

Morpho-physiological performance of *Mikania glomerata* Spreng. and *Mikania laevigata* Sch. Bip ex Baker plants under different light conditions

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ABSTRACT - (Morpho-physiological performance of *Mikania glomerata* Spreng. and *Mikania laevigata* Sch. Bip ex Baker plants under different light conditions). In tropical and subtropical zones, lianas play important roles in the process of ecological succession. This study aims to evaluate the photosynthetic and morpho-physiological performance between two lianas species from *Mikania* genus in response to different levels of radiation: full sun (I0), 25% (I25), 50% (I50), and 75% (I75) retention of solar radiation flux. Plants grown under I75 showed a reduced net photosynthetic rate (A). We observed dynamic photoinhibition at I0 during hours of high irradiation and temperature. The highest and lowest leaf chlorophyll content occurred at I75 and I0, respectively, while carotenoids/total chlorophyll and leaf thickness increased under I0. Total dry mass was higher in plants grown at I0 and I25. However, A values and biomass production of *Mikania laevigata* were higher at I25, while for *Mikania glomerata* greater biomass accumulation was observed between I0-I50. Therefore, we concluded that *M. laevigata* and *M. glomerata* have different morpho-physiological performances under same the radiation conditions. **Keywords:** chlorophyll a fluorescence, gas exchange, irradiation interference, lianas, photosynthesis

RESUMO - (Desempenho morfo-fisiológico de *Mikania glomerata* Spreng. e *Mikania laevigata* Sch. Bip ex Baker sob diferentes condições de luminosidade). Em regiões tropical e subtropical, lianas desempenham um papel importante no processo de sucessão ecológica. O objetivo deste estudo foi avaliar as respostas fotossintéticas e morfo-fisiológicas entre duas espécies de *Mikania* em diferentes níveis de radiação: sol pleno (I0), 25% (I25), 50% (I50) e 75% (I75) de retenção do fluxo da radiação solar. Plantas crescidas sob I75 mostraram reduzida taxa fotossintética (A). Nós observamos fotoinibição dinâmica em plantas crescidas sob I0 durante as horas de alta irradiação e temperatura. O maior e menor conteúdo de clorofila ocorreu em plantas sob I75 e I0, respectivamente; enquanto carotenoides/clorofila total e espessuras da epiderme e mesofilo aumentaram sob I0. A massa seca total foi maior em plantas crescidas sob I0 e I25. No entanto, os valores de A e a produção de biomassa de *M. laevigata* foram maiores sob I25; enquanto para *M. glomerata*, maior acúmulo de biomassa foi observado entre I0-I50. Portanto, nós concluímos que *M. laevigata* e *M. glomerata* apresentaram diferentes respostas morfo-fisiológicas sob mesma condição de radiação.

Palavras-chave: fluorescência da clorofila a, fotossíntese, interferência da irradiação, lianas, trocas gasosas

Introduction

Lianas are life forms frequently found in tropical and subtropical forests (Gentry & Dodson 1987) that explore all forest layers through different mechanisms of ascension, growth patterns, and physiological strategies (Schnitzer & Bongers 2002, Gerwing 2004, Kazda *et al.* 2009). Lianas stems are relatively thin and depends on external support to access the sunlight. Therefore, lianas species often allocate less carbon in stem growth, and more carbon in photosynthetic and vascular tissues (Putz 1984, Schnitzer 2005), leading to an advantage over trees due to higher growth rates (Zhu & Cao 2009). In addition, the ability to grow both laterally and vertically allows lianas to easily invade the canopy, extending long branches and reaching

adequate light conditions (Schnitzer & Bongers 2002, Toledo *et al.* 2003). Since radiation requirements of lianas are high, those species are often classified as gap-dependent pioneer species, presenting a similar distribution pattern of pioneer tree species (Putz 1984, Schnitzer & Bongers 2002). However, some lianas are also able to germinate and grow in the understory (Nabe-Nielsen 2002, Sanches & Válio 2002, Schnitzer *et al.* 2012), suggesting some level of shade tolerance (Gerwing 2004).

In the last decades, lianas abundance and productivity increased in tropical forests especially due to high rates of deforestation and human-induced climate change (Granados & Korner 2002, Phillips *et al.* 2002, Wright *et al.* 2004, Zhu *et al.* 2004, Schnitzer & Bongers 2011). In addition,

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evidences indicated that in mature and undisturbed forests the increased density of lianas may be consequence of changes in precipitation patterns (Schnitzer & Bongers 2011). Another important factor that influences lianas growth and distribution is light availability. For example, it is recognized that increased solar radiation improves lianas seedlings growth rates (Kurzel *et al.* 2006, Schnitzer & Bongers 2011). Furthermore, lianas may experience different amounts of radiation and spectral quality during growth. Therefore, it is expected a high phenotypic plasticity of lianas regarding photosynthetic and gas exchange adjustments in response to different light conditions (Bazzaz & Carlson 1982, Ribeiro *et al.* 2005). Stomatal opening level determines the trade-off between CO₂ absorption and water loss by transpiration (Caemmerer & Baker 2007). The adaptative success under different light conditions depends on adjustments of leaf morphology, anatomy, and photosynthetic apparatus. Therefore, adjustments ensure greater efficiency in the conversion of radiant energy into carbohydrates to sustain plant growth (Dias-Filho 1997, Campos & Uchida 2002, Gratani 2014).

Mikania glomerata Spreng. and *Mikania laevigata* Sch. Bip ex Baker are lianas species both native from Atlantic Forest in Brazil (Gasparetto *et al.* 2010). *Mikania* species belong to the Asteraceae family and are popularly known as “Guaco” and they both can benefit from Atlantic forest fragmentation. Within biodiversity hotspots around the world, the Atlantic forest is considered the most vulnerable ecosystem to deforestation and climate-change (Béllard *et al.* 2014). With a wide range of forest physiognomies (Myers *et al.* 2000, Ricketts *et al.* 2005, Metzger 2009), currently, only 28% of its original area remains (Rezende *et al.* 2018). Both species are similar regarding its morphology and are often indiscriminately used in traditional medicine to treat colds, flu, asthma, and, bronchitis because of the bronchodilator and expectorant properties (Moura *et al.* 2002, Graça *et al.* 2007, Bolina *et al.* 2009, Gasparetto *et al.* 2010). Recent studies demonstrated that *M. glomerata* and *M. laevigata* have a different chemical composition and therapeutic properties (Melo & Sawaya 2015, Almeida *et al.* 2016, Costa *et al.* 2017). However, no reports of photosynthetic proprieties and gas exchange behavior under different light conditions between both species are found in the literature.

Therefore, our objective was to evaluate the morpho-physiological performance of two *Mikania* species (*Mikania glomerata* and *Mikania laevigata*) under four different levels of retention of solar radiation flux: full sun (I0), and 25 (I25), 50 (I50), and 75% (I75). Our main hypothesis is that both *Mikania* species will be benefited from high radiation levels, resulting in enhanced photosynthesis and biomass production.

Materials and methods

Plant material and growth conditions - Experiments were carried out at the University of São Paulo at Ribeirão Preto campus (21° 10 '08.4" 102 S and 47° 51' 50.6" W), São Paulo, Brazil, which climate is tropical wet and dry (Aw) according to the Köppen-Geiger classification. For

further details of climatic conditions during the experiment (Supplementary material Table S1). We used the species *Mikania glomerata* Spreng. and *Mikania laevigata* Sch. Bip ex Baker. *Mikania* plantlets from each species were prepared cuttings from the middle of the branches of different parental plants of approximately 1 cm in diameter, 12 cm in length, a node at the top of the stake, and a pair of leaves (Lima *et al.* 2003). The plantlets were planted in 3-kg plastic bags containing a mixture of manure and soil (soil type redlatosol, 1:1) under greenhouse conditions. After rooting (approximately 60 days), the plants were transferred into 20 L pots containing soil and submitted to treatments for 150 days. The soil was fertilized with 1 g NPK (4-14-8) fertilizer per kg of soil. Plants were subjected to four light conditions: full sun condition (I0), and 25% (I25), 50% (I50), and 75% (I75) retention of solar radiation flux (Supplementary material Figure S1). To achieve the planned levels of solar radiation, special greenhouses were constructed with artificial shading of varying degrees of retention of solar radiation flux. During the experiment, pots were irrigated daily and were maintained at soil field capacity, using a sensor ML2× Theta Probe (Delta-T Devices, Cambridge, UK).

Gas exchange parameters, chlorophyll fluorescence, and photosynthetic pigment content were evaluated after 60, 90, 120, and 150 days after the treatments started (DAT). Leaf anatomy and biomass production were evaluated at 90 and 150 DAT. Three leaf samples per plant from upper, middle and lower canopy regions were collected.

Gas exchange measurements - Net photosynthesis rate (A, $\mu\text{mol m}^{-2} \text{s}^{-1}$), transpiration rate (E, $\text{mmol m}^{-2} \text{s}^{-1}$), and stomatal conductance (g_s , $\text{mol m}^{-2} \text{s}^{-1}$) were evaluated daily from 8:00 to 11:00 hours in fully expanded leaves using an infrared gas analyzer model LCpro+ (ADC BioScientific, Ltd., UK). The measurements were made under ambient conditions of radiation, [CO₂], and air temperature. The intrinsic water-use efficiency (i WUE; $\mu\text{mol mol}^{-1}$) was estimated from gas exchange (A/g_s) data.

Chlorophyll fluorescence - The maximum quantum yield of primary photochemistry (Fv/Fm) was measured in three fully expanded leaves using a portable fluorometer model OS-3P (ADC BioScientific, UK). Leaves were dark-adapted for 30 minutes and used to measure the dark fluorescence yield (Fo), maximum fluorescence yield (Fm), and variable fluorescence (Fv). Then, Fv/Fm ratio was calculated. We performed four Fv/Fm diurnal courses from 6:00 hours to 18:00 hours each two hours at 60, 90, 120, and 150 DAT. At sampling days, we monitored the relative humidity and ambient temperature using a hygro-thermometer, and the photosynthetic photon flux density (PPFD) using a quantum sensor connected to an irradiation meter model LI-250A (LI-COR, USA) (Supplementary material Figure S1).

Photosynthetic pigment analysis - Photosynthetic pigments were extracted and quantified following the methodology of Hendry and Price (1993). Leaf discs (0.1g) were ground in 80% acetone, and the absorbance was measured at 480, 645, and 663 nm using a spectrophotometer model Genesys 5Spectronic. Based on the absorbance value,

the concentration of total chlorophyll and carotenoids were calculated.

Leaf anatomy - Leaf fragments (1 cm²) were fixed in FAA 70% for 24 hours and dehydrated in ethanol series (Kraus & Arduin 1997). Then, samples were embedded in paraffin, cut in a microtome (8 μm), and stained with 1% toluidine blue. Samples were observed using a microscope QUIMIS (Q720 ED), photographed and the images were used to measure the leaf thickness, adaxial (AdE) and abaxial epidermis thickness (AbE), and palisade (PP) and spongy parenchyma (SP) thickness. Measurements were performed using the software AnatiQuanti 2.0 (Laboratory of Plant Anatomy/UFV). We found a hypodermic layer below the adaxial epidermis in both species, but since we did not find this tissue in all samples, it was not quantified.

For the epidermis analysis, lower epidermis (hypoostomatic leaves) were detached from mesophyll using the Jeffrey solution (10% chromic acid and 10% nitric acid, 1:1). Samples were stained with safranin for 30 seconds and mounted in glycerin 50% (Kraus & Arduin 1997). Samples were observed using a microscope QUIMIS (Q720 ED) and photographed. We counted the number of epidermal cells and stomata and calculated the stomatal density (SD) and stomatal index (SI) using the software AnatiQuanti 2.0 (Laboratory of Plant Anatomy/UFV).

Stomatal index (SI) was calculated according to the equation:

$$SI = \frac{SN}{SN + EC} * 100$$

where: SN = stomata number; EC = number of epidermal cells

Leaf area and biomass analysis - To measure leaf area (LA), we detached leaves from the whole plant and detached leaf discs of 1 cm² from basal, median, and apical regions of the leaves. Using the disc area, disc dry weight, and total leaf dry weight, we estimated the mean leaf area. Specific leaf area (SLA) was estimated as the ratio of leaf area to leaf dry mass (dm² g⁻¹).

For biomass, five plants from each treatment were collected. Samples were separated in roots, stems, petiole, and leaves. Then, plant material was dried at oven (70 °C) until constant mass. Subsequently, the dry weight of each organ was determined.

Experimental design and statistical analysis - The effects of irradiance interference, species, and their interactions were evaluated using analysis of variance (two-way ANOVA) using the SYSTAT software package (SPSS Inc., Chicago, IL) (P < 0.05 was accepted as statistically significant). Significant effects were further analyzed using Tukey's test. The ANOVA included two species (*M. glomerata* and *M. laevigata*) and four levels of sunlight retention of solar radiation flux treatment: 0% (I0), 25% (I25), 50% (I50), and 75% (I75).

Results

Gas exchange - The solar radiation level significantly affected stomatal conductance (g_s) and transpiration rate

(E) regardless of species, except at 150 DAT (figure 1 a-d; ANOVA in Supplementary material Table S2). E and g_s values were lower in plants grew under higher retention of solar radiation flux (I75). At 150 DAT, g_s showed the lowest values when compared to previous samplings. When species were compared, g_s was higher in *M. laevigata* than in *M. glomerata* (Supplementary material Table S2).

Net photosynthetic rate (A) from both *Mikania* species was significantly affected by solar radiance levels and species. However, no interactions between factors were observed for A (figure 1 e-f). Compared with plants grown under high solar radiances, a lower A was observed in plants grown under the greatest shading (I75) in both species (figures 1 e-f). At 150 DAT and under I75, A showed the lowest values and was 6.7 and 4.5-fold lower than plants under I25, in *Mikania glomerata* and *Mikania laevigata*, respectively. On average, in *M. glomerata* and *M. laevigata*, the highest A (12.69 and 14.87 μmol m⁻² s⁻¹, respectively) was observed in plants grown under I25 compared with other treatments (figure 1 e-f). During the entire experiment, the lowest A and g_s values were observed at 150 DAT, period with low air relative humidity, highest vapor pressure deficit (VPD), and increased air temperature (Supplementary material Table S3).

Intrinsic water-use efficiency (iWUE) of *M. glomerata* plants grown at I75 was higher than at I0 (figure 1 g). However, in *M. laevigata* plants grown under I75, iWUE showed the lowest values when compared to other treatments (figure 1 h). Lower values of iWUE were observed in *M. laevigata* plants when compared with *M. glomerata* plants (Supplementary material Table S2). This result might reflect the increased g_s observed in *M. laevigata* plants (figure 1 h).

Diurnal courses of chlorophyll a fluorescence - In both species, and under high shading conditions (I75), Fv/Fm values were higher compared with all other treatments (figure 2). We observed that in both species at 60 and 90 DAT only plants developed under I0 showed a decreased Fv/Fm ratio, with values below 0.75, indicating the occurrence of dynamic photoinhibition (figure 2 a-d). At 120 and 150 DAT, Fv/Fm reduction was observed under all treatments, except under I75 (figure 2 e-h). The recovery of this parameter was observed at 18:00 hours, except in *M. glomerata* plants grown under I0 and I25, and *M. laevigata* I0 (150 DAT), indicating a lower rate of recovery. In all treatments, the lowest Fv/Fm value was observed between 12:00 and 16:00 hours in plants grown under the full sun (figure 2).

Photosynthetic pigments - The effects of retention of solar radiation flux on the photosynthetic pigments were similar in both species. There were significant effects of solar radiances and species on total chlorophyll and carotenoids for both species (figure 3; Supplementary material Table S2). Pigments concentration increased under low light availability. Plants grew under I0 showed a reduction in chlorophyll and carotenoids content during the experiment (figure 3 a-d). For *M. glomerata*, 5.6 and 4.0-fold more chlorophyll and carotenoids were found for I75 than I0 treatments at the end of the experiment (150 DAT) (figure 3 a, c). In *M. laevigata* plants, this difference was between 4.9 and 3.6-fold. In this same sampling, both

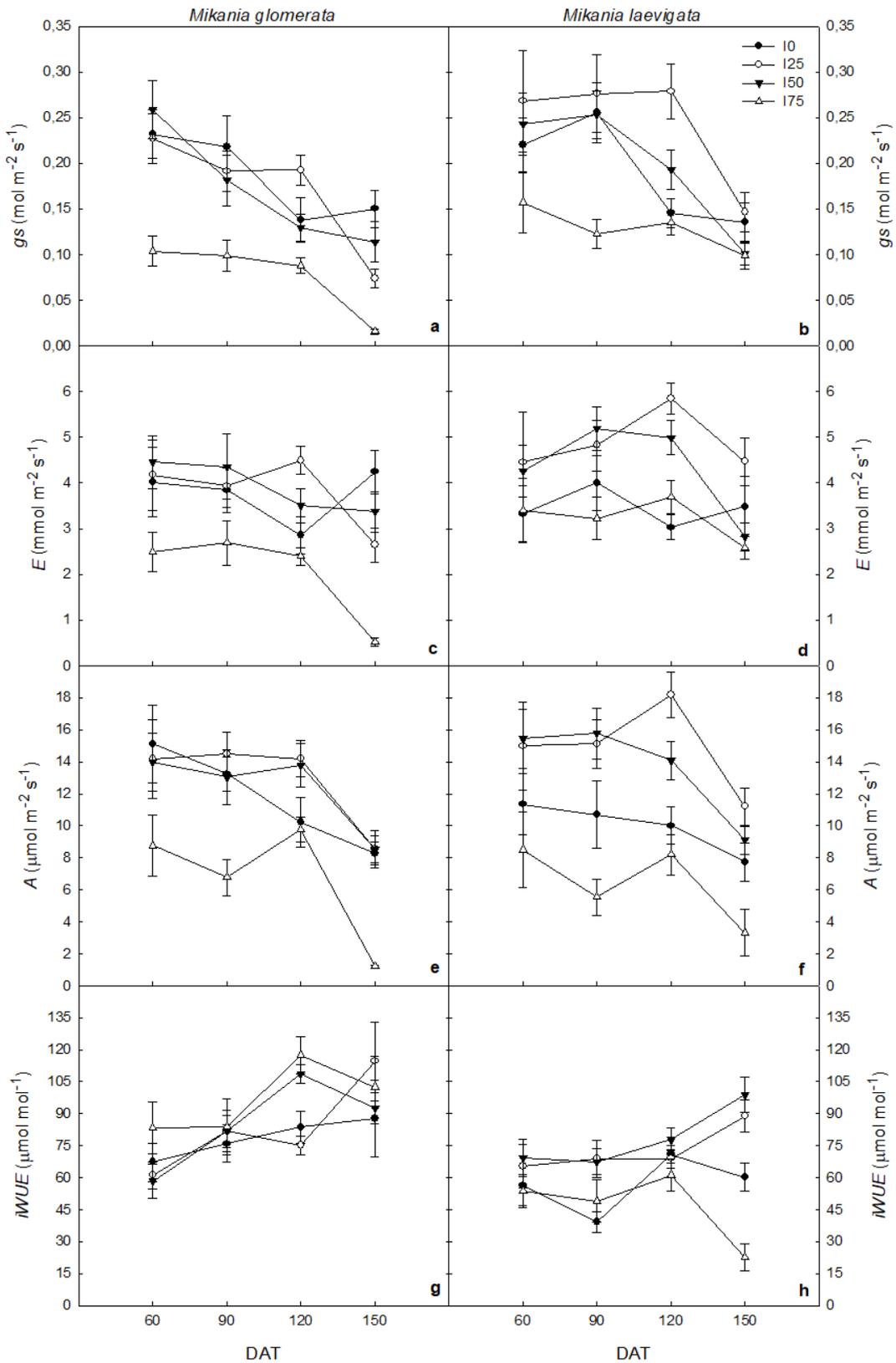


Figure 1. Gas exchange parameters: Stomatal conductance (g_s , a-b), transpiration rate (E , c-d), net photosynthesis (A , e-f), and intrinsic water use efficiency ($iWUE$, g-h) in *Mikania glomerata* Spreng. and *Mikania laevigata* Sch. Bip ex Baker, at 60, 90, 120 and 150 days after the treatment started (DAT), grown under sunlight irradiation interference treatments: 0% (I0), 25% (I25), 50% (I50) and 75% (I75). Data shown are the means (\pm SE) for measurements made on five plants per treatment.

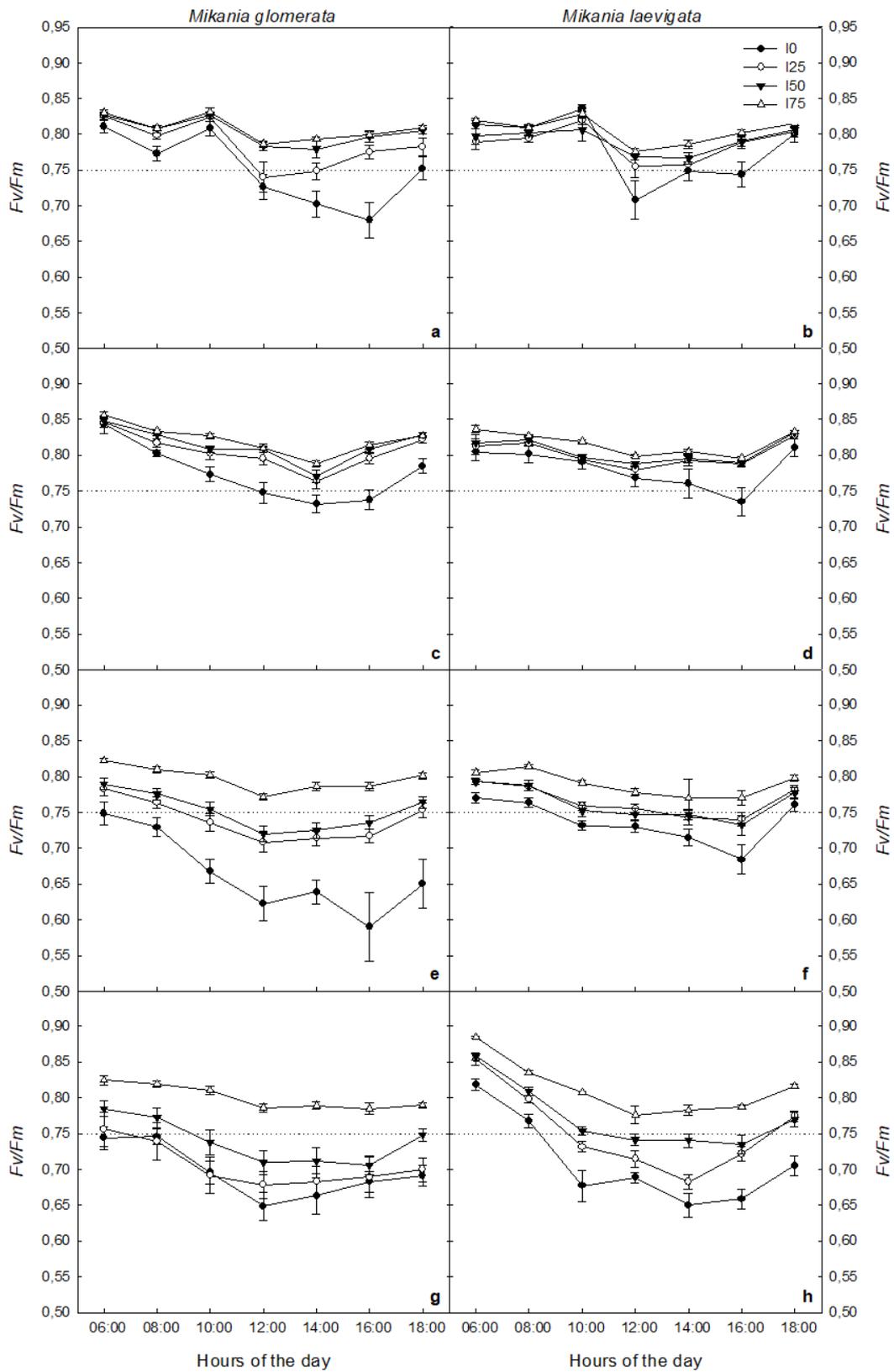


Figure 2. Diurnal course of maximum quantum efficiency of PSII (Fv/Fm) in *Mikania glomerata* Spreng. at 60 (a), 90 (c), 120 (e), and 150 (g) days after the treatment started (DAT) and *Mikania laevigata* Sch. Bip *ex* Baker at 60 (b), 90 (d), 120 (f), and 150 DAT (h), grown under sunlight irradiance interference treatments: 0% (I0), 25% (I25), 50% (I50), and 75% (I75). Data shown are the means (\pm SE) for measurements made on five plants per treatment.

species under I25 showed an average increase of 3-fold more chlorophyll and 2.5-fold more carotenoids than plants under I0 (figure 3 b, d). In both species, higher values of carotenoids/chlorophyll ratio were observed in plants grown in I0 compared with plants grown under high levels of shading (I75) (figure 3 e, f).

Leaf anatomy - The effects of retention of solar radiation flux on leaf anatomy were similar in both species. The thickness of the adaxial epidermis (AdE) and mesophyll (palisade and spongy parenchyma) decreased while the retention of solar radiation flux increased (table 1). The AdE was approximately 1.2 and 1.3-fold higher in I0 compared

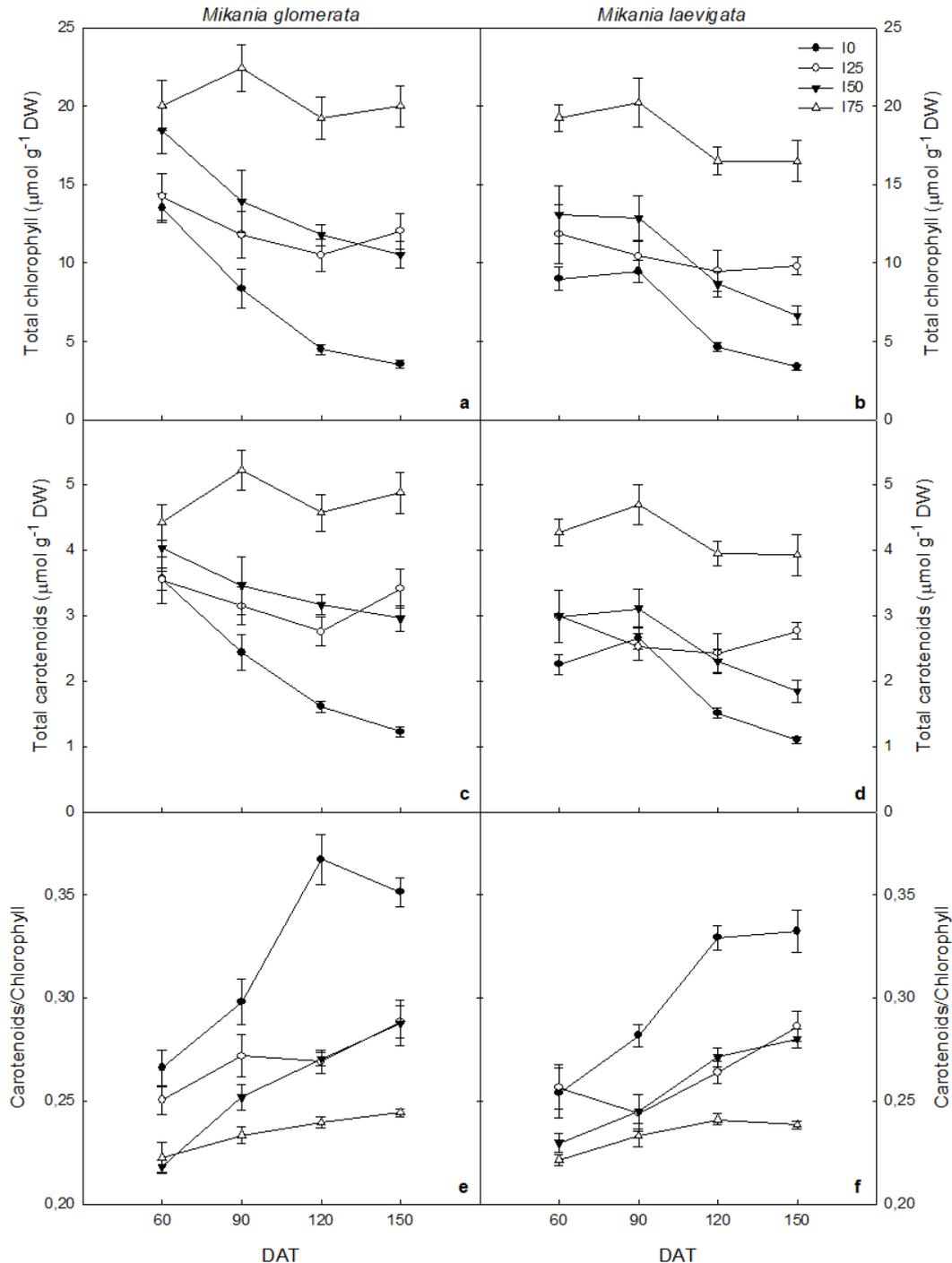


Figure 3. Photosynthetic pigments: Content of total chlorophyll (a-b), total carotenoids (c-d), and total carotenoids/chlorophyll ratio (e-f) in *Mikania glomerata* Spreng. and *Mikania laevigata* Sch. Bip ex Baker, at 60, 90, 120, and 150 days after the treatment started (DAT), grown under sunlight irradiation interference treatments: 0% (I0), 25% (I25), 50% (I50), and 75% (I75). Data shown are the means (\pm SE) for measurements made on five plants per treatment.

with I75 for *M. glomerata* and *M. laevigata*, respectively. Under I0, I25, and I50 the AdE was on average 1.4 and 1.7-fold higher in *M. laevigata* than *M. glomerata* at 90 and 150 DAT, respectively. Under I75, *M. laevigata* also showed a higher AdE. However, the values were 1.2 and 1.4-fold (90 and 150 DAT, respectively) higher than those observed in *M. glomerata*. Under I0 palisade and spongy parenchyma (150 DAT), were 10 and 17% higher in *M. laevigata* (table 1).

Stomatal index (SI) and stomatal density (SD) both increased according to the increased levels of radiation (table 2). The highest SI and SD values were observed at 150 DAT for both species under I0 and I25. In *M. glomerata* grown under I0, SI and SD were 18.05% and 256 stomata/mm², respectively, while for *M. laevigata* developed under I0, SI and SD were 14.41% and 176 stomata/mm², respectively. Regardless of the solar radiances level, *M. glomerata* showed the highest values of SI and SD when compared to *M. laevigata*.

Morphology and biomass partitioning - There was a significant effect of the retention of solar radiation flux and species for leaf area (LA). *M. glomerata* showed higher LA than *M. laevigata* at 90 and 150 DAT (figure 4). At 90 DAT, LA of plants developed under I0 was 50% and 12% higher than plants grew under I75 for *M. glomerata* and *M. laevigata*, respectively (figure 4 a). At 150 DAT, this difference was between 23% and 27%. In this same sampling, LA of *M. glomerata* developed under I0, I25, and I50 were 1.7-fold higher when compared to *M. laevigata* plants (figure 4 b). The SLA decreased with increased levels of solar radiances (figure 4 c, d). In both species (150 DAT) developed under I75, SLA was on average 1.4-fold higher when compared to plants grown under I0, I25, and I50 (figure 4 d).

The highest production of aboveground biomass was observed at 90 DAT in *M. glomerata* under I50 (45.37 g DW), and for *M. laevigata* under I0 (28.19 g DW) (figure 5 a). At 150 DAT, shoot biomass in *M. glomerata* was higher under I0, followed by plants grew under I25 and I50 (figure 5 b). However, in *M. laevigata* plants, the highest aboveground dry mass was observed in plants developed under I25 followed by I0. At 90 and 150 DAT, we observed that under I0, root dry mass was higher compared to other treatments and independent of species (figure 5). Both species at 90 DAT showed higher leaf biomass production when compared to other part plants, however, at 150 DAT, the stem biomass was higher than leaf dry mass (figure 5). At the end of the experiment (150 DAT), increased production of biomass was observed under I25 in *M. laevigata* compared with other solar radiances treatments. In *M. laevigata*, leaf, stem, and root biomass of plants grown under I75 were 38, 48, and 70%, respectively, lower when compared with plants grown under I25. Compared with full sun treatments (I0), root biomass showed a 54% and 69% reduction under I50 and I75 treatments, respectively (figure 5).

In *M. glomerata* plants, leaf and stem biomass were on average 40 and 48% higher under I0, I25, and I50 than under I75. Root biomass was 56, 40, and 70% higher under

I0 when compared to plants grown under I25, I50, and I75, respectively. Moreover, we observed that *M. glomerata* plants grown under I0, I25, and I50 had greater leaves and stems biomass than those observed in *M. laevigata* plants (figure 5).

Discussion

In this study, we unraveled the main morpho-physiological characteristics of two tropical lianas species in response to light availability. Our main hypothesis was not corroborated, since *Mikania glomerata* showed a better growth performance under I0, I25, and I50, while *Mikania laevigata* showed improved performance under I25. Plants grown under I0 and I25 showed greater g_s and E. The increased air temperature and VPD observed at 150 DAT presumably caused the reduction in g_s , E, and A in both *Mikania* species. Stomatal closure under high light conditions combined with high temperature, and low relative humidity it is a mechanism that decreases the water loss rate to the environment, but it also decreases the CO₂ influx into the leaves (Hsie *et al.* 2015). Stomatal regulation is an advantageous strategy, especially during the dry season. Under field conditions, improved \dot{WUE} due to the reduction of g_s and constant A values is the most effective mechanism for the continued growth of plants without experiencing dramatic water loss (Hanba *et al.* 2002).

Our data indicated that *M. glomerata* and *M. laevigata* plants grown under conditions of retention of solar radiation flux of up to 50% had higher A than plants grown under I75. Lower A under I75 is presumably the result of a combination of many factors such as thinner leaves, low stomatal density, decreased g_s and low light intensity. These factors may result in lower intercellular CO₂ concentration (C_i), Rubisco activity, and electron transporters (Lambers *et al.* 1998). Notably, plants grown under I25 exhibited higher A values than plants grown under full sunlight (I0). This effect was observed in both species, suggesting that the irradiation growth condition was too high at I0, which potentially induced dynamic photoinhibition. Fv/Fm is a parameter that indicates photoinhibition level, and smaller values than 0.75 indicate photoinhibition with a concomitant reduction of the maximum quantum efficiency of PSII (Bolh ar-Nordenkampf &  quist 1993). Under full sunlight conditions, the reduced Fv/Fm at noon and subsequent recovery at the end of the day is evidence of dynamic reversible photoinhibition (Bolh ar-Nordenkampf &  quist 1993). The dynamic photoinhibition (inactivation of PSII) is an efficient defense mechanism because the increase in non-photochemical dissipation when the influx of CO₂ is reduced (due to stomatal closure), prevents the formation of reactive oxygen species (ROS) and photooxidative damage (Choudhury & Behera 2001).

Differences in the level of total chlorophyll, carotenoids, and carotenoids/chlorophyll ratio are reported between the sun and shade-adapted leaves from other species (Demmig-Adams 1998; Lichtenthaler & Babani 2004, Lichtenthaler *et al.* 2007). The increased accumulation of chlorophyll in *Mikania* plants grown in the shade reflects a compensation mechanism to increase

Table 1. Anatomical Parameters: Adaxial epidermis thickness (AdE, μm), Abaxial epidermis thickness (AbE, μm), palisade parenchyma (PP, μm), spongy parenchyma (SP, μm), total leaf thickness (TL, μm) in *Mikania glomerata* Spreng. and *Mikania laevigata* Sch. Bip ex Baker exposed to 0% (I_0), 25% (I_{25}), 50% (I_{50}), and 75% (I_{75}) irradiance interference, at 90 and 150 days after the treatments started (DAT).

Species	Irradiance Interference	90 DAT					150 DAT				
		AdE	AbE	PP	SP	TL	AdE	AbE	PP	SP	TL
<i>Mikania glomerata</i>	I_0	23.27 ± 1.28	21.27 ± 0.63	91.66 ± 2.62	176.35 ± 4.65	312.54 ± 6.08	19.83 ± 0.64	16.47 ± 0.34	72.12 ± 1.38	145.07 ± 2.75	246.20 ± 5.26
	I_{25}	21.11 ± 1.04	21.40 ± 0.85	90.42 ± 3.58	171.21 ± 5.27	304.14 ± 8.19	17.66 ± 0.52	15.43 ± 0.31	67.30 ± 1.31	137.93 ± 2.66	231.46 ± 4.84
	I_{50}	20.57 ± 1.17	19.99 ± 0.67	94.82 ± 3.02	161.67 ± 5.19	297.06 ± 7.30	17.23 ± 0.44	17.65 ± 0.37	74.61 ± 1.73	137.28 ± 2.81	239.67 ± 5.46
	I_{75}	19.30 ± 0.91	20.41 ± 0.78	87.41 ± 3.91	159.70 ± 7.44	286.82 ± 10.86	15.74 ± 0.52	14.42 ± 0.33	64.47 ± 1.22	143.13 ± 2.90	230.91 ± 4.89
<i>Mikania laevigata</i>	I_0	33.75 ± 1.80	19.83 ± 0.82	111.66 ± 2.87	192.25 ± 3.61	357.48 ± 4.55	31.89 ± 0.99	16.66 ± 0.33	79.31 ± 1.43	176.39 ± 2.73	295.49 ± 5.52
	I_{25}	31.04 ± 1.43	20.30 ± 0.69	87.56 ± 3.23	172.37 ± 3.99	311.27 ± 3.98	30.62 ± 0.81	16.89 ± 0.38	74.83 ± 1.39	144.55 ± 2.55	259.21 ± 5.02
	I_{50}	28.80 ± 1.45	20.08 ± 0.64	90.02 ± 2.44	160.68 ± 4.26	299.58 ± 5.33	29.98 ± 0.63	13.83 ± 0.25	55.77 ± 1.03	129.72 ± 2.34	222.70 ± 4.26
	I_{75}	23.11 ± 1.27	19.09 ± 0.61	82.98 ± 2.46	153.73 ± 4.29	278.90 ± 5.37	22.52 ± 0.82	14.46 ± 0.31	66.51 ± 1.59	139.91 ± 2.54	236.40 ± 4.95
ANOVA	Species	**	ns	ns	ns	*	**	*	**	**	**
	Irradiance	**	ns	**	**	**	**	**	**	**	**
	Species x Irradiance	ns	ns	**	ns	**	**	**	**	**	**

Data are mean ± SD (n = 5), ns, not significant. * 0.05 > P > 0.001. ** P < 0.001

Table 2. Anatomical Parameters: stomatal index (SI, %) and stomatal density (SD, stomata/mm²) in *Mikania glomerata* Spreng. and *Mikania laevigata* Sch. Bip *ex* Baker exposed to 0% (I₀), 25% (I₂₅), 50% (I₅₀), and 75% (I₇₅) irradiance interference, at 90 and 150 days after the treatments started (DAT).

Species	Irradiance Interference	90 DAT		150 DAT	
		SI	SD	SI	SD
<i>Mikania glomerata</i>	I ₀	17.55 ± 0.54	248.68 ± 10.79	18.05 ± 0.32	256 ± 6.48
	I ₂₅	16.75 ± 0.64	248.02 ± 14.19	17.57 ± 0.30	242 ± 6.88
	I ₅₀	13.92 ± 0.62	192.46 ± 8.17	16.94 ± 0.31	209 ± 5.40
	I ₇₅	12.43 ± 0.47	157.41 ± 6.99	14.90 ± 0.28	154 ± 3.74
<i>Mikania laevigata</i>	I ₀	12.51 ± 0.38	165.34 ± 5.95	14.41 ± 0.29	176 ± 4.84
	I ₂₅	12.62 ± 0.45	178.57 ± 8.08	14.75 ± 0.32	166 ± 4.98
	I ₅₀	12.14 ± 0.50	154.10 ± 5.70	12.69 ± 0.28	141 ± 3.70
	I ₇₅	11.16 ± 0.36	136.90 ± 5.22	12.29 ± 0.24	123 ± 2.94
ANOVA	Species	**	**	**	**
	Irradiance	**	**	**	**
	Species x Irradiance	**	**	*	**

Data are mean ± SD (n = 5). ns, not significant. * 0.05 > P > 0.001. ** P < 0.001

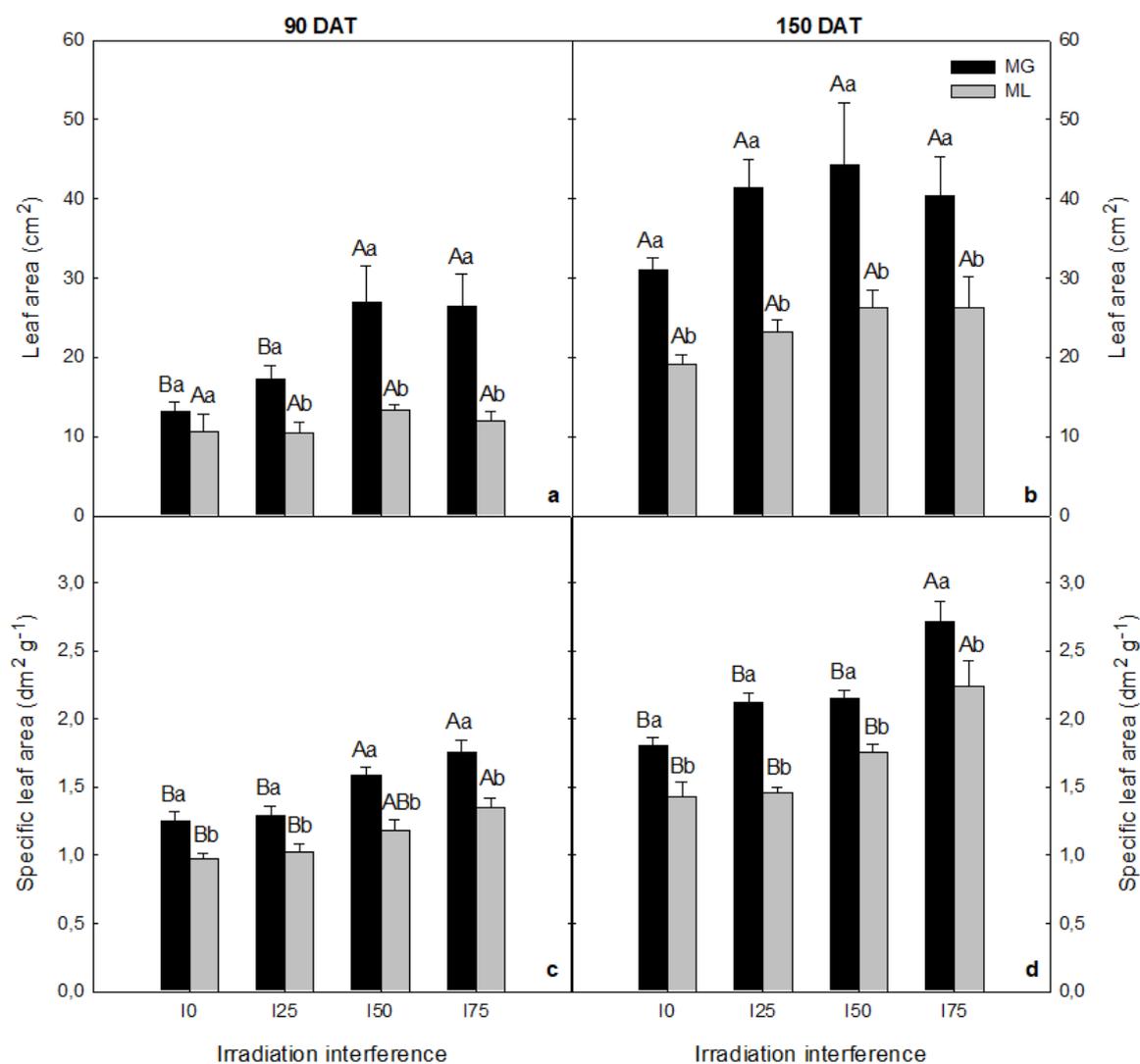


Figure 4. Leaf area (a) and specific leaf area (SLA; b) in *Mikania glomerata* Spreng. and *Mikania laevigata* Sch. Bip *ex* Baker, at 90 and 150 days after the treatment started (DAT), grown under sunlight irradiance interference treatments: 0% (I₀), 25% (I₂₅), 50% (I₅₀), and 75% (I₇₅). Data shown are the means (± SE) for measurements made on five plants per treatment. Different capital letters indicate significant differences (P < 0.05) among sunlight irradiance interference treatments. Different small letters indicate significant differences (P < 0.05) between species, according to the Tukey test.

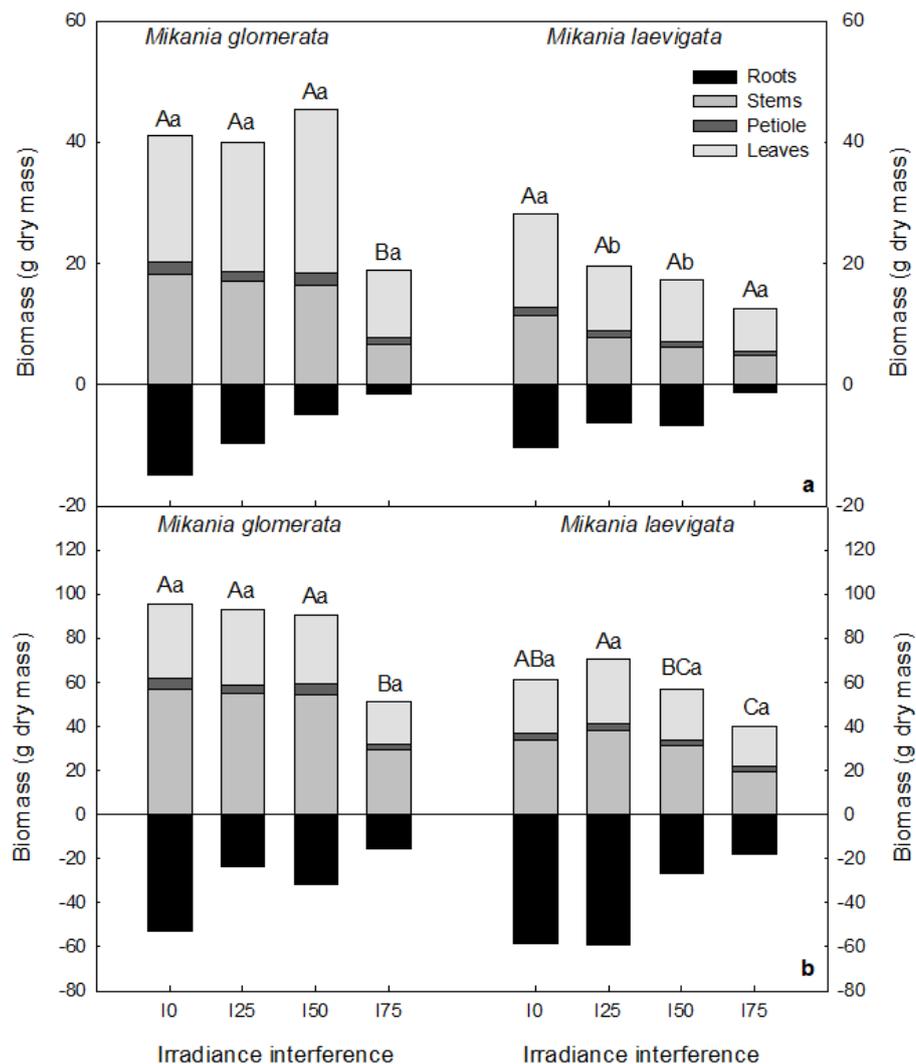


Figure 5. Biomass accumulation (g dry mass) of leaves, petiole, stem, and roots of *Mikania glomerata* Spreng. and *Mikania laevigata* Sch. Bip ex Baker, at 90 (a) and 150 (b) days after the treatment started (DAT), grown under sunlight irradiance interference treatments: 0% (I0), 25% (I25), 50% (I50), and 75% (I75). Data shown are the means (\pm SE) for measurements made on five plants per treatment. Different capital letters indicate significant differences ($P < 0.05$) among sunlight irradiance interference treatments. Different small letters indicate significant differences ($P < 0.05$) between species, according to the Tukey test.

the capture of light (Almeida *et al.* 2005). Chlorophyll is continuously synthesized and degraded (photo-oxidation) in the presence of light, but under conditions of high light intensity, the chlorophyll molecules are more likely to be photo-oxidized, and a balance is established under lower levels of irradiation (Kramer & Kozłowski 1979). The decreases of chlorophyll content and F_v/F_m values are indicators of oxidative stress, as we observed in plants developed under I0. However, in *M. laevigata* grew under I25 and I50, the lowest chlorophyll content compared to *M. glomerata* may help in photoprotection due to the reduced light interception, resulting in higher F_v/F_m in this species (Munné-Bosch & Alegre 2000).

High carotenoids levels under high light conditions are an essential mechanism for photoprotection since it prevents photooxidative damage of chloroplast pigments and avoids the formation of singlet oxygen (Demmig-Adams &

Adams 1992, Yamamoto & Bassi 1996, Gonçalves *et al.* 2001). The total number of molecules of carotenoid per chlorophyll molecule is typically higher in leaves grown in high irradiance environments when compared with leaves of shaded plants, which is in agreement with our results.

In addition to the increased chlorophyll content under shaded conditions, morphological alterations such as higher LA and SLA help plants in light interception process under shaded environments, as observed in *Mikania* plants under I75 (Artru 2018). In plants developed under I0, the SLA reduction enhance the protection and buffer the damages of excessive solar radiance may cause (Givnish *et al.* 2004, Matos *et al.* 2009, Wentworth *et al.* 2006). The small and dense leaves of plants developed under full sunlight is a morphological mechanism in order to avoid excessive water losses and protection of photosynthetic apparatus against

possible photo-oxidative damages caused by excessive solar radiances (Lima Junior *et al.* 2005).

Lowest SLA values indicate an increased leaf thickness as a result of thicker parenchyma and epidermis under high solar radiances levels. In *M. laevigata* plants, we observed a higher leaf thickness compared to *M. glomerata*. This increment in leaf thickness, mainly due to a thicker adaxial epidermis may explain the reduced photoinhibition and higher photosynthesis in plants developed under I25. Epidermis attenuates the UV radiation and allows photosynthetically active radiation (PAR) to diffuse into photosynthetic tissues. For example, Verdagner *et al.* (2017) found a thicker adaxial epidermis in plants stimulated by UV-A radiation. The development of a thicker adaxial epidermis, or hypoderm, seems to be a mechanism of protection of palisade parenchyma against the excessive visible light and a protection against leaf wilting when the plant is exposed to high solar radiances levels (Chazdon & Kaufmann 1993). Changes in leaf structure are crucial for plant growth under different environmental conditions (Hanba *et al.* 2002, Schlüter *et al.* 2003). The SD and SI in *Mikania* leaves were also affected by solar radiances levels. The synchronized increases in SD and SI indicated that solar radiances levels stimulated the process of epidermal cell differentiation in stomata and that SD and SI alterations were not caused by reductions in the size or number of epidermal cells (Woodward 1987). The increased SD and SI in plants with the lowest retention of solar radiation flux presumably resulted in an increased g_s and A.

In this study, we observed higher dry matter production under high solar radiances levels in both *Mikania* species. Similar results were observed in other lianas species, such as *Acacia kamerunensis*, *Loeseneriella rowlandii*, and *Afrobrunnichia erecta* (Toledo-Aceves & Swaine 2008), and *Plukenetia volubilis* (Cai 2011). Increased biomass accumulation highly depends on the transport of photoassimilates between organs, and patterns of cell division and cell expansion. In this study, high light levels induced A, presumably increasing the production of carbohydrates and dry matter. Our data showed that plants developed under low light availability conditions allocated a greater amount of assimilates to aerial organs in order to reach the light at the upper canopy layer, optimizing the photosynthetic process within an environment where low light might limit photosynthesis (Thompson *et al.* 1992, Walters *et al.* 1993). Leaf biomass in both *Mikania* species was higher in plants grown under higher light conditions, contradicting the results of Boeger *et al.* (2009), where authors showed that shading stimulated greater leaf biomass production in *M. glomerata*. In this study, we also observed higher root biomass at full sunlight conditions in both species. This response presumably allows greater absorption of water and nutrients, which may represent a strategy to increase the ability to withstand higher rates of photosynthesis and transpiration in bright environments (Poorter 2001, Mielke & Schaffer 2010).

Our data showed that plants developed under low light intensity showed an increased content of chlorophyll, LA, and SLA. However, A was small when compared to other treatments, presumably resulting in less aboveground dry

mass. In both *Mikania* species, plants developed under shaded environments (I75) showed lower performance than plants grown in other environments. Plants grown under I25 presented higher photosynthesis values than plants grown under I0, and this response may be associated with an increased amount of chlorophyll. Although both species are morphologically similar, *M. glomerata* and *M. laevigata* showed distinct responses to irradiance levels. *M. glomerata* had great biomass production under I0, I25, and I50 with low variation regarding photosynthetic rates. While in *M. laevigata* plants, the highest photosynthesis rate was observed under I25 and I50, while biomass production was higher under I25. In this species we also observed a higher investment in root growth under higher solar radiances levels (I0 e I25), resulting in a decreased aboveground biomass when compared to *M. glomerata*. While *M. glomerata* showed higher SI, SD, LA, and SLA, *M. laevigata* showed a thicker leaf. In addition to a thicker leaf, caused especially by an increased AdE, the lower chlorophyll content and higher photosynthesis rate observed under I25 and I50 presumably contributed to the reduced photoinhibitory effects (Fv/Fm) in this species when compared to *M. glomerata*, under the same solar radiances level.

Conclusions

We concluded that *M. laevigata* showed better performance under 25% of retention of solar radiation flux, and not under full sun, contradicting our main hypothesis. We also concluded that plants of both *Mikania* species show a low capacity of growing under shading conditions. However, under shading both species invested more in shoot biomass, a strategy presumably associate with enhanced growth to reach the canopy more quickly. Although lianas are known as light-dependent plants, the two species presented different responses under the same irradiance conditions. *M. laevigata* showed better growth under shading of 25% irradiation interference, while *M. glomerata* grows better from 0 to 50% irradiation interference.

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Authors contribution

Daniele Ribeiro Contin: carried out the experiments, collected and analyzed data and prepared the manuscript.

Eduardo Habermann: prepared the manuscript and contributed to the revision of the manuscript.

Vani Maria Alves: supervised anatomical analysis.

Carlos Alberto Martinez: supervised the study and contributed to the revision of the manuscript.

Conflicts of interest

The authors declare that there are no conflicts of interest

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Supplementary material

Supplementary material Table S1. Monthly climatological conditions in the study site during the experiment.

Month	Days of treatment	Maximum temperature (°C)	Minimum temperature (°C)	Average temperature (°C)	Precipitation (mm)
May	0	25.79	13.31	19.56	6.21
June	30	26.42	13.72	20.07	0.33
July	60	27.43	11.80	19.61	0.00
August	90	29.76	15.41	22.60	2.11
September	120	30.10	15.32	22.72	0.89
October	150	30.91	18.96	24.94	5.56

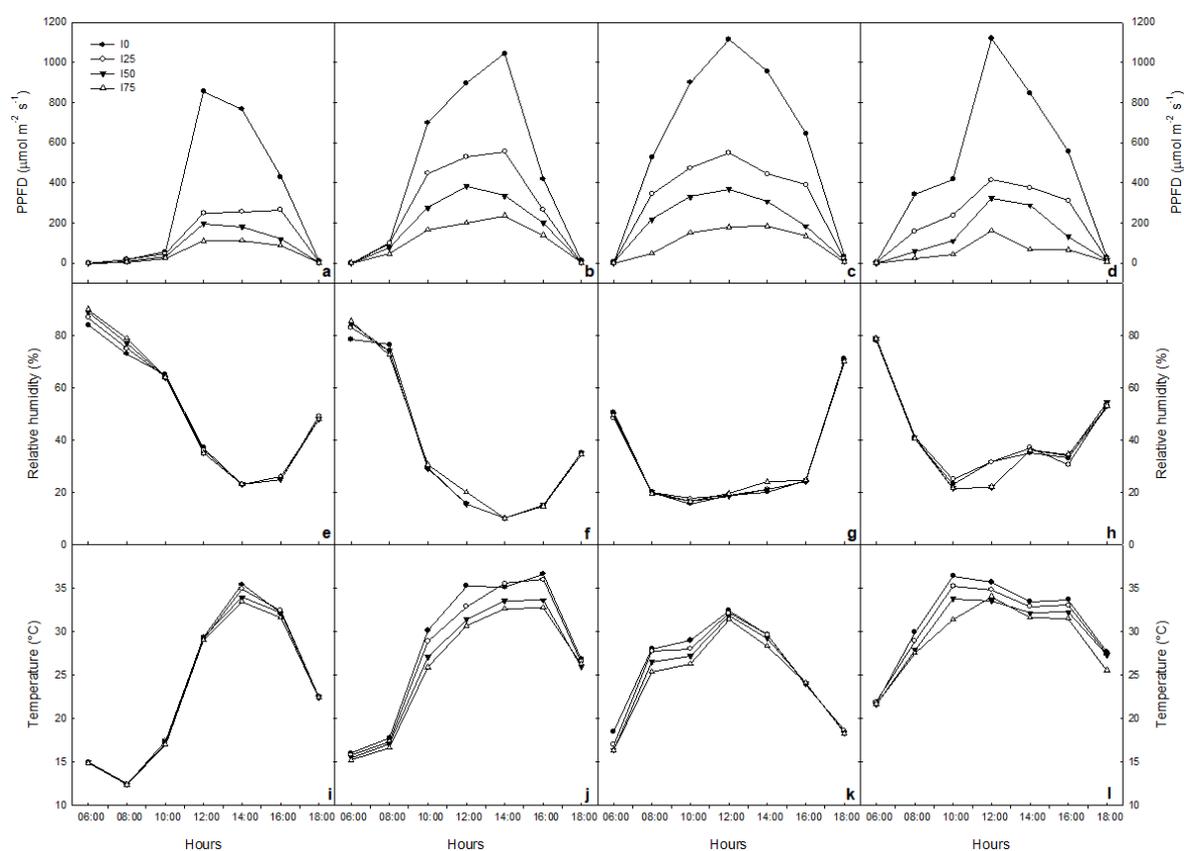
Supplementary material Table S2. The results of a two-way analysis of variance (ANOVA) with the species (S) and irradiance (I) as fixed factors with the interaction (S × I) is shown at each analyzed parameter.

Parameter	DAT											
	60			90			120			150		
	S	I	S x I	S	I	S x I	S	I	S x I	S	I	S x I
E	ns	ns	ns	ns	*	ns	**	**	ns	ns	**	**
gs	ns	*	ns	*	**	ns	*	**	ns	*	**	*
A	ns	*	ns	ns	**	ns	ns	**	ns	ns	**	*
iWUE	ns	ns	ns	**	ns	ns	**	*	**	**	**	*
Chlorophyll	*	**	ns	ns	**	ns	*	**	ns	**	**	ns
Carotenoids	*	**	ns	ns	**	ns	*	**	ns	**	**	ns
Car/Chl	ns	**	ns	*	**	ns	*	**	*	ns	**	ns

Data are mean ± SD (n = 5). ns, not significant. * 0.05 > P > 0.001. ** P < 0.001

Supplementary material Table S3. Average temperature (T, °C), relative humidity (RH, %) and vapor pressure deficit (VPD, kPa) in 0% (I0), 25% (I25), 50% (I50), and 75% (I75) irradiance interference.

Days of treatments	I0			I25			I50			I75		
	T	RH	VPD									
60	14.8	69.0	0.5	14.9	69.5	0.5	14.9	70.5	0.5	14.7	71.5	0.5
90	23.9	53.0	1.4	23.1	51.5	1.4	22.1	51.5	1.3	21.2	51.5	1.2
120	28.5	17.7	3.2	27.8	18.7	3.0	26.8	18.2	2.9	25.8	18.0	2.7
150	33.1	31.7	3.5	32.0	33.0	3.2	30.8	31.2	3.0	29.4	31.0	2.8



Supplementary material Figure S1. Diurnal course of relative humidity, ambient temperature, and the photosynthetic photon flux density (PPFD) at 60 (a, e, i), 90 (b, f, j), 120 (c, g, k), and 150 DAT (d, h, l). Treatments: 0% (I0), 25% (I25), 50% (I50), and 75% (I75) of irradiance interference.