

# FOLIAR PLASTICITY IN *SCHINUS TEREBINTHIFOLIA* (ANACARDIACEAE), A TROPICAL/SUBTROPICAL SPECIES COMMONLY USED IN REVEGETATION PROGRAMMES

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The study aimed to assess the influence of light on structural aspects of *Schinus terebinthifolia* sun and shade leaves. At the morphological level, leaf length, width, area and dry mass were measured. Anatomical analysis assessed the thickness and area of leaf blade and leaf tissues, histolocalised lipids and phenols, and calculated stomatal index. Sun leaves showed lower number of leaflets (22%) and lower leaf (50%) and leaflet area (30%). Petiole length and width were respectively 34 and 33% higher in shade leaves. Tissue proportion, stomatal index and stomatal density showed no variation between morphotypes. However, epidermal periclinal outer-wall thickness in sun leaflets was higher on both the adaxial (24%) and abaxial (27%) leaf surfaces, as was the total adaxial (11%) and abaxial (23%) epidermal thickness. Analogously, palisade and spongy parenchyma thicknesses were higher in sun leaflets (62 and 45%, respectively). Histochemical reaction for phenol detection was stronger in sun leaves. *Schinus terebinthifolia* showed high foliar phenotypic plasticity in response to different light conditions that occur even on a single-individual basis, across different crown regions.

Keywords: Ecological plant anatomy, leaf micromorphometry, light acclimation, phenotypic plasticity, sun and shade leaves

## INTRODUCTION

Light is a major determinant of plant growth and survival (Taiz et al. 2014, Yang et al. 2019). Depending on the species and its adaptability to the different levels of light intensity that reach the canopy, plants may have sun and shade leaves (Šimpraga et al. 2013, Bahamonde et al. 2018, Maslova et al. 2021, Thérroux-Rancourt et al. 2023). Accordingly, different morphological and anatomical traits can be found even among leaves of a same individual tree, as the tree canopy provides leaves with distinct environmental conditions (Hulshof & Swenson 2010, Ishii et al. 2018, Thérroux-Rancourt et al. 2023). Thereby, leaves growing on the upper and lower portions of the same plant may exhibit different morphological and anatomical traits (Zhang et al. 2019, Vega et al. 2020).

Adaptive responses in leaf structure to different light conditions are common in plants (Pereira et al. 2013, Campbell et al. 2018, Araújo et al. 2021). For instance, reduced shade periods lead to lower leaf area and leaf area ratio (i.e., leaf area/total plant dry mass) in *Neobalanocarpus heimii* seedlings (Sherzad et al. 2017). Several studies have emphasised the structural variations that occur in leaves of a single individual tree from a given species, due to exposure to different light intensities. Plant anatomy is used in those studies as a tool to elucidate physiological and structural aspects of such variations (Dias-Pereira et al. 2013, Campbell et al. 2018, Poorter et al. 2019, Hertel et al. 2021, Souza et al. 2022, Thérroux-Rancourt et al. 2023).

The use of native plant species in the rehabilitation of degraded or disturbed lands has grown over the years (Shono et al. 2007, Figueiredo et al. 2012, Leung et al. 2018, Figueiredo et al. 2021). Native tree species usually exhibit a broad range of responses to changes in light conditions, including anatomical, physiological and biochemical alterations, as demonstrated by dos Anjos et al. (2015). Therefore, plant growth efficiency may be related to the leaf adaptive capacity to light conditions in the surrounding environment.

*Schinus terebinthifolia* (Anacardiaceae) is native to Brazil, where it is commonly known as 'aroeira'. The species can reach up to 10 m, with a round canopy and alternate odd-pinnately compound leaves with a winged petiole and seven leaflets, each leaflet measuring ca. 3–7 cm long and 2–3 cm wide (Lorenzi 2020). Species occurrence ranges from Amapá state (northern Brazil) to the states of Mato Grosso do Sul (midwest) and Rio Grande do Sul (south) (Silva-Luz et al. 2022). *Schinus terebinthifolia* is an evergreen heliophytic pioneer species, commonly found in anthropic areas, grasslands, Cerrado (Brazilian savanna), riverine forests, gallery forests, seasonal semi-deciduous forests, tropical rain forests, mixed ombrophilous forests, mangroves and Restingas (Silva-Luz et al. 2022).

Studies have addressed the behavior of *S. terebinthifolia* in diverse ecological conditions (Rabelo et al. 2013, dos Anjos et al. 2015, Fernandes et al. 2021, Silva-Luz et al. 2022). However, in-depth information on the species primary responses to environmental variations in light intensity could aid further assessment of its full potential for rehabilitation of degraded lands. Thus, the study aimed to evaluate the influence of light on the structure of leaves developed under high and low light intensities in adult individuals of *S. terebinthifolia*. The main question we sought to answer was which are the morphological, micromorphological and anatomical traits that provide *S. terebinthifolia* with its well-known broad adaptive capacity to different light intensity conditions. In other words, which traits confer the species with high phenotypic plasticity. The study tested the hypothesis that *S. terebinthifolia* shows high adaptive capacity not only to remarkably disparate environments, as already shown by Souza et al. (2022), but also

to within-crown light variations that take place even on a single-individual basis.

## MATERIALS AND METHODS

Fully expanded sun and shade leaves (from the upper peripheral portion and lower inner portion of the canopy, respectively) were collected from four individuals of *Schinus terebinthifolia* (Anacardiaceae), all ca. 6 m high, located at Sítio Palmital, Viçosa municipality, Minas Gerais state, southeastern Brazil (20° 48' S and 42° 51' W). The criterion adopted to select the studied species was the round shape of its canopy, which favors the occurrence of distinct light conditions across it. The criterion for selection of individuals was their isolated occurrence in the area, which rendered leaf structure to be influenced by canopy shading only, while also ensuring that sun leaves had developed under full sunlight conditions.

Light measurements were recorded at noon, under clear sky conditions, using a quantum/radiometer/photometer.

For morphological analyses, four individuals were evaluated (n = 4). From each individual, five sun and five shade leaves were collected. Leaves were digitised and images were measured using Image Pro-Plus software version 4.1. Length and width of leaves and petioles, and area of leaves and leaflets, were measured. Leaf dry mass was determined with a precision balance, after oven drying leaf samples at 60 °C for 72 h. Leaf specific area was calculated following Gobbi (2011), using equation 1:

$$\text{Leaf specific area} = \frac{\text{leaf area (cm}^2\text{)}}{\text{leaf dry mass (g)}} \quad (1)$$

For all microscopic analyses, 0.5 cm<sup>2</sup> samples were obtained from the blade mid region of the third leaflet. For micromorphological analysis, leaf samples (n = 5) were fixed with 2% glutaraldehyde in pH 7.0 Sorenson's sodium phosphate buffer, and dehydrated in an ethyl series (Gabriel 1982). Samples were then critical-point dried with liquid CO<sub>2</sub>. After gold sputter coating, samples were photographically documented in a scanning electron microscope coupled with a digital camera.

For micromorphometric analysis, four individuals were evaluated (n = 4). From each individual, five sun and five shade leaves were

collected. Samples were fixed with FAA<sub>70</sub> (formalin, acetic acid, 70% ethanol, 1:1:18 v/v), dehydrated in an ethyl series and embedded in methacrylate. Cross-sections (7 µm thick) were stained with pH 4.0 toluidine blue and glass slides were mounted using Permount (O'Brien & McCully 1981). Thickness measurements were taken from the leaf blade, outer periclinal wall (including the cuticle) of pavement cells from the adaxial epidermis, adaxial epidermis, subepidermal layer, palisade parenchyma, spongy parenchyma, abaxial epidermis and outer periclinal wall (including the cuticle) of pavement cells from the abaxial epidermis. Area measurements in cross-section were taken from the aforementioned tissues/regions, as was their percentage in relation to total leaf area in cross-section.

Part of the cross-sectioned methacrylate-embedded samples (n = 4) was subjected to histolocalisation of non-structural phenolic compounds, using 10% ferric chloride solution, and lipids (for cuticle visualisation), using a Sudan black ethanolic solution (Johansen 1940, Pearse 1972).

Stomatal index was determined according to Cutter (1978), with leaf samples were obtained from four individuals (n = 4). From each

individual, three sun and three shade leaves were collected and subjected to epidermal dissociation using Jeffrey's solution (equal parts of 10% aqueous nitric acid and 10% aqueous chromic acid) (Johansen 1940). Data on thickness, area and stomatal index were obtained using Image-Pro Plus software version 4.1, in a total of nine, three and ten observations per replicate, respectively, for each evaluated leaf tissue, region or structure. Images were captured in a photomicroscope equipped with a U-Photo system.

Quantitative data was subjected to analysis of variance and means were compared at 5% probability by T-Test, using GraphPad Prism software.

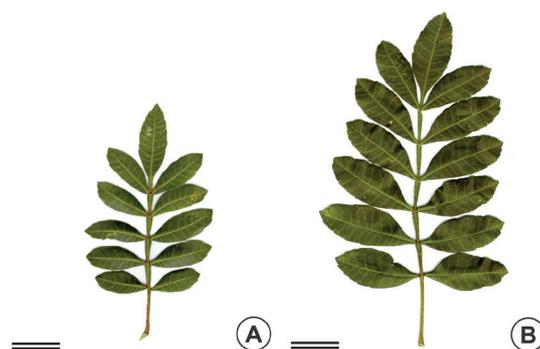
## RESULTS AND DISCUSSION

The different light conditions to which leaves from the outer (1500–1800 µmol m<sup>-2</sup> s<sup>-2</sup>) and inner (40–70 µmol m<sup>-2</sup> s<sup>-2</sup>) portions of the *S. terebinthifolia* canopy were exposed throughout their development induced pronounced morphoanatomical differences. Sun leaves showed lower number of leaflets (22%) than shade leaves (Table 1, Figure 1), and leaf (47%) and leaflet (30%) areas were

**Table 1** Morphological variables of *Schinus terebinthifolia* sun and shade leaves

Morphological variable	Sun leaves	Shade leaves
Number of leaflets	8.70 (± 0.58) b	11.19 (± 0.56) a
Leaflet area (cm <sup>2</sup> )	2.46 (± 0.20) b	3.53 (± 0.22) a
Leaf area (cm <sup>2</sup> )	21.07 (± 1.93) b	39.95 (± 2.06) a
Leaflet length/width ratio	2.44 (± 0.17) a	2.27 (± 0.09) a
Petiole length (cm)	2.09 (± 0.09) b	2.81 (± 0.09) a
Petiole width (cm)	0.12 (± 0.01) b	0.16 (± 0.01) a
Leaf specific area (cm <sup>2</sup> g <sup>-1</sup> )	68.46 (± 4.84) a	138.10 (± 41.37) a
Dry mass (g)	0.32 (± 0.03) a	0.36 (± 0.07) a

Means followed by different letters in the same line differ by T-test (p ≤ 0.05), values in parenthesis are standard error (n = 4)



**Figure 1** Sun (A) and shade (B) leaves of *Schinus terebinthifolia*, bar = 2 cm

higher in shade leaves in comparison with sun leaves. However, despite the size difference, no difference was found in length/width ratio (Table 1), which indicated that leaf shape was maintained (Figure 1). Alterations in leaf size may represent an efficient mechanism of acclimation to sun and shade, as they enable hydraulic and stomatal conductances to adjust to the contrasting evaporative demands under these two light conditions (Carins Murphy et al. 2012, Khan et al. 2020). Additionally, the higher photosynthetically active area of shade leaves allow for increased absorption of the lower amount of light available in the lower inner portion of the canopy (Syvertsen et al. 1995, Yiotis et al. 2006, Sellin et al. 2021).

Sun leaves usually have lower area and thicker yet more compact mesophyll than shade leaves (Metcalf & Chalk 1979, Fahn 1990, Paż-Dyderska et al. 2020). Other characteristic traits of sun-acclimated leaves may include higher photosynthetic protein content and higher photosynthetic capacity per unit area than shade-acclimated leaves (Oguchi et al. 2018, Th eroux-Rancourt et al. 2023).

Petiole length and width were respectively 34 and 33% higher in shade leaves (Table 1), revealing a reinforced structural support of shade-acclimated leaves, which, as stated above, also had larger blade areas than sun leaves. Interestingly, however, specific leaf area showed no significant difference between sun and shade leaves, neither did leaf dry mass (Table 1). Decrease in leaf area followed by increase in leaf tissue thickness was found in sun leaves, enabled by the more compact arrangement of tissues in the sun-leaf morphotype, especially of the palisade parenchyma (Dickison 2000). Other contributing factor was the reduced mesophyll porosity, i.e., reduced amount of intercellular spaces, in sun leaves (Coble & Cavaleri 2017, Th eroux-Rancourt et al. 2023). Both those features (i.e., higher compaction of tissue arrangement and lower mesophyll porosity) are related to the higher light intensity to which the canopy outer portion is exposed (Vega et al. 2020).

Leaf morphology and anatomy play a major role in shade acclimation and tolerance (dos Anjos et al. 2015, Zhang et al. 2019). In the Brazilian Cerrado, Mendonça

et al. (2020) found that *Dalbergia miscolobium* plants from a shade environment showed increased specific leaf area and decreased leaf thickness in contrast to plants from a full-sun environment. Similar results were found by dos Anjos et al. (2015) who studied the photosynthetic plasticity of tropical tree species subjected to different light intensities, simulating variations in canopy openings that occur naturally in tropical forests. It was reported that morphological, anatomical and physiological parameters are key factors in the plasticity of tropical species to light (dos Anjos et al. 2015). Ferreira et al. (2013) assessed foliar features of *Xylopia aromatica* populations growing in different vegetation types of the same biome, and found higher specific leaf area in individuals from Cerrad o (woodland savanna), a closed and shaded forest-like vegetation type, in comparison with individuals from Cerrado sensu stricto (savanna), an open vegetation type exposed to high light intensity. Changes in leaf size in response to distinct light conditions seem to be related to differences in epidermal cell size (Carins Murphy et al. 2012). However, when occurring in response to different values of ambient leaf-to-air vapor pressure difference (VPD), such leaf-size changes may owe to alterations in epidermal cell number rather than size (Carins Murphy et al. 2014). Leaf size plasticity in response to distinct abiotic factors is crucial to a species adaptive success to different environmental conditions (Ferreira et al. 2013, Mallik et al. 2013, Khan et al. 2020).

Abiotic factors such as light, water, temperature and photoperiod affect more intensely the developing leaf primordia than do genetically predetermined factors. Additionally, abiotic factors may induce alterations in leaf shape, particularly at later stages of foliar development, during leaf expansion and histogenesis (Dickison 2000, Bacelar et al. 2006, Thomas 2017). This is due to the leaf being an organ which adapts easily to the surrounding environment, given its form- and function-related traits that provide it with phenotypic plasticity. Because of such high adaptability, the leaf is the organ most frequently chosen to be analysed in studies on ecological plant anatomy (Dickison 2000, Cutler et al. 2007, dos Anjos et al. 2015, Mendonça et al. 2020).

Anticlinal walls of pavement cells from the adaxial epidermis were more sinuous in shade leaves (Figure 2A, B and C). Since the cuticle hardens slower in shade leaves than in sun leaves, epidermal cell walls in the former remain delicate and plastic for a longer period, thus favoring development of sinuosity (Watson 1942). Undulation in epidermal cell walls is generally more pronounced in leaves subjected to shaded environments, as demonstrated by Lauton et al. (2022) in *Justicia calycina* plants from forest environments with different crown openings. In full-sun environments, epidermal cells usually have straight or only slightly wavy anticlinal walls (Evert 2013, Lauton et al. 2022). In both sun and shade leaves of *S. terebinthifolia*, walls of pavement cells from the abaxial leaf surface were straight

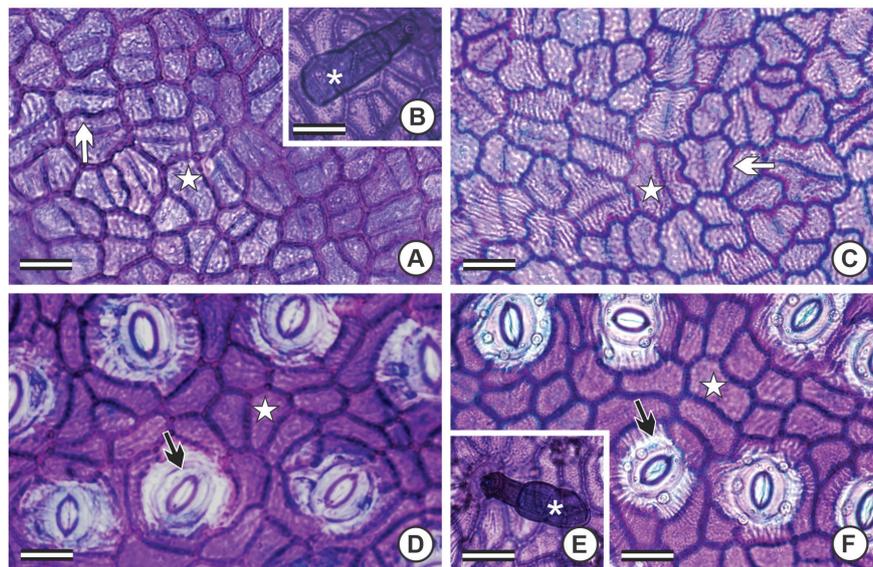
(Figure 2D and F), contrary to literature reports of a trend for pavement cells having more undulated margins on the leaf abaxial surface (Vófély et al. 2019). Alterations in cell shape might also be related to changes in the direction of cellulose microfibril deposition in the cell wall, along with localised differences in stiffness between walls from adjacent cells (Gao et al. 1987, Majda et al. 2017).

Trichomes occurred on both leaf surfaces (Figure 2B and E). The number of pavement cells varied significantly between sun and shade leaves in neither leaf surface (Table 2). In contrast, Martinez and Medri (1985) found, in a same individual of *Persea americana*, smaller epidermal cells on sun leaves and cells with more sinuous walls on shade leaves. Analogously, Carins Murphy et al. (2012) also found that acclimation to sun and shade in

**Table 2** Quantitative epidermal traits of *Schinus terebinthifolia* sun and shade leaves

Anatomical variable	Sun leaves	Shade leaves
Stomatal density (mm <sup>2</sup> )	70.14 (± 6.45) a	69.35 (± 7.24) a
Stomatal index (%)	7.70 (± 0.55) a	8.50 (± 0.31) a
Number of pavement cells	265.00 (± 25.37) a	227.90 (± 7.16) a

Means followed by different letters in the same line differ by T-test ( $p \leq 0.05$ ), values in parenthesis are standard error (n = 4)



**Figure 2** Leaf epidermal traits of *Schinus terebinthifolia* (light micrographs), A, B and D = sun leaf, C, E and F = shade leaf. A and C = straight (A) or sinuous (C) anticlinal walls (white arrows) of pavement cells (star), B = glandular trichome (asterisk) on the adaxial epidermis, D and F = stomata (black arrows) on the abaxial epidermis, E = glandular trichome (asterisk) on the abaxial epidermis; bars = 50  $\mu$ m

*Toona ciliata* involves changes in leaf size that are coordinated with alterations in epidermal cell size, which allows for an integrated response between vein and stomatal densities.

Stomal density did not vary significantly between sun and shade leaves (Table 2), conversely to other reports (Vega et al. 2020, Sellin et al. 2021). Changes in stomatal density are important for acclimation to different environmental conditions. Plants of the woody angiosperm *T. ciliata* grown under different levels of irradiance adjust stomatal and vein density so that water supply and transpirational demand remain proportional (Carins Murphy et al. 2012). Such leaf trait, however, must not be analysed singly. Carins Murphy et al. (2014), for instance, reported a small decrease in stomatal density in leaves grown under low values of ambient leaf-to-air vapor pressure difference, but they also found that the reduction in stomatal conductance necessary to maintain homeostasis was provided mainly by dynamic closure of stomata rather than by a reduction in stomatal density itself.

Similarly, stomatal index in *S. terebinthifolia* did not vary between sun and shade leaves (Table 2). This corroborates the findings of Silva and Anderson (1985) who studied the influence of light on leaf development in *Phaseolus vulgaris*, Paiva et al. (2003) who analysed the influence of light intensity on the leaf anatomical structure of *Tradescantia pallida* and Pires et al. (2015) who addressed the foliar morphoanatomical variations found across different canopy strata in *Schinus molle*. According to Cutter (1978), stomatal index is genetically established in plant species, as confirmed in *S. terebinthifolia*. Moreover light did not influence stomata differentiation in *S. terebinthifolia*, yet it did alter epidermal cell expansion, as reported by Silva and Anderson (1985) to *P. vulgaris*.

Scanning electron microscopy revealed fungal hyphae on both sun and shade leaves (Figure 3A–D). The outer periclinal wall (cuticle surface) of epidermal cells from the abaxial leaf surface has concentrically arranged striations around stomata (Figure 3A, B and D). Although cuticle ornamentation in the form of striae might increase leaf surface roughness and consequently decrease leaf wettability, the high vapor pressure in contact with leaves,

arising from high light intensity and high transpiration velocity, may propitiate favorable conditions for fungal growth (Salatino et al. 1986, Pott et al. 2007, Wang & Dai 2016). The phyllosphere is known to host a highly diverse microbiota, and it has been estimated to make up ca. 60% of the biomass on Earth (Leveau 2019, Koskella 2020).

Leaflets of *S. terebinthifolia* are hypostomatic and dorsiventral, with two layers of palisade parenchyma and three to four layers of spongy parenchyma (Figure 4A and B). The cuticle is thick, and beneath the adaxial surface there is a single-layered subepidermal tissue (Figure 4A and B). Idioblasts containing druses occur throughout the mesophyll, and secretory ducts occur associated with vascular tissues (Figure 4A and B). Both the druse idioblasts and secretory ducts had been previously described by Venning (1948) in *S. terebinthifolia* leaves, the latter being a characteristic trait of the Anacardiaceae (Metcalf & Chalk 1950, Tölke et al. 2021).

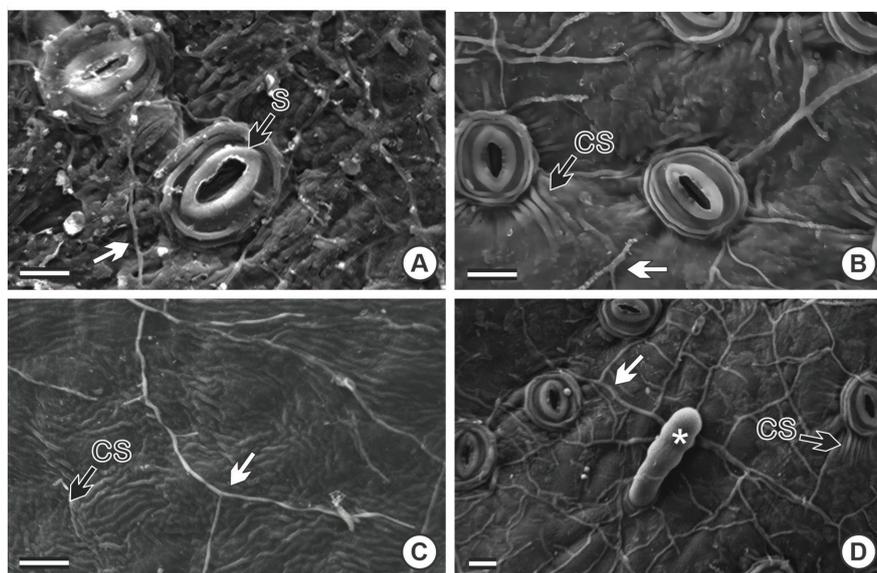
The outer periclinal wall of pavement cells from adaxial and abaxial leaf surfaces was 24 and 27% thicker, respectively, in sun leaves (Table 3). Accordingly, lipid deposition (i.e., forming the cuticle) on the outer periclinal wall of epidermal cells was shown to be quite conspicuous in sun leaves (Figure 4D and E), whereas in shade leaves it occurred in the form of a tenuous layer (Figure 4G and H). Increased cuticle thickness may be related to enhanced protection against excess water loss and enhanced reflection of excess light, which minimises leaf heating as well as other issues associated with light over-absorption (Dickison 2000, Evert 2013, Taiz et al. 2014, Campbell et al. 2018). Variations in light intensity, rather than in temperature or relative humidity (both of which are relatively less marked across the tree canopy), are the probable responsible factors for the increased cuticle thickness found on top-canopy leaves compared with bottom-canopy leaves (Bahamonde et al. 2018).

The epidermis of the adaxial and abaxial leaf surfaces were respectively 11 and 23% thicker in sun leaves (Table 3). Analogously, palisade and spongy parenchyma were respectively 62 and 43.5% thicker in sun leaves (Table 3). Similar results were reported by Mendonça et al. (2020) on the proportion of palisade

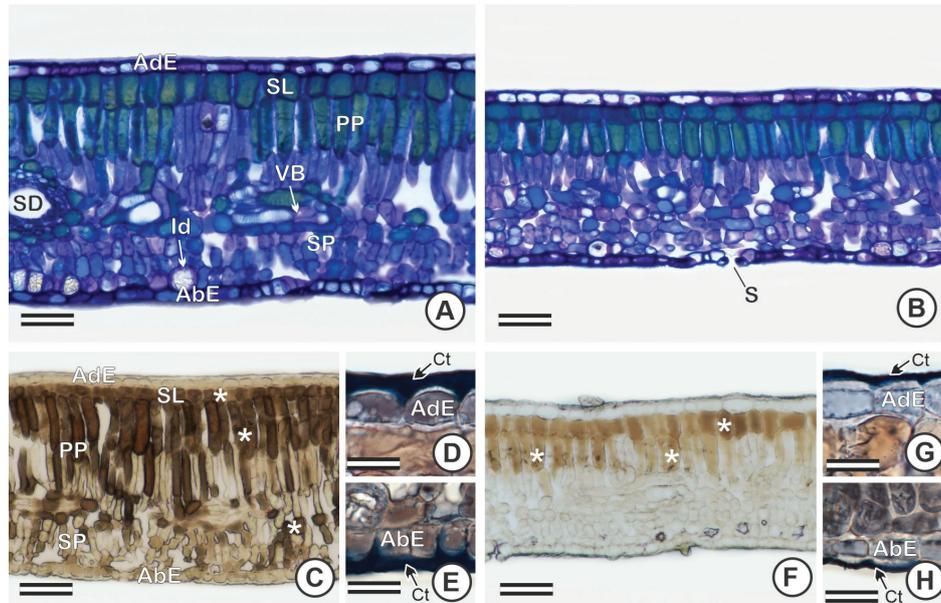
**Table 3** Mean thickness of the leaf blade and leaf tissues, and percentage of each leaf tissue in relation to the total leaf blade thickness, in *Schinus terebinthifolia* sun and shade leaves

Tissue/Region	Sun leaves	Shade leaves
OW-AdE ( $\mu\text{m}$ )	10.12 ( $\pm 0.15$ ) a	8.18 ( $\pm 0.46$ ) b
OW-AbE ( $\mu\text{m}$ )	9.98 ( $\pm 0.20$ ) a	7.84 ( $\pm 0.30$ ) b
AdE ( $\mu\text{m}$ )	29.21 ( $\pm 0.93$ ) a	26.26 ( $\pm 0.29$ ) b
AbE ( $\mu\text{m}$ )	25.44 ( $\pm 0.65$ ) a	20.68 ( $\pm 0.90$ ) b
SL ( $\mu\text{m}$ )	42.30 ( $\pm 3.13$ ) a	36.18 ( $\pm 1.33$ ) a
PP ( $\mu\text{m}$ )	213.50 ( $\pm 9.87$ ) a	131.60 ( $\pm 18.84$ ) b
SP ( $\mu\text{m}$ )	123.00 ( $\pm 8.97$ ) a	84.64 ( $\pm 8.73$ ) b
Leaf blade ( $\mu\text{m}$ )	435.00 ( $\pm 11.41$ ) a	298.00 ( $\pm 26.56$ ) b
OW-AdE (%)	2.33	2.74
OW-AbE (%)	2.29	2.63
AdE (%)	6.71	8.81
AbE (%)	5.85	6.94
SL (%)	9.72	12.14
PP (%)	49.08	44.16
SP (%)	28.28	28.40

Means followed by different letters in the same line differ by T-test ( $p \leq 0.05$ ), values in parenthesis are standard error ( $n = 4$ ); AdE = adaxial epidermis, AbE = abaxial epidermis, OW-AbE = outer periclinal wall of pavement cells from the abaxial epidermis, OW-AdE = outer periclinal wall of pavement cells from the adaxial epidermis, PP = palisade parenchyma, SL = subepidermal layer, SP = spongy parenchyma



**Figure 3** Leaf blade surface of *Schinus terebinthifolia* (scanning electron micrographs), A and C = sun leaf, B and D = shade leaf, A–C = white arrows indicate fungal hyphae on the abaxial (A, B and D) and adaxial (C) epidermis, D = glandular trichome (asterisk) on the abaxial epidermis, S = stomata, CS = cuticular striae; bars A–C = 20  $\mu\text{m}$ , D = 10  $\mu\text{m}$



**Figure 4** Leaf blade structure of *Schinus terebinthifolia* (light micrographs of cross-sections), A, C–E = sun leaf, B, F–H = shade leaf, C and F = histolocalisation of phenolic compounds with ferric chloride, positive reaction is shown by brown color (asterisk), D, E, G and H = histolocalisation of lipids with Sudan black, revealing the cuticle, positive reaction is shown by black stain, Ct = cuticle, SD = secretory duct, S = stomata, AdE = adaxial epidermis, AbE = abaxial epidermis, VB = vascular bundle, Id = druse idioblast, SP = spongy parenchyma, PP = palisade parenchyma, SL = subepidermal layer; bars A–C and F = 50  $\mu$ m, D, E, G and H = 20  $\mu$ m

and spongy parenchyma in leaves of *Dalbergia miscolobium* plants subjected to different light intensities. Thicker palisade parenchyma facilitates CO<sub>2</sub> absorption by mesophyll cells directly exposed to light (Sanches et al. 2017, Campbell et al. 2018). In shade leaves, increased intercellular spaces allow for higher diffusion of the low amount of available light, as radiation scatters when it reaches the interface between mesophyll cells and air spaces in the spongy parenchyma (Taiz et al. 2014, Th  roux-Rancourt et al. 2023) (Noda et al. 2020). Contribution of spongy parenchyma to leaf thickness and the ratio between palisade and spongy parenchyma thickness have been shown to be some of the traits (along with rubisco carboxylation capacity, contribution of collenchyma to leaf thickness, and specific leaf area) that provide *S. terebinthifolia* with photosynthetic plasticity (dos Anjos et al. 2015).

Visually, sun leaves showed higher number of cells with phenol accumulation than shade leaves, especially in the subepidermal layer and palisade parenchyma (Figure 4C and F). A

positive influence of light in phenol production has been reported to several plant species, for instance to three varieties of *Labisia pumila* and to *Hordeum vulgare* (Karimi et al. 2013, Hunt et al. 2021). Phenolic compounds form an important class of secondary metabolites that play a protective role in plants, owing to both their antioxidant activity and ability to attenuate excessive UV radiation (Marchiosi et al. 2020). This prevents the production of reactive oxygen species in plant cells, as well as the oxidative stress that might result from it (Klem et al. 2019, Hunt et al. 2021). Phenolic compounds also provide plants with protection and defense against other biotic and abiotic stress agents (Marchiosi et al. 2020).

Interestingly, the epidermis showed no positive reaction for histolocalisation of phenolic compounds. The subepidermal layer, on the other hand, did accumulate phenolic compounds in both sun and shade leaves (Figure 4C and F), despite having shown no alteration in thickness between the two leaf morphotypes (Table 3). Similarly, Hunt et al. (2021) reported accumulation of phenolic

compounds in the mesophyll of *H. vulgare*, mainly in tissues adjacent to the epidermis. Thus, the increased chlorenchyma thickness that was found in *S. terebinthifolia*, along with the seemingly increased accumulation of phenolic compounds in chlorenchyma cells, as indicated by the histochemical test, may represent adaptive advantages in environments subjected to high light intensity. These findings suggest that the subepidermal layer, which in *S. terebinthifolia* is formed by juxtaposed cells with high contents of phenolic compounds, may act as a filter, providing protection to subjacent mesophyll cells and their photosynthetic apparatus against the oxidative damage caused by excess irradiance, especially in sun leaves, which are exposed to higher light intensity (Marchiosi et al. 2020).

Sun leaves showed a 46% thicker leaf blade than shade leaves (Table 3), yet there was no alteration in the number of tissue layers that form the leaf (Figure 4A and B). This corroborates the reports by Oguchi et al. (2003) and Paiva et al. (2003) but contradicts the report by Vega et al. (2020), thus revealing that the responses to different light intensities across canopy height are species-specific.

Decreased leaf area and increased leaf-blade and cuticle thicknesses in more light-exposed leaves help prevent excess water loss (Dickson 2000, Evert 2013, Taiz et al. 2014). In addition, following the classic paradigm of the palisade parenchyma serving as a light guide toward the leaf interior, as opposed to the spongy parenchyma acting as a light diffuser, higher palisade parenchyma thickness enables light to be transmitted directly through the leaf and penetrate more deeply into the mesophyll, thus preventing photoinhibition (Taiz et al. 2014, Ichiro et al. 2016, Noda et al. 2020).

## CONCLUSION

Sun leaves of *S. terebinthifolia* have plastic morphoanatomical traits that allow for restricting excess water loss under unfavorable environmental conditions, whereas shade leaves have plastic traits that allow for optimising absorption of the reduced sunlight at the lower portion of the canopy. The phenotypic plasticity shown by *S. terebinthifolia* leaves in response

to different light conditions is pivotal for the species success in forest ecosystems. Such success is enabled by plastic morphological traits such as number of leaflets, leaf and leaflet area, petiole length and width, as well as plastic anatomical traits such as thickness of epidermal cells, of the outer periclinal wall of pavement cells, and of palisade and spongy mesophyll layers. The hypothesis was confirmed, insofar as *S. terebinthifolia* individuals showed high adaptive capacity not only to remarkably disparate environments, as previous studies have found, but also to within-crown light variations that take place even on a single-individual basis, as demonstrated in this study.

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## REFERENCES

- ARAÚJO I, MARIMON BS, SCALON MC ET AL. 2021. Intraspecific variation in leaf traits facilitates the occurrence of trees at the Amazonia–Cerrado transition. *Flora* 279: 151829. <https://doi.org/10.1016/j.flora.2021.151829>.
- BACELAR EA, SANTOS DL, MOUTINHO-PEREIRA JM, GONÇALVES BC, FERREIRA HF & CORREIA CM. 2006. Immediate responses and adaptive strategies of three olive cultivars under contrasting water availability regimes: changes on structure and chemical composition of foliage and oxidative damage. *Plant Science* 170: 596–605. <https://doi.org/10.1016/j.plantsci.2005.10.014>.
- BAHAMONDE HA, GIL L & FERNÁNDEZ V. 2018. Surface properties and permeability to calcium chloride of *Fagus sylvatica* and *Quercus petraea* leaves of different canopy heights. *Frontiers in Plant Science* 9: 494. <https://doi.org/10.3389/fpls.2018.00494>.
- CAMPBELL G, MIELKE MS, RABELO GR & DA CUNHA M. 2018. Key anatomical attributes for occurrence of *Psychotria schlechtendaliana* (Müll. Arg.) Müll. Arg. (Rubiaceae) in different successional stages of a tropical moist forest. *Flora* 246: 33–41. <https://doi.org/10.1016/j.flora.2018.07.004>.
- CARINS MURPHY MR, JORDAN GJ & BRODRIBB TJ. 2012. Differential leaf expansion can enable hydraulic acclimation to sun and shade. *Plant, Cell & Environment* 35: 1407–1418. <https://doi.org/10.1111/j.1365-3040.2012.02498.x>.

- CARINS MURPHY MR, JORDAN GJ & BRODRIBB TJ. 2014. Acclimation to humidity modifies the link between leaf size and the density of veins and stomata. *Plant, Cell & Environment* 37: 124–131. <https://doi.org/10.1111/pce.12136>.
- COBLE AP & CAVALERI MA. 2017. Vertical leaf mass per area gradient of mature sugar maple reflects both height-driven increases in vascular tissue and light-driven increases in palisade layer thickness. *Tree Physiology* 37: 1337–1351. <https://doi.org/10.1093/treephys/tpx016>.
- CUTTER EG. 1978. *Plant Anatomy. Part 1: Cells and Tissues*. 2<sup>nd</sup> Edition. William Clowes and Sons Limited, London.
- CUTLER DF, BOTHA CEJ & STEVENSON DW. 2007. *Plant Anatomy: An Applied Approach*. Blackwell Publishing, Oxford.
- DIAS-PEREIRA J, PIMENTA JA, MEDRI ME & STOLF-MOREIRA R. 2013. Avaliações morfológicas e micromorfométricas de folhas de sol e de sombra de *Lithraea molleoides* Vell. (Engl.) (Anacardiaceae) [morphological and micromorphometric analyses on sun and shade leaves of *Lithraea molleoides* Vell. (Engl.) (Anacardiaceae)]. *Evolução e Conservação da Biodiversidade* 4: 22–31.
- DICKISON WC. 2000. *Integrative Plant Anatomy*. Academic Press, San Diego.
- DOS ANJOS L, OLIVA MA, KUKI KN ET AL. 2015. Key leaf traits indicative of photosynthetic plasticity in tropical tree species. *Trees* 29: 247–258. <https://doi.org/10.1007/s00468-014-1110-2>.
- EVERT RF. 2013. *Anatomia das plantas de Esau: Meristemas, Células e Tecidos do Corpo da Planta*. 3<sup>rd</sup> Edition. São Paulo, Blucher, Portuguese.
- FAHN A. 1990. *Plant Anatomy*. 4<sup>th</sup> Edition. Pergamon Press, New York.
- FERNANDES MM, OLIVEIRA CM, SILVA AJ ET AL. 2021. The inoculation with arbuscular mycorrhizal fungi improved ecophysiological and growth parameters of *Schinus terebinthifolius* and *Caesalpinia ferrea* in degraded mining sites. *Environmental Challenges* 4: 100181. <https://doi.org/10.1016/j.envc.2021.100181>.
- FERREIRA GA, PRADO JÚNIOR JA, SCHIAVINI I & MELO C. 2013. Plastic responses in tree architecture and specific leaf area of *Xylopia aromatica* (Annonaceae): adaptations to environments with different light intensities. *Brazilian Journal of Botany* 36: 279–283. <https://doi.org/10.1007/s40415-013-0035-0>.
- FIGUEIREDO JM, ARAÚJO JM, PEREIRA ON, BAKKE IA, & BAKKE OA. 2012. Revegetation of degraded Caatinga sites. *Journal of Tropical Forest Science* 24: 332–343.
- FIGUEIREDO MA, MESSIAS MCTB, LEITE MGP & KOZOVITS AR. 2021. Direct seeding in the restoration of post-mined campo rupestre: Germination and establishment of 14 native species. *Flora* 276–277: 151772. <https://doi.org/10.1016/j.flora.2021.151772>.
- GABRIEL BL. 1982. *Biological Electron Microscopy*. Nostrand Reinhold Company, New York.
- GAO J, HOFSTRA G & FLETCHER RA. 1987. Anatomical changes induced by triazoles in wheat seedlings. *Canadian Journal of Botany* 66: 1178–1185. <https://doi.org/10.1139/b88-168>.
- 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 [specific leaf area and quantitative leaf anatomy of signalgrass and forage peanut submitted to shading]. *Revista Brasileira de Zootecnia*. 40: 1436–1444. <https://doi.org/10.1590/S1516-35982011000700006>.
- HERTEL MF, ARAÚJO HH, STOLF-MOREIRA R ET AL. 2021. Different leaf traits provide light-acclimation responses in two neotropical woody species. *Theoretical and Experimental Plant Physiology* 33: 313–327. <https://doi.org/10.1007/s40626-021-00213-1>.
- HULSHOF CM & SWENSON NG. 2010. Variation in leaf functional trait values within and across individuals and species: An example from a Costa Rican dry forest. *Functional Ecology* 24: 217–223. <https://doi.org/10.1111/j.1365-2435.2009.01614.x>.
- HUNT L, KLEM K, LHOTÁKOVÁ Z ET AL. 2021. Light and CO<sub>2</sub> modulate the accumulation and localization of phenolic compounds in barley leaves. *Antioxidants* 10: 385. <https://doi.org/10.3390/antiox10030385>.
- ICHIRO T, HIROKI O, TAKASHI F & RIICHI O. 2016. Light environment within a leaf. II. Progress in the past one-third century. *Journal of Plant Research* 129: 353–363. <https://doi.org/10.1007/s10265-016-0808-1>.
- ISHII HR, HORIKAWA S, NOGUCHI Y & AZUMA W. 2018. Variation of intra-crown leaf plasticity of *Fagus crenata* across its geographical range in Japan. *Forest Ecology and Management* 429: 437–448. <https://doi.org/10.1016/j.foreco.2018.07.016>.
- JOHANSEN DA. 1940. *Plant Microtechnique*. McGraw-Hill, New York.
- KARIMI E, JAAFAR HZE, GHASEMZADEH A & IBRAHIM MH. 2013. Light intensity effects on production and antioxidant activity of flavonoids and phenolic compounds in leaves, stems and roots of three varieties of *Labisia pumila* Benth. *Australian Journal of Crop Science* 7: 1016–1023.
- KHAN MNI, KHATUN S, AZAD MS & MOLLIK AS. 2020. Leaf morphological and anatomical plasticity in Sundri (*Heritiera fomes* Buch.-Ham.) along different canopy light and salinity zones in the Sundarbans mangrove forest, Bangladesh. *Global Ecology and Conservation*: e01127. <https://doi.org/10.1016/j.gecco.2020.e01127>.
- KLEM K, GARGALLO-GARRIGA A, RATTANAPICHAI W ET AL. 2019. Distinct morphological, physiological, and biochemical responses to light quality in barley leaves and roots. *Frontiers in Plant Science* 10: 1026. <https://doi.org/10.3389/fpls.2019.01026>.
- KOSKELLA B. 2020. The phyllosphere. *Current Biology* 30: R1143–R1146. <https://doi.org/10.1016/j.cub.2020.07.037>.
- LAUTON MB, GRESSLER E, OLIVEIRA JA, SIMIONI PF, RIBEIRO-JÚNIOR NG, YAMASHITA OM & SILVA, IV. 2022. Does the functional leaf anatomy of *Justicia calycina* (Acanthaceae) reflect variation across a canopy gradient in the Southern Brazilian Amazon? *Acta Botanica Brasilica* 36: e20210335. <https://doi.org/10.1590/0001-7595/20210335>.

- org/10.1590/1677-941X-ABB-2021-0335
- LEUNG FTY, YAN WM, HAU BCH, & THAM LG. 2018. Mechanical pull-out capacity and root reinforcement of four native tree and shrub species on ecological rehabilitation of roadside slopes in Hong Kong. *Journal of Tropical Forest Science* 30: 25–38. <https://doi.org/10.26525/jtfs2018.30.1.2538>.
- LEVEAU JH. 2019. A brief from the leaf: latest research to inform our understanding of the phyllosphere microbiome. *Current Opinion in Microbiology* 49: 41–49. <https://doi.org/10.1016/j.mib.2019.10.002>.
- LORENZI H. 2020. *Árvores brasileiras: Manual de Identificação e Cultivo de Plantas Arbóreas Nativas do Brasil [Brazilian Trees: A Handbook for the Identification and Cultivation of Native Arboreal Plants from Brazil]*. Volume 1, 8<sup>th</sup> Edition. Plantarum, Nova Odessa.
- MAJDA M, GRONES P, SINTORN IM ET AL. 2017. Mechanochemical polarization of contiguous cell walls shapes plant pavement cells. *Developmental Cell* 43: 290–304: e4. <https://doi.org/10.1016/j.devcel.2017.10.017>.
- MALLIK AU, KREUTZWEISER DP, SPALVIERI CM & MACKERETH RW. 2013. Understory plant community resilience to partial harvesting in riparian buffers of central Canadian boreal forests. *Forest Ecology and Management* 289: 209–218. <https://doi.org/10.1016/j.foreco.2012.09.039>.
- MARCHIOSI R, SANTOS WD, CONSTANTIN RP ET AL. 2020. Biosynthesis and metabolic actions of simple phenolic acids in plants. *Phytochemistry Reviews* 19: 865–906. <https://doi.org/10.1007/s11101-020-09689-2>.
- MARTINEZ MM & MEDRI ME. 1985. Alguns aspectos da anatomia-ecológica de *Persea americana* Mill (abacateiro) e interpretações fisio-ecológicas [some aspects of the ecological anatomy of *Persea americana* Mill (avocado plant) and physio-ecological interpretations]. *Semina* 6: 140–146.
- MASLOVA NP, KARASEV EV, XU S ET AL. 2021. Variations in morphological and epidermal features of shade and sun leaves of two species: *Quercus bambusifolia* and *Q. myrsinifolia*. *American Journal of Botany* 108: 1441–1463. <https://doi.org/10.1002/ajb2.1706>.
- MENDONÇA AMC, LIRA JMS, RODRIGUES M, MONTEIRO VFC, SIMÃO E & BARBOSA JPRAD. 2020. Anatomical, physiological and allometric contrasts of the Cerrado tree *Dalbergia miscolobium* in full sun and shade environments. *Southern Forests: a Journal of Forest Science*, 82: 104–111. <https://doi.org/10.2989/20702620.2019.1649348>.
- METCALFE CR & CHALK L. 1950. *Anatomy of Dicotyledons*. Volume 2. Clarendon Press, Oxford.
- METCALFE CR & CHALK L. 1979. *Anatomy of the Dicotyledons: Systematic Anatomy of The Leaf and Stem*. Volume 1, 2<sup>nd</sup> Edition. Clarendon Press, Oxford.
- NODA HM, MURAOKA H & NASHIHARA KN. 2020. Phenology of leaf optical properties and their relationship to mesophyll development in cool-temperate deciduous broad-leaf trees. *Agricultural and Forest Meteorology*: 108236. <https://doi.org/10.1016/j.agrformet.2020.108236>.
- O'BRIEN TP & MCCULLY ME. 1981. *The Study of Plant Structure: Principles and Selected Methods*. Termarcaphi Pty. Ltd, Melbourne.
- OGUCHI R, HIKOSAKA K & HIROSE T. 2003. Does the photosynthetic light-acclimation need change in leaf anatomy? *Plant, Cell & Environment* 26: 505–512. <https://doi.org/10.1046/j.1365-3040.2003.00981.x>.
- OGUCHI R, ONODA Y, TERASHIMA I & THOLEN D. 2018. Leaf anatomy and function. Pp 97–139 in Adams IIIW & Terashima I (eds) *The Leaf: A Platform for Performing Photosynthesis. Advances in Photosynthesis and Respiration (Including Bioenergy and Related Processes)*. Volume 44. Springer, Cham. [https://doi.org/10.1007/978-3-319-93594-2\\_5](https://doi.org/10.1007/978-3-319-93594-2_5).
- PAIVA EAS, ISAIAS RMS, VALE FHA & QUEIROZ CGS. 2003. The influence of light intensity on anatomical structure and pigment contents of *Tradescantia pallida* (Rose) Hunt. cv. purpurea Boom (Commelinaceae) leaves. *Brazilian Archives of Biology and Technology* 46: 617–624. <https://doi.org/10.1590/S1516-89132003000400017>.
- PAŻ-DYDERSKA S, DYDERSKI MK, NOWAK K & JAGODZIŃSKI AM. 2020. On the sunny side of the crown – quantification of intra-canopy SLA variation among 179 taxa. *Forest Ecology and Management* 472: 118254. <https://doi.org/10.1016/j.foreco.2020.118254>.
- PEARSE AGE. 1972. *Histochemistry: Theoretical and Applied*. 3<sup>rd</sup> Edition. The Williams & Wilkins Company, Baltimore.
- PEREIRA TAR, SILVA LC, AZEVEDO AA, FRANCINO DMT, COSER TS & DIAS-PEREIRA J. 2013. Leaf morpho-anatomical variations in *Billbergia elegans* and *Neoregelia mucugensis* (Bromeliaceae) exposed to low and high solar radiation. *Botany* 91: 327–334. <https://doi.org/10.1139/cjb-2012-0276>.
- PIRES MF, PEREIRA MP, CASTRO EM, BARBOSA S & PEREIRA FJ. 2015. Micromorfometria foliar de *Schinus molle* L. (Anarcadiaceae) em diferentes alturas na copa [leaf micromorphometry of *Schinus molle* L. (Anarcadiaceae) in different canopy heights]. *Cerne* 21: 17–25. <https://doi.org/10.1590/010477602015210111530>.
- POTT C, KRINGS M & KERP H. 2007. A surface microrelief on the leaves of *Glossophyllum florinii* (Ginkgoales) from the Upper Triassic of Lunz, Austria. *Botanical Journal of the Linnean Society* 153: 87–95. <https://doi.org/10.1111/j.1095-8339.2007.00569.x>.
- POORTER H, NIINEMETS U, NTAGKAS N, SIEBENKÄS A, MÄENPÄÄ M, MATSUBARA S & PONS TL. 2019. A meta-analysis of plant responses to light intensity for 70 traits ranging from molecules to whole plant performance. *New Phytologist* 223: 1073–1105. <https://doi.org/10.1111/nph.15754>.
- RABELO GR, VITÓRIA AP, SILVA MVA ET AL. 2013. Structural and ecophysiological adaptations to forest gaps. *Trees* 27: 259–272. <https://doi.org/10.1007/s00468-012-0796-2>.
- SALATINO A, MONTENEGRO G & SALATINO MLF. 1986. Microscopia eletrônica de varredura de superfícies foliares de espécies lenhosas do cerrado [scanning electron microscopy of leaf surfaces of woody species from the Brazilian savanna]. *Revista Brasileira de Botânica* 9: 117–124.
- SANCHES MC, MARZINEK J, BRAGIOLA NG & NASCIMENTO ART.

2017. Morpho-physiological responses in *Cedrella fissilis* Vell. submitted to changes in natural light conditions: implications for biomass accumulation. *Trees* 31: 215–227. <https://doi.org/10.1007/s00468-016-1474-6>.
- SELLIN A, ALBER M, JASIŃSKA AK & ROSENVALD K. 2021. Adjustment of leaf anatomical and hydraulic traits across vertical canopy profiles of young broadleaved forest stands. *Trees* 36: 67–80. <https://doi.org/10.1007/s00468-021-02181-0>.
- SHERZAD OH, MOHD ZAKI H, HAZANDY AH, & MOHAMAD AZANI A. 2017. Effect of different shade periods on *Neobalanocarpus heimii* seedlings biomass and leaf morphology. *Journal of Tropical Forest Science* 29: 457–464. <https://doi.org/10.26525/jtfs2017.29.4.457464>.
- SHONO K, DAVIES SJ & CHUA YK. 2007. Performance of 45 native tree species on degraded lands in Singapore. *Journal of Tropical Forest Science* 19: 25–34.
- SILVA EAM & ANDERSON CE. 1985. Influência da luz no desenvolvimento foliar do feijoeiro (*Phaseolus vulgaris* L.) [the influence of light on leaf development of bean plant (*Phaseolus vulgaris* L.)]. *Ceres* 32: 1–11.
- SILVA-LUZ CL, PIRANI JR, PELL SK & MITCHELL JD. 2022. *Anacardiaceae in Flora e Funga do Brasil*. Jardim Botânico, Rio de Janeiro. <https://floradobrasil.jbrj.gov.br/FB15471>.
- ŠIMPRAGA M, VERBEECK H, BLOEMEN J ET AL. 2013. Vertical canopy gradient in photosynthesis and monoterpenoid emissions: an insight into the chemistry and physiology behind. *Atmospheric Environment* 80: 85–95. <https://doi.org/10.1016/j.atmosenv.2013.07.047>.
- SYVERTSEN JP, LLOYD J, MCCONCHIE C, KRIEDEMANN PE & FARQUHAR G. 1995. On the relationship between leaf anatomy and CO<sub>2</sub> diffusion through the mesophyll of hypostomatous leaves. *Plant, Cell & Environment* 18: 149–157. <https://doi.org/10.1111/j.1365-3040.1995.tb00348.x>.
- SOUZA EV, ANDRADE GC, ARAÚJO HH & DIAS-PEREIRA J. 2022. Structural plasticity in leaves of *Schinus terebinthifolius* (Anacardiaceae) populations from three contrasting tropical ecosystems. *Journal of the Torrey Botanical Society* 149: 187–193. <https://doi.org/10.3159/TORREY-D-22-00001.1>.
- TAIZ L, ZEIGER E, MÖLLER IM & MUPHY A. 2014. *Plant Physiology & Development*. 6<sup>th</sup> Edition. Sinauer Associates Inc, Sunderland.
- THÉROUX-RANCOURT G, HERRERA JC, VOGGENEDER K ET AL. 2023. Analyzing anatomy over three dimensions unpacks the differences in mesophyll diffusive area between sun and shade *Vitis vinifera* leaves. *Annals of Botany Plants* 15: 2. <https://doi.org/10.1093/aobpla/plad001>.
- THOMAS B. 2017. *Leaf Development. Encyclopedia of Applied Plant Sciences*. 2<sup>nd</sup> Edition, Volume 1. Academic Press, Cambridge, Massachusetts.
- TÖLKE ED, LACCHIA APS, LIMA EA, DEMARCO D, ASCENSÃO L & CARMELLO-GUERREIRO SM. 2021. Secretory ducts in Anacardiaceae revisited: updated concepts and new findings based on histochemical evidence. *South African Journal of Botany* 138: 394–405. <https://doi.org/10.1016/j.sajb.2021.01.012>.
- VEGA C, GONZÁLEZ G, BAHAMONDE HA, VALBUENA-CARABAÑA M, GIL L & FERNÁNDEZ V. 2020. Effect of irradiation and canopy position on anatomical and physiological features of *Fagus sylvatica* and *Quercus petraea* leaves. *Plant Physiology and Biochemistry* 152: 232–242. <https://doi.org/10.1016/j.plaphy.2020.05.007>.
- VENNING FD. 1948. The ontogeny of the laticiferous canals in the Anacardiaceae. *American Journal of Botany* 35: 637–644. <https://doi.org/10.2307/2438062>.
- VÓFÉLY RV, GALLAGHER J, PISANO GD, BARTLETT M & BRAYBROOK SA. 2019. Of puzzles and pavements: a quantitative exploration of leaf epidermal cell shape. *New Phytologist* 221: 540–552. <https://doi.org/10.1111/nph.15461>.
- WANG LF & DAI ZD. 2016. Effects of the natural microstructures on the wettability of leaf surfaces. *Biosurface and Biotribology* 2: 70–74. <https://doi.org/10.1016/j.bsbt.2016.06.001>.
- WATSON RW. 1942. The effect of cuticular hardening on the form of epidermal cells. *New Phytologist* 41: 223–229. <https://www.jstor.org/stable/2428729>.
- YANG B, TANG J, YU Z, KHARE T, SRIVASTAV A, DATIR S & KUMAR V. 2019. Light stress responses and prospects for engineering light stress tolerance in crop plants. *Journal of Plant Growth Regulation* 38: 1489–1506. <https://doi.org/10.1007/s00344-019-09951-8>.
- YIOTIS C, MANETAS Y & PSARAS GK. 2006. Leaf and green stem anatomy of the drought deciduous Mediterranean shrub *Calicotome villosa* (Poiret) Link. (Leguminosae). *Flora* 201: 102–107. <https://doi.org/10.1016/j.flora.2005.04.007>.
- ZHANG X, JIN G & LIU Z. 2019. Contribution of leaf anatomical traits to leaf mass per area among canopy layers for five coexisting broadleaf species across shade tolerances at a regional scale. *Forest Ecology and Management* 452: 117569. <https://doi.org/10.1016/j.foreco.2019.117569>.