SCIENTIA PLENA VOL. 20, NUM. 11 2024
www.scientiaplena.org.br doi: 10.14808/sci.plena.2024.110203

Rhythms of nature: exploring the phenology of *Guapira opposita* (Vell.) Reitz in southeast Espírito Santo, Brazil

Ritmos da natureza: explorando a fenologia de *Guapira opposita* (Vell) Reitz no sudeste do Espírito Santo, Brasil

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(Recebido em 25 de maio de 2024; aceito em 06 de novembro de 2024)

Plant phenology allows us to understand the dynamics of vegetative and reproductive events in response to environmental conditions that act upon populations and communities in a given habitat. Moreover, this knowledge enriches the ecological aspects of species and supports ecosystem development, restoration, and conservation. In this sense, the present study aimed to characterize the phenology of *Guapira opposita* (Vell.) Reitz and verify the influence of climatic variables on the different phenophases of the species, which originated from two forest remnants located in the southeast of Espírito Santo state. Phenological data were collected monthly throughout 2014, and the methods of Fournier intensity index, percentage intensity, and Rayleigh test were used in the analyses. For the Rayleigh test, the months of the year were converted into circular angles to assess the seasonality of leaf change events. The results showed low variation in the vegetative behavior of the species, classifying it as deciduous. The Rayleigh test indicated extremely strong seasonality in Serra do Valentim, with leaf change events concentrated in a specific period of the year (test statistic = 1, p-value = 0), while in Cafundó, seasonality was also significant but less intense (test statistic = 0.8609, p-value = 0.002). No reproductive phenophases were observed, possibly due to climatic factors. The water deficit during the sampling year was probably the limiting factor for the absence of reproductive phenophases in the species.
Keywords: phenophases, Atlantic Forest, climatic variables.

A fenologia das plantas permite compreender a dinâmica dos eventos vegetativos e reprodutivos em resposta às condições ambientais que atuam sobre as populações e comunidades em um determinado habitat. Além disso, esse conhecimento enriquece os aspectos ecológicos das espécies e subsidia o desenvolvimento, a recuperação e a conservação dos ecossistemas. Nesse sentido, objetivou-se no presente estudo caracterizar a fenologia de *Guapira opposita* (Vell.) Reitz e verificar a influência das variáveis climáticas nas diferentes fenofases da espécie, oriunda de dois remanescentes florestais localizados no sudeste do estado do Espírito Santo. Os dados fenológicos foram coletados mensalmente durante o ano de 2014 e os métodos do Índice de intensidade de Fournier, da intensidade percentual e o teste de Rayleigh foram utilizados nas análises. Para o teste de Rayleigh, os meses do ano foram convertidos em ângulos circulares para avaliar a sazonalidade dos eventos de mudança foliar. Os resultados mostraram baixa variação no comportamento vegetativo da espécie, classificando-a como decídua. O teste de Rayleigh indicou uma sazonalidade extremamente forte na Serra do Valentim, com os eventos de mudança foliar concentrados em um período específico do ano (estatística de teste = 1, p-valor = 0), enquanto em Cafundó, a sazonalidade também foi significativa, mas menos intensa (estatística de teste = 0,8609, p-valor = 0,002). Não foram observadas fenofases reprodutivas, possivelmente devido a fatores climáticos. O déficit hídrico ocorrido no ano de amostragem foi, provavelmente, o fator limitante para a não ocorrência das fenofases reprodutivas da espécie.
Palavras-chave: fenofases, Floresta Atlântica, variáveis climáticas.

1. INTRODUCTION

Brazil has the world's greatest biodiversity, with around 20% of the planet's species [1]. Among Brazilian biomes endowed with high diversity and endemism, the Atlantic Forest stands out, with an area of 1.1 million km², which represents around 13% of Brazil's total surface area [2]. Despite many endangered species and endemic species with great biological value [2, 3], the Atlantic Forest natural cover is extremely vulnerable to degradation [4].

Thus, to promote recovery and conservation of this biome, it is necessary to know the vegetative and reproductive development of its species. In this sense, phenology seeks to study the patterns of periodic phenomena of living beings, their relationship with environmental conditions and correlation with morphological aspects [5]. Phenophases, for instance, are the visible biological activity of organisms and allows interpreting their reaction to climatic variables, especially temperature and precipitation. In this way, such studies support the understanding about forest dynamics and gather information that provides subsidies for actions related to ecosystem recovery and preservation [6, 7].

Due to the considerable diversity and complexity of tropical forest ecosystems, there is variability in the response to climatic conditions among species and individuals and, consequently, among remnants [8, 9], which is one of the biggest challenges of phenological studies. Many plant species are able to occupy environments with different characteristics, such as *Guapira opposita* (Vell.) Reitz, which belongs to Nyctaginaceae family and is abundant in sandy and undulating terrain [10]. This tree or shrub species is frequent in the phytogeographic domains of the Atlantic Forest, Amazon, Caatinga and Cerrado [11]. *G. opposita* has high morphophysiological plasticity, once the selection filters of the environment are not able to restrict its survival and establishment in different communities [12].

Given the above, studies about the influence of climatic factors on the phenological responses of tropical forest species are of great importance [13]. Thus, this study aims to characterize the phenology of *G. opposita* and verify the influence of climatic factors over its phenophases, in two distinct environments: Montane Dense Ombrophilous Forest (FODM) and Seasonal Semideciduous Forest (FES), southeast of the state of Espírito Santo. It is believed that variations in climatic conditions between the two forest remnants studied (FES and FODM) result in different intensities and phenological patterns in the species *Guapira opposita*. The higher altitude and humidity of the FODM are expected to result in more synchronized and intense phenology compared to the FES, where climatic conditions are drier and less favorable.

2. MATERIAL AND METHODS

2.1. *Guapira opposita*

The species was selected due to its occurrence in both study areas. *Guapira opposita* is an early secondary tree species from the Nyctaginaceae family, commonly found in tropical forests of South America, especially in the Atlantic Forest [14]. Its leaves are simple, opposite, coriaceous, and elliptical [11]. The flowers are small, greenish to whitish, and the species is dioecious, meaning it has separate male and female plants. The fruit is a fleshy drupe, reddish to purple when ripe, and is primarily dispersed by birds. Pollination is carried out by insects and wind [15]. *Guapira opposita* plays a crucial role in the recovery of degraded areas and the maintenance of biodiversity due to its ability to colonize open areas and stabilize the soil [14].

2.2. Study areas

The study was carried out in "Cafundó" Private Reserve of Natural Heritage (RPPN), which is located in Cachoeiro de Itapemirim municipality, between geographic coordinates 20°43'S and 41°13'W, and in Serra do Valentim, which extends itself between municipalities of Iúna and Muniz Freire, 41º28'26"W and 20º21'59"S, in the southeast of the state of Espírito Santo, Brazil.

Cafundó RPPN has a total area of 517 ha and Cwa climate according to Köppen classification. The average annual temperature in the region is 24.2°C [16], while the predominant vegetation is classified as Submontane Seasonal Semideciduous Forest (FES) [17].

Serra do Valentim, on the other hand, has approximately 50 ha. According to Köppen adapted by Alvares et al. (2013) [18] classification, the region climate is classified as Cfb, with mild summer and no dry season. The vegetation in this mountainous region, in turn, is classified as Montane Dense Ombrophilous Forest (FODM) [19] (Figure 1).

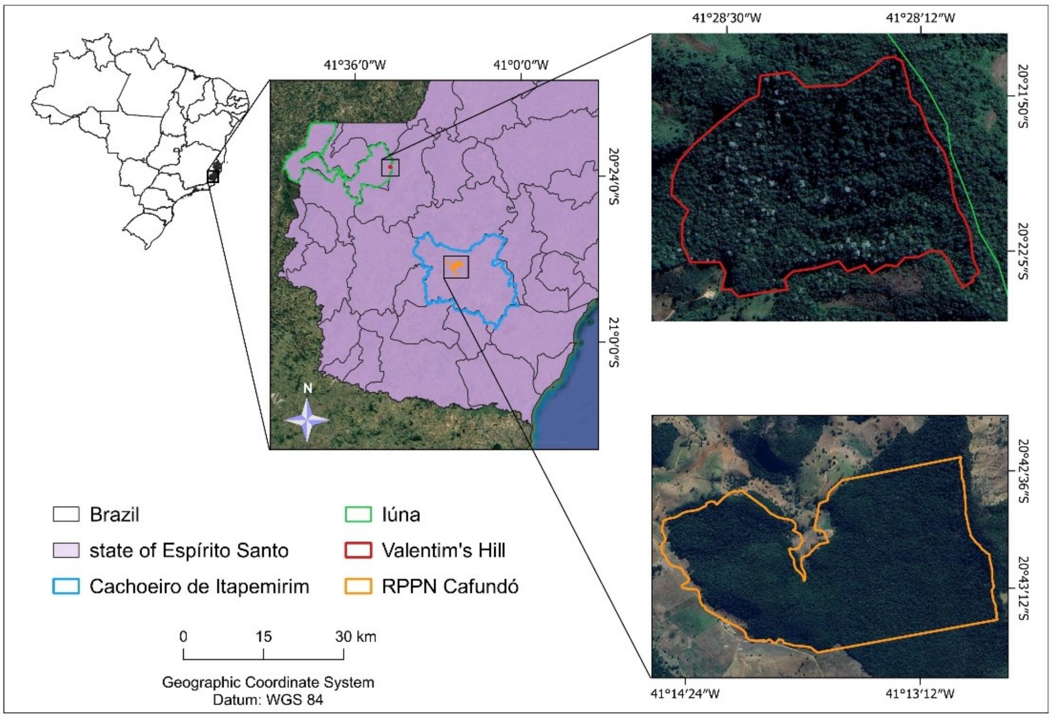

Figure 1: Location map of the study areas Private Reserve of Natural Heritage (RPPN) Cafundó and Valentim's Hill, in southeast of the state of Espírito Santo, Brazil.

2.3. Climate and Meteorological Data

Despite both studied areas being apart by only 46 km (in a straight line) and within the same hydrographic basin, they are located at different altitudes. Cafundó RPPN (FES) is located at 160m of altitude, while Farm Aristides (FODM) at 1,165 m. This factor makes temperature and precipitation to be different throughout the year in those areas. Thus, FODM-data concerning those variables were obtained from the meteorological station of the National Institute of Meteorology (INMET), located in Alegre municipality, while FES-data were obtained from the meteorological station of Capixaba Institute for Research, Technical Assistance and Rural Extension (Incaper), located in Muniz Freire.

The lowest precipitation value during sampling year (2014) occurred in the month of June in FES, while the highest precipitation occurred in the month of November. Comparing 2014 monthly precipitation with 2013's, it was found a reduction from 1,862.2 mm to 902.4 mm, which corresponds to 51.5% decrease in the expected annual precipitation values between successive those years. When comparing total annual precipitation for the study year with historical data from Alegre municipality, it is clear that 2014 was the one with the lowest precipitation in the last 38 years.

The average temperature observed for FES during 2014 (23.9°C), in turn, was close to the average temperature for the year 2013 (23.6°C). The lower average minimum temperatures in 2014 occurred in the months of June (15.9°C), July (16.0°C) and August (14.9°C), while the higher average maximums in January (34.9°C), February (35.1°C) and December (34.1°C).

Analyzing monthly precipitation in FODM during 2014, it was observed its predominance in the months of April, October and November. However, there was a significant reduction in total annual precipitation when comparing the study year (1,091.80 mm) with the previous one (1,637.50 mm), which corresponds to, approximately, 33% reduction. When comparing total annual precipitation for the study year (2014) with historical data from Muniz Freire municipality, it was noticed that 2014 was the 6th year with lowest annual precipitation in the last 38 years.

The lower average minimum temperatures occurred in August (13.2°C), June (14.4°C) and July (14.4°C), while the higher average maximums in January (32°C) and February (31.3°C). When comparing monthly mean temperature for study year (22.4°C) with previous year (22.3°C), it is observed a small difference between them.

2.4. Sampling

In FODM, 16 individuals of *G. opposita* were selected for monthly phenological observations. At FES, 20 individuals were selected for monthly phenological observations. Factors such as the good condition of the individuals in terms of health, pest attacks, crown visibility, as well as easy access to them were considered for selection. It is noteworthy that despite *G. opposita* occurrence in other plots, factors such as individuals' health, pest attack, crown visualization and accessibility were taken into account during plot selection. Diameter at Breast Height (DBH), total height and geographic coordinates composed the data collected from the 36 observed individuals in both areas (FES and FODM).

2.5. Phenological observation and data analysis

Monthly phenological observations [20, 21] were carried out, from January to December 2014 and, when necessary, binoculars were used to assist in the visualization of phenophases. Data on flowering, fruiting, budding and leaf fall were also recorded (Table 1).

Table 1. Description of phenophase progression observed in the field, which was used to calculate the activity index for both study areas in southeast Espírito Santo, Brazil. Source: Modified from Fournier (1974) [22].

Phenophase	Code	Progression
Flowering	1	Floral buds or inflorescences present
	2	Early flowering or fully flowering tree
	3	Flowering finished or finishing
Fruiting	4	New fruits present
	5	Ripe fruit present
Leaf change	6	Release of new leaves (budding)
	7	Old leaves fall

Each individual had its phenophase quantified according to methodology proposed by Fournier (1974) [22], where the occurrence intensity of each phenophase in the population is estimated through a semi-quantitative interval scale of five categories (0 to 4), with 25% intervals between each of them.

The activity index was considered, which indicates the presence or absence of phenophase, regardless of its intensity [23]. This method has a qualitative character and indicates the percentage of individuals within population that are manifesting a certain phenological event, thus determining the population's synchrony. It allows to highlight phenological peaks with great precision and refinement, better representing the species phenological dynamics.

These indices are generally used together to describe the phenology of tree species [24]. Considering vegetative phenology, the species was also classified according to leaf production and its falling behavior (deciduous, semideciduous and evergreen), as proposed by Borchert (1994) [25].

To assess the seasonality of leaf change events in the two study areas, the Rayleigh test was applied, which detects the presence of a preferred direction in circular data. The months of the year were converted into circular angles (0° for January, 30° for February, 60° for March, etc.), and the events were analyzed to determine the existence of a significant concentration throughout the annual cycle.

2.6. Herbarium records

To complement the phenological data of the study, herbarium records from the state of Espírito Santo were consulted, including those from the CAP (Federal University of Espírito Santo), MBML (Mello Leitão Biological Museum), SAMES (Federal University of São Mateus), and VIES (Federal University of Espírito Santo). These records provided information on the fruiting and flowering phases of *G. opposita*. All specimens that mentioned these phenological phases were reviewed, aiming to gather secondary data that could contribute to the construction of a phenological pattern for the species. The collection of these data was essential, as the fruiting and flowering phases were not directly observed during the field period of this study.

3. RESULTS AND DISCUSSION

3.1 Vegetative phenology

During the study period, *G. opposita* did not present great variation of vegetation behavior between studied areas, FODM and FES (Figure 2). In sampling, it was observed that, from the 36 studied individuals, 18 exhibited leaf-sprouting phenophase. The Activity Index of individuals producing new leaves and leaf shoots was seasonal for both phytophysiognomies, with the highest percentage of activity occurring within rainy season months (January, February and March) and with high temperatures (> 25°C).

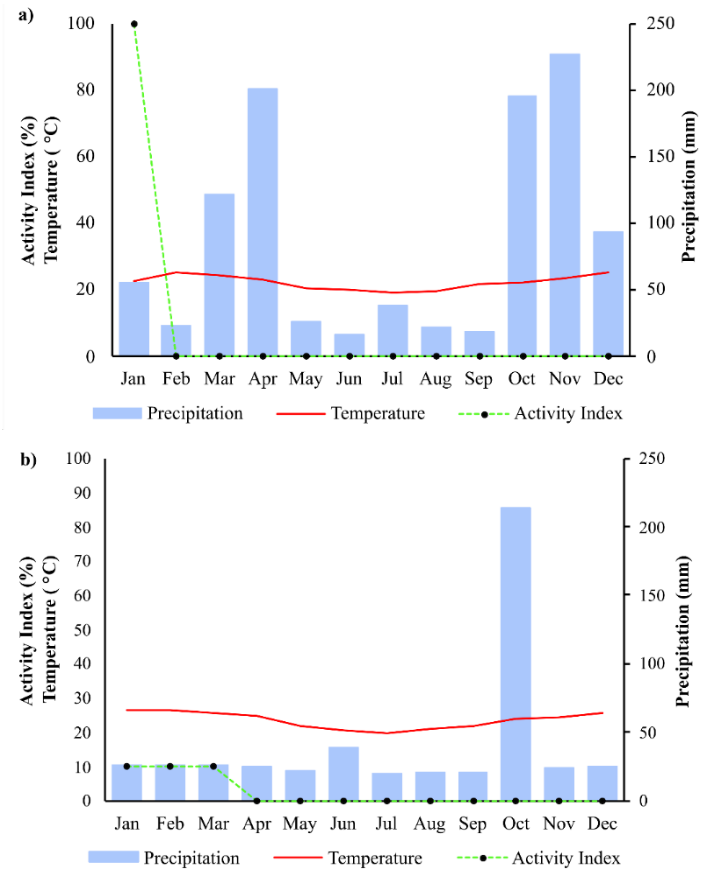

Figure 2: Activity index of leaf budding phenophase, mean temperature and precipitation observed for Montane Dense Ombrophilous Forest (a) and Semideciduous Seasonal Forest (b), in southeast Espírito Santo, Brazil.

By analyzing both populations together, it was possible to observe that leaf budding was more pronounced in January, which corresponds to one of the warmest months within rainy season for both study areas. Several other studies carried out in seasonal forests have also shown that the manifestation of leaf budding occurs at the end of the dry season and the beginning of the wet season [26, 27]. However, more accentuated results for the month of January may be a characteristic of G. opposita's phenology, as they were observed in both locations. In woody species like G. opposita, the emergence of new leaves during the dry season can be associated with the presence of deep root systems, which allow the plant to access water from lower soil layers, alleviating water stress [28]. G. opposita is considered a nurse plant and, as well as forming a symbiosis with ectomycorrhizal fungi, it benefits from this association by improving nutrient absorption and accessing water from the deeper layers of the soil, which helps to alleviate water stress in the plant [29].

Qin et al. (2023) [30] stated that the seasonality of environmental variables, such as, temperature and precipitation, alter nutritional and water characteristics of the soil, influencing the plant physiology and, consequently, being able to determine phenological patterns of forest species. Therefore, it is prudent to admit that the combination of precipitation and high temperatures favored leaf sprouting (Figure 2).

Nunes et al. (2006) [31], when studying Guazuma ulmifolia phenology in a Seasonal Deciduous Forest, north of Minas Gerais State, observed that there was no significant correlation of leaf budding with precipitation or temperature during analyzed period, which may relate to the low degree of synchrony found for the species. Marques and Oliveira (2004) [32], when carrying out a phenology study in two Restinga Forests, located in southern Brazil, observed that G. opposita manifested leaf budding phenophase in the period of higher rainfall intensity and temperature. Another study conducted in ferruginous rocky fields yielded similar results, showing that leaf budding of G. opposita was more pronounced in December, a month characterized by higher precipitation [28]. These findings corroborate the present study results concerning the species' phenological behavior during rainy and hot season.

By observing the Activity Index of each G. opposita population, it was possible to verify a high degree of synchrony in the Dense Ombrophilous Forest, where all individuals manifested the leaf budding phenophase at same time. In contrast, the population within Semideciduous Seasonal Forest was characterized as asynchronous because only 10% of its individuals manifested leaf budding phenophase. When comparing both populations, it was possible to verify a different behavior between them. In FODM, budding phenophase manifested itself only in January and with greater intensity, while in FES, budding occurred during the months of January, February and March (Figure 3).

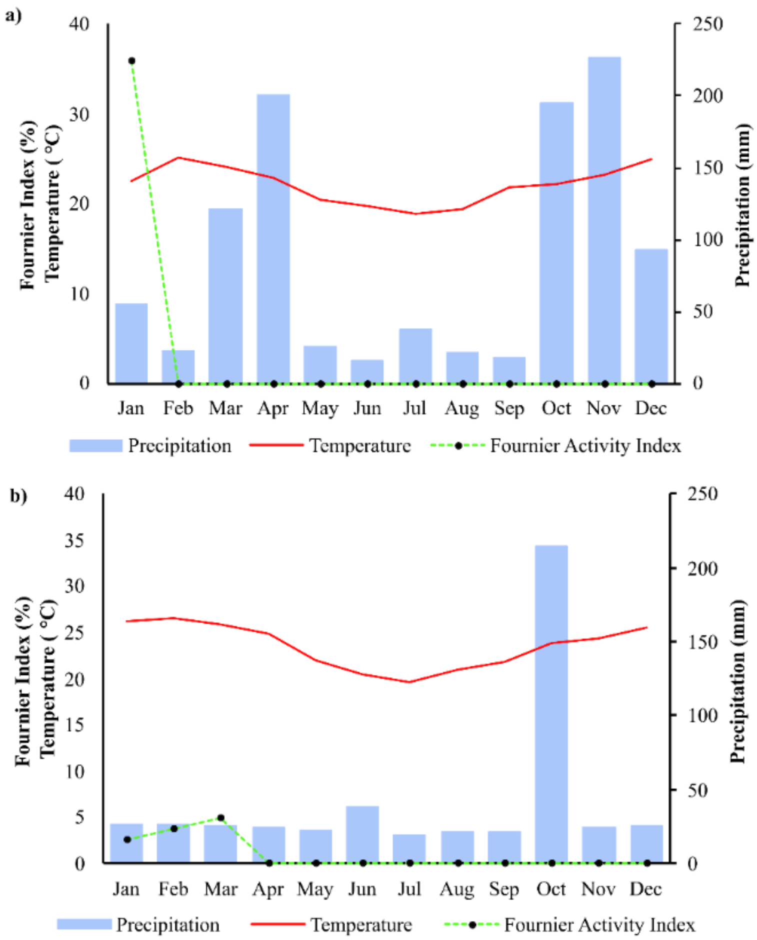

Figure 3: Fournier Intensity Percentage for leaf budding phenophase, mean temperature and precipitation observed for Montane Dense Ombrophilous Forest (a) and Seasonal Semideciduous Forest (b), in southeast Espírito Santo, Brazil.

Fournier Intensity Percentage for leaf sprouting in FODM was regular (35.9%) compared to the low value (2.5 to 5.0%) verified for FES population, showing that its intensity for the first, where budding occurred in just one month, was higher than the second, where budding took place along three months.

Leaf falling was not observed in FODM population during sampling period. In FES, it was very discrete, with only 5% of sampled individuals manifesting it, along with a low Fournier Intensity Percentage (Figure 4). According to Nunes et al. (2006) [31] species inserted in places that concentrate rainfall in a certain period of the year (marked seasonality), manifest the leaf fall phenophase during dry and cold seasons. In the Quadrilátero Ferrífero, similar results were observed, with *G. opposita* showing greater leaf loss during the dry season, and in this study area, the seasons are well-defined [28].

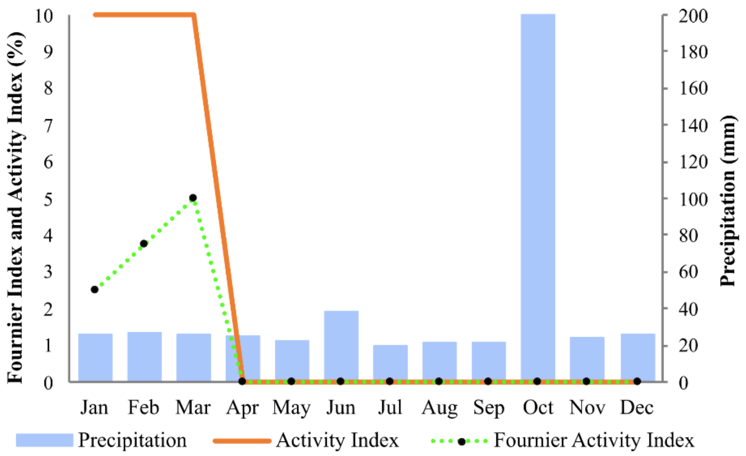

Figure 4: Fournier Intensity Percentage and Activity Index for leaf fall concerning the Guapira opposita population within Semideciduous Seasonal Forest. Precipitation is also shown, in southeast Espírito Santo, Brazil.

In this study, leaf fall phenophase of *G. opposita* was recorded in April, which coincides with the rainy season as well as the beginning of cold season for the study year. Bencke and Morellato (2002) [24] observed the same pattern for *Marlierea obscura*, when losing few leaves throughout year, but with greater abscission intensity during super-humid season months.

As leaf fall was not very expressive in the studied populations, it is not possible to infer about the main factor acting over this phenophase. However, several studies concerning community and population phenology show that leaf fall generally has a significant negative correlation with precipitation, that is, the greater the precipitation, the less expressive will be the phenophase [32, 33].

It was observed that studied populations of *G. opposita* showed a synchronous behavior for leaf fall phenophase [28]. Sampled individuals in both phytophysiognomies (FES and FODM) presented mature leaves during practically the entire studied year, and, therefore, the species of this study can be classified as deciduous. The same classification was considered by other authors [28, 32, 34]. However, the same species has been previously considered deciduous in another phenology study [28].

At Serra do Valentim, the Rayleigh test resulted in a test statistic of 1 and a p-value of 0, indicating extremely strong seasonality, with leaf change events concentrated in a specific period of the year. At Cafundó, the test statistic was 0.8609, with a p-value of 0.002, also suggesting significant seasonality, although with a slightly less intense concentration.

These results show that both areas exhibit well-defined seasonal patterns for leaf change, but with different intensities. Serra do Valentim demonstrates a greater concentration of events in January, while at Cafundó, the events are seasonal but slightly more distributed over the early months of the year (January, February, March, and April). These differences may be attributed to variations in environmental conditions between the studied areas.

3.2. Reproductive phenology

During sampling period of *G. opposita*, the reproductive phenophases (flowering and fruiting) were not observed. Some factors that may have interfered with the non-occurrence of reproductive phenophases were the timing between data collections (performed monthly) and the sampling period (one year). Phenological studies carried out with forest species use periods of observation for different phenophases, which can be weekly, fortnightly and, in the great majority, monthly [35, 36].

Studies that perform monthly collections usually extend the collection time to more than two years, which was not possible in this study. However, Talora and Morellato (2000) [37], when studying 46 tree species, including *G. opposita*, in a lowland forest in the state of São Paulo, performed monthly phenological observations during one sampling year (July 1993 to June 1994), which was sufficient to determine the phenological characteristics of the species in that environment. According to Diez et al. (2012) [38], climatic seasonality may be the most important factor in determining phenological patterns of plant species. Forests with marked seasonality, dry season commonly determines the species' phenology, limiting plant growth and reproduction during this period [39, 40].

According to IPCC (2023) [41], all phenological events are intrinsically linked and related with climatic factors, as well as with the acclimatization of each individual to the place where it is inserted. Marques and Oliveira (2004) [32], when studying canopy and understory species phenology of two "Restinga" forests (flooded forest and non-flooded forest) in Ilha do Mel (Paranaguá municipality – PR State, southern Brazil), showed that *G. opposita* flowered (November to February) and fructified (January and February) during rainy season in non-floodable forest. Morellato et al. (2000) [42], when studying the phenology of trees and shrubs in a seasonal semideciduous forest, southeastern Brazil, also sampled some individuals of *G. opposita*, which manifested flowering in the transitional and rainy period, whereas fruiting only in rainy period.

Although in these studies flowering and fruiting phenophases did not occur exactly in the same months, they all correspond to periods with high precipitation indices within own regions, thus maintaining a reproductive pattern for the species. Thus, it can be inferred that the reproduction of *G. opposita* is closely linked with the rainy period of the region where it is inserted. Given the above, it can be accepted that low rainfall was one of the limiting factors for the non-occurrence of reproductive phenophases (flowering and fruiting) in the studied areas, since its average reduction during sampling year was 40% of the total rainfall observed in the previous year.

According to Morellato et al. (2000) [42], and Marques and Oliveira (2004) [32], *G. opposita* is classified with annual flowering pattern, which was not confirmed by the sampling of this work. Yet, it is noteworthy that the non-confirmation may have been due to climatic variables, the characteristics of individuals and the studied regions.

3.3. Herbarium records

The results obtained from the herbarium records in the state of Espírito Santo showed a seasonal pattern (Figure 5). The number of flowering individuals peaks in February, October, and November, suggesting that these months are favorable for reproductive development. Flowering is more prominent than fruiting, with the highest number of individuals occurring in February. The months between April and July show low phenological activity, with few individuals flowering or fruiting, and no flowering recorded between August and September. In a study conducted in a Cerrado area in the state of São Paulo, *G. opposita* showed flowering between October and November and fruiting throughout the year, with a peak in December [43].

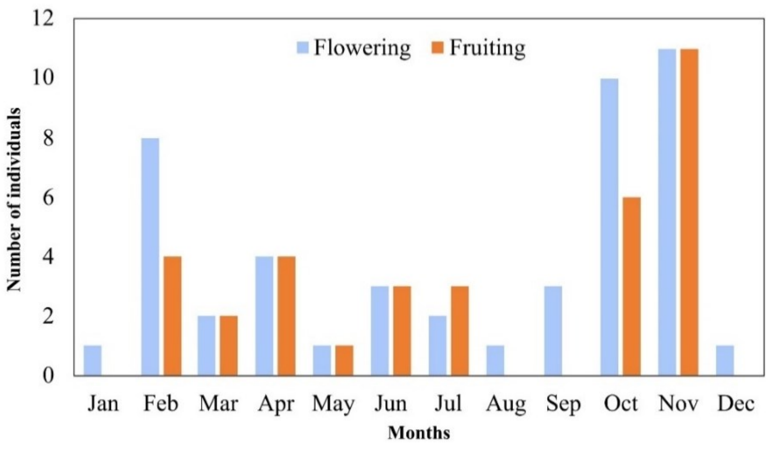

Figure 5: Flowering and fruiting of herbarium records of the state of Espírito Santo, Brazil.

Fruiting, though less intense, peaks in February and November, partially coinciding with the flowering peaks. This coincidence suggests that environmental conditions during these months may be conducive to both flowering and fruiting for some species. However, fruiting is more sparsely distributed throughout the year, with smaller occurrences in other months, such as March, April, June, July, October, and December.

Studies indicated that fruiting peaks generally coincided with the rainy season, which provided the necessary moisture for fruit development. In the case of *G. opposita*, fruiting peaks in February and November aligned with the flowering peaks, suggesting favorable environmental conditions. However, fruiting was less intense and more sporadic throughout the year, with small occurrences in March, April, June, July, October, and December [44]. The fruiting has also been recorded in other fragment of Atlantic Forest during the periods of July to December and April to May, in addition to the month of February [45].

These phenological patterns indicate a strong seasonal influence on flowering and fruiting events, likely related to climatic factors such as temperature and precipitation. The predominance of flowering over fruiting throughout the year may reflect different reproductive strategies of the species studied, in response to seasonal environmental variations.

4. CONCLUSIONS

Guapira opposita's vegetation behavior in the Dense Ombrophilous Forest and in the Seasonal Semideciduous Forest had little variation, occurring only during rainy and hot seasons (January, February and March).

Leaf sprouting occurred in months with the highest temperatures in both study areas. In Montana Dense Ombrophilous Forest, the budding activity was synchronous, encompassing all individuals, while in Semideciduous Seasonal Forest it was asynchronous.

Leaf fall was observed only in the Seasonal Semideciduous Forest during the rainy season and at the beginning of the cold season, while no leaf fall was recorded in the Dense Ombrophilous Forest.

The vegetative phenological behavior of *G. opposita* was similar to patterns found in other studies with longer sampling periods. In both study areas, the individuals of the species maintained mature leaves practically throughout the year, classifying *G. opposita* as deciduous.

The non-occurrence of reproductive phenophases may be closely related with the water deficit that occurred in the sampling year. This reinforces the significant influence of climatic factors, such as precipitation, on the reproductive phenology of the species. While the hypothesis proposed that different climatic conditions between the two remnants would result in distinct phenological patterns, this was only partially confirmed.

Although leaf sprouting was more intense and synchronized in the Dense Ombrophilous Forest compared to the Seasonal Semideciduous Forest, both areas exhibited similar vegetative patterns, with minimal reproductive activity due to the overall water deficit during the study year. Therefore, while climatic conditions influenced the intensity and synchronization of vegetative phenophases, the absence of reproductive phases suggests that factors beyond altitude and humidity, such as annual precipitation levels, are fundamental in the phenology of *G. opposita*.

5. ACKNOWLEDGEMENTS

We thank the Coordination of Higher Education Personnel Improvement (CAPES) for granting the Master's scholarship to the first author of this work, which was of paramount importance to carry out the same.

We thank the Federal University of Espírito Santo and the Postgraduate Program in Forest Sciences – PPGCF for the opportunity to do science and contribute to research in the area of ecology in our country.

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