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RESEARCH PAPER

Can light intensity influence the tolerance of *Synedrellopsis grisebachii* to glyphosate?

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Plant susceptibility to herbicides is related to several factors, including the environmental conditions under which the plants develop. Two experiments were carried out using *Synedrellopsis grisebachii* plants in two different developmental stages (vegetative and reproductive), with the goal of studying plant susceptibility to the herbicide, glyphosate, and the dependence of this susceptibility on light intensity (full sunlight and 70% shading), correlated with leaf anatomy. The experimental design for both experiments was completely randomized, with a 2 × 7 factorial scheme, with two light intensities and seven different doses of glyphosate (0D, 1/4D, 1/2D, D, 2D, 4D and 6D, where D is the recommended dose of 1800 g ae ha^{-1}) as the factors and four replicates per treatment. The leaf anatomy was characterized with optical and scanning electron microscopy. The plants that were grown in full sunlight were more tolerant of glyphosate because of thickening of the adaxial epidermis, parenchyma and main vein structures, which required higher glyphosate doses for effective weed control. The plants that were in the reproductive stage were more tolerant of glyphosate, probably because of lower glyphosate absorption and translocation to the reproductive organs.

Keywords: chemical control, developmental stage, leaf anatomy, light intensity.

Glyphosate (N-[phosphonomethyl] glycine) is the most commonly used herbicide worldwide due to its wide range of action, low cost, low toxicity and rapid degradation in the environment. Glyphosate is the only herbicide that inhibits 5-enolpyruvylshikimate 3-phosphate synthase (EPSP), a key enzyme in the shikimate pathway, which produces the aromatic amino acids, phenylalanine, tyrosine and tryptophan (Duke & Powles 2008; Tzin & Galili 2010). The inhibition of EPSP also interferes with the biosynthesis of secondary compounds (i.e. lignin, phenols and proteins), causing decreased plant growth and leading to plant death (Schonbrunn *et al.* 2001).

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For glyphosate to be effective, it needs to be retained at the leaf surface, absorbed and translocated in lethal amounts to the site of action and then to inhibit the target enzyme (Monquero *et al.* 2004). The leaves are the main absorption pathway of herbicides that are applied following plant emergence. Several leaf functional structures affect herbicide entrance through the epidermis, such as waxes, the stomata, trichomes and the cuticle.

Environmental conditions directly influence leaf morpho-anatomical changes. Light is one of the main factors that are responsible for changes in leaf development, size and thickness (Raven *et al.* 2001). In general, leaves that have developed under high light, or sun leaves, are smaller and thicker than leaves that have developed under low light, or shade leaves (Milaneze-Gutierre *et al.* 2003). Sun leaves also tend to exhibit a greater level of development of the palisade and lacunose parenchyma at the mesophyll than shade leaves because of higher photosynthesis rates under high light-saturation conditions (Chatelet *et al.* 2013).

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The capacity to adapt and acclimate to environmental stresses results from several integrated events, occurring at all levels of organization, from the morphological and anatomical to the cellular, biochemical and molecular (Taiz & Zeiger 2010).

Another factor that affects herbicide absorption and its efficacy is the plant's developmental stage at the moment of application. Leaves under development (young) present a greater capacity for herbicide absorption than fully developed leaves (adult) because of a higher level of thickening of the epidermal cells with age (Passos & Mendonça 2006). Marques *et al.* (2012) studied the anatomy of *Brachiaria decumbens* Stapf. and *Brachiaria plantaginea* (Link) Hitch and observed that adult plants in the beginning of flowering presented higher percentages of sclerenchyma, vascular bundles and parenchyma than plants in the initial developmental stages (four-to-six leaves on the stem), making herbicide absorption and transport in the plant more difficult for adults.

Synedrellopsis grisebachii (Hieron. & Kuntze) is a perennial weed belonging to the family Asteraceae. It is frequently found in the pastures of the Center-West and Center-South regions of Brazil and is also increasingly present in perennial crops (Nakajima *et al.* 2014). This weed also occurs in other countries of South America, such as Bolivia, Paraguay and northern Argentina (Cabrera 1978). *Synedrellopsis grisebachii* exhibits a prostate growth habit, easily dominating forage grasses, especially when grazed (Yamauti *et al.* 2012), and possesses a high degree of plasticity, developing both under full-sunlight and shade conditions. This species is tolerant to glyphosate (Procópio *et al.* 2006; Giancotti *et al.* 2012) and therefore is difficult to control in areas where glyphosate is used for weed management, such as areas of pasture desiccation for no-tillage seeding and perennial crops, especially fruit trees. In fruit trees, such as citrus, *S. grisebachii* seems to grow in both interrow spacing with full sunlight and under canopy trees, with shading.

Given its characteristics, aggressiveness and difficulty to control, the goal of the present study was to correlate the tolerance of *S. grisebachii* to glyphosate and its leaf anatomy under different light conditions and at two developmental stages.

MATERIALS AND METHODS

Experimental setting and design

Two experiments were carried out using *S. grisebachii* plants in two different developmental stages: vegetative (two-to-four nodes on the main branch) and reproductive (full-flowering), according to the classification by Bleiholder *et al.* (1991).

The seeds of *S. grisebachii* were sown in plastic boxes (34 cm × 43 cm), filled with a mix of soil and agricultural substrate (3:1, v/v). When they had two fully expanded leaves, the plants were transferred into 3 L plastic boxes (15 cm × 15 cm) filled with the same substrate, leaving two plants per pot. The chemical analysis of the substrate revealed the following characteristics: pH ($CaCl_2$) = 6.2; organic matter = 15 g dm^{-3}; P = 96 mg dm^{-3}; K = 5.7 $mmol_c$ dm^{-3}; Ca = 54 $mmol_c$ dm^{-3}; Mg = 23 $mmol_c$ dm^{-3}; H and Al = 10 $mmol_c$ dm^{-3}; sum of the bases = 82.7 $mmol_c$ dm^{-3}; cation exchange capacity = 92.7 $mmol_c$ dm^{-3}; and base saturation = 89%.

The experimental design for both experiments was completely randomized, with a 2 × 7 factorial scheme and four replicates per treatment. The factors were two light intensities (full sunlight: 980 μmol m^{-2} s^{-1}; 70% shading: 320 μmol m^{-2} s^{-1}) and seven glyphosate doses (0D, 1/4D, 1/2D, D, 2D, 4D and 6D, where D is the recommended dose of 1800 g ae ha^{-1}). The application was sprayed at 2.8 kgf cm^{-2} constant pressure (CO_2) with a backpack sprayer that was equipped with a spray wand and two XR 110.02 spray nozzles, spaced at 0.5 m, and with a spray volume of 200 L ha^{-1}.

The level of plant damage was evaluated visually at 14, 21, 28 and 35 days after application (DAA) of the herbicide, according to a percentage grading scale, where 0 is "no damage" and 100 is "plant death", according to the Brazilian Society of Weed Science (1995).

Analysis of the leaf anatomy

For both experiments, *S. grisebachii* leaves that were grown under the two tested light intensities were collected on the day of herbicide application and at 7 DAA.

The leaf anatomy analysis was carried out on the middle portion (3 mm × 3 mm) of the leaves that were collected from the third node of the main branch. Four replicates were analyzed, with five leaves per replicate. The leaf samples were fixed in FAA 50 (formaldehyde + acetic acid + 50% ethanol) for ~48 h and stored in 70% ethanol and the permanent slides were prepared according to Johansen (1940). The histological cross-sections were cut by using a rotary microtome that was mounted on slides, using Mayer's albumin as an adhesive (Bissing 1974).

The anatomical structures were observed by using an optical microscope (Bel Photonics, Monza, Italy) at ×10 and ×40 magnification and they were measured with an ocular micrometer. Drawings were made using a camera lucida coupled to the microscope, equipped with an automated photography system. The following leaf anatomical characteristics were quantified: the thickness of the adaxial and abaxial epidermis and parenchyma, width

of the main vein and length and width of the vascular bundles.

Frontal view observations of the leaf adaxial and abaxial epidermis were carried out on leaf samples that were collected on the day of herbicide application. Samples measuring ~3 mm × 3 mm were fixed in 25% glutaraldehyde and 0.1 molar potassium phosphate buffer, with a pH of 7.4, and dehydrated in a graded ethanol series (30, 50, 70, 85 and 95%, then 100% three times, for 20 min each time). Then, the samples were critical-point dried to remove the ethanol, replacing it with liquid CO_2, and then to change the liquid CO_2 to vapor (CPD 050; Balzers, Swiss). Following drying, the samples were placed on aluminum stubs using double-faced carbon tape and were gold-coated (20 nm gold) with a coater (SCD005; Baltec).

The image recording and analysis were carried out with a scanning electron microscope (SEM) (Phillips, Amsterdam, Netherlands). The images were scanned and processed using Adobe Photoshop CS 5.1 software in order to prepare the figures.

Data analysis

The weed-control data, obtained by using the percentage grading scale, were subjected to ANOVAs with the *F*-test. When significant differences were found, the data were fitted to a log-logistic dose–response model (Seefeldt *et al*. 1995) by using Origin Pro 8.0 software (Northampton, England):

$$y = \mathrm{A2} + \left([\mathrm{A1} - \mathrm{A2}]/[1 + (x/x_o)]^p\right),$$

where y is the level of weed control (%), A2 is the lower limit of the curve (average response at higher herbicide doses), A1 is the upper limit of the curve (average response of the control plants), x_o is the glyphosate dose required to cause damage in 50% of the plants (EC_{50}) and p is the curve slope around x_o.

The quantitative anatomical data, obtained through an analysis of the cross-sections, were subjected to ANOVAs with the *F*-test, followed by Tukey's post-hoc test, at $P < 0.05$, by using Sisvar software (Lavras, Brazil).

A multivariate analysis of the mean quantitative anatomical values was carried out, including a principal component analysis (PCA) (Sneath & Sokal 1973), using STATISTICA 7.0 software (StatSoft, Inc., Tulsa, OK, USA).

The PCA was used to test the discriminatory power of the traits that were originally used for clustering, reducing the set of traits into two new uncorrelated variables, called "principal components" (Y1 and Y2). The result of this analysis is presented as a scatter plot.

RESULTS

Dose–response curves

The percentage of weed control of *S. grisebachii* increased with increasing glyphosate doses in both of the experiments. However, different responses were observed for the plants that were grown under the different light intensities (Figs 1 and 2). Higher glyphosate doses were needed to control the plants that were grown under full sunlight, compared to the plants under shading.

For the plants in the vegetative stage and treated with 1800 g ae ha^{-1} of glyphosate, weed control was 79% for the plants that were grown under shading and 53% for the plants that were grown under full sunlight at 14 DAA (Fig. 1a), 91% under shading and 73% under full sunlight at 21 DAA (Fig. 1b) and 94% under shading and 79% under full sunlight at 28 DAA (Fig. 1c). Weed control that was >90% was observed only for the last evaluation (35 DAA) for the plants that were grown under both light conditions (Fig. 1d). Also at 35 DAA, the EC_{50}-value under full sunlight was 1277.10 g ae ha^{-1}, whereas under shading, the required dose was 454.05 g ae ha^{-1} (Table 1). The plants at the vegetative stage that were grown under full sunlight therefore exhibited a 2.81 tolerance factor; that is, their control required a glyphosate dose of 2.81-fold higher than for the plants that were grown under shading.

The same weed-control pattern was observed for the plants at the reproductive stage. However, the doses that were necessary for efficient weed control of the reproductive-stage plants were higher than for the plants at the vegetative stage for both of the tested light conditions. For the plants that received the glyphosate dose that is recommended by the manufacturer (1800 g ae ha^{-1}), at 14 DAA the weed control was 48% under shading and 37% under full sunlight (Fig. 2a). For the remaining evaluations (21, 28 and 35 DAA), the level of weed control was >80% under shading and <60% under full sunlight (Fig. 2b,c,d). Weed control that was >90% was observed only with 3600 g ae ha^{-1} in both light conditions. The EC_{50}-value at 35 DAA was also higher than observed in the same period for the plants in the vegetative stage. The EC_{50}-value at 35 DAA was 778.39 and 1720.71 g ae ha^{-1} under shading and full sunlight, respectively (Table 2), ~41 and ~25% higher than observed for the plants at the vegetative stage. The *S. grisebachii* plants at the reproductive stage that were grown under full sunlight therefore exhibited a 2.21 tolerance factor; that is, their control required a glyphosate dose that was 2.21-fold higher than for the plants that were grown under shading.

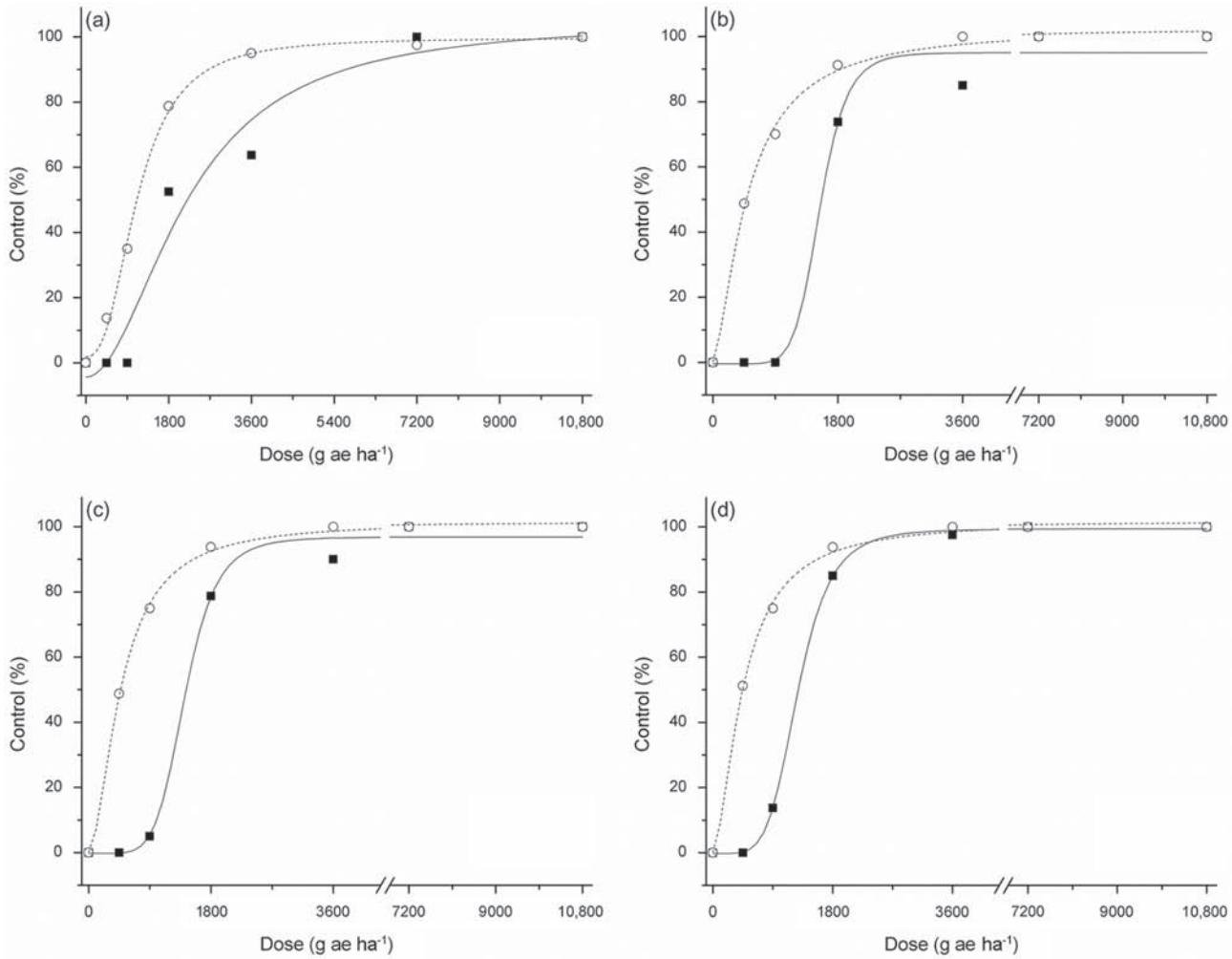

Fig. 1. Percentage control of *Synedrellopsis grisebachii* in the vegetative stage, grown under two light conditions – full sunlight and shading – at (a) 14, (b) 21, (c) 28 and (d) 35 days after glyphosate application in Jaboticabal, Sao Paulo, Brazil, during 2014. (—), Sun; (---), shade.

Characterization of the leaf anatomy before the herbicide application

Leaf blade

The epidermis in frontal view (SEM) featured polygonal cells with curved walls, with anisocytic stomata, that were distributed randomly at the adaxial and abaxial leaf surfaces (amphistomatic) (Figs 3 and 4). Tector trichomes, unbranched, uniseriate and with different sizes, occurred randomly throughout the leaf blade, on both leaf surfaces, and were covered with wax crystals. In the plants that were grown under full sunlight, there was the formation of small quantities of epicuticular wax at the adaxial leaf surface, which was more evident in the plants at the reproductive stage (Figs 3 and 4).

The epidermal cells in cross-section (light microscopy) featured rectangular and flat shapes, formed by a single cell layer (uniseriate) on both surfaces of the leaf blade, and covered by a thin cuticle (Figs 5A,C and 6A,C).

The mesophyll featured dorsiventral organization, being different for the plants that were grown under full sunlight and for those under shading. For the plants that were grown under full sunlight, the mesophyll was formed by two layers of discretely differentiated palisade parenchyma and two or three layers of lacunose parenchyma. Under shading, the plants featured only one layer of palisade parenchyma and two or three layers of lacunose parenchyma. In the plants that were grown under full sunlight (vegetative and reproductive stages),

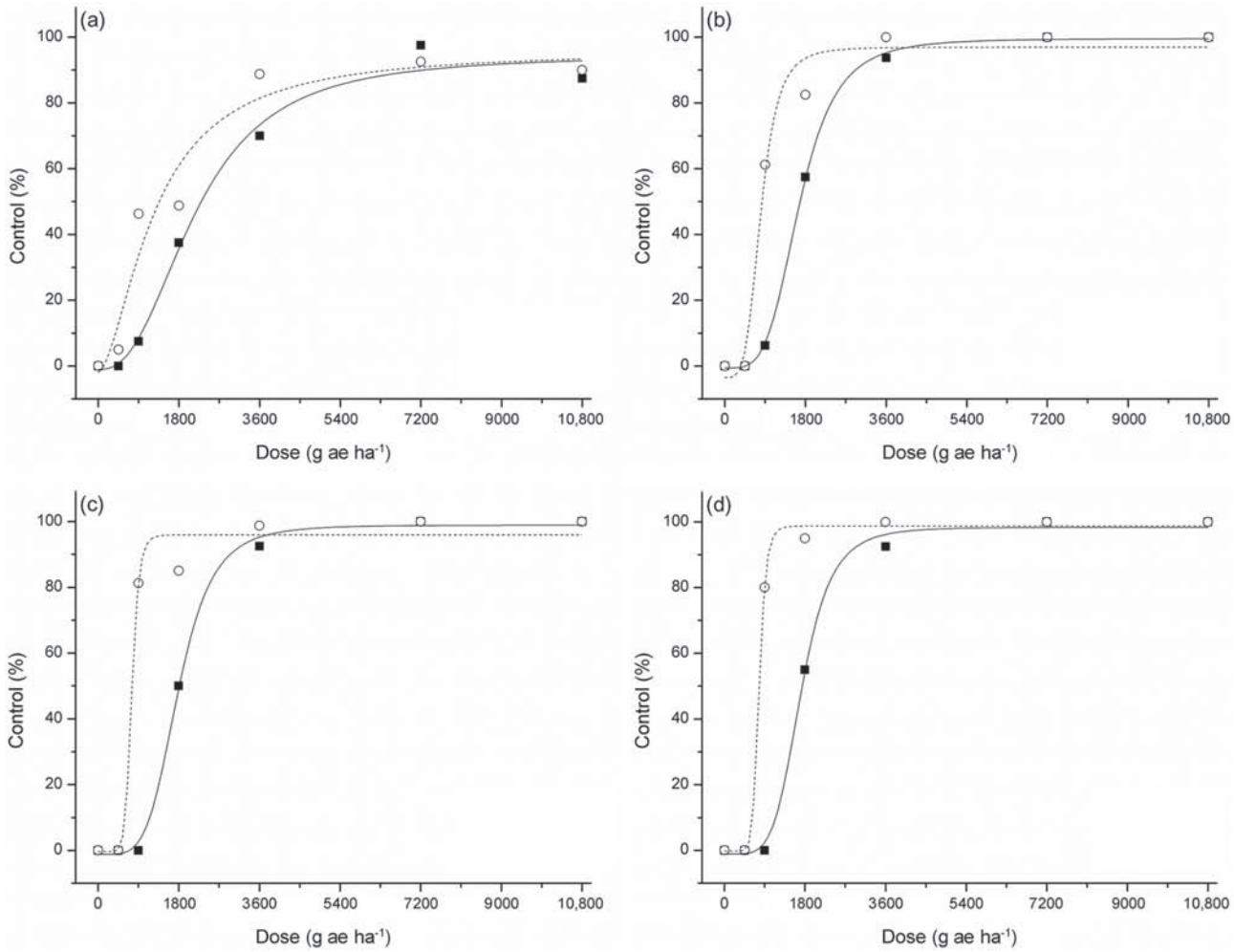

Fig. 2. Percentage control of *Synedrellopsis grisebachii* in the reproductive stage, grown under two light conditions – full sunlight and shading – at at (a) 14, (b) 21, (c) 28 and (d) 35 days after glyphosate application in Jaboticabal, Sao Paulo, Brazil, during 2014. (—), Sun; (---), shade.

the palisade and lacunose parenchymal cells were tightly arranged (Figs 5A and 6A), whereas in the plants that were grown under shading, these cells were loosely arranged, showing intracellular spaces (Figs 5C and 6C). The vascular bundles at the mesophyll were surrounded by endodermal cells.

Main vein

The main vein, at the middle third of the leaf blade, in cross-section featured a biconvex shape, with a more prominent abaxial arc, under both of the tested light conditions. The epidermis of both surfaces featured a rectangular-to-elliptical shape, also covered by a thin cuticular layer. The angular collenchyma was subjacent to the epidermis, consisting of approximately three cell layers on the adaxial surface and one on the abaxial surface. The secretion ducts were distributed throughout the fundamental parenchyma and the vascular system was composed of a single collateral bundle (Figs 5B,D and 6B,D).

As a result of the anatomic similarity between the plants that were grown under full sunlight and those that were grown under shading, quantitative analyses of the evaluated anatomical parameters were carried out.

The ANOVA revealed significant differences in the adaxial epidermal thickness, parenchymal thickness, main vein width and length and vascular bundle width between the full-sunlight and shading treatments (Table 3).

The mean thickness of the adaxial epidermis, parenchyma and main vein was significantly higher, and the

Table 1. Parameters of the log-logistic dose–response model for the percentage weed control after glyphosate application on *Synedrellopsis grisebachii* plants at the vegetative stage in Jaboticabal, Sao Paulo, Brazil, in 2014

Parameter	Weed control (%)			
	14 DAA	21 DAA	28 DAA	35 DAA
Full sunlight				
A1	−4.39	−0.42	−0.21	−0.24
A2	104.85	95.07	96.85	99.35
x_0	2215.93	1541.67	1419.60	1277.10
p	1.98	7.99	6.12	5.17
R^2-value	0.925⋆	0.979⋆⋆	0.991⋆⋆	0.999⋆⋆
Shading				
A1	1.84	0.16	0.08	0.09
A2	99.69	102.59	101.45	101.71
x_0	1122.30	497.59	476.95	454.05
p	2.55	1.50	1.76	1.65
R^2-value	0.995⋆⋆	0.995⋆⋆	0.998⋆⋆	0.997⋆⋆
Tolerance factor	1.97	3.09	2.98	2.81

A1, the upper limit of the curve (average response of control plants); A2, the lower limit of the curve (average response at higher herbicide doses); DAA, days after application of the herbicide; p, the curve slope around x_0; x_0, the glyphosate dose required to cause damage in 50% of the plants.
⋆ Significant at a <0.05; ⋆⋆ Significant correlation at a <0.01.

Table 2. Parameters of the log-logistic dose–response model for the percentage of weed control after glyphosate application on *Synedrellopsis grisebachii* plants at the reproductive stage in Jaboticabal, Sao Paulo, Brazil, in 2014

Parameter	Weed control (%)			
	14 DAA	21 DAA	28 DAA	35 DAA
Full sunlight				
A1	−1.05	−0.56	−1.19	−1.09
A2	94.54	99.55	98.92	98.38
x_0	2174.6	1669.88	1794.09	1720.71
p	2.49	4.04	4.68	5.13
R^2-value	0.981⋆⋆	0.999⋆⋆	0.997⋆⋆	0.996⋆⋆
Shading				
A1	−1.99	−3.49	−0.36	−0.2
A2	96.06	96.96	95.95	98.76
x_0	1263.39	804.47	752.05	778.39
p	1.66	4.18	9.53	10.01
R^2-value	0.904⋆⋆	0.968⋆⋆	0.974⋆⋆	0.997⋆⋆
Tolerance factor	1.72	2.08	2.39	2.21

A1, the upper limit of the curve (average response of control plants); A2, the lower limit of the curve (average response at higher herbicide doses); DAA, days after application of the herbicide; p, the curve slope around x_0; x_0, the glyphosate dose required to cause damage in 50% of the plants.
⋆ Significant at a <0.05; ⋆⋆ Significant correlation at a <0.01.

vascular bundle length and width were significantly lower, under full sunlight. The plants that were grown under full sunlight exhibited a parenchyma that was ~30% thicker than that of the plants grown under shading, in both the vegetative and reproductive stages, suggesting a possible dilution of glyphosate at the leaf mesophyll for *S. grisebachii* plants under full sunlight.

The PCA of the leaf anatomical traits that were analyzed showed differences between the full-sunlight and shading treatments (Fig. 7). The PCA resulted in data

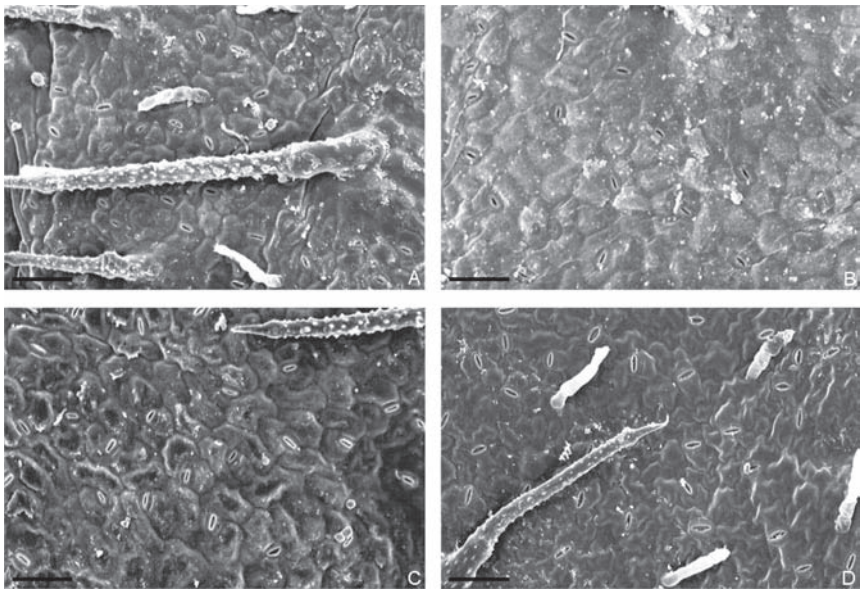

Fig. 3. Leaf surface of *Synedrellopsis grisebachii* in the vegetative stage: (A) abaxial and (B) adaxial leaf surface under full sunlight; (C) abaxial and (D) adaxial leaf surface under shading. Bar: 50 μm.

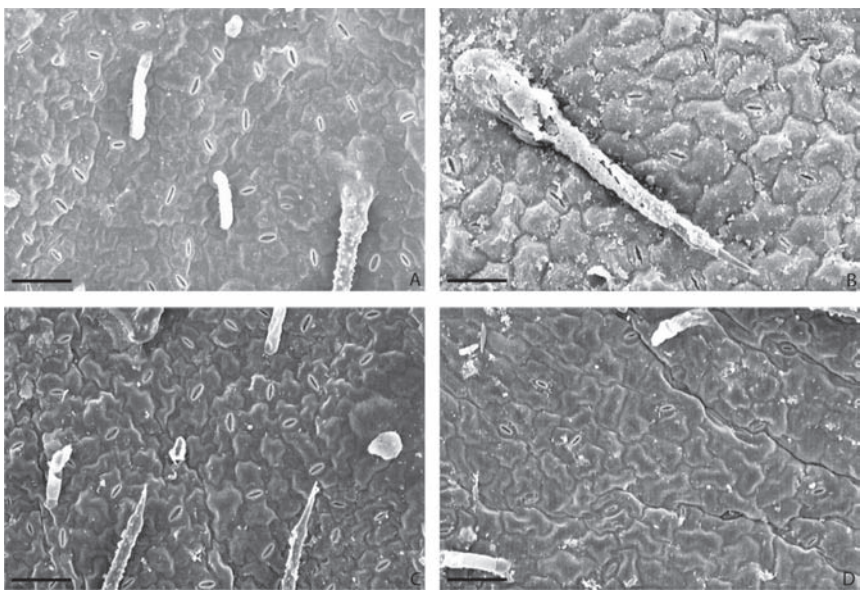

Fig. 4. Leaf surface of *Synedrellopsis grisebachii* in the reproductive stage: (A) abaxial and (B) adaxial leaf surface of a plant grown under full sunlight; (C) abaxial and (D) adaxial leaf surface of a plant grown under shading. Bar: 50 μm.

distribution on a bidimensional plane that was defined by two principal components, PC1 and PC2 (Fig. 7), which accounted for 75.48% of the observed variation: 55.91% for PC1 and 19.57% for PC2. This finding is in accordance with Sneath and Sokal (1973), according to whom the number of PCs used should explain a minimum 70% of the total variance that is observed.

The most important variables for PC1 were the main vein thickness ($r = 0.89$), adaxial epidermal thickness ($r = 0.63$) and parenchymal thickness ($r = 0.83$), which were positively correlated; whereas, the vascular bundle length ($r = -0.82$) and width ($r = -0.87$) were negatively correlated. The abaxial epidermis ($r = -0.87$) exhibited different behavior from the remaining analyzed variables, being isolated in PC2. PC1 indicated a wide distribution of anatomical traits of the *S. grisebachii* leaves, whereas PC2 presented a narrower distribution of the same set of characteristics.

The PCA analysis revealed a high degree of dissimilarity between the plants that were grown in full sunlight and those that were grown in shading, for both of the developmental stages. This difference was because the sun plants presented a greater parenchymal and main vein thickness and lower vascular bundle length and

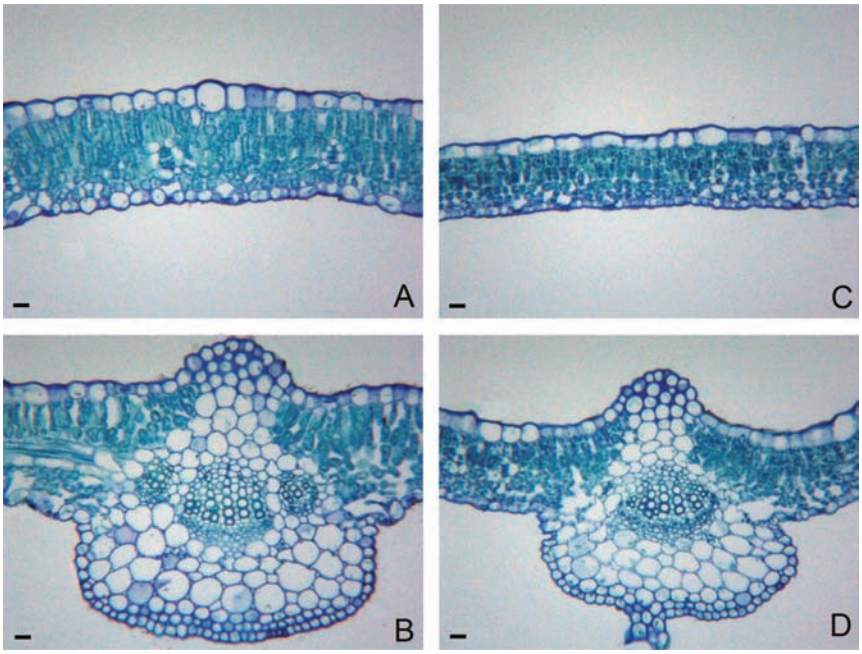

Fig. 5. Cross-section of a leaf limb of *Synedrellopsis grisebachii* in the vegetative stage: (A) leaf mesophyll and (B) main vein of a plant grown under full sunlight; (C) leaf mesophyll and (D) main vein of a plant grown under shading. Bar: 20 μm.

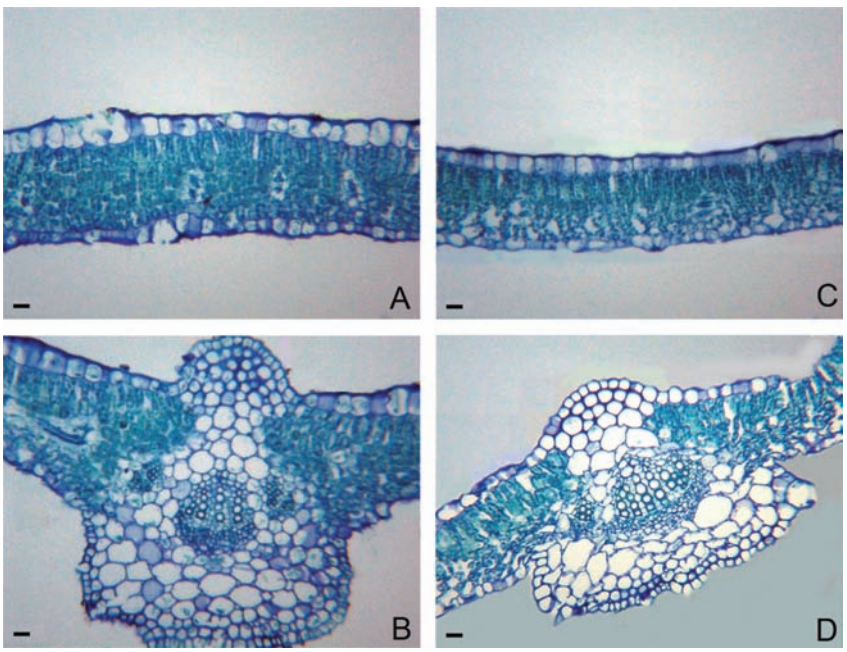

Fig. 6. Cross-section of a leaf limb of *Synedrellopsis grisebachii* in the reproductive stage: (A) leaf mesophyll and (B) main vein of a plant grown under full sunlight; (C) leaf mesophyll and (D) main vein of a plant grown under shading. Bar: 20 μm.

width. These variables also were different for the two developmental stages, being higher for the plants at the reproductive stage than for the plants at the vegetative stage, resulting in the formation of four different groups. This discrimination between sun and shade leaves and different developmental stages is presented in Table 3.

Characterization of the leaf anatomy after the herbicide application

Differences in the leaf anatomy between the plants that were treated with herbicide and the control plants were observed at 7 DAA. For both of the glyphosate doses tested, 1800 and 3600 g ae ha^{-1}, the full-sun plants

Table 3. Means and standard deviations of the anatomical traits of *Synedrellopsis grisebachii* plants that were grown under two light conditions and at two developmental stages in Jaboticabal, Sao Paulo, Brazil, in 2014

Developmental stage	ADET	ABET	PT	MVT	VBL	VBW
Vegetative						
Sun	26.0 ± 0.41a	14.4 ± 0.25a	102.1 ± 0.49a	399.9 ± 0.55a	66.1 ± 0.50b	59.9 ± 0.24b
Shading	21.9 ± 0.33b	16.2 ± 0.24a	73.0 ± 0.68b	242.5 ± 0.77b	107.2 ± 0.17a	119.1 ± 0.30a
F_{cal}	5.976*	2.196^{NS}	247.040**	2142.79**	545.45**	1978.61**
CV (%)	15.69	17.65	4.72	2.37	4.55	3.32
Reproductive						
Sun	24.2 ± 0.22a	14.7 ± 0.12a	113.6 ± 0.57a	400.9 ± 0.35a	102.7 ± 2.43b	102.7 ± 0.11b
Shading	22.6 ± 0.16b	14.0 ± 0.13a	81.1 ± 0.18b	309.9 ± 1.04b	113.4 ± 0.50a	142.8 ± 0.50a
F_{cal}	5.546*	1.042^{NS}	311.292**	97.09**	22.25**	171.33**
CV (%)	6.46	9.78	4.23	5.81	4.71	5.59

ABET, abaxial epidermal thickness (μm); ADET, adaxial epidermal thickness (μm); CV, coefficient of variation; MVT, main vein thickness (μm); NS, not significant; PT, parenchymal thickness (μm); VBL, vascular bundle length (μm); VBW, vascular bundle width (μm). * Significant at a $p < 0.05$; ** Significant correlation at a $p < 0.01$. Mean values with the same letter in the column are not significantly different at $p < 0.05$.

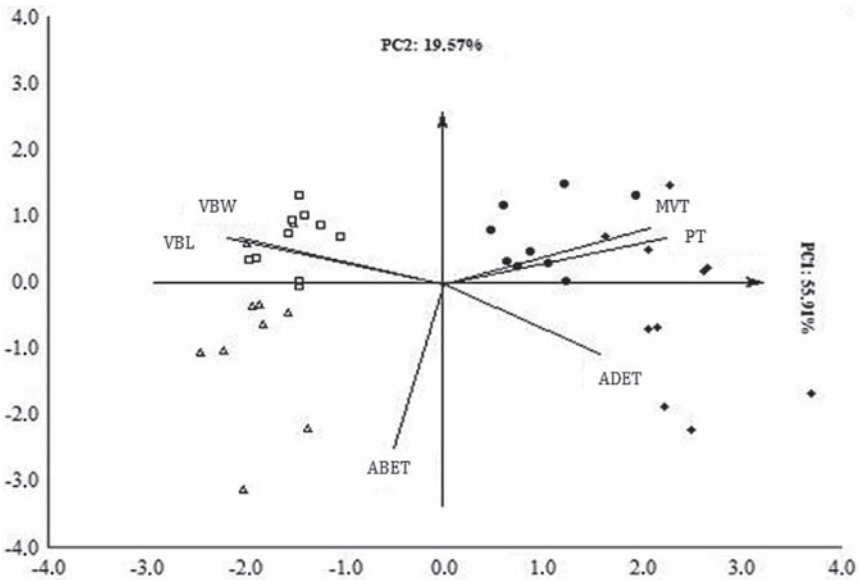

Fig. 7. Principal component analysis (two principal components: PC1 and PC2) of the following variables: abaxial epidermis (ABET), adaxial epidermis (ADET), main vein thickness (MVT), parenchymal thickness (PT), vascular bundle length (VBL) and vascular bundle width (VBW). The plants were in the vegetative (I) or reproductive (F) stage and were grown under shading (Shade) or full sunlight (Sun). (◆), Sun-I; (Δ), Shade-I; (●), Sun-F; (□), Shade-F.

exhibited less damage to the leaf mesophyll tissues than the shade plants, for both of the developmental stages (Figs 8 and 9).

The glyphosate application caused plasmolysis of the parenchymal cells, resulting in tissue rupture and necrosis, increasing the intercellular spaces. The plants that were grown under full sunlight maintained a higher degree of integrity of their leaf tissues, whereas the cells of the plants that were grown under shading were completely damaged by the herbicide.

DISCUSSION

In the present study, the tolerance of *S. grisebachii* to glyphosate was observed to depend on the light conditions under which the plant is grown. The *S. grisebachii* plants that were grown under high light presented a higher level of tolerance to glyphosate than did the plants that were grown under low light. This tolerance also depended on the plants' developmental stage at the moment of glyphosate application. The plants in the

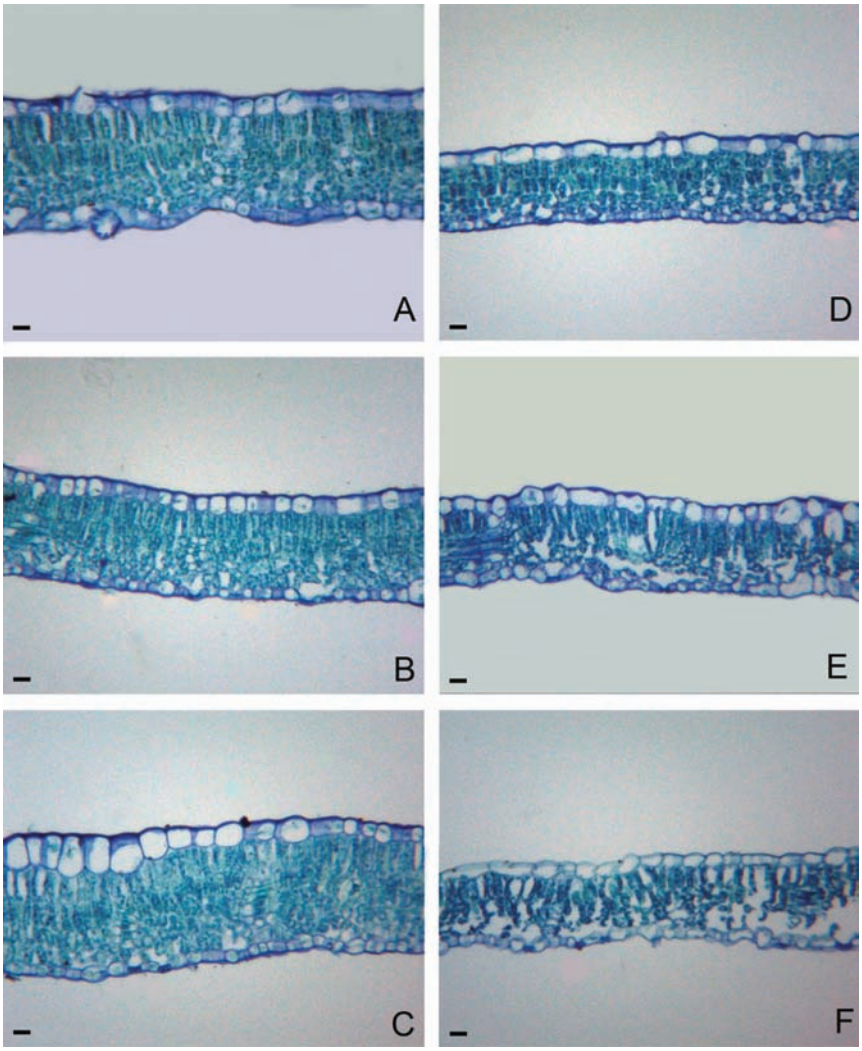

Fig. 8. Cross-section of a leaf limb of *Synedrellopsis grisebachii* in the vegetative stage at 7 days after glyphosate application. The plants were grown under full sunlight (A, B and C) or shading (D, E and F) and given 0, 1800 and 3600 g ae ha^{-1} of glyphosate, respectively. Bar: 20 μm.

reproductive stage were less sensitive to glyphosate. Young plants present leaves with less epicuticular wax and thinner cuticles, which facilitates the absorption of herbicides (King & Radosevich 1979).

Herbicide translocation into reproductive plant organs also could have contributed to the lower level of weed control that was observed for the plants at the reproductive stage. At this stage, there is a higher water and nutrient demand for the reproductive organs, which function as sinks. The herbicide might follow the photoassimilate flow to the seeds (Chachalis *et al.* 2001; Hennigh *et al.* 2005) and be translocated into the fruits, resulting in lower quantities of herbicide being allocated to its sites of action (Schuster *et al.* 2007).

The plant anatomical adaptations to full-sunlight conditions are one of the main factors that are responsible for the observed differences in susceptibility between the sun and the shade plants. Plants under high light intensity tend to decrease their leaf area and increase their cuticle thickness as a strategy for decreasing water loss. The cuticle plays a critical role in the decrease in water loss, decrease in temperature and reflection of solar radiation by leaves (Fermino *et al.* 2004). The cuticle also functions as a barrier to the entrance of microorganisms and chemicals (Procópio *et al.* 2003), interfering with the absorption of herbicides that are applied following plant emergence.

Hydrophilic herbicides, such as glyphosate, present a higher level of difficulty in entering the cuticle as a result of the cuticle's lipophilic nature. The absorption of these herbicides can occur through the pores (Procópio *et al.* 2003) and/or pectin filaments that are present at the cuticular layer, which, when well hydrated, could favor the transport of herbicides into the leaf. This process explains the higher level of weed control that was observed for the plants that were grown under shading.

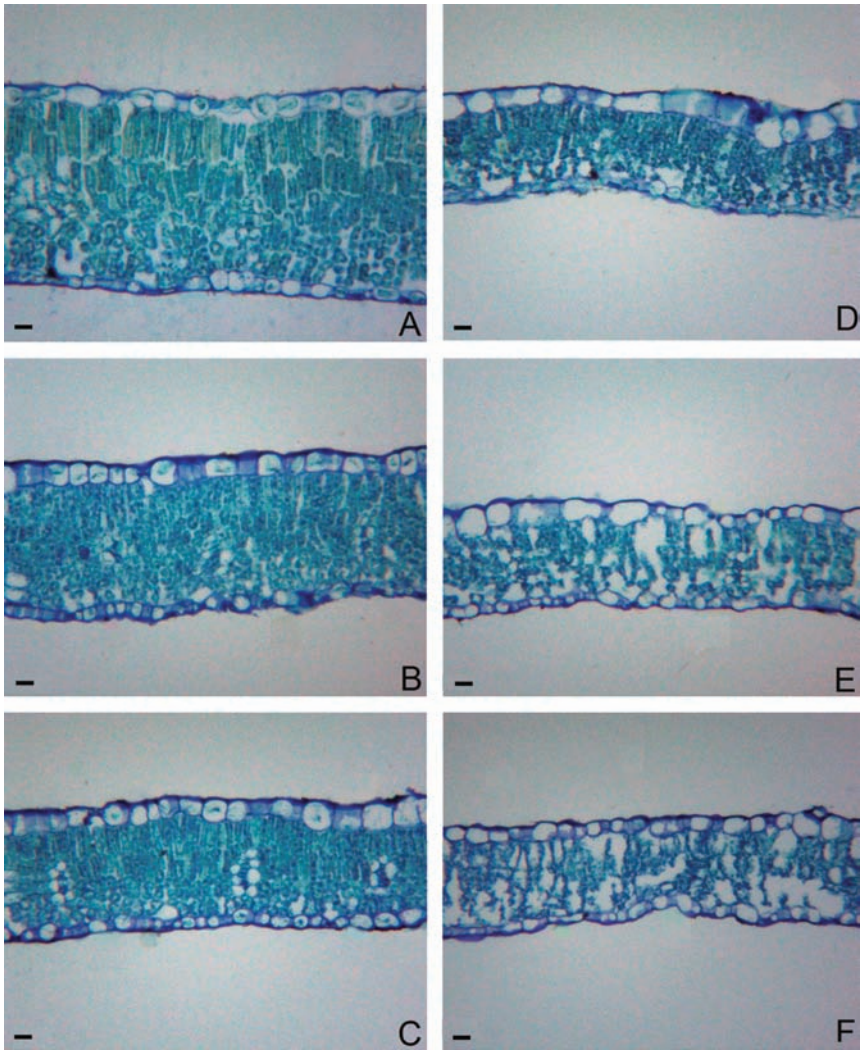

Fig. 9. Cross-section of a leaf limb of *Synedrellopsis grisebachii* in the reproductive stage at 7 days after glyphosate application. The plants were grown under full sunlight (A, B and C) or shading (D, E and F) and given 0, 1800 and 3600 g ae ha^{-1} of glyphosate, respectively. Bar: 20 μm.

The lower temperature and higher relative air humidity under shading favored the absorption of glyphosate by the plants through the hydrated cuticle.

Differences in the leaf limb thickness are also common between sun and shade plants. In general, leaf limb thickness is proportional to the light intensity and seems to be associated with an increased thickness of the palisade (Cao 2000; Mendes *et al.* 2001; Pimenta-Barrios & Ramírez-Hernández 2003; Dickson 2006) and/or lacunose parenchyma (Dickson 2006). An increased level of thickness of the palisade parenchyma under high light has been reported previously (Espindola *et al.* 2009; Pollastrini *et al.* 2011; Xiao-Xue *et al.* 2013), which is in accordance with the present results. This variation in internal leaf structure is related to light capture because the increase in palisade parenchyma and the columnar arrangement of the parenchymal cells allow light to be transmitted more directly, therefore avoiding photoinhibition (Taiz & Zeiger 2010).

This quantitative difference in the parenchymal tissues also could have contributed to a lower level of weed control under the condition of full sunlight. After passing the leaf cuticle and epidermis, the herbicide needs to be absorbed into the cytoplasm in order to then be translocated to its site of action (Satichivi *et al.* 2000). Once glyphosate is within the intercellular spaces, the glyphosate molecules need to cross the internal cuticle coating the cell walls, reaching the apoplast, and then be absorbed through the plasma membrane and into the symplast (Velini *et al.* 2009). Therefore, with a greater number of cells because of an increased parenchymal thickness in the sun leaves, there are more barriers to the absorption of the herbicide and the herbicide might be more easily lost. Thus, lower quantities of herbicide might have been absorbed under full sunlight, explaining the lower level of weed control that was observed, compared to the plants that were grown under shading, when smaller doses of herbicide were applied.

The 30% increase in parenchymal tissues under full sunlight contributed to the herbicide dilution at the leaf mesophyll, resulting in a decreased allocation of herbicide to the cells. Glyphosate should be absorbed by all photosynthesizing tissue (parenchymal) cells containing EPSP. This factor, together with the lower herbicide absorption, contributed to the lower level of weed control that was observed under the high-light condition.

The level of weed control that was observed with the glyphosate doses that were >3600 g ae ha^{-1}, for both developmental stages, was related to the higher glyphosate concentrations because increased external solute concentrations result in increased absorption rates (Velini *et al.* 2009).

The full-sunlight treatments were positively correlated with the thickness of the parenchyma, main vein and adaxial epidermis and negatively correlated with the vascular bundle length and width. The first three characteristics affect the herbicide absorption by the plant, whereas the last two affect the herbicide's translocation.

In conclusion, the observed results showed that the *S. grisebachii* plants that grew under high light intensity were more tolerant of glyphosate than the plants that grew under low light intensity. Thus, in field conditions, the dose that is required to control these plants under shade needs to be lower than under the sun condition.

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