, 10SA	100B	100B
<i>Myrcia neoregeliana</i>	A	B	B	100A	100B	100B
<i>Myrcia sylvatica</i>	A	I	P	100A	100I	100P
<i>Myrciaria floribunda</i>	A	E	P	100A	10BI, 60I, 30IE	10BP, 90P
<i>Myrciaria glanduliflora</i>	A	B	P	90A, 10SA	100B	90B, 10BP
<i>Psidium brownianum</i>	A	I	S	100A	80B, 20I	100S

displayed “steady state” flowering (few flowers per day over a long period) (Tab. 1). The patterns were similar among congeneric species, except in *Myrcia* spp., in which half of the species were classified as having “pulsed bang” (*Myrcia amazonica* and *M. sylvatica*) and the other half “big bang” strategies (*M. blanchetiana* and *M. neoregeliana*) (Tab. 1). *Psidium brownianum* was the only species to show “steady state” flowering (at both population and individual levels), with a small number of flowers available each day (approximately 30 flowers). This number was less than the set of flowers from a single inflorescence of *Myrcia neoregeliana* or *Myrcia sylvatica*.

#### Seasonality, synchrony, and environmental triggers

In general, the flowering periods of the studied Myrtaceae species were concentrated during the rainy season (November to April). Circular statistics revealed that the flowering patterns were seasonal and synchronous, with exceptions being observed with *Myrcia neoregeliana* and *Myrciaria glanduliflora* in 2006 (Tab. 2 and Fig. 3). For the *Myrcia* species, the mean flowering date was from October to November. For *Eugenia* species, the mean flowering peak was between March and April, while for *Myrciaria floribunda* it was between February and March. The flowering

peaks of *Blepharocalyx salicifolius*, *Myrciaria glanduliflora*, and *Psidium brownianum*, occurred in the same months in both research years (*B. salicifolius* and *P. brownianum* in December, and *M. glanduliflora* in January) (Tab. 2). The differences between the peak dates in 2005 and 2006 were not significant. However, there were significant variations in peak dates among the species evaluated, with greater variation observed in 2005 than in 2006 (Appendix S2, available on supplementary material <10.6084/m9.figshare.25923958>).

Flowering phenophases responses of Myrtaceae species to environmental factors were complex (Tab. 3). Except for *Myrciaria floribunda*, which did not respond to any of the environmental variables, all other species studied responded positively or negatively to photoperiod and/or rainfall variation. Only *E. gracillima* and *E. puniceifolia* responded negatively to variations in environmental factors (rainfall and photoperiod). The other species responded positively to photoperiod (Tab. 3).

#### Floral biology

The flowers of the species studied here were small (2–6 mm long), with calyces that were either open (*Blepharocalyx salicifolius*, *Eugenia puniceifolia*, and *Myrcia sylvatica*) or closed



**Table 2** – Results of the circular analyses of the occurrence of seasonality in the flowering of Myrtaceae species during two years (2005 - top row, and 2006 - bottom row). N corresponds to the total frequency (total number of records) of the phenophase per year of study.  $r > 0.5$  indicates synchrony.  $P < 0.05$  indicates a statistic difference, according to Watson-Williams test (W). \* = signal no significant values ( $p > 0.05$ ).

Species	Statistic parameters					
	N	Mean angle	Mean date	Mean vector length ( $r$ )	Rayleigh test ( $p$ )	W ( $p$ )
<i>Blepharocalyx salicifolius</i>	30	344.23°	14 dez	0.81	< 0.01	1.49
	23	351.69	21 dez	0.86	< 0.01	(0.48)
<i>Eugenia gracillima</i>	39	72.39°	14 mar	0.76	< 0.01	1.93
	35	72.26°	14 mar	0.62	< 0.01	(0.38)
<i>Eugenia puniceifolia</i>	30	89.14°	31 mar	0.82	< 0.01	5.65
	20	114.68°	25 abr	0.86	< 0.01	(0.06)
<i>Myrcia amazonica</i>	34	282.23°	12 out	0.86	< 0.01	0.15
	11	272.84°	02 out	0.92	< 0.01	(0.93)
<i>Myrcia blanchetiana</i>	30	287.74°	18 out	0.72	< 0.01	0.58
	8	296.10°	27 out	0.92	< 0.01	(0.75)
<i>Myrcia neoregeliana</i>	10	330.00°	30 nov	1.00	< 0.01	0.00
	2	330.00°	30 nov	1.00*	> 0.05	(1.00)
<i>Myrcia sylvatica</i>	27	294.65°	25 out	0.78	< 0.01	5.61
	17	335.41°	05 dez	0.79	< 0.01	(0.06)
<i>Myrciaria floribunda</i>	54	51.57°	21 fev	0.52	< 0.01	2.80
	44	60.69°	01 mar	0.68	< 0.01	(0.25)
<i>Myrciaria glanduliflora</i>	34	3.37°	04 jan	0.68	< 0.01	2.00
	1	30.00°	31 jan	1.00*	> 0.05	(0.37)
<i>Psidium brownianum</i>	23	342.18°	12 dez	0.85	< 0.01	0.61
	17	352.26°	22 dez	0.93	< 0.01	(0.74)

(*Myrcia neoregeliana* and *Psidium brownianum*). The calyx had an operculum (*P. brownianum*) and four (*B. salicifolius* and *E. puniceifolia*) or five (*M. neoregeliana* and *M. sylvatica*) lacinia. The corollas were white, with four (*B. salicifolius*, *E. puniceifolia*, and *P. brownianum*) or five petals (*M. sylvatica*), hermaphroditic, and androecium formed by many stamens distributed around an erect central style. The flowers are arranged in terminal panicles (e.g., *M. neoregeliana*), axillary dichasia (e.g., *B. salicifolius*), or axillary panicles (e.g., *M. sylvatica*), with 3–5 flowers and a reduced peduncle (*E. puniceifolia*), or with 1–4 flowers and opposite and solitary peduncles.

The anthesis of *P. brownianum* initiated near 06:00 h, while the other species initiated anthesis earlier (04:30–05:00 h). The process of opening lasted approximately 15 minutes, with

longitudinal fissures (*M. neoregeliana*) giving rise to equal calyx lobes by transverse rupture of the calyptra, followed by irregular longitudinal fissures in the hypanthium, separation of the petals (*P. brownianum*), and separation of sepals and petals in the other species. The anthesis of *E. puniceifolia* was of the “*Psidium*” type, in which the stamens and style expanded as the sepals and petals opened. In the other species, anthesis was of the “*Myrcia*” type, in which the stamens remained completely curved while the style expanded together with the sepals and petals (or only the sepals, e.g., *M. neoregeliana*).

The flowers of all species lasted for one day and provided pollen to visitors. The availability of pollen and receptivity of the stigma preceded the total opening of the flowers, coinciding with the onset of visitor activity (04:30–06:30 h). The

**Table 3** – Summary of generalized linear model analysis of climatic variables predicting the flowering phenologies of Myrtaceae species.

Species	Photoperiod		Rainfall	
	$\beta$	z or F value	$\beta$	z or F value
<i>Blepharocalyx salicifolius</i>	9.964	95.12***	0.0006949	0.037
<i>Eugenia gracillima</i>	-0.3226	0.18	0.014171	25.60***
<i>Eugenia puniceifolia</i>	-1.8339	5.77*	0.005217	2.43
<i>Myrcia amazonica</i>	2.707	7.24*	-0.002709	0.37
<i>Myrcia blanchetiana</i>	2.844	9.09**	-0.0006439	0.03
<i>Myrcia neoregeliana</i>	409.3	0.01***	0.0007845	0.30
<i>Myrcia sylvatica</i>	4.130	17.93***	-0.004662	1.19
<i>Myrciaria floribunda</i>	0.2777	0.13	0.006941	3.95
<i>Myrciaria glanduliflora</i>	3.440	6.86*	0.009136	5.32*
<i>Psidium brownianum</i>	13.041	33.98***	-0.002517	0.25

\*\*\* =  $p < 0.001$ ; \*\* =  $p < 0.01$ ; \* =  $p < 0.05$

flowers gave off a sweet scent in the early morning, which was emitted from the base of the petals, as indicated in tests with neutral red. All of the floral structures tested with ammonium hydroxide contained UV-absorbing pigments.

#### Floral visitors

We recorded nine visitor species during our observations, eight of which were Hymenoptera (Tab. 4). All visitors acted as pollinators, as they came into contact with both floral reproductive structures. Six of these species were regarded as effective pollinators based on their visitation frequencies and foraging behaviors (Tab. 4). Only *Xylocopa grisescens*, *Epicharis* sp., and Syrphidae species were considered occasional (or rarely observed) pollinators. The species *Apis mellifera*, *Melipona quadrifasciata anthidioides*, *Trigona spinipes*, and *Partamona* sp. were common visitors to all of the Myrtaceae species studied (Tab. 4).

Maximum foraging activity occurred 5–10 minutes after full flower anthesis and abruptly decreased approximately 30 minutes before the end of the feeding period. On dry days, anthers (except *P. brownianum*) were completely emptied before 07:00 h. *Megalopta* sp. visited *E. puniceifolia* flowers even earlier, concluding before 05:30 h. The duration of visits to *P. brownianum* were longer, possibly because of the small number of flowers available per day (Tab. 4).

#### Breeding systems

Fruit production by all of the species was significantly higher among flowers subjected to experimental cross-pollination than among those subjected to the other breeding system treatments (Tab. 5). Fruit production by *P. brownianum* by cross-pollination, for example, was almost triple that resulting from open pollination. Fruit production by *M. sylvatica* after open pollination was comparable to the number of fruits produced by experimental self-pollination. The Self-Incompatibility Index was lower than 0.2 in all of the species, except *Myrcia sylvatica* (Tab. 5). We observed the abortion of many fruits during their development.

In the experimental self-pollination and cross-pollination tests with flowers of *B. salicifolius*, *E. puniceifolia*, *M. sylvatica*, and *P. brownianum*, pollen tubes developed within 24 h (Fig. 4a,c). Fertilization, however, was observed only in cross-pollination experiments (Fig. 4b). Few pollen tubes resulting from experimental self-pollination reached the ovaries (Fig. 4d). We observed bundles of pollen tubes growing through the style of *P. brownianum* and reaching the ovary less than 24 h after experimental self-pollination and cross-pollination (Fig. 4e). More fertilized ovules (Fig. 4f) were observed after cross-pollination than after experimental self-pollination. The ovules of

**Table 4** – Floral visitors of Myrtaceae in a Gallery Forest, Chapada Diamantina, Brazil: species, visitation frequency, and period. Pollinator categories (according to Inouye 1980), based on visitor behaviors and frequencies: E = effective pollinator; O = occasional pollinator. \* = Observed by 1-2 researchers.

Species	Observations			Visitors		
	Total hours (days)	Schedules	Effective visiting hours*	Species	Frequency (%)	Pollinator category
<i>Blepharocalyx salicifolius</i>	15:30 (3)	5:30-5:45	11:30	<i>Apis mellifera</i>	4.0 (33.8)	E
		and 8:15-8:30		<i>Melipona quadrifasciata anthidioides</i>	3.1 (26.5)	E
				<i>Partamona</i> sp. - <i>Trigona spinipes</i>	4.5 (38.2)	E
				<i>Xylocopa grisescens</i>	0.2 (1.5)	O
<i>Eugenia puniceifolia</i>	13:00 (2)	5:00-5:15	6:00 (for 1 m <sup>2</sup> )	<i>Syrphidae</i>	0.2 (1.5)	O
		and 6:15-6:30		<i>Apis mellifera</i>	2.7 (10.1)	E
				<i>Melipona quadrifasciata anthidioides</i>	16.7 (63.3)	E
				<i>Partamona</i> sp. - <i>Trigona spinipes</i>	3.0 (11.4)	E
				<i>Xylocopa grisescens</i>	0.7 (2.5)	O
<i>Myrcia neoregeliana</i>	13:45 (3)	5:00-5:15	9:30 (5:00 for 0.5 m <sup>2</sup> )	<i>Megalopta</i> sp.	3.3 (12.7)	E
		and 7:30-7:45		<i>Apis mellifera</i>	10.2 (47.1)	E
				<i>Melipona quadrifasciata anthidioides</i>	1.8 (8.3)	E
				<i>Partamona</i> sp. - <i>Trigona spinipes</i>	8.8 (40.8)	E
				<i>Xylocopa grisescens</i>	0.6 (2.9)	O
<i>Myrcia sylvatica</i>	10:00 (2)	5:00-5:15	5:00 (2:30 for 1 m <sup>2</sup> )	<i>Epicharis</i> sp.	0.2 (1.0)	O
		and 7:15-7:30		<i>Apis mellifera</i>	7.4 (30.1)	E
				<i>Melipona quadrifasciata anthidioides</i>	6.6 (26.8)	E
				<i>Partamona</i> sp. - <i>Trigona spinipes</i>	8.8 (35.8)	E
				<i>Xylocopa grisescens</i>	0.6 (2.4)	O
<i>Psidium brownianum</i>	30:00 (3)	6:00-6:15	18:00	<i>Pseudaugochlora</i> sp.	1.2 (4.9)	E
		and 9:45-10:00		<i>Apis mellifera</i>	2.8 (18.4)	E
				<i>Melipona quadrifasciata anthidioides</i>	2.5 (16.5)	E
			<i>Partamona</i> sp. - <i>Trigona spinipes</i>	9.8 (65.1)	E	

all of the species were fertilized within 24 h. Seed formation in *M. neoregeliana* was initiated within 24 h of cross-pollination.

## Discussion

Our results indicated that the Myrtaceae species studied here evidenced annual, intermediate, synchronous, and seasonal flowering patterns, which mostly responded to photoperiod variations; with massive blooming, discontinuous, and concentrated in approximately seven days (with intervals). Its pollen flowers opened at dawn, had

bees as effective pollinators, and were mostly self-incompatible and allogamous.

In general, the Myrtaceae species studied here produced numerous flowers that were available for only a few consecutive or interspersed days. This strategy has likewise been observed in other Myrtaceae species (Proença & Gibbs 1994; Silva & Pinheiro 2007; Londe *et al.* 2021; Oliveira *et al.* 2021) and may be a widespread feature of the family. Most of the species evaluated (except *Eugenia gracillima* and *Myrciaria glanduliflora*) did not show strong correlations with precipitation

**Table 5** – Fruiting resulting from different pollination treatments of Myrtaceae flowers in a Gallery Forest, Chapada Diamantina, Brazil. The parentheses contain the number of plants used/fruited in each treatment. SII = self-incompatibility index (ratio of fruit set from self vs. crossed flowers). AI = autogamy index (product of fruit/flower ratio by autonomous self-pollination vs. hand cross-pollination). Self = self-pollination; cross = cross-pollination; natural (open) = natural (open) pollination.

Species / Treatment	Fruit / Flower	Fruit-set (%)	SII	AI
<i>Blepharocalyx salicifolius</i>			0.167	0.127
Autonomous self	2(1) / 79(2)	2.5		
Hand self-	1(1) / 30(1)	3.3		
Hand cross-	7(1) / 35(1)	20.0		
Natural (open)	0 / 33(1)	0.0		
<i>Eugenia punicifolia</i>			0	0
Autonomous self-	0 / 37(2)	0.0		
Hand self-	0 / 28(2)	0.0		
Hand cross-	11(1) / 29(1)	37.9		
Natural (open)	0 / 50(1)	0.0		
<i>Myrcia neoregeliana</i>			0.074	0.17
Autonomous self-	6(1) / 85(2)	7.1		
Hand self-	2(1) / 65(2)	3.1		
Hand cross-	22(1) / 53(3)	41.5		
Natural (open)	0 / 64(2)	0.0		
<i>Myrcia sylvatica</i>			0.314	0
Autonomous self-	0 / 78(2)	0.0		
Hand self-	2(2) / 52(2)	3.8		
Hand cross-	6(2) / 49(2)	12.2		
Natural (open)	2(1) / 62(1)	3.2		
<i>Psidium brownianum</i>			0.067	0.067
Autonomous self-	1(1) / 20(3)	5.0		
Hand self-	1(1) / 20(3)	5.0		
Hand cross-	15(3) / 20(3)	75.0		
Natural (open)	8(3) / 30(5)	26.7		

rates. In riparian areas, where water is not a limiting factor, it is not surprising that other abiotic variables operate as flowering triggers (Borchert *et al.* 2004; Zimmerman *et al.* 2007). Similarly, when analyzing reproductive phenologies in gallery forests, Silva *et al.* (2011) found that most species flowered and fruited during the rainy season. Nonetheless, as demonstrated by Funch *et al.* (2002), the phenologies of riparian species appear to respond only weakly to soil moisture.

Thus, variables linked to day length appear to be the main signals for reproduction in riparian forests (Borchert *et al.* 2005; Silva *et al.* 2011).

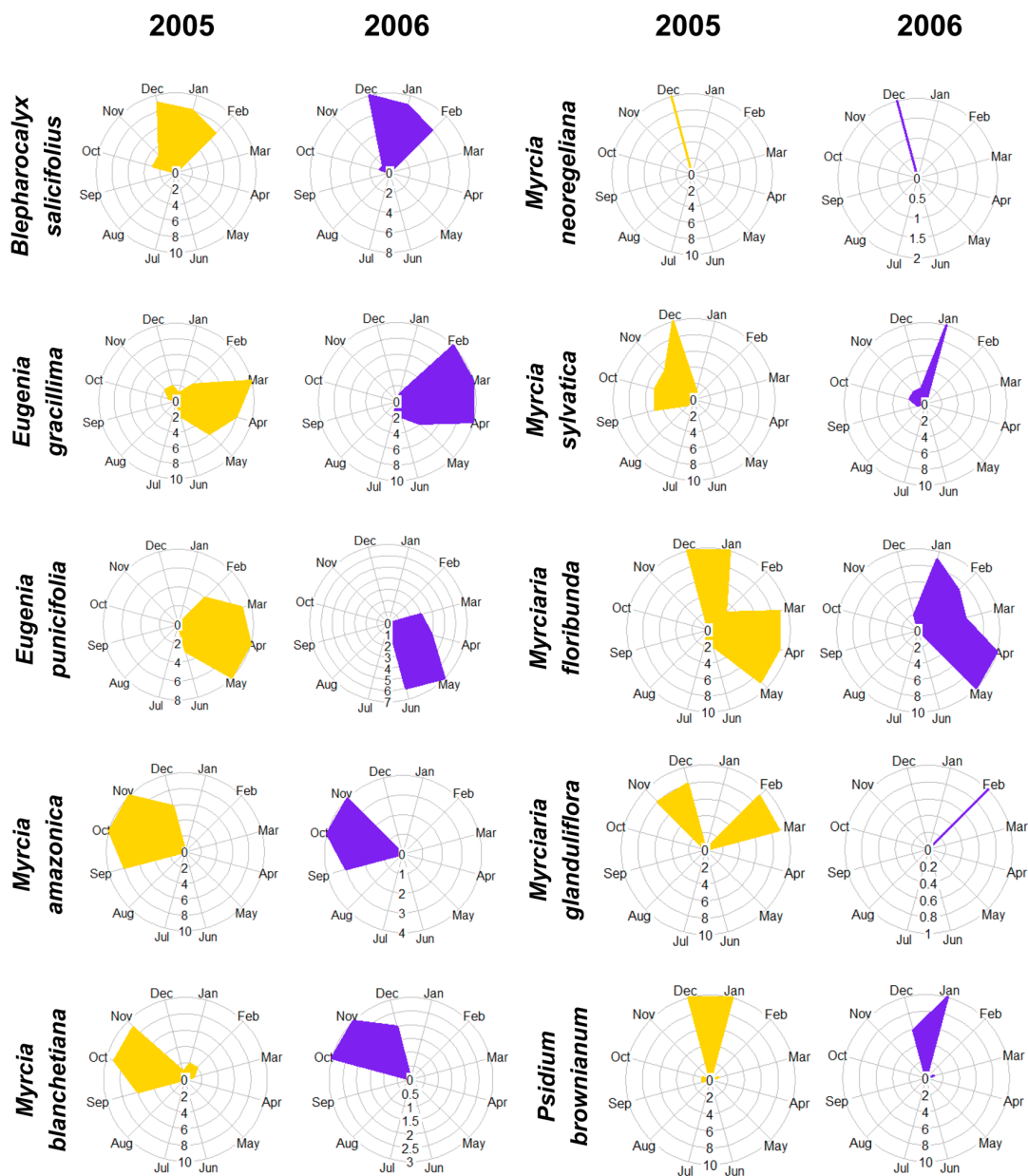
Only the phenologies of *Eugenia gracillima* and *Myrciaria floribunda* were not significantly correlated with photoperiod. The interannual photoperiod constancy would therefore explain, at least in part, the small interannual phenological variations observed, that is, the greater synchrony between years (Borchert *et al.* 2005; Luna-Nieves *et al.* 2017). When dealing with Myrtaceae, the possible conservation of the reproductive phenological niche must be considered as a possible result of the evolutionary inheritances of closely related species tending to flower under similar environmental conditions (Staggemeier *et al.* 2010,



2015), as verified by Londe *et al.* (2021) for *Myrcia amazonica*.

Despite the lack of correlation between the flowering of most species and precipitation, the observed seasonality of phenophases (rainy season) may be linked to the period in which pollinators and dispersers are abundant (van Schaik *et al.* 1993; Fidalgo & Kleinert 2009; Maia-Silva *et al.* 2015; Cortés-Flores *et al.* 2017; Bergamo *et al.* 2020a),

suggesting facilitation interactions (Rathcke & Lacey 1985; van Schaik *et al.* 1993; Bergamo *et al.* 2020a, b; Genini *et al.* 2021). Thus, considering that most Myrtaceae species do not have a species-specific pollination mechanism (Gressler *et al.* 2006; Geethika & Sabu 2017), these interactions could be enhanced by the greater abundance of generalist pollinators during the rainy season (Hansman 2001), when the species studied here



**Figure 3** – Circular distribution of flowering Myrtaceae species in a Gallery Forest, Chapada Diamantina, Brazil. Observed and modeled circular distribution of flowering events for 2005–2006.

regularly bloom. In the case of montane tropical ecosystems, such as our study area in the Chapada Diamantina Mountains, this strategy of temporal synchronization of plant flowering with the greatest availability of pollinators would tend to increase the reproductive success of the Myrtaceae species studied, especially in light of natural limitations of pollinators in these environments (Bergamo *et al.* 2021).

As stated by Primack (1987) and Singh & Kushwaha (2005), flowering and fruiting are dependent events, and the beginning of flowering usually determines the moment of fruiting. Environmental seasonality that is favorable to dispersal may therefore determine the flowering season. The seasonal white flowers of the Myrtaceae species studied here, for example, may promote greater pollinator attraction (*i.e.*, bees), as suggested by Lunau *et al.* (2011), Cordeiro *et al.* (2019), Aguiar *et al.* (2020), and Martins *et al.* (2021).

*Blepharocalyx salicifolius*, *Myrcia neoregeliana*, *Eugenia puniceifolia*, and *Psidium brownianum* were classified as self-incompatible and allogamous according to their SII and AI values, which were corroborated by pollen tube growth analyses (gametophytic self-incompatibility) (Richards 1997; Charlesworth 2010). The SII of *Myrcia sylvatica* indicated it as a self-compatible species, although its low fruit production suggests this result is not conclusive. Moreover, tests showed that despite pollen grain germination, pollen tube growth was aborted in the style, indicating gametophytic self-incompatibility (Takayama & Isogai 2005; Charlesworth 2010). Additionally, the absence of autogamy implies dependence on pollinators, which, according to Wolowski *et al.* (2016) and Bergamo *et al.* (2021), would be expected in tropical montane forests. Self-incompatible and synchronous flowering species increase their chances of reproductive success through the increased possibility of pollen transfer (Pires *et al.* 2013), possibly constituting an evolutionary response to biotic pressures (Augspurger 1981; van Schaik *et al.* 1993). Despite not being the focus of this research, we noticed certain aggregated spatial distributions of these populations in the study area associated with mass flowering (recorded for 90% of the evaluated Myrtaceae). Such spatial aggregation would facilitate crosses between neighboring plants (Carneiro *et al.* 2007; Moura *et al.* 2009) and may explain the lack of reproductive isolation over time, even for less synchronous species.

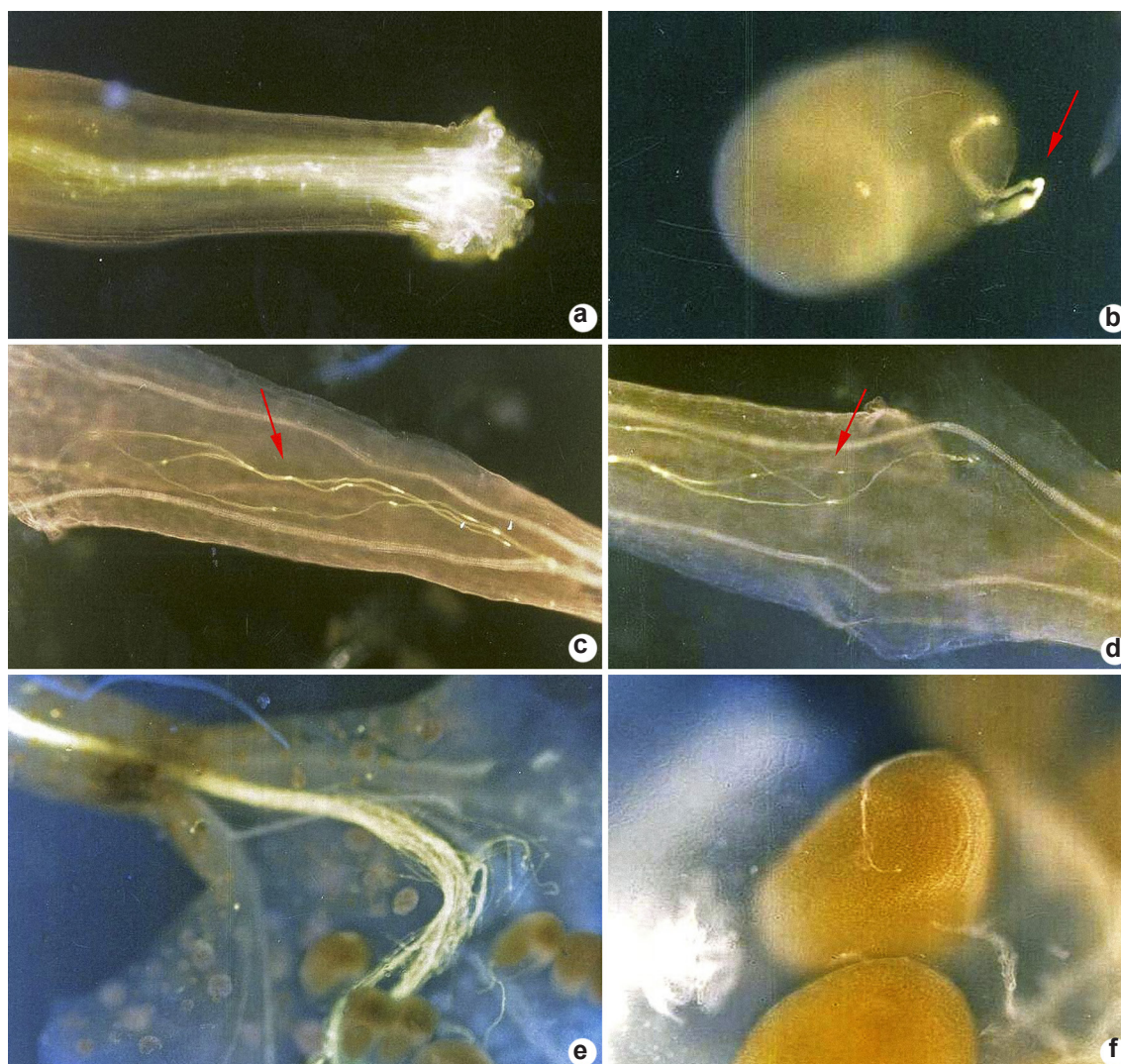
This combination of mass flowering and spatial aggregation of the Myrtaceae species evaluated can provide long-distance signaling of resource availability to bees (Wester & Lunau 2017), facilitating intense exploration of their pollen (Nadra *et al.* 2018). Within just a few hours after floral anthesis, bees had transported almost every grain of pollen. The same has been observed with other Myrtaceae species (Proença & Gibbs 1994; Silva & Pinheiro 2007; Fidalgo & Kleinert 2009; Cordeiro *et al.* 2017; Geethika & Sabu 2017; Guollo *et al.* 2021; Mudiana & Aryanti 2021; Oliveira *et al.* 2021). We can therefore assume that flowering in many Myrtaceae species does not occur within a few days, but rather within a few hours. From the perspective of the pollinators, this aspect certainly potentiates the disadvantages of foraging on distant individual plants (Nadra *et al.* 2018). Bergamo *et al.* (2020a, b), evaluating the facilitation mediated by pollination associated with flowering density and floral traits at the community level, showed that similar species (*i.e.*, synchronous species having the same colored flowers) facilitate interspecific pollination and increase reproductive success, as suggested by the Sargent & Ackerly (2008) community structuring theory.

The broad predominance of self-incompatibility in Myrtaceae species in this gallery forest is in line with the results obtained by Proença & Gibbs (1994), where all Myrtaceae species in the same phytophysiology in another location were self-incompatible, with greater variation in the reproductive system of species growing in more open areas. The variation of the reproductive systems in the family are very great, however (Nic Lughada & Proença 1996; Torezan-Silingardi & Oliveira 2002), and the influences of the phylogenetic and environmental components in the determining the occurrence of self-incompatibility or any other such reproductive mode are not clear. The massive flowering of these species, however, associated with the low density of woody species typically observed in tropical forests, tends to result in high rates of pollinator-mediated self-pollination, and can lead to high selection pressure favoring exclusive allogamy. The inbreeding depression hypothesis (Charlesworth & Willis 2009) can also explain fruit production of less than 50% by experimental cross-pollination in *Blepharocalyx salicifolius*, *Myrcia neoregeliana*, and *Eugenia puniceifolia*, as the treatments involved plants growing close to each other (because of mass flowering). Crosses between related plants increase the chances of deleterious recessive alleles being

expressed and, consequently, lower fruit production (Richards 1997). The large number of aborted fruits observed with *Myrcia neoregeliana* could reflect that situation (Charlesworth & Willis 2009), although the analyses of the reproductive systems evidenced more abortions in self-pollination tests.

In contrast, the small number of flowers produced each day by each *Psidium brownianum* plant may contribute to cross-pollination. This hypothesis is supported by open pollination fruit

production. Augspurger (1981) and Smith-Ramírez *et al.* (1998) claimed that pollinators exert selective pressure on flowering, affecting the intensity, timing, synchrony, and productivity of plants. The numerous flowers per inflorescence in *Myrcia* facilitate restricted foraging, especially by medium and large bees that visit groups of flowers (Fidalgo & Kleinert 2009; Pires & Souza 2011). The opposite is true for *Psidium*, whose flowers at anthesis are both spatially and temporally spread.



**Figure 4** – a. Bundles of pollen tubes growing through the style of *Eugenia puniceifolia* (hand cross-pollination after 24 h). b. fertilized ovule of *Blepharocalyx salicifolius* (hand cross-pollination after 24 h). c. three pollen tubes growing inside the style of *Myrcia sylvatica* (hand self-pollination after 24 h). d. two pollen tube endings their growth in the style of *M. sylvatica* (hand self-pollination after 24 h). e. bundles of pollen tubes reaching the ovary of *Psidium brownianum* (hand self-pollination after 24 h). f. fertilized ovule of *P. brownianum* (hand cross-pollination after 24 h).



These aspects support the proposition that the “big bang” and “pulsed bang” flowering strategies of Myrtaceae can have serious implications for their reproduction and, consequently, for population evolution. If these strategies reduce gene flow between neighboring plants, that flow is likely to occur via seed dispersal. Therefore, it is necessary that the phenological behaviors of the “new members” matches those of the rest of the population. In cases of inter-population migration, temporally divergent flowering can serve as an efficient barrier to gene flow (Hauser & Weidema 2000). A dispersal study conducted on these same Myrtaceae populations (Fonseca 2008) indicated that, for most species, there is no (or only low) seed dispersal. If there is replication between neighboring populations, these groups may become isolated.

Finally, we concluded that: 1) the populations studied differed in terms of the flowering behaviors of their individual members; 2) flowering was synchronous, seasonal, and correlated with the photoperiod for most species; 3) the intrapopulation flowering strategy is related to the pattern of inflorescence development and bud production (uniform or gradual); 4) mass flowering for a few days (successive or not), together with spatial distribution patterns and pollinator behaviors, favor crossing between phenologically similar plants; 5) these strategies (“big bang” and “pulsed bang”), under these conditions, make intra- and especially interpopulation gene flow difficult; 6) the Myrtaceae species studied did not have conspecific groups that were reproductively isolated over time; and 7) most (or even all) of the species are self-incompatible, reducing autogamy and biparental inbreeding in massive flowering individuals. This may have future reproductive and evolutionary implications for populations, as partially suggested by the intrapopulation phenological similarities and the diversity of Myrtaceae species. Future studies on spatial distribution and phenological behaviors, gene flow, and spatial genetic structure may confirm these hypotheses.

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### Data availability statement

In accordance with Open Science communication practices, the authors inform that all data are available within the manuscript.

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