Seasonality and the Relationships Between Reproductive and Leaf Phenophases In Myrtaceae Using Field and Herbarium Data

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Abstract
Phenological studies are considerably complemented through field data and herbarium collection databases. We examined the seasonality and relationships between leafing and reproductive phenophases using field observations and herbarium data of *Campomanesia eugenioides* var. *desertorum*, *Eugenia punicifolia*, and *Psidium schenckianum*, all native species of Myrtaceae. Field observations were performed in a caatinga remnant in Bahia State, Brazil; HUEFS herbarium collections were examined. In general, all species showed seasonal reproductive phases and ase seasonal leaf phases, and Spearman correlations were observed between those phases. Field observations and herbarium collections were similar and complementary, providing clues about seasonality and the relationship between leaf and reproductive phases of Myrtaceae species.

Keywords: phenology, tropical plants, *Campomanesia eugenioides*, *Eugenia punicifolia*, *Psidium schenckianum*.

Myrtaceae comprises approximately 5000 species distributed in tropical and subtropical regions, with South America being one of its main centers of diversity (Wilson, 2011). Twenty-three genera and 1027 species have been recorded for Brazil, with *Eugenia* P. Micheli ex L., *Myrcia* DC. ex Guill., and *Psidium* L. being the most abundant genera (Flora do Brasil 2020 under construction). Species richness occurs mainly in the Atlantic Forest and the caatinga domain (Sobral et al., 2013). The economic importance is recognized in terms of the production of wood, cellulose, substances with medicinal properties, and food resources (Souza & Lorenzi, 2012).

Tropical phenological studies have gathered considerable qualitative and quantitative data concerning focal trees with great utility for understanding forest ecosystem functioning (Mendoza et al., 2018; Pires et al., 2016). Phenological seasonality is the association between the occurrence of a certain phenophase and a season (Newstron et al., 1994). As Myrtaceae is an important arboreal component of tropical forests, the phenology of the family has been the subject of several studies and evidenced flowering and fruiting seasonality (Staggemeier et al., 2010; 2015; 2016). Most species have been treated as evergreen, although leaf phenology and relationships between leaf and reproductive phenophases (Fonseca, 2008; Moraes et al., 2017) have received little attention.

Herbarium collections represent potential sources of phenological information on tropical tree species and have long been utilized to supplement phenological field studies (e.g., Borchert, 1996; Mori et Prance, 1986), to understand patterns of plant phenology (e.g., Souza et Funch, 2017), and can contribute to conservation efforts (e.g., Banaszak et al., 2020) – but have only been used to examine reproductive phenophases, never leaf phases. We sought to examine the seasonality of native species of Myrtaceae, and the relationships between their leaf and reproductive phenophases, using field and herbarium data.

The focal species were shrubs or small trees 1.5-8 m tall: *Campomanesia eugenioides* var. *desertorum* (DC.) Landrum, (“cambuí”, “araçai”, and “arácarico”), which produces edible fruits and occurs in restinga, caatinga, and cerrado vegetation formations from Santa Catarina to Pernambuco (Oliveira et al., 2012);
*Eugenia punicifolia* (Kunth) DC. ("pitanga-do-campo"), widely distributed in cerrado, caatinga, campo rupestre, and forests in Brazil, Paraguay, and Venezuela (Sobral, 1987); and *Psidium schenckianum* Kiaersk. ("araçá"), common in caatinga and dry forest phytophysiognomies (Sobral, 2013; Landrum, 2017). Their populations were in caatinga remnants on the campus of the Bahia State University at Feira de Santana – UEFS, Brazil, inserted within a dry to sub-humid region with a mean annual temperature of 23.5°C and mean annual rainfall of 802 mm (Source: UEFS weather station). Phenological observations were performed monthly from September/2011 to September/2012 on marked individuals of *C. eugenioides* var. *desertorum* (*n* = 44), *E. punicifolia* (*n* = 59), and *P. schenckianum* (*n* = 50). The phenophases of young leaves (including leaf budding), mature leaves, flowering (budding and flowering), and fruiting (immature and ripe fruits) were monitored. Herbarium data were obtained through reviews of 458 vouchers deposited at the UEFS (HUEFS) [(*C. eugenioides* var. *desertorum* (*n* = 87 specimens), *E. punicifolia* (*n* = 278), and *P. schenckianum* (*n* = 93)]. The selection of herbarium specimens considered: (1) the professionals responsible for their identification; (2) the presence of both young and mature leaves (considering leaf expansion and texture), flowers, and fruits; (3) collection location and date. We examined seasonality for both field and herbarium phenological data using circular statistics using Oriana 4.02 software (Kovach Computing Services, http://www.kovcomp.co.uk). The frequency of each phenophase was calculated based on the total number of individuals/vouchers showing the phases per month. Months were converted into angles at 30° intervals. The mean angles and *r* vector lengths were calculated. Angle significance was tested using the Rayleigh test (*Z* for circular distributions (Zar, 2010). The phenological events with significant mean angles (*p* < 0.05) were transformed into mean dates. Phenophases whose vector lengths (*r*) were > 0.5, and which the Rayleigh test indicated as significant, were considered seasonal (Morellato et al., 2010). We examined correlations between leaf and reproductive phenophases for both field and herbarium phenological data using Spearman’s correlation coefficients (*rs*) calculated using R software 1.2.1335 (R core Team 2019). The normality of the phenological data was tested based on Shapiro & Wilk (Zar 2010). All three species demonstrated seasonality in their reproductive phenophases in both the field and in herbarium collections, except *E. punicifolia*, which did not appear seasonal in the herbarium data; leaf phenophases were generally not seasonal, except for young leaves in *P. schenckianum* (field) and young and mature leaves in *C. eugenioides* var. *desertorum* (herbarium) (Table 1; Figure 1). The greatest flowering activities (budding and flowering) occurred simultaneously with the production of young leaves (including leaf budding) in *C. eugenioides* var. *desertorum*, *E. punicifolia*, and *P. schenckianum* in both field and herbarium data (Figure 1). There were correlations between young leaves and flowering and fruiting phenophases, principally in *C. eugenioides* var. *desertorum* and *P. schenckianum* in terms of both field and herbarium data (Table 2). The results of the analysis of the collections housed at the HUEFS herbaria indicated a tendency towards seasonality of both the flowering and fruiting periods among the Myrtaceae species, although this behavior was not found to be as pronounced for fruiting as for flowering. The results of our field studies likewise indicated that flowering was associated with specific periods of the year (between November to January), corroborating earlier phenological studies (Fonseca, 2008; Staggemeier et al., 2010; 2015). The herbarium data from the present study indicated that fruiting is less seasonal than flowering, which could be explained by differences in fruit size and the length of the period required for maturation (Smith-Ramirez et al., 1998; Staggemeier et al., 2010; 2015; 2016). The relationship between phases was evidenced, mainly in species showing more restricted distributions such as *C. eugenioides* var. *desertorum* and *P. schenckianum* - both more frequent in caatinga environments (Oliveira et al., 2012; Landrum, 2017), where seasonal water restrictions are strong drivers of plant ecological strategies (Neves et al., 2017). The field data of *E. punicifolia* reveals the initial development of young leaves, with flower bud development occurring slightly later (Moraes et al., 2017). Our field observations of a small number of trees in a limited area, added to herbarium collections from a species’ entire range, were similar and complementary, providing clues about seasonality and the relationships between leaf and reproductive phases of the Myrtaceae studied species.
Table 1. Circular analysis of phenological events of *Campomanesia eugenioides* var. *desertorum* (DC.) Landrum, *Eugenia punicifolia* (Kunth) DC. and *Psidium schenckianum* Kiaersk. from field and herbarium data. Observations were performed in caatinga remnants at the campus of Universidade Estadual de Feira de Santana (UEFS), in Feira de Santana, Bahia, Brazil. Herbarium data were obtained from the collection of the HUEFS.

### Campanemasia eugenioides var desertorum

| Phenophase   | Field (°) | Herbarium (°) | Field (°) | Herbarium (°) | Field (°) | Herbarium (°) |
|--------------|-----------|---------------|-----------|---------------|-----------|---------------|
| Flowering    | 315.615°  | 342.00°       | 157.58°   | 45.90°        | 265.98°   | 187.57°       |
| Fruiting     | 342.00°   | 157.58°       | 265.98°   | 187.57°       | 106.58°   | 24.93°        |
| Young leaf   | 15.9°     | 0.61          | 0.33      | 0.08          | 0.56      | 0.05          |
| Mature leaf  | 45.90°    | 265.98°       | 187.57°   | 106.58°       | 24.93°    | 10.66          |

Rayleigh Test (z): 16.01, 1453.82, 22.49, 1716.96, 4.72, 1845.55, 2.20, 2138.36
Rayleigh Test (p): < 1.11E-07, < 1E-12, < 1.70E-10, < 1E-12, 0.009, < 1E-12, 0.11, < 1E-12

Grand Mean group: October, December, January, February, September, January, April, January

### Eugenia punicifolia (Kunth) DC

| Phenophase   | Field (°) | Herbarium (°) | Field (°) | Herbarium (°) | Field (°) | Herbarium (°) |
|--------------|-----------|---------------|-----------|---------------|-----------|---------------|
| Flowering    | 353.10°   | 99.63°        | 52.88°    | 146.24°       | 246.08°   | 127.56°       |
| Fruiting     | 246.08°   | 127.56°       | 127.06°   | 127.06°       |           |               |
| Young leaf   | 154.68°   | 1118.30       | 71.40°    | 1073.07       | 0.00      | 2003.10       |
| Mature leaf  | 1118.30   | 71.40         | 1073.07   | 0.00          |           |               |

Rayleigh Test (z): 63.01, 1534.56, 154.68, 1118.30, 71.40, 1073.07, 0.00, 2003.10
Rayleigh Test (p): < 1E-12, < 1E-12, < 1E-12, < 1E-12, < 1E-12, < 1E-12, < 1E-12, < 1E-12

Grand Mean group: December, April, February, May, September, May

### Psidium schenckianum

| Phenophase   | Field (°) | Herbarium (°) | Field (°) | Herbarium (°) | Field (°) | Herbarium (°) |
|--------------|-----------|---------------|-----------|---------------|-----------|---------------|
| Flowering    | 282.16°   | 340.45°       | 67.89°    | 83.85°        | 242.77°   | 357.18°       |
| Fruiting     | 340.45°   | 67.89°        | 83.85°    | 242.77°       | 123.69°   | 64.48°        |
| Young leaf   | 79.14°    | 129.14        | 1460.62   | 133.11        | 381.4     | 593.49        |
| Mature leaf  | 129.14    | 1460.62       | 133.11    | 381.4         | 0.09      | 593.49        |

Rayleigh Test (z): 92.34, 791.60, 129.14, 1460.62, 133.11, 381.4, 0.09, 593.49
Rayleigh Test (p): < 1E-12, < 1E-12, < 1E-12, < 1E-12, < 1E-12, < 1E-12, 0.91, < 1E-12

Grand Mean group: October, December, May, March, September, December, May, March

*p > 0.5 indicates synchrony; p < 0.05 indicates differences using the Watson-Williams test; * value may not be reliable due to low concentrations (p > 0.05)*

Figure 1. Circular histograms of the individual frequencies of flowering (Fl), fruiting (Fr), young leaves (Yl), and mature leaves (Ml) of *Campanemasia eugenioides* var. *desertorum* (DC.) Landrum, *Eugenia punicifolia* (Kunth) DC., and *Psidium schenckianum* Kiaersk. based on field observations (F) and herbarium (H) data. To the right, images of the corresponding species showing flowers, fruits, and leaves. For details of the analyses, see Table 1.
Table 2. Spearman correlation between leaves and reproductive phenophases of *Campomanesia eugenioides* var. *desertorum* (DC.) Landrum, *Eugenia punicifolia* (Kunth) DC. and *Psidium schenckianum* Kiaersk. based on field and herbarium data.

| Species                        | YL / FL | YL / FR | ML / FL | ML / FR |
|--------------------------------|---------|---------|---------|---------|
| *Campomanesia eugenioides*     | 0.5599  | 0.5874  | 0.7133  | 0.7055  | 0.6633  |
| *Eugenia punicifolia*         |         | -0.6831 |         |         |         |
| *Psidium schenckianum*        | 0.8984  | 0.6244  | -0.6733 | 0.7018  | 0.6770  | 0.5417  |

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