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# Organization of leaf vascular system and gas exchange in seedlings of *Guazuma ulmifolia* Lam. in different light conditions

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**Abstract.** In tropical forests, different physiological characteristics of leaves in tree species are evidenced by variations in different incident light conditions. We aim to evaluate gas exchange and organization of leaf vascular system in *Guazuma ulmifolia* Lam. seedlings under different light conditions. Seedlings were obtained from seeds germinated under greenhouse conditions with controlled environment. Ninety days after germination, seedlings in 8kg pots were transferred to the experimental site to allow acclimatization under sun and shade conditions. The experimental design was completely randomized, with two treatments: full sunlight and artificial shading, limiting the luminosity to about 5% of irradiance. Seedlings were maintained under these conditions for 120 days before measuring gas exchange parameters. We measured photosynthetic rate, stomatal conductance, sub-stomatic CO<sub>2</sub> concentration, transpiration, and chlorophyll content. Anatomical analysis measured distance between veins, distance from veins to abaxial epidermis, distance from veins to adaxial epidermis, distance from veins to stomata, total leaf thickness, abaxial epidermis thickness, adaxial epidermis thickness, palisade parenchyma thickness, and spongy parenchyma thickness of foliar gas exchange of *G. ulmifolia* which presented significant differences between light environments. Photosynthetic rate and stomatal conductance were reduced by 78% and 39%, respectively, in shade, while stomatal conductance increased by 31% in full sunlight. Transpiration showed no significant difference between the two treatments, but chlorophyll content was 30% lower in full sunlight. Distance between veins and distance from veins to stomata showed no difference between treatments, but the other parameters increased in full sunlight. Thus, the results showed that the pioneer species *G. ulmifolia* presented leaf gas exchange acclimated to environments with high luminosity.

**Keywords:** leaf anatomy, acclimatization, vascular bundle, photosynthesis

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## Introduction

In tropical forests, different structural characteristics of leaves in tree species are evidenced by variations in different conditions of incident light (Givnish, 1988; Goldstein et al., 2016). Tropical forests are dynamic communities with a high frequency of disturbance. The uneven and discontinuous nature of canopy results in highly variable patterns of light availability in understory and clearings. Forests can be considered as a mosaic of patches at different stages of growth with each stage receiving different amounts of light and other resources (Chazdon & Fetcher, 1984).

Leaf morphological diversity is related to water transport (Sack and Holbrook, 2006) and gas exchange (Flexas et al., 2013). Allometric studies of leaf tissues (epidermis, mesophyll and vascular bundles) have brought insight to their relationship with water flow and CO<sub>2</sub> (John et al., 2013).

The entire hydraulic system of the plant is limited by the leaf hydraulic system as the main resistance bottleneck limiting gas exchange and biomass production (Sack et al., 2003). Water that reaches the leaves meets a resistance of at least 30% against water flow (Sack & Holbrook, 2006).

Leaves are extremely variable in morphology and anatomy, e.g., the arrangement and size of mesophyll tissues, such as palisade and spongy parenchyma. Among all plants, angiosperms evolved to an exceptional diversity of leaf vein types, nonvascular anatomical features, and other features characterizing leaf morphology and physiology. Among species, the branching and arrangement of the main vein present in leaves are governed by highly variable vascular systems. Generally, the first order vein enters the leaf from the petiole. The second and third order veins branch out in smaller veins, i.e., veins of third to fifth orders, incorporated within the blade forming a continuous mesh with the larger veins (Sack et al., 2015).

A positive correlation between leaf hydraulic properties and photosynthetic capacity was found among the taxa. This finding highlights the role of hydraulic performance in plants. For this reason, higher vein densities allow greater water flow to the stomata where gas exchange occurs (Zwieniecki & Boyce, 2014). Stomata are like valves that react to turgor that control water loss and CO<sub>2</sub> capture during photosynthesis. Thus, water flow and accumulation of plant biomass are strongly influenced by stomatal functioning (Camargo & Marengo, 2011).

“The main role of leaf venation is to supply water across the photosynthetic surface to keep stomata open and allow access to atmospheric CO<sub>2</sub> despite evaporative demand. The optimal uniform delivery of water occurs when the distance between veins equals the depth of vein placement within the leaf away from the evaporative surface” (Zwieniecki & Boyce, 2014). Therefore, by experimental testing on artificial leaves, as well as theoretical considerations, a significant reduction in water potential through the leaf epidermis would be achieved when the distance between veins ( $d$ ) is

equal to vein depth out of the surface of leaf that stomata occur, i.e., abaxial surface in most cases ( $\delta$ ) (Noblin, 2008). The violation of this relationship would not come without significant costs for photosynthetic rates and / or water use efficiency. If veins are too far apart, but close to leaf surface ( $d > \delta$ ), then transpiration and assimilation rates would be greater only close to the veins where the higher stomatal conductance ( $G_s$ ) would coincide with shortest diffusion path. This means that leaf tissue farther from veins would experience water scarcity, resulting in a drop in  $G_s$  and CO<sub>2</sub> absorption. In this case, photosynthetic gains and losses would occur on all surfaces of leaves; however, they would not be balanced (Zwieniecki & Boyce, 2014).

Photosynthetic gains in gas exchanges close to veins would be at the point of decreasing the return of water used, while photosynthetic losses would be of greater risk (i.e., where the return in carbon gain for a greater use of water would be greater). Thus, excessively long distances between veins, for a given vein depth ( $d > \delta$ ) are disadvantageous, leading to a general drop in leaf productivity and lower water use efficiency. The opposite anatomical relationships ( $d < \delta$ ) would also be problematic. The presence of more veins, so that distance between them was less than depth of the vein ( $d < \delta$ ) would not increase stomatal conductance, as it is close to its maximum at  $d = \delta$ , in fact, we can expect productivity the leaf's global value would decrease in such a situation, as the space available for photosynthetic mesophilic cells is lost to non-productive veins (Zwieniecki & Boyce, 2014).

Studies show that thickness of leaf blade, mesophyll, palisade parenchyma, spongy parenchyma and epidermis on both sides gradually increases with increase in light intensity. Species that grow in environments with high solar radiation, tend to have thicker leaves, thus guaranteeing greater water storage and thus buffering great variations in plant water potential (Aragão et al., 2014).

In this context, in our study we selected a plant of wide natural occurrence throughout Brazil, *Guazuma ulmifolia* Lam. (Colli-Silva, 2020), and we aim to understand how anatomy and gas exchange are related, and how seedlings of *G. ulmifolia* behaves in response to variations in different light environments.

## Methods

### Sampling

*Guazuma ulmifolia* seeds were available by Forestry Institute. According to Colli-Silva (2020), *G. ulmifolia* is native, not endemic to Brazil, popularly known as araticum-bravo, cabeça-de-negro ou mutamba, the species occurs naturally in all regions of Brazil, and in different types of vegetation. The study was carried out at Plant Ecophysiology Laboratory of the Universidade do Oeste Paulista (CEVOP), located in Presidente Prudente City, São Paulo State, Brazil (22°07' 32"S, 51°23'20"W, elevation 472 m). From seeds we produce seedlings

germinated in greenhouse conditions with controlled environment, using a tray containing washed sand, covered with vermiculite. After germinating and reaching a pair of leaves stage, seedlings were transplanted into 8 kg pots containing eutrophic red / yellow clayey soil with a medium-sandy texture, and maintained in field capacity conditions, adding water daily. A soil analysis was carried out for correction. Ninety days after germination, pots were transferred to experiment site to allow acclimatization of seedlings at different levels of irradiance.

#### Experimental design

Experimental design used was in randomized blocks, completely randomized, with two treatments of light availability (control: 100% of incident irradiance; low irradiance: 5% of irradiance), and 15 repetitions. The shading was built in a greenhouse covered by a black screen, "sombrite", the irradiance levels were calibrated using a portable photosynthetically active radiation meter (LI-191R). Seedlings were kept in these conditions for 120 days before parameter measurements. The seedlings were constantly kept in field capacity until the end of the experiment.

#### Physiological analyses

Gas exchange measurements were carried out on healthy leaves and completely expanded using an infrared gas analyzer (Ciras-2, PPSYSTEM, UK), under irradiance in full sunlight of  $1500 \mu\text{mol m}^{-2} \text{s}^{-1}$  and under the shade irradiance of  $80 \mu\text{mol m}^{-2} \text{s}^{-1}$ , with a concentration of  $\text{CO}_2$  in the sampling chamber of  $400 \mu\text{mol CO}_2 \text{ mol}^{-1}$  of air. Under these conditions, the following gas exchange parameters were measured: photosynthetic rate (Pr), stomatal conductance (Sc), sub-stomatic  $\text{CO}_2$  concentration (Ssc), transpiration (T). To obtain the chlorophyll content (Cc), a portable chlorophyll meter (model CCM-200, OPTI-SCIENCE, USA) was used, making three measurements per leaf per seedling.

#### Anatomical analyses

We collected leaves between the 3rd and 6th knots of all individuals sampled. The leaves were fixed in F.A.A. 70% for 48 hours and conserved in

70% ethanol (Johansen, 1940). Leave samples (20 x 20 mm) from median region were used and embedded in Histo-resina Leica® (prepared as instructed by the manufacturer) to perform transverse sections, and stained with Toluidine Blue (O'Brien et al., 1964). In transverse sections of the medial region of the leaf blade, the parameters were measured with the aid of the ImageJ software - National Institutes of Health USA, after transversal anatomical cut, the parameters: distance between veins (Dv-v), distance from veins to abaxial epidermis (Dv-abe), distance from veins to adaxial epidermis (Dv-ade), distance from veins to stomata (Dv-s), total leaf thickness (Tlt), abaxial epidermis thickness (Abet), adaxial epidermis thickness (Adet), palisade parenchyma thickness (Ppt), and spongy parenchyma thickness (Spt).

#### Data analyses

We initially undertook descriptive statistical analysis and used Box Plot graphics to detect outliers. Thus, values 1.5 times higher than the 3rd quartile and values 1.5 times lower than the 1st quartile were excluded from the analysis. Normality tests were performed to check the distribution of data, and when a normal distribution was not observed, data were square root-transformed. Then, a parametric analysis of variance (one-way analysis of variance (ANOVA)) was performed. When a significant difference was observed, Tukey's test was used to identify pairs of significantly different means.

#### Results and discussion

Transpiration showed no difference between treatments. At full sunlight, photosynthetic rate, sub-stomatic  $\text{CO}_2$  concentration were higher than shade. While higher values of stomatal conductance and chlorophyll content occurred in the shade (table 1).

In anatomical features most of variables analyzed showed differences between two treatments, and was higher at full sunlight, with exception to distance between veins, and distance from veins to stomata (tables 2).

**Table 1.** Average values and standard deviations of physiological features of *Guazuma ulmifolia* under different lighting conditions.

Features	Full sunlight	Shade
Pr	9.01 ± 2.92 A	1.97 ± 0.26 B
Sc	258.92 ± 35.11 B	338.92 ± 20.59 A
Ssc	143.42 ± 63.33 A	87.83 ± 37.16 B
T	2.15 ± 0.86 A	1.73 ± 0.64 A
Cc	7.86 ± 1.26 B	11.29 ± 4.52 A

Photosynthetic rate (Pr), stomatal conductance (Sc), sub-stomatic  $\text{CO}_2$  concentration (Ssc), transpiration (T) chlorophyll content (Cc).

**Table 2.** Mean values and standard deviations of anatomical features of *Guazuma ulmifolia* under different lighting conditions.

Features	Full sunlight	Shade
Dv-v	320.20 ± 95.82 A	287.00 ± 47.88 A
Dv-abe	60.03 ± 7.93 A	38.49 ± 5.04 B
Dv-ade	73.43 ± 13.75 A	46.61 ± 4.35 B
Dv-s	85.63 ± 22.73 A	100.21 ± 39.70 A
Tlt	130.45 ± 17.66 A	80.17 ± 9.04 B
Abet	18.10 ± 3.72 A	13.13 ± 3.70 B
Adet	25.64 ± 5.89 A	18.50 ± 5.49 B
Ppt	45.56 ± 10.70 A	17.76 ± 1.79 B
Spt	36.13 ± 10.04 A	26.65 ± 5.04 B

Distance between veins (Dv-v), distance from veins to abaxial epidermis (Dv-abe), distance from veins to adaxial epidermis (Dv-ade), distance from veins to stomata (Dv-s), total leaf thickness (Tlt), abaxial epidermis thickness (Abet), adaxial epidermis thickness (Adet), palisade parenchyma thickness (Ppt), and spongy parenchyma thickness (Spt).

The different luminous environments influenced the physiological and anatomical parameters. Several authors have observed the effect of luminosity on these parameters in tree species (Larcher et al., 2015; Felseburgh et al., 2016; Dardengo et al., 2017), and *Guazuma ulmifolia* is considered a pioneer species tolerant to greater light (Lorenzi, 1992). We observed that *G. ulmifolia* showed characteristics such as increased photosynthetic rate, stomatal conductance and anatomical features such as thickness of parenchyma tissues, which refer to pioneer species tolerant to high light (Aragão et al., 2014; Dardengo et al., 2017).

A higher chlorophyll content is linked to a greater absorption of light. Since in shaded environment, light is less abundant, requiring greater efficiency in the plant's absorption (Larcher, 2004). According to Engel and Poggiani (1991) studying *Erythrina speciosa* and *Tabebuia avellanadae*, and Rego and Possamai (2011) in *Cariniana legalis*, chlorophyll content presents a positive correlation with shading and a negative one in full sunlight, similar results that we observed in *G. ulmifolia* seedlings (Table 1).

Considering light as one of the most limiting factors for photosynthesis (Chazdon & Fetcher, 1984), seedlings submitted to full sunlight showed greater photosynthesis (Table 1). Larcher et al. (2015), obtained similar results in *Ligustrum lucidum*, where it demonstrated lower values of photosynthesis in the shade.

The decrease in sub-stomatic CO<sub>2</sub> concentration at full sunlight is linked to greater photosynthesis (Table 1). Pereira et al. (2011), studying *Coffea arabica*, observed a 15% increase in sub-stomatic CO<sub>2</sub> concentration in shade plants.

The fact that *G. ulmifolia* did not show any difference in transpiration between two treatments, demonstrates high capacity that species has to acclimatize to variable sunlight, showing phenotypic plasticity. Souza et al. (2017) and Silva et al. (2002) studying young plants of *Bertholletia excelsa* and *Theobroma grandiflorum*, found a difference in the values of transpiration in their treatments, respectively. However, Ribeiro et al. (2005), studying tropical tree species in contrasting

irradiance environments, also found no difference in transpiration in *G. ulmifolia*.

Stomatal conductance is the factor most associated with carbon assimilation (Rossatto et al., 2010). Similar to Rossatto et al. (2010), who evaluated functional characteristics of leaves of sun and shade of tree species in a gallery forest in Federal District (Brazil), resulting in a stomatal conductance in leaves in full sunlight of 51% greater than in leaves in the shade. Lichtenthaler et al. (2007), also observed the highest stomatal conductance in leaves in full sunlight. However, Silva et al. (2002) in *Theobroma grandiflorum* obtained a higher stomatal conductance in species in a shaded environment. An explanation for this phenomenon is given by the natural environment in which these species are found, since *T. grandiflorum* is found in understory (Silva et al., 2002), and *G. ulmifolia* is a pioneer species.

The transpiration, stomatal conductance, photosynthetic rate and sub-stomatic CO<sub>2</sub> concentration of *G. ulmifolia*, presented here, demonstrate relationship of the balance between adaptations and light intensity condition, which plant individuals are exposed.

According to Zwieniecki and Boyce (2014), angiosperms have an optimal morphospace in the mesophyll, i.e., their veins have a pattern that is related to the distance they are from the leaf's gas exchange surface. *Guazuma ulmifolia* did not present veins pattern (distance between veins equal to distance from vein to abaxial epidermis) considered optimal. The pattern presented was that of "under investment" in two treatments, showing underinvestment in vein density, where veins are closer to abaxial epidermis. This ensured statistically equivalent transpiration in both treatments (Table 1). In addition to low vein densities in thick leaves, they contribute to high water efficiency.

Due to greater thickness of upper epidermis and palisade parenchyma (Table 2), it appears that under conditions of high luminosity changes are induced at the morphological and anatomical level (Queirós et al., 2014). Similar results were found by Larcher et al. (2015), in *Ligustrum lucidum*. The thicker spongy parenchyma increases the diffusion of light within the leaf, resulting in multiple reflections

and a longer length of light path, increasing probability of absorption (Larcher et al., 2015).

The greater thickness of palisade parenchyma is important to avoid photoinhibition in leaves with exposure to greater light intensity (Taiz & Zeiger, 2006). The ratio of spaces area of spongy parenchyma to total area of same parenchyma in shadow leaflets allows for a greater use of light incident on the lower internal portion (Dardengo et al., 2017).

The lower total thickness of leaves seen in low light (Table 2) allows high gas exchange rates, but they are at a greater risk of drying out outside the protected environments in which they are universally common (Zwieniecki & Boyce, 2014).

## Conclusions

In general, pioneer species tend to have greater tolerance in environments with high light conditions. The results obtained based on the analysis of gas exchange and leaf anatomy demonstrated that *Guazuma ulmifolia* (pioneer species) corroborated the results of its successional class and showed acclimatization in environments with high luminosity.

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