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Influence of shading on the leaf morphoanatomy and tolerance to glyphosate in *Commelina benghalensis* L. and *Cyperus rotundus* L.

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Abstract

The efficiency of the herbicide glyphosate in weed control is associated with leaf morphology that is a character influenced by environmental factors such as light. This study evaluated the leaf morpho-anatomy and control of *Commelina benghalensis* and *Cyperus rotundus* cultivated under shading and treated with glyphosate. The study was conducted under area without vegetation and weeds were grown in pots. One assay was conducted per species with a 3×3 factorial design, with factor 1 represented by the doses of 0, 720 and 1440 g ha^{-1} of glyphosate and factor 2 by three shading levels: 0, 50 or 70% of shade. The assays were conducted in randomized blocks with five replicates, with each replicate consisting of one pot with two plants of *C. benghalensis* or *C. rotundus*. Both species showed a 50% reduction in tolerance to glyphosate when they were grown under shade compared to plants grown in the sun. Plants of *C. benghalensis* and *C. rotundus* showed a significant increase in leaf area (161 and 46 %, respectively) when cultivated in the shade. However, shading caused reduction on leaf thickness in both species. In *C. benghalensis*, exposure to glyphosate promoted turgor loss in the trichomes; plants cultivated in 70% shading showed trichome cuticle ruptures. The changes on leaf morphoanatomy are related to the increased efficiency of glyphosate in controlling these two species when they are cultivated in shaded environments. Our results suggest that reduced doses of glyphosate (50%) are required for controlling weed in shaded environments.

Keywords: shaded environments, micromorphometric analyses, herbicide, weed, bengaldayflower, nutsedge.
Abreviations: ANOVA_analysis of variance; BT_blade thickness; Ct_Cuticle rupture; DAA_days after application; HAB_abaxial epidermis thickness; HAD_adaxial epidermis thickness; MT_mesophyll thickness; PP_palisade parenchyma; SEM_scanning electron microscope; SP_spongy parenchyma; Tt_Tector trichomes.

Introduction

The morphophysiology, growth and development of weeds are directly influenced by the environment and characteristics related to these processes reveal relevant information regarding weed management in different agroecosystems (Lima JR. et al., 2006; Gondim et al., 2008; Santos Júnior et al., 2013). Among weed control practices, the use of herbicides is the most prominent because of the volume of product used, treatment area, and risk of negative impacts on the environment and non-target organisms. Glyphosate is one of the most widely used herbicides in agriculture worldwide because of its broad spectrum of action, low cost, and lesser impact to the environment compared to other products (Malik et al., 1989). However, other studies indicate that it can have adverse effects on the ecosystem if used indiscriminately how contamination risks to non-target organisms are also reported in the literature, such as damage to crops as a result of drift (Koger et al., 2005; Londo et al., 2011; Tuffi-Santos et al., 2011), adverse effects on soil microbiota (Malty et al., 2006; Lane et al., 2012), selection of resistant plants, and damage to beneficial insects (Buckelew et al., 2000; Jackson and Pitre, 2004; Menezes et al., 2012). In addition, highlighting the risks of contamination of workers and consumers is important (Thongprakaisang et al., 2013). Therefore, studies need to be performed to assess the employment of techniques aimed at reducing the amount of herbicides used. The dose of glyphosate needs to be reduced such that satisfactory levels of weed control are achieved in shaded agroecosystems and the product can be used safely, especially for controlling hard-to-control and widely distributed species in cultivated areas. Bengaldayflower (*Commelina benghalensis* L.), belonging to the Commelinaceae family, and nutsedge (*Cyperus rotundus* L.), belonging to the Cyperaceae family, are weeds commonly found in annual and perennial crops (Adegas et al., 2010; Pragada and Venkaiah, 2012; Nagaraju et al., 2014). These species are considered to be difficult to control; however, when grown in the shade, they show reduced tolerance to glyphosate unlike plants grown in the sun (Santos Júnior et al., 2013). In this context, studies on plant anatomy are of fundamental importance to elucidate the plant characteristics that might contribute to resistance, tolerance, or sensitivity to herbicides (Tuffi-Santos et al., 2009; Lorentz et al., 2011). Increases in the index and density of stomata, reduction in leaf thickness (Lima Jr. et al., 2006; Gondim et al., 2008; Taiz and Zeiger, 2009), and increase in leaf area are the changes that result from low light intensity (Pires et al., 2011; Pires et al., 2012), and these changes

might influence the interception, penetration, and absorption of herbicides applied on the leaves. Therefore, these characteristics are relevant parameters for analyzing the efficiency of herbicides such as glyphosate-based products used in post-emergence of weeds. Anatomical changes related to reduced tolerance to glyphosate in *C. benghalensis* and *C. rotundus* grown in shaded environments remain unknown. Thus, this study aimed to evaluate the anatomy, foliar micromorphology, and glyphosate weed control of *C. benghalensis* and *C. rotundus* cultivated under different conditions of shading.

Results

Control of Cyperus rotundus and Commelina benghalensis

C. rotundus plants cultivated in shaded environments were less tolerant to glyphosate than those grown in the sun (Tables 1 and 3). This behavior was observed at 10 and 20 DAA (Table 1). And this species, when cultivated in the shade, was completely controlled by the herbicide regardless of the dose applied, with control scores of over 95% at 30 DAA of glyphosate. However, when grown in the sun, this weed could not be controlled was considered sufficiently even with the application of 1440 g ha^{-1} of glyphosate.
Similarly, in *C. benghalensis* the control by glyphosate was more efficient in shaded environments (Table 2). At 30 DAA of glyphosate, the rates of control of *C. benghalensis* plants cultivated in 70% shading were significantly higher those for plants maintained under the sun and 50% shading (Table 3). The herbicide control reached values greater than 80% only in shaded environments (Table 3).

Leaf blade area, and anatomical and micromorphological analyses

C. benghalensis plants cultivated under 50 and 70% shading had 131.86 and 161.36% greater leaf blade area, respectively, than those of plants grown in the sun (Fig. 1). The leaf blade areas of *C. rotundus* cultivated under 50 and 70% shading were of 21.45 and 46.05% higher, respectively, than those of plants cultivated under the sun (Fig. 1). *C. benghalensis* leaves are amphistomatic and have dorsiventral mesophyll with palisade and spongy parenchyma toward the adaxial and abaxial sides, respectively (Figs. 3A and C). *C. rotundus* leaves are hypostomatic with homogenous mesophyll (Figs. 3B and D). The tissues of the leaves of *C. benghalensis* plants exposed to shading were adversely affected. A similar effect was observed when the plants were exposed to glyphosate (Fig. 3). However, the leaf blade thickness was influenced by the interaction between the cultivation environment and the glyphosate dose; the highest values were observed in individuals cultivated under the sun regardless of the herbicide dose applied (Table 4). Plants maintained under 50 and 70% shading, untreated or treated with 720 gha^{-1} glyphosate, did not show a difference in leaf thickness. However, plants exposed to the highest herbicide dose (1440 gha^{-1}) and cultivated under the highest light restriction showed the lowest leaf thickness (Table 4). *C. rotundus* plants cultivated under 50 and 70% shading had 20.7 and 32.7% higher leaf blade thickness, respectively, than those of plants exposed to the sun (Fig. 4A). However, the application of glyphosate had no significant effect on leaf tissues, except on HAD (Fig. 4B), which is the tissue that is directly exposed to the application of the herbicide. The leaf blade area and control rate at 10, 20, and 30 DAA of glyphosate in *C. rotundus* showed positive correlations with each other (Table 5). However, the correlation was negative for the leaf blade thickness (Table 5). A similar relationship was observed in *C. benghalensis* at 10 DAA of glyphosate alone (Table 5).
In *C. benghalensis*, the micromorphological damages caused by the herbicide were associated with trichomes (Figs. 5B–D) and epidermal cells near the stomata (Figs. 6B–D.). Turgor loss was observed mainly in the cells at the base of trichomes; cuticle rupture was observed in plants cultivated under 70% shading (Figs. 5D and 6D). In *C. rotundus*, no damages were observed in response to glyphosate and levels of shading (Figs. 7). The control of *C. benghalensis* and *C. rotundus* by using glyphosate was more efficient when the plants were cultivated under shading; these results indicate the possibility of a 50% reduction in the dose of glyphosate recommended by the manufacturer (1440 g ha^{-1}). Shading promotes area increase and thickness reduction of the leaf blade of *C. benghalensis* and *C. rotundus*. The trichomes in *C. benghalensis* leaves are the epidermal structures that are the most affected by glyphosate and show the most significant adverse effects observed in plants cultivated in shaded environments. The increase in area and reduction in the thickness of leaf blades associated with the most intense micromorphological damage were associated with the greatest efficiency of glyphosate in controlling *C. benghalensis* and *C. rotundus*.

Discussion

The control of *C. rotundus* treated with glyphosate was more efficient in plants that were cultivated in shaded environments (Tables 1 and 3). At 30 DAA of glyphosate, plants maintained in shaded environments showed no differences in herbicide control rate at 720 and 1440 g ha^{-1} doses (Table 3). According to the manufacturer's information described in the package insert of the product, the recommended dose to control this species is 1440 g ha^{-1} of glyphosate. *C. rotundus* is controlled with a 25% reduction in the recommended dose when cultivated under shading (Santos Júnior et al., 2013). However, in this study, the results showed that the amount of glyphosate could be reduced to 50% (Tables 1 and 3). Controlling the level of incident light in an open field with agricultural or forestry crop is difficult. However, applying a dose of 720 g ha^{-1} glyphosate for plants cultivated under 50% shading is feasible. There were no differences in the control of plants cultivated in 50 and 70% shading (Table 3). The control of *C. benghalensis* was also more efficient in plants cultivated in 70% shading (Tables 2 and 3). At 30 DAA of glyphosate, there was no difference in the control rates in relation to the glyphosate dose tested in plants maintained under the highest level of shading (Table 3). The recommended dose to control *C. benghalensis* is also 1440 g ha^{-1}; in this study, we showed that this value could also be reduced to half. This reduction in glyphosate doses that are required for an efficient control of weeds in shaded environments, results in lower cost and less harm to the environment. The adverse effects of glyphosate are directly linked to the dose used. The lower thickness (Table 4 and Figs. 4 and 5) and higher leaf blade area (Fig. 1), as well as the morphological damages observed using an SEM (Figs. 5 and 7), suggested the sensitivity of these species to the studied herbicide; higher control rates were found in *C. rotundus* (Tables 1and 3) and *C. benghalensis* (Tables 2 and 3) in shaded environments. Leaf thickness was reduced in plant species cultivated under shaded conditions (Morais et al., 2003; Morais et al., 2004; Lima Jr. et al., 2006, Gondim et al., 2008). In general, the leaf blade is thicker and leaf area is smaller in plants cultivated under high luminosity

Table 1. Control of *Cyperus rotundus* cultivated under different levels of shading at 10 and 20 days after application of glyphosate.

10 DAA				
Doses of glyphosate (g ha^{-1})	Cultivation environments			Mean value to Dose
	0% of shade	50% of shade	70% of shade	
720	15 ± 2.92	30 ± 3.39	45 ± 2.92	30 b
1440	30 ± 3.32	45 ± 6.04	55 ± 4.74	45 a
Mean value to Shading	22.5 C	37.5 B	50 A	-
20 DAA				
	0% of shade	50% of shade	70% of shade	Mean value to Dose
720	25 ± 2.74	65 ± 5.70	80 ± 4.06	65 b
1440	40 ± 1.00	95 ± 5.24	95 ± 3.74	95 a
Mean value to Shading	32.5 B	80 A	87.5 A	-

Mean values ± standard error. The uppercase letters in rows testing the mean values to shading at by Tukey's test. And lowercase letters in columns testing mean values to Dose by F's test. Means that do not share a letter are significantly different (P≤ 0.05).

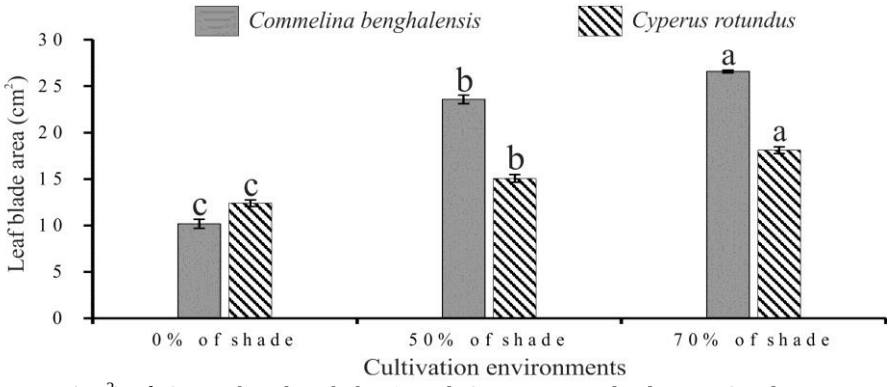

Fig 1. Average blade area (cm^2) of *Commelina benghalensis* and *Cyperus rotundus* leaves. Overlapping averages with different letters in the same species differ by the Tukey's test at 5% probability. Bars showed ± SE.

Table 2. Control of *Commelina benghalensis* cultivated under different levels of shading at 10 and 20 days after application of glyphosate

10 DAA				
Doses of glyphosate (g ha^{-1})	Cultivation environments			Mean value to Dose
	0% of shade	50% of shade	70% of shade	
720	30 ± 4.18	25 ± 6.12	35 ± 4.18	30 b
1440	40 ± 11.40	35 ± 9.08	55 ± 5.70	40 a
Mean value to Shading	35 B	30 B	45 A	-
20 DAA				
	0% of shade	50% of shade	70% of shade	Mean value to Dose
720	65 ± 10.37	55 ± 8.37	65 ± 7.58	65 b
1440	65 ± 10.84	75 ± 16.36	90 ± 5.70	75 a
Mean value to Shading	65 B	65 B	77.5 A	-

Mean values ± standard error. The uppercase letters in rows testing the mean values to shading at by Tukey's test. And lowercase letters in columns testing mean values to Dose by F's test. Means that do not share a letter are significantly different (P≤ 0.05).

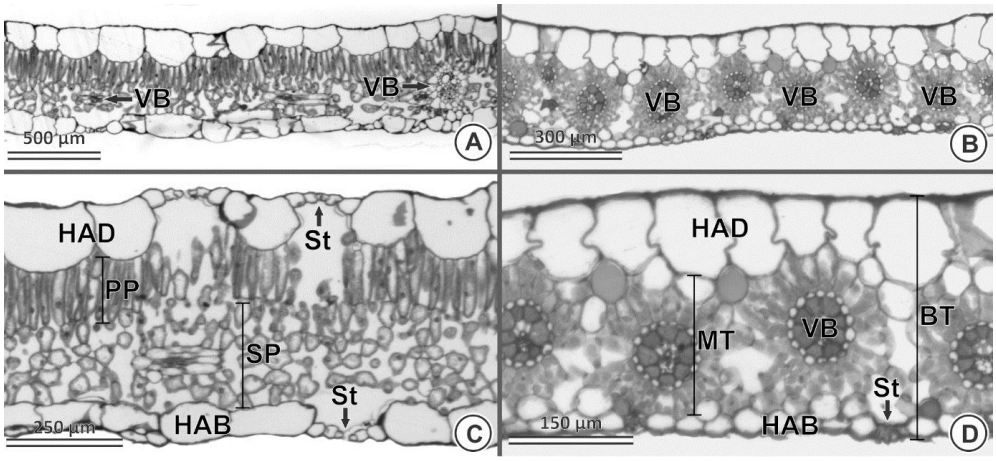

Fig 2. Leaf anatomy of *Commelina benghalensis* (A and C) and *Cyperus rotundus* (B and D). Abbreviations: VB: Vascular bundle, HAD: Adaxial epidermis, PP: Palisade parenchyma, SP: Spongy parenchyma, HAB: Abaxial epidermis, MT: Mesophyll thickness, BT: Leaf blade thickness, and St: Stomata.

Table 3. Control of *Cyperus rotundus* and *Commelina benghalensis* cultivated under different levels of shading at 30 days after application of glyphosate

Cyperus rotundus			
Doses of glyphosate (g ha^{-1})	Cultivation environments		
	0% of shade	50% of shade	70% of shade
720	30 ± 3.74 Bb	95 ± 1.58 Aa	100 ± 0.00 Aa
1440	50 ± 2.00 Ba	100 ± 1.22 Aa	100 ± 0.00 Aa
Commelina benghalensis			
Doses of glyphosate (g ha^{-1})	Cultivation environments		
	0% of shade	50% of shade	70% of shade
720	65 ± 6.52 Ba	60 ± 4.47 Bb	80 ± 4.18 Aa
1440	70 ± 6.52 Ba	80 ± 2.74 Aa	90 ± 7.58 Aa

Mean values ± standard error followed by the same uppercase letters in rows, and lowercase letters in columns differ significantly by Tukey's test (P≤ 0.05).

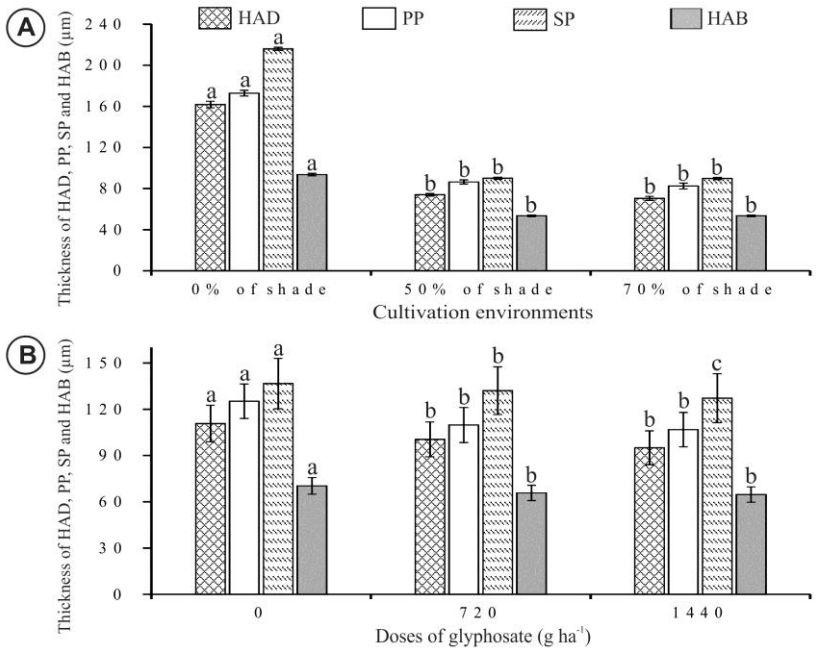

Fig 3. Effects of shading (A) and glyphosate (B) on the thickness of the adaxial (HAD) and abaxial (HAB) faces of the epidermis, palisade parenchyma (PP), and spongy parenchyma (SP) in *Commelina benghalensis* leaves. Overlapping averages followed by the same letters in the same tissue do not differ by the Tukey's test at 5% probability. Bars showed ± SE.

than those of plants exposed to shading. This is because of the mesophyll expansion and reduction in intercellular spaces in the mesophyll, which is associated with the regulation of diffusion of light and gas inside the leaves. This eventually leads to maximized photosynthetic efficiency (Terashima et al., 2006). The absorption and translocation of herbicides inside the plants represent essential processes for determining the effectiveness of the herbicides (Galon et al., 2013). Glyphosate moves through the phloem and xylem, follows the route of photosynthesis products (Shaner, 2009), and reaches the vascular bundles after crossing the epidermis both via apoplast and symplast, from where it is distributed to the different parts of a plant (Yanniccari et al., 2012). Thus, this study provides evidence that glyphosate can easily and efficiently reach the vascular bundles in thinner leaves because of the shorter path that needs to be traversed. The increase in leaf blade area allows an increased interception of products such as glyphosate applied on this region during post-emergence. The positive correlation between leaf area and control rate (Table 5) corroborates the influence of this variable on the efficiency of glyphosate because, in shaded environments, where plants showed higher leaf blade areas, the efficiency of the product was also higher. Nevertheless, SEM observations showed that the damage caused by glyphosate at the base of the trichomes in *C. benghalensis* was more pronounced in the plants cultivated in shaded environments. The cuticle at the base of trichomes is more permeable (Sargent and Blackman, 1962). And the efficiency of glyphosate to control *C. benghalensis* is related to the presence of trichomes (Santos et al., 2001). These structures contribute to greater interception of the product's drops that trickle down and accumulate at its base, causing the effects shown in Figure 5. Thus, the base of trichomes appears to be an important route of herbicide absorption by the leaves. This explains why the control rates are higher with increased shading levels during cultivation (Tables 2 and 3). Despite the lack of symptoms caused by glyphosate on the surface of *C. rotundus* leaves cultivated in shaded environments (Figs. 7), the product's efficiency in these environments is clear (Tables 1 and 3). *C. rotundus* is known to be a C_4 metabolism plant (Kranz anatomy; Fig. 2D), and plants with this anatomy are known to have high photosynthetic rates (Taiz and Zeiguer, 2009). The observation of a cross-section from the *C. rotundus* leaf blade (Fig. 2B.) showed that the density of vascular bundles was greater than that in *C. benghalensis* (Fig. 2A). Because glyphosate is systemically transported via

Table 4. Blade thickness of *Commelina benghalensis* leaves cultivated under shading conditions and treated with glyphosate.

Cultivation environments	Doses of glyphosate (g ha^{-1})		
	0	720	1440
0% of shade	678.48 Aa	634.72 Ba	619.84 Ca
50% of shade	324.79 Ab	298.92 Bb	288.33Bb
70% of shade	325.53 Ab	290.55 Bb	272.78 Cc

Means followed by the same uppercase letters in rows, and lowercase letters in columns differ significantly by Tukey's test (P≤ 0.05).

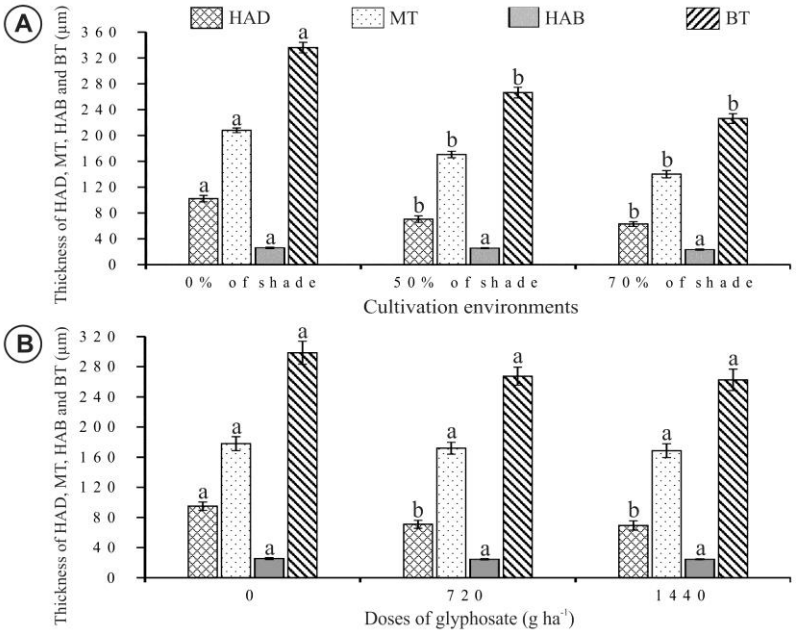

Fig 4. Effects of shading (A) and glyphosate (B) on the thickness of the adaxial (HAD) and abaxial (HAB) faces of the epidermis, mesophyll (MT), and blade (BT) in *Cyperus rotundus* leaves. Overlapping averages followed by the same letters in the same variable do not differ by the Tukey's test at 5% probability. Bars showed ± SE.

Table 5. Correlation between the area and thickness variables in the foliar blade and glyphosate control rate at 10, 20, and 30 DAA.

Variable	Assessments on phytotoxicity (DAA)		
	10	20	30
	Commelina benghalensis		
leaf blade area	0.40*	0.15^{ns}	0.22^{ns}
blade thickness	-0.31*	-0.55 ^{ns}	-0.15 ^{ns}
	Cyperus rotundus		
leaf blade area	0.52**	0.60**	0.67**
blade thickness	-0.31*	-0.48**	-0.58**

Pearson correlation coefficients values (r). **Significant at 1% probability. *Significant at 5% probability by the t test. ^{ns}Non-significant. DAA: days after application of glyphosate.

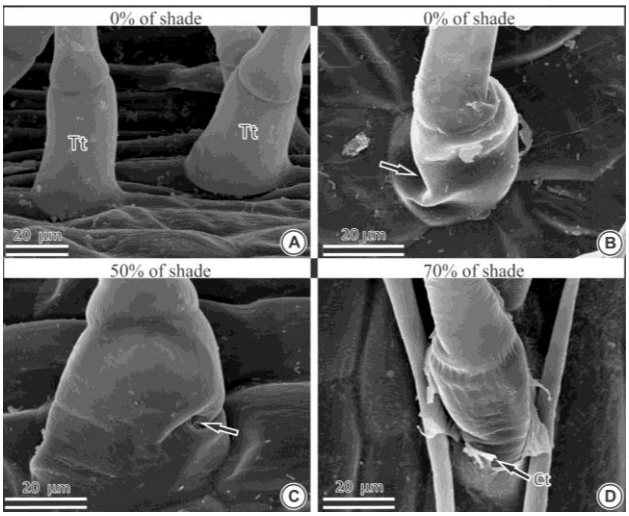

Fig 5. Scanning electron microscopy: effects of glyphosate on the adaxial face of *Commelina benghalensis* leaves. **A.** without glyphosate application. **B–D.** Plant exposed at 720 g ha^{-1} glyphosate. **A.** Tector trichomes (Tt) turgid. **B–C.** Loss of turgor in the basal cells (arrow). **D.** Cuticle rupture (Ct).

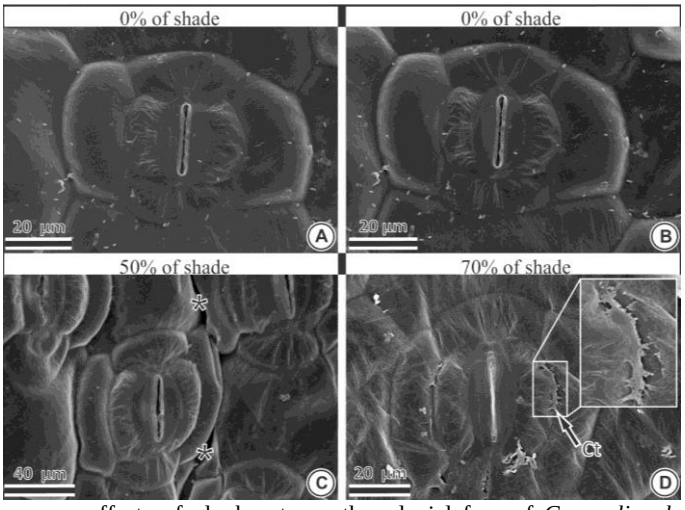

Fig 6. Scanning electron microscopy: effects of glyphosate on the adaxial face of *Commelina benghalensis* leaves. **A.** without glyphosate application. **B–D.** Plant exposed at 720 g ha^{-1} glyphosate. **A–B.** Turgid cells of the stomatal complex. **C**. Presence of depressions (*). **D.** Cuticle rupture (Ct).

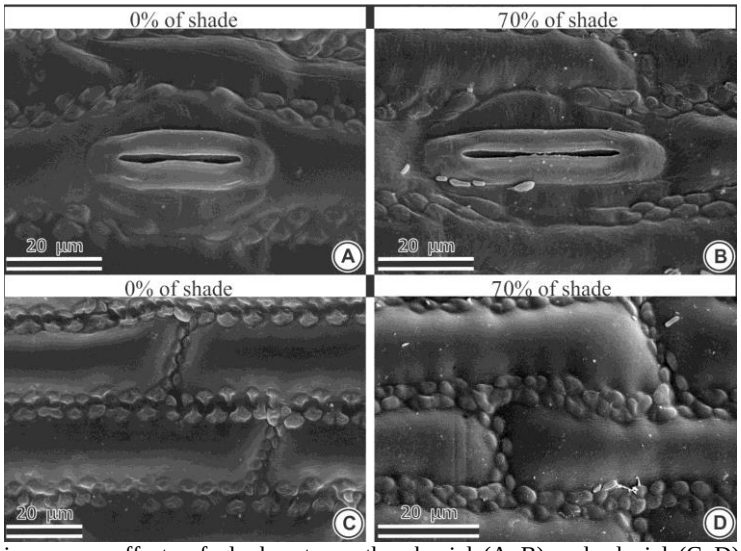

Fig 7. Scanning electron microscopy: effects of glyphosate on the abaxial (A–B) and adaxial (C–D) faces of *Cyperus rotundus* leaves. **A** and **C.** without glyphosate application. **B** and **D.** Plants exposed at 720 g ha^{-1} glyphosate.

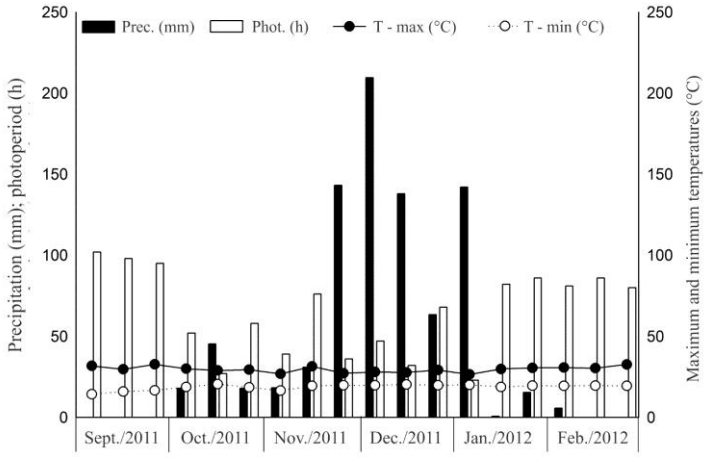

Fig 8. Decennial averages of precipitation (mm), photoperiod (h), and maximum and minimum temperatures (°C) obtained during the field experiment.

the route of the products from photosynthesis (Shaner, 2009), the translocation of the products in *C. rotundus* was likely the process that most contributed to the greatest efficiency of glyphosate in controlling this species. However, studies on the photosynthetic rate and product translocation in this species are necessary to confirm this assumption. The results of the present study suggest that shaded environments stimulate the development of anatomical and micromorphological features in the studied weeds, increasing the effectiveness of glyphosate. In forest crops, fruit-growing areas, native forests, and regions with some agricultural crops of plant species that are tolerant to glyphosate, where shading is common and present most of the time, the amount of glyphosate to be applied needs to be reassessed to combat invasive plants.

Materials and Methods

Experimental outline

The experiment was conducted in the municipality of Montes Claros, MG, Brazil, at the geographical coordinates of 43°50′15.90″W and 16°40′57.59″S and an altitude of 620 m. The climate data for the study period in the field are presented in Figure 8. The study was conducted under field for approximately seven months. Both species was chosen because are hard-to-control and widely distributed species in cultivated areas in Brazil and world. Seedlings of *Commelina benghalensis* were produced from the cuttings obtained from healthy branches having two nodes and a couple of expanded leaves cut in half. Seedlings of *Cyperus rotundus* were obtained from healthy tubers collected from the field. Two *C. benghalensis* or *C. rotundus* seedlings were transplanted into 12 L pots filled with a substrate consisting of soil, sand, cured cow manure (3:1:1), and 10 kg of a mixture of nitrogen, phosphorus and potassium (NPK, 4:30:10) for each 0.9 m^3 of the substrate. The pots were distributed in their respective cultivation environments. One assay was conducted per species with a 3 × 3 factorial design, with factor 1 represented by the doses of 0, 720 and 1440 g ha^{-1} of glyphosate, and factor 2 represented by three cultivation environments with shading levels of 0 (complete sun exposure) and 50 or 70% shade. The assays were conducted in randomized blocks with five replicates, with each portion consisting of one vase containing two plants of either *C. benghalensis* (assay 1) or *C. rotundus* (assay 2). The shaded environments were created using black polypropylene screens to produce average value (mean ± standard error) equivalent 50 ± 4.2 or 70 ± 3.8 % of shading. Light interception in different levels of shading were measured in 10 replicates for shaded environments using AccuPAR Linear PAR/LAI ceptometer, Model - LP 80 (Decagon Devices). The plants were irrigated twice daily in the morning and evening. The *C. benghalensis* and *C. rotundus* plants were pruned at 100 days after transplanting to standardize the vegetative development.

Application of herbicide and assessments of phytotoxicity

The herbicide spray was applied 25 days after pruning by using a sprayer equipped with a single-spout bar (model Teejet AI110015) and a constant pressure regulator valve at 150 KPa with the output set to apply 100 L ha^{-1} of spray.
The control evaluations were performed at 10, 20, and 30 days after application (DAA) of glyphosate; control samples were visually evaluated by using a scale from 0-100%, where 0=no control and 100=complete plant death (Frans et al. 1986).

Sampling for measurement of leaf blade area and anatomical and micromorphological analyses

Twelve fully expanded leaves were collected from each plot to determine the area of leaf blade immediately before the application of glyphosate. The leaves were digitalized and submitted to image analysis by using the Image-Pro Plus version 4.1 software for Windows® (Media Cybernetics, Silver Spring, MD, USA) for the calculation of the blade foliar area. At 4 DAA of glyphoshate, leaf fragments were collected and fixed in Karnovsky solution (Karnovsky, 1965) for the quantitative and qualitative evaluation of leaf anatomy of *C. benghalensis* and *C. rotundus* as a function of availability of light and application of glyphosate. Leaf pieces (0.5 × 1.0 cm) were removed from the median vein region from fully expanded leaves. The selected leaves did not show any visual symptoms of toxicity caused by glyphosate or other biotic or abiotic agents used. The samples were dehydrated in an ethanol series (30, 50, 70, 90 and 100%) and embedded in methacrylate-type acrylic resin for anatomical analysis. Cross-sections of 7 µm thickness were obtained using an auto-advance rotatory microtome (model RM2255; Leica Microsystems Inc., Deerfield, Illinois, USA) and stained with 0.05% toluidine blue at pH 4.7 (O'Brien & McCully, 1981). The slides were mounted on Eukitt (Eukitt Mounting Medium; Sigma-Aldrich Corporation, USA) and photographed using a light microscope (model AX70RF; Olympus Optical, Tokyo, Japan) equipped with a U-Photo photographic system and coupled with a digital camera (model Spot Insight color 3.2.0; Diagnostic Instruments Inc., New York, USA). Images were analyzed using Image Pro Plus, version 4.1 software for Windows® (Media Cybernetics, Silver Spring, MD, USA). The following parameters were measured: blade thickness (BT), palisade parenchyma (PP), spongy parenchyma (SP), and thickness of the adaxial (HAD) and abaxial face (HAB) of the epidermis. The mesophyll thickness (MT) was measured in *C. rotundus* because this species possesses a homogenous mesophyll layer. Twenty-seven measurements were obtained per tissue/replicate, leading to a total of 135 and 108 measurements per replicate for *C. benghalensis* and *C. rotundus*, respectively. The micromorphological analysis was performed using a scanning electron microscope (SEM) by using those samples from the test and control plants of the two studied species that did not show visible symptoms after the application of 720 g ha^{-1} glyphosate. Plants that received the highest dose (1440 g ha^{-1}) already exhibited initial signs of chlorosis, and thus were not used for microscopic characterization. The plant material was post-fixed in 1% osmium tetroxide, dehydrated in an acetone series (30, 50, 70, 90, and 100%), and dried to the critical point (model Balzers CPD 030). The samples were fixed in "stubs," metallized with gold (metalizador Balzers SCD 050), and analyzed using a SEM (LEO model 435-VP). Images were digitally captured.

Statistical analysis

The data were subjected to analysis of variance (ANOVA) and, when relevant, averages were compared using Tukey's test at a significance level of 5%. The variables of leaf blade area and thickness and the control rate were subjected to Pearson correlation coefficient.

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