



# Revista Brasileira de Geografia Física

Homepage: <https://periodicos.ufpe.br/revistas/rbgfe>



## Environmental Influence on the Leaf Morphoanatomical Characteristics of *Myrcia splendens* (Sw.) DC. (Myrtaceae)<sup>1</sup>

Isabela Santiago Carneiro da Costa<sup>2</sup>, Ítalo Antônio Cotta Coutinho<sup>3</sup>, Oriel Herrera Bonilla<sup>4</sup>, Eliseu Marlônio Pereira de Lucena<sup>5</sup>

<sup>1</sup>Part of the Master's research, performed by the first and guided by the last author, under the Postgraduate Program in Natural Sciences of the State University of Ceará, Fortaleza-CE, Brazil. <sup>2</sup>Master student, Academic Master in Natural Sciences, State University of Ceará, Itaperi Campus, CEP 60.714-903, Fortaleza, CE. E-mail: isabelasantiago819@gmail.com <sup>3</sup>Post-Doctor, Adjunct Professor of the Biological Sciences Course, the Postgraduate Program in Ecology and Natural Resources and the Postgraduate Program in Systematics, Use and Conservation of Biodiversity, Federal University of Ceará, Pici Campus, CEP 60.440-900, Fortaleza, CE. E-mail: italo.coutinho@ufc.br <sup>4</sup>PhD, Associate Professor of the Biological Sciences Course and the Postgraduate Program in Natural Sciences, State University of Ceará, Itaperi Campus, CEP 60.714-903, Fortaleza, CE. E-mail: oriel.herrera@uece.br <sup>5</sup>Post-Doctor, Associate Professor of the Biological Sciences Course and the Postgraduate Program in Natural Sciences, State University of Ceará, Itaperi Campus, CEP 60.714-903, Fortaleza, CE. E-mail: eliseu.lucena@uece.br - corresponding author

Artigo recebido em 08/06/2020 e aceito em 09/08/2020

### ABSTRACT

*Myrcia splendens* (Sw.) DC. is a native and endemic species of Brazil. Plants can have ecological plasticity due to environmental influences and studies on the ecological anatomy of leaves in the genus *Myrcia* DC. are scarce. In this sense, the present study aimed to verify the effect of seasonality and luminosity on the leaf morphoanatomical characteristics of *Myrcia splendens* (Sw.) DC., in order to contribute with information about the species responses to the natural abiotic factors of its occurrence. The botanical material for the study was collected in the São Gonçalo do Amarante Botanic Garden, Ceará, in the rainy and dry seasons, fully expanded leaves exposed to the sun and shade, which were subsequently subjected to laboratory procedures to obtain the paradermal and cross sections of the leaves, in order to verify how *Myrcia splendens* (Sw.) DC. responds to seasonal variations in the availability of water and light in a coastal environment. As a result, most leaf structures of *Myrcia splendens* (Sw.) DC. presented greater thicknesses, areas or densities when in the dry season (water deficit) and submitted to intense sunlight, that is, in more stressful environmental conditions. Therefore, it's concluded that the changes in the anatomy of *Myrcia splendens* (Sw.) DC. demonstrated the acclimatization of the leaves in response to abiotic factors (water and light), thus contributing to the survival of the species in the Vegetation Complex of the Coastal Zone of Ceará.

Keywords: Ecological anatomy, season, lightness, plasticity, Vegetation Complex of the Coastal Zone.

## Influência Ambiental nas Características Morfo-anatômicas Foliaves de *Myrcia splendens* (Sw.) DC. (Myrtaceae)

### RESUMO

*Myrcia splendens* (Sw.) DC. é uma espécie nativa e endêmica do Brasil. As plantas podem ter plasticidade ecológica devido às influências ambientais e estudos sobre a anatomia ecológica das folhas no gênero *Myrcia* DC. são escassos. Neste sentido, o presente estudo objetivou verificar o efeito da sazonalidade e da luminosidade nas características morfo-anatômicas foliaves de *Myrcia splendens* (Sw.) DC., a fim de contribuir com informações sobre as respostas da espécie aos fatores abióticos naturais de sua ocorrência. O material botânico para o estudo foi coletado no Jardim Botânico de São Gonçalo do Amarante, Ceará, nas estações chuvosa e seca, folhas totalmente expandidas expostas ao sol e à sombra, as quais posteriormente foram submetidas aos procedimentos laboratoriais para obtenção dos cortes paradermicos e transversais das folhas, a fim de verificar como a *Myrcia splendens* (Sw.) DC. responde às variações sazonais em relação à disponibilidade de água e luz em ambiente costeiro. Como resultado, a maioria das estruturas foliaves da *Myrcia splendens* (Sw.) DC. apresentou maiores espessuras, áreas ou densidades quando na estação seca (déficit hídrico) e submetidas à luz solar intensa, ou seja, em condições ambientais mais estressantes. Portanto, concluiu-se que as alterações na anatomia da *Myrcia splendens* (Sw.) DC. demonstraram a aclimação das folhas em resposta aos fatores abióticos (água e luz), contribuindo assim para a sobrevivência da espécie no Complexo Vegetacional da Zona Litorânea cearense.

Palavras-chave: Anatomia ecológica, estação, luminosidade, plasticidade, Complexo Vegetacional da Zona Litorânea.

## Introduction

Light intensity and water are abiotic factors that exerts great influence on leaf modification (Smith, Bell, & Shepherd, 1998; Larcher & Boeger, 2009). As a result of abiotic stresses leaves may go through acclimation in response to environmental changes (Sultan, 2000; Mantuano, Barros, & Scarano, 2006; Li et al., 2009; Olsen, Caudle, Johnson, Baer, & Maricle, 2013; Oguchi, Onoda, Terashima, & Tholen, 2018). Therefore, plants may undergo physiological, morphological and anatomical changes as adaptive strategies to the environment changes (Devi et al., 2017; Melo Júnior & Boeger, 2017). Leaf is the plant organ commonly used in ecological studies which may easily respond to environmental changes (Wyka, Robakowski, & Zytkowskiak, 2007). Leaf tissues such as chlorenchyma, epidermis, parenchyma as well as vascular bundles may undergo phenotypic plasticity and register the influence of abiotic factor on plants (Dardengo, Rossi, Silva, Pessoa, & Silva, 2017; Devi et al., 2017; Lemos et al., 2018).

Research focusing on ecological leaf anatomy has been reported in the Myrtaceae family for genera such as *Eucalyptus* L'Hér. (Ali et al., 2009), *Eugenia* L. (Alves, Tresmondi, & Longui, 2008; Esposito-Polesi, Rodrigues, & Almeida, 2011; Lemos, Lucena, Bonilla, & Edson-Chaves, 2019), *Metrosideros* (Amada, Onoda, Ichie, & Kitayama, 2017), *Myrceugenia* O.Berg (Retamales & Scharaschkin, 2015), *Myrcia* DC. (Amorim & Melo Júnior, 2016; Lemos, Lucena, Bonilla, Edson-Chaves, & Freitas, 2020; Costa, Lucena, Bonilla, Radosavljevic, & Coutinho, 2020) and *Syzygium* Steud. (Zhu, Chen, Cao & Ye, 2015; Abdulrahman, Ali, Fatihah, Khandaker & Mat, 2018). However, studies with leaf anatomy as a response to seasonality, that is, water stress and light intensity on the genus *Myrcia* are still scarce.

*Myrcia* is a neotropical genus of the family Myrtaceae (McVaugh, 1968) with 333 spp. occurring in Brazil (The Brazil Flora Group [BFG], 2018). *Myrcia splendens* (Sw.) DC. is a medicinal plant with antioxidant activity (Nakamura, Monteiro, Bizarri, Siani, & Ramos, 2010; Scio et al., 2012; Pontes, Abdalla, Imatomi, Fuentes, & Gualtieri, 2019) and is popularly known as 'viuvinha' (Lucena, Major, & Bonilla, 2011). This species occurs in several biomes, being well distributed throughout all the Brazilian territory (BFG, 2018).

*Myrcia splendens* (Sw.) DC. is often found at sandy coastal plains (Castro, Moro, & Menezes, 2012; Moro, Macedo, Moura-Fé, Castro, & Costa, 2015). The sandy coastal plains are areas susceptible to great water availability, salinity, strong winds and exposure to high incident light (Rosado & Mattos, 2007; Moro et al., 2015; Melo Júnior & Boeger, 2017). In addition, sandy coastal plains environments presents deep and leachate soils and with drought periods up to six months per year (Castro et al. 2012). Such environmental stresses reflect on the occupation of these areas by plants as well as on plant morphoanatomical characteristics (Rosado & Mattos, 2007; Kuster, Meira, & Azevedo, 2018) that change in response to the abiotic factors so plants may survive under such harsh conditions (Devi et al., 2017; Melo Júnior & Boeger, 2017).

Despite *Myrcia splendens* (Sw.) DC. being native and endemic to Brazil (BFG, 2018), little is known about the leaf morphoanatomic changes of this species for its survival under the various conditions imposed by the environment, especially in coastal regions of the Northeast of the country. Thus, the hypothesis of this research was that there is a correlation between the anatomy of *Myrcia splendens* (Sw.) DC. leaves and the environmental conditions to which they are exposed.

Therefore, evergreen plants such as *Myrcia splendens* (Sw.) DC. inhabiting sandy coastal plains areas make up a good model to understand the possible anatomical responses of plants to environmental stresses. In this sense, the present study aimed to verify the effect of seasonality and luminosity on the leaf morphoanatomical characteristics of *Myrcia splendens* (Sw.) DC., in order to contribute with information about the species responses to the natural abiotic factors of its occurrence.

## Materials and Methods

### Study Area

The material was collected in the São Gonçalo do Amarante Botanic Garden (3°34'07.0"S and 38°53'12.8"W) located in the municipality of São Gonçalo do Amarante, located in the state of Ceará, Brazil (Figure 1).

This area is a Vegetation Complex of the Coastal Zone (Figure 2) (Castro et al., 2012) with average annual precipitation in 2018 of 1772.9 mm, lowest rainfall rate between the months of

September to November (3.3, 5.4 and 5.0 mm, respectively) and highest rainfall rate between February to May (308.7, 246.7, 265.9 and 237.4 mm, respectively) (Table 1) (Universidade Federal do Ceará [UFC], 2018).

### *Botanical Material*

*Myrcia splendens* (Sw.) DC. are treelets and tress with leaves lanceolate or elliptic-lanceolate, pubescent on the abaxial side, opposite and absent stipules (Figure 3a) (Morais & Lombardi, 2006; Amorim & Alves, 2011; Amorim & Alves, 2012; Araujo, Lucena, Sampaio, Bonilla, & Pinheiro, 2020). It's a medicinal plant (Scio et al., 2012) with edible fruits, popularly known as 'viuvinha' (Figure 3b) (Lucena et al., 2011) with leaf extracts presenting antioxidant activity (Scio et al., 2012; Moresco et al. 2014; Pontes et al., 2019), which is a common feature in Myrtaceae (Santos et al., 2020).

Fully developed leaves from *Myrcia splendens* (Sw.) DC. specimens from the 3<sup>rd</sup> to 5<sup>th</sup> node exposed to sun or shade were collected in March/2018 (wet season - average temperature of 27.5 °C and average relative humidity of 79%) and October/2018 (dry season - average temperature of 27.9 °C and average relative humidity of 70%) (Table 1) (UFC, 2018).

A voucher material for the population was deposited in the Prisco Bezerra Herbarium (EAC), acronyms according to Thiers (2019), of the Federal University of Ceará, under the registration number EAC62243 (V.S. Sampaio, E.M.P. Lucena, & L.F. Pinheiro, 170, 26.X.2017).

### *Light Microscopy Study*

The botanical material collected was fixed in a solution containing 4% paraformaldehyde and 1% glutaraldehyde in 0.2 M phosphate buffer at pH 7.2 (Karnovsky, 1965). For leaf epidermal characterization, fragments from the middle third of leaves fixed in Karnovsky were dissociated according to Jeffrey's Method (Johansen, 1940). The dissociated fragments from the abaxial and adaxial side of the epidermis were stained with 1% Astra blue and 0.05% safranin for 12 hours and mounted on glass varnish.

For the anatomical characterization of leaves in cross sections, fragments from the middle third of leaves fixed in Karnovsky were dehydrated in increasing ethyl alcohol series up to 95% ethanol and embedded in methacrylate resin

according to the recommendations (Leica, Heidelberg, Germany). Cross sections at 3-5 µm were made in an automatic rotary microtome (Leica RM 2065, Leica Instruments GmbH, Nussloch, Germany) with glass blades. Sections were stained in a solution composed of 1% Astra blue and 0.05% safranin for 12 hours. The excess of staining was removed by dipping the slides in distilled water for about 15 min. Permanent slides were dried at 37 °C and mounted with glass varnish. Observations and photographs were taken using a digital camera Olympus UC 30 (Hamburg, Germany) equipped with light microscope Olympus BX 41TF (Tokyo, Japan).

### *Analysis of the Effect of Seasonality and Luminosity on Leaves*

Quantitative analysis of the seasonal variation in leaf anatomy was performed by comparing the leaves fully expanded collected during the wet and dry seasons of plants exposed to sun and shade. In paradermal sections of leaf blade the parameters: trichome density (number of trichomes/area), stomatal density (number of stomata/area), frequency (number of stomata/number of epidermal cells), index [number of stomata/(number of epidermal cells + number of stomata) x 100] and area (µm<sup>2</sup>) were quantitatively evaluated. The area of density of trichomes and stomata were 0.0024 cm<sup>2</sup> and 31.3 cm<sup>2</sup>, respectively. For cross sections were evaluated the leaf blade region, mesophyll, palisade and spongy parenchyma, epidermis (with cuticle) of the adaxial and abaxial face thickness. In the midrib were measured the midrib thickness (µm), vascular bundle, xylem, phloem and fibers area (µm<sup>2</sup>). The structures were measured using the Image J program (Abràmoff, Magalhães, & Ram, 2004).

### *Statistical Analysis*

The experimental design was completely randomized, with 2 (wet and dry) x 2 (sun and shade) factorial and 15 replicates. Results were submitted to normality test, followed by analysis of variance observing the significance by the F test and when significant, the Tukey test was performed at 5% probability level using the Statistical Analysis System V. 1.0 (ESTAT) software.

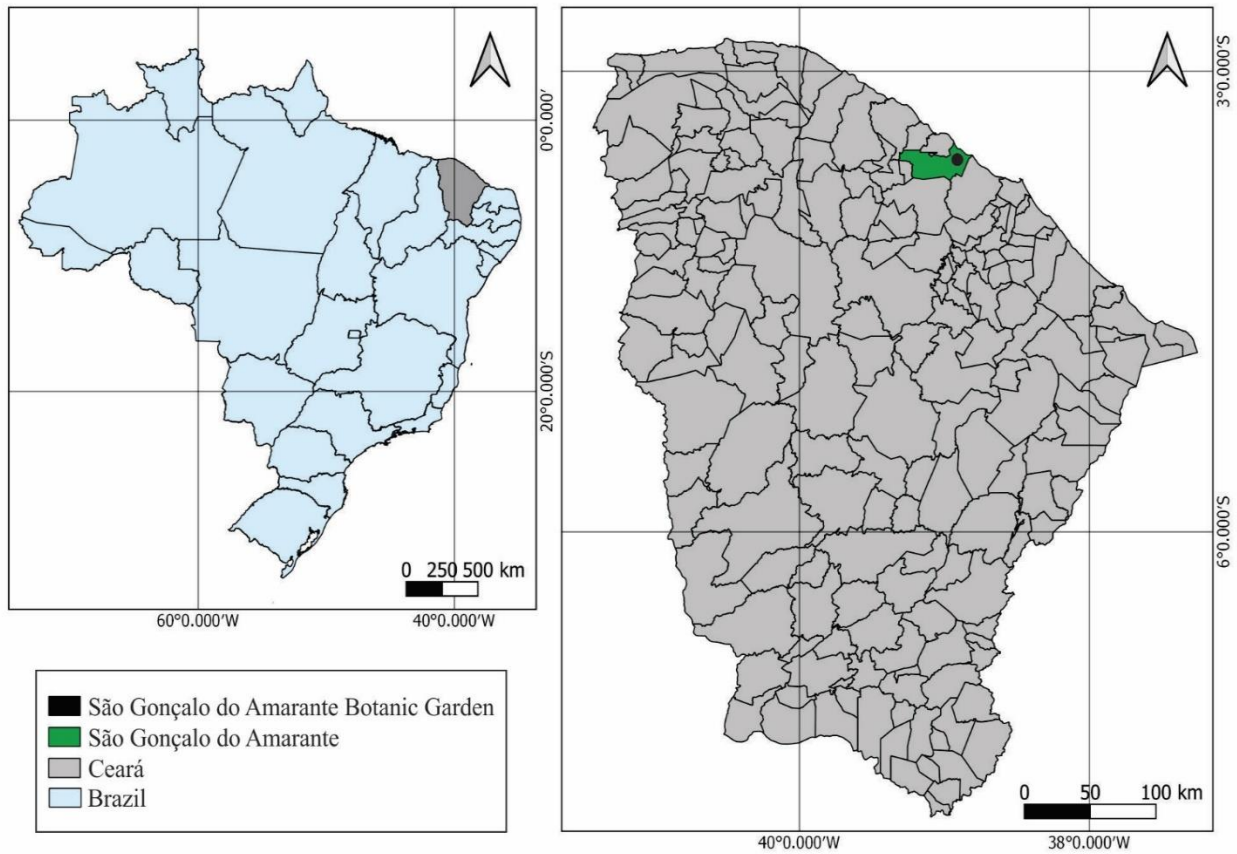


Figure 1. Location of the São Gonçalo do Amarante Botanic Garden, São Gonçalo do Amarante, Ceará, Brazil.



Figure 2. Vegetation Complex of the Coastal Zone located in São Gonçalo do Amarante Botanic Garden, São Gonçalo do Amarante, Ceará, Brazil.

Table 1. Weather data collection for São Gonçalo do Amarante Botanic Garden in 2018 (UFC, 2018).

Month 2018	Total precipitation (mm)	Average temperature (°C)	Average relative humidity (%)	Insolation (h/month)
Jan	208.4	26.9	77	210.6
Fev	308.7	26.6	84	125.0
Mar	246.7	27.5	79	220.6
Abr	265.9	27.0	81	179.7
May	237.4	26.6	85	192.1
Jun	44.7	27.1	72	266.6
Jul	160.1	26.5	73	272.1
Ago	28.4	27.0	72	302.6
Set	3.3	27.2	76	307.3
Out	5.4	27.9	70	314.7
Nov	5.0	28.4	70	279.8
Dez	258.9	27.6	78	236.9

Weather data was taken from the nearest weather station, Agrometeorological Station at the Federal University of Ceará (Ceará State, Brazil), ~60 km from São Gonçalo do Amarante Botanic Garden.

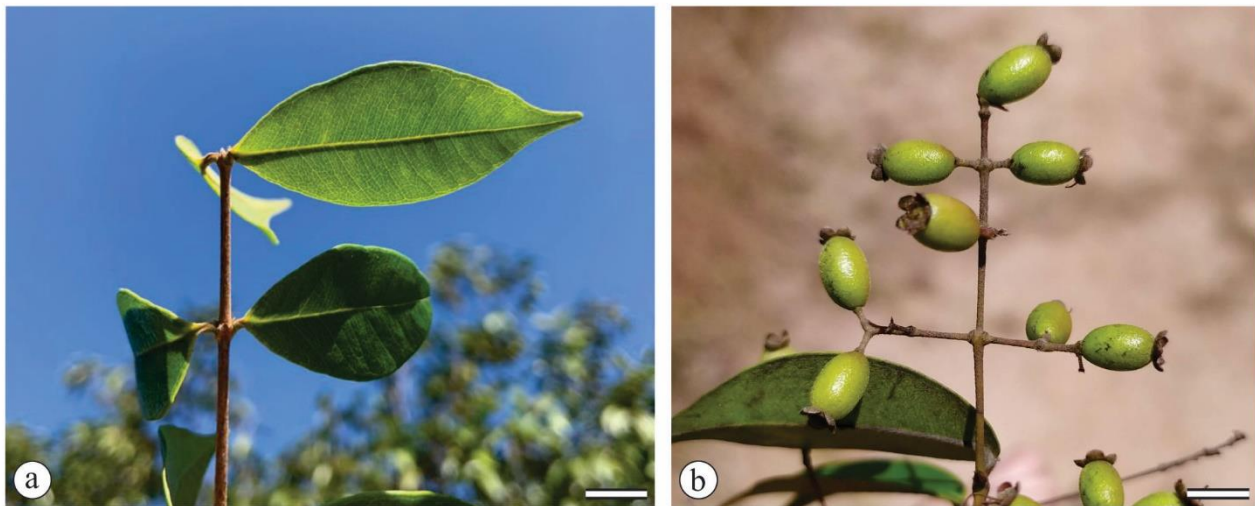


Figure 3. *Myrcia splendens* in field conditions. a. Opposite leaves. b. Unripe fruits. Scale bars. 500 µm.

## Results

### Morphoanatomical Characteristics

*Myrcia splendens* (Sw.) DC. leaf blades observed in frontal view presented markedly sinuous anticlinal cell walls on both leaf sides (Figure 4a–f). Simple non-secretory trichomes with a pointy apex and paracytic stomata (hypostomatic leaf) were observed only on the abaxial side (Figure 4a–e). On the adaxial leaf side, among the ordinary epidermal cells, two cells (cap cells) that fit together similar to two jigsaw puzzle pieces were observed. Such cells were always covering a subepidermal secretory cavity (Figure 4f).

In cross sections of the leaf blade, it was possible to observe a single-layered epidermis on both leaf sides in which the adaxial epidermis is

thicker than the abaxial epidermis (thickened external periclinal wall). The mesophyll is composed of both spongy and palisade parenchyma (dorsiventral mesophyll) and palisade parenchyma presents a single-layered while the spongy parenchyma was composed by 6-8 cell layers (Figure 4g–j). In the mesophyll there are predominantly secretory cavities in the palisade parenchyma (adaxial face) and collateral vascular bundles with two caps (upper and lower) of sclerenchymatic fibers (Figure 4h–j).

At the midrib region, the leaf blade was convex on the abaxial side and slight concave on the adaxial side (Figure 4k–n). On the adaxial side the palisade parenchyma was interrupted by collenchyma (Figure 4k–n). Phloem was placed on both sides of xylem (bicollateral vascular bundles) and the whole vascular bundles were

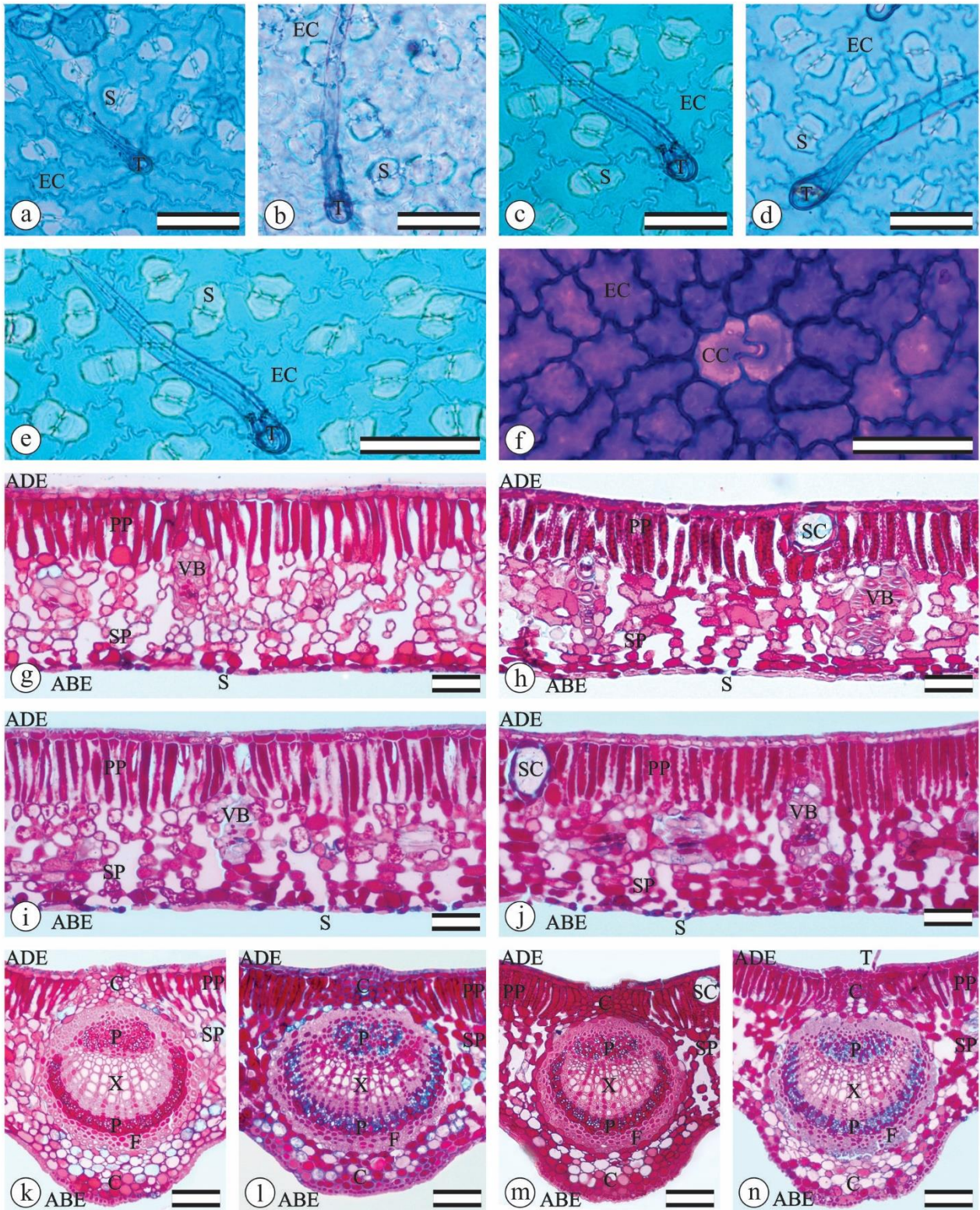


Figure 4. Anatomical sections of the *Myrcia splendens* (Sw.) DC. leaves. a-e. Paradermal sections of abaxial epiderms. f. Paradermal section of adaxial epiderms. g-n. Cross sections of leaf and midrib. Treatments: a,g,k. Wet/shade; b,h,l. Wet/sun; c,i,m. Dry/shade; d,j,n. Dry/sun. Abbreviations: CC – Cap cell; C – colenchyma; EC – epidermal cell; ABE – abaxial epidermis; ADE – adaxial epidermis; F – fibers; P – phloem; SP – spongy parenchyma; PP – palisade parenchyma; SC – secretory cavity; S – stomata; T – trichome; VB – vascular bundle; X – xylem. Scale bars: a–d. 50 µm; e–f. 10 µm; g–n. 20 µm.

surrounded by fibers. At abaxial side, the spongy parenchyma was interrupted by of collenchyma and parenchyma (Figure 4k–n). On some occasions secretory cavities were observed in the mesophyll near the vascular bundles. Simple trichomes were also observed on adaxial side of the midrib region above the collenchyma (Figure 4n).

### *Environmental Influence*

Trichome density showed no statistical difference between the seasons (wet season density 3986.1111; dry season density 4138.8889) while for light intensity there was a greater value of trichome density for shade leaves (4375.0) than in leaves subjected to the sun (3750.0) (Table 2). There was interaction between the two treatments evaluated (season and lightness) and a higher density of shade leaves was observed in the wet season (4805.5556) (Table 3).

All stomata criteria (density, frequency, index and area) showed higher values in the dry season (Table 2). In these criteria, the values obtained in dry season were 0.2641, 0.4944, 33.6784%, 455.5458  $\mu\text{m}^2$ , respectively (Table 2). In the wet season, the lower values of 0.2076, 0.3800, 28.2554% and 390.3109  $\mu\text{m}^2$ , respectively (Table 2). In terms of luminosity, all criteria were higher for sun leaves, except for the stomatal area (Table 2). The stomatal area in shade leaves was 425.6951  $\mu\text{m}^2$ , in contrast the sun leaves the area was 420.1617  $\mu\text{m}^2$  (Table 2). Stomatal density, frequency and index showed higher values in sun leaves 0.2491, 0.4838 and 32.8798%, respectively, than shade leaves (0.2225, 0.3906 and 29.054%, respectively) (Table 2). Stomatal area demonstrated an interaction between seasonality and lightness (Table 3). Shade leaves from dry season showed greater area (480.6323  $\mu\text{m}^2$ ) when compared to other treatments (Table 3). The other stoma criteria had no interaction between season and lightness (Table 2).

In the cross sections, the leaf blade, spongy parenchyma and adaxial epidermis thickness obtained high values in sun lightness and dry season leaves (Table 2) with interaction between the treatments only in leaf blade and adaxial epidermis thickness (Table 3). The values in sun lightness of leaf blade, spongy parenchyma and adaxial epidermis thickness were 507.78, 119.3662 and 15.7596  $\mu\text{m}$ , respectively. Shadow leaves had lower values (Table 2). As for the seasonality values in these same criteria, higher values can be observed in leaves of the dry season (496.59, 126.798 and 16.2916  $\mu\text{m}$ , respectively)

(Table 2). While the wet season presented lower values of thickness (483.68, 104.7794 and 14.5044  $\mu\text{m}$ , respectively) (Table 2). Regarding the season *versus* lightness interaction the leaf blade was thicker in leaves exposed to the sun in the dry season (528.81  $\mu\text{m}$ ) when compared to the other treatments (Table 3). The same was observed in the adaxial epidermis thickness parameter (16.9705  $\mu\text{m}$ ) (Table 3).

The mesophyll thickness showed no statistical difference between the leaves of the dry (221.4006  $\mu\text{m}$ ) and wet season (217.7305  $\mu\text{m}$ ) (Table 4). As for lightness, the sun leaves presented thicker (222.5936  $\mu\text{m}$ ) leaves when compared to shade leaves (216.5375  $\mu\text{m}$ ) (Table 2). As regards interaction between the season and lightness, the mesophyll it was thicker in sun leaves in the wet season (225.3962  $\mu\text{m}$ ) and shade leaves in the dry season (223.0102  $\mu\text{m}$ ) (Table 3). Palisade parenchyma thickness showed high values in wet season (102.8377  $\mu\text{m}$ ), while the leaves in sun (97.6026  $\mu\text{m}$ ) and shade (98.4499  $\mu\text{m}$ ) lightness didn't differ from each other (Table 2). In this characteristic was interaction between season and lightness (Table 3) in which the palisade parenchyma was thicker in leaves of sun (104.3863  $\mu\text{m}$ ) and shade (101.2891  $\mu\text{m}$ ) from the wet season (Table 3). As for the abaxial epidermis thickness, there was no statistical difference between treatments (Table 2), however, there was an interaction between season and lightness, being higher for dry season in the sun and wet season in the shade (Table 3).

All parameters evaluated in the cross sections in the midrib of *Myrcia splendens* (Sw.) DC. leaves presented higher values in dry season (Table 2). While in the lightness the results varied. The midrib thickness, vascular bundle and phloem area presented higher values in sun lightness leaves (Table 2). While the xylem presented greater area in shade leaves (28226.7037  $\mu\text{m}^2$ ) (Table 2) and fibers area presented no statistical difference in areas of sun (25458.6874  $\mu\text{m}^2$ ) and shade (24863.9519  $\mu\text{m}^2$ ) lightness (Table 2). *Myrcia splendens* (Sw.) DC. leaves presented larger areas of phloem in dry season (33732.1589  $\mu\text{m}^2$ ) and sun lightness (29617.0597  $\mu\text{m}^2$ ) when compared to wet season (24056.8982  $\mu\text{m}^2$ ) and shade lightness leaves (28171.9974  $\mu\text{m}^2$ ), respectively (Table 2). As for midrib thickness and vascular bundle area the leaves showed higher values in dry season (496.5936  $\mu\text{m}$  and 92323.7985  $\mu\text{m}^2$ ) and sun lightness (507.7802  $\mu\text{m}$  and 81969.6525  $\mu\text{m}^2$ ) (Table 2). The midrib thickness and vascular bundle area of the midrib was the parameters in the midrib that presented

Table 2. Quantitative parameters of the paradermal and cross sections of the *Myrcia splendens* (Sw.) DC. leaves in wet and dry seasons, as well as in the sun and shade lightness.

Parameters	Season		Lightness	
	Wet	Dry	Sun	Shade
Trichome density*	3986.1111 a	4138.8889 a	3750.0000 b	4375.0000 a
Stomatal density	0.2076 b	0.2641 a	0.2491 a	0.2225 b
Stomatal frequency	0.3800 b	0.4944 a	0.4838 a	0.3906 b
Stomatal index (%)	28.2554 b	33.6784 a	32.8798 a	29.0540 b
Stomata area ( $\mu\text{m}^2$ )*	390.3109 b	455.5458 a	420.1617 b	425.6951 a
Leaf blade thickness ( $\mu\text{m}$ )*	483.6800 b	496.5900 a	507.7800 a	472.5000 b
Mesophyll thickness ( $\mu\text{m}$ )*	217.7305 a	221.4006 a	222.5936 a	216.5375 b
Spongy parenchyma thickness ( $\mu\text{m}$ )	104.7794 b	126.7980 a	119.3662 a	112.2112 b
Palisade parenchyma thickness ( $\mu\text{m}$ )*	102.8377 a	93.2148 b	97.6026 a	98.4499 a
Adaxial epidermis thickness ( $\mu\text{m}$ )*	14.5044 b	16.2916 a	15.7596 a	15.0364 b
Abaxial epidermis thickness ( $\mu\text{m}$ )*	9.9418 a	10.0304 a	9.9226 a	10.0496 a
Midrib thickness ( $\mu\text{m}$ )*	483.6867 b	496.5936 a	507.7802 a	472.5001 b
Vascular bundle area of the midrib ( $\mu\text{m}^2$ )*	68466.6827 b	92323.7985 a	81969.6525 a	78820.8287 b
Xylem area of the midrib ( $\mu\text{m}^2$ )	25406.8930 b	28736.3975 a	25916.5869 b	28226.7037 a
Phloem area of the midrib ( $\mu\text{m}^2$ )	24056.8982 b	33732.1589 a	29617.0597 a	28171.9974 b
Fibers area of the midrib ( $\mu\text{m}^2$ )	24069.5503 b	26253.0890 a	25458.6874 a	24863.9519 a

Means followed by the same letter in season or lightness don't differ statistically by the Tukey's test at 5% probability level. Asterisk indicates interaction between season and lightness, more details in Table 3.

Table 3. Quantitative parameters of *Myrcia splendens* (Sw.) DC. leaves: interaction between season and lightness.

Season	Lightness	
	Sun	Shade
Trichome density		
<b>Wet</b>	3166.6667 Bb	4805.5556 Aa
<b>Dry</b>	4333.3333 Aa	3944.4444 Bb
Stomata area ( $\mu\text{m}^2$ )		
<b>Wet</b>	409.8641 Ab	370.7578 Bb
<b>Dry</b>	430.4593 Ba	480.6323 Aa
Leaf blade thickness ( $\mu\text{m}$ )		
<b>Wet</b>	486.74 Ab	480.62 Aa
<b>Dry</b>	528.81 Aa	464.37 Bb
Mesophyll thickness ( $\mu\text{m}$ )		
<b>Wet</b>	225.3962 Aa	210.0649 Bb
<b>Dry</b>	219.7909 Ab	223.0102 Aa
Palisade parenchyma thickness ( $\mu\text{m}$ )		
<b>Wet</b>	104.3863 Aa	101.2891 Aa
<b>Dry</b>	90.8189 Bb	95.6107 Ab
Adaxial epidermis thickness ( $\mu\text{m}$ )		
<b>Wet</b>	14.5487 Ab	14.4601 Ab
<b>Dry</b>	16.9705 Aa	15.6127 Ba
Abaxial epidermis thickness ( $\mu\text{m}$ )		
<b>Wet</b>	9.4207 Bb	10.4629 Aa
<b>Dry</b>	10.4246 Aa	9.6362 Bb
Midrib thickness ( $\mu\text{m}$ )		
<b>Wet</b>	486.7450 Ab	480.6284 Aa
<b>Dry</b>	528.8154 Aa	464.3717 Bb
Vascular bundle area of the midrib ( $\mu\text{m}^2$ )		
<b>Wet</b>	65831.2980 Bb	71102.0675 Ab
<b>Dry</b>	98108.0070 Aa	86539.5900 Ba

Means followed by the same uppercase letter in the row and lowercase in the column don't differ statistically by the Tukey's test at 5% probability level.



interaction between lightness and season (Table 3). The biggest midrib thickness and vascular bundle area was in sun leaves in the dry season (528.8154  $\mu\text{m}$  and 98108.0070  $\mu\text{m}^2$ , respectively) (Table 3).

## Discussion

### *Morphoanatomical Characteristics*

*Myrcia splendens* (Sw.) DC. the epidermis is unistratified with markedly sinuous and anticlinal walls were similarly found in other species of Myrtaceae (Retamales & Scharaschkin, 2015) like those found in the genus *Eugenia* (Lemos et al., 2018; Lemos et al., 2019) and *Myrciaria* (Pacheco-Silva & Donato, 2016). Among the epidermal cells are found the cap cells, cellular structures that cover the subepidermal secretory cavities (Ciccarelli, Pagni, & Andreucci, 2003; Costa et al., 2020).

On the abaxial face non-glandular unicellular trichomes are present and scars are sometimes observed (Fontenelle, Costa, & Machado, 1994; Armstrong, Duarte, & Miguel, 2012; Retamales & Scharaschkin, 2015) this characteristic is often found in the family Myrtaceae, as well as paracytic stomata (Retamales & Scharaschkin, 2015; Lemos et al., 2018; Lemos et al., 2019; Lemos et al., 2020). The presence of stomata on one side of the leaves (hypostomatic) guarantees the plant a water saving in periods of water scarcity and intense light (Esposito-Polesi et al., 2011).

In *Myrcia splendens* (Sw.) DC. cross sections, the epidermis on both sides is composed of a layer and flattened cells as reported in other Myrtaceae species (Arruda & Victório, 2011; Lemos et al., 2019). The mesophyll of *Myrcia splendens* (Sw.) DC. is characterized by elongated and uniseriate cells of palisade parenchyma, this anatomical feature is frequent in some species in Myrtaceae (Cardoso, Proença, & Sajo, 2009; Lemos et al., 2018) and the spongy parenchyma is composed of 6-8 layers and many intercellular spaces is similar to other species of the genus *Myrcia* (Cardoso et al., 2009). Collateral vascular bundles is distributed in the mesophyll (Kantachot, Chantaranonthai, & Thammathaworn, 2007; Cardoso et al., 2009; Esposito-Polesi et al., 2011) and dorsiventral mesophyll is common in Myrtaceae species (Donato & Morretes, 2009; Larcher & Boeger, 2009; Pacheco-Silva & Donato, 2016; Lemos et al., 2019).

In the midrib, the phloem is present in the upper and lower part of the xylem and these

vascular tissues are surrounded by fibers (Pacheco-Silva & Donato, 2016) and sometimes are the presence of secretory cavities (Cardoso et al., 2009; Lemos et al., 2019). The *M. splendens* presents collenchyma in the midrib, it interrupts the palisade parenchyma on the adaxial face and is composed of several layers like other species of Myrtaceae (Lemos et al., 2018; Lemos et al., 2019). The midrib is convex in abaxial surface (Donato & Morretes, 2007; Alves et al., 2008; Donato & Morretes, 2009; Donato & Morretes, 2011).

### *Environmental Influence*

Species adapted to specific climatic conditions must have adapted some structural adaptations (Nawazish et al., 2006). In *Myrcia splendens* (Sw.) DC., the trichome density no showed statistical difference between seasons, but higher density in shade leaves, as this finding is not consistent with the literature. Trichome density is generally higher in plants from sun-exposed areas and in plants without water availability (not irrigated) (Pérez-Estrada, Cano-Santana, & Oyama, 2000) or water deficit (Bañon et al., 2004). In addition, leaves with trichomes showed a lower rate of transpiration than leaves without trichomes (Pérez-Estrada et al., 2000). *Myrcia splendens* (Sw.) DC. presented trichomes on abaxial face, despite the fact that it didn't have a response of higher density values in sun leaves is a way of adapting environmental stress conditions.

*Myrcia splendens* (Sw.) DC. presents stomata on abaxial face. This characteristic is frequent in plants adapted to xeric environments, as it reduces respiration, allowing the plant to save water (Esposito-Polesi et al., 2011). Stomatal density can be affected by several abiotic environmental factors. In this studied species the leaves of sun and leaves in the dry season presented greater stomatal density. Sun leaves with higher stomatal densities are frequent (Klich, 2000; Morais et al., 2004; Donato & Morretes, 2009).

Leaves that are under more severe environmental conditions have higher stomatal densities (Rabelo et al., 2013). Water deficit (Batista, Guimarães, Pereira, Carvalho, & Castro, 2010; Vile et al., 2012), exposure to light (Gamage, Ashton, & Singhakumara, 2003; Donato & Morretes, 2009) and high temperature increases stomatal density corroborating with this study, which dry season leaves showed higher densities. Another study indicates that plants

subjected to water stress have higher stomatal density (Bañón et al., 2004) corroborating with this study.

In *Myrcia splendens* (Sw.) DC. the stomatal index and density showed higher values in dry season leaves and sun lightness. Different results were found in a study with leaves that, when subjected to lower temperatures presented a higher stomatal index than plants subjected to high temperatures (Vile et al., 2012). Regarding water availability, stomatal index tended to increase in leaves with water deficit (Vile et al., 2012).

Larger stomatal area may be related to greater availability of CO<sub>2</sub> (James & Bell, 2001). Stomatal area in *Myrcia splendens* (Sw.) DC. there was interaction between season and lightness in which shade leaves in the dry season showed larger stomatal areas. This no corroborates with Batista et al. (2010) study. Research has found that when water availability decreases, there is a reduction in stoma size. This occurs so that the plant loses less water in the transpiration process (Batista et al., 2010). In addition, intense light is related to a high rate of photosynthesis and leaves transpiration. High temperatures are also related to increased sweating transpiration (Stewart et al., 2017).

Greater leaf thickness in sun leaves was perceived in *Myrcia splendens* (Sw.) DC. and is ratified by other studies (Klich, 2000; Oguchi, Hikosaka, & Hirose, 2003; Larcher & Boeger, 2009; Lemos et al., 2019). On the other hand, some authors disagree with this result, because thicker leaves are common in shaded environments (Melo Júnior & Boeger, 2016). Higher leaf thickness contributes to maximizing light and carbon absorption (Rabelo et al., 2013) and a greater allocation of chloroplasts, thus increasing photosynthesis (Oguchi et al., 2003; Amorim & Melo Júnior, 2017). However, a different result was observed in *Eugenia florida* DC. that leaves exposed to the sun showed greater leaf thickness (Donato & Morretes, 2009) and high light intensity and temperature can generate thicker leaves (Smith, Vogelmann, DeLucia, Bell, & Shepherd, 1997).

The leaves of *Myrcia splendens* (Sw.) DC. in the shade during the dry season, as well as the leaves of sun in the wet season have the greatest mesophyll thickness. Therefore, what induces the thickening of the mesophyll is stress, whether due to excess light or lack of water. Leaves more exposed to luminosity have greater mesophyll thickness and corroborates with the research carried out with leaves of *Chenopodium album* L. (Oguchi et al., 2003), *Alocasia macrorrhiza* Schott

(Sims & Percy, 1992) and *Eugenia florida* DC. (Donato & Morretes, 2009). In this regard, other studies also have observed that the mesophyll of leaves exposed to high light intensity was thicker than leaves exposed to low light (Klich, 2000; Hanba, Kogami, & Terashima, 2002; Oguchi et al., 2003; Dardengo et al., 2017). Larger areas of the mesophyll cells is a way to use water efficiently with a greater capture of CO<sub>2</sub> than loss of water during transpiration (Smith et al., 1997), which corroborates with the present research when leaf was in the shade, but there was no water.

*Myrcia splendens* (Sw.) DC. showed greater thickness of the adaxial epidermis in sun lightness leaves and dry season. In this regard, in Myrtaceae species the sun leaves obtained greater thickness in sun lightness leaves and wet season (Lemos et al., 2018; Lemos et al., 2019). Increasing the thickness of the adaxial epidermis can be a protection against high luminosity on the leaves (Chazdon & Kaufmann, 1993). The result in *Myrcia splendens* (Sw.) DC. shows that there is an interaction of seasonality and lightness on the leaf, indicating a response to the plants most exposed to environmental variations (Mantuano et al., 2006). Greater thickness of the adaxial epidermis is considered a xeromorphic characteristic, which is a response to avoid water loss when plants are subjected to high temperatures and high sun incidence (Fahn & Cutler, 1992).

As for the abaxial epidermis, there was no difference between seasons and luminosity. The study with leaf anatomy of *Elaeagnus angustifolia* L. corroborates in this research, because there is no difference in abaxial epidermis in different luminosities (Klich, 2000). On the other hand, there was an interaction between season and lightness, being greater for dry season in the sun and rainy season in the shade, which fully corroborates with *Myrcia guianensis* (Aubl.) DC. (Lemos et al., 2020), in part with *Eugenia luschnathiana* (O.Berg) Klotzsch ex B.D.Jacks., since the epidermis is more thick in leaves in the sun for both seasons (Lemos et al., 2018) and differs from *Eugenia puniceifolia* (Kunth) DC. in that the interaction was greater in the wet season leaves and sun lightness (Lemos et al., 2019).

In arid environments, generally the adaxial epidermis is thicker than the abaxial epidermis (Lemos et al., 2018; Lemos et al., 2019; Lemos et al. 2020), in order to avoid water loss, which was found in the present study.

The spongy parenchyma thicker in sun leaves has been verified in other works (Larcher & Boeger, 2009; Dardengo et al., 2017; Lemos et

al., 2018) and corroborates with *Myrcia splendens* (Sw.) DC. leaves in which it presented thicker spongy parenchyma in plants submitted to light and in the dry season. On the other hand, this result is not corroborated by DeLucia, Nelson, Vogelmann e Smith (1996), who found that the spongy parenchyma is thicker in shadow leaves, as they need to increase the luminous absorption responsible for photosynthetic activity. As for the thickness of the palisade parenchyma greater in wet season leaves, being corroborated by Lemos et al. (2019). However, sun leaves are more exposed to light (high luminous intensity) and generally have a thicker palisade parenchyma (Sims & Pearcy, 1992; Klich, 2000; Larcher & Boeger, 2009). Thus, the more uniform distribution of light on the leaf by the palisade parenchyma is important, since the thickness of this tissue in sun leaves could be a problem in the distribution of light (Vogelmann, 1993).

Sun leaves with thicker midrib than shade leaves are cited in the literature (Dardengo et al., 2017) and corroborates the result recorded in *Myrcia splendens* (Sw.) DC. More developed midrib is a response to water deficit (Batista et al., 2010). On the other hand, another study found that decreasing midrib thickness is an drought adaptation (Olsen et al., 2013). Vascular bundle area in *Myrcia splendens* (Sw.) DC. is higher in sun and in dry season leaves corroborates with *Eugenia luschnathiana* (O.Berg) Klotzsch ex B.D.Jacks. study (Lemos et al., 2018).

Xylem and phloem area were higher values in dry season *Myrcia splendens* (Sw.) DC. leaves, as for luminosity xylem presents larger areas on shade leaves and phloem higher areas in sun leaves. In *Arabidopsis thaliana* Schur leaves in environments controlled it was found that high light intensity and temperature favored the increase in the area of xylem, as the high solar intensity increased areas of the phloem (Stewart et al., 2017). These data corroborate almost entirely with what was observed in *Myrcia splendens* (Sw.) DC., indicating that the plasticity of acclimatization of leaf vascular bundles in response to the environment conditions (Stewart et al., 2017).

Fibers are strong tissues sclerenchyma that surround vascular tissue (Yu, Liu, Shen, Jiang, & Huang, 2015). It is known that fibers are a possible form of adaptation against water scarcity, which corroborates with the present work, since a larger fibers area of the midrib was found in the dry season.

In *Myrcia splendens* (Sw.) DC the only parameters of the midrib that there was an interaction of the treatments were the midrib

thickness and vascular bundle area of the midrib. This indicates that these parameters responded to the abiotic factors tested (season and luminosity).

Thus, this study evaluated 16 structures of which nine presented interaction of luminosity and season. This demonstrates the influence of abiotic factors on *Myrcia splendens* (Sw.) DC. leaf plasticity. Under natural environmental conditions, water availability and temperature variation can influence independently or together the characteristics and distribution of plant species (Vile et al., 2012). The plasticity of plants is qualified as environmental conditions like water and luminosity influence (Smith et al., 1998). These conditions can be responsible for morphological and anatomical changes in the leaves (Larcher & Boeger, 2009; Defaveri, Arruda, & Sato, 2011). Besides that, water availability is a limiting factor for plant occupation in coastal environments (Rosado & Mattos, 2007).

## Conclusions

Based on the results obtained, it can be concluded that:

1. It was observed that the anatomical phenotypic plasticity in the leaves of *Myrcia splendens* (Sw.) DC is related to environmental variations throughout the year, since most of the analyzed parameters responded to abiotic factors (water and light);

2. This study contributed to the understanding of the environmental influence on morphoanatomy and ecological plasticity of the species *Myrcia splendens* (Sw.) DC in the Vegetation Complex of the Coastal Zone of Ceará.

## Acknowledgments

We thank Fundação Cearense de Apoio ao Desenvolvimento Científico e Tecnológico (FUNCAP) for granting a Master's fellowship to the first author. Conselho Nacional de Pesquisa e Desenvolvimento (CNPq) for financial support. São Gonçalo do Amarante Botanic Garden for logistical support.

## References

- Abdulrahman, M. D., Ali, A. M., Fatihah, H. N. N., Khandaker, M. M., & Mat, N. (2018). Morphological and anatomical studies of *Syzygium polyanthum* (Wight) Walp.

- (Myrtaceae). *Malayan Nature Journal*, 70(3), 309–322.
- Abràmoff, M. D., Magalhães, P. J., & Ram S. J. (2004). Image processing with Image J. *Biophotonics International*, 11(7), 36–42.
- Ali, I., Abbas, S. Q., Hameed, M., Abbas, S. Q., Hameed, M., Naz, N., . . . Kanwal, S. (2009). Leaf anatomical adaptations in some exotic species of *Eucalyptus* L'hér. (Myrtaceae). *Pakistan Journal of Botany*, 41(6), 2717–2727.
- Alves, E. S., Tresmondi, F., & Longui, E. L. (2008). Análise estrutural de folhas de *Eugenia uniflora* L. (Myrtaceae) coletadas em ambientes rural e urbano, SP, Brasil. *Acta Botanica Brasilica*, 22(1), 241–248. doi:10.1590/S0102-33062008000100023
- Amada, G., Onoda, Y., Ichie, T., & Kitayama, K. (2017). Influence of leaf trichomes on boundary layer conductance and gas-exchange characteristics in *Metrosideros polymorpha* (Myrtaceae). *Biotropica*, 0(0), 1–11. doi:https://doi.org/10.1111/btp.12433
- Amorim, B. S., & Alves, M. (2011). Flora da Usina São José, Igarassu, Pernambuco: Myrtaceae. *Rodriguésia*, 62(3), 499–514. doi:10.1590/2175-7860201162306
- Amorim, B. S., & Alves, M. (2012). Myrtaceae from lowland Atlantic forest areas in the state of Pernambuco, Northeastern Brazil. *Phytotaxa* 40(1), 33–54. doi:10.11646/phytotaxa.40.1.6
- Amorim, M. W., & Melo Júnior, J. C. F. (2017). Plasticidade morfoanatômica foliar de *Tibouchina clavata* (Melastomataceae) ocorrente em duas formações de restinga. *Rodriguésia*, 68(2), 545–555. doi:10.1590/2175-7860201768217
- Amorim, M. W., & Melo Júnior, J. C. F. (2016). Plasticidade morfológica de *Myrcia splendens* (Sw.) DC. (Myrtaceae) ocorrente em Mata Atlântica e Cerrado. *Iheringia Série Botânica*, 71(3), 261–268.
- Araujo, R. O., Lucena, E. M. P., Sampaio, V. S., Bonilla, O. H., & Pinheiro, L. F. (2020). Levantamento florístico do Jardim Botânico de São Gonçalo do Amarante, Ceará, Nordeste do Brasil. *Revista Brasileira de Geografia Física*, 13(3), 1162-1176, 2020. doi: 10.26848/rbfg.v13.3.p1162-1176
- Armstrong, L., Duarte, M. R., & Miguel, O. G. (2012). Morpho-anatomy of the leaf and stem of *Eugenia pyriformis*. *Brazilian Journal of Pharmacognosy*, 22(3), 475–481. doi:10.1590/S0102-695X2012005000022
- Arruda, R. C. O., & Victório, C. P. (2011). Leaf secretory structure and volatile compounds of *Eugenia copacabanensis* Kiaersk. (Myrtaceae). *Journal of Essential Oil Research*, 23(5), 1–6. doi:10.1080/10412905.2011.9700472
- Bañon, S., Fernandez, J. A., Franco, J. A., Torrecillas, A., Alarcón, J. J., & Sánchez-Blanco, M. J. (2004). Effects of water stress and night temperature preconditioning on water relations and morphological and anatomical changes of *Lotus creticus* plants. *Scientia Horticulturae*, 101(3), 333–342. doi:10.1016/j.scienta.2003.11.007
- Batista, L. A., Guimarães, R. J., Pereira, F. J., Carvalho, G. R., & Castro, E. M. (2010). Anatomia foliar e potencial hídrico na tolerância de cultivares de café ao estresse hídrico. *Revista Ciência Agronômica*, 41(3), 475–481. doi:10.1590/S1806-66902010000300022
- Boeger, M. R. T., & Wisniewski, C. (2003). Comparação da morfologia foliar de espécies arbóreas de três estádios sucessionais distintos de floresta ombrófila densa (Floresta Atlântica) no Sul do Brasil. *Revista Brasileira de Botânica*, 26(1), 61–72. doi:10.1590/s0100-84042003000100007
- Cardoso, C. M. V., Proença, S. L., & Sajo, M. G. (2009). Foliar anatomy of the subfamily Myrtoideae (Myrtaceae). *Australian Journal of Botany*, 57(2), 148–161. doi:10.1071/BT07176
- Castro, A. S. F., Moro, M. F., & Menezes, M. O. T. (2012). O complexo vegetacional da zona litorânea no Ceará: Pecém, São Gonçalo do Amarante. *Acta Botanica Brasilica*, 26(1), 108–124. doi:10.1590/S0102-33062012000100013
- Chazdon, R. L., & Kaufmann, S. (1993). Plasticity of leaf anatomy of two rain forest shrubs in relation to photosynthetic light acclimation. *Functional Ecology*, 7(4), 385–394. doi:10.2307/2390025
- Ciccarelli, D., Pagni, A. M., & Andreucci, A. C. (2003). Ontogeny of secretory cavities in vegetative parts of *Myrtus communis* L. (Myrtaceae): an example of schizolysigenous

- development. *Israel Journal of Plant Sciences*, 51(3), 193–198. doi:10.1560/12F4-M3YH-WD2D-NF3B
- Costa, I. S. C., Lucena, E. M. P., Bonilla, O. H., Radosavljevic, A., & Coutinho, I. A. C. (2020). Ontogenesis, histochemistry and seasonal and luminous environmental characterization of secretory cavities in leaves of *Myrcia splendens* (Myrtaceae). *Botany*, 98(9), 479-495. doi:10.1139/cjb-2020-0060
- Dardengo, J. F. E., Rossi, A. A. B., Silva, I. V., Pessoa, M. J. G., & Silva, C. J. (2017). Análise da influência luminosa nos aspectos anatômicos de folhas de *Theobroma speciosum* Willd ex Spreng. (Malvaceae). *Ciência Florestal*, 27(3), 843–851. doi:10.5902/1980509828634
- Defaveri, A. C. A., Arruda, R. C., & Sato, A. (2011). Leaf anatomy and morphology of *Eugenia rotundifolia* applied to the authentication of the “abajurú” commercially sold. *Brazilian Journal of Pharmacognosy*, 21(3), 373–381. doi:10.1590/S0102-695X2011005000029
- DeLucia, E. H., Nelson, K., Vogelmann, T. C., & Smith, W. K. (1996). Contribution of intercellular reflectance to photosynthesis in shade leaves. *Plant, Cell and Environment*, 19(2), 159–170. doi:10.1111/j.1365-3040.1996.tb00237.x
- Devi, E. L., Kumar, S., Singh, T. B., Sharma, S. K., Beemrote, A., Devi, C. P., . . . Wani, S. H. (2017). Adaptation strategies and defence mechanisms of plants during environmental stress. In M. Ghorbanpour & A. Varma (Eds), *Medicinal plants and environmental challenges*. (Ch. 20, pp. 359-413) Cham: Springer. doi:10.1007/978-3-319-68717-9\_20
- Donato, A. M., & Morretes, B. L. (2007). Anatomia foliar de *Eugenia brasiliensis* Lam. (Myrtaceae) proveniente de áreas de restinga e de floresta. *Brazilian Journal of Pharmacognosy*, 17(3), 426–443. doi:10.1590/S0102-695X2007000300018
- Donato, A. M., & Morretes, B. L. (2009). Anatomia foliar de *Eugenia florida* DC. (Myrtaceae). *Revista Brasileira de Farmacognosia*, 19(3), 759–770. doi:10.1590/S0102-695X2009000500019
- Donato, A. M., & Morretes, B. L. (2011). Morfo-anatomia foliar de *Myrcia multiflora* (Lam.) DC. - Myrtaceae. *Revista Brasileira de Plantas Medicinai*s, 13(1), 43–51. doi:10.1590/S1516-05722011000100007
- Esposito-Polesi, N. P., Rodrigues, R. R., & Almeida, M. (2011). Anatomia ecológica da folha de *Eugenia glazioviana* Kiaersk (Myrtaceae). *Revista Árvore*, 35(2), 255–263. doi:10.1590/S0100-67622011000200010
- Fahn A., & Cutler D. F. (1992). *Xerophytes*. Berlin: Gebrüder Borntraeger.
- Fontenelle, G. B., Costa, C. G., & Machado, R. D. (1994). Foliar anatomy and micromorphology of eleven species of *Eugenia* L. (Myrtaceae). *Botanical Journal of the Linnean Society*, 116(2), 111–133. doi:10.1111/j.1095-8339.1994.tb00426.x
- Gamage, H. K., Ashton, M. S., & Singhakumara, B. M. P. (2003). Leaf structure of *Syzygium* spp. (Myrtaceae) in relation to site affinity within a tropical rain forest. *Botanical Journal of the Linnean Society*, 141(3), 365–377. doi:10.1046/j.1095-8339.2003.00138.x
- Hanba, Y. T., Kogami, H., & Terashima, I. (2002). The effect of growth irradiance on leaf anatomy and photosynthesis in *Acer* species differing in light demand. *Plant, Cell and Environment*, 25(8), 1021–1030. doi:10.1046/j.1365-3040.2002.00881.x
- James, A. S., & Bell, D. T. (2001). Leaf morphological and anatomical characteristics of heteroblastic *Eucalyptus globulus* ssp. *globulus* (Myrtaceae). *Australian Journal of Botany*, 49(2), 259–269. doi:10.1071/BT99044
- Johansen, D. A. (1940). *Plant microtechnique*. New York: McGraw-Hill.
- Kantachot, C., Chantaranothai, P., & Thammathaworn, A. (2007). Contributions to the leaf anatomy and taxonomy of Thai Myrtaceae. *The Natural History Journal of Chulalongkorn University*, 7(1), 35–45.
- Karnovsky, M. J. (1965). A formaldehyde-glutaraldehyde fixative of high osmolality for use in electron microscopy. *The Journal of Cell Biology*, 27, 137–138.
- Klich, M. G. (2000). Leaf variations in *Elaeagnus angustifolia* related to environmental heterogeneity. *Environmental and*

- Experimental Botany*, 44(3), 171–183. doi:10.1016/S0098-8472(00)00056-3
- Kuster, V. C., Silva, L. C., Meira, R. M. S. A., & Azevedo, A. A. (2018). Structural adaptation and anatomical convergence in stems and roots of five plant species from a “Restinga” sand coastal plain. *Flora*, 243, 77–87. doi:10.1016/j.flora.2018.03.017
- Larcher, L., & Boeger, M. R. T. (2009). Arquitetura foliar de *Odontonema strictum* (Nees) O. Kuntze (Acanthaceae) em duas condições de luminosidade. *Hoehnea*, 36(2), 321–327. doi:10.1590/S2236-89062009000200009
- Lemos, V. O. T., Lucena, E. M. P., Bonilla, O. H., & Edson-Chaves, B. (2019). Ecological anatomy of *Eugenia puniceifolia* (Kunth) DC. (Myrtaceae) in the restinga region, state of Ceará. *Revista Brasileira de Fruticultura*, 41(6), 1–11. doi:10.1590/0100-29452019503
- Lemos, V. O. T., Lucena, E. M. P., Bonilla, O. H., Edson-Chaves, B., Castro, M. A., & Sampaio, V. S. (2018). Ecological anatomy of *Eugenia luschnathiana* (O. Berg) Klotzsch ex B. D. Jacks. (Myrtaceae) leaves in the Restinga region, state of Ceara. *Revista Brasileira de Fruticultura*, 40(4), 1–14. doi:10.1590/0100-29452018696
- Lemos, V. O. T., Lucena, E. M. P., Bonilla, O. H., Edson-Chaves, B., & Freitas M. A. (2020). Anatomia ecológica foliar de *Myrcia guianensis* (Aubl.) DC. na Restinga Cearense. *Ciência Florestal*, 30(2), 307–322. doi:10.5902/1980509830997
- Li, W-D., Biswas, D. K., Xu, H., Xu, C-Q., Wang, X-Z., Liu, J-K., & Jiang, G-M. (2009). Photosynthetic responses to chromosome doubling in relation to leaf anatomy in *Lonicera japonica* subjected to water stress. *Functional Plant Biology*, 36(9), 783–792. doi:10.1071/FP09022
- Lucena, E. M. P., Major, I., & Bonilla, O. H. (2011). *Frutas do litoral cearense*. Fortaleza: EdUECE.
- Mantuano, D. G., Barros, C. F., & Scarano, F. R. (2006). Leaf anatomy variation within and between three “restinga” populations of *Erythroxylum ovalifolium* Peyr. (Erythroxylaceae) in Southeast Brazil. *Revista Brasileira de Botânica*, 29(2), 209–215. doi:10.1590/S0100-84042006000200002
- McVaugh, R. (1968). The genera of American Myrtaceae: an interim report. *Taxon*, 17(4), 354–418. doi:10.2307/1217393
- Melo Júnior J. C. F., & Boeger M. R. T. (2016). Leaf traits and plastic potential of plant species in a light-edaphic gradient from Restinga in southern Brazil. *Acta Biológica Colombiana*, 21(1), 51–62. doi:10.15446/abc.v21n1.47621
- Melo Júnior, J. C. F., & Boeger, M. R. T. (2017). Functional traits of dominant plant species of the Brazilian sandy coastal plain. *International Journal of Current Research*, 9(1), 45585–45593.
- Morais, H., Medri, M. E., Marur, C. J., Caramori, P. H., Ribeiro, A. M. A., & Gomes, J. C. (2004). Modifications on leaf anatomy of *Coffea arabica* caused by shade of pigeonpea (*Cajanus cajan*). *Brazilian Archives of Biology and Technology*, 47(6), 863–871. doi:10.1590/S1516-89132004000600005
- Morais, P. O., & Lombardi, J. A. (2006). A família Myrtaceae na Reserva Particular do Patrimônio Natural da Serra do Caraça, Catas Altas, Minas Gerais, Brasil. *Lundiana*, 7(1), 3–32.
- Moresco, H. H., Pereira, M., Bretanha, L. C., Micke, G. A., Pizzolatti, M. G., & Brighente, I. M. C. (2014). Myricitrin as the main constituent of two species of *Myrcia*. *Journal of Applied Pharmaceutical Science*, 4(2), 1–7. doi:10.7324/JAPS.2014.40201
- Moro, M. F., Macedo, M. B., Moura-Fé, M. M., Castro, A. S. F., & Costa, R. C. (2015). Vegetação, unidades fitoecológicas e diversidade paisagística do estado do Ceará. *Rodriguésia*, 66(3), 717–743. doi:10.1590/2175-7860201566305
- Nakamura, M. J., Monteiro, S. S., Bizarri, C. H. B., Siani, A. C. R., & Mônica, F. S. (2010). Essential oils of four Myrtaceae species from the Brazilian southeast. *Biochemical Systematics and Ecology*, 38(6), 1170–1175. doi:10.1016/j.bse.2010.11.003
- Nawazish, S., Hameed, M. & Naurin, S. (2006). Leaf anatomical adaptations of *Cenchrus ciliaris* L. from the Salt Range, Pakistan against drought stress. *Pakistan Journal of Botany*, 38(5), 1723–1730.
- Oguchi, R., Hikosaka, K., & Hirose, T. (2003). Does the photosynthetic light-acclimation need

- change in leaf anatomy? *Plant, Cell and Environment*, 26(4), 505–512. doi:10.1046/j.1365-3040.2003.00981.x
- Oguchi, R., Onoda, Y., Terashima, I., & Tholen, D. (2018). Leaf anatomy and function. In W. W. Adams III. & I. Terashima (Eds), *The leaf: a platform for performing photosynthesis*. (Ch. 6, pp. 97–139). Cham: Springer. doi:10.1007/978-3-319-93594-2\_5
- Olsen, J. T., Caudle, K. L., Johnson, L. C., Baer, S. G. & Maricle, B. R. (2013). Environmental and genetic variation in leaf anatomy among populations of *Andropogon gerardii* (Poaceae) along a precipitation gradient. *American Journal of Botany*, 100(10), 1957–1968. doi:10.3732/ajb.1200628
- Pacheco-Silva, N. V., & Donato, A. M. (2016). Morpho-anatomy of the leaf of *Myrciaria glomerata*. *Brazilian Journal of Pharmacognosy*, 26(3), 275–280. doi:10.1016/j.bjp.2015.12.002
- Pérez-Estrada, L. B., Cano-Santana, Z., & Oyama, K. (2000). Variation in leaf trichomes of *Wigandia urens*: environmental factors and physiological consequences. *Tree Physiology*, 20(9), 629–632. doi:10.1093/treephys/20.9.629
- Pontes, F. C., Abdalla, V. C. P., Imatomi, M., Fuentes, L. F. G., & Gualtieri, S. C. J. (2019). Antifungal and antioxidant activities of mature leaves of *Myrcia splendens* (Sw.) DC. *Brazilian Journal of Biology*, 79(1), 127–132. doi:10.1590/1519-6984.179829
- Rabelo, G. R., Vitória A. P., Silva, M. V. A., Cruz, R. A., Pinho, E. I. B., Ribeiro, D. R., . . . Cunha, M. (2013). Structural and ecophysiological adaptations to forest gaps. *Trees*, 27(1), 259–272. doi:10.1007/s00468-012-0796-2
- Retamales, H. A., & Scharaschkin, T. (2015). Comparative leaf anatomy and micromorphology of the Chilean Myrtaceae: taxonomic and ecological implications. *Flora*, 217, 138–154. doi:10.1016/j.flora.2015.10.005
- Rosado, B. H. P., & Mattos, E. A. (2007). Variação temporal de características morfológicas de folhas em dez espécies do Parque Nacional da Restinga de Jurubatiba, Macaé, RJ, Brasil. *Acta Botanica Brasilica*, 21(3), 741–752. doi:10.1590/S0102-33062007000300020
- Santos, L. S., Alves Filho, E. G., Zocolo, G. J., Silva, S. M., Lucena, E. M. P., Alves, R. E., & Brito, E. S. (2020). Chemotaxonomic evaluation of different species from the Myrtaceae family by UPLC-qToF/MS-MS coupled to supervised classification based on genus. *Biochemical Systematics and Ecology*, 90, 104028/1-10. doi:10.1016/j.bse.2020.104028
- Scio, E., Mendes, R. F., Motta, E. V. S., Bellozi, P. M. Q., Aragão, D. M. O. A., Mello, J., . . . & Bouzada, M. L. M. (2012). Antimicrobial and antioxidant activities of some plant extracts. In V. Rao (Ed.), *Phytochemicals as nutraceuticals: global approaches to their role in nutrition and health*. (1st ed., Ch. 2, pp. 21-42) Rijeka: InTech. doi:10.5772/27308
- Sims, D. A., & Pearcy, R. W. (1992). Response of leaf anatomy and photosynthetic capacity in *Alocasia macrorrhiza* (Araceae) to a transfer from low to high light. *American Journal of Botany*, 79(4), 449–455. doi:10.1002/j.1537-2197.1992.tb14573.x
- Smith, W. K., Bell, D. T., & Shepherd, K. A. (1998). Associations between leaf structure, orientation, and sunlight exposure in five Western Australian communities. *American Journal of Botany*, 85(1), 56–63. doi:10.2307/2446554
- Smith, W. K., Vogelmann, T. C., DeLucia, E. H., Bell, D. T., & Shepherd, K. A. (1997). Leaf Form and Photosynthesis. *BioScience*, 47:785–793. doi:10.2307/1313100
- Stewart, J. J., Polutchko, S. K., Adams, W. W., Cohu, C. M., Wenzl, C. A., & Demmig-Adams, B. (2017). Light, temperature and tocopherol status influence foliar vascular anatomy and leaf function in *Arabidopsis thaliana*. *Physiologia Plantarum*, 160(1), 98–110. doi:10.1111/ppl.12543
- Sultan, S. E. (2000). Phenotypic plasticity for plant development, function and life history. *Trends in Plant Science*, 5(12), 537–542. doi:10.1016/S1360-1385(00)01797-0
- The Brazil Flora Group. (2018). Brazilian Flora 2020: innovation and collaboration to meet Target 1 of the Global Strategy for Plant Conservation (GSPC). *Rodriguésia*, 69(4), 1513–1527. doi:10.1590/2175-7860201869402
- Thiers, B. (2019). *Index Herbariorum: a global directory of public herbaria and associated*

- staff. New York: New York Botanical Garden's Virtual Herbarium. Retrieved Sep 18, 2019, from <http://sweetgum.nybg.org/science/ih/>
- Universidade Federal do Ceará. (2018). *Estação Agrometeorológica*. Retrieved Nov 20, 2019, from <https://ppgea.ufc.br/pt/infraestrutura/estacoes-experimentais/estacao-agrometeorologica/>
- Vile, D., Pervent, M., Belluau, M., Vasseur, F., Bresson, J., Muller, B., . . . Simonneau, T. (2012). *Arabidopsis* growth under prolonged high temperature and water deficit: independent or interactive effects? *Plant, Cell & Environment*, 35(4), 702–718. doi:10.1111/j.1365-3040.2011.02445.x
- Vogelmann, T. C. (1993). Plant Tissue Optics. *Annual Review of Plant Physiology and Plant Molecular Biology*, 44(1), 231–251. doi:10.1146/annurev.pp.44.060193.001311
- Wyka, T., Robakowski, P., & Zytowskiak, R. (2007). Acclimation of leaves to contrasting irradiance in juvenile trees differing in shade tolerance. *Tree Physiology*, 27(9), 1293–1306. doi:10.1093/treephys/27.9.1293
- Yu, H., Liu, R., Shen, D., Jiang, Y., & Huang, Y. (2005). Study on morphology and orientation of cellulose in the vascular bundle of wheat straw. *Polymer*, 46(15), 5689–5694. doi:10.1016/j.polymer.2005.05.052
- Zhu, S-D., Chen, Y-J., Cao, K-F., & Ye, Q. (2015). Interspecific variation in branch and leaf traits among three *Syzygium* tree species from different successional tropical forests. *Functional Plant Biology*, 42, 423–432. doi:10.1071/FP14201