

Gas Exchange and Plant Growth after Defoliation on *Leandra lacunosa*, a Cerrado Woody Species with Continuous Leaf Production

Maria Angélica Damascos^{1,2}, Carlos Cesar Ronquim¹ and Carlos Henrique Britto Assis Prado^{1*}

¹Universidade Federal de São Carlos; Centro de Ciências Biológicas e da Saúde; Departamento de Botânica; Laboratório de Fisiologia Vegetal; 13565-905; São Carlos - SP - Brasil. ²Universidad Nacional del Comahue; Departamento de Ecología; Quintral 1250; (8400); San Carlos de Bariloche - Argentina

ABSTRACT

Morphological and physiological leaf traits were studied on control and defoliated potted Leandra lacunosa young plants. After defoliation, remaining bottom leaves showed no significant change in net photosynthesis and stomatal conductance values with time, but the leaf transpiration rate was higher. The production of the new leaves was greater after defoliation in top defoliated plants. Bottom defoliated plants increased stem extension rates and net photosynthesis transitorily. Two months after defoliation, control and defoliated plants did not differ in stem length and root dry mass values. In spite of smaller area and photosynthetic capacity, remaining bottom leaves were able to compensate top leaves elimination. The effectiveness of this compensatory response, however, depended on the ability of new leaves to escape from herbivores.

Key words: Compensatory photosynthesis, herbivory, leaf age, leaf phenology, *Leandra lacunosa*

INTRODUCTION

Herbivores decrease plant carbon gains because they reduce the size of the carbon source. Plant defoliation experiments have demonstrated that plant growth and reproduction are negatively affected by herbivores (Aide, 1993) but plants can replace leaf area loss through new leaf production or by increasing the photosynthetic rate of the remaining leaves (Chabot and Hicks, 1982). This compensatory photosynthesis normally occurs over a period of days and involves an enhancement of carboxylation efficiency, presumably increasing nutrient and/or hormones supply for surviving leaves (Mooney, 1989).

Since insect damage is higher on top not-expanded young leaves (Coley and Barone, 1996) the survival bottom leaves of species with continuous leaf production, should compensate the loss of the more productive young leaf area increasing their photosynthesis in order to maintain plant carbon balance. However, vertical differences in shading and leaf age affect photosynthesis rate of overtopped leaves (Kikuzawa, 1995; Kikuzawa et al., 1996). Photosynthetic capacity decreases with leaf age because old leaves redistribute resources to younger leaves, improving the whole shoot photosynthetic income (Field and Mooney, 1983; Kitajima et al., 1997). It was indicated that leaf position and orientation seem to be more important

* Author for correspondence

in determining photosynthetic capacity than leaf age (Ackerly, 1999). Overtopped shaded leaves reduce photosynthetic capacity as the result of their enzyme content, which decreases in concert with lower light level (Money and Gulmon, 1982). These factors would determine that after new leaf insect herbivory the remaining bottom leaves of species with continuous leaf production show reduced photosynthetic capacity recuperation.

This study analyze if the remaining old leaves on new leaf-defoliated plants are able to overcome the herbivory effects maintaining the plant growth throughout the following months. The consequences of differential plant defoliation (elimination of top or bottom plant leaves) were studied on young individuals of *Leandra lacunosa* Cong. (Melastomataceae), a native shrub of the Brazilian cerrado. Leaf production on *Leandra lacunosa* follows a continuous pattern throughout six or seven months. Yearly, insects cause a loss of about 41-68% of leaves produced on adult plant shoots during wet season, 3% of leaves formed on the same shoots during dry season, whereas 18% of leaf herbivory occurs on ramifications emerged on main shoots during dry period (Damascos, unpubl. data). Insect preference by *Leandra lacunosa*'s not-expanded young leaves accounted for 74 to 84% of total leaf herbivory and the remaining leaves, which escaped from insects, survived for 4 to 13 months on plants (values from two consecutive years, Damascos, unpubl. data).

Before defoliation, this study analyzed the vertical photosynthetic gradient of leaves attached to different positions of the stem, as well as the structural (leaf size, specific leaf mass) and physiological (gas exchange) differences between top and bottom *L. lacunosa* leaves. Short-term effects of top or bottom plant leaf elimination on gas exchange of remaining leaves and stem extension were determined by periodic measurements. Root and stem biomass and production of new leaves were compared between defoliated plants and control plants at the end of the experiment.

MATERIALS AND METHODS

Leandra lacunosa Cogn. (Melastomataceae) is a woody species occurring in Brazilian cerrado physiognomies (Ratter et al., 1996; Mendonça et al., 1998, Castro et al., 1999), an area of

Neotropical savanna where dry period corresponds to winter. Adult plants reach 1.5 m in height and grow in sites with some shading, protected by taller shrubs or trees. Leaf insertion is opposite and decussate, and adult plant shoots produce terminal inflorescences. Leaf production occurs between October (wet season) and March or April (beginning of dry season) on main shoots, and during the dry season on new branches. Flowering happens between February and October, and fruiting between July and November (Damascos, unpubl. data).

In November 2000 field collected seeds were sown in pots with sifted cerrado soil. This soil presented 26 g dm⁻³ of total organic matter, pH in CaCl₂ centimolar solution was 3.9 and the cation exchange capacity value was 63.3 mmol_c dm⁻³. Available nutrients included 4 mg dm⁻³ of phosphorus, 1.3, 3.0, and 1.0 mmol_c dm⁻³ of potassium, calcium and magnesium, respectively. Soil analysis was carried out in FertLab in São Paulo State University (UNESP-Jaboticabal). Plants were grown in a greenhouse under 72% of transmittance (maximum values of photosynthetic photon flux density at noon varied between 1300 and 1600 μmol m² s⁻¹), daytime air relative humidity varied between 59 and 67%, and air temperature was 25 ± 2 °C.

At the end of August 2001, thirty six-month-old plants showing similar leaf number and size were randomly assigned to three treatments (n = 10 individuals per treatment): a) top defoliated plants (TD), b) bottom defoliated plants (BD), c) control plants (CO). Top and bottom leaves were identified on each individual according to striking differences in leaf visual characteristics (size, thickness) and by separating plants into two parts (top and bottom) with a colored label around the stem. On September 3rd, plants were defoliated according to the schema shown on Fig. 1.

Leaf gas exchange was measured with a portable infrared gas analyzer ADC, LCA-4, Hoddesdon, UK. Photosynthetic Photon Flux density (PPFD) was maintained at 1500 μmol m⁻²s⁻¹ by a PLU-002 system, ADC, and leaf temperature was held at 25-27 °C by a Peltier system, ADC, set at the head of the leaf chamber (PLC-N, ADC).

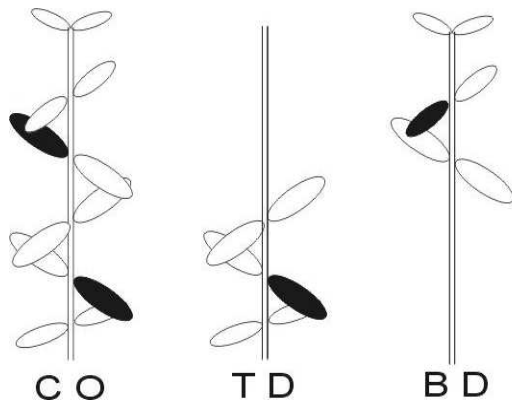


Figure 1 - Schematic representation of the defoliation treatment for *Leandra lacunosa* young plants. CO: control plants, TD: top defoliated plants, BD: bottom defoliated plants. The black color shows marked leaf for gas exchange measurements.

The curve of net photosynthesis (A) as function of PPFD was determined for one selected top leaf and one selected bottom leaf of the same intact (CO) plant. The leaf showing the highest photosynthetic capacity in an individual was chosen to perform A-PPFD curves. Values in A-PPFD curves were taken at regular intervals $100 \mu\text{mol m}^{-2}\text{s}^{-1}$ of PPFD between 2200 and $0 \mu\text{mol m}^{-2}\text{s}^{-1}$. The equation used to adjust the values of A-PPFD curve was that used by Prado and Moraes (1997). The light compensation point (LCP), dark respiration (R_e) and the light saturating net photosynthesis (LSP) were obtained from those A-PPFD curves. The last value was used as reference to PPFD during gas exchange measurements before and after defoliation experiment ($1500 \mu\text{mol m}^{-2}\text{s}^{-1}$).

The vertical gradient of net photosynthesis was measured on all leaves inserted above the second or third node from plant bases on five CO plants. The lowest pair of leaves could not be measured because there was no space between the leaf and the pot to fit the leaf chamber.

The methodology used by Meyer (1998) was considered for gas exchange measures after defoliation, on defoliated plants, as follows. On September 1st, one leaf of the plant part that did not suffer defoliation was labeled for future periodic gas exchange measurements on six plants of each treatment (Fig. 1). Two leaves were marked on each of the six control plants, one top leaf and one bottom leaf (Fig. 1). Gas exchange was measured before defoliation, on September 2nd

and 3rd, and after defoliation, on September 6th, 12th and 20th (four, ten and 18 days after defoliation, respectively). Three measures were made for each marked leaf and the mean values were calculated. Measurements were done during the morning, between 7:00 am and 10:00 am.

Detached leaves during plant defoliation of six BD and six TD plants were scanned and their area was measured by using the image-pro software for windows 4.0 (Media Cybernetics, USA). Leaves were dried up in stove at $60 \text{ }^\circ\text{C}$ until constant weight, and weighted individually. The specific leaf mass (SLM, $\text{g}\cdot\text{m}^{-2}$) values of top and bottom leaves detached from defoliated plants were determined by using the area and the dry weight values of each detached leaf.

Plant height was measured before defoliation (on September 10th) with a millimeter ruler from soil surface to the last leaf insertion point and the total leaf number was counted on the ten individuals of each treatment. Linear stem length of the thirty plants was measured every ten days, from September 20th (eight days after defoliation) until November 11th. On this last day the new leaves emerged on the upper part of the TD, BD, and CO plants (n =ten individuals for each treatment) were counted.

At the end of the experiment (60 days after defoliation) leaves, stems and roots of all TD, BD and CO plants ($n = 30$) were harvested and dried up in stove at $60 \text{ }^\circ\text{C}$ until constant weight. Leaves, stems and roots were weighted separately.

Leaf biomass, leaf area, SLM, and total leaf biomass values of top and bottom leaves detached from TD and BD plants were compared with the Student t test (Zar, 1999). The same test was used to compare leaf photosynthesis (A), stomatal conductance (g_s), and leaf transpiration (E) between leaves of defoliated and control plants on each sampling date. Gas exchange values of leaves inserted at the most basal, the medium, and the distal stem part of the five control plants were compared with the one-way ANOVA (Zar, 1999). The same test was used to compare all other studied variables (stem linear extension, root biomass, new leaf re-growth number, stem mass/stem length ratio, stem/root mass ratio) among TD, BD, and CO plants, at the end of the experiment.

RESULTS

Bottom expanded leaves presented lower mean values of morphological traits than those for top expanded leaves (Table 1). Maximum rate of A and dark respiration obtained from A-PPFD curves was higher for top (11.40 and $0.90 \mu\text{mol m}^{-2} \text{s}^{-1}$,

respectively) than for bottom (7.90 and $0.65 \mu\text{mol m}^{-2} \text{s}^{-1}$, respectively) leaves while values of light compensation point (LCP) were similar for both kinds of leaves (29 and $32 \mu\text{mol m}^{-2} \text{s}^{-1}$).

Table 1 - Morphological traits (mean \pm SE, n = 6 individuals) of top and bottom expanded leaves of *Leandra lacunosa* young plants before defoliation. Values followed by different letters indicate significant differences (Student t-test, $p < 0.05$) between top and bottom expanded leaves. SLM: specific leaf mass.

Morphological Traits	Top leaves	Bottom leaves
Leaf mass (g)	1.10 ± 0.16 a	0.96 ± 0.13 b
Dry mass/leaf (g)	0.31 ± 0.02 a	0.19 ± 0.02 b
Area/leaf (cm^2)	44.14 ± 2.88 a	30.61 ± 2.25 b
SLM (g m^{-2})	71.23 ± 2.16 a	61.53 ± 1.90 b

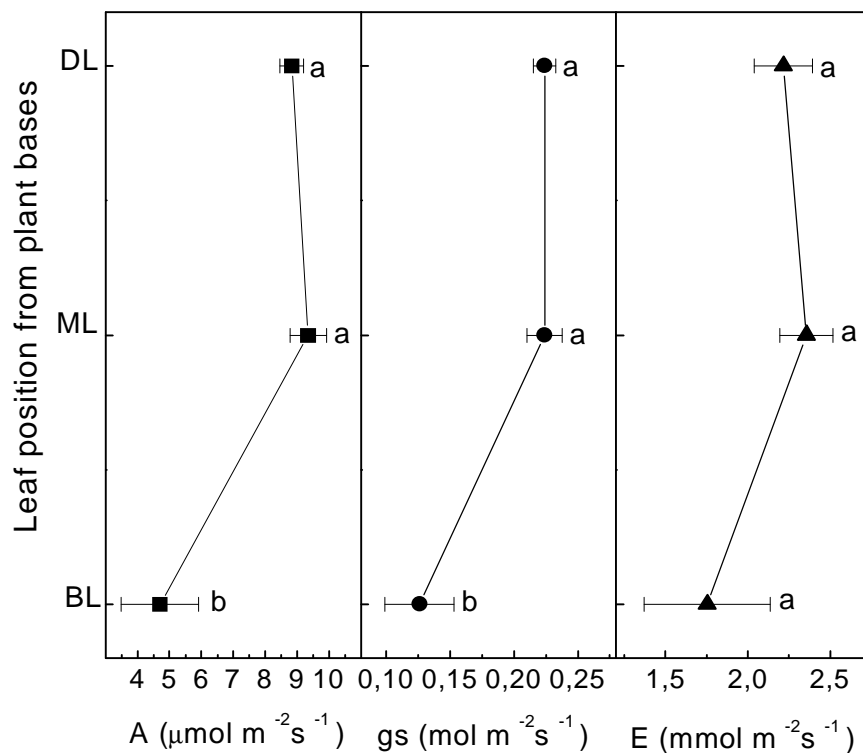


Figure 2 - Leaf gas exchange values (mean \pm SE) for *Leandra lacunosa* young intact control plants (n=5). A: net photosynthesis, gs: stomatal conductance, E: transpiration. BL: most basal measured expanded leaf, ML: middle leaf, DL: most distal expanded leaf. Values followed by different letters indicate significant differences (one-way ANOVA, $p < 0.05$) among leaves attached at each position.

Fig. 2 shows the A, gs and E mean values of leaves attached to three different positions along the stem of five intact CO plants. Net photosynthesis (A), and gs mean values measured on the lowest leaf (basal leaf) were smaller ($p < 0.05$) than those on the middle and distal expanded leaves (Fig. 2). Leaf transpiration was similar among leaves of three studied positions. Leaves of BD and CO plants showed significant differences in photosynthesis rate 18 days after defoliation (Fig. 3). Stomatal conductance did not present significant variation among treatments and control plants, or among sampling dates in same treatment (Fig. 3). Leaf transpiration increased four days after defoliation for TD plants (Fig. 3).

BD and TD plants showed, respectively, higher and lower linear stem extension than CO plants at 20 days after defoliation (Fig. 4). However, final stem linear extension at the end of the experiment (60 days after defoliation) was similar among treatments and control (Table 2).

The production of new leaves after defoliation was higher for TD than for BD plants (Table 2). However, only two TD plants produced new distal branches at the end of the experiment.

Final root dry biomass, dry stem mass/stem length ratio and stem/root dry mass ratio measured at the end of the experiment did not differ between TD, BD, and CO plants (Table 2).

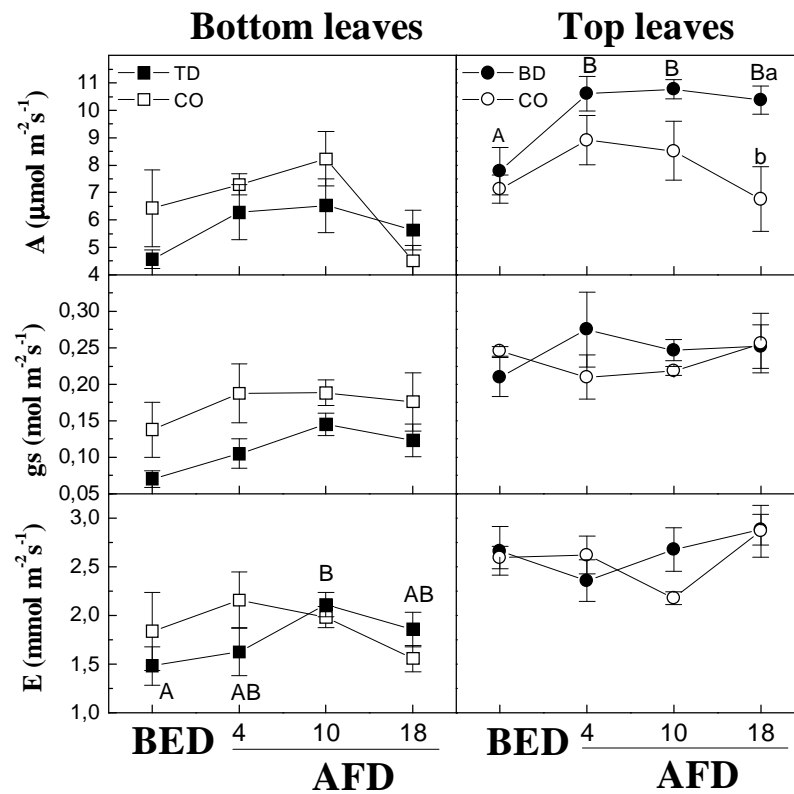


Figure 3 - Periodic variations of gas exchange values (mean \pm SE) for same leaves of top defoliated (TD), bottom defoliated (BD) and control (CO) *Leandra lacunosa* young plants ($n=6$), measured before defoliation (BED) and at 4, 10 and 18 days after defoliation (AFD). A: net photosynthesis, gs: stomatal conductance, E: transpiration. Values followed by different lowercase letters (Top leaf, top panel) indicate statistical significant differences (Student t test, $p < 0.05$) between BD and CO plants on each date. Values followed by different capital letters indicate statistical significant differences (one-way ANOVA, $p < 0.05$) among dates for same treatment.

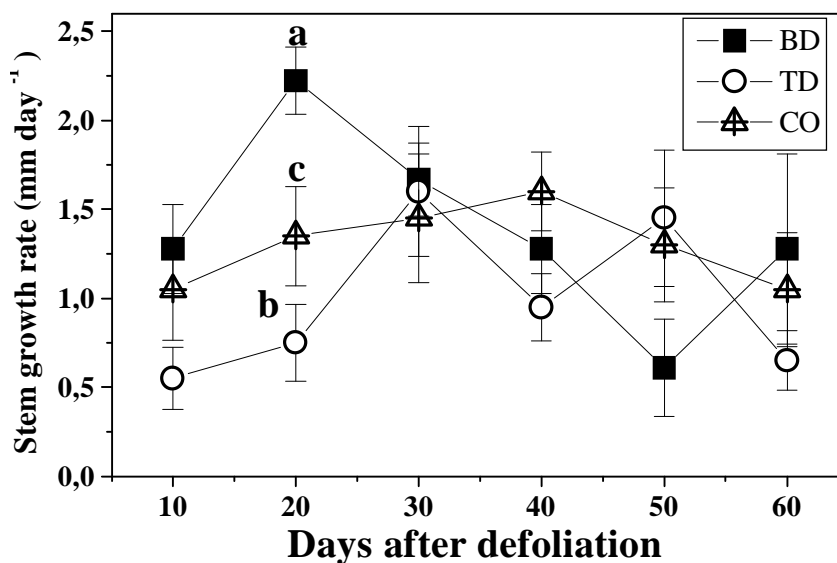


Figure 4 - Linear stem extension rate of *Leandra lacunosa* young plants measured every ten days during the two months following defoliation (September 3). BD: bottom defoliated, TD: top defoliated, CO: control plants. Values followed by different letters indicate statistical significant differences (one-way ANOVA, $P < 0.05$) between plants of the three treatments on each date. $n = 10$.

DISCUSSION

Upper and bottom leaves of *L. lacunosa* young plants presented age differences of one to five months because they were produced in a continuous pattern, but all of them were expanded leaves. The fact that SLM values were lower for bottom leaves than for top leaves was a morphological difference associated with the structural component loss in old leaves, and the lower development of structural tissues caused by shade (Jurik, 1986). The smaller size of bottom leaves in relation to leaves attached to the upper plant part may be determined in this species by endogenous growth limitations of leaves formed during the first months of plant life. In spite of opposite and decussate leaf insertion, bigger top leaves would impose some shade to bottom small plant leaves.

Hence, functional characteristics of the two leaf groups reflect consequences of leaf age, shading conditions and endogenous features.

It is known that photosynthetic rate on area basis decreases and the LCP increases with leaf age and self-shading because of resource redistribution from old to new younger leaves (Kitajima et al.,

1997). In general terms, our results were in accordance with these authors because old bottom expanded leaves of *L. lacunosa* plants showed lower average values of A, gs and dark respiration than top expanded leaves. However, the LCP obtained from A-PPFD curves was similar for both leaf groups. In addition, the differences observed for top, middle and bottom leaves on young *L. lacunosa* plants (Fig. 2) did not seem to indicate senescence of bottom leaves. Similar relations were also found in erect shoots of adult *L. lacunosa* plants growing under field conditions. Photosynthesis measurements (mean \pm SE) on the most distal expanded leaf ($10.54 \pm 0.99 \mu\text{mol s}^{-1}$) and on the oldest basal leaf ($6.13 \pm 1.60 \mu\text{mol m}^{-2} \text{s}^{-1}$) of adult plant shoots were also significantly different (Damascos, unpubl. data). Kikuzawa (1995) postulated that in species with continuous leaf production a new leaf emerges when the photosynthetic capacity of the leaf immediately inferior decreases and leaf becomes senescent.

However, a reduction in photosynthetic rates in old leaves of both adult and young *L. lacunosa* plants represents less than a half in relation to the activity of the top expanded leaves. Consequently, four- to six-months-old leaves shaded by top

bigger leaves would maintain medium A values and remain active after insect top leaf elimination. It also occurs with some herbaceous species that might benefit from retaining a reserve of foliage to replace top leaves eaten by grazing animals (Thomas and Sadras, 2001).

Remaining bottom leaves of *L. lacunosa* TD plants did not improve A and g_s values, but they increased their transpiration four days after top leaf elimination probably as a consequence of the new condition of great leaf exposure. However,

these old leaves maintained growth throughout the following two months after defoliation and allowed greater recovery of new leaves than BD plants. All growth studied variables on TD *L. lacunosa* plants (linear stem extension, stem biomass/stem length, stem/root dry biomass, and root biomass) did not vary in relation to undamaged plants or to those BD plants. Thus, old leaves could maintain stem and root growth at least during a short period of time (two months).

Table 2 - Mean values (\pm SE) of the final linear stem extension, the production of new leaves, the dry mass/length stem ratio, the stem/root dry mass ratio and the total root biomass of young *Leandra lacunosa* plants 60 days after defoliation. TD: top defoliated plants, BD: bottom defoliated plants, CO: control plants. Values followed by different letters indicate statistical significant differences (one-way ANOVA, $p < 0.05$) between the three treatments. $n = 10$.

	TD	BD	CO
Stem linear extension (cm)	7.7 \pm 0.7 a	6.2 \pm 1.0 a	7.8 \pm 1.3 a
Stem dry mass/length (mg/cm)	87.8 \pm 23.3 a	59.4 \pm 7.7a	92.8 \pm 23.1 a
Production of new leaves	7.0 \pm 0.3 a	5.4 \pm 0.4 b	6.2 \pm 0.2 ab
Root biomass (g)	1.4 \pm 0.3 a	2.7 \pm 0.5 a	2.22 \pm 0.4 a
Stem/root dry mass	0.6 \pm 0.2 a	0.7 \pm 0.2 a	0.5 \pm 0.1 a

The elimination of less productive bottom leaves improved plant physiological conditions and allowed transiently higher A values of top leaves. However, at the end of the experiment BD, TD and CO plants showed similar stem extension and stem and root biomass. This indicated that the main compensatory effects occurred in plants that only conserved their old leaves.

In conclusion, bottom leaves conserved photosynthesis values of about 60 % in relation to those of top leaves but after different age leaf elimination both leaf groups were able to maintain plant growth at similar intensity at least during a short period of time. Only top leaves on the studied species showed compensatory photosynthesis after bottom leaf elimination. This positive response did not cause substantial short-term advantages on plant growth in relation to the other two studied treatments (TD and CO plants). Remaining bottom leaves after defoliation of the species with continuous leaf production compensated the elimination of more productive top leaves. It probably made possible faster production of new leaves on the defoliated part of

the stem. The effectiveness of this strategy would depend on the number of these new leaves that could escape from herbivores. In terms of anti herbivores defense, it could be more advantageous for the plant to have leaves of different ages, which assures both constant and differential (structural and physiological) offer to herbivores. That would allow less attractive mature leaves escape and support plant growth, recovering the new leaf area within short time.

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RESUMO

Características morfológicas e fisiológicas foliares foram estudadas em indivíduos jovens envasados de *Leandra lacunosa* que sofreram desfolhamento e em indivíduos controle. Após o desfolhamento, as folhas remanescentes da parte inferior não apresentaram mudanças significativas dos valores de fotossíntese e condutância estomática. Entretanto, a taxa de transpiração foliar foi maior quando comparada com plantas desfolhadas em porções inferiores. A rebrota de folhas foi maior após o desfolhamento da parte superior. Nas plantas desfolhadas em porções inferiores, ocorreu, momentaneamente, aumento da taxa fotossintética e da extensão do caule. Após dois meses de desfolhamento as plantas controle e desfolhadas não diferiram quanto aos valores do comprimento do caule e massa seca da raiz. Portanto, apesar da menor área foliar e menor capacidade fotossintética, as folhas na parte inferior foram capazes de compensar a eliminação das folhas superiores. A efetividade desta resposta compensatória, no entanto, dependerá da habilidade das novas folhas emitidas escaparem do ataque dos herbívoros.

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