Leaf senescence of Christella hispidula (Decne.) Holttum throughout climatic seasons in an urban park with a remnant of the Atlantic Forest

Senescência foliar da *Christella hispidula* (Decne.) Holttum ao longo das estações climáticas em um parque urbano com remanescente de Floresta Atlântica

Senescencia foliar de Christella hispidula (Decne.) Holttum a lo largo de las estaciones en un parque urbano con remanentes de Mata Atlántica

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Abstract
The term senescence is used mainly for plants to define genetically programmed degenerative physiological changes. Leaf senescence is the result of internal and external environmental signals as well as information on leaf age. *Christella hispidula* (Decne.) Holttum is a species of fern characterized by its adaptation ease and consequent broad occupation of different environments. The aim of the present study was to investigate the phenology of *C. hispidula* in a remnant of the Atlantic Forest to gain knowledge on abiotic and biotic factors associated with leaf senescence throughout the seasons. Multiple regression analysis was used to estimate correlations between leaf senescence and both the occurrence of herbivory and presence of sori in the population studied. Positive correlations were found with these two variables. Senescence increased significantly with each new sign of herbivory on the plant (p = 0.46) and with the emergence of sori on the plant (p = 0.02). The multiple coefficients of determination (R^2= 0.47) explained approximately 47% of the dependence of senescence on herbivory and sori. The findings reveal that the seasons exert an influence on leaf senescence in *C. hispidula*.
Keywords: Leaf phenology; Leaf necrosis; Pteridophyte; Fern.

Resumo
O termo senescência é usado principalmente em plantas para definir as alterações fisiológicas degenerativas geneticamente programadas. A senescência foliar é resultado de sinais ambientais internos e externos em informações sobre a idade foliar. *Christella hispidula* (Decne.) Holttum é uma espécie caracterizada por sua facilidade de adaptação e consequente ampla ocupação de suas populações. O objetivo geral deste trabalho foi entender a fenologia da *C. hispidula* em um remanescente de Floresta Atlântica, buscando compreender suas relações abióticas e bióticas a partir da senescência foliar ao longo das estações sazonais. Para estimar a correlação entre a senescência foliar e o valor os dados de herbivoria e soros na população estudada, foi realizado o teste de regressão múltipla. Assim, foi observada a ocorrência da correlação positiva entre a senescência foliar e as duas variáveis supracitadas, onde a cada novo sinal de herbivoria na planta a senescência aumenta (p=0.46), bem como, para surgimento de soros na planta, a senescência foliar tem aumento estatisticamente significativo (p=0.02). O coeficiente de determinação múltipla (R^2=0.47) explica aproximadamente 47% da dependência da senescência diante da herbivoria e soros. Assim, conclui-se que as estações influenciam na senescência foliar em *C. hispidula*.
Palavras-chave: Fenologia foliar; Necrose foliar; Pteridófita; Samambaia.

Resumen
El término senescencia se utiliza principalmente para las plantas para definir cambios fisiológicos degenerativos programados genéticamente. La senescencia de la hoja es el resultado de señales ambientales internas y externas, así como información sobre la edad de la hoja. *Christella hispidula* (Decne.) Holttum es una especie de helecho que se

caracteriza por su facilidad de adaptación y consecuentemente una amplia ocupación de diferentes ambientes. El objetivo del presente estudio fue investigar la fenología de *C. hispidula* en un remanente de Mata Atlántica para conocer los factores abióticos y bióticos asociados con la senescencia de las hojas a lo largo de las estaciones. Se utilizó un análisis de regresión múltiple para estimar las correlaciones entre la senescencia de las hojas y la ocurrencia de herbivoría y la presencia de soros en la población estudiada. Se encontraron correlaciones positivas con estas dos variables. La senescencia aumentó significativamente con cada nuevo signo de herbivoría en la planta (p = 0,46) y con la aparición de soros en la planta (p = 0,02). El coeficiente de determinación múltiple (R^2 = 0,47) explicó aproximadamente el 47% de la dependencia de la senescencia de la herbivoría y los soros. Los hallazgos revelan que las estaciones ejercen una influencia en la senescencia de las hojas en *C. hispidula*.
Palabras clave: Fenología foliar; Necrosis de hojas; Pteridófito; Helecho.

1. Introduction

With broad distribution in the tropics, a considerable diversity of seedless vascular plants – known as ferns and lycophytes – is found in Brazil, with 1253 species recorded (PPG I, 2016). The main characteristic of this group is the preference for moist, shaded habitats (Zuquin *et al.*, 2008). Thus, a large part of the species richness of these plants is found in tropical forests (Prado *et al.,* 2015).

Families of seedless vascular plants are distributed throughout the entire extension of the forest. One example is *Christella hispidula* (Decne.) Holttum, which belongs to the family Thelypteridaceae and is characterized by its adaptation ease, with the consequent distribution of populations in the neotropics and throughout all regions of Brazil (Silvestre & Xavier, 2013).

Ferns and lycophytes do not depend on pollinators or dispersers to reproduce. The climate regulates their phenological events, with phenophases defined in accordance with seasonality (Lieth *apud* Müller & Schmitt, 2019).

Living organisms naturally age, leading to the senescence of cells, tissues, organs and finally the organism itself (Borniego, 2018). The term senescence is mainly used in plants to define degenerative physiological changes (Woo *et al.*, 2019). Gan (2003) describes two types of senescence: (1) mitotic, which occurs in the meristematic tissue following cell division, and (2) and post-mitotic, which occurs in plant organs, such as the leaves, after differentiation and maturation, completing the genetically programmed degeneration process (Liu *et al.,* 2017).

Leaves constitute the main photosynthetic organ of plants, which is fundamental to the absorption of energy and production of nutrients in the growth and maturation phases. When leaves enter the phase of senescence, the cells undergo a sequential disorganization of organelles as well as changes in metabolism and gene expression (Müller & Schmitt, 2019).

During foliar expansion, nutrients are transferred to young leaves, which induces senescence in more mature leaves (Borniego, 2018). According to Himelblau and Amasino (2001), leaf senescence enables the plant to recover nutrients that would naturally be lost. During the nutrient transition process, the cells of mature leaves undergo genetically mediated changes in structure and metabolism, resulting in leaf death (Borniego, 2018). Thus, leaf senescence occurs in three stages: (1) initiation, (2) degradation of macromolecules (and degradation of degradation products) and (3) programmed cell death (Noodén *et al.,* 1997).

Leaf senescence is the result of internal and external environmental signals (*e.g.*, hormonal signals, nutritional signals, water quality and light regime) as well as information on leaf age (Buchanan-Wollaston *et al.,* 2003, Lim *et al.,* 2007).

In a review study on the phenology of ferns and lycophytes in Brazil, Müller and Schmitt (2019) report that a warm, moist climate favors the development of perennial plants. Ferns in tropical regions generally have greater foliar resistance in comparison to those in subtropical regions. However, the leaves of ferns in subtropical regions and regions with considerable alternance between dry and wet periods tend to have similar patterns as those found in temperate regions.

The Arruda Câmara Zoological and Botanical Park is known for its tropical forest and numerous plant species. *C. hispidula* is found at the edges of the forest and seen by the visitors of the park. Studies on the species contribute fundamental ecological information for programs directed at habitat use and conservation (Farias & Xavier, 2011). Thus, there is a need to understand the leaf senescence pattern of this species and associations with abiotic factors in a remnant of the Atlantic Forest in northeastern Brazil located in an urban area with considerable visitor traffic.

Phenology is the study of the temporality of cyclic biological events and associations with environmental conditions, such as temperature, humidity and precipitation (Guariguata & Kattan, 2002), enabling knowledge on vegetative and reproductive aspects of species as well as adaptations to the environment.

The aim of the present study was to investigate the phenology of *Christella hispidula* in the Arruda Câmara Zoological and Botanical Park, analyzing the influence of abiotic factors on leaf senescence in periods with different rainfall indices.

2. Material and Methods

Study Area

The Arruda Câmara Zoological and Botanical Park is considered the oldest park in the city of João Pessoa (state of Paraíba, northeastern Brazil), according to the Municipal Atlantic Forest Conservation and Recovery Plan (2017). Inaugurated in 1922, the park has an area of 26.8 hectares and constitutes a remnant of the Atlantic Forest. The park currently has more than five hundred species of animals and diverse species of flora, receiving approximately 100,000 visitors annually (Costa, 2009). The average annual temperature is 25 °C and the climate is wet tropical, with an average precipitation of 1888 mm (Alvares *et al.,* 2013).

According to Francisco *et al.* (2015), November is the driest month (precipitation: 35 mm) and April is the month of the greatest rainfall (average of 169 mm). Moreover, January is the warmest month of the year (average temperature: 26.6 °C) and August is the month with the lowest temperature (23.5 °C) (Clima Tempo, 2020).

The park has a relatively diversified flora composed of secular plant species, including large native trees. The location in a depression of the topology contributes to the maintenance of the plant biota (Municipal Atlantic Forest Conservation and Recovery Plan, 2017).

Biological Material

Christella hispidula is a species of pantropical fern, with distribution in Africa, Asia, Oceania and the Americas and is found in all regions of Brazil (Ponce *et al.,* 2013). According to the author cited, *C. hispidula* is characterized by an erect or creeping rhizome, an ovate or triangular shape; monomorphic, polystichous leaves, ranging in size from 0.40 to 1.20 meters in length; an elliptical blade, tapering at the base, with a width of 10 to 30 cm; pilous frond with scales at the base; circular, medial sori; reniform indusia with dense hairs of different lengths, rarely glabrous; the sporangium has a unicellular capitate trichome at the pedicel; besides triangular linear pavilions with an acute or attenuated apex with two to four small proximal pairs, the plant sometimes has elongate-crenate acroscopically basal segments, glandular-pubescent middle vein; triangular linear segments, oblique, acute or obtuse, with 6 to 10 pairs of veins, the basal pair united, forming a vein that exceeds the sinus; 1-3 mm long pubescent adaxial surface on veins, margin; abaxial surface completely glandular-pubescent; hyaline acicular capitate trichomes, 0.2-0.5 mm in length, not uniform.

Data Collection

Monthly visits were made to monitor the development of the individuals of two subpopulations of *Christella hispidula* in different environments between February 2018 and February 2020. The development of the individuals in the two plots was compared in different seasons of the year with regards to the senescence period.

For the study, a plot measuring 1.5 m^2 was established. Changes in the number of foliar blades of each specimen were recorded monthly, along with precipitation data obtained from the site of the *Instituto Nacional de Meteorologia* (INMET [National Meteorological Institute]) (Farias & Xavier, 2011). A total of 30 specimens were visited.

The observation method was employed for the determination of senescence. Senescent blades were those with a brown tone covering more than 50% of the blade. No removal of the senescent leaves was performed after the monthly counts; the decision was made to maintain each individual as little affected by anthropic impact as possible. Only leaves with aspects of relatively new senescence were counted in the monthly data, as those with a high level of degradation had been recorded as senescent the previous month.

Data were collected monthly on the occurrence of fiddleheads and fertile leaves. Young leaves were considered fiddleheads and foliar blades with spores on the abaxial part were considered fertile leaves.

Besides the *in situ* observations during the data collection, a thermohygrometer was used to record the temperature data per specimen.

Data Analysis

The Shapiro-Wilk test was used to test the normality of the variables (plant size, frond size, fiddleheads, herbivory, fertile leaves and leaf senescence) in the different periods of the year. For such, each variable was divided into two groups: (1) those at a distance of less than 30 meters from the stream; and (2) those at a distance of more than 30 meters from the same stream. Variables with a p-value ≥ 0.05 were submitted to the t-test and those with a p-value ≤ 0.05 were submitted to the Wilcoxon-Mann-Whitney test (Costa *et al.,* 2018; Farias & Xavier, 2011). R-Studio version 1.4 was used for these analyses.

The correlation test was performed to determine possible correlations between environmental variables and senescence. Variables with a significant correlation were incorporated into the multiple linear regression model, in which the dependent variable was senescence. The t-test was used to determine the significance of the coefficients of the model. These analyses were performed with the aid of the BioEstat 5.0 statistical package.

The Cox-Stuart test was used to determine a tendency toward an increase in senescence over time. The level of significance was set at $\alpha = 0.05$. These analyses were performed with the aid of the R-Studio version 1.4 statistical package.

3. Results

Leaf senescence was observed in all periods. A statistically significant difference was found between the dry and rainy period ($p < 2.2\ e^{-16}$), indicating a significant influence of rainfall on senescence. Despite the constant occurrence of leaf senescence, differences were found in the mean quantity of all dead leaves, with a monthly mean of 2.32 (Table 1).

Fertile leaves and fiddleheads were recorded in all months of the year independently of seasonality. Significant differences were found in the comparison of means of fertile leaves and fiddleheads throughout the seasonal variations, with higher means found in the rainy period (p = 0.76 and 0.15, respectively). No significant difference in frond size was found throughout the seasonal variations (p = ns).

Table 2 shows significant direct correlations between senescence and herbivory as well as between senescence and sori, indicating the senescence increases with the increase in herbivory as well as the increase in sori. Considering these correlations, a multiple linear regression model was run to analyze whether the behavior of senescence could be explained by herbivory and sori (Table 3).

Table 1 - Mean, standard deviation (SD) and precipitation of all senescent leaves of *Christella hispidula* throughout monthly sampling.

Month/year	Mean of leaves	SD	Precipitation (mm)
Feb/18	1.22	0.6	138.7
Mar/18	0.6	0.42	195.6
Apr/18	1.3	0.14	368.5
May/18	0.87	0.18	233.6
Jun/18	0.34	0.39	103.7
Jul/18	0.64	0.64	207.2
Aug/18	1.42	0.68	81.8
Sep/18	2	0.28	32.1
Oct/18	2.89	0.6	1.9
Nov/18	3.8	0.85	16.3
Dec/18	3.88	0.73	56.9
Jan/19	4	0.1	72.6
Feb/19	4	1.22	219.9
Mar/19	4.51	0.58	239.8
Apr/19	4.41	0.3	128.7
May/19	3.25	0.7	204.5
Jun/19	2.7	0.28	659.6
Jul/19	3.66	1.08	354.7
Aug/19	1.98	2.81	84.6
Sep/19	1.25	1.77	62.6
Oct/19	1.45	2	29.4
Nov/19	1.05	1.48	0
Dec/19	2.95	0.07	24.6
Jan/20	1.85	2.62	29.5
Feb/20	1.55	2.19	157.3
Mean	2.32		148.16

Source: Authors (2022).

Table 2 - Correlation coefficients between senescence and other variables collected during two years of sampling.

Source of variation	Degrees of freedom	Sum of squares	Mean square	F	P-value
Regression	2	19.91	9.96	9.76	0.0012
Residual	22	22.45	1.02	-	
Total	24	42.36	-	-	

Source: Authors (2022).

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Table 3 - Analysis of variance of significant variables displayed in Table 2 (herbivory and sori) using data collected.

Variable	Coefficient	P-value
Temperature	0.33	(0.1008)
Size	0.22	(0.2995)
Leaf	-0.15	(0.4619)
Humidity	-0.23	(0.2720)
Fiddlehead	0.16	(0.4432)
Herbivory	0.57	(0.0026)
Sori	0.67	(0.0002)

Source: Authors (2022).

Table 3 displays significant results in the multiple regression analysis for the variables cited (p = 0.0012), suggesting that the behavior of senescence seems to be explained by herbivory and sori. The t-test was used to determine the validity of the coefficients of the model, the results of which are displayed in Table 4.

Table 4 - Results of t-test for significance of coefficients of multiple linear regression model of herbivory and sori using data collected.

Coefficient of model	Estimate	Statistic of test	p-value
Intercept	1.33	t = 4.06	p = 0.0005
Herbivory	0.12	t = 0.74	p = 0.4645
Sori	0.59	t = 2.41	p = 0.0248

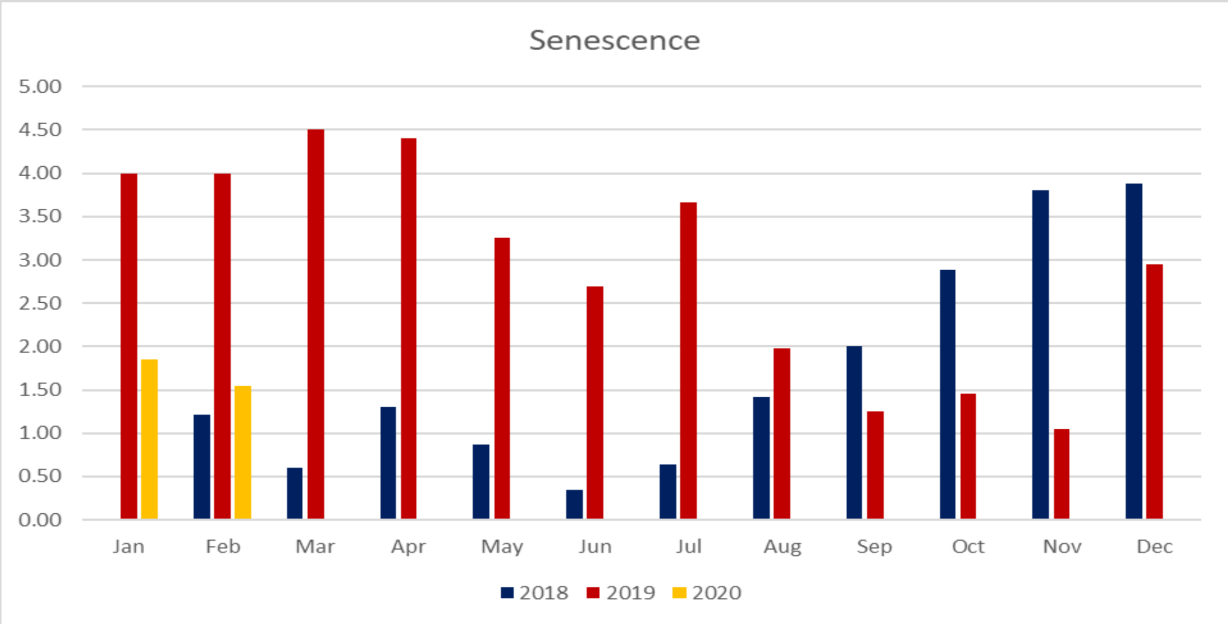
Source: Authors (2022).

Table 4 shows that the B1 coefficient, which refers to herbivory, did not have a significant result. However, as a variable of extreme importance for the biological model, the decision was made to maintain it. Thus, the following multiple linear regression model was found: Y = 1.33 + 0.12 X1 + 0.59 X2. For each new sign of herbivory on the plant, leaf senescence increased 0.12, but the increase was nonsignificant in the model (p = 0.4645). For the emergence of fertile leaves on the plant, senescence increased 0.5898, which was statistically significant (p = 0.0248).

The coefficient of determination ($R^2 =$ 0.47; p < 0.0001) suggests that the multiple regression model explains approximately 47% of senescence as a function of herbivory and sori.

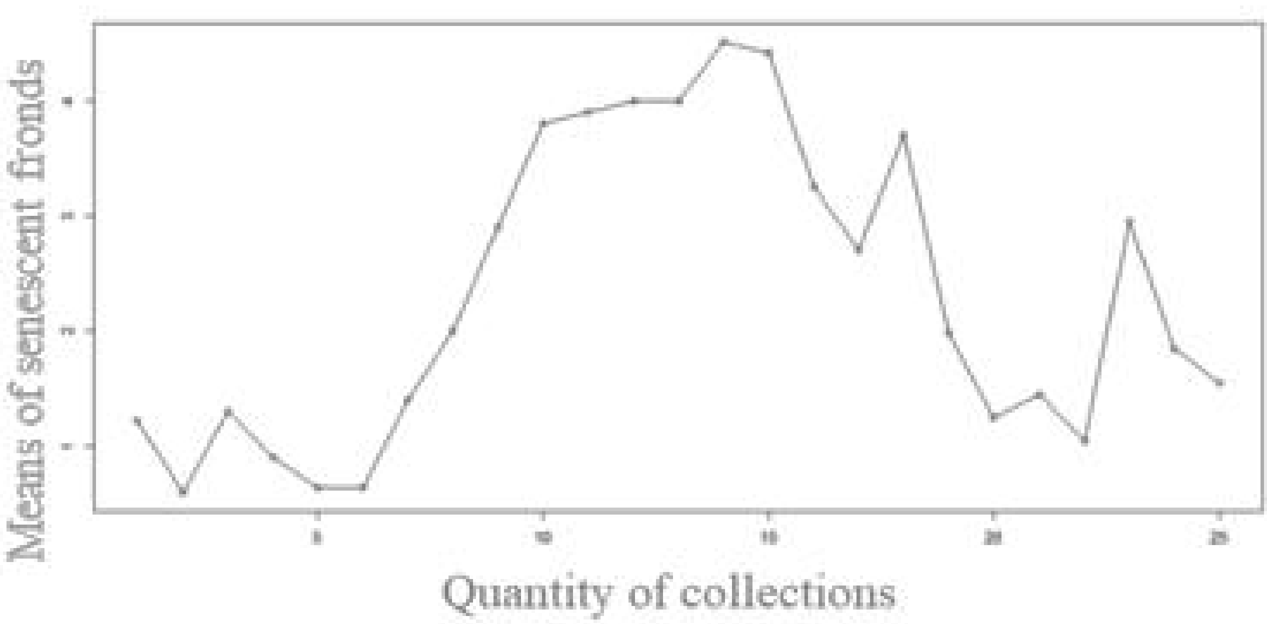
The constant variation in senescence shown in Figure 1 indicated the need to perform a trend test to evaluate whether there is an increase in senescence. For such, the Cox-Stuart test (p =1.123) was used, which confirmed the growth trend in the series between February 2018 and February 2020, as shown in Figure 2.

Figure 1 - Graph of population frequency senescence.



Samples of the senescence frequency of the *C. hispidula* population throughout the research. Source: Authors (2022).

Figure 2- Graph of trend of leaf senescence.



Variation of the senescence leaves of the *C. hispidula* population throughout February 2018 and February 2020. Source: Authors (2022).

4. Discussion

Leaf senescence in *Christella hispidula* was strongly stimulated by rainfall and the condition of the micro-habitat, which is an area close to streams. Thus, senescence peaked in the months of March and April 2019, which were periods with high rainfall indices (239.8 mm). In studies on *Blechnum serrulatum* Rich., Farias & Xavier (2013) report that rainfall is capable of altering the micro-habitat by increasing the moisture content of the soil and enabling greater interaction between the soil and stream, resulting in an increase in the availability of nutrients for the plant and a consequent increase in leaf production. This increase was also recorded in *Blechnum occidentale* L. and *Blechnum brasiliense* Desv. in the Serrana Forest in the state of Pernambuco (Miranda, 2008).

The rainy period was also associated with an increase in herbivory. Zuluaga *et al.* (2013) compared the occurrence of herbivory in an area of coastal vegetation and rocky coast on Barra do Una Beach in the state of São Paulo, Brazil from the standpoint of the Water Availability Hypothesis, which states that plants invest less in protection from herbivory in environments with a high availability of nutrients, where they generally invest their resources in leaf growth and development.

The monthly mean number of blades on *C. hispidula* was much lower than that reported for populations of *Acrostichum danaeifolium* Langsd. & Fisch. and *Thelypteris serrata* (Cav.) Alston in a remnant of the Atlantic Forest in the state of Paraíba, Brazil (mean: 76 and 35 blades, respectively). However, no significant differences between the dry and rainy season were found in these populations (Farias & Xavier, 2011).

The association between rainfall and leaf senescence in *C. hispidula* was similar to that found in a population of *Alsophila firma* (Baker) D. S. Conant, for which leaf mortality increased significantly in rainy months in a lower montane forest in Mexico (Mehltreter, 2008). However, a reduction in leaf senescence in the rainy period was found for *Lygodium volubile* Sw, *Thelypteris serrata* (Cav.) Alston. and *Adiantum serratodentatum* Willd (Dias-Filha, 1989, Ranal, 1995, Farias & Xavier, 2011, Costa *et al.,* 2018). Precipitation can induce leaf production and senescence, especially in areas with alternance between the dry and wet period due to the small root system, which hinders the obtainment of water in periods of less rainfall (Mehltreter, 2006, Müller & Schmitt, 2019).

The months from August to November 2019 and January to February 2020 (Figure 1) had mean senescence of 0.25 due to the falling of a tree in the collection plot, which affected 10 individuals in this population and impeded data collection in the period. In January and February 2020, 12 individuals died due to the increase in herbivory in this plot.

The occurrence of fertile leaves and fiddleheads in all months of the year, independently of seasonality, was also reported by Souza *et al.* (2007) for *Anemia tomentosa* (Sav.) Sw. var. *anthriscifolia* (Schrad.) and *A. danaeifolium* Langsd. & Fisch. in a semi-deciduous forest in the state of Pernambuco, Brazil.

A senescence pattern in *C. hispidula* was found in all periods and the incidence of fiddleheads and new leaves accompanied this same pattern. Some researchers, such as Landi *et al.* (2014), Mehltreter *et al.* (2008) and Schmitt *et al.* (2009), indicate a direct association between leaf senescence and leaf production in ferns and herbaceous plants due to the continuous flow of nutrients, which are transported to young leaves.

The association between senescence and the production of fertile leaves may be explained by the cycling of nutrients. Sharma & Sharma (2004) define the dynamics of nutrients, such as nutritional translocation from senescent leaves to growing leaves, especially with regards to the mobile elements (N, P and K), which are found in greater quantity in young leaves, whereas the opposite behavior is found for the concentration of Ca.

5. Conclusion

Based on the present findings, senescence, herbivory as well as the production of fiddleheads and fertile leaves occurs in all months of the year in *C. hispidula,* independently of rainfall incidence, but greater intensity is found in periods of higher rainfall. This senescence pattern is also commonly found in other ferns.

Senescence in *C. hispidula* occurs mainly in mature leaves. This may be due to the cycling of nutrients, which controls the natural cycle of foliar life.

Senescence also depends on rainfall and seasonality, as phenophases varied between periods of greater rainfall and less rainfall. The senescence of *C. hispidula* tends to increase in rainy periods, influencing the entire metabolism of the plant, indicating that *C. hispidula* maintains its phenophases in all seasons of the year.

Some plants alter this metabolism and their phenophases on different environments and there are still many studies on *C.* hispidula so new studies are needed to verify whether the phenophases change in different biomes and environmental condictions.

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