

## Morphological Plasticity and Gas Exchange of *Ligustrum lucidum* W.T. Aiton in Distinct Light Conditions

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### ABSTRACT

*The objective of this study was to understand the different morpho-physiological strategies of Ligustrum lucidum, an invasive species occurring in Brazilian forest fragments under heterogeneous light conditions. Ten individuals of L. lucidum were selected and evaluated for morphological of the leaves and physiological traits. For morphological parameters were evaluated: length, width, area, angle, petiole length, dried mass, total thickness, palisade parenchyma and spongy parenchyma thickness, abaxial and adaxial epidermis thickness, stomata density, leaf density and specific leaf area. The physiological traits were vapor-pressure deficit, assimilation rate, CO<sub>2</sub> substomata concentration, intrinsic water-use efficiency, transpiration rate and stomatal conductance. All the physiological variables and most morphological variables presented significant differences between light conditions. Phenotypic plasticity indexes were not high as expected. However, phenotypic integration among the morphological and physiological attributes appeared to explain better these results, as observed on the relationship among assimilation rates, palisade parenchyma thickness and SLA. Phenotypic integration could increase the species adaptive responses efficiency, making it more competitive to occupy and to establish in new niches.*

**Key words:** invasive plants, phenotypic integration, assimilation rates; glossy privet

### INTRODUCTION

The ability that plants possess to respond to different environmental pressures may be the key factor of plants colonization potential (Gratani 2014). Plastic phenotypic responses to light are involved on the invasion capacity of some plants (Durand and Goldstein 2001), since allows it to adjust morphology and physiology to a certain light intensity range (Sultan 2000; Delagrangue et al. 2004). The morphological and physiological adjustments developed, maximize light efficiency capture, and thus photosynthetic rate. These characteristics are crucial to plant establishment and growth (Valladares and Pearcy 1998). Based on this concept, phenotypic plasticity has often been associated with plant invasion processes, and

it is defined as the property of a given genotype to express different phenotypes in different environments (Gratani 2014). Greater plasticity can result in variations of the morphology, physiology or development of species, ensuring adaptation success in many environments (González and Gianoli 2004; Richards et al. 2006; Davidson et al. 2011).

The nested morphological organization of plants allows plastic responses of individual metamers according to the specific environmental conditions applied to each metamer (Navas and Garnier 2002). Branches bearing leaves can be interpreted as the metamers and they respond directly to light variation, adjusting their morphology, anatomy and physiology to the local light condition (Givnish 1988; Smith et al. 1998; Brites and

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Valladares 2005). Variations in leaf format, size and structure suggests the existence of a wide range in light response mechanisms. Several studies have shown variations on leaf thickness, stomata density, leaf size, leaf area (Valladares and Niinemets 2008), leaf inclination (Myers et al. 1997) according to different light intensities that reaches the intracanopy (Dias et al 2007; Gratani 2014). Intracanopy plasticity can contribute to the whole canopy performance due to their effects on the energy and water balance of individual leaves (Gratani 2014). Several studies highlight that sun leaves tend to have sharp angles, smaller leaf area and stem lengths while leaf mass and blade thickness tend to be higher in shade leaves (Brites and Valladares 2005; Larcher and Boeger 2009).

Physiological adjustments are also expected to exhibit plastic responses to light variations (Valladares et al. 2002; Gratani 2014). Gas exchange parameters, such as photosynthetic rate, are responsive to light changes and useful in understanding light adaptation mechanisms (Lichtenthaler et al. 2007). Environmental conditions, mainly temperature and light, affect plant physiological processes and induce the mechanisms development, allowing environmental acclimation (Searle et al. 2011). Some of these mechanisms are stomata conductance and photosynthetic rate regulation to make an efficient use of available light (Aasamaa and Söber 2011). Although the interest in biological invasions prevention and control has generated extensive information about the invasion process (Gurvich et al. 2005; Richards et al. 2006), ecophysiological aspects related to these invasions are poorly understood (Niinemets et al. 2003). The complex relationship between the invasion process and niche opportunity differ among the species and space (Gurvich et al. 2005), generating several responses that are not yet elucidated.

*Ligustrum lucidum* W. T. Aiton (Oleaceae) is a native tree from China, 10 m high, with simple and opposite, lanceolate shape and entire margin leaves. This species is considered an invasive in North and South America, Asia and Oceania (Aragón and Groom 2003). *L. lucidum* has a remarkable dispersion by the birds (Ayup et al. 2014) and occupation ability of different habitats, with potential to fill gaps and forest edges (Backes and Irgang 2004; Hoyos et al. 2010). Previous floristic and phytosociological surveys in the studied area indicated that *L. lucidum* presented an

aggregate distribution occupying forest edges, but some individuals were also found at the inner fragment areas. In this fragment, this species had a high importance index value (Reginato et al. 2008), probably because it was largely used in urban afforestation (Guidini et al. 2014).

This study aimed to understand the different strategies that made *L. lucidum* a good competitor in the forest environments, even in heterogeneous light conditions within the canopy. Morphological, anatomical and gas exchange parameters of sun and shade leaves of *L. lucidum* were evaluated to detect leaf plasticity.

## MATERIAL AND METHODS

### Study area

Experiment was conducted in a 55 ha of Mixed Ombrophilus Forest fragment (Araucaria Forest) at Centro Politecnico of Universidade Federal do Parana, located east of Curitiba, Parana State (25° 25 'S and 49° 17 'W 900 m). Region climate is Cfb (Köppen classification), representing a subtropical humid mesothermic, without dry season, with mild summers and winters with frequent frosts. According to the provided data by SIMEPAR for 2013, annual mean temperature was between 17 and 19°C. The annual average relative humidity was 81.4% and annual rainfall was 1426.7 mm.

### Leaf morphology and anatomy analysis

Ten *L. lucidum* individuals, with estimated height from 7 to 10 m, were marked. Sun (Photosynthetic Active Radiation =  $1874.7 \pm 1027.7 \mu\text{mol.m}^{-2}.\text{s}^{-1}$ ) and shade (Photosynthetic Active Radiation =  $79.1 \pm 34.7 \mu\text{mol.m}^{-2}.\text{s}^{-1}$ ) branches were selected. In each branch, five leaves between the 3<sup>rd</sup> and the 6<sup>th</sup> node from the apex were collected for each light condition, totaling 100 leaves. Leaf angles were measured with a protractor. Leaf length and width, were measured with a millimeter tape and the petiole length, with a digital caliper. Selected leaves were dried at 60°C until constant weight. Dry mass (g) was estimated using an analytical scale. Leaf area (cm<sup>2</sup>) was calculated from a scanned image in a flatbed scanner with the help of Sigma Scan software (version 4.0, SPSS Inc., Chicago, IL, USA). Specific leaf area (SLA, cm<sup>2</sup>.g<sup>-1</sup>) was estimated by the leaf area and dry mass ratio. Leaf density (mg.mm<sup>-3</sup>) were estimated by the leaf mass/leaf area  $\times$  1/leaf thickness ratio (Witkowski and

Lamont 1991). Stomata density was estimated from modeling with colorless nail polish on the abaxial face of the epidermis of dry leaves. Stomata density ( $\text{n.mm}^{-2}$ ) was determined from the clear nail polish prints and from the median epidermal surface of leaves and leaflets, using coupled camera light microscope.

Ten leaves were fixed in FAA 70%, for 48 h, and kept in ethanol 70% (Johansen 1940). For anatomical studies,  $0.5 \text{ cm}^2$  samples were taken from the median region of leaf blades, sectioned with a razor and stained with toluidine blue 0.05% (Feder and O'Brien 1968), and mounted in glycerin. Blade, epidermis, palisade and spongy thickness were measured in transverse sections using micrometric ocular coupled in a light microscope Olympus-BX 41.

#### Gas exchange and photosynthetic rates

Gas exchange measurements were performed during summer of 2013, around noon (11:00 to 13:00 h) in sunny days under natural humidity and temperature conditions. Three sunlight exposed leaves and three shade mature leaves were analyzed from five selected individuals. A portable infrared gas analyzer system (IRGA CI-340 model, BioScience) with open system were used, analyzing  $\text{CO}_2$  concentration, air and leaf temperature, relative humidity and photosynthetically active radiation (PAR). From these data and considering atmospheric  $\text{CO}_2$  concentration of approximate  $380 \mu\text{mol mol}^{-1}$ , assimilation rate ( $A$ ,  $\mu\text{mol m}^{-2}\text{s}^{-1}$ ), stomatal conductance ( $g_s$ ,  $\text{mol m}^{-2}\text{s}^{-1}$ ), transpiration rate ( $T$ ,  $\text{mmol H}_2\text{O m}^{-2}\text{s}^{-1}$ ), vapor-pressure deficit ( $VPD$ ,  $\text{Kg.Pa}$ ) and internal  $\text{CO}_2$  concentration ( $C_i$ , ppm) were calculated. Intrinsic water-use efficiency ( $iWUE$ ,  $\text{mmol CO}_2 \text{ mol}^{-1} \text{ H}_2\text{O}$ ) was calculated as the ratio  $A_{max}/g_s$  (Farquhar et al. 1982).

#### Phenotypic plasticity index and data analysis

For all the quantitative variables, phenotypic plasticity index were calculated (PPI, *sensu* Valladares et al. 2006), according to the following formula:  $\text{PPI} = (\text{maximum mean value} - \text{minimum mean value}) / (\text{mean maximum value})$ . The index varies from zero to one and allows comparisons

among traits with different units (Valladares et al. 2000a). Means and respective standard deviations for all quantitative variables were calculated. Analyzed traits mean values in the two light conditions were compared by T Test at  $\alpha = .05$ , using the software Past 2.17 (Hammer et al. 2001).

## RESULTS

Significant differences were observed in morphological and anatomical traits between sun and shade leaves for nine variables (Table 1). Among them, only SLA and leaf angle were higher on shade leaves (48 and 30%, respectively). The following variables showed higher values on the sun leaves: dry mass (43%), total thickness (38%), leaf density (28%), palisade and spongy parenchyma thickness (46 and 22%, respectively), palisade/spongy parenchyma ratio (28%). Leaf length, leaf width, length/width ratio, petiole length, leaf area and stomata density did not differ between sun and shade leaves. All physiological traits exhibited significant differences between distinct light conditions. PAR was higher in the shade leaves (96%), leaf temperature differed about  $10^\circ\text{C}$ , and the highest value occurred in sun leaves (Table 1).

Assimilation rate, transpiration rate and intrinsic water-use efficiency differed approximately 60% between light conditions, being higher in the sun leaves. Vapor-pressure deficit was 57% higher in the sun leaves while stomata conductance and internal  $\text{CO}_2$  concentration was higher in shade leaves (18% and 14%, respectively). The uppermost PPI values were observed for gas exchange variables (vapor-pressure deficit; transpiration temperature; internal  $\text{CO}_2$  concentration and intrinsic water-use efficiency). SLA presented highest PPI value among morphological variables, while palisade parenchyma thickness showed highest value among anatomical traits. However, all traits presented a PPI lower than 0.5, which meant low plasticity of evaluated traits (Table 1).

**Table 1** – Morphological, anatomical and physiological traits from sun and shade leaves means, respective standard deviations and phenotypic plasticity index (IPF). Legends: PAR (photosynthetic active radiation); Temp<sub>leaf</sub> (leaf temperature); A (assimilation rate); T (Transpiration rate); *gs* (stomata conductance); *iWUE* (intrinsic efficiency of water use); *VPD* (vapor-pressure deficit); *Ci* (internal CO<sub>2</sub> concentration); PPI (phenotypic plasticity index)\*. Means followed by different letters in the same row are statistically significant, by T test, p<0.05.

| Traits  | Full sun      | Shade         | PPI         |
|---|---------------|---------------|-------------|
| <b>Morphology</b>   |               |               |             |
| Angle (°)   | 71.6 ± 14.6b  | 102.8 ± 13.0a | 0.30        |
| Leaf area (cm <sup>2</sup> )  | 36.2 ± 11.6a  | 36.5 ± 9.6a   | 0.01        |
| Leaf dry mass (g)   | 0.8 ± 0.2a    | 0.4 ± 0.2b    | 0.43        |
| Leaf density (mg.mm <sup>-3</sup> )                                   | 0.5 ± 0.08a   | 0.3 ± 0.07b   | 0.27        |
| Length (cm)   | 10.9 ± 1.6a   | 11.1 ± 1.6a   | 0.01        |
| Width (cm)  | 5.2 ± 1.1a    | 5.5 ± 0.7a    | 0.05        |
| Length/width ratio  | 2.1 ± 0.4a    | 2.0 ± 0.2a    | 0.06        |
| Petiole length (cm)   | 1.5 ± 0.3a    | 1.3 ± 0.4a    | 0.13        |
| Specific leaf area (cm <sup>2</sup> .g <sup>-1</sup> )                | 49.6 ± 11.4b  | 96.2 ± 29.1a  | 0.48        |
| <b>PPI Mean</b>   |               |               | <b>0.37</b> |
| <b>Anatomy</b>  |               |               |             |
| Palisade parenchyma thickness (µm)                                    | 260.6 ± 55.2a | 140.4 ± 32.2b | 0.46        |
| Spongy parenchyma thickness (µm)                                      | 144.1 ± 25.8a | 112.2 ± 35.2b | 0.22        |
| Rate palisade/spongy parenchyma                                       | 1.9 ± 0.5a    | 1.3 ± 0.4b    | 0.28        |
| Stomata density (n.mm <sup>-2</sup> )                                 | 220.9 ± 33.9a | 204.1 ± 54.9a | 0.08        |
| Total thickness (µm)  | 467.5 ± 65.8a | 305.1 ± 51.2b | 0.35        |
| <b>PPI Mean</b>   |               |               | <b>0.28</b> |
| <b>Gas Exchange</b>   |               |               |             |
| Temp <sub>leaf</sub> (°C)   | 38.8 ± 4.2a   | 25.9 ± 0.3b   | --          |
| A (µmol CO <sub>2</sub> m <sup>-2</sup> .s <sup>-1</sup> )            | 3.4 ± 2.9a    | 1.4 ± 1.4b    | 0.58        |
| <i>Ci</i> (ppm)   | 301.3 ± 57.4b | 348.5 ± 37.5a | 0.14        |
| <i>gs</i> (mol H <sub>2</sub> O m <sup>-2</sup> .s <sup>-1</sup> )    | 67.4 ± 29.6b  | 82 ± 24.6a    | 0.18        |
| <i>iWUE</i> (µmol CO <sub>2</sub> mol <sup>-1</sup> H <sub>2</sub> O) | 0.05 ± 0.04a  | 0.02 ± 0.02b  | 0.59        |
| <i>T</i> (mmol.m <sup>-2</sup> .s <sup>-1</sup> )                     | 2.8 ± 0.8a    | 1.2 ± 0.3b    | 0.58        |
| <i>VPD</i> (KgPa)   | 4.4 ± 1.7a    | 1.9 ± 0.1b    | 0.57        |
| <b>PPI Mean</b>   |               |               | <b>0.44</b> |

\* PPI mean values were calculated only with significant traits (p<0.05).

## DISCUSSION

The studied individuals responded to light availability and presented higher plasticity, e.g. SLA, leaf density, chlorophyll parenchyma thickness, assimilation rates, transpiration rate and intrinsic water-use efficiency. Other variables such as leaf area, petiole length and stomata density were expected to show some variation since they were known as sensitive to light (Niinemets 2010). However, no change was observed under different light conditions.

SLA and leaf density expresses higher area per unit mass investment and, generally, greater values occur in low light availability environments (Gutschick 1999; Vendramini et al. 2002). SLA and leaf density variations are usually result of a

leaf area, dry mass and thickness adjustment in response to light condition (Niinemets 2010). At higher light environments, specific leaf area and leaf density tended to decrease (Takenaka et al. 2001), as observed in the present study indicating a greater investment in photosynthetic tissue by increasing chlorophyll parenchyma thickness. However, sun and shade leaves did not present leaf area variation but only blade thickness and dry mass. This result did not follow the trend found in most morphological leaf plasticity in response to light studies (Vogelmann and Gorton 2014).

Chlorophyll parenchyma and total thickness in sun leaves could be associated to the increase in cells length and number of layers of both palisade and spongy parenchyma (Myers et al. 1997). In sun leaves, the quality of light reaching leaves

produces morphological adjustments. When sunbeams reach the leaf, specialized morphology canalizes available light allowing its best utilization by palisade cells. Thicker spongy parenchyma enhances light diffusion inside the leaf, resulting in multiple reflections and higher light path length, increasing absorption probability (Delucia et al. 1991; Vogelmann and Gorton 2014).

Leaf angle is also important to improve light capture. Sun leaves showed a vertical arrangement, which is a recognized protection mechanism against damage to the photosynthetic apparatus from excessive light interception (Falster and Westoby 2003; Mullen et al. 2006). These adjustments in the leaf inclination angle are a tradeoff between light absorption and prevention to stressful conditions (Myers et al. 1997; Van Zanten et al. 2010). In addition, in the leaves with a vertical arrangement, the total carbon gain may increase due to the efficiency of light absorption by abaxial surface when the sun is near horizon line (Delucia et al. 1991, Falster and Westoby 2003). The horizontal position concomitant to thinner blades presented by shade leaves appeared to be a strategy to capture most of diffused sunbeams and to compensate the low light availability (Pearcy et al. 2005).

The physiological characteristics analyzed also responded to light conditions. Sun leaves exhibited lower values of stomata conductance and internal CO<sub>2</sub> concentration. However, higher assimilation rates expressed by these leaves could be interpreted as a higher carbon assimilation capacity through a set of morpho-anatomical features associated to light processing, e.g., thicker chlorophyll parenchyma represented higher investment in photosynthetic machinery (Klich 2000), which resulted in more efficient photosynthesis.

When compared to other studies, both sun and shade leaves assimilation rates of *L. lucidum* were lower than expected (Zhang et al. 2013), probably due to the measurements being held around noon. Previous studies involving the photosynthetic traits showed that this species displayed a photosynthetic depression as a protection strategy to the photosynthetic system at that time of day (Liang et al. 2008; Fang et al. 2012). This mechanism is widely known for the species that occur under water stress, as in desert, grasslands, and savanas (Lüttge 2008), but it was not expected to exist in subtropical humid climate conditions, as

observed in this study. At higher temperature and low CO<sub>2</sub> concentration, enzymatic activity in the leaves can be changed, thus preventing photosynthetic apparatus damage (Dias and Marengo 2007). In the case of *L. lucidum*, it seems to be an inherent strategy, since it occurs regardless temperature and light availability (Fang et al. 2012) and can be linked to the species-invasive success.

The balance between the transpiration rate and stomata conductance represent the tradeoff among carbon incorporation and water availability. In this study, sun leaves showed the most conservative water use (increased *iWUE*), which would be expected for the species under water stress. In general, intrinsic water-use efficiency may be related to environmental conditions and it increases with decreased water availability (Lüttge 2008). High light availability and consequent high temperature can trigger these adaptations. However, it is difficult to pinpoint if differences in intrinsic water-use efficiency are related to vapor-pressure deficit between the leaf and air, or if differences are associated with evaporation and leaf temperature, considering that all the factors interact (Evans and Loreto 2000).

Under higher temperature and irradiance conditions, vapor-pressure deficit between leaf and air increased, producing adjustments in assimilation rates, transpiration rates, stomata conductance, and consequently, the intrinsic water-use efficiency (Lüttge 2008; Duursma et al. 2014), as found in this study. Sun leaves showed higher intrinsic water-use efficiency, lower stomata conductance and transpiration rate. In response to greater vapor-pressure deficit, plants tend to close their stomata to balance water vapor loss through transpiration and water flow inside guard cells (Yang et al. 2012). Stomata conductance appears to be linked to the variation of light radiation by regulating the process of gas exchange maintenance necessary for photosynthesis; still, it is also regulated by the tradeoff between leaf water status and water loss by transpiration (Evans and Loreto 2000). In this study, stomata conductance decreased according to the increase in evaporation demand, which represented higher photo protection and water conservation, despite the lower instant carbon gain (Valladares and Pearcy 1997; Marengo et al. 2006; Dias and Marengo 2007). Under conditions of intense light and high vapor-pressure deficit, higher transpiration rates were expected, as found

in sun leaves. This adjustment was associated to individuals' thermoregulation and prevention against overheating of the photosynthetic system (Gutschick 1999; Yang et al. 2012). In addition, transpiration rate and stomata conductance adjustment, vapor-pressure deficit seems to be related to internal CO<sub>2</sub> concentration. In sun leaves, internal CO<sub>2</sub> concentration values were lower compared to shade leaves. Probably, the stomata closure lead to lower stomata conductance and internal CO<sub>2</sub> concentration decrease (Dias and Marengo 2007). However, sun leaves had higher assimilation rates than shade leaves, even with lower internal CO<sub>2</sub> concentration, due to morpho-anatomical feature combination, like greater palisade and spongy parenchyma thickness.

The uppermost PPI values were found for the dry mass, specific leaf area, and palisade parenchyma thickness supported the premise that biomass investment presented by the sun leaves was directly involved in adaptive responses to the light (Valladares et al. 2000a; Funk 2008) and provided a better use of this resource (Meziane and Shipley 1999). The photosynthetic tissue investment influenced the highest observed values in assimilation rate and intrinsic water-use efficiency, expressed in higher values of PPI (Vogelmann and Gorton 2014). The major physiological attributes PPI values of *L. lucidum* also influenced growth capacity and establishment in the areas with intense radiation (Valladares et al. 2002). Many invasive species have their success linked to the traits such as high photosynthetic rates, high SLA values, low root/stem ratio, high fertility and high relative growth rate (Aragón and Groom 2003), since these characteristics express a phenotypic advantage in several environments (Godoy et al. 2012).

Although some PPI values indicated plasticity for this species (Valladares et al. 2002, Valladares et al. 2000b), taking into account average indexes for morphology, anatomy and physiology, PPI values were not considered high for most analyzed variables. Therefore, PPI values found for *L. lucidum* in this area did not characterize the species as highly plastic as expected for the invasive species. The plasticity in the invasive species seemed to be a determinant factor for invasion success in different environments because this allowed them to be better competitors (Zou et al 2007). On the other hand, the PPI analyzed a solo attribute, without contemplating the co-variation of attributes and how they were

depending on each other. When considering integration between morphological and physiological attributes, plasticity index values could explain better these findings for this species. The functional attributes integration is defined as phenotypic integration (Pigliucci 2003) and indicates that an integrated phenotype can respond to environmental change more efficiently, producing adaptive responses to the environment (Godoy et al. 2012). The correlation among traits can be interpreted the integrated function of growth, morphology, life history and physiology (Arntz and Delph 2001). For example, traits as assimilation rate, palisade parenchyma thickness and SLA are highly correlated and have faster plastic responses, but not necessarily cause changes in other characteristics of higher organization levels (Godoy et al. 2012). During the invasion process, phenotypic integration can be more important than plasticity of isolated features, because faster adaptive responses may increase species suitability, allowing a wide range of resource utilization and improving competitive ability to occupy new niches (Godoy et al. 2012). This integration may be crucial to survival in heterogeneous and variable conditions (Gratini 2014). Our results suggest also that the combined expression of these features is independent of climate conditions, since they are present in an area with climate conditions distinct from those observed in its native area. Thus, the presence of an integrated response to environment may explain part of *L. lucidum* success as an invasive species.

## CONCLUSIONS

The isolated PPI value analysis for *L. lucidum* did not support the hypothesis that this species was highly plastic, as expected for the invasive species. The highest plasticity values were found for the physiological characteristics, such as assimilation rate and intrinsic water-use efficiency, followed by specific leaf-area and palisade parenchyma thickness. Together, these characteristics demonstrated a higher investment in photosynthetic tissue in the sun leaves, which influenced the growth in the areas with intense radiation. Conversely, when considering the integration between the morphological and physiological attributes, plasticity index values seemed to explain better these findings, e.g., plasticity index integration of assimilation rate,

SLA and palisade parenchyma thickness. These findings suggested that invasive species invested in the traits that enabled fast growth (high SLA and high A), allowing them to be more successful invaders and competitive in heterogeneous environments such as ombrophilous forests.

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