Original Paper Phenological patterns of *Oreopanax fulvus* (Araliaceae) in remnants of Araucaria Rainforest in Paraná, Brazil

Marcelo Bosco Pinto^{1,3}, Santiago José Elías Velazco², Felipe Manzoni Barbosa^{1,4}, Kyvia Pontes Teixeira das Chagas^{1,5} & Christopher Thomas Blum^{1,6,7}

Abstract

Oreopanax fulvus is a species endemic to the Brazilian Atlantic Forest, assessed as rare and vulnerable in the states of Paraná and Rio Grande do Sul, respectively. This adverse situation requires conservation actions that must be supported by the understanding of its autecology. We aimed to identify the phenological patterns of *O. fulvus* populations from two Araucaria Rainforest remnants in Paraná. Monthly phenology monitoring of 19 trees in each site was performed over 24 months. We quantified the vegetative (i.e., leaf flushing, mature leaves, and leaf-senescence) and reproductive phenophases (i.e., buds production, blooming, immature, and mature fruits). Circular statistics based on activity index were applied to identify the phenophases seasonality. Spearman correlations were performed among phenophases and meteorological parameters. Leaf flushing and mature leaves were recorded in all months, with a decrease of mature leaves during November and December. Leaf-senescence was seasonal, occurring from May to December. The reproductive phenophases were seasonal: blooming from January to May; and fruiting from March to November, with fruits starting to mature in September. Vegetative and reproductive phenophases were highly influenced by average temperature and photoperiod. Although there were dissimilarities in reproductive phenological patterns, the different populations studied had similar phenological performances.

Key words: circular statistics, phenophase, seasonal performance, threatened species.

Resumo

Oreopanax fulvus é uma espécie endêmica da Mata Atlântica brasileira, avaliada como rara e vulnerável nos estados do Paraná e Rio Grande do Sul, respectivamente. Essa situação adversa requer ações de conservação que devem ser apoiadas pelo entendimento de sua autoecologia. O objetivo foi identificar os padrões fenológicos de populações de *O. fulvus* de dois remanescentes de Floresta com Araucária no Paraná. O monitoramento mensal da fenologia de 19 árvores em cada local foi realizado durante 24 meses. Quantificamos as fenofases vegetativas (brotos, folhas maduras e senescência foliar) e reprodutivas (produção de botões, floração, frutos imaturos e maduros). Estatísticas circulares baseadas no índice de atividade foram aplicadas para identificar a sazonalidade das fenofases. Correlações de Spearman foram realizadas entre fenofases e parâmetros meteorológicos. Brotos e folhas maduras foram registrados em todos os meses, com diminuição das folhas maduras nos meses de novembro e dezembro. A senescência foliar foi sazonal, ocorrendo de maio a dezembro. As fenofases reprodutivas foram sazonais: floração de janeiro a maio; e frutificação de março a novembro, com frutos começando a amadurecer em setembro. As fenofases vegetativas e reprodutivas foram altamente influenciadas pela temperatura média e pelo fotoperíodo. Embora tenha havido diferenças nos

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¹ Universidade Federal do Paraná, Prog. Pós-graduação em Engenharia Florestal, Av. Pref. Lothário Meissner 632, Jardim Botânico, 80210-170, Curitiba, PR, Brasil.

² Universidad Nacional de Misiones-CONICET, Inst. Biología Subtropical, N3370BFA, Puerto Iguazú, Misiones, Argentina. ORCID: https://orcid.org/0000-0002-7527-0967>.

³ORCID: https://orcid.org/0000-0003-0833-9546. ⁵ORCID: https://orcid.org/0000-0003-1361-3204>.

⁶ ORCID: <https://orcid.org/0000-0002-1898-3311>

⁷Corresponding author: ctblum.ufpr@gmail.com

padrões fenológicos reprodutivos, as diferentes populações estudadas apresentaram desempenhos fenológicos semelhantes.

Palavras-chave: estatística circular, fenofase, desempenho sazonal, espécie ameaçada.

Introduction

The Araucaria Rainforest is typical of the highlands of Southern Brazil, with an estimated area of 25,379,300 ha (Ribeiro *et al.* 2009). However, intensive logging activities and agriculture have drastically affected this ecosystem, resulting in a landscape reduced to small fragments, where only about 3.1% of the remaining forest is protected (Ribeiro *et al.* 2009).

The processes of fragmentation and suppression of the Araucaria Rainforest affect the maintenance of their plant populations and prompt risk of extinction of many plant families (Medeiros et al. 2005). This is the case of Araliaceae, which in Brazil occurs mainly in the Atlantic Forest (Martinelli & Moraes 2013), a high degraded Brazilian biome (Guerra et al., 2020; Rezende et al., 2018)The majority of its species are poorly studied regarding their ecological aspects, like phenology or pollination and seed dispersal; moreover, several of them are threatened (Martinelli & Moraes 2013). Oreopanax fulvus Marchal is a fast-growing species endemic to the Atlantic Forest's understory in Southern and Southeastern Brazil (Fiaschi 2015; Pscheidt et al. 2018; Fockink et al. 2020). This species has been assessed as rare and vulnerable for the states of Paraná and Rio Grande do Sul, respectively (SEMA 1995; SEMA 2014).

Effective conservation actions for threatened populations mainly rely on understanding the species ecology (Peres et al. 2013). In this context, phenology is one of the sciences that contributes to the knowledge of autecology, as it reveals the relationship patterns between vegetative and reproductive cycles with climate variables and natural population changes in ecosystems (Ferrera et al. 2017). Understanding species phenology's patterns in different populations is important for comprehending the regional variability driven by local adaptation, phenotypic plasticity, and how populations are affected by local climate (Park et al. 2019). The scarcity of this information represents a limitation to conservation strategies, such as forest restoration initiatives (Silva et al. 2019).

Due to the high plant diversity in the Brazilian territory (BFG 2018), research studies on the phenology of native species are still scarce (Mendoza *et al.* 2017), even in the Araucaria Rainforest (Souza & Hattemer 2003; Liebsch & Mikich 2009; Danner *et al.* 2010; Milani *et al.* 2013; Hoffmann *et al.* 2015; Milani *et al.* 2015; Andreacci *et al.* 2017). An identical scenario is observed in Araliaceae, with a few examples: phenology of *Schefflera rodriguesiana* Frodin (Camacho & Orozco 1998); evaluation of the effect of fire on the vegetative and reproductive phenophases of *Schefflera vinosa* (Cham & Schltdl.) Frodin & Fiaschi (Lucena *et al.* 2015); and reproductive biology and seed biology of *Panax pseudoginseng* Wall. (Jamir *et al.* 2016).

We analyzed the phenological patterns of two populations of *O. fulvus* from remnants of the Araucaria Rainforest in the State of Paraná, generating information about its autecology that will support species conservation. The present study aims to answer the following questions: (1) are there differences between the phenological patterns of the populations?; (2) which meteorological variables have the greatest influence on the phenophases patterns?

Materials and Methods

Study areas

The study was carried out on two remnants of Araucaria Rainforest, in intermediary to advanced stages of succession, about 130 km apart from each other, located in the State of Paraná (see supplementary material 1: https:// doi.org/10.6084/m9.figshare.12485423.v3>). The soil is mainly characterized as Cambisols (EMBRAPA 2013). Climate is Cfb, according to Köppen's classification, i.e., humid subtropical mesothermal, with occasional frosts in winter and usually lower temperatures from May to August, as well as a slight reduction in precipitation during the same period, but not characterized as a dry season (Alvares et al. 2013). One study area, with 19 ha, is in the municipality of Curitiba (25°26'53"S and 49°14'25"W), at an altitude of 920 m.a.s. The annual accumulated precipitation and average temperature in this area in 2014 and 2015 were 1584.2-1861.0 mm and 18.45-18.48 °C. This fragment is in the middle of an urban landscape matrix, with only three forest patches

nearby. The other study area, with 101 ha, is in the municipality of Fernandes Pinheiro (25°32'49"S and 50°28'37"W) at an altitude of c. 900 m.a.s, within a rural landscape and more continuous forest areas. The annual accumulated precipitation and average temperature in this area in 2014 and 2015 were 1728.9-2249.3 mm and 18.07-18.44 °C.

We assume that the two study sites represent different populations presupposing a reduced gene exchange between the areas because: (i) the study areas are 130 km far from each other; (ii) the pollination vectors of *O. fulvus* are insects, and its dispersion is made by birds (Carvalho 2014); (iii) the forest fragmentation, which characterizes the landscape of our study regions, is a factor that can be a barrier for gene flow (Bacles & Jump 2011).

Species characteristics

The species reaches 6 to 10 m in height, is polygamous-dioecious with inflorescence in capitulum and globular infructescence, in which 10 to 16 indehiscent fruits develop, becoming purple when mature (Fiaschi *et al.* 2007; Pinto *et al.* 2016). The seeds have phanerocotylar-epigeal germination, which occurs only from mature fruits (Pinto *et al.* 2016). The species occurs in the states of Minas Gerais, São Paulo, Rio de Janeiro, and in the South region of Brazil (Fiaschi *et al.* 2007), being represented by 123 records in herbarium collections (SpeciesLink 2020).

Phenological data collection

The monthly evaluations were carried out between January 2014 and December 2015, with 19 individuals observed in each study area. Individuals were selected for monitoring through the trail method (D'eça-Neves & Morellato 2004), considering the following criteria (Morellato 2004), considering the following criteria (Morellato *et al.* 2000): healthy adult individuals; similar in size (diameter to breast height 10.9–29.3 cm, this interval was chosen to select trees that were from the same time range, excluding young and old individuals); located in the intermediate vertical stratum and in a similar topographic situation.

Both vegetative (i.e., leaf flushing, mature leaves, and leaf-senescence) and reproductive (i.e., buds production, blooming, immature, and mature fruits) phenophases were monitored. The intensity of the vegetative phenophases was assessed through Fournier's (1974) semi-quantitative method, with the following interval scale of intensity: 0) absence of phenophase; 1) 1-25%; 2) 26-50%; 3) 51-75%; 4) 76-100%. These values were divided

by the maximum possible value (i.e., the number of individuals multiplied by four) and multiplied by 100. For the reproductive phenophases, the intensity was represented by the ratio between the number of panicles and the number of branches (Mazza *et al.* 2011), considering that, in each branch, only one terminal panicle can be formed.

Meteorological data and photoperiod

Monthly meteorological data were provided by the Instituto Agronômico do Paraná - Estação Fernandes Pinheiro (IAPAR), the Sistema Meteorológico do Paraná - Estação Curitiba (SIMEPAR 2015), and the Instituto Nacional de Meteorologia - Estação Irati e Curitiba (INMET), where the meteorological variables used were: minimum, average, and maximum monthly temperature, and accumulated monthly precipitation (Fig. 1). The photoperiod was calculated by , where: D = photoperiod in hours and tenths of an hour; $\boldsymbol{\varphi}$ = latitude; and $\boldsymbol{\delta}$ = solar declination angle (Soares & Batista 2004). Climatic proximity between the areas was tested through comparisons of the meteorological data of each site based on Spearman Correlations (ρ) in PAST software (Hammer et al. 2001). Such comparisons pointed out similar weather conditions between areas, with significant correlations of maximum temperature ($\rho = 0.77$; p < 0.001), precipitation $(\rho = 0.82; p < 0.001)$, minimum temperature ($\rho =$ 0,89; p < 0,001), average temperature ($\rho = 0.97$; p < 0.001), and photoperiod ($\rho = 1.00$; p < 0.001).

Data analysis

The occurrence of phenophase periods, their concentration, seasonality, and duration, was analyzed with circular statistics in ORIANA 3 (Kovach 2004), using the activity index which evaluates the presence or absence of phenologic events (Bencke & Morellato 2002). We evaluated the number of individuals in each phenophase and calculated the proportion of individuals with a determined phenophase presence. This proportion was used in circular statistics. For this analysis, months were converted to angles of 0-360°. Each month was represented by an interval of 30°, assigning the value of 90° to January (Morellato et al. 2000). Additionally, the average angle, the standard error of the angular mean, and the average vector length (r) were calculated. The significance of the average angle was assessed by Rayleigh's (z) test (Zar 1999). The phenophases with a statistically significant average angle (denoting



Figure 1 – Photoperiod, monthly temperatures, and accumulated precipitation recorded during the phenological monitoring period in Curitiba (a) and Fernandes Pinheiro (b).

seasonality) were converted to average date of occurrence of each phenophase (Morellato *et al.* 2000). The average date provides information on the concentration of the population's phenological activities. This allows a complete description of how individuals are structured and how they respond to ecological factors.

Populations' phenological patterns from both areas were compared by the average angles of each phenophase, using the Watson-Williams (F) test (Zar 1999) and the Wallraff test (Wallraff 1979). If confirmed difference between areas, the analysis was carried out Therefore, each area was analyzed independently. Z and F tests were performed with the *circular* package in R software v.3.0.3 (R Core Team 2014). We used Spearman Correlations (ρ) to test the relationship between meteorological variables and the presence-absence of phenophases.

Results

We found a seasonal pattern in leaf senescence and all reproductive phenophases in both areas, according to the Rayleigh's test (p>0.05; see supplementary material 2: https://doi.org/10.6084/m9.figshare.12485423.v3). For both study sites, the average date of leaf senescence was

significant (Figs 2a,b; S2). It occurred in Curitiba and Fernandes Pinheiro on 27 and 24 September, respectively. A partial seasonality was denoted by medium *r* values, ranging from 0.485 in Fernandes Pinheiro to 0.671 in Curitiba. The average dates of leaf flushing and mature leaves did not exhibit a significant average value for any study place, showing the lowest *r* values, with dates distributed uniformly through the year (Figs 2c-f, S2).

We verified a high seasonality for buds production, denoted by the elevated r values, ranging from 0.826 in Fernandes Pinheiro to 0.812 in Curitiba with average dates occurring on 06 March and 28 February, respectively. Blooming had the same r values pattern, ranging from 0.994 in Fernandes Pinheiro to 0.973 in Curitiba. The average dates for blooming in Fernandes Pinheiro and Curitiba occurred in 14 and 7 April, respectively (Figs 3a-d, S2). The average dates of immature and mature fruits had a significant seasonality in both sites. The highest values of rwere in Fernandes Pinheiro with 0.672 and 0.948 for immature and mature fruits, respectively, on 03 June and 20 October. Curitiba had 0.621 and 0.930 for immature and mature fruits, respectively, on 20 July and 21 October (Figs 3e-h, S2).

We observed differences in the phenological patterns of leaf senescence, buds production, and blooming between the studied areas, according to Watson-Williams and Wallraff's tests (*p*<0.05; Figs. 2; 3; see supplementary material 3: https://doi.org/10.6084/m9.figshare.12485423.v3).

We observed that the cold season with shorter photoperiod increased the leaf-senescence process (Figs. 1; 4a), that started in May in Fernandes Pinheiro and in June in Curitiba (Fig. 3a,b). The populations showed similar peaks in leafsenescence in 2014. In Curitiba, leaf-senescence was observed for five months, July to December, with peaks at the beginning and end of this period. While for Fernandes Pinheiro it was beginning in May, reached its peak in August, and also ended in December. However, in November 2015, it was 25% higher in the Curitiba population (Fig. 4a). In 2014 the population of Curitiba showed the highest values of leaf flushing during January to May, with 100% intensity. However, in 2015 the peak with 100% intensity occurred from February to April. The pattern for Fernandes Pinheiro in 2014 was similar, the peak started in January and



Figure 2 – Vegetative phenological patterns of *Oreopanax fulvus* in the Curitiba (a; c; e) and Fernandes Pinheiro (b; d; f) populations during 24 months of observations. The bars represent the number of individuals. The error bar represents the average angle, average vector length (r), and standard mean error. ^{ns} = not significant, * = significant p < 0.05.



Figure 3 – Reproductive phenological patterns of *Oreopanax fulvus* in the Curitiba (a; c; e; g) and Fernandes Pinheiro (b; d; f; h) populations during 24 months of observations. The bars represent the number of individuals. The error bar represents the average angle, average vector length (r), and standard mean error. ^{ns} = not significant, * = significant p < 0.05.24 month.

lasted until April. The intensity increase of leaf flushing started with the raise of temperature and photoperiod, following the same pattern in both areas in 2014 (Fig. 1); while in 2015 it was 25% higher in Curitiba, from February to April (Fig. 4b). Mature leaves showed intensity above 75%, in the months of the first half of 2014 for both areas, whereas in 2015 this pattern extended higher than 75% until October (Fig. 4c).

We observed that in the Fernandes Pinheiro population, photoperiod (ρ = -0,52; p= 0,01) and average temperature (ρ = -0,50; p= 0,01) showed a negative relationship with Leaf flushing; the same relationship was observed between average temperature and Mature leaves (ρ = -0,41; p= 0,04). The Curitiba population had a negative relationship between photoperiod and Mature leaves (ρ = -0,46; p= 0,02; see supplementary material 4: <https://doi. org/10.6084/m9.figshare.12485423.v3 >).

For both areas, the production of floral buds occurred for five months in 2014 with a peak in March, and four months in 2015 with peak in February. The floral buds showed greater expression of the activity index in the Fernandes Pinheiro population (Figs. 3a,b). Blooming was recorded between March and April with a peak in April, and the fruiting season in Curitiba occurred from April to November while in Fernandes Pinheiro it started in March and ended in August, lasting 8 and 6 months respectively (Figs. 3c-f). The first mature fruits appear in October (Fig. 3gh). The mature fruits period lasted three months and was concentrated in October for the Fernandes Pinheiro population, and November for the Curitiba population (Figs. 3g-h).

The intensity expression of floral buds has been higher in the Curitiba population, the blooming patterns were inverse, being higher in Fernandes Pinheiro population (Fig 5a,b). The buds production occurred in the same month in different years, but less than 10% in both areas. However, immature fruiting intensity was higher in the Curitiba population, particularly in 2015 (Fig. 5 c). Most of the mature fruits were observed during October/November in both areas and showed a small intensity, less than 5% (Fig. 5d).

We found positive correlation (p<0,05) of photoperiod (ρ = 0,48; ρ = 0,45), maximum (ρ = 0,50; ρ = 0,51), and average temperatures (ρ = 0,54; ρ = -0,49), with buds production in Curitiba and Fernandes Pinheiro respectively. This also happens for minimal temperature (ρ = 0,58) in the Curitiba population. The production of immature fruits



Figure 4 – Intensity of vegetative phenological events of *Oreopanax fulvus* in the Curitiba and Fernandes Pinheiro populations. a – Leaf flushing, b – Mature leaves, c – Leaf-senescence.

correlated negatively (p<0,05) with the minimum (ρ =-0,82; ρ =-0,87), average (ρ =-0,84; ρ =-0,66), and maximum temperatures (ρ =-0,76; ρ =-0,45), and with the photoperiod in Curitiba and Fernandes Pinheiro respectively. No correlation between the phenophases and precipitation was observed. Blooming and mature fruits showed no correlation with any of the meteorological variables in both areas (see supplementary material 5: <https://doi.org/10.6084/m9.figshare.12485423.v3>).

Discussion

Even though both areas have similar environmental characteristics, we observed that the species' phenological patterns change in some parameters (e.g., leaf-senescence, buds production and blooming). Nonetheless, leaf flushing and production of mature leaves occurred throughout the observation period in both areas. Circular statistics tests suggested that Leaf senescence and all observed reproductive phenophases are seasonal. Analysis of populations phenology in





Figure 5 – Intensity of the reproductive phenological events of *Oreopanax fulvus* in the Curitiba and Fernandes Pinheiro populations. a – Buds production, b – Blooming, c – Immature fruits, d – Mature fruits.

different locations is important to better understand species' patterns since they can respond to specific environmental conditions (Castro *et al.* 2007). In this context, circular analyzes are useful for comparing populations phenology (Morellato *et al.* 2010).

The climatological similarities observed between the study areas were expected since they are at the same latitude (Soares & Batista 2004) and both belong to Cfb climate classification, which is a humid subtropical climate with temperatures between -3 and 18 °C in the coldest months with a temperate summer (Alvares *et al.* 2013). Nevertheless, the differences in the phenological patterns of some phenophases might result from slight variations related to microenvironmental differences, water availability, and competition between pollinators and dispersers (Bulhão & Figueiredo 2002; Danner *et al.* 2010; Felippi *et al.* 2012). Oreopanax fulvus can be considered an evergreen species due to the constant leaf flushing and production of mature leaves, which, in its turn, implies in a lower photosynthetic capacity and longevous leaves (Wright *et al.* 2006; Kanieski *et al.* 2013; Oliveira *et al.* 2015). The seasonality observed in leaf-senescence might have been influenced by the colder season, concentrated from May to September (Alvares *et al.* 2013; Fig. 1), which is consistent with other species occurring in the Araucaria Rainforest (Marques *et al.* 2004; Milani *et al.* 2013).

The significative influence of the average temperature and photoperiod on the production of new and mature leaves might be associated with the fact that, in regions further from the equator, phenological patterns are more influenced by changes in photoperiod and temperature than by precipitation (Marques *et al.* 2004; Mendoza *et al.* 2017). In addition, it is important to note

that precipitation in the Araucaria Rainforest is distributed throughout the year and vary considerably, therefore is not a good predictor of phenology patterns in this region (Marques *et al.* 2004).

Previous studies suggested that leaf changes are progressively less dependent on rainfall when trees are in microenvironments with higher soil water supply. These microenvironments protect the plant from hydric stress (Borchert 1999), as documented in *O. fulvus* (Higuchi *et al.* 2014). This corroborates the results of previous research studies performed in the same region, with *Guarea macrophylla* Vahl. and *Cedrella fissilis* Vell, where no correlation between phenological variables and precipitation was observed (Andreacci *et al.* 2017; Müller & Schmitt 2018).

In the same way as observed for leaf flushing, the buds production occurred in the period with higher temperatures and photoperiods, which provides favorable conditions for the better performance of these phenophases (Marques *et al.* 2004; Marchioretto *et al.* 2007; Mendoza *et al.* 2017).

We have characterized the production of floral buds and flowers in Oreopanax fulvus as seasonal, during our 2-year observations in both populations. With only one major reproductive cycle per year, it is classified as an annual species (Newstrom 1994). Blooming was recorded as less intense and shorter in time length than the production of floral buds. This difference may be due to a short blooming period, combined with the monthly interval of observations or the abortion/ loss of floral buds (Pereira et al. 2008). Some species may present inter-annual variations in the number of produced flowers, so that this product may contribute or not with resources to the community (Pirani et al. 2009; Franco et al. 2017; Ivanov et al. 2018).

Generally, species in the Araucaria Rainforest concentrate their blooming between September and November (Marques *et al.* 2004; Kanieski *et al.* 2013; Milani *et al.* 2013; Bauer *et al.* 2014; Pires *et al.* 2014). Nonetheless, this was not observed for *O. fulvus*, in which blooming was concentrated in April, a period of reduced rainfall (Fig. 1). This change in the blooming season may be a positive strategy since the non-synchronized blooming production reduces pollinator competition (Oleques *et al.* 2017). Furthermore, the intensity peak of immature fruits in Curitiba population was greater than the intensity peak of blooming. It is probably because of our monthly interval observations, which may have been performed when blooming had already occurred.

Immature fruits were recorded for seven to eight months, overlapping with the appearance of mature fruits in the last three months, thus representing the longer reproductive phenophase. Both phenophases were considered seasonal. Commonly, for several species, the fruiting period lasted from two to four months (Mazza *et al.* 2011; Milani *et al.* 2013; Bauer *et al.* 2014; Pires *et al.* 2014), which differs from *O. fulvus*.

The long fruiting period, although characterized by immature fruits for most of its length, in *O. fulvus*, can be a common feature of tree species of Araliaceae, such as *Schefflera rodriguesiana* in Costa Rica, which present continuous fruiting throughout the year (Camacho & Orozco 1998). For *Dendropanax arboreus* (L.) Decne. & Planch., in a tropical forest of Mexico, the presence of fruit lasted for six months, with intervals (Ochoa-Gaona *et al.* 2008).

Overall, in both areas, few individuals were registered with mature fruits, always with low intensity. The small number of observed mature fruits may be related to their short permanence on the trees, due to the great demand by local fauna for food, as already documented for other Araliaceae species (Purificação *et al.* 2015; Silva 2015; Cruz-Tejada *et al.* 2018). The fruits of *O. fulvus* are an important food source for several frugivorous birds in the montane forests (Parrini *et al.* 2017). Therefore, we recommend an increase in frequency of visits to study areas during the fruiting period to expand seed collection.

The season of mature fruits in O. fulvus coincides with other zoochoric species found in the Araucaria Rainforest, such as Campomanesia xanthocarpa (Mart.) O.Berg, Eugenia involucrata DC., E. pyriformis Cambess., E. uniflora L. (Myrtaceae, Danner et al. 2010) and Drimys brasiliensis Miers (Winteraceae, Mariot et al. 2014). Alternatively, only 10% of the species in the Araucaria Rainforest produce mature fruits from June to November, which is caused by the seasonality of the reproductive phenophases (Liebsch & Mikich 2009; Mazza et al. 2011; Felippi et al. 2012; Kanieski et al. 2013; Bauer et al. 2014). Thus, O. fulvus has a critical ecological role in this forest formation since it supplies the fauna, mainly birds (Parrini et al. 2017), with food resources in lean periods.

Oreopanax fulvus has shown similar phenological patterns across the studied populations. The average temperature and the photoperiod influenced the vegetative phenophases. Conversely, the minimum, average and maximum temperatures, and the photoperiod affected the reproductive phenophases. The synchrony observed in the reproductive phenophases and their periods of occurrence can be used to predict the best moment for seed collection. The increased access to viable seeds would greatly facilitate seedlings production, aiming at conservation strategies for *O. fulvus*.

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12 de 12

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