Leaflet phenotypic plasticity in three woody species in two strata of a gallery forest

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ABSTRACT

Background: Along the vertical canopy profile, natural light availability is variable and may induce leaves morphophysiological changes due to phenotypic plasticity. Morphological changes in juvenile and adult leaflet of compound leaves of plant species is still poorly understood, including in woody species of gallery forests. The present study evaluated the leaflet morphophysiological characteristics and plasticity index of three woody species in canopy and understory leaves in a gallery forest.

Results: All morphophysiological characteristics varied within species (Myracrodruon urundeuva Allemão, Copaifera langsdorffii Desf. and Tapirira guianensis Aubl.). The leaflets of canopy trees showed significantly higher values of petiole length (PL) and diameter (PD), leaflet thickness (fresh - FT and dry - DT), specific leaf mass (SLM), in relation to understory leaflets. On the other hand, relative water content (RWC) did not change between adult trees and saplings, except for T. guianensis. The plasticity index of leaflet characteristics varied significantly within species. Plasticity index of three morphophysiological characteristics (PL, PD and DT) varied between species. PL and PD had the highest plasticity index values across species, whereas RWC had the lowest plasticity.

Conclusion: Plasticity index of the tree species associates these leaflet morphophysiological traits (PL, PD and DT) with variations in the physical environment between strata of a gallery forest.

Keywords: Brazilian savanna, leaf structure, specific leaf mass, relative water content

HIGHLIGHTS

Leaflets morphological traits are influenced by light variations of a gallery forest. Petiole traits (length and diameter) and dry leaflet thickness vary between species.
INTRODUCTION

A type of riparian forest, known as gallery forest, covers the humid areas in the Brazilian savanna, occupying the banks of streams and small rivers, forming closed corridors above them (Ribeiro and Walter, 2008). Although the gallery’s forests occupy less than 10% of the savanna area, they are home to an enormous diversity of flora and fauna (Oliveira-Filho and Ratter, 2002). Basic differences of the manually measured variables between formations (forested savanna, woodland savanna (rainy and dry) highlight that the gallery forest had the tallest canopy, greatest trunk volumes and biomass, even when there is a lower density of individuals (Zimbres et al., 2020). Besides, the Brazilian Forest Code regulates the protection of Riparian Zones, which are categorized as riparian permanent protection areas (RPPAs). According Forest Code, RPPAs prescribes a minimum 30 meters forest buffer around narrow streams (less than 10 m wide), to ensure water supply, erosion control, and biodiversity conservation (Federal Law 12.651/2012). In this context, it is important to increase knowledge about gallery forest, both from the biotic and abiotic component.

The first includes the survival and maintenance of gene flow between populations of animal species, while the abiotic part comprises hydrological functions, protection of the riparian zone, filtration of sediments and nutrients, control of nutrient and chemical inputs to water courses, erosion control of channel banks and the control of changes in temperature in the aquatic ecosystem (Durigan and Silveira 1999; Silva et al., 2017).

Microclimatic conditions below the closed canopy of gallery forests also provide shading for topsoil (Ribeiro and Walter, 2008; Gignoux et al., 2016). It is well known that tropical forests with denser canopies reduce incidence of solar radiation to plants in the lower stratum of the forest (Mendes et al., 2013). In heterogeneous light environments, leaves from different forest strata may have altered leaf morphophysiological characteristics (Rozendaal et al., 2006; da Silveira et al., 2015). Morphophysiological characteristics such as stomatal density, dry mass, total thickness, leaf angle, leaf shape and petiole and internode length (da Silveira et al., 2015; Marenco et al., 2017; Boeger et al., 2018) may alter fitness across species and determine divergence among species of the forest ecosystem. These changes promoted by the environment can be crucial for the survival of understory plants under heterogeneous conditions.

Despite that, morphological changes in juvenile and adult leaflet of compound leaves of plant species is still poorly understood, including in woody species of gallery forests. The structure of compound leaves provides flexibility for morphological change by variation in shape, size and arrangement of leaflets (Sanches et al., 2009; Klingenberg et al., 2012). These characteristics are important for increasing light capture and maintaining photosynthetic rates, especially in understory plants. The differences between mature leaves from a young plant and mature leaves from a fully grown tree of the same species may suggest that the microclimate influences leaf formation during plant growth.

The ability to adjust the expression of various phenotypes according to environmental conditions is known as phenotypic plasticity (Nicotra et al., 2010; Gratani, 2014). Quantifying the phenotypic plasticity is important to understand how species can or cannot adjust to environmental changes (Valladares et al., 2000). Considering changes in natural light conditions, it has been hypothesized that species may modify morphophysiological characteristics, and consequently they present high phenotypic plasticity in order to survive in different light environments (Rozendaal et al., 2006; Marenco et al., 2017). Thus, the aim of this study was to evaluate the leaflet morphological characteristics and plasticity index of Myracrodruon urundeuva Allemão (Anacardiaceae), Copaifera langsdorffii Desf. (Leguminosae) and Tapirira guianensis Aubl. (Anacardiaceae) in canopy and understory leaves of a gallery forest, at County Jatá, State Goiás, Brazil.

MATERIAL AND METHODS

The study was carried out in a fragment of a Gallery forest (17º55’S to 17º56’S and 51º42’W to 51º42’W), at County Jatá, State Goiás, Brazil, with an approximate area of 10.9 ha. The study area is located in the Cerrado, with an mean annual rainfall of 1,645 mm and an mean annual temperature of 23.7ºC, located at an altitude of 670 m (INMET, 2017). The climate in the region is tropical savanna (Awa in the Köppen classification). The predominant soil is a Dystrophic Red Latosol (Oxisol), with a very clay texture; it is deep, very porous and permeable, and with low susceptibility to erosion (Scopel and Silva, 2001).

In the study area, canopy trees (adult tree) and small understory saplings of three species with compound leaves were selected. The selected species were Myracrodruon urundeuva, Copaifera langsdorffii e Tapirira guianensis. These woody species were selected in a previous survey based on their Importance Value Index obtained from a phytosociological study. Five replications per species were collected between 7:00 and 9:00 h from each forest stratum (canopy and understory) and five leaflets per leaf were selected. Morphological data were obtained in November 2014 (rainy season). Saplings height and diameter were obtained with a millimeter rule and calipers, respectively (Tab. 1). In adult trees, diameter at breast height (1.30 m above the ground) was determined with mechanical calipers and height was estimated using a retractable ruler (Tab. 1).

The plant material (branches) was collected in the study area, properly packed in plastic bags and then taken to the laboratory for the determination of morphophysiological leaflet characteristics. From each individual studied, five leaflets fully expanded were selected. They had an adequate phytosanitary condition and were located in the middle third of the branches.

Petiole length and diameter (PL and PD, mm) data were measured using digital calipers. The fresh and dry leaflet thickness (FT and DT, mm) was measured with a digital micrometer. Specific leaf mass (g·cm⁻²) was calculated as the ratio of dry mass (g) to leaf disc area – cm² (SLM = DM/A). Relative water content (RWC) was determined by extracting...
two leaf discs per leaf, with a hollow metal cylinder with 14.15 mm in diameter. The weight of fresh matter (FM) of the samples was obtained with a precision balance (accuracy of 0.0001 g). Then, the leaf discs were transferred to a 35-mm Petri dish, where they were submerged for 36 hours in water, in order to obtain the weight of the turgid material (TM). Subsequently, the discs were oven-dried at 65ºC, 72 h, to determine the dry matter (DM). The RWC was determined by the formula: RWC (%) = 100 (FM - DM)/(TM - DM).

The phenotypic plasticity index was obtained from the leaflet morphological characteristics, calculated as the maximum value minus the minimum value divided by the maximum value, with a scale varying between 0 and 1, as proposed by Valladares et al. (2000). The phenotypic plasticity index was calculated for each species, considering the different forest strata, and for each morphological characteristic.

Mean values and respective standard deviations were calculated for the variables studied. Leaflet morphological characteristics were compared using the t-test (α = 0.05). The data were subjected to analysis of variance (ANOVA) and transformed when they did not meet the statistical assumptions (dividing the observed value by the maximum value found in each species). The morphological characteristics were correlated with each other using Pearson’s correlation. Rbio statistical analysis software was used.

RESULTS

The respective of forest stratum, morphological leaflet characteristics showed differences between species (p < 0.01; Fig. 1), except for dry thickness (DT) in canopy

**Tab 1** Tree diameter (cm) and height (m) of three species from canopy and understory of a Gallery forest fragment at County Jataí, State Goiás, Brazil.

<table>
<thead>
<tr>
<th>Species</th>
<th>Canopy cover</th>
<th>Understory cover</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Diameter (cm)</td>
<td>Height (m)</td>
</tr>
<tr>
<td>Myracrodruon urundeuva</td>
<td>11.0 – 24.0</td>
<td>17.0 – 20.0</td>
</tr>
<tr>
<td>Copaifera langsdorffii</td>
<td>14.4 – 30.0</td>
<td>18.0 – 31.0</td>
</tr>
<tr>
<td>Tapirira guianensis</td>
<td>13.6 – 40.2</td>
<td>21.0 – 24.0</td>
</tr>
</tbody>
</table>

Fig. 1 Relationship between canopy opening (CO), leaf area index (IAF) and diffuse fraction of photosynthetically active absorbed radiation (FAPARdif) and the number of harvested trees (DBH > 30 cm). * indicates a significant Person linear coefficient (p = 0.05).
leaflets ($p = 0.16, F = 2.11$) and the relative water content (RWC) of canopy ($p = 0.33, F = 1.21$) and understory leaflets ($p = 0.09, F = 2.90$).

*C. langsdorffii* (Cla) petiole was smaller in canopy and understory leaflets (Fig. 1), in relation to others species. *Tapirira guianensis* (Tgu) presented larger petiole diameter (PD) and fresh thickness (FT) in canopy and understory leaflets. Petiole diameter (DT) of *M. urundeuva* (Mur) from understory leaflets was lower than that of the other species, as well as the specific leaf mass (SLM) of this species irrespective of forest stratum. The RWC was higher in the saplings than in adult tree, except in *T. guianensis*.

Petiole length (PL) was greater in canopy leaflets than in understory leaflets (Fig. 1), except in *C. langsdorffii*. In *M. urundeuva* and *T. guianensis*, PL from understory leaflets decreased 29.4 and 42.5%, respectively, in comparison with canopy leaflets. In each species studied, PD showed significant difference ($p < 0.05$) between canopy and understory leaflets (Fig. 1). In comparison with canopy leaflets, PD decreased 22.1% in *M. urundeuva*, 21.6% in *C. langsdorffii* and 32.7% in *T. guianensis* in understory leaflets.

Fresh thickness (FT) of *M. urundeuva* leaflets showed no significant changes ($p > 0.05$) in different forest strata (Fig. 1), while *C. langsdorffii* showed an increase of 19.37% and *T. guianensis* 17.68% in canopy leaflets compared with understory leaflets. Taking the understory as the baseline, DT increased 27.45% in *M. urundeuva*, 26.38% in *C. langsdorffii* and for *T. guianensis* there was no significant difference between strata (Fig. 1).

In the three studied species, SLM varied significantly ($p < 0.05$) between leaflets of the forest strata, with the understory leaflets showing lower SLM than canopy leaflets (Figure 1). In canopy leaflets, SLM was 36.17% higher for *M. urundeuva*, 25.87% for *C. langsdorffii* and 22.49% for *T. guianensis* in comparison with understory leaflets.

Relative water content (RWC) in canopy and understory leaflets did not differ between species (Fig. 1, $p = 0.00$), the values ranged from 76.6 to 88.6%.

We found a positive correlation between SLM and PL, PD, FT or DT (Tab. 2), but there was no significantly correlation between RWC and other variables (Tab. 2). The correlation was greater between FT and PD ($0.84$).

In all species, the plasticity index of morphophysiological leaflet characteristics varied significantly within species (Fig. 2, $p = 0.00$). PL and PD plasticity had the greater values and RWC, FT and DT plasticity the lower.

The plasticity index for FT, SLM and RWC did not differ between species (Fig. 3), however the plasticity index for PL, PD and DT varied between species (Figure 3). While *C. langsdorffii* (Cla) leaflets presented greater plasticity index in relation to PL and PD leaflets, *T guianensis* (Tgu) leaflets showed lower plasticity index in dry thickness.

### Tab. 2 Pearson’s correlation coefficient between morphophysiological characteristics analyzed (petiole length and diameter (PL and PD, mm), leaflet thickness (fresh and dry – FT and DT, mm), specific leaf mass (SLM, mg cm$^{-2}$) and relative water content (RWC, %).

<table>
<thead>
<tr>
<th></th>
<th>PL</th>
<th>PD</th>
<th>FT</th>
<th>DT</th>
<th>SLM</th>
<th>RWC</th>
</tr>
</thead>
<tbody>
<tr>
<td>PL</td>
<td>-</td>
<td>-0.22</td>
<td>-</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>PD</td>
<td>-0.18</td>
<td>0.84**</td>
<td>-</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>FT</td>
<td>0.27</td>
<td>0.40*</td>
<td>0.52**</td>
<td>-</td>
<td></td>
<td></td>
</tr>
<tr>
<td>DT</td>
<td>0.53**</td>
<td>0.47**</td>
<td>0.57**</td>
<td>0.69**</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td>SLM</td>
<td>0.16</td>
<td>0.12</td>
<td>0.12</td>
<td>0.13</td>
<td>0.05</td>
<td>-</td>
</tr>
</tbody>
</table>

**Fig. 2** Phenotypic plasticity index of leaflet morphological characteristics within species (Petiole diameter and length - PD and PL, leaflet thickness (fresh and dry – FT and DT), specific leaf mass - SLM and relative water content - RWC) of three tree species (*Myracrodruon urundeuva* Allemão, *Copaifera langsdorffii* Desf. and *Tapirira guianensis* Aubl.) in canopy and understory of a gallery forest fragment.
Phenotypic plasticity index of leaflet morphological variables between species (Petiole length and diameter, leaflet thickness (fresh and dry), specific leaf mass and relative water content) of three tree species (*Myracrodruon urundeuva* - Mur, *Copaifera langsdorffii* - Cla e *Tapirira guianensis* - Tgu) in canopy and understory of a gallery forest fragment.

Petiole length (PL) had the highest plasticity index values (0.44) across species (Fig. 4), whereas RWC had the lowest plasticity (0.13).

**DISCUSSION**

*Myracrodruon urundeuva*, *Copaifera langsdorffii* and *Tapirira guianensis* leaflets in canopy and understory of a gallery forest fragment exhibited differences in morphophysiological characteristics. Our results are in agreement with those reported by Barros et al. (2012), Marenco et al. (2017), suggesting genetic differences. On the other hand, mean values for dry thickness (DT) of canopy leaflets did not differ significantly between species, which led us to conclude that this trait is less responsive to variations in environmental factors. The relative water content (RWC) values, in canopy and understory leaflets, did not differ between species, which suggests that data collected during the rainy season led all species to have high RWC. Goldsmith et al. (2013) hypothesize that foliar water uptake occurs independent of phylogeny, morphology...
and growth habit; however, more information is required to actually identify plant anatomy traits that favor this process. To maximize their photosynthetic rates, tropical plants may have modified their leaf anatomy to cope with frequent leaf wetness. By contrast, leaf anatomy traits in savanna sites are more likely to have evolved to conserve water (Aparecido et al., 2017).

Rainfall alone does not define water availability to trees (Adams et al., 2019). In the tropics especially, seasonal distributions of rainfall and evaporative demand ensure that soil water storage plays a major role in year-round water availability, which in turn is reflected in seasonal variation in photosynthetic productivity of tropical forests (Guan et al., 2015). Broadly speaking, tropical regions with pronounced dry seasons (typically savanna ecosystems) have seasonal changes in leaf area and transpiration, when compared with regions that have more uniform rainfall (Guan et al., 2015). In this respect, even in tropical rainforest changes in transpiration rates can also be observed over the year depending on environmental conditions (Antezana-Vera and Marenco, 2020).

Morphophysiological characteristics varied among strata (Boeger et al., 2004; da Silveira et al., 2015; Marenco et al., 2017). In comparison with canopy leaflets, petiole length and diameter (PL and PD), fresh and dry thickness of leaflets (FT and DT), and specific leaf mass (SLM) were lower in understory leaflets, in the present study. As expected, SLM increase with leaf development for all species, hence it is used as an indicator of leaf softness, with low values of SLM indicating soft leaves (Kursar and Coley, 2003). Understory leaves had higher leaf area and lower leaf dry mass and thickness, in comparison with canopy leaves. Under low light availability, tree species have developed strategies to capture diffuse light (Boeger et al., 2006). In Caesalpinia echinata Lam., for example, SLM of leaflets increased from 38.6 g·m⁻² at the sapling stage to 55.0 g·m⁻² in adult trees (Sanches et al., 2009), with is consistent with higher leaves thickness in canopy leaves.

Regarding the forest profile, leaves of tree species grown in the lower stratum of the forest presents leaf thickness lower than leaves from upper strata (da Silveira et al. 2015). With respect to anatomical leaf traits, leaf thickness of sun and shade leaves of Corylus avellana L. (Betulaceae) were 97.6 and 77.7 µm thick, respectively (Catoni et al., 2015a). In the first leaves of Eugenia hiemalis Cambess. (Myrtaceae), leaf thickness increased with increasing light availability (Nascimento et al., 2015). An increase in leaves thickness is often associated with an increase in palisade parenchyma thickness and values of net photosynthetic rates (Valladares et al., 2000; Catoni et al., 2015a; da Silva et al., 2015, Marenco et al., 2017). Canopy leaves have more photosynthetic cells per unit leaf area, as well as greater amounts of photosynthetic enzymes, which indicates that canopy leaflets had mesophyll cells that are well developed. In contrast, shade leaves had more spongy parenchyma and the leaves are thinner (Sanches et al., 2009; Catoni et al., 2015b; Marenco et al., 2017).

However, contrary to our initial expectations, PL and PD did not increase with reduction in light availability. Beside contributing to avoid overlapping of leaves (self-shading) and providing mechanical support, the length and diameter of the petiole may reflect the influence of light interception. King (1999) found shorter petiole in leaves with thinner leaves, and generally, leaves of individuals in the understory have thinner leaves, because they have been established under low light conditions.

Relative water content (RWC) has been commonly used to assess the level of water stress, as it directly reflects changes in photosynthetic rates (Lin et al., 2015). RWC reduction to values below 70% slows the photosynthetic CO₂ assimilation rate, promoting a decline in the photosynthetic leaf rate due to stomatal closure (Lawlor and Cornic, 2002). In cells, the reduction in CRA affects cell physiology in several ways, including changes in the position of intracellular organelles, and modifications in transport channels and enzyme biochemistry, as well as cell wall contraction (Lambers et al., 1998; Lawlor and Cornic, 2002).

Just as FT was related to PD, all leaflet characteristics revealed a relationship with SLM. Under different strata conditions, especially light-related influences, the relationships were expected, which was previously discussed. Contrary to expectations, RWC was not significantly related to leaflet characteristics, probably because RWC data showed little variation between strata.

Phenotypic plasticity was expressed in leaflets from different forest strata in response to different light availability and ontogeny. Its expression occurs differently in petioles (length and diameter) and leaf dry thickness. Phenotypic plasticity can be valuable in climate change studies, because genetic adaptation is slow (Valladares et al., 2014; Duputié et al., 2015). Plant morphological plasticity may be greater than physiological and anatomical plasticity index (Catoni et al., 2015a). The mean plasticity index of petiole length and diameter were higher than other morphophysiological characteristics, with no significant differences between species. Our results showed that species studied respond differently to changing light environments, affecting the success of understory species in forest dynamics.

In their study based on the 25 leaf traits, dos Anjos et al. (2015) identified the traits most related to high and low plasticity on young plants of five tropical tree species for six months under different light conditions. Catoni et al. (2015a) showed the high plasticity index (0.33) of C. avellana plants growing in different light conditions. This study shows plasticity index related to shade tolerance of M. urundeuva, C. langsdorffii and T. guianensis, which were responsive to light variations.

## Conclusion

Morphological characteristics of M. urundeuva, C. langsdorffii and T. guianensis leaflets of canopy and understory grown saplings are influenced by light variations, of the heterogeneous environment of a gallery forest fragment. The phenotypic plasticity of morphological variables associated with petiole traits (length and diameter) and dry leaflet thickness vary between species.
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Processing: JS, DPD
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Writing: JS, RAM, DPD
Review: RAM, WCF, DPD

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