

# Clethodim and photosystem inhibitors on sourgrass control as a function of application time<sup>1</sup>

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**Abstract** - This study aimed to evaluate the effects of application time and addition of diuron to clethodim and diquat on the control of sourgrass. Two experiments were conducted with sourgrass plants, applying spray solutions comprising the herbicide clethodim (216 g a.i. ha<sup>-1</sup>), clethodim + diquat (216 + 400 g a.i. ha<sup>-1</sup>), and clethodim + diquat + diuron (216 + 400 + 400 g a.i. ha<sup>-1</sup>) three times (08:00, 12:00, and 16:00) in addition to the control. Control and mass evaluations of sourgrass plants were performed in both experiments, and the relative rate of linear electron transport by PSII and leaf accumulation of hydrogen peroxide were evaluated in the greenhouse experiment. The results indicated an increase in sourgrass control with the addition of diuron to the mixture, resulting in 100% control in the application performed at 16:00. Spray solutions containing photosystem inhibitors should be applied at 08:00 and 16:00 to improve plant control. These mixtures applied under lower light intensity showed greater inhibition of the relative electron transfer rate and increased H<sub>2</sub>O<sub>2</sub> concentrations with greater accumulation of this reactive oxygen species (ROS; 17894.17 nmol g<sup>-1</sup> FM) after the application of clethodim + diquat + diuron at 16:00. The addition of diuron was necessary for total plant control, resulting in greater inhibition of photosynthetic activity and accumulation of ROS.

**Key words:** *Digitaria insularis* (L.) Fedde; Oxidative stress; Graminicides; Tank mixture; Bipyridyliums.

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## INTRODUCTION

Sourgrass (*Digitaria insularis* (L.) Fedde) is native to the American continent and is present in cultivation systems in Brazil, mainly infesting cereal and oilseed fields. The invasive behavior of this species is associated with its biological characteristics, such as its ability to propagate through rhizomes and seeds (OREJA; DE LA FUENTE; FERNANDEZ-DUVIVIER, 2017), which are light, hairy, and easily dispersed by wind (GOMES *et al.*, 2017), with a germination rate > 90%. Another characteristic that makes sourgrass control difficult is its resistance to the herbicide glyphosate (HEAP, 2022), which is present on approximately 12.7 million hectares of Brazilian crops (LUCIO *et al.*, 2019). Therefore, using herbicides with different mechanisms of action is necessary, whether in isolated, mixed, or sequential applications (ANDREOTTI *et al.*, 2019).

Acetyl-CoA carboxylase (ACCase) inhibitors are the main herbicides used to control sourgrass (ANDRADE *et al.*, 2018). The herbicide clethodim is a cyclohexanedione (DIM) with mobility through the phloem and xylem, and is widely used to control weeds from the *Poaceae* family, such as sourgrass (SHANER, 2014). Another option for controlling this species is using herbicides that inhibit the electron transport chain in photosystem I (FSI inhibitors), such as diquat. However, these herbicides have low mobility, and their efficiency is restricted to plants at the early developmental stage (TAHMASEBI *et al.*, 2018), or in sequential applications associated with systemic herbicides (ANDREOTTI *et al.*, 2019).

The post-emergence application of contact and systemic herbicides typically results in antagonism (BESANÇON; PENNER; EVERMAN, 2018). This is due to the rapid necrotic action caused by the accumulation of reactive oxygen species (ROS) after inhibition of the electron transport chain by photosynthesis inhibitors. In this way, the systemic herbicide is not translocated, and its efficiency is reduced. However, the accumulation of ROS depends on the presence of light, and applications under low light intensity can reduce or delay oxidative stress and allow herbicide translocation, thus increasing weed control (MONTGOMERY *et al.*, 2017). Another method to reduce the rapid production of ROS by diquat is to combine it with photosystem II (FSII) inhibitor herbicides, such as diuron. Diuron inhibits the photosynthetic process before diquat causes cell destruction and inhibits its translocation, similar to other herbicides in the spray (HAYWARD; COLBY; PARHAM, 1988).

Although the sequential application of systemic herbicides (e.g., clethodim) followed by the application of bipyrindyls constitutes one of the most efficient measures to

control sourgrass (MELO *et al.*, 2012), the intensification of cultivation systems does not always allow this application modality to be conducted. Furthermore, sequential applications require greater operational capacity and increased labor, fuel, and machinery costs. Based on the above, this study hypothesized that the application of clethodim associated with diquat controls sourgrass as long as it is applied at the appropriate time and/or associated with the herbicide diuron. Therefore, this study aimed to evaluate the effects of application time and the addition of diuron to clethodim and diquat on the control of sourgrass.

## MATERIAL AND METHODS

This study was conducted in an experimental area in the state of Paraná, Brazil. Two experiments were conducted in 2019, one in the field and the other in a greenhouse.

### Origin and Production of Plant Material

#### Experiment I – Field

The experiment was conducted in plots 6.0 m in length and 4.0 m in width (24 m<sup>2</sup>), with the central area of each plot being considered a useful area for evaluations, disregarding 1 m of border on each side of the parcel. The soil in the experimental area is dystrophic red Oxisol comprising 12% sand, 36% silt, and 52% clay; pH (CaCl<sub>2</sub>) 4.80; 20.10 g dm<sup>-3</sup> of organic matter; and 8.8 cmolc dm<sup>-3</sup> of cation exchange capacity. The experiment area was fallow with a natural infestation of sourgrass (*D. insularis*). During the experiment (October and November), the total rainfall was 150.30 mm and the average air temperature was 27.34 °C.

#### Experiment II – Vegetation House

The experiment was conducted in plastic pots with a capacity of 0.5 dm<sup>3</sup>, filled with sieved dystrophic red Oxisol comprising 30% sand, 25% silt, and 45% clay; pH (CaCl<sub>2</sub>) 5.30; 20.10 g dm<sup>-3</sup> of organic matter; and 9.90 cmolc dm<sup>-3</sup> of cation exchange capacity. Sourgrass seeds were collected from the same area where the field experiment was conducted and sown in plastic trays containing a 0.1 m layer of organic substrate. After germination, three sourgrass seedlings were transplanted per experimental unit. The plants were irrigated periodically, maintaining the humidity close to field capacity.

#### Experimental Design and Treatments

In both experiments, a completely randomized design was used, with four replications, and treatments organized in a 3 × 3 factorial scheme, in addition to the control without the application of herbicide as an additional

treatment. Factor A comprised the herbicides clethodim, clethodim + diquat, and clethodim + diquat + diuron. Factor B corresponded to the application of the herbicide sprays at 08:00, 12:00, and 16:00. The herbicides clethodim (Select® 240 EC, 240 g a.i. L<sup>-1</sup>, Arysta LifeScience), diquat (Reglone®, 200 g a.i. L<sup>-1</sup>, Syngenta), and diuron (Herburon 500 BR®, 500 g a.i. L<sup>-1</sup>, Adama) were used, in doses of 216, 400, and 400 g a.i. ha<sup>-1</sup>, respectively, with 0.5% (v.v) of phosphoric acid ethoxylated alkyl ester adjuvant (Lanzar®, Arysta LifeScience).

#### Application of Treatments

The herbicide was applied using a CO<sub>2</sub> pressurized knapsack sprayer equipped with flat jet nozzles with pre-orifices (model ADI 11002, spaced 0.5 m apart and positioned 0.5 m from the surface of the plants). The working pressure was 0.414 MPa, the displacement speed was 1.0 m s<sup>-1</sup>, resulting in an application rate equivalent to 150 L of mixture per ha<sup>-1</sup>. At the time of application, in both experiments, the sourgrass plants were 0.15 m tall and had three to four tillers. The treatments were performed under the conditions listed in Table 1.

#### Assessments

Visual control assessments were performed 7 and 15 days after treatment (DAT). In each evaluation, scores were assigned on a percentage scale, where 0 and 100 represented the absence of injury and death of the plants, respectively (FRANS; CROWLEY, 1986). The plants were collected and weighed at 15 DAT to determine the fresh mass of the shoot (MFPA). Subsequently, the samples were placed in paper bags and placed in an oven with forced air circulation at 60 °C until they reached a constant mass to determine the dry mass of the aerial part (MSPA). In the field experiment,

evaluations to determine the MFPA and MSPA were conducted on three plants per experimental unit, randomly selected from the useful area of the plots.

In the greenhouse experiment, FSII activity and H<sub>2</sub>O<sub>2</sub> accumulation were evaluated in addition to control and mass evaluations. FSII activity was assessed using chlorophyll fluorescence measured in leaves from the middle third of each plant using an OS1p fluorometer (Opti-Sciences, Hudson, NH, USA). Evaluations were performed 24 h after the application of the herbicides at each application time and in the control. The relative rate of linear electron transport by FSII was calculated as the relative electron transfer rate (rETR) =  $\Delta F/F_m' \times PAR \times 0.5 \times 0.84$ , where PAR is the photosynthetically active radiation; 0.5 is the partition of light between FS; and 0.84 is the leaf absorption coefficient (BAKER, 2008).

ROS accumulation was assessed by determining the H<sub>2</sub>O<sub>2</sub> content in the leaves. After 24 h of treatment applications, one leaf was collected from the middle third of each experimental unit. At the time of collection, the samples were placed individually in aluminum foil envelopes, immediately immersed in liquid nitrogen, and transferred to a biofreezer at -80 °C until analysis. Leaves (0.1 g) were macerated in a crucible with liquid nitrogen and extracted with 1.4 mL of trichloroacetic acid (0.2%) diluted in methanol. After centrifugation at 15,645 × g at 4 °C for 5 min, the supernatant was used to measure H<sub>2</sub>O<sub>2</sub> via reaction with 1 M potassium iodide in 0.1 M potassium phosphate buffer (pH 7.5). Absorbance readings were performed using a spectrophotometer at 390 nm, and H<sub>2</sub>O<sub>2</sub> levels were calculated using a standard curve prepared with known H<sub>2</sub>O<sub>2</sub> concentrations and expressed in nmol per gram of fresh leaf mass (nmol g<sup>-1</sup> FM) (ALEXIEVA *et al.*, 2001).

**Table 1** - Meteorological conditions at the time of applying clethodim, clethodim + diquat, and clethodim + diquat + diuron at different application times under field and greenhouse conditions. Par: photosynthetically active radiation; T: temperature; Ur: relative humidity

Experiment I – Field			
Application time	Par (μmol.m <sup>-2</sup> s <sup>-1</sup> )	T (°C)	Ur (%)
08:00	149.1	24.7	74.5
12:00	1351.2	31.3	59.1
16:00	57.9	26.8	65.2
Experiment II – Vegetation House			
Application time	Par (μmol.m <sup>-2</sup> s <sup>-1</sup> )	T (°C)	Ur (%)
08:00	178.2	23.5	71.2
12:00	1320.0	29.0	50.3
16:00	73.4	27.2	53.2

## Statistical Analysis

In both experiments, the data were analyzed using descriptive statistics to obtain measures of central tendency, dispersion, and verification of the presence of discrepant data. After exploratory analysis, the normality of the errors, homoscedasticity of the variances, and independence of the errors were verified using the Shapiro–Wilk, Bartlett, and Durbin–Watson tests, respectively ( $p < 0.05$ ). After accepting these assumptions, an analysis of variance was performed. When a significant effect was found, the means were compared using Tukey’s test ( $p < 0.05$ ). In the visual control evaluation, data from the control group without herbicide application were excluded from the statistical analyses to meet the assumptions of the analysis of variance. Data from the control evaluations at 7 and 15 DAT, MFPA, MSPA, and the accumulation of hydrogen peroxide ( $H_2O_2$ ) were compared with those of the control using Dunnett’s test ( $p < 0.05$ ). The Pearson correlation coefficient ( $r$ ) ( $p < 0.05$ ) was used to evaluate the variables.

## RESULTS AND DISCUSSION

### Experiment 1 – Field

In the field experiment, there was an interaction between herbicides and application time for all variables evaluated. At 7 DAT, the best results were obtained by applying clethodim + diquat + diuron at 16:00, with 95.6% control (Figure 1A). The same herbicide combination resulted in inferior controls of 5% and 18.1% when applied at 08:00 and 12:00, respectively. At 15 DAT (Figure 1B), the application of clethodim + diquat + diuron at 12:00 resulted in 85% control, which was lower than that at other times. The application at 08:00 generated 95% control, which did not differ statistically from the application at 16:00. However, there was only plant mortality (100%) at 16:00, which ruled out the possibility of regrowth.

In general, the addition of diuron to the mixture significantly increased sourgrass control. Applications of clethodim associated with diquat performed at 08:00 and 16:00 did not differ significantly, with controls of 82.5% and 84.4% at 7 DAT, and 85.6% and 90% at 15 DAT, respectively. However, they were higher by 11.3% and 13.1% in the plant control at 7 DAT, and by 8.8% and 13.1% at 15 DAT, respectively, when compared to the application at 12:00.

The efficiency of isolated clethodim was not influenced by the time of application in the evaluations at 7 DAT and 15 DAT. This treatment presented the lowest control rates compared to the other sprayed mixtures, regardless of the time of application, with an average control rate of 25.4% at 7 DAT and 56.3% at 15 DAT.

The MFPA (Figure 1C) and MSPA (Figure 1D) results corroborated those observed in the control evaluations. In other words, the lowest accumulation of MFPA was observed in the treatment with clethodim + diquat + diuron, particularly when the application was conducted at 16:00 with 1.3 g. For MSPA, the application of clethodim + diquat + diuron also resulted in lower values, however, there was no difference between applications at 08:00 and 16:00.

For the application of clethodim + diquat, MFPA and MSPA were lower when the herbicides were applied at 08:00 and 16:00, whereas for the isolated application of clethodim, the application time did not have a significant effect on mass accumulation. In the mass evaluations, all herbicide treatments differed from the control, which presented an MFPA of 20.7 g and a MSPA of 6.3 g.

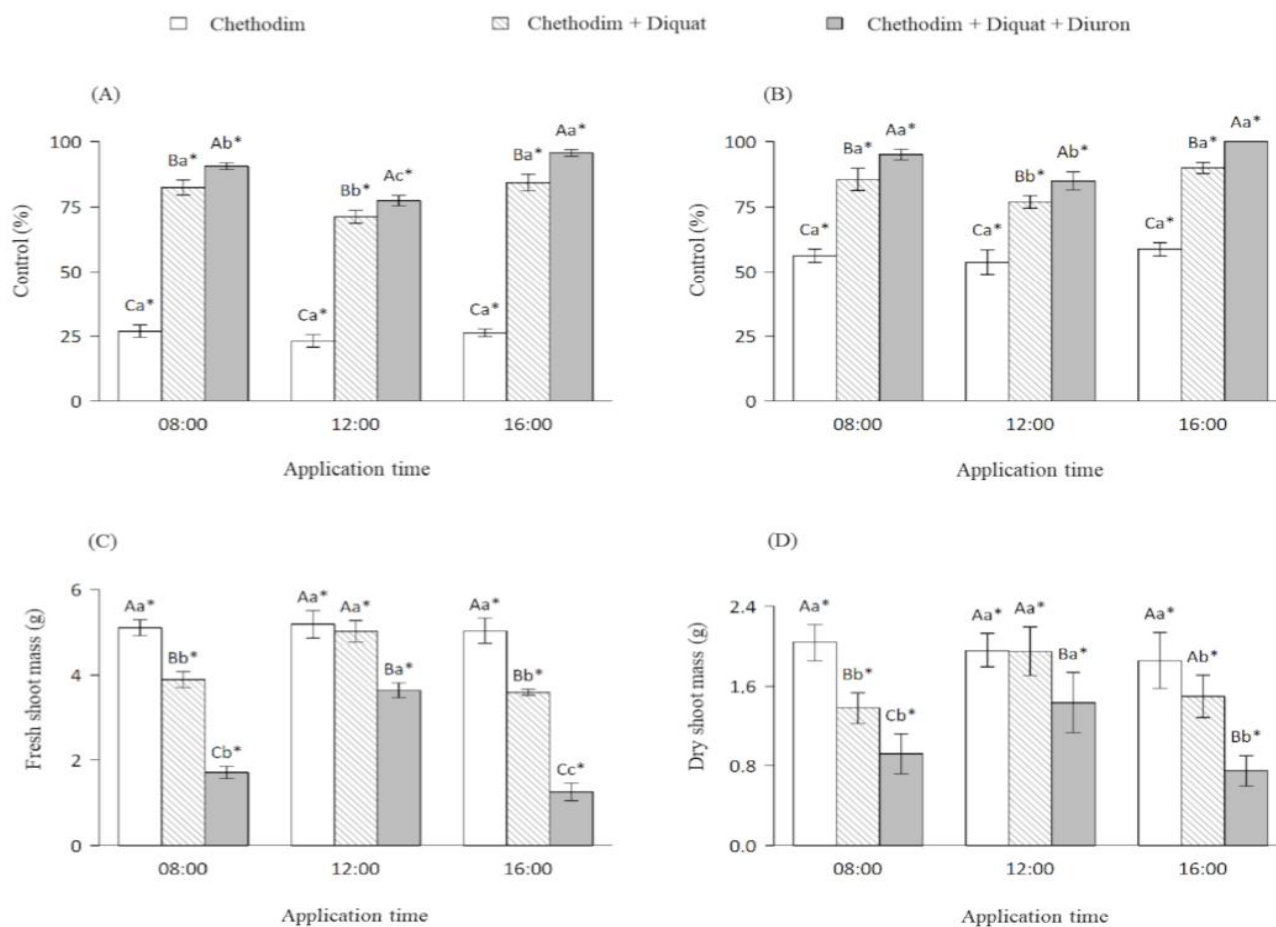
### Experiment II – Vegetation house

Similar to that observed in the field, there was a significant interaction between the factors evaluated for all variables. The controls at 7 DAT (Figure 2A) and 15 DAT (Figures 2B and 3) of sourgrass plants in the greenhouse showed responses statistically equal to those in the field experiment. Thus, clethodim + diquat + diuron applied at 16:00 resulted in better control rates of 98.8% and 100% at 7 and 15 DAT, respectively. This treatment led to plant death without the occurrence of regrowth (Figure 3I). Clethodim + diquat resulted in better control rates when applied at 08:00 and 16:00 at 7 DAT and 15 DAT. However, at 15 DAT, the control was 93.8%, with green tissues remaining, enabling plant regrowth even when applied at 16:00 (Figure 3F).

In the MFPA (Figure 2C) and MSPA (Figure 2D) evaluations, the clethodim + diquat + diuron treatment showed the best results, with the lowest mass indices in applications performed at 08:00 and 16:00, with reductions of 26.3% and 37.5% in MFPA, and 26.7% and 42.7% in MSPA, respectively, compared to the application at 12:00. Treatment with clethodim associated with diquat reduced MFPA and MSPA compared to clethodim alone at the three evaluation times. The evaluated mass variables of the herbicide treatments differed from that of the control, which showed a MFPA of 10.0 g pot<sup>-1</sup> and a MSPA of 3.3 g pot<sup>-1</sup>.

The rETR evaluations indicated that the photosynthetic activity of sourgrass plants treated with herbicide combinations was lower than that of the control, regardless of the application time (Figure 4). The greatest inhibition was observed in treatments containing FS inhibitors. Clethodim + diquat + diuron applied at 8:00 and 16:00 reduced the rETR by 76.2% and 83.2%, respectively, compared with the application at 12:00 (Figure 4). For the clethodim + diquat treatment, there was greater inhibition of rETR when applied at 16:00,

**Figure 1** - Control (%) at 7 days after treatment (DAT) (A) and at 15 DAT (B); fresh shoot mass (MFPA) (C) and dry shoot mass (MSPA) (D) of sourgrass plants in response to the application of clethodim, clethodim + diquat and clethodim + diquat + diuron at different application times under field conditions. The same capital letters between the sprays within each application time do not differ from each other, according to Tukey's test ( $p < 0.05$ ). Lowercase letters that are the same between application times do not differ from each other, according to Tukey's test ( $p < 0.05$ ). \*Differs from the control using Dunnett's test ( $p < 0.05$ ). The vertical bars indicate standard deviations



which was 53.5% and 74.9% lower than applications carried out at 08:00 and 12:00, respectively.

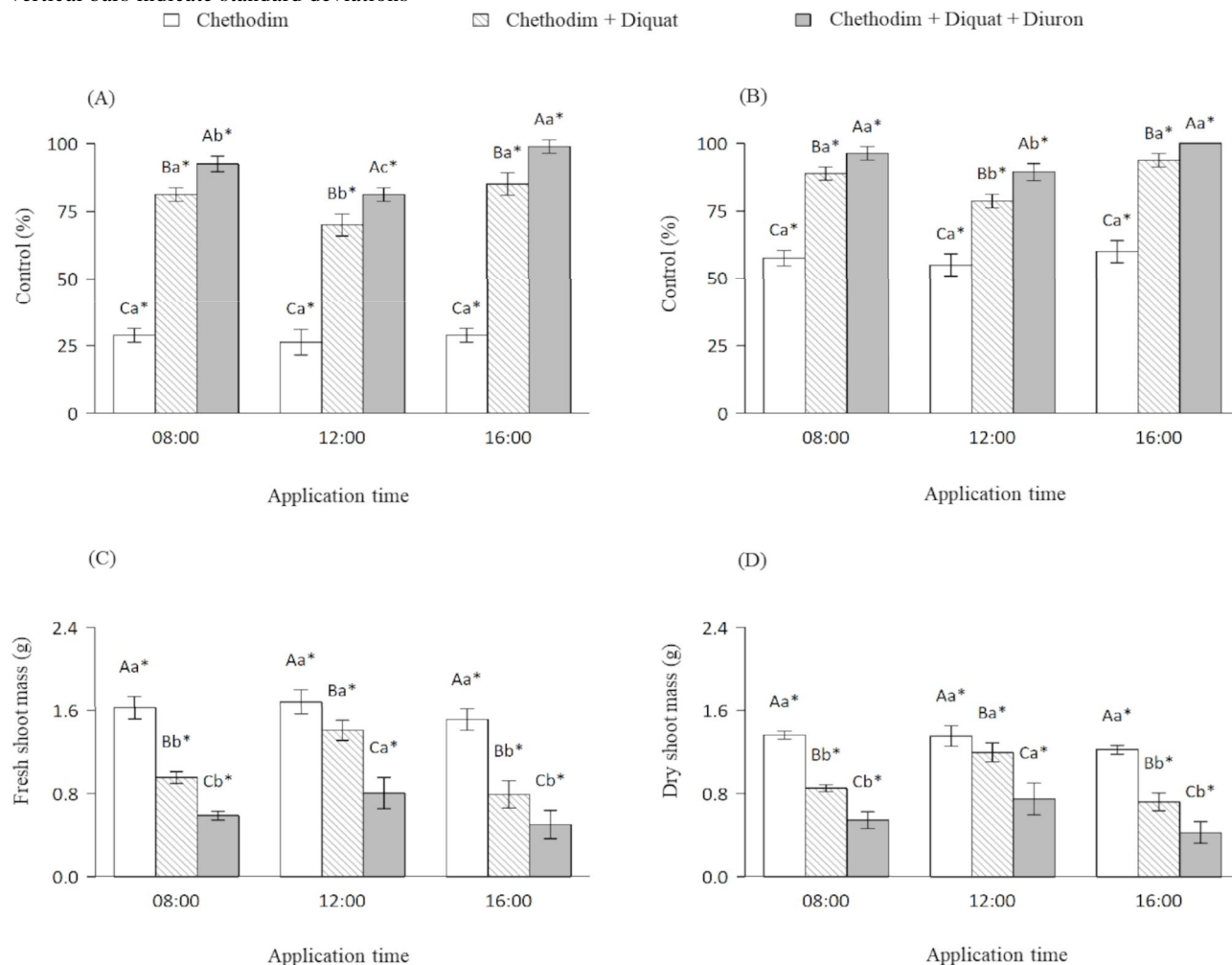
Unlike in the control and mass evaluations of sourgrass plants, there was a difference depending on the application time in the clethodim treatment alone. This herbicide applied at 08:00 resulted in greater inhibition of the rETR than that at other times. However, there was no significant difference between applications at 12:00 and 16:00. The evaluation time influenced the control without herbicide, with the lowest rETR at 8:00 and the highest at 12:00.

The evaluation of  $H_2O_2$  accumulation indicated that the addition of diuron to the mixture potentiated oxidative stress in sourgrass plants. The highest  $H_2O_2$  concentration was obtained with the application of clethodim + diquat + diuron at 16:00 (Figure 5). The

same herbicide combination resulted in lower concentrations of 19.9% and 32.3% when applied at 08:00 and 12:00, respectively. For the clethodim + diquat treatment, the highest accumulation of  $H_2O_2$  was observed in applications at 08:00 and 16:00, being 17% and 13% higher than that at 12:00 h. Oxidative stress in plants exposed to the herbicide clethodim alone was not influenced by the application time. This treatment resulted in the lowest  $H_2O_2$  concentrations compared to sprays containing FS inhibitors, regardless of the application time. All herbicide treatments differed from the control, which had the lowest  $H_2O_2$  concentration (approximately 6416.95 nmol  $g^{-1}$  FM).

The control evaluations negatively correlated with the fresh and dry masses of the aerial parts of the sourgrass plants measured at the end of the experiment (Figure 6). Furthermore, negative correlations were observed between the control percentages at 7 and 15 DAT and the rETR. However,

**Figure 2** - Control (%) at 7 days after treatment (DAT) (A) and at 15 DAT (B); fresh shoot mass (MFPA) (C) and dry shoot mass (MSPA) (D) of sourgrass plants in response to the application of clethodim, clethodim + diquat, and clethodim + diquat + diuron at different application times under greenhouse conditions. The same capital letters between the sprays within each application time do not differ from each other, according to Tukey's test ( $p < 0.05$ ). Lowercase letters that are the same between application times do not differ from each other, according to Tukey's test ( $p < 0.05$ ). \*Differs from the control using Dunnett's test ( $p < 0.05$ ). The vertical bars indicate standard deviations



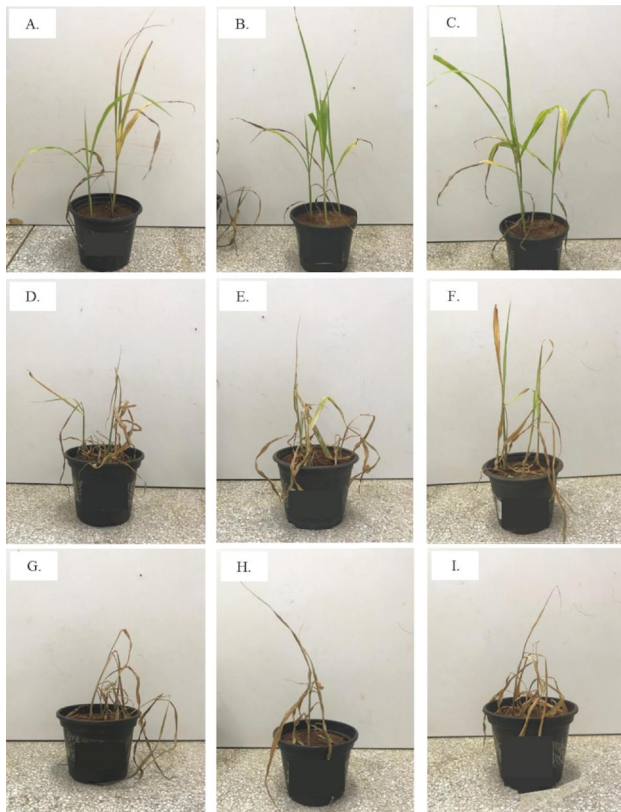
there was a positive correlation between control evaluations and leaf  $H_2O_2$  concentrations. The physiological variables, rETR and mass assessment, were positively correlated.

The results demonstrate that the mixtures containing diquat were more efficient when applied under lower light intensities (08:00 and 16:00). Thus, at 7 DAT (Figures 1A and 2A) and 15 DAT (Figures 1B and 2B), sourgrass plants exposed to mixtures containing diquat at 12:00 had lower controls than those exposed at others times in both experiments.

Diquat is a bipyridyl that acts as an electron acceptor in FSI and diverts electrons from ferredoxin to alternative receptors, such as oxygen, favoring the formation of ROS (HESS, 2000), which damages phospholipid membranes and culminates in cell death (LIMA-MELO *et al.*, 2019).

Its physicochemical characteristics (log Kow -4.26) allow it to be translocated through the phloem and the xylem (RODRIGUES; ALMEIDA, 2018). However, when applied under high light intensities (12:00), it causes tissue damage, which limits its translocation; thus, the accumulation of ROS is limited to the contact points of the herbicide with the plant (BRUNHARO; HANSON, 2017). In applications under lower light intensities (08:00 and 16:00), there is greater translocation of herbicides, and oxidative stress is more comprehensive (OLIVEIRA *et al.* 2022a, b), as observed 24 h after application (Figure 5). Therefore, the lower  $H_2O_2$  concentration observed when applying the clethodim + diquat mixture at 12:00 (Figure 5) corroborates the results obtained with the lower control of sourgrass plants compared to other times (Figures 1A, 1B, 2A, and 2 B).

**Figure 3** - Sourgrass plants in response to the application of clethodim, clethodim + diquat, and clethodim + diquat + diuron at different time points under greenhouse conditions. Symptoms were recorded 15 days after treatment (DAT). A: clethodim (08:00), B: clethodim (12:00), C: clethodim (16:00), D: clethodim + diquat (08:00), E: clethodim + diquat (12:00), F: clethodim + diquat (16:00), G: clethodim + diquat + diuron (08:00), H: clethodim + diquat + diuron (12:00), and I: clethodim + diquat + diuron (16:00)



Application of clethodim + diquat at 08:00 and 16:00 resulted in lower photosynthetic activity (Figure 4) than that at 12:00. The results for the control at 12:00 also proved that there was a greater rETR under greater light intensity, regardless of the application of herbicide sprays. However, at times of lower light intensity, the flow of electrons is slower, which allows the translocation of the syrup to the growth points (rhizomes and meristems) of the plants through conductive vessels (phloem), as these are not immediately damaged (PITELLI *et al.*, 2011). When exposed to greater light intensities, the flow of electrons in the FSI increased, starting the oxidative process (SEJIMA *et al.*, 2014), as observed in the ROS assessments; the highest H<sub>2</sub>O<sub>2</sub> concentration in plants exposed to diquat treatments was seen at 16:00 (Figure 5).

The combination of systemic herbicides and diquat caused foliar lesions more rapidly. This association initially appears to be effective; however, plant regrowth may occur over the long term (WEHTJE; ALTLAND; GILLIAM, 2008).

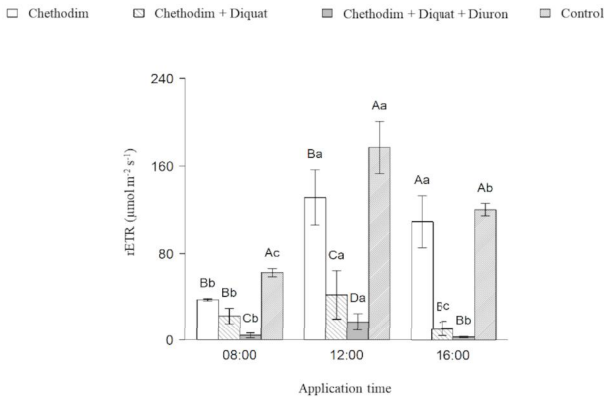
In addition to manipulating the application time, the addition of diuron also resulted in an increased control, thereby preventing plant regrowth (Figure 3I). The solution associated with diuron provided greater plant control at 7 DAT (Figures 1A and 2A) and 15 DAT (Figures 1B and 2B) than that with clethodim + diquat, regardless of the application time. Diuron acts on the FSII reaction center, preventing the transport of electrons to plastoquinone, which results in inhibited photosynthesis. The accumulation of electrons in FSII triggers ROS formation. This rapid inhibition of photosynthesis prevents the immediate action of diquat and damages cell membranes, favoring translocation to places where there is no contact with the sprayed droplets (HAYWARD; COLBY; PARHAM, 1988). Thus, in diuron applications, there was greater inhibition of rETR at 08:00 and 12:00 (Figure 4). Therefore, the correlations between the percentages of the control at 7 DAT or 15 DAT and the rETR were negative; that is, the higher the control indices, the lower the photosynthetic activity of the plants (Figure 6).

When comparing the application times of the clethodim + diquat + diuron combination, application at 08:00 and 16:00 resulted in greater plant control than that at 12:00 (Figures 1B and 2B). At times of lower light intensity, there is less activity of antioxidant enzymes, such as catalase, making the plant more susceptible to the application of photosystem-inhibiting herbicides (DALAZEN; MEROTTO JUNIOR, 2016). LAI *et al.* (2012) observed that in *Arabidopsis thaliana* (L.), catalase and H<sub>2</sub>O<sub>2</sub> synthesis is regulated by the circadian clock (CCA1 gene). Transcription of CCA1 is lower during times of low light intensity, which favors greater plant control when herbicides are applied under these conditions.

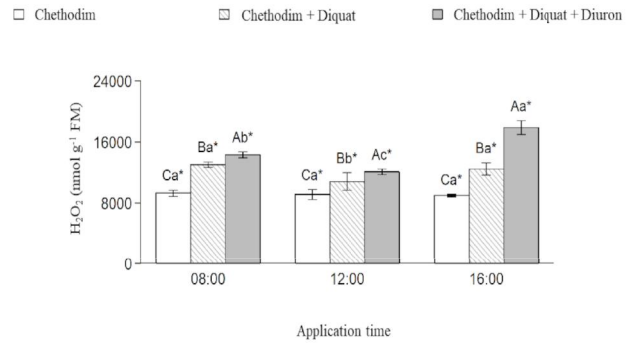
The clethodim treatment alone showed the lowest control of sourgrass plants at 7 DAT (Figures 1A and 2A) and 15 DAT (Figures 1B and 2B), regardless of the application time. Clethodim inhibits the ACCase enzyme, interrupting fatty acid synthesis, and consequently reducing lipid production in cell membranes. However, because it is characterized as a systemic herbicide, it requires a longer period to cause plant mortality.

The results of this study demonstrate that the addition of diuron makes enables the combination of clethodim and diquat without reducing the efficiency of sourgrass control

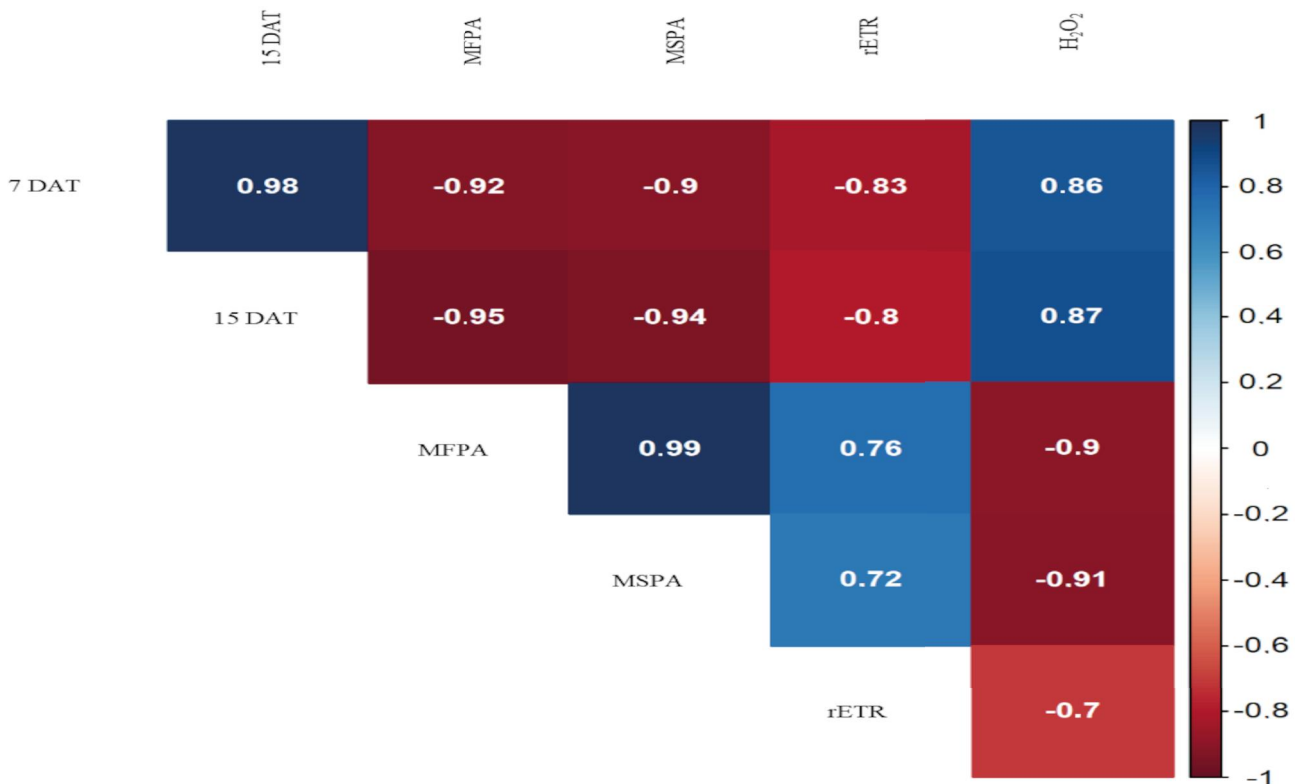
**Figure 4** - Relative rate of linear electron transport by photosystem II (rETR) in sourgrass plants in response to the application of clethodim, clethodim + diquat, and clethodim + diquat + diuron at different application times under greenhouse conditions. The same capital letters between the sprays within each application time do not differ from each other, according to Tukey's test ( $p < 0.05$ ). Lowercase letters that are the same between application times do not differ from each other, according to Tukey's test ( $p < 0.05$ ). The means used to construct Tukey's test were obtained by transforming the data using the square root ( $\sqrt{\quad}$ ). The vertical bars indicate standard deviations



**Figure 5** - Concentration of hydrogen peroxide ( $H_2O_2$ ) in the leaves of sourgrass plants in response to the application of clethodim, clethodim + diquat, and clethodim + diquat + diuron at different application times under greenhouse conditions. The same capital letters between the sprays within each application time do not differ from each other, according to Tukey's test ( $p < 0.05$ ). Lowercase letters that are the same between application times do not differ from each other, according to Tukey's test ( $p < 0.05$ ). \*Differs from the control using Dunnett's test ( $p < 0.05$ ). The vertical bars indicate standard deviations



**Figure 6** - Pearson correlation coefficient ( $r$ ) of the control variables (%) at 7 days after treatment (DAT) and at 15 DAT, fresh shoot mass (MFPA), dry shoot mass (MSPA), rate relative linear electron transport by photosystem II (rETR) and foliar concentration of hydrogen peroxide ( $H_2O_2$ ) of sourgrass plants in response to the application of clethodim, clethodim + diquat, and clethodim + diquat + diuron at different application times under greenhouse conditions. \*Significant difference ( $p < 0.05$ )





using three to four tillers. This herbicide combination resulted in 100% control and prevented plant regrowth, particularly if applied at 16:00 (Figures 1B, 2B, and 3I). In practice, the possibility of applying clethodim + diquat in a mixture with diuron can optimize the operational performance of cultivation systems by reducing the number of applications to control sourgrass, thus avoiding the use of clethodim in the first application followed by a sequential application with diquat. However, further studies on the more advanced stages of sourgrass development are needed.

## CONCLUSIONS

1. The application of diquat in association with clethodim under a lower light intensity (08:00 or 16:00) resulted in better control of sourgrass from three to four tillers compared to the application at 12:00. However, to avoid total plant control and regrowth, the addition of diuron is necessary, resulting in greater inhibition of photosynthetic activity and accumulation of ROS.

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