

EFFECTS OF SUN AND SHADE ON LEAF STRUCTURE AND SCLEROPHYLLY OF *SEBASTIANIA MYRTILLOIDES* (EUPHORBIACEAE) FROM SERRA DO CIPÓ, MINAS GERAIS, BRAZIL

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Abstract - (Effects of sun and shade on leaf structure and sclerophylly of *Sebastiania myrtilloides* (Euphorbiaceae) from Serra do Cipó, Minas Gerais, Brazil). The relationships among several attributes of the leaves of *Sebastiania myrtilloides* were studied: leaf sclerophylly, area, mass, volume, density, thickness, and anatomy in plants under natural conditions in a sun-exposed cerrado environment and in a shaded understorey riparian forest in Serra do Cipó, Brazil. The area, mass, density and thickness of the leaves varied significantly between sunny and shady habitats. Variations in leaf sclerophylly were influenced by leaf mass and density. Sun exposed leaves were denser and thicker due to both an increase in the length of adaxial epidermis, and the palisade and spongy parenchyma cells. *S. myrtilloides* presented an increase of 64% in stomatal density in the sunny habitat. The species showed an increase of 57% and 46% in trichome density under light conditions on the adaxial and abaxial epidermis, respectively. *S. myrtilloides* showed significant leaf structure plasticity, which reflected on its capability to explore both cerrado and riparian forest environments.

Resumo - (Efeitos do sol e da sombra na estrutura foliar e esclerofilia de *Sebastiania myrtilloides* (Euphorbiaceae) da Serra do Cipó, Minas Gerais, Brasil). As relações entre vários atributos foliares de *Sebastiania myrtilloides* foram estudados: esclerofilia, área, massa, densidade, espessura foliar e anatomia foliar de plantas em condições naturais sob completa exposição solar (cerrado) e sob sombreamento (mata ripária) na Serra do Cipó. A área, massa, densidade e espessura foliar variaram significativamente entre os habitats ensolarado e sombreado. As variações na esclerofilia, expressada em massa foliar específica (MFE), foram influenciadas pela massa e densidade foliar. As folhas expostas à luz solar mostraram-se mais densas e espessas devido ao aumento na espessura da epiderme adaxial e dos parênquimas paliádico e esponjoso. *S. myrtilloides* apresentou um aumento de 64% na densidade de estômatos no habitat ensolarado e um aumento de 57% e 46% na densidade de tricomas sob condições de luz na epiderme abaxial e adaxial, respectivamente. A espécie mostrou uma significativa plasticidade estrutural na folha, que é refletida na sua capacidade de explorar tanto ambientes de cerrado como de mata.

Key words: Euphorbiaceae, leaf anatomy, leaf specific mass, *Sebastiania myrtilloides*, Serra do Cipó.

Introduction

Leaf plasticity may be adaptative as it can provide additional mechanisms to adapt to different environments (Grime *et al.* 1986). Leaves of many plant species are known for their large growth plasticity. They vary in morphology, anatomy, as well as in physiology (Witkowski & Lamont 1991, Sims & Pearcy 1992, Rôças *et al.* 1997). Under high irradiance and low soil moisture availability, plants produce thicker and sclerophyllous leaves which have shorter aerial spaces (Witkowski & Lamont 1991, Thompson *et al.* 1992). In sunny and arid habitats water availability limits plant growth. Leaf thickening through the addition of more layers of cells increase net carbon

gain without increasing the cost of transpiration (Turner 1994). Otherwise, in low irradiance and high moisture and nutrients availability, plants present thinner leaf blades and large leaf area (Witkowski & Lamont 1991).

Sclerophylly may have several advantages under a wide range of environmental conditions (Turner 1994). Sclerophylly is a character which has been associated with plants growing in arid habitats with high irradiance as Mediterranean ecosystems and savannas (*e.g.*, Crawford 1989). The cerrado vegetation of Brazil is known for its generally large incident radiation (Eiten 1972, Oliveira-Filho & Ratter 1995) and by a lack of available water, at least during the dry season when much of the ground layer appears desiccated (Lopes & Cox 1977). Nevertheless, the

cerrado is composed of a mosaic of neighboring habitats where light penetration is reduced due to the size and architectural complexity of the vegetation. A common feature of the cerrado is the presence of narrow riparian forests along river, washes, and creeks (Meguro *et al.* 1996). In these riparian forests, light intensity is lower on shrub and herb strata and water and nutrients availability is higher. These features facilitate the study of leaf sclerophylly under different environmental conditions in habitats separated by a few meters.

The aim of the present work was to determine the variations in sclerophylly and structure in leaves of *Sebastiania myrtilloides*, a species widely distributed in sunny and shady habitats in Serra do Cipó. Several leaf attributes were studied concomitantly with sclerophylly: area, mass, volume, density, thickness, and anatomy, in an attempt to evaluate their relationships with habitat type.

Materials and Methods

The study was performed in Serra do Cipó National Park, MG, southeastern Brazil in the southern end of the Espinhaço Mountains (19° 20'S, 43° 44'W) at 800 m of altitude above sea level in October 1996. The species studied, *Sebastiania myrtilloides* (Mart.) Pax (Euphorbiaceae) was selected by its abundance in the area. This species is frequently found in open and sunny cerrado, as well as in the shaded understory of riparian forest, hence providing a natural experiment in which sun and shade adaptations can be studied at adjacent sites.

Habitats studied were selected by their distinctiveness in edaphic and climate characteristics (Table 1). The two habitats presented large differences in light intensity. They were categorized by photosynthetic photon flux density (PPFD) as: a) sunny habitat (integrated PPFD of 70 $\mu\text{mol m}^{-2} \text{day}^{-1}$), and b) shady habitat (integrated PPFD of 6.8 $\mu\text{mol m}^{-2} \text{day}^{-1}$). All climatic data were obtained between 7 am and 5 pm with 30 minutes of intervals. Relative humidity (%) and vapor pressure deficit (Kpa) of the air were estimated by temperature (°C) obtained by dry and wet bulb thermometers of a psychrometer (ICOTERM). Light intensity was measured with quantum

sensors (LI-189) at ten points randomly selected in each habitat.

Soil fertility was evaluated at two depths (10 and 20cm). Soil water content (SWC) was estimated by the percentage of soil moisture of six soil samples of 20cm³ at each depth by habitat. The percentage of soil moisture was calculated through the difference between fresh and dry soil. The dry soil was obtained by uniform drying at 70-80°C for 72 h. The amount of soil organic matter (SOM) was determined after the incineration of three soil samples at 500 °C for two hours, where the percentage of SOM represents the difference between the weights. The pH was determined by the Raij and Quaggio (1983) method.

In each habitat, five random samples of mature leaves (5th node under shoot apex) of ten plants were taken. Leaves were oven-dried at 70°C for a week to obtain leaf mass (mg) and leaf area (mm²). Leaf area was obtained with an area meter (model MK2, Delta T Devices). Leaf specific mass (LSM), the most widely used index of sclerophylly, was calculated based on leaf mass and area, while leaf density ($\mu\text{g} \cdot \text{mm}^{-3}$) was calculated by dividing LSM ($\mu\text{g} \cdot \text{mm}^{-2}$) by leaf thickness (Witkowski & Lamont 1991). In order to avoid major leaf veins, leaf thickness (μm) was obtained from fresh leaves and measured midway between the margin and the midrib at the widest part of the leaf. The measures were done under a microscope with a micrometer eyepiece.

Some leaves were fixed in alcohol 70% and then transferred to Jeffrey's solution (Johansen 1940) for epidermis dissociation, stomatal counting, and measurements of trichome density. Ten fields of view of known area (1mm²) were examined per leaf and the mean counting converted to stomata and trichome density. Mesophyll comparisons were done by leaf transverse sections in paraffin. Cross sections 10mm thick were prepared with a microtome, and the cuts were stained with Astra-Blue and Fuchsin (see Roeser 1962). Leaf anatomical features were also quantified under a microscope with a micrometer eyepiece. Cell counting (cells.mm⁻²) was done for palisade and spongy parenchyma on ten different sections from each species in both sunny and shady sites.

Table 1. Soil and climate characteristics of sunny and shady habitats in the Serra do Cipó, Brazil. Means \pm SE.

Traits	Cerrado (Sun)	Riparian Forest (Shade)
Soil (10 – 20cm)		
Organic Matter (%)	1.07 \pm 0.001 - 0.81 \pm 0.001	1.31 \pm 0.03 - 1.13 \pm 0.01
Soil Relative Water (%)	3.00 \pm 0.38 - 3.00 \pm 0.57	3.96 \pm 0.09 - 4.35 \pm 0.53
pH	4.99 \pm 0.01 - 4.95 \pm 0.04	4.81 \pm 0.01 - 5.03 \pm 0.03
Environment (min - max)		
Air Relative Humidity (%)	37 - 65	43 - 73
Vapor Pressure Deficit (KPa)	2.06 - 4.25	1.94 - 2.99
Air Temperature (°C)	18.0 - 31.5	17.0 - 24.0
Integrated 11-HR PPFD (mol.m ⁻² .day ⁻¹)	70.0	6.8

Pearson linear correlations were used to analysis the relationship between LSM and the leaf attributes. The Student t test was applied to compare all the leaf traits under the two distinct environments as the data were normally distributed (Zar 1984).

Results

Sebastiania myrtilloides showed consistent differences in leaf area, mass, LSM, density, and thickness between the sunny and shady habitats (Table 2). The leaf area of plants in the shady habitat was 217% greater than leaf area of plants in the sunny habitat. The dry mass of leaves of *S. myrtilloides* in the shady habitat was 118% greater than of leaves in the sunny habitat. Leaves of plants in the sunny habitat had higher LSM and density, and were thicker when compared to leaves of plants in the shady habitat (Table 2). Leaf specific mass (LSM) was highly correlated with leaf dry mass and leaf density in both habitats (Table 3). However, LSM did not correlate with leaf area. Nevertheless correlated significantly with thickness in leaves of shady habitat ($r = 0.289$).

The differences in LSM were influenced by changes in the thickness of the cuticle, adaxial epidermis, palisade, and spongy parenchyma. All measured anatomical parameters differed between sunny and shady habitats (Figure 1). Leaves of *S. myrtilloides* plants in the sunny habitat were 22% thicker than leaves of plants in the shade. This increase was influenced by the thickness of the palisade and spongy parenchyma. Palisade parenchyma was 19% thicker, because of increased cell length while the spongy parenchyma was 24% because of increased number of cells in leaves in the sunny habitat. Leaves in the sunny habitat showed cuticle and adaxial epidermis

thicker compared to leaves in the shady habitat (Table 4). Spongy parenchyma of leaves in the sunny habitat had more cells per unit leaf area (18%) than leaves in the shady habitat.

The species is hypostomata in both shady and sunny habitats. The number of stomata per leaf area in plants in the shady habitat was significantly lower than those in the sunny habitat (Table 4, Figure 2). Trichome density of leaves in the sunny habitat was significantly higher than on leaves of shady habitat (Table 4, Figure 2). Nevertheless, the adaxial leaf epidermis had lower trichome density than the abaxial leaf epidermis.

Discussion

Sclerophylly is readily recognizable, although not precisely definable, on their mechanical properties and anatomy (Turner 1994). *Sebastiania myrtilloides* had larger leaf thickness, density, and LSM in sunlight-exposed habitat of the cerrado, where moisture and nutrient availability were lower. These results agree with several experimental studies (e.g., Boardman 1977, Sims & Pearcy 1992, Witkowski & Lamont 1991). Plants in the understory of the riparian forest had thinner leaves resulting in greater area but smaller density and LSM, as reported by Corre (1983).

No significant relationship between leaf LSM and thickness was found in individuals in the cerrado. However, for individuals in the understory LSM was positively correlated with the leaf thickness. Contrasts between the correlations of these two variables were reported in several studies. Dijkstra and Lamber (1989) found a 29% difference in LSM in two inbred lines of *Plantago major*, but no difference in leaf thickness, while van Arendonk

Table 2. Leaf traits ($\bar{X} \pm SE$) of *Sebastiania myrtilloides* (Euphorbiaceae) in sunny and shady habitats. LSM denotes leaf specific mass. (t Test, * = $P < 0.05$, ns = not statistically significant).

Leaf Traits	<i>Sebastiania myrtilloides</i>	
	Sun	Shade
Area (mm ²)	75 ± 3	238 ± 10 *
Dry Mass (mg)	8.5 ± 0.4	18.5 ± 0.8 *
LSM (µg.mm ⁻²)	113 ± 2	78 ± 2 *
Density (µg.mm ⁻³)	624 ± 13	555 ± 14 *
Thickness (µm)	162 ± 2	134 ± 2 *

Table 3. Linear (Pearson product moment) correlations between leaf specific mass (LSM) and various other leaf traits of *Sebastiania myrtilloides* (Euphorbiaceae) in sunny and shady habitats. (* = $P < 0.05$, ns = not statistically significant).

Leaf Traits	<i>Sebastiania myrtilloides</i>	
	Sun	Shade
Area	0.082 ns	-0.242 ns
Volume	0.128 ns	-0.080 ns
Dry Mass	0.482 *	0.350 *
Density	0.817 *	0.712 *
Thickness	0.216 ns	0.289 *

Table 4. Anatomical quantitative informations ($\bar{X} \pm SE$, n=10 plants) of *Sebastiania myrtilloides* (Euphorbiaceae) in sunny and shady habitats (* = $P < 0.005$, ns = not statistically significant).

Leaf Traits	<i>Sebastiania myrtilloides</i>	
	Sun	Shade
Cuticle thickness (µm)	2	1
Adaxial epidermis thickness (µm)	18 ± 1	11 ± 1 *
Abaxial epidermis thickness (µm)	10 ± 1	11 ± 1 ns
Mesophyll thickness (µm)	132 ± 2	111 ± 2 *
Palisade Parenchyma		
Thickness (µm)	70 ± 3*	61 ± 1 *
Cell number (mm)	84 ± 2	82 ± 2 ns
Cell width (µm)	13 ± 1	13 ± 1 ns
Cell length (µm)	70 ± 2	59 ± 1*
Spongy Parenchyma		
Thickness(µm)	62 ± 2	50 ± 1 *
Cell number (mm ²)	2150 ± 76	1820 ± 84 *
Cell width (µm)	20 ± 1	25 ± 2 ns
Cell length (µm)	16 ± 1	15 ± 1 ns
Stomatal density (stomata.mm ⁻²)	121 ± 6	74 ± 3 *
Trichome density (trichomes.mm ⁻²)		
Adaxial epidermis	222 ± 16	144 ± 3 *
Abaxial epidermis	510 ± 10	458 ± 19 *

and Poorter (1994) did not find any relationship between LSM and thickness in 14 species of grasses that differed in relative growth rate. Garnier and Laurent (1994) did not observe any significant relationship between LSM and leaf thickness in seven annual-perennial pairs of grasses. Finally, Kebede *et al.* (1994) reported that two *Lycopersicon* species showed high LSM and thin leaves.

Therefore, the species studied showed LSM positively correlated with leaf mass and leaf density in both habitats that agree with the data of Pammenter *et al.* (1986), Kebede *et al.* (1994), and Garnier and Laurent (1994). These authors plays a major role of leaf density in the determination of LSM. The effects of thickness and/or leaf density on LSM have also clearly been shown in the study of Witkowski and Lamont (1991), who studied various combinations in shrub species growing in different environments. Leaf density results mainly from the amount of cell wall per unit volume (Garnier & Laurent 1994). *S. myrtilloides* showed increased amount of mesophyll cells which reflected in thickness and density of the mesophyll;

therefore contributing to either an increase in the amount of the cellular walls. We must be reminded, however, that leaf sclerophylly reflects a general increase in the proportion of cellular wall tissue (Turner *et al.* 1993).

Leaf thickness is influenced by variations in the cuticle thickness, epidermis (number of layers), hypodermis, mesophyll (number of layers and length of the palisade and spongy tissue), as well as by increased cell size (Esau 1977, Fahn & Cuttler 1992, Garnier & Laurent 1994, Sims & Pearcy 1992, Thompson *et al.* 1992, Witkowski & Lamont 1991). Chazdon & Kaufmann (1993) demonstrated variation in epidermal thickness in response to a natural gradient in light availability suggesting a role protection against high irradiance in thicker epidermis. Ours results showed an increase in thickness of cuticle and adaxial epidermis of leaves in sunny habitat. The increase of the cuticle and/or upper epidermis thickness, and outer periclinal wall thickness of epidermal cells, as well as in the height of cells, should improve the reflectance layer. Thus, the data indicate that *S. myrtilloides* presented anatomical

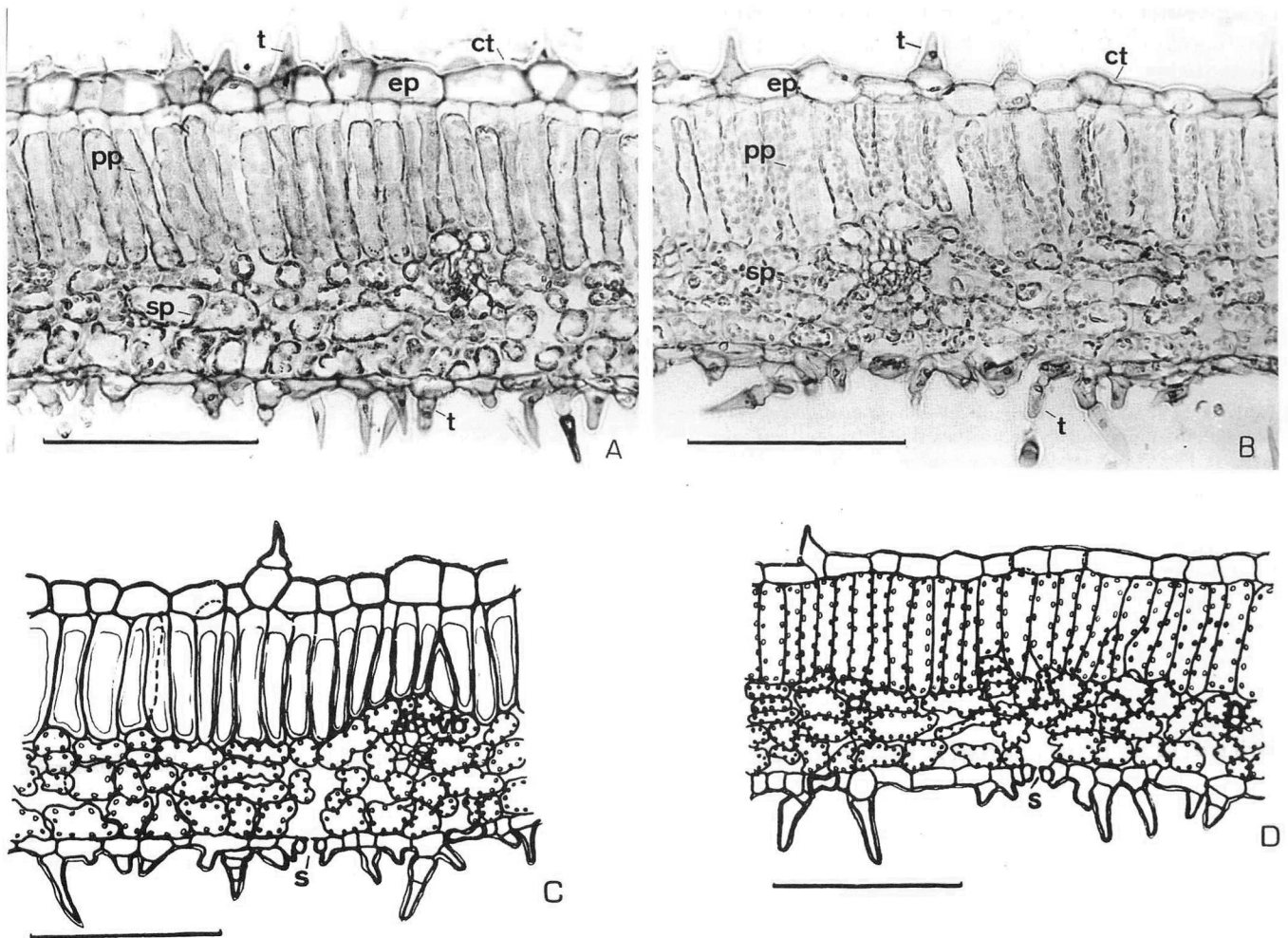


Figure 1 . Cross section of *Sebastiania myrtilloides* leaves in sunny habitat (A, C) and in shady habitat (B, D). ct, cuticle; ep, epidermis; pp, palisade parenchyma; s, stomata; sp, spongy parenchyma; t, trichome; vb, vascular bundle. Bar = 100 μm.

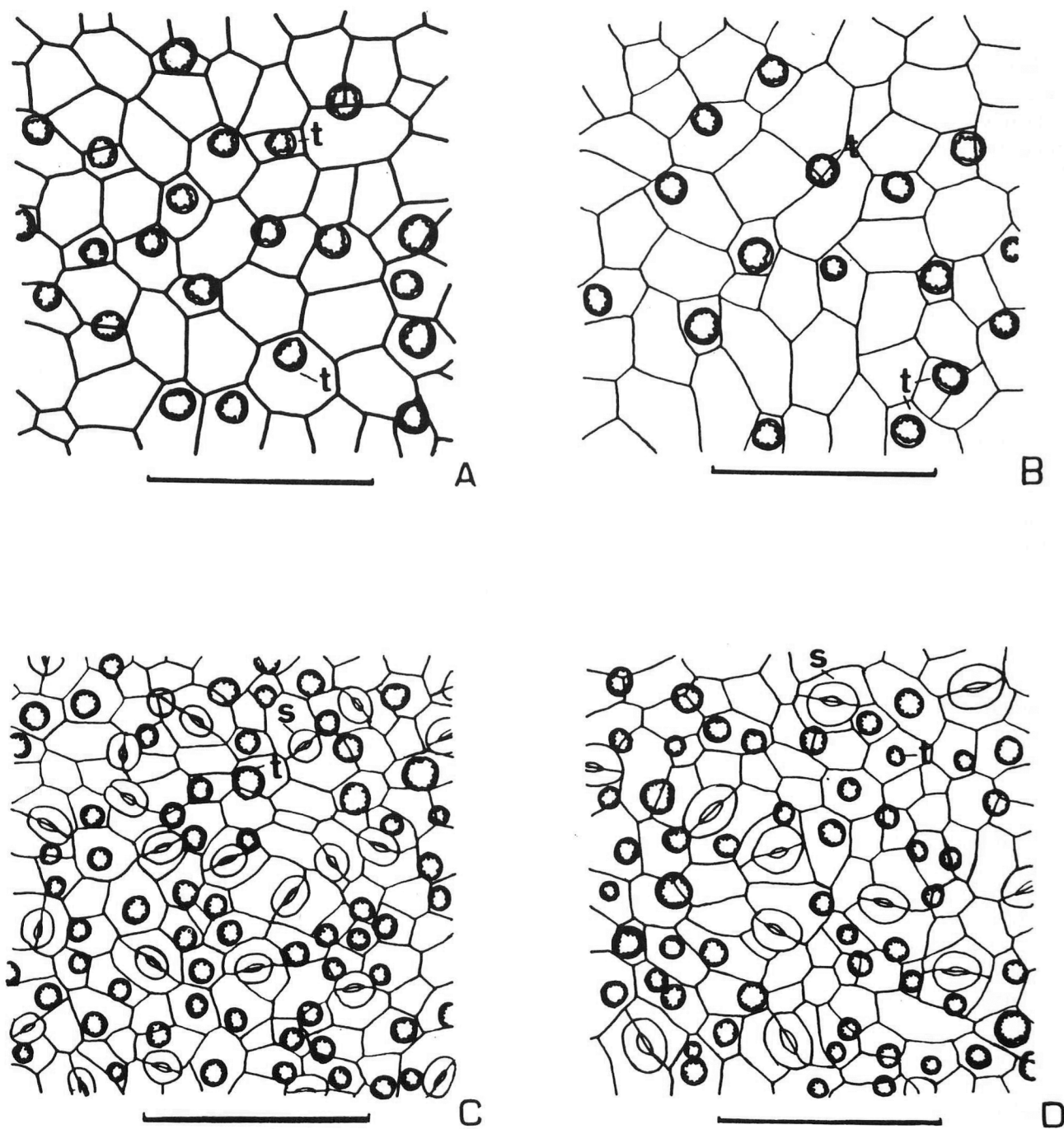


Figure 2 . Leaf surface of *Sebastiania myrtilloides*: adaxial epidermis in sunny habitat (A) and in shady habitat (B); abaxial epidermis in sunny habitat (C), and in shady habitat (D). s, stomata; t, trichome. Bar = 100µm.

characteristics that suggest more protection against light irradiation in the sunny habitat than shady habitat.

Sebastiania myrtilloides showed an increase in the thickness in the palisade and spongy parenchyma in sun-exposed plants. Thompson *et al.* (1992) discussed that high light irradiance induces the development of the palisade and spongy parenchyma, while under low light irradiance leaf cells are smaller and densely packed.

Leaves of plants in cerrado of all species showed higher stomatal density than leaves of understorey riparian forest. The increased number of stomata per unit area favors higher leaf conductance under high irradiance conditions (Mott *et al.* 1982, Mott & Michaelson 1991). Trichome density was also influenced by light and low soil moisture availability. As expected, trichome density was higher in exposed cerrado than in understorey of *S. myrtilloides*. The increase in trichome density plays an important role in water regulation through an increase in the boundary layer. Higher trichome density decrease the vapor pressure between the leaf tissues and the microclimate just above the leaf lamina. Furthermore it reduces water loss (e.g. Ehleringer & Mooney 1978, Harrington & Clark 1989, Johnson 1975, Upadhyaya & Furness 1994).

These results supports the interpretation of Witkoswisk and Lamont (1991) that sclerophyllous leaves may respond independently to resource and other gradients. For *S. myrtilloides* the leaf sclerophylly in plants in understorey of the riparian forest change mainly by variations of the leaf thickness. Conversely, leaf sclerophylly those of plants in the sun-exposed cerrado habitat reflected mainly the variations in leaf density and leaf mass.

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References

- BOARDMAN, N.K. 1977. Comparative photosynthesis of sun and shade plants. *Ann. Rev. Pl. Physiol.* 28: 335-377.
- CHAZDON, R.L. & KAUFMANN, S. 1993. Plasticity of leaf anatomy of two rain forest shrubs in relation to photosynthetic light acclimation. *Funct. Ecol.* 7: 385-394.
- CORRE, W.J. 1983. Growth and morphogenesis of sun and shade plants. I. The influence of light intensity. *Acta Bot. Neerl.* 32: 49-62.
- CRAWFORD, R.M.M. 1989. *Studies in plant survival*. Blackwell, Oxford.
- DIJKSTRA, P. & LAMBER, H. 1989. Analysis of specific leaf area and photosynthesis of two inbred lines of *Plantago* major differing in relative growth rate. *New Phytol.* 113: 283-290.
- EHLERINGER, J.R. & MOONEY, H.A. 1978. Leaf hairs: effects on physiological activity and adaptative values to a desert shrub. *Oecologia* 37: 183-200.
- EITEN, G. 1972. The cerrado vegetation of Brazil. *Bot. Rev.* 38: 201-341.
- ESAU, K. 1977. *Anatomy of seed plants*. 2^a ed. Wiley, New York.
- FAHN, A. & CUTTLER, D.F. 1992. *Xerophytes*. Gebrüder Borntraeger, Berlin.
- GARNIER, E. & LAURENT, G. 1994. Leaf anatomy, specific mass and water content in cogenetic annual and perennial grass species. *New Phytol.* 128: 725-736.
- GRIME, J.O., CRICK, J.C. & RINCON, J.E. 1986. The ecological significance of plasticity. In D.H. Jennings & J. Trewavas (eds) *Plasticity in plants*. Cambridge. Symposia of the Society for Experimental Biology Company of Biologists, p. 5-29.
- HARRINGTON, D.F. & CLARK, C. 1989. Reduction in light reflectance of leaves of *Encelia densifolia* (Asteraceae) by trichome wetting. *Madroño* 36: 180-186.
- JOHANSEN, D.A. 1940. *Plant Microtechnique*. Mc-Graw-Hill, New York.
- JOHNSON, H.B. 1975. Plant pubescence: an ecological perspective. *Bot. Rev.* 41: 233-258.
- KEBEDE, H., MARTIN, B., NIENHUIS, J. & KING, G. 1994. Leaf anatomy of two *Lycopersicon* species with contrasting gas exchange properties. *Crop Sci. (Madison)* 34: 108-113.
- LOPES, A.S. & COX, F.R. 1977. Cerrado vegetation in Brazil: an edaphic gradient. *Agron. J.* 69: 828-831.
- MEGURO, M., PIRANI, J.R., MELLO-SILVA, R. & GIULIETTI, A.M. 1996. Estabelecimento de matas ripárias e capões nos ecossistemas campestres da Cadeia do Espinhaço, Minas Gerais. *Bol. Bot. Univ. São Paulo* 15: 1-12.
- MOTT, K.A., GIBSON, A.C. & O'LEARY, J.W. 1982. The adaptive significance of amphistomatic leaves. *Pl. Cell Environm.* 5: 455-460.
- MOTT, K.A. & MICHAELSON, O. 1991. Amphistomy as an adaptation to high light intensity in *Ambrosia cordifolia* (Compositae). *Amer. J. Bot.* 78: 76-79.
- OLIVEIRA-FILHO, A.T. & RATTER, J.A. 1995. A study of the origin of the central Brazilian forest by the analysis of plant species distribution pattern. *Edinburgh J. Bot.* 52: 141-194.
- PAMMENTER, N.W., DRENNAN, P.M. & SMITH, V.R. 1986. Physiological and anatomical aspects of photosynthesis of two *Agrostis* species at a sub-antarctic island. *New Phytol.* 102: 143-160.
- RAIJ, B.V. & QUAGGIO, J.A. 1983. *Métodos de análise de solo para fins de fertilidade*. Boletim Técnico - 81. Instituto Agronômico de Campinas. São Paulo.
- RÔÇAS, G., BARROS, C.F. & SCARANO, F.R. 1997. Leaf anatomy plasticity of *Alchornea triplinervia* (Euphorbiaceae) under distinct light regimes in a Brazilian montane Atlantic rain forest. *Trees* 11: 469-473.
- ROESER, K.R. 1962. Die Nadel der schwarzkiefer-massenprodukt und kunstwert der natur. *Mikrokosmos* 61: 33-36.
- SIMS, D.A. & PEARCY, R.W. 1992. Response of anatomy and photosynthetic capacity in *Alocasia macrorrhiza* (Araceae) to a transfer from low to high light. *Amer. J. Bot.* 79: 449-455.
- THOMPSON, W.A., KRIEDEMANN, P.E. & CRAIG, I.E. 1992. Photosynthetic response to light and nutrients in sun-tolerant and shade-tolerant rainforest trees. I. Growth, leaf anatomy and nutrient content. *Austral. J. Pl. Physiol.* 19: 1-18.
- TURNER, J.M., CHOONG, N.F., TAN, H.T.W. & LUCAS, P.W. 1993. How tough are sclerophylls? *Ann. Bot.* 71: 343-345.

- TURNER, J.M. 1994. Sclerophylly: primarily protective? *Funct. Ecol.* 8: 669-675.
- UPADHYAYA, M.K. & FURNESS, N.H. 1994. Influence of light intensity and water stress on leaf surface characteristics of *Cynoglossum officinale*, *Centaurea* spp., and *Tragopogon* spp. *Canad. J. Bot.* 72: 1379-1386.
- VAN ARENDONK, J.J.C.M. & POORTER, H. 1994. The chemical composition and anatomical structure of leaves of grass species differing in relative growth rate. *Pl. Cell Environm.* 17: 962-970.
- WITKOWSKI, E.T.F. & LAMONT, B.B. 1991. Leaf specific mass confounds leaf density and thickness. *Oecologia* 88: 486-493.
- ZAR, J.H. 1984. *Biostatistical analysis*. 2^a ed. Prentice Hall. New Jersey.