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ECOSYSTEMS

Phenotypic plasticity in *Copaifera langsdorffii* Desf. in different forest fragments in São Paulo state, Brazil

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Abstract: Forest fragments are susceptible to environmental shifts and this demands high phenotypic plasticity of the species growing in these areas. In this context, the objective of the present work was to study the phenotypic plasticity of copaíba (*Copaifera langsdorffii* Desf.) based on morphological and anatomical metrics of the leaflets of plants from six forest fragments. The leaflets of *C. langsdorffii* individuals of the different fragments did not show qualitative differences, nonetheless, they demonstrated quantitative plasticity. Stomatal density ($p = 0.017$), specific leaf area ($p = 0.009$), palisade parenchyma ($p = 0.008$) and relative water content ($p = 0.002$), indicated a high luminous, water and nutritional influence on the development of leaflets. Based on the dry mass of the leaflets and the thickness of the palisade parenchyma, the principal component analysis explained 57.43% of the differences found between the variables. The data presented here provides evidence of the phenotypic plasticity of *C. langsdorffii* which, although occurring in similar soils, showed significant quantitative differences in its morphoanatomical characters.

Key words: Atlantic forest, Forest fragments, leaf anatomy, morphoanatomy, Tropical forests, vegetation types.

INTRODUCTION

Much of the current knowledge about phenotypic plasticity comes from plant studies that document the variety of phenotypes that can be produced by individual genotypes in response to contrasting conditions (Sultan 2000). Species with high phenotypic plasticity have higher survival chances in unstable, heterogeneous or transitional environments due to their ability to acclimate morphologically, physiologically and biochemically, and to overcome environmental stressors (Olguin et al. 2020). The data obtained so far have been instrumental in understanding not only the high number of ecosystem environmental factors (Gratani 2014), but also how the impacts associated with climate change can be decisive to select the genotypes more adapted to a new condition (Arnold et al. 2019).

Plasticity studies may involve functional, morphological, physiological and phenological characterization (Violle et al. 2007). In a broader concept, functional characteristics are those associated with species' responses to changes (*e.g.* climate, soil resources, fire, etc.) in the environment in which they live (Lavorel & Garnier 2002). Leaf-related characteristics, for example, play crucial roles in their physiology and phenology because they are to constant biotic and abiotic pressures in the environment in which the plant grows (Pringle et al. 2011).

Water seasonality and accentuated irradiation are the dominant ecophysiological parameters in tropical forests, which results in physiological, phenological, structural and biochemical acclimation of plants (Lüttge 2008). Thus, leaf deciduity in species in these regions is a strategy to withstand prolonged periods of drought or heat, significantly reducing water loss through transpiration (Lüttge 2008, Tomlinson et al. 2013).

Another determining factor in the composition of plant communities is the availability of nutrients in the soil, over which plants play an important role in the cycling of organic compounds (Gmach et al. 2020). Plant-nutrient relations have been intensively studied (*e.g.*, Aerts & Chapin 2000, Pereira-Silva et al. 2012), and analyses of variation in phenotypic characteristics along gradients in communities, especially temperate ones, have led to the recognition of characteristic profiles of rich and poor soils (Paoli 2006). Climate and soil are important factors in phenotypic plasticity of trees (Souza et al. 2018), and their continuous quantification is essential for the development of new models to assess the effects of climate changes (Ordoñez et al. 2009).

One of the main tools for the study of phenotypic plasticity in plants that suffer different environmental pressures is the morphological and anatomical analysis of the leaves (Castro et al. 2009). Studies on leaf morphology and anatomy have described a great variation in leaf tissues of tree species related to light variations, soil nutrients and the effects of seasonality (Rossatto et al. 2008, Somavilla & Ribeiro 2011). The evaluation of such characteristics is based on the understanding of the relationship between the environment and the leaf structure (Vieira et al. 2014), with the objective of identifying ecophysiological responses to environmental stress within a given community or landscape (Gratani 2014).

Despite the high deforestation rates, São Paulo state houses forest fragments of high floristic diversity (Mangueira et al. 2021). The midwest part of the state is characterized by the occurrence of physiognomies of the two phytogeographic domains found in São Paulo – the Cerrado (Central Brazilian Savanna) and Floresta Atlântica (Atlantic Rainforest), where important transitional areas are found (SMA 2017). The forest fragments in this region are represented by patches of Cerradão and Floresta Estacional Semidecidual (Semideciduous Seasonal Forest), their distribution being related mostly to edaphic factors (Oliveira-Filho & Ratter 1995), but they are both characterized by an expressive leaf deciduity in individuals of certain species during part of the year (IBGE 2012).

Transitional areas are poorly studied in plant ecology, leading to a scarcity in data regarding acclimation processes of species. Indeed, the factors related to phenotypic plasticity in transitional areas are still very poorly known, and are in general related to climate and geomorphology, including edaphic characteristics such as fertility, granulometry and drainage (Askew et al. 1970, Ruggiero et al. 2002, Cavassan 2013). Studies that produce data about phenotypic plasticity of widely distributed species in areas such as the Cerrado might help the comprehension of the extension of such plasticity (Goulart et al. 2011).

Copaifera langsdorffii Desf. (Fabaceae) is a tree species with a broad geographical distribution, with medicinal importance and a valuable element in the restoration of degraded areas. “Copaíba” – as it is popularly known – is particularly frequent in savannas and seasonal forests but is also found in several physiognomies of nearly all Brazilian phytogeographic domains (Costa 2020), thus suggesting a great capacity for acclimatization of the most diverse environmental conditions. For this reason, the species was chosen for an analysis of phenotypic plasticity, based on the observation of the morphological and anatomical differences of the leaflets, with the objective of providing subsidies in the understanding of how the species responds to different environmental pressures.

MATERIALS AND METHODS

Description of the area and collection of samples

Sampling took place in the midwest region of São Paulo state (southeastern Brazil) in areas of Semideciduous Seasonal Forest (a physiognomy of the Atlantic Forest domain) and its transitions to Cerradão (a physiognomy of the Cerrado phytogeographic domain). These domains are two Brazilian hotspots for biodiversity conservation (Myers et al. 2000). The climate is humid tropical with dry winters and hot summers, with air temperatures in the hottest month above 22°C; the average rainfall is less than 60 mm in at least one of the months of the season (Alvares et al. 2013). The sampled areas comprised secondary, regenerating and ecotone forests from six forest fragments in public and private areas, located in four municipalities of the state: Agudos, Bauru, Gália and Pederneiras (Figure 1). The Bauru Botanical Garden (BBG), Legal Reserve of UNESP - *campus* Bauru (LRU) and Pederneiras State Forest (PSF) fragments are covered by patches of Cerradão and Seasonal Semideciduous Forest, thus corresponding to important ecotone regions. The other sampled areas (Aimorés Forest Park - AFP, Caetetus Ecological Station - CES and Duratex Legal

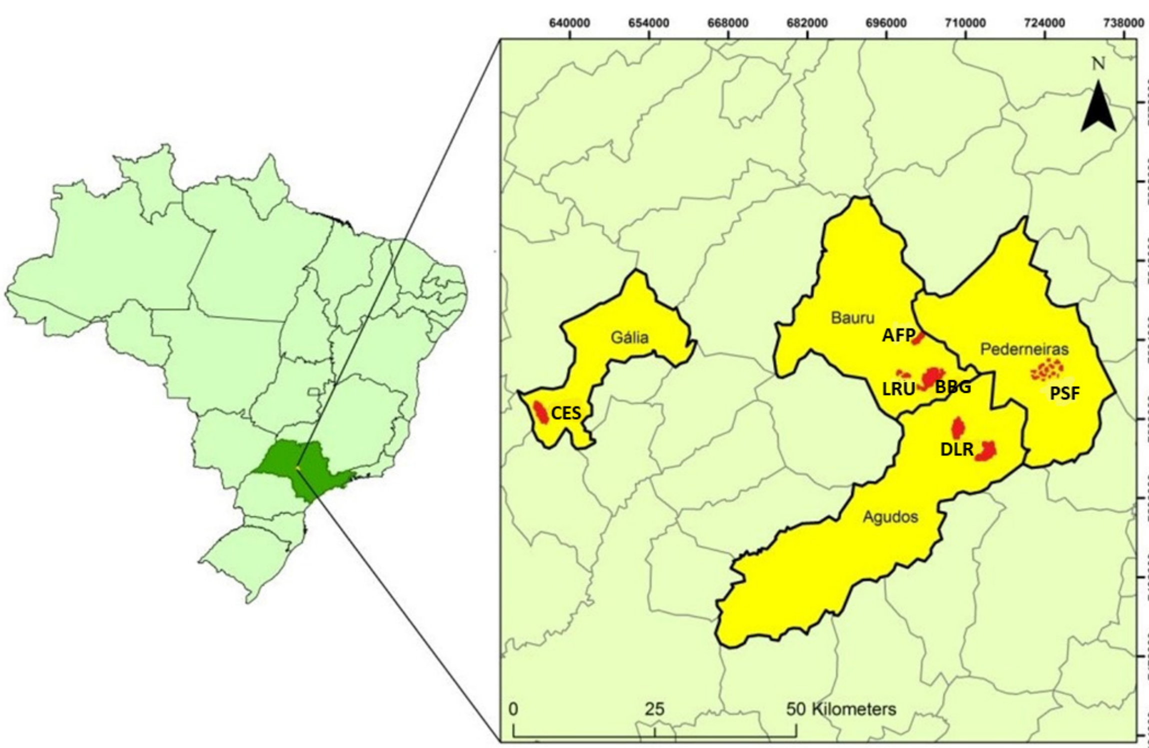

Figure 1. Collection sites of samples of copaíba (*Copaifera langsdorffii*) in four municipallities of midwestern São Paulo state (BBG: Bauru Botanical Garden; LRU: Legal Reserve of UNESP – campus Bauru; AFP: Aimorés Forest Park; PSF: Pederneiras State Forest; DLR: Duratex Legal Reserve; CES: Caetetus Ecological Station).

Reserve - DLR) comprise only Semideciduous Seasonal Forests, and DLR stands out for being a private area in process of natural regeneration (Table I; Figure 1).

Five fully expanded leaves were collected in the median region (between the 3rd and 4th nodes) of the lower branches of the canopy of adult individuals of *C. langsdorffii*, and the leaflets were fixed in FAA_{50} and preserved in ethanol 70%. A total of 95 adult trees from 6 populations were sampled between May and June 2015, prioritizing reproductive individuals with a minimum distance of 10 m between them and with different environmental growing conditions (Figure 1, Table I).

Biometric analyses

Fresh weight, dry weight and area of leaflets were evaluated from 475 samples. The total fresh mass and the total dry mass (g) were obtained by weighing material on an analytical balance (Shimadzu, model AY220) from the recently collected leaflets and after drying at 120˚C for 48 hours in an oven (FANEM, model 315 SE). The leaf area (cm^2) was obtained using the Image J software (Schneider et al. 2012) from scanned images of the leaflet. The specific leaf area (AFE) was determined through the ratio between the leaf area and the dry mass found in the leaflets (Wilson et al. 1999). The relative water content (%) was calculated using the formula proposed by Barrs (1968).

Anatomical and micromorphometric analyses

The material for anatomical analysis was fixed in FAA_{70} (formalin, glacial acetic acid and 70% ethanol in the proportion of 1:1:18) and stored in 70% ethanol (Johansen 1940).

Transverse sections from the middle region of the leaflets and paradermic sections of the abaxial and adaxial surfaces of the epidermis were obtained freehand and clarified in sodium hypochlorite (20%), washed several times in distilled water and stained with 0.05% Toluidine Blue in acetate buffer, pH 4.7 (O'Brien et al. 1964, modified). Slides mounted in glycerin water were analyzed and photographed using a Nikon® eclipse 80i optical photomicroscope.

Two slides per leaflet were used for micromorphometric analyses. The evaluated parameters were stomatal density, stomatal index, polar and radial diameter of the stomata, thickness of epidermis on adaxial and abaxial surfaces, palisade (PP) and spongy (SP) parenchyma and mesophyll. For the measurement of stomata (polar and radial diameter), two measurements were obtained by photomicrography, using two photomicrographs per leaflet; for stomata counting (density and stomatal index) four photomicrographs per

Table I. Collection sites of *C. langsdorffii* in midwestern São Paulo state (Ce: Cerradão; SSF: Semideciduous Seasonal Forest).

Colletion sites	Geographic coordinates	Area (ha)	Number of individuals sampled	Vegetation
Bauru Botanical Garden (BBG)	22°20'30''S, 49°00'30''W	321	29	Transition Ce/SSF
Legal Reserve of UNESP - *campus* Bauru (LRU)	22°20'46''S, 49°01'05''W	132	20	Transition Ce/SSF
Pederneiras State Forest (PSF)	22°06'46"S, 48°55'00"W	430	14	Transition Ce/SSF
Aimorés Forest Park (AFP)	22°17'55''S, 49°01'33''W	5.424	11	SSF
Caetetus Ecological Station (CES)	22°24'11''S, 49°42'05''W	2.178	10	SSF
Duratex Legal Reserve (DLR)	22°06'49''S, 49°55'00''W	2.000	11	SSF

leaflet were used; for the measurement of the epidermis, palisade and spongy parenchyma and mesophyll, two photomicrographs per leaflet were used. The quantitative anatomical parameters were analyzed using the Image-pro Plus 5.1 software.

Soil analyses

Soil samples were collected at 20 and 40 cm depth, at 10 different points of each sampled area, in a way that best represented the fragment. The extraction points were determined in areas with registers of *C. langsdorffii* without human disturbance. The pH was determined and the contents of organic matter (O.M.), calcium (Ca), potassium (K) and magnesium (Mg) were quantified for chemical analysis; soil texture was identified for physical analysis. The respective samples were analyzed according to the methods referring to the IAC Soil Analysis System (Malavolta et al. 1997, Raij et al. 2001).

Statistical analyses

The data obtained were subjected to the calculation of the mean of the respective morphological and anatomical variations. The data collected in the vegetation fragments were compared to each other by means of analysis of variance and Tukey's post-hoc test at the 5% significance level. Subsequently, the results passed the Pearson correlation test at 1% significance for analysis of the correlation between the morphological and anatomical components of the leaflets.

The analysis of variance and the Tukey and Pearson correlation tests were performed using the Past 3.24 software (Hammer et al. 2001). Analysis of variance of the main components was performed using the Origin 2018 software (OriginLab Corporation, Northampton, MA, USA.) using the morphological and anatomical variables of the leaflets and the chemical variables of the soils of the fragments.

RESULTS

Morphoanatomical analyses

The epidermis is uniseriate on both surfaces, with the thickest cuticle on the adaxial surface (Figures 2a-c). The epidermal cells on the abaxial surface are smaller than those on the adaxial surface, which are cubic to rectangular. The leaflet is hypoestomatic with paracitic stomata (Figures 2a and d). There are a few single-celled tector trichomes (Figure 2e).

The mesophyll is of the dorsiventral type, with a single layer of palisade parenchyma and two to five layers of spongy parenchyma with irregularly shaped cells (Figure 2a).

Secretory structures were observed in the mesophyll, with its round-shaped cavity in cross section, delimited by a single layer of secretory cells. They are located between the two parenchyma constituents of the mesophyll, occupying a median position (Figure 2a).

Collateral vascular bundles were found immersed in the mesophyll, surrounded by lignified thick-walled fibers, and crystalline idioblasts containing prismatic calcium oxalate crystals (Figure 2f). In the highest caliber leaf vein region, in addition to these characteristics, there is a uniseriate epidermis on both surfaces and two or three layers of collenchyma internally to the epidermis on both surfaces.

Observing these results, it is noted that in all forest fragments the leaflets of *C. langsdorffii* were anatomically similar.

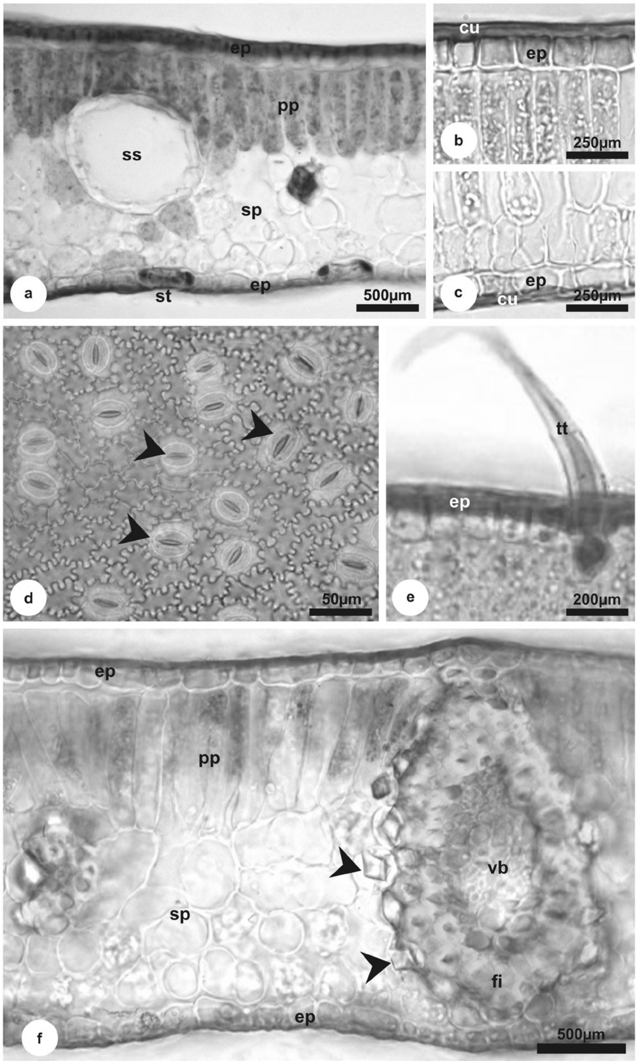

Figure 2. Anatomy of *Copaifera langsdorffii* leaflets. Cross sections (a-c; e-f). Paradermal section (d). Caetetus Ecological Station (a; f). Bauru Botanical Garden (b-d; f). Legal Reserve of UNESP – *campus* Bauru (E). a. General aspect. b. Detail of the epidermis and cuticle of adaxial surface. c. Detail of the epidermis and cuticle of abaxial surface. d. Paracytic stomata (arrow). e. Detail of the epidermis with tector trichome. f. Vascular bundle surrounded by fibers and prismatic crystals (arrow). (cu = cuticle; ep = epidermis; st = stomata; fi = fiber; vb = vascular bundle; sp = spongy parenchyma; pp = palisade parenchyma; ss = secretory structure; tt = tector trichome).

Micromorphometric analyses

The soil collected in the fragments presented mainly the sandy texture, except for the soil of the CES fragment, which presented a sandy-clay texture. The analysis of the environmental data showed that the type soils Red Latosol and Dark Red Latosol are predominant in the sampled areas. From the pH results, the soils of the six fragments are acidic, with an average value of 3.6 (Table II). The highest pH, organic matter and magnesium value was found in the DLR fragment (Table II). The analysis of the main components of the soil (Figure 4) explains 93.18% of the variance found between the studied fragments.

Stomatal density in *C. langsdorffii* individuals differed significantly between forest fragments (Table II). The highest stomatal density was observed in the leaflets of BBG individuals (92 stomata/mm²), while the lowest density was observed in PSF individuals (79 stomata/mm²). The equatorial and polar diameters of the stomata did not differ significantly between the areas (Table II).

Correlation tests showed an average negative relationship (Cohen 1988) between stomata density and the equatorial diameter in the CES fragment (r = - 0.37), and only in that fragment there was no relationship between the

Table II. Means of micromorphometric analyses performed on *Copaifera langsdorffii* leaflets. Means indicated by the same lowercase letter on the line do not differ by Tukey's test at 5% significance. (BBG: Bauru Botanical Garden; LRU: Legal Reserve of UNESP – campus Bauru; AFP: Aimorés Forest Park; PSF: Pederneiras State Forest; DLR: Duratex Legal Reserve; CES: Caetetus Ecological Station; 1: component 1 - dry mass of the leaflets; 2: component 2 - thickness of the palisade parenchyma).

PLANT VARIABLES	COLECTION SITES						ANOVA		PCA	
	Transition Ce/SSF			SSF						
	BBG	LRU	PSF	AFP	DLR	CES	F value	p- value	1	2
Fresh mass (g)	0.095	0.116	0.146	0.140	0.139	0.141	1.065	0.404 (>0.05)	-0.430	0.253
Dry mass (g)	0.049	0.076	0.071	0.070	0.076	0.087	1.316	0.290 (>0.05)	-0.449	0.035
Leaf area (cm²)	5.871	7.475	6.784	5.768	6.286	9.755	1.501	0.226 (>0.05)	-0.339	-0.317
Specific leaf area (cm² g^{-1})	133.23 a	98.0 ab	96.73 b	81.99 b	83.295 b	105.049 ab	4.336	0.009 (<0.05)	0.293	-0.411
Relative water content (%)	0.49 ab	0.34 c	0.52 a	0.51 a	0.46 abc	0.35 bc	5.228	0.002 (<0.05)	0.174	0.249
Adaxial surface of epidermis (μm)	13	16	14	14	14	14	0.795	0.556 (>0.05)	-0.158	0.166
Abaxial surface of epidermis (μm)	12	13	12	12	13	13	0.460	0.805 (>0.05)	0.271	-0.264
Palisade parenchyma (μm)	50 b	58 ab	53 ab	58 ab	62 a	53 ab	3.382	0.008 (<0.05)	-0.123	0.447
Spongy parenchyma (μm)	74	72	69	72	79	78	1.264	0.287 (>0.05)	0.171	0.355
Mesophyll (μm)	129	127	123	132	141	126	0.869	0.505 (>0.05)	0.179	0.336
Stomata density (mm²)	92 a	84.5 ab	79 ab	90.5 ab	90.5 ab	80 b	2.888	0.017 (<0.05)	0.435	0.255
Equatorial diameter of the stomata (μm)	15	16	13	14	14	14	1.233	0.298 (>0.05)	0.059	0.098
Polar diameter of the stomata (μm)	21	21	18	20	20	21	0.832	0.529 (>0.05)	0.119	0.016
SOIL VARIABLES	BBG	LRU	PSF	AFP	DLR	CES	F value	p-value	1	2
pH	3.55	3.6	3.5	3.55	4.1	3.7	1.583	0.18 (>0.05)	0.363	-0.659
Organic matter (g dm$^{3\ -1}$)	14.5	19	13	11.5	14.5	20	2.757	0.027 (<0.05)	0.361	0.566
K (mmolc dm$^{3\ -1}$)	0.85 ab	0.95 ab	0.95 ab	0.65 b	1.05 ab	1.75 a	2.434	0.046 (<0.05)	0.466	0.375
Ca (mmolc dm$^{3\ -1}$)	3.5	3	2	1.5	9.5	14.5	2.682	0.030 (<0.05)	0.520	0.039
Mg (mmolc dm$^{3\ -1}$)	1.5	1.5	1	1	6	4.5	2.305	0.057 (>0.05)	0.500	-0.320
Texture	sandy	sandy	sandy	sandy	sandy	sandy-clay				

equatorial diameter and the polar diameter in the analyzed stomata (r = - 0.19).

The specific leaf area (SLA) showed a significant difference between the fragments analyzed. BBG had a higher mean (133.2 cm² / g) and was significantly different from the AFP, PSF and DLR fragments (Table II). From Pearson's correlation analysis at 1%, it was possible to observe that the SLA of the forest fragment BBG was negatively correlated with the dry mass (r = - 0.90) and the mesophyll (r = - 0.67) found in the leaflets of this fragment.

The thickness of the mesophyll and the spongy parenchyma did not show significant variations, differently from the palisade parenchyma (PP) (Table II). The thickness of the palisade parenchyma among the six forest fragments showed significant differences between BBG (50 μm) and DLR (62 μm). In the DLR fragment, the individuals of *C. langsdorffii* presented PP with the highest thickness values found among the fragments (62 μm) and is negatively related (r = - 0.73) to the spongy parenchyma. In addition, the palisade parenchyma showed an average positive relationship with the equatorial diameter of the stomata (r = + 0.31). In the BBG fragment, the thickness of the palisade parenchyma correlated with the organic matter index of the soil (r = + 0.52), different from that found in the DLR fragment where such correlation was not verified.

The relative water content of the leaflets varied significantly between the fragments. Using the Tukey test (5%), LRU differed from BBG, AFP and PSF. PSF was the one that most statistically distanced itself from LRU. CES differed from AFP and PSF, and AFP was more distant from CES. However, LRU and CES were similar to each other and had the lowest values of relative water content in the leaflets, 0.34% and 0.35%, respectively (Table II). AFP and PSF obtained the highest relative water content, with 0.51% and 0.52%, respectively (Table II), and demonstrated a negative relationship with the leaf area and dry mass, as verified in the correlation tests.

Principal component analysis (PCA) indicated a 57.43% correlation between the morphological and anatomical characteristics of the leaflets between vegetation fragments from two main components: dry weight of the leaflets (1) and thickness of the palisade parenchyma (2). The first component (dry mass of leaflets) showed a correlation between dry weight, fresh weight, leaf area, thickness of the adaxial surface of the epidermis and thickness of the palisade parenchyma with the morphological and anatomical characteristics of the leaflets (Figure 3).

As observed in the correlation analysis, the CES fragment is at the opposite end of the vector that represents the density of stomata found in the leaflets. In addition, it is the only vegetation fragment where a negative correlation is found between the density of the stomata and the equatorial diameter of the stomata. This finding is evident in Figure 3, where *C. langsdorffii* populations at CES have the largest numerical distance from stomata density together with the equatorial diameter of the stomata. The BBG forest fragment showed a positive relationship with the specific leaf area and opposite the quadrant of the dry mass, represented by the first component, as previously observed by the Pearson correlation test at 1% (Figure 3).

Regarding the physical and chemical characteristics of the soils (Table II), the CES fragment showed a soil that was richer in potassium, which significantly differentiated it from AFP. The K and Ca ions are positively related to the dry mass (r = + 0.48 and r = + 0.44) and to

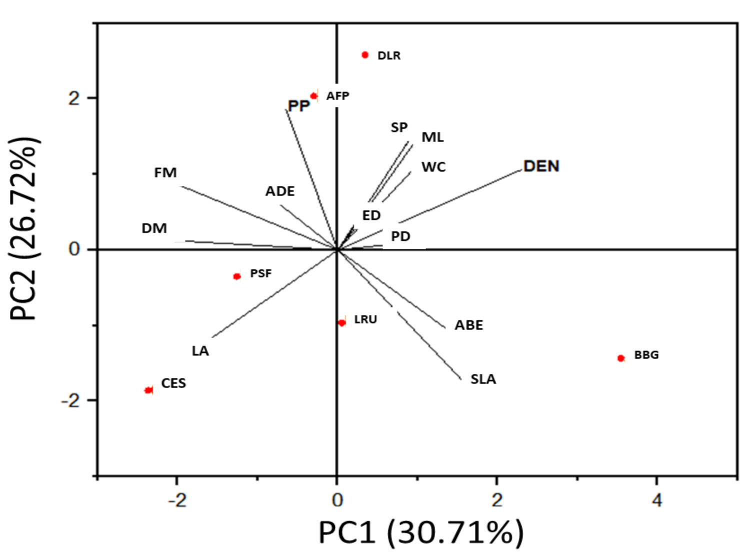

Figure 3. **Principal Components Analysis of morphological, anatomical and physical characteristics of *Copaifera langsdorffii* leaflet (LA: leaf area; SLA: specific leaf area; ED: equatorial diameter; DEN: density of stomata; PD: polar diameter; ABE: abaxial surface of epidermis; ADE: adaxial surface of epidermis; DM: dry mass; ML: mesophyll; FM: fresh mass; PP: palisade parenchyma; SP: spongy parenchyma, WC: water contest) distributed in six fragments (BBG: Bauru Botanical Garden; LRU: Legal Reserve of UNESP – campus Bauru; AFP: Aimorés Forest Park; PSF: Pederneiras State Forest; DLR: Duratex Legal Reserve; CES: Caetetus Ecological Station). PC1: dry mass of leaflets and PC2: thickness of palisade parenchyma.**

the leaf area (r = + 0.75 and r = + 0.60) found in the fragment, and negatively with the relative water content (r = - 0.58 and r = - 0.49).

The variance found in the main components shows that the soil of the CES fragment has a higher content of organic matter, potassium ions and calcium ions. These characteristics are opposite to those found in fragments BBG and AFP. The DLR showed a greater variance of pH, magnesium, potassium and calcium, opposite to what was found in the LRU and PSF forest fragments (Figure 4).

DISCUSSION

The anatomy described for the *C. langsdorffii* leaflets is similar reported for the same species by Moreira-Coneglian & Oliveira (2006) and Nascimento et al. (2014), and the anatomical organization of the leaflets is similar to that found in other species of the Fabaceae family (Metcalfe & Chalk 1950, Mendes & Paviani 1997, Duarte & Debur 2003, Lima et al. 2003, Francino et al. 2006).

The presence of thick cuticle on the adaxial surface of the epidermis and stomata restricted to the abaxial surface of the epidermis of the leaflets are mechanisms involved in decreasing water loss (Müller & Riederer 2005, Esposito-Polesi et al. 2011, Simioni et al. 2017). This is the most common pattern of stomata distribution in terrestrial plants and is considered an important adaptation to water savings due to the greater exposure to the sun on the adaxial surface of the epidermis (Lleras 1977, Smith & McClean 1989), which also explains the thicker cuticle on this surface.

The variation in stomatal density observed in individuals from the different fragments analyzed in this study may be related to water availability and luminosity (Pearce et al. 2006, Gobbi et al. 2011). The high stomatal density observed in the leaflets of *C. langsdorffii* of the BBG fragment is generally observed in leaves of plants exposed to environmental stresses and may be an indication of the acclimation mechanism of these plants to the conditions of low water availability in the soil, which may help to increase control over the rates of water loss and carbon dioxide absorption (Souza et al. 2019). A higher stomatal frequency per unit area has been observed in regions with low

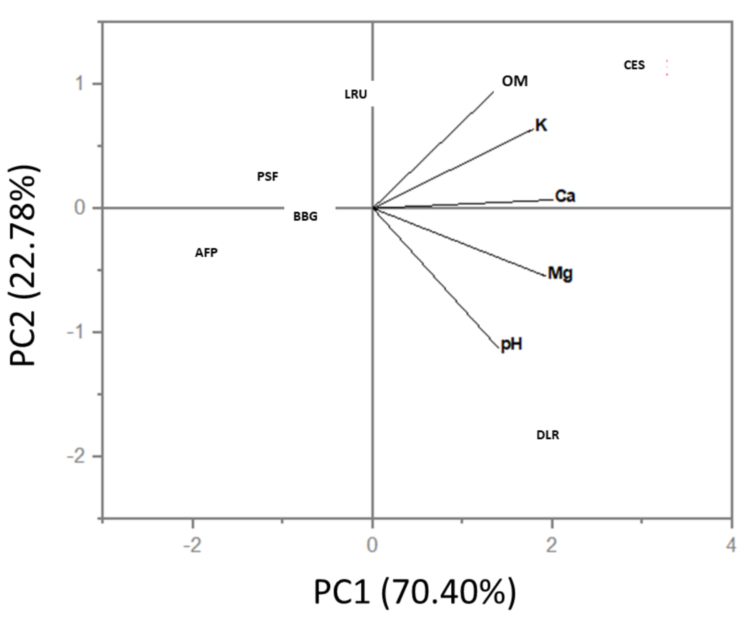

Figure 4. Principal Components Analysis of physico-chemical characteristics of soil (OM: organic material; K: potassium; Ca: calcium; Mg: magnesium, pH) in six vegetation fragments (BBG: Bauru Botanical Garden; LRU: Legal Reserve of UNESP – campus Bauru; AFP: Aimorés Forest Park; PSF: Pederneiras State Forest; DLR: Duratex Legal Reserve; CES: Caetetus Ecological Station). PC1: dry mass of leaflets and PC2: thickness of palisade parenchyma.

water availability (Lleras 1977, Souza et al. 2010, Machado et al. 2015), which has been associated with a more efficient gas exchange in periods of higher humidity, when the stomata can remain open without the risk of excessive dehydration, and this might explain the higher stomatal density in the transition areas between SSF and Cerradão.

Although there are differences in stomatal density, there are no significant differences between the polar diameter and the equatorial diameter of the stomata of the leaflets of the individuals analyzed in the different vegetation fragments. However, the polar and equatorial diameters of the stomata of the leaflets of individuals located in BBG and LRU (transition areas between SSF and Cerradão and, therefore, drier) are numerically higher. As the size of the stomata is related to its functionality, larger diameters can mean more efficient gas exchange, which favors photosynthesis and the existence in drier places, where the opening of the stomata can lead to excessive water loss (Lleras 1977, Souza et al. 2010), justifying the larger diameters found in individuals from the BBG and LRU fragments.

The similarity of the values of stomata functionality, that is the relationship polar diameter/equatorial diameter, points to similarity between the transition and SSF areas. According to Rocha (2005), the relationship between the polar and equatorial diameters provides a good indication of the shape of the stomata, being that the greater this relationship, the more ellipsoid is the stomatal shape and the greater its functionality, as well as, the smaller this relationship is less ellipsoid and less functional is the stomata, indicating that, although there are differences in stomatal density, there are no differences in stomata functionality between the individuals of *C. langsdorffii* in the different fragments.

There were no significant variations in the thickness of the mesophyll and the spongy parenchyma of the leaflets between individuals of *C. langsdorffii* in the different fragments, but significant differences were found in the thickness of the palisade parenchyma. The difference in thickness between the BBG (50 μm) and DLR (62 μm) fragments corroborates the indication of low light incidence in the BBG compared to the other vegetation fragments. The negative correlation (r = - 0.73) between the PP and the SP found in the DLR fragment shows a strong indication of a decrease in the intracellular spaces of the leaflet, resulting from the plastic adaptability of plants to drier environments (Esau 1974). The positive correlation of PP with the equatorial diameter of the stomata (r = + 0.52), indicates that there is a change in the shape of the stomata due to the favorable conditions for photosynthetic efficiency, which may indicate an increase in their functionality, once the more ellipsoid the stomata shape, the greater functionality, since increases in polar and equatorial dimensions promote greater stomatal conductance (Martins et al. 2009, Aragão et al. 2014, Eburneo et al. 2017).

The highest value of specific leaf area and the lowest thickness of the palisade parenchyma found in the leaves of *C. langsdorffii* collected in the BBG fragment in relation to the values observed in the individuals of DLR and CE fragments, may be related to the lower light incidence. According to Esau (1974) and Dickison (2000), in environments with high luminosity the leaves tend to be smaller and thicker to regulate the light radiation and the diffusion of carbon dioxide. Oguchi et al. (2003), Justo et al. (2005), Aragão et al. (2014) and Fernandes et al. (2014), also described an increase in the thickness of the limbus due to the increase in luminosity.

Melo Júnior et al. (2012) explained the structural variations found in the mesophyll of the individuals of *C. langsdorffii* studied as resulting from different conditions of exposure to the sun, which was also reported by Voltan et al. (1992) for *Coffea arabica* in which it was concluded that under high radiation conditions there is leaf thickening induced by the expansion of mesophyll cells and by the cell elongation of the palisade parenchyma. This also explains the greater thickness of the palisade parenchyma in trees located in DLR, where individuals were more exposed to the sun than in BBG, where they were under more shading conditions.

According to Nascimento et al. (2014), the temperature can also interfere in the stomatal density and thickness of the mesophyll and palisade and spongy parenchyma, however, the distance in which the sampled fragments are found suggest that there is not a significant temperature differences to the point of reflecting in the anatomy of the individuals.

As for the leaf area, although there were no significant differences between the individuals of the different fragments, there was a greater leaf area in the individuals of CES and a smaller leaf area in individuals of BBG, which may be related to the different degrees of exposure to solar radiation, since, according to Dickison (2000) and Larcher (2000), a reduction in leaf area is expected in plants more directly exposed to the sun, which was also observed by Melo Júnior et al. (2012) for *C. langsdorffii*.

Leaf area reduction can also be a water conservation strategy in plants growing in soils with lower water holding capacity and low nutrient availability (Brünig 1973), which may also explain the smaller leaf area found in BBG, which presents sandy soil and with a lower amount of organic matter, K, Ca and Mg than the CES soil, where the leaf area found in *C. langsdorffii* was much larger.

Considering that phenotypic plasticity is the ability of a single genotype to produce different phenotypes in multiple environmental conditions (Sultan 2000), the data presented here provide support regarding the high phenotypic plasticity of *C. langsdorffii* which has significant quantitative differences in the specific leaf area, in the thickness of palisade parenchyma and in the stomata density. This phenotypic plasticity is probably related to the wide distribution of *C. langsdorffii* and its versatility in occupying different environments.

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REFERENCES

AERTS R & CHAPIN III FS. 2000. The mineral nutrition of wild plants revisited: a re-evaluation of processes and patterns. Adv Ecol Res 30: 1-67.

ALVARES CA, STAPE JL, SENTELHAS PC, GONÇALVES JLM & SPAROVEK G. 2013. Köppen's climate classification map for Brazil. Meteorologische Zeitschrift 22: 711-728. https://doi.org/10.1127/0941-2948/2013/0507.

ARAGÃO DS, LUNZ AMP, OLIVEIRA, LC, RAPOSO A & FERMINO JR PCP. 2014. Efeito do sombreamento na anatomia foliar de plantas jovens de Andiroba (*Carapa guianensis* Aubl.). Rev Árvore 38(4): 631-639.

ARNOLD PA, KRUUK LEB & NICOTRA AB. 2019. How to analyse plant phenotypic plasticity in response to a changing climate. New Phytol 222(3): 1235-1241.

ASKEW GP, MOFFATT DJ, MONTGOMERY RF & SEARL PL. 1970. Interrelationships of soils and vegetation in the Savanna-Forest boundary zone of north-eastern Mato Grosso. Geograph J 136(3): 370-376. http://www.jstor.org/stable/1795187.

BARRS HD. 1968. Effect of cyclic variations in gas exchange under constant environmental conditions on the ratio of transpiration to net photosynthesis. Physiol Plant 21: 918-922.

BRÜNIG EF. 1973. Species richness and stand diversity in relation to site and succession forests in Sarawak and Brunei (Borneo). Amazoniana 4(3): 293-320.

CASTRO EM, PEREIRA FJ & PAIVA R. 2009. Histologia vegetal: estrutura e função de órgãos vegetativos, 1a ed., Universidade Federal de Lavras, 244 p.

CAVASSAN O. 2013. Bauru: terra de cerrado ou floresta? Ci Geogr 17: 46-54.

COHEN J. 1988. Statistical power analysis for the behavioral sciences. 2nd ed, Hillsdale: Erlbaum, 567 p.

COSTA JAS. 2020. *Copaifera in* Flora do Brasil 2020 under construction. Jardim Botânico do Rio de Janeiro. Available at: <http://floradobrasil.jbrj.gov.br/reflora/floradobrasil/FB22896>. Accessed on 07 Apr. 2020.

DICKISON WC. 2000. Integrative plant anatomy. 1st ed., San Diego: Harcourt Academic Press, 533 p.

DUARTE MR & DEBUR MC. 2003. Caracteres morfo-anatômicos de folha e caule de *Bauhinia microstrachya* (Raddi) J. F. Macbr. (Fabaceae). Rev Bras de Farmacogn 13(1): 7-15.

EBURNEO L, RIBEIRO-JÚNIOR NG, KARSBURG IV, ROSSI AAB & SILVA IV. 2017. Anatomy and micromorphometric analysis of leaf *Catasetum* x *apolloi* Benelli & Grade with addition of potassium silicate under different light sources. Braz J Biol 77: 140-149.

ESAU K. 1974. Anatomia das plantas com sementes. São Paulo: Editora Blucher, 293 p.

ESPOSITO-POLESI NP, RODRIGUES RR & ALMEIDA M. 2011. Anatomia ecológica da folha de *Eugenia glazioviana* Kiaersk (Myrtaceae). Rev Árvore 35(2): 255-263.

FERNANDES VF, BEZERRAL LA, MIELKEL MS, SILVA DC & COSTA LCB. 2014. Anatomia e ultraestrutura foliar de *Ocimum gratissimum* sob diferentes níveis de radiação luminosa. Ciênc Rural 44(6): 1037-1042.

FRANCINO DMT, SANT'ANNA-SANTOS BF, SILVA KLF, THADEO M, MEIRA RMSA & AZEVEDO AA. 2006. Anatomia foliar e caulinar de *Chamaecrista trichopoda* (Caesalpinioideae) e histoquímica do nectário extrafloral. Planta Daninha 24(4): 695-705.

GMACH MR, CHERUBIN MR, KAISER K & CERRI CEP. 2020. Processes that influence dissolved organic matter in the soil: a review. Sci Agr 77(3): e20180164.

GOBBI KF, GARCIA R, VENTRELLA MC, GARCEZ NETO AF & ROCHA GC. 2011. Área foliar específica e anatomia foliar quantitativa do capim-braquiária e do amendoim-forrageiro submetidos a sombreamento. R Bras Zootec 40: 1436-1444.

GOULART MF, LOVATO MB, BARROS FV & LEMOS-FILHO JP. 2011. Which Extent is Plasticity to Light Involved in the Ecotypic Differentiation of a Tree Species from Savanna and Forest? Biotropica 43(6):695-703.

GRATANI L. 2014. Plant phenotypic plasticity in response to environmental factors. Advances in Botany, Article ID 208747, 17 p.

HAMMER O, HARPER DAT & RYAN PD. 2001. PAST: Paleontological statistic software package for education and data analysis. Paleontol Eletron 4(1): 1-9.

IBGE. 2012. Manual técnico da vegetação brasileira. 2ª ed. Manuais técnicos em geociências. IGBE, Rio de Janeiro.

JOHANSEN DA. 1940. Plant microtechnique. New York: McGraw-Hill, 523 p.

JUSTO CF, SOARES AM, GAVILANES ML & CASTRO EM. 2005. Plasticidade anatômica das folhas de *Xylopia brasiliensis* Sprengel (Annonaceae). Acta Bot Brasilica 19(1): 111-123.

LARCHER W. 2000. Ecofisiologia vegetal. São Carlos: RiMa, 439 p.

LAVOREL S & GARNIER E. 2002. Predicting changes in community composition and ecosystem functioning from plant traits: revisiting the Holy Grail. Funct Ecol 16: 545-556.

LLERAS E. 1977. Differences in stomatal number per unit area within the same species under different microenvironmental conditions: a working hypothesis. Acta Amazonica 7(4): 473-476.

LIMA AK, AMORIM ELC, AQUINO TM, LIMA CSA, PIMENTEL RMM, HIGINO JS & ALBUQUERQUE UP. 2003. Estudo farmacognóstico de *Indigofera microcarpa* Desv. (Fabaceae). Rev Bras Ciênc Farm 39(4): 373-379.

LÜTTGE U. 2008. Physiological ecology of tropical plants. Chapter 5 - Tropical forests. III. Ecophysiological responses to drought. 2nd ed. Berlin, Heidelberg: Springer-Verlag, p. 149-164.

MACHADO JL, FREITAS T, VIANA MTR, MATOS NMS, BOTENHO CE & GUIMARÃES RJ. 2015. Diferenças nas características estomáticas de genótipos de cafeeiro. IX Simpósio de Pesquisa dos Cafés do Brasil, Curitiba – PR.

MALAVOLTA E, VITTI GC & OLIVEIRA SA. 1997. Avaliação do estado nutricional das plantas, princípios e aplicações. 2nd ed. Piracicaba: Potafos, 319 p.

MANGUEIRA JRSA, VIEIRA LTA, AZEVEDO TN, SABINO APS, FERRAZ, KMPMB, FERRAZ SFB, ROTHER DC & RODRIGUES RR. 2021. Plant diversity conservation in highly deforested landscapes of the Brazilian Atlantic Forest. Perspect Ecol Conser 19(1): 69-80.

MARTINS JR, ALVARENGA AA, CASTRO EM, SILVA APO, OLIVEIRA C & ALVES E. 2009. Leaf Anatomy of alfavaca-cravo plants cultivated under colored nets. Cienc Rural 39(1): 82-87.

MELO JÚNIOR JCF, BONA C & CECCANTINI G. 2012. Anatomia foliar de *Copaifera langsdorffii* Desf. (Leguminosae): interpretações ecológicas em diferentes condições edáficas de Cerrado. Biotemas 25(4): 29-36.

MENDES ICA & PAVIANI TI. 1997. Morfo-anatomia comparada das folhas do par vicariante *Plathymenia foliolosa* Benth. e *Plathymenia reticulata* Benth. (Leguminosae – Mimosoideae). Rev Bras Bot 20(2): 185-195.

METCALFE CR & CHALK L. 1950. Anatomy of the Dicotyledons. Oxford: Clarendon Press, 288 p.

MOREIRA-CONEGLIAN IR & OLIVEIRA DMT. 2006. Anatomia comparada dos limbos cotiledonares e eofilares de dez espécies de Caesalpinioideae (Fabaceae). Rev Bras Bot 29(2): 193-207.

MÜLLER C & RIEDERER M. 2005. Plant surface properties in chemical ecology. J Chem Ecol 31(11): 2621-2651.

MYERS N, MITTERMEIER RA, MITTERMEIER CG, FONSECA GAB & KENT J. 2000. Biodiversity hotspots for conservation priorities. Nature 403: 853-858. https://doi.org/10.1038/35002501.

NASCIMENTO ME, BERTOLUCCI SKV, SANTOS FM, SANTOS JR JM, CASTRO EM & PINTO JEBP. 2014. Avaliação morfológica de plantas jovens de *Copaifera langsdorffii* Desf. desenvolvidas em diferentes temperaturas. Rev Bras Plantas Med 16(4): 931-937.

O'BRIEN TP, FEDER N & MCCULLY ME. 1964. Polychromatic staining of plat cell walls by Toluidine Blue O. Protoplasma 59(2): 368-373.

OGUCHI R, HIROSAKA K & HIROSE T. 2003. Does the photosynthetic light acclimation need change in leaf anatomy? Plant Cell Environ 26(4): 505-512.

OLGUIN FY, MORETTIB AP, PINAZOC M, GORTARID F, BAHIMAB JV & GRACIANOA C. 2020. Morphological and physiological plasticity in seedlings of *Araucaria angustifolia* and *Cabralea canjerana* is related to plant establishment performance in the rainforest. Forest Ecology and Management 460: 117867. https://doi.org/10.1016/j.foreco.2020.117867.

OLIVEIRA-FILHO AT & RATTER JA. 1995. A study of the origin of Central Brazilian forests by the analysis of plant species distribution patterns. Edinburgh J Bot 52(2): 141-194.

ORDOÑEZ JC, VAN BODEGOM PM, WITTE J-PM, WRIGHT IJ, REICH PB & AERTS R. 2009. A global study of relationships between leaf traits, climate and soil measures of nutrient fertility. Glob Ecol Biogeogr 18: 137-149.

PAOLI GD. 2006. Divergent leaf traits among congeneric tropical trees with contrasting habitat associations on Borneo. J Trop Ecol 22: 397-408.

PEARCE DW, MILLARD S, BRAY DF & ROOD SB. 2006. Stomatal characteristics of riparian poplar species in a semi-arid environment. Tree Physiol 26: 211-218.

PEREIRA-SILVA EFL, HARDT E & FERNANDES AO. 2012. The soil-plant relationship of nitrogen use in three tropical tree species. Web Ecol 12: 57-64.

PRINGLE EG, ADAMS RI, BROADBENT E, BUSBY PE, DONATTI CI, KURTEN EL, RENTON K & DIRZO R. 2011. Distinct leaf-trait syndromes of evergreen and deciduous trees in a seasonally dry. Biotropica 43(3): 299-308.

RAIJ BV, ANDRADE JC, CANTARELLA H & QUAGGIO JA. 2001. Análise química para avaliação da fertilidade de solos tropicais. Campinas: Instituto Agronômico, 285 p.

ROCHA HS. 2005. Luz e sacarose na micropropagação da bananeira "Prata Anã": alterações morfoanatômicas. 2005. Dissertação (Mestrado em Fisiologia vegetal), Universidade Federal de Lavras, Lavras, 98 p.

ROSSATTO DR, TONIATO MTZ & DURIGAN G. 2008. Flora fanerogâmica não arbórea da Estação Ecológica de Assis, SP. Rev Bras Bot 31: 409-424.

RUGGIERO PGC, BATALHA MA, PIVELLO VR & MEIRELLES ST. 2002. Soil-vegetation relationships in cerrado (Brazilian savanna) and semideciduous forest, Southeastern Brazil. Pl Ecol 160: 1-16.

SCHNEIDER CA, RASBAND WS & ELICEIRI KW. 2012. NIH Image to ImageJ: 25 years of image analysis. Nat Methods 9: 671-675.

SIMIONI PF, EISENLOHR PV, PESSOA MJG & SILVA IV. 2017. Elucidating adaptive strategies from leaf anatomy: Do amazonian savannas present xeromorphic characteristics? Flora 226: 38-46.

SMA. 2017. Resolução SMA Nº 146, de 08 de Novembro de 2017. Institui o Mapa de Biomas do Estado de São Paulo, e dá outras providências. Available from: http://arquivos.ambiente.sp.gov.br/legislacao/2017/11/resolucao-sma-146-2017.pdf.

SMITH WK & MCCLEAN TM. 1989. Adaptative relationship between leaf water repellency, stomatal distribution and gas exchange. Am J Bot 76(3): 465-469.

SOMAVILLA NS & RIBEIRO DG. 2011. Análise comparativa da anatomia foliar de Melastomataceae em ambiente de vereda e cerrado sensu stricto. Acta Bot Bras 25: 764-775.

SOUZA ML, DUARTE AA, LOVATO MB, FAGUNDES M, VALLADARES F & LEMOS-FILHO JP. 2018. Climatic factors shaping intraspecific leaf trait variation of a neotropical tree along a rainfall gradient. PLoS ONE 13(12): e0208512.

SOUZA JP, MELO NMJ, HALFELD AD, VIEIRA KIC & ROSA BL. 2019. Elevated atmospheric CO_2 concentration improves water use efficiency and growth of a widespread Cerrado tree species even under soil water deficit. Acta Bot Bras 33(3): 425-436.

SOUZA TC, MAGALHÃES PC, PEREIRA FJ, CASTRO EM, SILVA JÚNIOR JM & PARENTONI SN. 2010. Leaf plasticity in successive selection cycles of 'Saracura' maize in response to periodic soil flooding. Pesq Agropec Bras 45(1): 16-24.

SULTAN SE. 2000. Phenotypic plasticity for plant development, function and life history. Trends Plant Sci 5: 537-542.

TOMLINSON KW, POORTER L, STERCK FJ, BORGHETTI F, WARD D, DE BIE S & VAN LANGEVELDE F. 2013. Leaf adaptations of evergreen and deciduous trees of semi-arid and humid savannas on three continents. J Ecol 101: 430-440.

VIEIRA WL, BOEGER MRT, COSMO NL & COAN AI. 2014. Leaf morphological plasticity of tree species from two developmental stages in Araucaria Forest. Braz Arch Biol Technol 57(4): 476-485.

VIOLLE C, NAVAS M-L, VILE D, KAZALOU E, FORTUNEL C, HUMMEL I & GARNIER E. 2007. Let the concept of trait be functional! Oikos 116: 882-892.

VOLTAN RBQ, FAHL JI & CARELLI MLC. 1992. Variação na anatomia foliar de cafeeiros submetidos a diferentes intensidades luminosas. R Bras Fisiol Veg 4(2): 99-105.

WILSON PJ, THOMPSON K & HODGSON JG. 1999. Specific leaf area and leaf dry matter content as alternative predictors of plant strategies. New Phytol 143: 155-162.

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