Efficiency of glyphosate and carfentrazone-ethyl in the control of *Macroptilium atropurpureum* (DC.) Urb. under different light intensities

Gustavo Amaral Costa[a], Leonardo David Tuffi-Santos[a,*], Samuel Alves dos Santos[a,*], Leandro Roberto da Cruz[a], Bruno Francisco Sant'Anna-Santos[b], Izabela Thais dos Santos[a], Francisco André Ossamu Tanaka[c]

[a] *Universidade Federal de Minas Gerais (UFMG) - Campus Regional de Montes Claros.* Av. Universitária, 1.000. Bairro Universitário. Montes Claros, MG CEP: 39.404-547, Brazil
[b] *Universidade Federal do Paraná (UFPR) - Laboratório de Anatomia e Biomecânica Vegetal, Departamento de Botânica, Setor de Ciências Biológicas, Universidade Federal do Paraná (UFPR), Curitiba CEP: 81.531-990, PR, Brazil*
[c] *Departamento de Fitopatologia, Escola Superior de Agricultura Luiz de Queiroz (ESALQ). Av. Pádua Dias, 11 - Cx. Postal 9, Piracicaba, SP CEP: 13418-900, Brazil*

A R T I C L E   I N F O

A B S T R A C T

Variation in environmental conditions, such as light availability, can affect herbicide efficacy by altering leaf morphoanatomy. Accordingly, the aim of the present study was to evaluate the influence of light level and herbicide application on leaf anatomy and herbicide efficacy, using *Macroptilium atropurpureum* as a model weed species. The experiment involved a factorial design (3 × 4), using three light levels (0, 50, or 70% shadow) and four herbicide treatments (no herbicide, glyphosate, carfentrazone-ethyl, or a combination of glyphosate and carfentrazone-ethyl). When combined, the herbicides were efficient in controlling *M. atropurpureum* under all light levels. However, when applied independently, the herbicides failed to satisfactorily control plants cultivated in full sunlight, and plants cultivated in shaded environments were more sensitive to glyphosate application than those cultivated in full sunlight. The greater efficacy of the herbicides on plants grown under shaded conditions can likely be attributed to changes in leaf morphoanatomy. Plants grown under restricted light conditions exhibit greater average leaf area, lower trichome density, reduced epicuticular wax deposition, thinner leaves, and lower dry mass accumulation, characteristics which are closely correlated to chemical control. The combination of the herbicides presents an additional effect in the control of *M. atropurpureum*. Also, changes in leaf anatomy caused by light restriction reduce the tolerance of *M. atropurpureum* to the herbicides.

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1. Introduction

Macroptilium atropurpureum (DC.) Urb. (Fabaceae) is a pubescent, perennial legume that possesses a tap root system, is well adapted to low-moisture conditions and different soil types (Valentim, 2010), and generally inhabits shaded environments (Lorenzi, 2008) considered to have low or moderate tolerance to shade (Baig et al., 2005; Valentim, 2010). In Brazil, this species has been reported to occur as a weed in a variety of crops, such as sugarcane, soy, peanut, fruit growing and reforestation areas (Lorenzi, 2008), and is considered a problem weed, owing to its high seed production capacity, vigor, size, and negative effect on crop productivity (Lorenzi, 2008; Oliveira and Freitas, 2008). In particular, the voluminous habit of the plant interferes with the growth of cultivated plants and impedes both cultivation practices and harvesting. Furthermore, the species has been reported to exhibit glyphosate tolerance (Costa et al., 2018).

Understanding the mechanisms underlying herbicide tolerance is fundamental for establishing weed management strategies that can be used under a variety of environmental conditions. The combined effects of the environment and serial repeated herbicide use may encourage the selection of resistance to one or more herbicide modes of action (Hanson et al., 2013). The combined use of herbicides with different modes of action improves the spectrum of action and efficacy of herbicides (Walsh et al., 2014; Kumar and Jha, 2015; McCullough et al., 2015; Walsh et al., 2015). In addition, as plant tolerance to post-emergence herbicides is often related to leaf morphology and anatomy (Tuffi-Santos et al., 2006; Santos et al., 2015), changes in environment conditions, such as light incidence or availability, may affect the sensitivity of weeds to herbicides.

Low light incidence can cause changes in leaf characteristics (e.g., trichome density, the chemistry and structure of epicuticular waxes,

* Corresponding authors.
E-mail addresses: ltuffi@ufmg.br (L.D. Tuffi-Santos), samuelalves.ufmg@gmail.com (S.A.d. Santos), brunofrancisco@ufpr.br (B.F. Sant'Anna-Santos), faotanaka@ig.com.br (F.A.O. Tanaka).

and stomatal density) that may also inhibit the diffusion of herbicides into the epidermis, thereby reducing herbicide damage (Procópio et al., 2003). Therefore, it is important to consider leaf anatomy and ultrastructure during the development of weed management strategies, especially in plants that exhibit phenotypic plasticity in response to changes in light availability (Pires et al., 2012; Santos et al., 2015). Studies of *M. atropurpureum* biology or its response to chemical control are scarce. Accordingly, the aim of the present study was to evaluate the influence of light level and herbicide application on leaf anatomy and herbicide efficacy, using *M. atropurpureum* as a model weed species.

2. Material and methods

2.1. Experimental design

The present study was conducted in the municipality of Montes Claros, Minas Gerais State, Brazil (43°50′15.90″W, 16°40′57.59″S; 620 m altitude), which possesses an Aw-Tropical savanna-type climate (Köppen, 1948).

Macroptilium atropurpureum seeds were collected from plants in the field and sown in styrofoam trays containing commercial substrate (Plantmax®, Eucatex Agro; Brazil) which included coconut fiber, pine bark, vermiculite, rice husk, and nutrients. The trays were kept open-air on wooden benches for growth of *M. atropurpureum* seedlings. Voucher specimens (HMCMG-2106, HMCMG-2107, and HMCMG-2108) were deposited at Herbarium HMC (State University of Montes Claros). After 30 days, the most developed and uniform seedlings were transplanted to pots (12 dm^3 diameter) that contained a substrate with a 3:1:1 mixture of soil, sand, and cured cow manure and 10 kg of nitrogen, phosphorus, and potassium (NPK-4:30:10) per 0.9 m^3 of substrate. The substrate possessed the following characteristics: pH (water): 4.7; organic matter content: 2.37 dag kg^{-1}; P: 0.47 mg dm^{-3}; K: 35 mg dm^{-3}; Ca: 0.20 $cmol_c$ dm^{-3}; Mg: 0.10 $cmol_c$ dm^{-3}; Al: 0.60 $cmol_c$ dm^{-3}; H+Al: 2.90 $cmol_c$ dm^{-3}; effective CTC: 0.99 $cmol_c$ dm^{-3}.

To meet the macro- and micronutrient demands of the plants, the plants were fertilized with foliar mineral fertilizer (Nutrigarden)® every two weeks throughout the period of cultivation, and soil humidity was maintained near its field capacity using irrigation, which was performed twice a day.

The experiment involved a factorial design (3 × 4), using three light levels (0, 50, or 70% shadow) and four herbicide treatments (no herbicide; glyphosate, 1440 g e.a. ha^{-1}; carfentrazone-ethyl, 40 g e.a. ha^{-1}; or a combination of glyphosate and carfentrazone-ethyl, 1080 and 30 g e.a. ha^{-1}, respectively), with five replicates. Experimental plots consisted of individual pots, each of which contained a single plant. The commercial formulations Roundup Original® and SPOT-LIGHT® were used for the application of glyphosate and carfentrazone-ethyl, respectively.

The light was intercepted using black polypropylene shade cloth, which produced environments with 50 ± 4.2% (mean ± standard error) or 70 ± 3.8% shade. Light intensity was calculated for each environment using measurements (*n* = 10) taken using an AccuPAR Linear PAR/LAI Ceptometer (Model LP-80; Decagon Devices, Inc.,

Pullman, VA, USA). The mean irradiance during the experiment was 1170 μmol m^{-2} s^{-1} of PAR to full sunlight. The minimum and maximum temperatures of each light condition for the study period are shown in Table 1.

2.2. Leaf area and epicuticular wax content

At 45 days after planting (DAP) and immediately prior to the application of herbicides, two fully expanded leaves were collected from the upper third of each plant stem, and the leaf area of each stem was calculated using scanned images and Image-Pro Plus (Media Cybernetics, Inc., Rockville, MD, USA). Each sample was then transferred to a Petri dish that contained 100 mL chloroform and gently agitated for 20 s. The resulting extracts (wax + chloroform) were filtered through filter paper, transferred to 25 mL test tubes of known weight, and incubated in a hot water bath until the chloroform had completely evaporated. The solid residue that adhered to the walls of each tube was then quantified, and the wax content per unit leaf area (μg cm^{-2}) was calculated for each stem sample. The epicuticular wax extraction method was adapted from Hamilton (1995).

2.3. Herbicide application and efficacy assessment

At 45 DAP the herbicides were applied (100 L ha^{-1}) at 150 kPa pressure using a backpack sprayer equipped with a single tip rod (Teejet AI110015; TeeJet Technologies, Glendale Heights, IL, USA). The aerial part of each plant was then monitored daily for possible morphological changes; at 4, 16 and 28 days after application (DAA) control with respect to the no herbicide treatment were determined. In this visual analysis the control was considered using the proportional scale proposed by Frans et al. (1986), where 0% corresponds to the absence of visible leaf and stem damage and 100% corresponds to plant death. Control for the plot was calculated based on the arithmetic mean of data obtained using three evaluators. At 28 DAA, each plant was cut at the root collar, dried using a forced-air oven (65 °C for 72 h), and weighed (g pot^{-1}).

2.4. Analysis of leaf anatomy and ultrastructure

Samples of the middle region of fully expanded leaves were collected at 24 and 48 h after herbicide application and fixed in Karnovsky solution (Karnovsky, 1965). Part of each sample was dehydrated using a graduated ethanol series (30, 50, 70, 90, and 100%) and subsequently polymerized in acrylic resin. Cross sections (5 μm) were then obtained using a manual rotary microtome, stained using toluidine blue (pH 4.0; O'Brian and McCully, 1981), and mounted in Entellan®. Finally, the histological slides were photographed using a light microscope (Cousin Star; Carl Zeiss Microscopy, GmbH, Jena, Germany) and an attached digital camera (AxioCam ERc5s, Carl Zeiss, Germany), and Image-Pro Plus (Version 4.1; Media Cybernetics, USA) was used to measure the thickness of the palisade, spongy parenchyma, adaxial epidermis, and abaxial epidermis of the leaves. Total leaf thickness was calculated as the sum of the four tissues.

Table 1
Minimum and maximum temperatures (°C) of cultivation environments of the Macroptilium atropurpureum during the period of study.

Sunlight intensity	February		March		April		May	
	T. max.	T. min.	T. max.	T. min.	T. max.	T. min.	T. max.	T. min.
Full sun	31.77	20.03	30.63	20.67	31.17	19.47	30.10	16.90
50% of shade	29.55	20.32	27.49	20.97	28.99	19.75	28.00	17.06
70% of shade	28.65	20.41	27.03	21.26	28.11	19.83	27.19	17.13

The other part of each leaf sample was dehydrated using a graduated acetone series (30, 50, 70, 90, and 100%), dried to the critical point (Balzers CPD 030) using CO_2 as a drying medium, assembled in "stubs," and plated with gold (Balzers SCD 050). After preparation, the structure of leaf surface was analyzed for each sample using a scanning electron microscope (model LEO 435-VP; Carl Zeiss). Mean trichome density was calculated from 0.05 mm^2 sections (n = 10) of scanning electron microscope images using Image-Pro Plus.

2.5. Statistical analysis

Data were subjected to analyses of variance (ANOVA) and then to the Tukey test (Cecon et al., 2012), using a 5% probability of error. Average leaf area, epicuticular wax quantity, trichome density, leaf thickness, dry matter production, and chemical control were analyzed using the Pearson correlation test (Cecon et al., 2012). All the statistical analyses were performed using the R software version 3.4.1 (R Development Core Team, 2017). The morphological data and leaf anatomy and ultrastructure of M. atropurpureum were presented descriptively.

3. Results

3.1. Effect of light and herbicide treatment on plant growth

Plants that were not exposed to herbicides were well developed, regardless of light intensity, and all plants (i.e., regardless of herbicide treatment) accumulated less biomass under shaded conditions (Table 2).

The application of glyphosate alone failed to provide satisfactory control of plants cultivated under full sunlight (~40% control at 28 d after application; Figs. 1C and 2D) but yielded 95% control at 16 d after application for plants grown under restricted light levels (Fig. 1B). Meanwhile, the application of carfentrazone-ethyl failed to satisfactorily control plants, regardless of light level (Figs. 1A−C and 2G−I). However, the addition of carfentrazone-ethyl to the glyphosate treatment enhanced the control of M. atropurpureum, even in plants cultivated under full sunlight (Figs. 1A−C and 2J−L). In fact, the herbicide mixture provided more than 70% control (Fig. 1A), regardless of light level, and reduced the residual biomass in the post-control (Table 2).

The application of carfentrazone-ethyl alone failed to reduce the biomass of M. atropurpureum plants grown under full sunlight conditions, and the shoot dry mass of carfentrazone-ethyl-treated plants was similar to that of control plants (Table 2). Meanwhile, glyphosate, alone or mixed with carfentrazone-ethyl, reduced plant biomass, and the effect was observed regardless of light intensity, although the reduction was greater in plants grown under restricted light levels (Table 2).

3.2. Average leaf area and epicuticular wax content of M. atropurpureum

Average leaf area was directly affected by light intensity (Fig. 3A), and plants grown under full sunlight conditions accumulated more
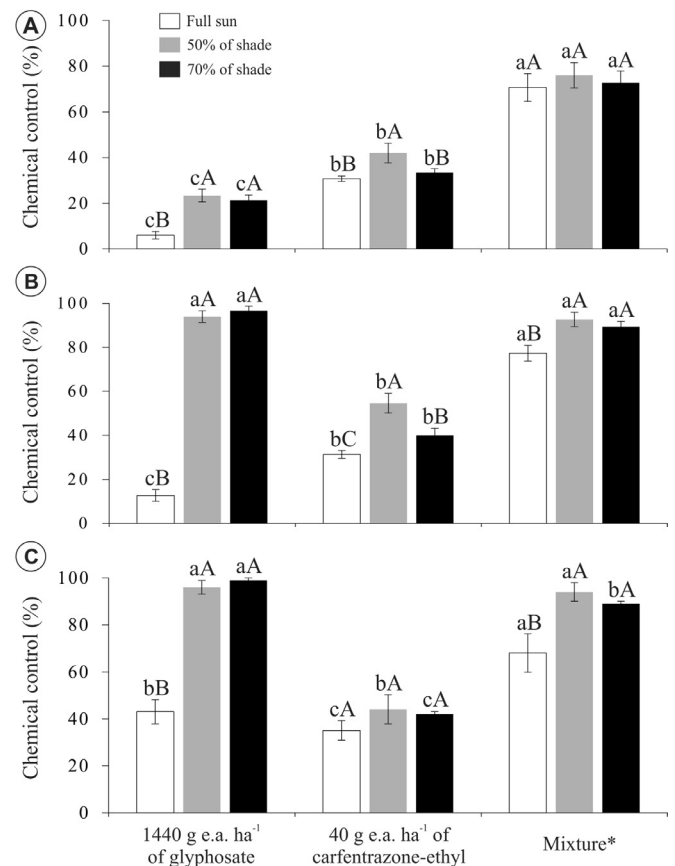

Fig. 1. Chemical control of Macroptilium atropurpureum at 4, 16, and 28 days after application of herbicides (A, B, and C, respectively). Lowercase letters compare the averages within the same light intensity and capital letters within the same herbicide. Averages ± standard error in a row followed by the same letters do not differ according to the Tukey test at 5% probability. *Mixture = 1080 g e.a. ha^{-1} of glyphosate + 30 g e.a. ha^{-1} of carfentrazone-ethyl.

epicuticular wax per unit leaf area, with three times greater more wax than those cultivated under restricted light conditions (Fig. 3B).

3.3. Leaf anatomy and ultrastructure of M. atropurpureum as a function of light intensity and herbicide application

Among epidermal cells, trichomes were the most affected by herbicide application. Control plants possessed intact trichomes with typical turgor pressure characteristic (Fig. 4A), whereas herbicide-treated plants exhibited loss of turgor pressure (Fig. 4B). The density of trichomes on both the adaxial and abaxial leaf surfaces was lower in plants grown under restricted light conditions than in those grown under full sunlight (Figs. 4D, E, G, and H; Table 3). At 24 h after the application of glyphosate, the plants grown under 50 and 70% shade exhibited trichomes with lower turgor pressure in the base (Fig. 5E

Table 2
Shoot dry mass (g pot^{-1}) of Macroptilium atropurpureum at 28 days after application of herbicides.

Sunlight intensity	Chemical control			
	Without herbicide	1440 g e.a. ha^{-1} of glyphosate	40 g e.a. ha^{-1} of carfentrazone-ethyl	Mixture *
Full sun	30.64 ± 1.29 aA	22.03 ± 0.57 bA	30.85 ± 2.08 aA	13.04 ± 1.07 cA
50% of shade	24.18 ± 2.48 aB	4.88 ± 1.33 cB	9.13 ± 0.98 bC	5.93 ± 1.06 cB
70% of shade	19.59 ± 1.39 aB	2.59 ± 0.68 dB	12.93 ± 0.97 bB	5.22 ± 0.77 cB

Averages ± standard error followed by the same lowercase letter in the same line and uppercase in the column do not differ from each other according to the Tukey test at 5% probability.
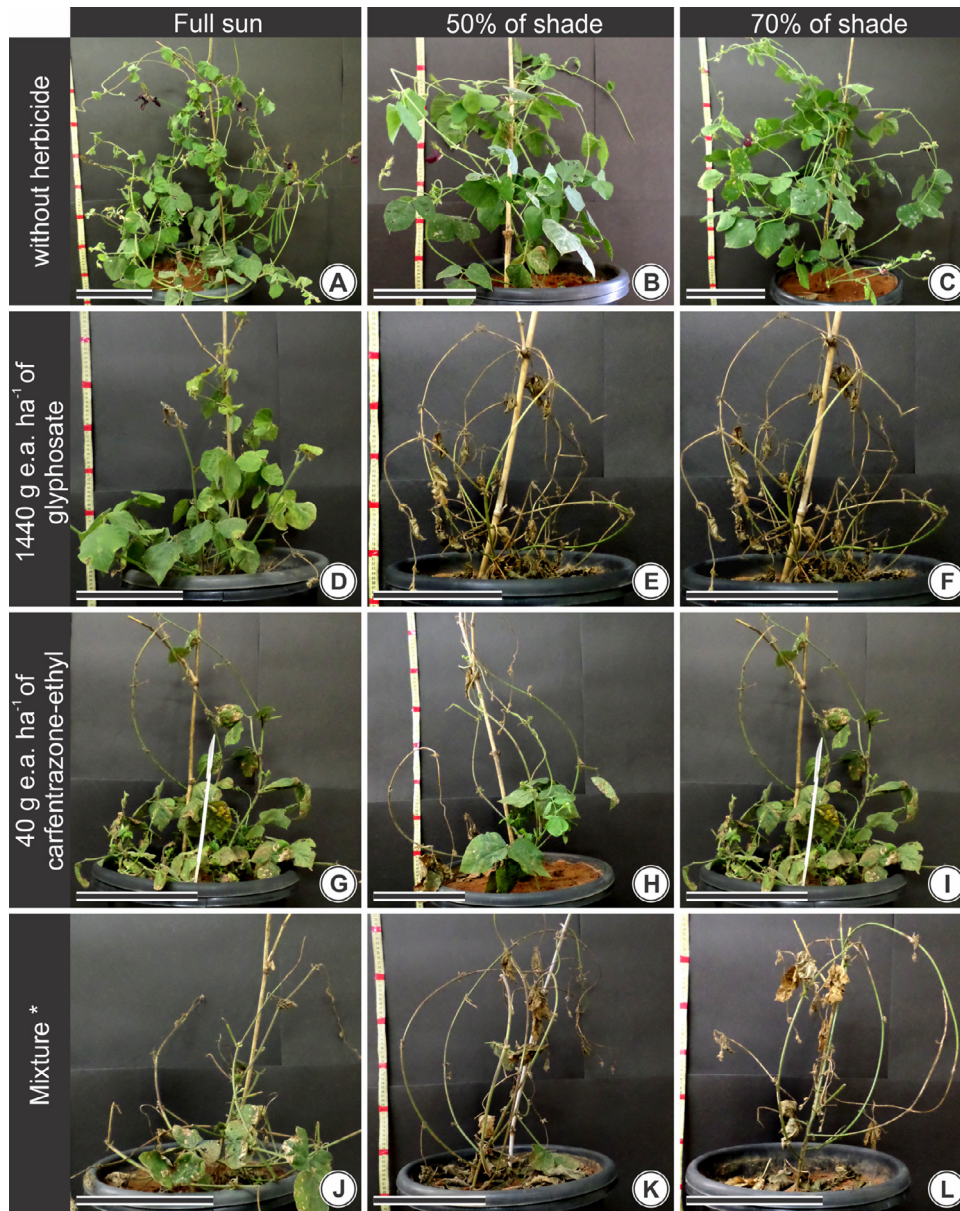* Mixture = 1080 g e.a. ha^{-1} of glyphosate + 30 g e.a. ha^{-1} of carfentrazone-ethyl.

Fig. 2. *Macroptilium atropurpureum* plants at 28 days after application of herbicides. A−C. Plants not exposed to herbicides. G−L. Plants with moderate herbicide injury. E, F, J, K, and L. Plants with high herbicide injury. Scale bars = 25 cm. *Mixture = 1080 g e.a. ha^{-1} of glyphosate + 30 g e.a. ha^{-1} of carfentrazone-ethyl.

and F), when compared to the trichomes of plants grown under full sunlight.

Plants treated with carfentrazone-ethyl exhibited epicuticular wax erosion and cuticle rupture, especially in those grown under restricted light conditions (Fig. 5G−I), and plants treated with the herbicide mixture exhibited epicuticular wax erosion and cuticle rupture, as well as a loss of turgor pressure (Fig. 5J−L), although less pronounced than in plants exposed to carfentrazone-ethyl alone.

The interaction between light restriction and herbicide treatment had a significant effect on leaf thickness (Table 4). The leaves of plants grown under restricted light conditions were 30% thinner than those of plants grown under full sunlight, and in plants grown under full sunlight or 50% shade, the application of carfentrazone-ethyl, alone or in combination with glyphosate, also reduced leaf thickness, when compared to those of control or glyphosate-treated plants (Table 4).

All the measured variables were closely correlated with chemical treatment (Table 4). Chemical control was positively associated with average leaf area but negatively associated with amount of epicuticular wax, trichome density, and leaf thickness (Table 5).

Pearson correlation coefficient values (r). **Significant at 1% probability and *Significant at 5% probability according to the *t*-test, comparing values Pearson correlacion among chemical control and Average leaf area, epicuticular wax quantity, trichome density, leaf thickness and dry matter. Mixture = 1080 g e.a. ha^{-1} of glyphosate + 30 g e.a. ha^{-1} of carfentrazone-ethyl.

4. Discussion

The present study demonstrates that restricted light conditions affect the leaf anatomy and ultrastructure of *M. atropurpureum* and increase the sensitivity of *M. atropurpureum* plants to glyphosate. Plants grown under restricted light conditions exhibit greater average leaf area, lower trichome density, reduced epicuticular wax deposition, thinner leaves, and lower dry mass accumulation, characteristics which are closely correlated to chemical control (Table 5).

In the present study, glyphosate and carfentrazone-ethyl combined had increased control of *M. atropurpureum*, regardless of light
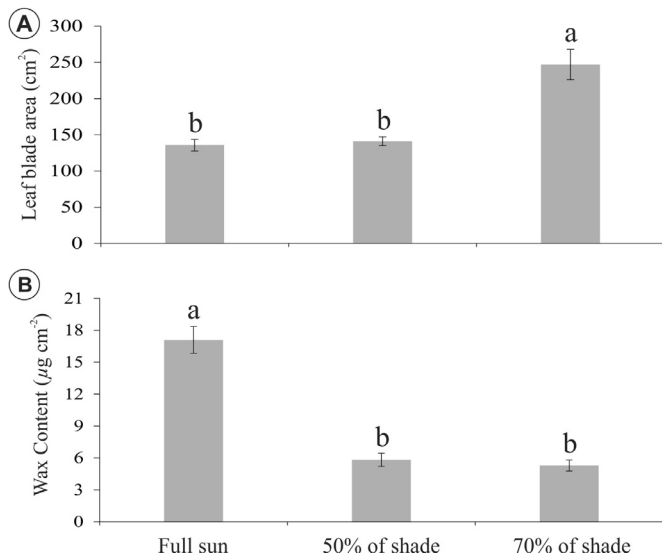
Fig. 3. The average leaf area (A) and epicuticular wax content (B) in *Macroptilium atropurpureum* grown under different light intensities. Averages ± standard error in a row followed by the same letter indicates no significant difference according to the Tukey test at 5% probability.

Table 3
Density of trichomes (trichomes mm^{-2}) in *Macroptilium atropurpureum* leaves grown under different light intensities.

Sunlight intensity	Epidermal surface	
	Adaxial	Abaxial
Full sun	23.12 ± 0.54 a	41.90 ± 2.38 a
50% of shade	16.13 ± 0.49 b	23.86 ± 1.22 b
70% of shade	14.55 ± 0.70 b	23.37 ± 1.33 b

Averages ± standard error followed by the same letter indicates no significant difference according to the Tukey test at 5% probability.

availability. The greater efficacy of glyphosate under restricted light conditions has been reported previously. For example, *Commelina benghalensis* and *Cyperus rotundus,* which are regarded as difficult weeds to control, were reportedly controlled using only 25% (Santos Junior et al., 2013) and 50% (Santos et al., 2015) of the recommended dose when the plants were grown under restricted light conditions.

Glyphosate is a systemic post-emergent herbicide that primarily targets leaves. When herbicides are applied to pubescent plants, trichomes, which are positioned at a higher level than other cells of the epidermis, are the first epidermal structures to be contacted. Therefore, the main effects of herbicide application are perceived in these structures or nearby (Fig. 5). Drops of herbicide that are intercepted by the trichomes may never reach the cells of the epidermis, which are the main route of entry. Therefore, the greater density of trichomes observed in plants grown in full sunlight (Table 3; Fig. 4C and F) function as a protective barrier, thereby contributing to glyphosate tolerance. Meanwhile, the lower density of trichomes in plants grown under restricted light conditions may favor the absorption of herbicides, as a greater amount of herbicide is able to reach the leaf surface, thereby contributing to the greater efficacy of glyphosate in plants grown under restricted light conditions.

The greater wax deposition observed in plants grown under full sunlight conditions functions to reduce excessive water loss and
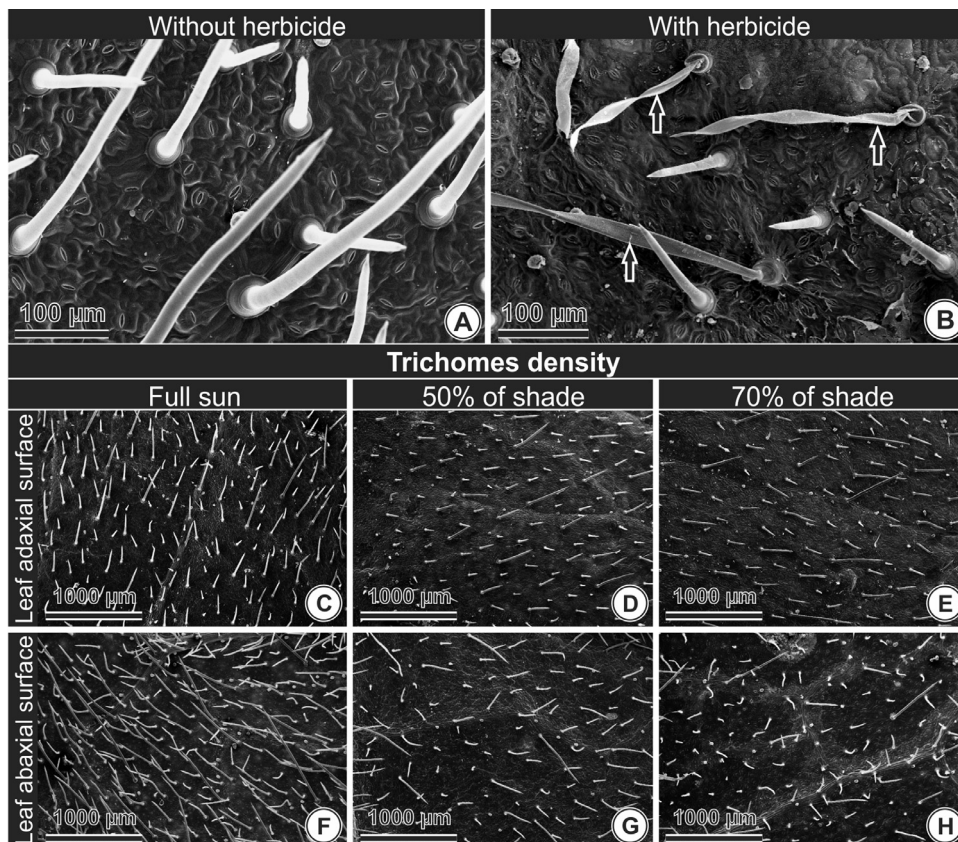

Fig. 4. Effects caused by herbicides in trichomes (A, B) and density of trichomes (C−H) in *Macroptilium atropurpureum* leaves. A. Intact trichomes with characteristic turgor pressure. B. Arrows indicate trichomes with loss of turgor pressure. C and F. Greater density of trichomes. D, E, G, and H. Lower density of trichomes.
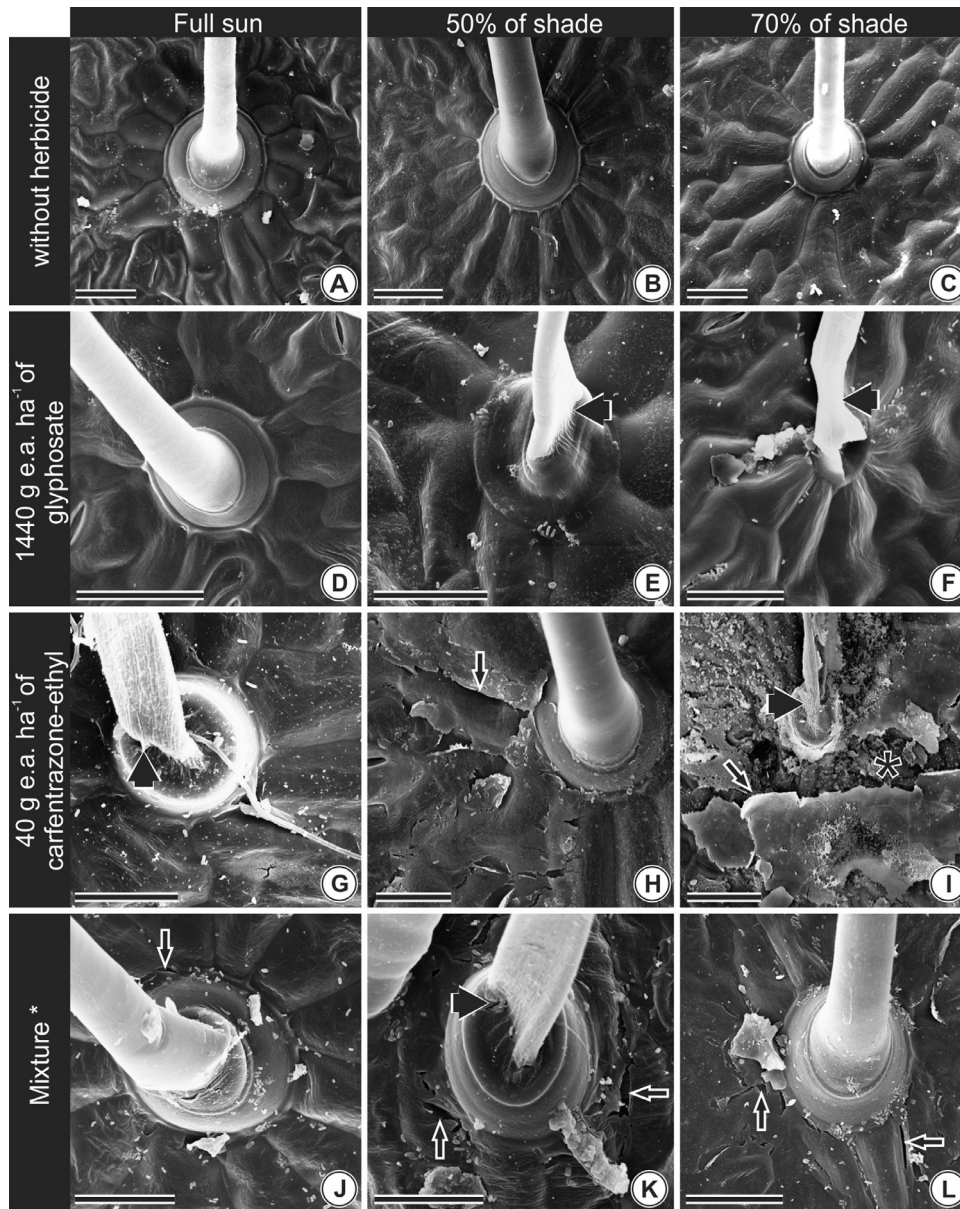
| | Full sun | 50% of shade | 70% of shade |

without herbicide — A, B, C

1440 g e.a. ha⁻¹ of glyphosate — D, E, F

40 g e.a. ha⁻¹ of carfentrazone-ethyl — G, H, I

Mixture * — J, K, L

Fig. 5. Damage caused by glyphosate and carfentrazone-ethyl, individually or in combination, on the adaxial leaf surface of *Macroptilium atropurpureum* grown under different light intensities 24 h after application. A−D. Trichomes with turgor pressure characteristic of the species. E−L. Arrowheads indicate trichomes with loss of turgor pressure; arrows indicate cuticle rupture; asterisk indicates complete rupture of the cuticle and exposure of the epidermal cells. Scale bars = 25 μm. *Mixture = 1080 g e.a. ha^{-1} of glyphosate + 30 g e.a. ha^{-1} of carfentrazone-ethyl.

possibly to reduce excessive irradiation (Viana et al., 2010; Boutin et al., 2012; Razeq et al., 2014). The amount and chemical composition of epicuticular waxes can reduce herbicide absorption and promote herbicide tolerance (Taylor, 1996) since they are hydrophobic substances, whereas most herbicides, including glyphosate and

carfentrazone-ethyl, are applied in aqueous solutions (Rodrigues and Almeida, 2011). The greater wax deposition in *M. atropurpureum* grown in full sunlight likely limited the absorption of glyphosate, thereby conferring tolerance. However, when combined with carfentrazone-ethyl, the rapid absorption and mode of action of the

Table 4
Leaf blade thickness (μm) in *Macroptilium atropurpureum* grown under different light intensities 24 h after herbicide application.

Sunlight intensity	Chemical control			
	Without herbicide	1440 g e.a. ha-1 of glyphosate	40 g e.a. ha^{-1} of carfentrazone-ethyl	Mixture*
Full sun	225.1 ± 9.4 aA	239.6 ± 6.0 aA	180.14 ± 10.5 bA	195.50 ± 5.5 bA
50% of shade	162.9 ± 5.0 aB	168.0 ± 11.9 aB	146.32 ± 8.7 bB	144.17 ± 2.1 bB
70% of shade	164.3 ± 7.5 aB	153.7 ± 5.9 aB	141.07 ± 11.7 abB	145.48 ± 3.9 aB

Averages ± standard error followed by the same lowercase letter in the same line and uppercase within the column do not differ according to the Tukey test at 5% probability.
* Mixture = 1080 g e.a. ha^{-1} of glyphosate + 30 g e.a. ha^{-1} of carfentrazone-ethyl.

Table 5
Correlation between the variables analyzed and the control of *Macroptilium atropurpureum* at 28 days after application of herbicides.

Variable	Chemical control		
	1440 g e.a. ha^{-1} of glyphosate	40 g e.a. ha^{-1} of carfentrazone-ethyl	Mixture
Leaf area	0.84**	0.77**	0.80**
Wax content	−0.98**	−0.59*	−0.81**
Trichomes density	−0.96**	−0.55*	−0.78**
Leaf thickness	−0.85**	−0.73**	−0.76**
Dry mater	−0.98**	−0.55*	−0.87**

* Significant at 5% probability according to the *t*-test.
** Significant at 1% probability according to the *t*-test.

herbicide changed the cuticle structure, which may have favored the absorption of glyphosate (Fig. 5D, G, and J).

The greater efficacy of glyphosate in plants grown under restricted light conditions may also be related to larger leaf surface area and thinner leaves. Larger leaves intercept a greater amount of herbicide, which can directly influence the amount of product absorbed. Plants grown under restricted light conditions generally develop larger leaves to compensate for the light deficit (Taiz and Zeiger, 2009). Reduced leaf thickness has also been reported to occur in plants grown under low light intensity (Morais et al., 2003, 2004; Lima et al., 2006; Gondim et al., 2008; Santos et al., 2015; Tuffi-Santos et al., 2015).

The absorption and translocation of glyphosate are essential processes for herbicide efficacy (Vila-Ajub et al., 2012; Yerka et al., 2013; Adu-Yeboah et al., 2014). Glyphosate is transported through both phloem and xylem, following the route of photosynthates (Shaner, 2009). After passing through the epidermis, glyphosate reaches the vascular bundles, *via* both the apoplast and symplast, where it is distributed to different parts of the plant (Yanniccari et al., 2012). The results presented herein suggest that glyphosate can reach the vascular bundles with greater ease and speed in thinner leaves due to the shorter path to be followed, as indicated by the high negative correlation between leaf thickness and herbicide control in the present study (Table 5).

The mixture of carfentrazone-ethyl and glyphosate was able to sufficiently control *M. atropurpureum* plants grown in full sunlight. This combination increases the spectrum of action for species tolerant to glyphosate alone (Werlang and Silva, 2002; Sharma and Singh, 2007). Glyphosate is the most widely used and most studied herbicide worldwide, partly because of its low cost, wide spectrum of action and large areas under glyphosate-resistant crops. In areas where glyphosate is continuously applied, plants have been reported to evolve resistance to glyphosate over time (Neve et al., 2003; Vargas et al., 2007; Duke and Powles, 2008; Powles, 2008; Beckie, 2011; Norsworthy et al., 2011; Green, 2012; Shaner et al., 2012). The use of two or more herbicides with different modes of action is useful in resistance management and can be used to improve the spectrum of controlled weed species (Walsh et al., 2014; Kumar and Jha, 2015; McCullough et al., 2015; Walsh et al., 2015) and circumvent the development of tolerant or resistant biotypes (Hanson et al., 2013).

Both the visible herbicide damage and images of plants at the end of the experiment (Fig. 2), associated with the reduction in the amount of dry matter caused by the chemical control, substantiate the greater efficacy of chemical control against *M. atropurpureum* grown under reduced light conditions. The strong correlation between the variables evaluated and herbicide efficacy demonstrates that anatomical plasticity, resulting from restricted light conditions, explains, at least in part, the lower tolerance of *M. atropurpureum* to the herbicides tested. However, despite this strong evidence, studies on the absorption and translocation of herbicide molecules in *M. atropurpureum* grown in environments with different light levels will further elucidate the mechanisms involved in plant tolerance to glyphosate and carfentrazone-ethyl.

5. Conclusions

Sufficient chemical control of *M. atropurpureum* with glyphosate was only achieved in plants grown under restricted light conditions, and sufficient control of *M. atropurpureum* grown in full sunlight was only achieved using a mixture of glyphosate and carfentrazone-ethyl. The effects of reduced light conditions on the leaf morphology and anatomy of *M. atropurpureum* are closely related to the species' reduced herbicide tolerance when grown under such conditions.

The mixture of glyphosate and carfentrazone-ethyl is a viable alternative for controlling *M. atropurpureum* in crops with high light availability for weed growth. In shaded environments glyphosate is recommended for the management of *M. atropurpureum*.

Declaration Competing of Interest

None.

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