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Phenology of *Aloysia hatschbachii* cultivated in a subtropical region

Fenologia de Aloysia hatschbachii sob cultivo em região subtropical

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ABSTRACT

Aloysia hatschbachii is an endangered species that occurs endemically in Paraná and has potential for essential oil production. Expanding research on threatened species could serve as a tool to ensure their conservation, management and reproduction. In this study, we sought to evaluate phenological phases (phenophases) for species Aloysia hatschbachii in the years 2018 and 2019 by conducting monthly checks of a plant population consisting of 5 individuals, identifying the phenological events of budding, mature leaves, flowering, fruiting, leaf senescence and leaf fall, using the percent index of intensity as well as the index of activity in the sampled individuals regarding the occurrence of such phenomena, and then correlating phenological data with meteorological variables. The phenological phases showed synchrony in activity throughout most of the assessed period, but were not always synchronous in intensity. Budding occurred between July and April, and mature leaves were present all year round. The patterns of leaf senescence and leaf fall revealed that the species has nondeciduous characteristics. These phenophases were influenced by relative air humidity. Flowering occurred between November and April, while fruiting lasted from December to May, with the reproductive stage being influenced by air temperature and by incident solar radiation.
Keywords: Aromatic plant; Budding; Phenopases

RESUMO

A espécie Aloysia hatschbachii, com potencial de produção de óleo essencial, ocorre de forma endêmica no estado do Paraná. Pesquisas envolvendo espécies que se encontram em risco de extinção, servem de ferramenta para sua conservação, manejo e reprodução. Desta forma, buscou-se avaliar as fases fenológicas da espécie Aloysia hatschbachii, nos anos de 2018 e 2019, através da avaliação mensal da população de plantas (5 indivíduos), identificando as fases fenológicas (fenofases) de brotamento, folhas maduras, floração, frutificação, folhas senescentes e queda foliar, através do percentual de intensidade e índice de atividade fenológica dos indivíduos e correlacionando a fenologia com as variáveis meteorológicas. As fases fenológicas apresentaram sincronismo de atividade na maior parte do período avaliado, porém a intensidade em muitos casos foi baixa. O brotamento ocorre entre

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os meses de julho a abril, além disso as folhas maduras estão presentes durante todo o ano. Pela senescência e a queda foliar se constatou que a espécie não apresenta característica decídua. Estas fenofases são influenciadas pela umidade relativa do ar. A floração ocorre entre os meses de novembro a abril e a frutificação de dezembro a maio, sendo o período reprodutivo influenciado pela temperatura do ar e pela radiação solar incidente.
Keywords: Planta aromática; Fenofases; Brotamento

1 INTRODUCTION

Increasing consumption of medicines made from medicinal plants and aromatic herbs is clearly evident, and in some countries the volume of sales has been boosted by about 500% in only 10 years (GODOY *et al*., 2013). As a result, manufacturers in this sector have evolved into a highly sophisticated and competitive industry while trying to follow the market trends. So, today, there is a need to prioritize research into the use and effects of such plants to ensure their correct use, by encouraging ethnobotanical and ethnopharmacological studies and by compiling information on the biodiversity of the existing local species (FIRMO *et al*., 2011).

Family Verbenaceae includes a large variety of aromatic plants and is considered cosmopolitan, spreading mainly across tropical and subtropical regions and comprising around 100 genera and 2,000 species of trees, shrubs and herbs (LIRA, 2016). Belonging in that family, genus Aloysia is distributed across the Americas, from United States to Patagonia, and includes around 30 species (BOTTA, 1979; RICCIARD *et al*., 2000; MÚLGURA *et al*., 2003; SIEDO, 2006). This genus includes mainly aromatic shrubs considered of great importance and used widely in both traditional and folk medicines (RICCO *et al*., 2010). The phytochemistry of this genus has detected presence of many monoterpenes and sesquiterpenes in its most commonly studied species (VANDRESEN *et al*., 2010; MORAIS *et al*., 2012; PROCHNOW *et al*., 2017; SGARBOSSA *et al*., 2019), which has been attracting growing interest for its potential for essential oil production.

Species Aloysia hatschbachii Moldenke, originally from Brazil (SIEDO, 2006; LU-IRVING, 2014), is known to be present in Paraná state, more precisely in the towns of Piên and Rio Negro, and is considered a spontaneous, endemic species of that region

(SEGECIN, 1995; SIEDO, 2006; MÚLGURA, 2007). A new occurrence of the species has been cited in Rio Grande do Sul state, more precisely in Caracol Park, which is in the town of Canela (CRESPAM, 2010), and a possible occurrence has also been cited in northern Pará state (MORONI and O'LEARY, 20..). According to Brazil's National Flora Conservation Center - CNCFlora, Aloysia hatschbachii is categorized as an endangered species (EN), since the area of its natural occurrence is less than 5,000 km^2 and subjected to intense forest and agricultural activity (CNCFlora, 2012).

Native species from regions with seasonal climate usually show variations in their leaf, flower and fruit production, which determines their adaptations to biotic and abiotic factors (VAN SCHAIK *et al.*, 1993). These adaptations relate to structural and functional characteristics, which in turn are analyzed by phenology. Keeping records of phenological data is critical because it serves as an indicator of plant responses to climate conditions while contributing information on the annual cycle of species, and these annual cycles are directly related to climate conditions and to the adaptive characteristics of each species (ANDREIS *et al.*, 2005).

Phenological studies are crucially important in that they help understand the reproduction process (FISCH *et al.*, 2000) and domestication, besides helping define cultivation techniques (PEREIRA *et al.*, 2008). Also, they have proved useful for taxonomists and for departments engaged in reforestation projects (KUARAKSA *et al.*, 2012). And, more recently, research on phenological events has drawn the attention of scientists following confirmation of the existing connections of phenology with climate change, serving as an indicator of climate change due to the alterations it causes to the life cycle of plants (CHAMBERS *et al.*, 2013; RICHARDSON *et al.*, 2013; ANWAR *et al.*, 2015).

Elements of climate that affect phenophases or at least have some type of relationship with phenological manifestation, include precipitation, temperature, relative humidity and photoperiod (ATHAYDE *et al.*, 2009; PIRANI *et al.*, 2009; AZEVEDO *et al.*, 2014). In semi-arid regions, fruit development usually is closely related to rainfall (LIMA and RODAL, 2010), while in subtropical regions such as in southern Brazil, the reproductive period of plants is affected by temperature and by photoperiod (ATHAYDE *et al*., 2009). Some researchers, however, argue that phenological events are genetically determined, which means they are induced by life forms and are thus independent of abiotic factors (WRIGHT and CALDERON 1995).

Since data on phenological events are nonexistent for Aloysia hatschbachii and since researching them is of critical importance to understanding how the species functions and to verifying the occurrence of patterns in its annual cycle, the objective of this study is to evaluate the phenological events of aromatic species Aloysia hatschbachii, correlating them with meteorological variables, in an attempt to contribute toward the conservation and management of this endangered species.

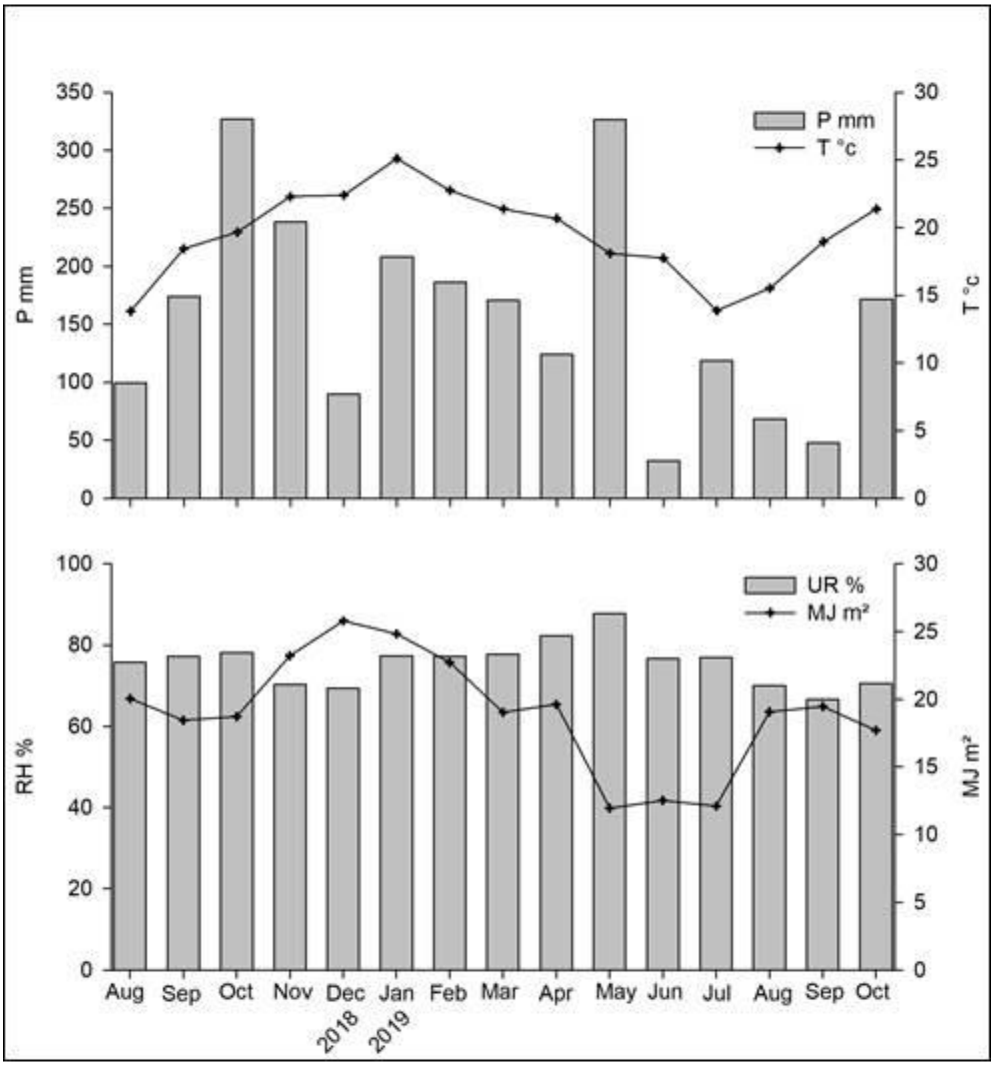
2. MATERIALS AND METHODS

2.1 Characteristics of the study site

The study was conducted in the experimental site of the Federal University of Santa Maria, Frederico Westphalen Campus, located at latitude 27°23’26”S and longitude 53°25’43”N, at an altitude of 461m, in the municipality of Frederico Westphalen, Rio Grande do Sul state.

According to the Köppen climate classification, the local climate is described as Cfa, which means well-defined seasons throughout the year and well-distributed rainfall, around 100 to 170 mm each month (ALVARES *et al*., 2013). Meteorological data for the assessed period are depicted in Figure 1, with variations found throughout the experiment as follows: the lowest monthly average air temperature was 13.8°C in August 2018, against 25.1°C in January 2019 as the highest; the lowest monthly average relative air humidity was 66% in September 2019, against 87% in May 2019 as the highest; the lowest cumulative monthly precipitation was 32.40 mm in June 2019, against 327 mm in October 2018 as the highest; and the lowest monthly average global incident solar radiation was 11.95 MJ m^2 in May 2019, against 25.79 MJ m^2 in December 2018 as the highest. O presente estudo foi desenvolvido na área experimental da Universidade Federal de Santa Maria, Campus Frederico Westphalen, localizado na latitude 27°23'26"S; longitude 53°25'43"N e altitude de 461m, no munícipio de Frederico Westphalen – RS.

Figure 1 - Meteorological variables for the period of phenological assessments (August 2018 to October 2019) of species Aloysia hatschbachii (P mm = cumulative monthly precipitation) T °C = average monthly air temperature; RH % = average monthly relative humidity; MJ m² = average incident monthly global solar radiation).



2.2 Phenological observations

Observations were recorded of field-grown adult plants subjected to suitable phytosanitary conditions and manually irrigated using a watering can. The sample specimens were obtained from a parent plant which had been donated by the Botanical Garden of Curitiba, then propagated vegetatively using cuttings.

Tests were performed every fifteen days, on five plant specimens, from August 2018 to October 2019, to a total of 28 observations. According to Fournier & Charpantier (1975), that count of individuals and observations is considered sufficient for carrying out a study on plant phenology.

The characterization of the phenophases of Aloysia hatschbachii comprised assessments from the vegetative growth period through to the reproductive period, and include: (1) Budding: emergence of leaf buds through to new fully expanded leaves; (2) Mature leaves: dark-green fully expanded leaves; (3) Leaf senescence: progressively yellowed leaves; (4) Flowering: emergence of flower blossoms through to the last fully open flowers (anthesis); (5) Fruiting: end of anthesis and subsequent confirmation of seed formation/presence; (6) Leaf fall: confirmation of fallen leaves, missing leaves on the branches, and leaves on the ground below the plant specimen. At this point, observations are recorded as to presence or absence of each phenophase (GOMES, 2017).

To quantify the intensity of each phenophase, we used the methodology proposed by Fournier (1974), known as Fournier's percent index of intensity. It quantitatively estimates stages such as budding, flowering, fruiting, leaf formation and leaf fall, using a simple method to assess distinct specimen characteristics at different dates, and accurately defining performance patterns to then produce phenological representations. This method is commonly used to provide phenological description of trees, understory shrubs and aromatic plant species (MORELLATO *et al*., 2000; BENKE e MORELLATO, 2002; MARTIN-GAJARDO e MORELLATO, 2003; OLIVEIRA JÚNIOR *et al*., 2007; LOPES e JARDIM, 2008; CARVALHO JÚNIOR *et al*., 2011; GOULART *et al*., 2013; MACHADO, 2013; GOMES, 2017).

The methodology proposed by Fournier (1974) consists of individual assessments using a scale of five categories with scores from 0 to 4, with a 25% interval between categories, where: 0 = absence of a phenological event; 1 = presence of a phenological event within an interval from 1 to 25%; 2 = presence of a phenological event within an interval from 26 to 50%; 3 = presence of a phenological event within an interval from 51 to 75%; 4 = presence of a phenological event within an interval from 76 to 100%. Each month, intensity scores for all individuals are brought together, divided by the possible maximum (number of individuals multiplied by four), then multiplying the result by 100 to turn it into a percentage, as provided by the formula below (BENKE e MORELLATO, 2002; MARTIN-GAJARDO e MORELLATO, 2003).

$$\% \text{ de Fournier} = \frac{\sum \text{Fournier}}{4.\text{N}}.100 \quad (1)$$

To verify synchrony between individuals in each phenophase, we used the index of activity (percent of individuals), which simply consists of keeping records as to presence or absence of a phenological event in each individual and indicating the percent of individuals manifesting that event, by using the method described by Benke & Morellato (2002), assigning the following magnitudes to manifestation of a given phenological event: 20% or less = asynchrony; 20 to 60% = weak synchrony; 60% or more = synchrony.

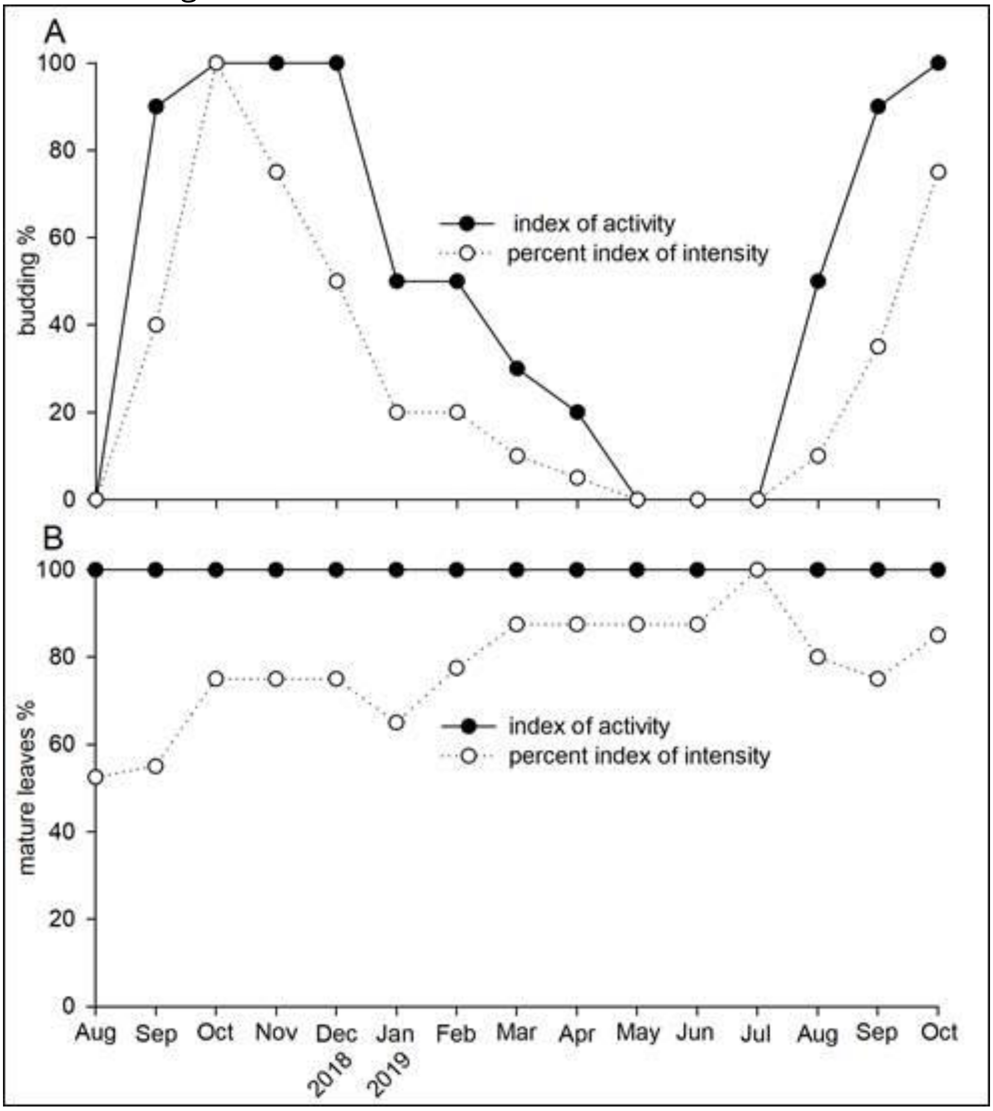
The phenological data was tabulated and then merged with the meteorological data that had been collected by the institution's Agrometeorology Laboratory from an automatic weather station located in the Frederico Westphalen Campus. The phenological and meteorological data were then subjected to Pearson correlation analysis using statistical software Genes (CRUZ, 2013).

3. RESULTS

Results in Figure 2-A show that, in 2018, species Aloysia hatschbachii started the differentiation process of vegetative buds in August, a time when mild temperatures still predominate. Buds started to emerge in September, though with low intensity still. The peak intensity of this phenological event occurred in October (100%) and from then on there was a decline, though intensity remained above 20% until January. Through May, June and July the event was not observed. In the following year (2019), buds started to emerge a month earlier, in August, compared to 2018. Despite early, the budding event shows weak synchrony, as it was not manifested by all individuals.

The index of activity (solid line) illustrates phenophase synchrony, that is, the count of individuals in the plant population manifesting a physiological event. The budding phase of A. hatschbachii showed synchrony (>60%) among individuals from September to December in 2018 and from September to October in 2019, but for the remainder of the assessed period it showed weak synchrony.

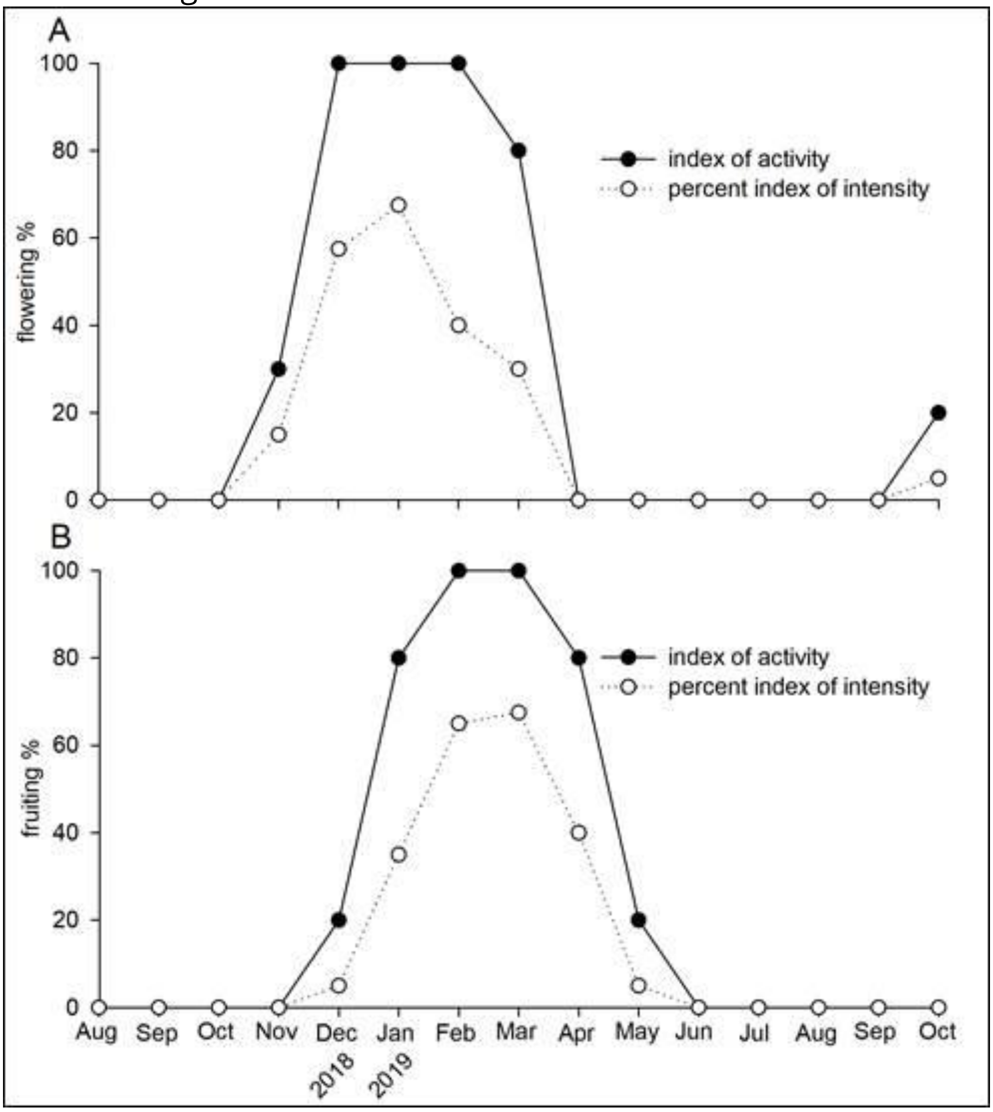
Figura 2 - Index of activity (solid line) and percent index of intensity (dotted line) for budding (a) and mature leaves (b) in a population of Aloysia hatschbachii evaluated between August 2018 and October 2019.



Mature leaves (Figure 2-B) was the most noticeably present phase over the assessment period, showing a peak maximum index of activity that reveals synchrony, with presence of mature leaves for a large part of the year. The percent index of intensity for this phase was invariably above 50%, reaching a peak maximum in July (100%).

The flowering phase (anthesis), depicted in Figure 3-A, started in November and had its last occurrence in April. The index of activity reveals weak synchrony in this phase (between 20 and 60%), although there was synchrony from December to March. The peak percent index of intensity was just above 60%, in January, that is, despite the flowering phase being present in all individuals, the intensity of the event was not high. In 2019, the flowering phase was observed a month earlier, in October, and revealed asynchrony (20% or <).

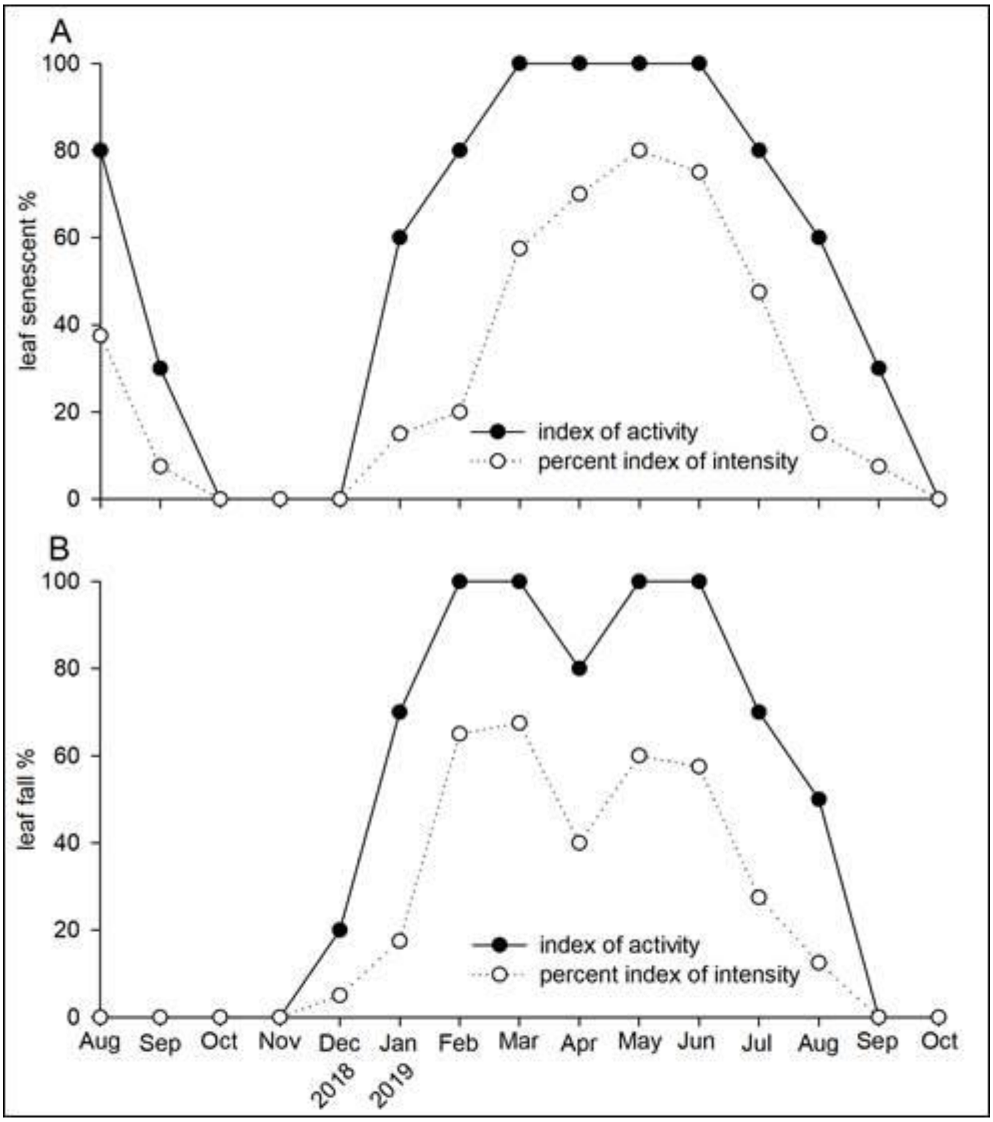
Figura 3 - Index of activity (solid line) and percent index of intensity (dotted line) for flowering (a) and fruiting (b) in a population of Aloysia hatschbachii evaluated between August 2018 and October 2019.



The fruiting phase (Figure 3-B), regarded as the period when fruits ripen, started in December and lasted until May. During this phase, the index of activity reveals synchrony from January to April, and asynchrony in the remainder of the period. The percent index of intensity, similarly to the flowering phase, was not high, with a peak maximum of just above 60% in February and March, but dropping to below 40% in the remaining period, thus representing uneven ripening of fruits.

The senescence phase (Figure 4-A), similarly to leaf fall, is represented by yellowed leaves in the process of about to fall off. This phenophase was observed from January to September and proved the most synchronous phase, since throughout virtually the entire assessed period, the index of activity was 60% or over, except in September of 2018 and 2019, when it reveals weak synchrony. The percent index of intensity reached a peak maximum between April, May and June (70 to 80%). After June, the intensity of this phenomenon declined due to increasing emergence of young leaves (budding). From observations, it can be said that the decline in intensity was only a prolonged senescence of older leaves, usually found at the base portion of the plant.

Figura 4 - Index of activity (solid line) and percent index of intensity (dotted line) for leaf fall (a) and leaf senescence (b) in a population of Aloysia hatschbachii evaluated between August 2018 and October 2019.



The representation of leaf fall (Figure 4-B), defined as occasional dropping of some leaves or leaves missing in-between nodes, in other words, occasional fall of some leaves (but not all), reveals that this phenophase occurred between December and September. The index of activity shows synchrony between January and August, asynchrony in December 2018 and weak synchrony in September 2019. The percent index of intensity reached a peak in February and March, just above 60%, and another again in May and June, near 60%. Leaf fall was not observed in September, October or November, which coincides with the time of intensive budding, hence no leaf fall.

Tables 1 and 2 illustrate Pearson correlation to explain the effect of meteorological variables on the phenological phases of species Aloysia hatschbachii. A significant correlation confirms there to be a relationship between the potential effect of environmental conditions on the phenological events expressed by the population.

Table 1: Pearson correlation between meteorological variables and phenological phases, for index of activity. (T = average monthly air temperature; RH = average monthly relative humidity; SR = average incident monthly solar radiation; bu = budding; fl = flowering; fr = fruiting; lf = leaf fall; ls = leaf senescence)

Weather variables	**Phenological phases**				
	bu	fl	fr	lf	ls
T (°C)	0.339^{ns}	0.740^{**}	0.584^{*}	0.082^{ns}	-0.309^{ns}
m T (°C)	0.247^{ns}	0.696^{**}	0.622^{*}	0.181^{ns}	-0.215^{ns}
M T (°C)	0.414^{ns}	0.787^{**}	0.540^{*}	-0.013^{ns}	-0.389^{ns}
RH (%)	-0.632^{*}	-0.111^{ns}	0.379^{ns}	0.607*	0.641^{**}
SR (MJ m²)	0.438^{ns}	0.681^{**}	0.365^{ns}	-0.331^{ns}	-0.458^{ns}

*Significant (0.01 %) by the t test; ** Significant (0.05%) by the t test; ^{ns}Not significant.

Table 2: Pearson correlation between meteorological variables and phenological phases, for percent index of intensity. (T = average monthly air temperature; RH = average monthly relative humidity; SR = average incident monthly solar radiation; bu = budding; ml = mature leaves; fl = flowering; fr = fruiting; lf = leaf fall; ls = leaf senescence)

Weather variables	**Phenological phases**					
	bu	ml	fl	fr	lf	ls
T (°C)	0.343^{ns}	-0.068^{ns}	0.732**	0.519*	0.093^{ns}	-0.323^{ns}
m T (°C)	0.289^{ns}	-0.001^{ns}	0.691**	0.547*	0.196^{ns}	-0.199^{ns}
M T (°C)	0.355^{ns}	-0.131^{ns}	0.782**	0.488^{ns}	-0.020^{ns}	-0.454^{ns}
RH (%)	-0.427^{ns}	0.188^{ns}	-0.116^{ns}	0.326^{ns}	0.620*	0.718**
SR (MJ m²)	0.314^{ns}	-0.533*	0.713**	0.317^{ns}	-0.362^{ns}	-0.641**

*Significant (0.01 %) by the t test; ** Significant (0.05%) by the t test; ^{ns}Not significant.

Average air temperature (T °C) had a significant positive correlation with flowering and fruiting, strong and moderate respectively, both for index of activity (Table 1) and for percent index of intensity (Table 2), but no correlation with the other phenological phases. Average minimum and maximum temperatures followed the same trend, having significant positive correlations with flowering and fruiting, again strong and moderate respectively, for index of activity. As for the percent index of intensity, average minimum temperature had a moderate positive correlation with flowering and fruiting, while average maximum temperature had a significant strong positive correlation only with flowering, which suggests that flowering is induced by increasing temperatures.

Relative air humidity (RH%) had a moderate negative correlation with budding, negatively affecting the index of activity, that is, there is antagonism between the two (Table 1). But it had a moderate positive correlation with leaf fall (lf) and with leaf senescence (ls), indicating that the index of activity increases with increasing RH%. As for percent index of intensity, results reveal a positive correlation with leaf fall and with leaf senescence, moderate and strong respectively.

Average monthly global incident solar radiation had a significant moderate positive correlation with flowering, for index of activity only (Table 1). As for percent index of intensity (Table 2), solar radiation had a moderate negative correlation with mature leaves and with leaf senescence but a strong positive correlation with flowering, which indicates that flowering intensifies with increasing global incident solar radiation.

4. DISCUSSION

The budding phase in species A. hatschbachii was found to be negatively correlated with relative air humidity for index of activity, that is, most of the budding event occurs in the months with low relative air humidity. The budding phase had a peak index of activity and peak intensity between October and December but ceased in periods of high relative humidity. Likewise, Vernonia polyanthes and some other tree species from southeastern Brazil also revealed a negative correlation between humidity and budding (GOMES, 2017; AZEVEDO *et al.*, 2014). In 2019, budding started earlier, in August, while in 2018 it was observed in September, noting that relative humidity levels in August 2019 were lower, which possibly may have caused the budding phase to start earlier. A phenological study on grasslands of southern Brazil revealed that growth rates decline during the cold season due to low temperatures and reduced daylight, combined with excess humidity (TRENTIN & FONSECA, 2011). In addition to influences the expression of phenology, weather variables can influence the yield (PROCHNOW *et al*., 2019).

Length of daylight may be a determinant of how long a leaf stays attached to the branch. Some authors found that leaf longevity is reduced with increasing light intensity (HARLOW *et al*., 2005; VINCENT, 2006), though the reverse may also be true, as intense shading could induce leaf senescence (MARENCO *et al*., 2019). Studying the phenology of Amazonian plant Pseudobombax munguba, the above authors also found that leaf fall could be related to increasing insolation. and, consequently, to solar radiation. What happens is that the aging process in leaves subjected to low light intensity takes longer, a result of slow photosynthetic metabolism (VICENT, 2006). The mature leaves phase of A. hatschbachii reached a peak percent index of intensity when the global incident solar radiation was less intense, i.e. June, July and August, which may have favored a slow photosynthetic metabolism and consequently caused mature leaves to stay longer attached to the branch. Increased senescence in around May, in association with reduced global incident solar radiation, may also be related to the life span of leaves.

In subtropical regions such as southern Brazil, reproductive phenophases (flowering and fruiting) may be associated with stimulus from rising temperatures and photoperiod (TALORA & MORELLATO, 2000; FERRERA *et al*., 2017). While photoperiod is a critical factor for annual plants, temperature affects flower development particularly in woody species and in perennial herbs adapted to a temperate climate (RATHCKE & LACEY, 1985). Talora & Morellato (2000) found flowering induction to be associated with air temperature in some Atlantic Forest species. Some authors reported peak flowering rates in November in some forest species of southern Brazil, which is when air temperature rises and the length of daylight is longer, having found a significant correlation of these two meteorological variables with reproductive phenophases (ATHAYDE *et al*., 2009). Also, Ferrera *et al*. (2017) found that the anthesis phase (fully open flowers) had a correlation with length of daylight hours and with air temperature. The onset of the flowering phase in A. hatschbachii, which occurred in November, coincides both with rising temperatures and with increasing global incident solar radiation, revealing a significant positive correlation. Siedo (2006) also compiled data on the flowering phase of this species and found that it occurs from November to February. Other plant material occurring occasionally in Rio Grande do Sul was found to manifest flowering and fruiting events in October and from January to March (CRESPAM, 2010), which coincides with the findings in this study. Another study in a native forest of Argentina on the flowering phase of species A. virgata and A. gratíssima, both belonging in the same genus, revealed that both species manifested the phenophase between November and April (CABRERA *et al.*, 2013).

The fruiting phenophase in A. hatschbachii was found to reach peak intensity in February and March, immediately following the peak flowering period. The phenophase started in December and extended until April, despite showing uneven fruit ripening, which may otherwise have a positive side as an indicator of an extended period for seed production and thus a good opportunity for natural propagation of the species. The correlation between fruiting and climate variables was significant for the parameter temperature which, similarly to flowering, was observed in the period of high temperatures.

Similarly to budding, leaf fall and leaf senescence were found to correlate with relative air humidity, intensifying in activity and in intensity with rising relative air humidity throughout the assessed period. Increasing relative humidity affects plant transpiration in a decreasing order (KUPPER *et al.*, 2011), as it likely disturbs the absorption of mineral nutrients by plant roots, diminishing nutrient supply to the leaves and perhaps even leading to a decline in photosynthesis (PARTS *et al.*, 2013; SELLIN *et al.*, 2013). It is important to mention that the phenophase of occurrence date of a year will not influence the phenophase of the date of occurrence of the following year. That this variation is attributed to the temperature variation and internal regulators of the plant itself (JIANG *et al.*, 2020). Probably, for this same reason, the start dates of some phenophases (for example fruiting) were different for Aloysia hatschbachii.

When it comes to aromatic plants, phenology can assist in the search for the ideal constitution and higher yield of essential oil. Variations in yield and composition of the essential oil of C. triflorus between the vegetative , flowering and fruit phenofase, the extraction of essential oil during flowering had the highest yield, besides the composition, the concentration of monoterpenes (DAGHBOUCHE *et al*., 2020), beyond to other studies emphasizing this influence of phenophases in the essential oil of aromatic plants (MOISA *et al*., 2019; REAISI *et al*., 2019), thus demonstrating that this study can serve as a basis to collecting material for extraction at different phenological stages.

5. DISCUSSION

The budding phase occurred throughout virtually the entire assessed period in species Aloysia hatschbachii, except between May and July, when it ceased. Budding revealed synchrony between September and December and was influenced by relative air humidity.

The mature leaves phase was present all year round in the plant specimens, with an index of activity revealing high synchrony, and the phase was influenced by incident solar radiation.

Leaf senescence and leaf fall in Aloysia hatschbachii had a low percent index of intensity, indicating leaf permanence on the branch throughout the assessment period, which points to its nondeciduous characteristic, being influenced by relative air humidity and by global incident solar radiation.

The flowering phase in Aloysia hatschbachii occurred from November to April, while the fruiting phase occurred from December to May, both showing synchrony, for two and three months respectively. The reproductive period was influenced by air temperature and by incident solar radiation.

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