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Levels of shading and application of glyphosate and carfentrazone-ethyl in the control of *Macroptilium atropurpureum*

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Key words:

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ABSTRACT

Variations in environmental conditions, such as the availability of light, can affect the efficacy of herbicides because they alter the biological characteristics of plants including those that are related to the plant's sensitivity to herbicides. Therefore, the objective of the present study was to assess the influence of environments with different light availabilities, and of the application of glyphosate and carfentrazone-ethyl (separately or in combination) on the morphophysiology and control of *Macroptilium atropurpureum*. An experimental design of randomized blocks with five replicates was used, with treatments arranged in a split-plot design. The plots were composed of three levels of shading (full sunlight, 50% shading, and 70% shading); within each plot there were sub-plots, one for each of the two herbicides (glyphosate and carfentrazone-ethyl), used either separately or in combination. The doses of glyphosate + carfentrazone-ethyl applied in the treatments were 0+40 g ha⁻¹ of carfentrazone-ethyl, 1.440+0 g ha⁻¹ of glyphosate, 1.080+30 g ha⁻¹ of glyphosate + carfentrazone-ethyl, and there was an additional treatment that did not include the application of an herbicide. Plants of *M. atropurpureum* cultivated under shading exhibited higher sensitivity to the herbicides, greater leaflet area, and lower photosynthetic rates than plants cultivated under full sunlight conditions. Under shading, both herbicides applied separately or in combination were effective in controlling *M. atropurpureum*, which indicates a greater susceptibility of this species to these herbicides in environments with light restriction. The species was tolerant to the two tested herbicides, used either alone or in combination, when grown under full sunlight conditions.

Palavras-chave:

fisiologia
siratro
taxa fotossintética
área foliar

Níveis de sombreamento e aplicação de glifosato e carfentrazone-etil no controle de *Macroptilium atropurpureum*

RESUMO

Variações nas condições ambientais, como a disponibilidade luminosa, podem afetar a eficiência de controle dos herbicidas por alterar características biológicas das plantas relacionadas à sensibilidade aos herbicidas. Desse modo, objetivou-se avaliar a influência do cultivo em ambientes com diferentes disponibilidades luminosas e da aplicação isolada ou em mistura de glifosato e carfentrazone-etil, sobre a morfofisiologia e o controle de *M. atropurpureum*. O delineamento adotado foi o de blocos casualizados, em esquema de parcelas subdivididas com 5 repetições. As parcelas foram constituídas de três níveis de sombreamento (pleno sol, 50 e 70% de sombra) e as subparcelas de dois herbicidas (glifosato e carfentrazone-etil) combinados ou não. As doses de glifosato + carfentrazone-etil utilizadas foram 0+40 g ha⁻¹ de carfentrazone-etil, 1,440+0 g ha⁻¹ de glifosato, 1,080+30 g ha⁻¹ de glifosato + carfentrazone-etil e mais um tratamento sem aplicação de herbicida. Plantas de *M. atropurpureum* cultivadas sob sombreamento apresentaram maior sensibilidade aos herbicidas, maior área dos folíolos e menor taxa fotossintética em comparação ao seu cultivo em ambiente a pleno sol. Em sombreamento, tanto os herbicidas aplicados isoladamente quanto a mistura são efetivos no controle do *M. atropurpureum*, o que indica maior susceptibilidade dessa espécie aos herbicidas em ambientes com restrição luminosa. A espécie é tolerante aos dois herbicidas testados, isolados ou em mistura, quando crescida a pleno sol.



INTRODUCTION

Macroptilium atropurpureum is a legume that occurs naturally in several tropical regions that are difficult to grow in, owing to their certain characteristics, such as low fertility requirements and tolerance to both water stress and poorly drained soils (Skerman, 1977; Santos et al., 2000). It is a plant with forage potential but also considered a weed, being characterized by a high capacity for seed production, tolerance to stress conditions, and a variable growth habit (Lorenzi, 2008).

In addition to the intrinsic characteristics of *M. atropurpureum*, it is also important to consider the interference of the crop environment with the management of the species, including its sensitivity to herbicides. Abiotic factors are modified by the crop environment as a result of the microclimate created by the crop canopy. These environmental conditions promote modifications in the anatomy, morphology, and physiology of weeds, which influences the interception, absorption, translocation, and detoxification of herbicides in the plants (Cieslik et al., 2013; Santos Júnior et al., 2013; Matzenbacher et al., 2014; Kucharski & Kieloch, 2015; Bagavathiannan et al., 2017).

Environmental conditions resulting from the frequent use of the same herbicide can favor the selection of individuals that are tolerant and/or resistant to one or more mechanisms of action of the herbicide (Santos et al., 2013; 2015), or of plants that are better adapted to specific environmental conditions. Combining herbicides with different and synergistic mechanisms of action is an interesting strategy for increasing the spectrum of action and efficiency of chemical control (Braz et al., 2013).

The action of herbicides on plants grown under shade is quite variable, depending on the mechanism of action of the herbicide and on the response of the weed species to radiation restriction (Fonseca et al., 2016; Besançon et al., 2017). Thus, knowledge about the effect of herbicides with distinct mechanisms of action in plants established in environments with different levels of insolation is essential to develop improved management strategies.

Research on the biology of *M. atropurpureum* and its responses to chemical control are scarce in the literature, especially in terms of data obtained from distinct growth environments. Therefore, the objective of the present study was to assess the effect of environments with different light availabilities and of the application of glyphosate and carfentrazone-ethyl (separately or in combination) on the morphophysiological characteristics and control of cultivated *M. atropurpureum*.

MATERIAL AND METHODS

The experiment was conducted between February and May 2014 in Montes Claros, MG, Brazil (43° 50' 18.31" W, 16° 40' 59.22" S, altitude of 650 m). The climate of the region is tropical with wet winters (As), according to the Köppen classification (Alvares et al., 2014). Figure 1 shows climatic data during the experimental period.

Seedlings of *M. atropurpureum* were initially produced by semiferous propagation in Styrofoam trays filled with

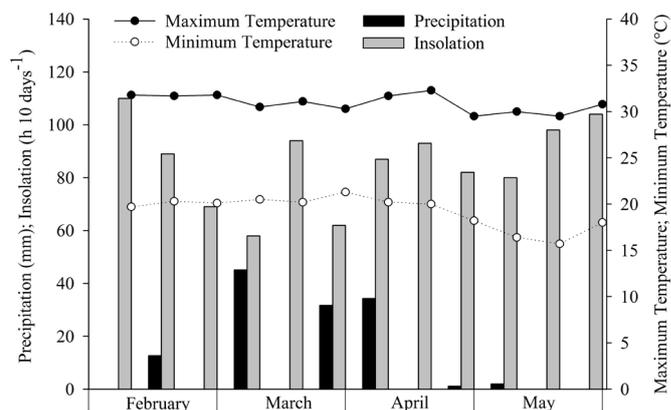


Figure 1. Ten-day means of insolation (h day^{-1}), precipitation (mm), and minimum and maximum temperatures ($^{\circ}\text{C}$) relative to the period from February to May of 2014, Montes Claros, Minas Gerais

commercial substrate. The seeds that were used were collected from naturally-grown plants present in a commercial orange grove. Thirty days after planting, two plants were transferred to 12 dm^3 pots containing substrate consisting of sandy soil plus fertilizers. These plants were selected to obtain a standardized number of plant leaves and height. The soil used in the experiment was collected in a way so as to avoid removing the superficial layer so that contamination by weeds did not occur; the chemical characteristics of the collected soil were as follows: 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 $\text{cmol}_c \text{dm}^{-3}$; Mg: 0.10 $\text{cmol}_c \text{dm}^{-3}$; Al: 0.60 $\text{cmol}_c \text{dm}^{-3}$; H+Al: 2.90 $\text{cmol}_c \text{dm}^{-3}$; ECEC: 0.99 $\text{cmol}_c \text{dm}^{-3}$; BS: 11.84%. The substrate was fertilized with single superphosphate, potassium chloride, and urea, according to the recommendations of Cantarutti et al. (2007). Soil moisture content was maintained near field levels through irrigation performed twice a day.

An experimental design of randomized blocks with five replicates was used. The treatments were arranged in a split-plot design, in which the levels of shading (0, 50, or 70% of shade) were allocated to the plots and the herbicide treatments (applied separately or in combination), i.e., 40 g ha^{-1} of carfentrazone-ethyl, 1.080 + 30 g ha^{-1} of glyphosate + carfentrazone-ethyl, and 1.440 g ha^{-1} of glyphosate and the treatments without herbicide were allocated to the sub-plots.

The environments providing shade had $50 \pm 4.2\%$ ($1.560 \mu\text{mol m}^{-2} \text{s}^{-2}$) and $70 \pm 3.8\%$ ($890 \mu\text{mol m}^{-2} \text{s}^{-2}$) of light interception, which was achieved by using black polypropylene shade screens (sombrite) with light interception corresponding to 50 and 70%, as per the manufacturer's specifications. Light interception was measured at noon, with 10 spot measurements in each environment, using a linear ceptometer (AccuPAR Linear PAR/LAI ceptometer, Model LP 80; Decagon Devices).

Forty-five days after transplanting, when more than 50% of the plants were starting to bud, the herbicides were applied using a backpack sprayer equipped with a wand with a TeeJet AI110015 nozzle and a regulating valve to maintain a constant pressure at 150 kPa, with the volume of applied herbicide solution being 100 L ha^{-1} . The applications were performed in the morning to avoid high temperatures.

Photosynthetic rates (A) were assessed between the first and third completely expanded leaves in the upper third of the

plants, two and six days after herbicide application (DAA). The evaluation period was determined based on the time required for the stress caused by the herbicides to manifest in the plants, while the plants were still fit to be assessed. A portable infrared gas analyzer (IRGA) (model LCA 4, Analytical Development Co. Ltd, Hoddesdon, UK) was used. It measured leaf gas exchange based on CO₂ variations and relative humidity inside the device's chamber.

The dry biomass of the aerial part of the plants was determined 28 DAA by collecting all the living plant materials that remained in the pot and transferring it to a forced air circulation oven at 55 °C until a constant weight was reached. The mean leaflet area was determined by collecting the completely expanded leaves of the upper third part of the plants in the treatments without herbicide application in each crop environment. These were scanned and subjected to image analysis using the ImagePro Plus software, version 4.1, for Windows® (Media Cybernetics, Silver Spring, MD, USA).

Visual assessments were performed at 28 DAA to evaluate weed control, using a scale of 0 to 100%, where 0% is the absence of toxic effects caused by the herbicide and 100% is the total death of plant tissues, according to the method of ALAM (1974).

The data on weed control and leaflet area were analyzed descriptively using means and standard errors. The data on the remaining variables were subjected to analysis of variance by the F test, and when the result was significant the means were compared by Tukey's test at 5% probability, using the Proc Mixed procedure of the SAS statistical package (SAS Institute, 2002).

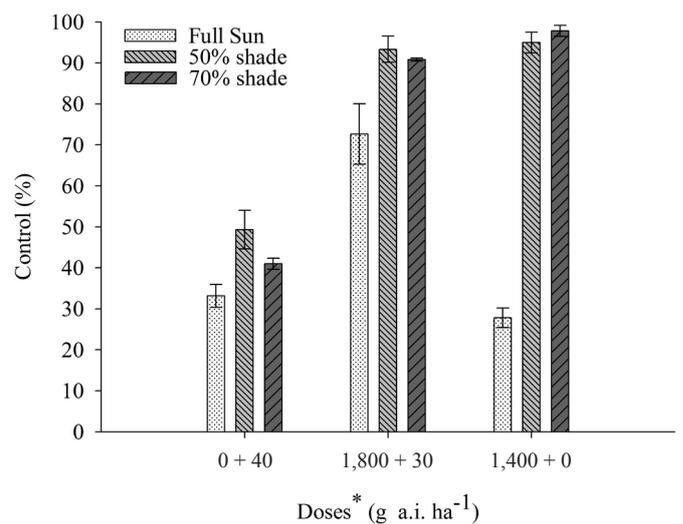
RESULTS AND DISCUSSION

The application of glyphosate separately or in combination with carfentrazone-ethyl was more effective in the control of *M. atropurpureum* cultivated under shading. Under full sunlight conditions, the mixture provided greater control than the herbicides applied separately; however, the control was unsatisfactory for the effective management of this weed. Under these conditions, the plants were more tolerant to the herbicides (Figure 2). Weed control with the use of one herbicide alone under full sunlight conditions was less than 35%, thus indicating that the species tolerates the tested herbicides.

Under shade, weed control was greater than 90%, i.e. very good, when glyphosate was applied alone or in combination with carfentrazone-ethyl, and there was no additive effect of the herbicide mixture (Figure 2). The control of *M. atropurpureum* was poor when carfentrazone-ethyl was applied separately, regardless of the environment (Figure 2).

The interaction of the herbicides with the crop environment affected the dry mass of the aerial part of *M. atropurpureum* ($p < 0.0001$). The herbicides were more effective in reducing the aerial part when the plants were cultivated in environments with restricted light, regardless of the level of shading (Table 1).

The poor control of *M. atropurpureum* observed with the application of glyphosate and carfentrazone-ethyl, separately or in combination, in an environment under full sunlight



* Doses of glyphosate + carfentrazone-ethyl (0 + 40 g ha⁻¹ of carfentrazone-ethyl; 1.440 + 0 g ha⁻¹ of glyphosate; and 1.080 + 30 g ha⁻¹ of glyphosate + carfentrazone-ethyl)

Figure 2. Control of *Macroptilium atropurpureum* at 28 days after herbicide application

Table 1. Dry mass of the aerial part (g per pot) of *Macroptilium atropurpureum* treated with doses of glyphosate and/or carfentrazone-ethyl, depending on the growth environment, at 28 days after herbicide application

Environment	Doses of Glyphosate + Carfentrazone-ethyl (g a.i. ha ⁻¹)			
	0 + 0	0 + 40	1.440 + 0	1.080 + 30
Full sunlight	30.56 Aa	30.85 Aa	21.94 Ab	12.95 Ac
50% shading	24.46 Ba	9.15 Bb	4.84 Bb	5.95 Bb
70% shading	19.55 Ba	13.11 Bb	2.59 Bc	5.24 Bc
CV(%): 20.8				

Means followed by the same letters, uppercase in the column and lowercase in the line, do not differ by Tukey's test at 0.05 probability

conditions (Figure 2) translated into a greater accumulation of plant dry mass (Table 1). The application of carfentrazone-ethyl separately under full sunlight conditions did not lead to a reduction in the accumulated biomass of *M. atropurpureum*, in contrast to the results from when the plants were kept under shading, regardless of the level of shading (Table 1). The application of glyphosate, alone or in combination, caused a reduction in the biomass of *M. atropurpureum* relative to the control plants in the crop environments. The mobility of the herbicide carfentrazone-ethyl in the plant is reduced, and, for effective weed control to occur, the applied herbicide must cover a major part of the leaf area. Sometimes, however, the solution only reaches the upper part of the canopy, causing the so-called "umbrella" effect, in which the upper leaves receive the largest volume of herbicide and the lower leaves remain protected (Rocha et al., 2007).

The additive effect provided by the glyphosate + carfentrazone-ethyl mixture has been reported by previous authors, and the compatibility between them depends on the doses of the herbicides used in the mixture and on the species of weed under assessment (Werlang & Silva, 2002). The increase in glyphosate efficacy by its association with herbicides sharing the mechanism of action of carfentrazone-ethyl has been observed in previous studies (Pereira & Crabtree, 1986; Wells & Appleby, 1992). The mixture of low doses of lactofen with glyphosate increased the accumulation of shikimate in the tissues of plants of *Malva parviflora* (Wells & Appleby, 1992).

These authors believe that lactofen promotes the entry of glyphosate into the cells. Pereira & Crabtree (1986) reported an improvement in the absorption and translocation of glyphosate in plants of *Cyperus esculentus* after the addition of oxyfluorfen. The increase in the accumulation of shikimate and in the absorption, cell penetration, and translocation of glyphosate as a result of adding herbicides that inhibit PPO may explain the increased efficacy of the mixture glyphosate + carfentrazone-ethyl under full sunlight conditions. Concenço et al. (2012) observed that the application of glyphosate alone was effective in the control of weeds even when it was performed at a later stage; however, carfentrazone-ethyl was only effective when applied after initial emergence. In the present study, the herbicides were applied whilst the plants were budding, which may be the reason for the poor control of *M. atropurpureum* observed when carfentrazone-ethyl was applied.

The effect of glyphosate, applied separately or in combination with carfentrazone-ethyl, was greater when the plants were cultivated under shade, with a mean reduction in dry mass of 80% and weed control of more than 90% relative to the control plant. The lower availability of light led to a reduction in the plants' dry mass and photosynthetic rate (Tables 1 and 2), which potentiated the effect of the herbicides (Figure 1). Shading reduces the amount of photosynthetically active radiation and compromises photochemical efficiency, the rate of electron transportation, and the activity of Rubisco (Li et al., 2010; Mu et al., 2010; Baldi et al., 2012). These changes are responsible for the decrease in photosynthetic rate and, consequently, for the lower accumulation of dry mass in the plants. An increase in the efficacy of *Commelina benghalensis* and *Cyperus rotundus* control by glyphosate under shading was also observed by Santos Júnior et al. (2013).

Application of the herbicides decreased the photosynthetic rate of *M. atropurpureum* at 2 ($p < 0.0001$) and 6 ($p < 0.0001$) DAA relative to the control plant, in all the environments tested (Table 2). At 2 DAA, the reduction in photosynthetic rates was greater in the shaded environments for the 1.440 + 0 g ha⁻¹ dose of glyphosate, and there was no difference between the environments at the other applied doses. At 6 DAA, the trend in photosynthetic rates was the same, with greater reductions in the shaded environments for the 0 + 40 g ha⁻¹ dose of carfentrazone-ethyl and the 1.440 + 0 g ha⁻¹ dose of glyphosate;

Table 2. Photosynthetic rates ($\mu\text{mol m}^{-2}\text{s}^{-2}$) of *Macroptilium atropurpureum* treated with doses of glyphosate and/or carfentrazone-ethyl at 2 and 6 DAA, depending on the growth environment

Environment	Doses of Glyphosate + Carfentrazone-ethyl (g a.i. ha ⁻¹)			
	0 + 0	0 + 40	1.440 + 0	1.080 + 30
2 DAA				
Full sunlight	30.87 Aa	4.84 Ab	9.27 Ab	2.43 Ab
50% shading	14.83 Ba	3.17 Ab	3.14 Bb	1.81 Ab
70% shading	10.28 Ba	3.42 Ab	2.48 Bb	1.63 Ab
CV (%): 34.2				
6 DAA				
Full sunlight	29.28 Aa	19.26 Ab	12.97 Ab	2.41 Ac
50% shading	15.00 Ba	3.85 Bb	0.63 Bb	0.90 Ab
70% shading	12.55 Ba	2.46 Bb	0.74 Bb	1.72 Ab
CV (%): 34.8				

Means followed by the same letters, uppercase in the column and lowercase in the row, do not differ by Tukey's test at 0.05 probability

there were no differences for the 1.080 + 30 g ha⁻¹ dose of glyphosate + carfentrazone-ethyl.

Under full sunlight conditions, the combined use of the herbicides led to a greater reduction in the photosynthetic rates at 6 DAA. When glyphosate and carfentrazone-ethyl were used separately in this environment, the physiological recovery from herbicide treatment occurred at 6 DAA, compared to 2 DAA. The differences in photosynthetic rates explain the reduction in the dry mass of the plants under shading when the herbicides were applied.

Carfentrazone-ethyl acts by inhibiting a precursor of chlorophyll, protoporphyrinogen oxidase (Protox) (Dayan et al., 1997), which results in the accumulation of protoporphyrin IX (PpIX) in the cytosol (Jacobs & Jacobs, 1993). In the presence of light, PpIX converts oxygen into singlet oxygen, which is responsible for the plant's death through membrane peroxidation (Jung et al., 2008). Fausey & Renner (2001) reported an increase in the control of *Amaranthus retroflexus* by carfentrazone-ethyl of up to 15 times when the plants were exposed to radiation of 1.000 $\mu\text{mol m}^{-2}\text{s}^{-2}$ after herbicide application, than when they were exposed to radiation of 4 $\mu\text{mol m}^{-2}\text{s}^{-2}$. This indicates that radiation restriction, at the magnitudes tested in the present study, does not limit the formation of singlet oxygen or the efficacy of carfentrazone-ethyl in shaded environments.

It has been found, however, that reduced radiation increases the synthesis of chlorophyll (Lopes et al., 2017), which may contribute to an increase in PpIX in plants that receive carfentrazone-ethyl. Thus, plants are more sensitive to the herbicide when grown under shade because of the increase in PpIX, which, in the presence of oxygen, promotes membrane peroxidation. These results are in line with those obtained by Thompson & Nissen (2002), who assessed intoxication by sulfentrazone in soybean plants under 80% shade (200 $\mu\text{mol m}^{-2}\text{s}^{-2}$).

The greater susceptibility to glyphosate of plants grown under shade may be associated with the greater absorption of the herbicide as a result of anatomical changes. These plants have a thinner epidermis with less cutin deposition (Skoss, 1955). Another mechanism that may contribute to the synergistic effect of shading on weed control by glyphosate is the reduction in nitrogen assimilation. Glyphosate phytotoxicity is caused by, among other factors, an increase in nitrate concentration in the tissues (Bellaloui et al., 2006). In this context, Neel et al. (2016) observed a similar effect of shading on nitrogen assimilation, with an increase in nitrate concentration in temperate climate grasses. Thus, plants under shading are more prone to accumulating nitrate and are, therefore, more sensitive to glyphosate.

Another factor that favors the action of herbicides in shaded environments is related to the greater probability of interception of sprayed chemicals by the plants because of the increased leaflet area in environments with light restriction (Figure 3).

Under full sunlight conditions, the leaflets of *M. atropurpureum* exhibited a mean area of 46.65 cm², whereas under 50 and 70% shading the areas were 51.79 and

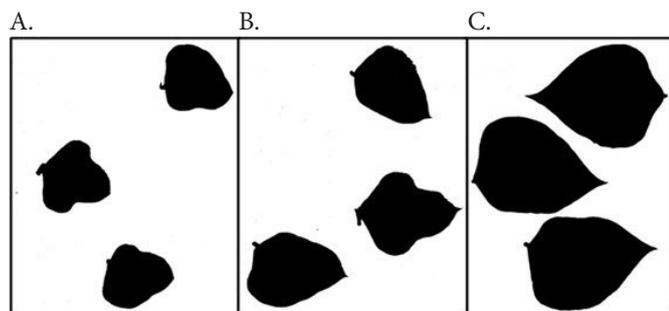


Figure 3. Area and shape of *Macroptilium atropurpureum* leaflets in different environments. (A) Under full sunlight conditions; (B) 50% shading; (C) 70% shading

72.75 cm², respectively (Figure 3). According to Li et al. (2010), leaf expansion is stimulated in plants under shading as a way of acclimatizing to the environment, to allow greater interception of incident radiation.

CONCLUSIONS

1. *Macroptilium atropurpureum* grown under full sunlight conditions showed a high tolerance to carfentrazone-ethyl and glyphosate. Mixing the two herbicides promoted improved results in the control of the plants under this condition.

2. Under shading, glyphosate applied alone or in combination with carfentrazone-ethyl was effective in controlling *M. atropurpureum*, which indicates a higher susceptibility of this species to the herbicides in environments with light restriction.

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