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GROWTH INHIBITION OF SOURGRASS AS A FUNCTION OF PERIOD OF DARKNESS AFTER DIQUAT APPLICATION

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Abstract

Photosystem-inhibiting herbicides, such as diquat, act by inducing oxidative stress. However, oxidative damage impairs translocation, resulting in regrowth of the plants. The aim was to evaluate the effectiveness of diquat in controlling the growth of sourgrass exposed to different periods of darkness after application of the herbicide, as well as to evaluate the photosynthetic activity and the production of reactive oxygen species. Two experiments (field and greenhouse) were conducted by applying diquat (200 g a.i. ha^{-1}) on sourgrass plants at the 3 to 4 tiller stage. The treated plants were subjected to different periods of darkness after diquat application (0, 1, 2, 3, 4, 5, and 6 h), in addition to the control treatment without any application. Growth inhibition and mass evaluations of the sourgrass plants were performed in both experiments, whereas photosynthetic activity and H_2O_2 accumulation in the leaves were evaluated in the greenhouse experiment. The results showed an increase in the sourgrass growth inhibition with an increase in the period of darkness after application. There was a need for a minimum of 6 h of darkness after diquat application to fully inhibit growth (100%) of the sourgrass, whereas plants that remained in the sun since application exhibited less than 50% inhibition. The increase in the period of darkness after diquat application resulted in a reduction in photosynthetic activity and, consequently, lower accumulation of H_2O_2. Thus, the maintenance of sourgrass in the dark for at least 6 h enables total control of the growth of the plants, preventing regrowth.

Keywords: Application time. *Digitaria insularis* (L.) Fedde. Oxidative stress. Photosystem I inhibitors. Reactive oxygen species.

1. Introduction

Sourgrass (*Digitaria insularis* (L.) Fedde) is a weed member of Poaceae, which propagates through rhizomes and seeds (Machado et al. 2008). The production of light and hairy seeds facilitates dispersion by wind over long distances. Dispersal through the transport of contaminated agricultural machinery, makes sourgrass a weed occuring in the cultivation systems of much of the Brazilian territory and other countries of South America (Gomes et al. 2017; Lopez Ovejero et al. 2017). In addition, the advent of genetically modified cultivars like Roundup Ready® (RR) led to the indiscriminate use of glyphosate, which resulted in the selection of resistant biotypes (Heap 2021), requiring the use of herbicides with different mechanisms of action (Gilo et al. 2016).

One of the methods for controlling this weed is through application of graminicides, such as inhibitors of the enzyme, acetyl-CoA carboxylase (ACCase) (Lopez Ovejero et al. 2017; Andrade et al. 2018). However, rotation of herbicides with different mechanisms of action is necessary to avoid the emergence of resistant biotypes. Thus, the use of photosystem I (PSI) inhibitors, such as paraquat and diquat, is also an option to control sourgrass, mainly in desiccation operations (Cobb and Reade 2010) and in sequential applications after systemic herbicides (Andreotti et al. 2019). However, with the ban on the commercialization and use of paraquat in Brazil (Anvisa 2017), the herbicide diquat is the only option left.

Diquat 6,7-dihydrodipyrido[1,2-a:2',1'-c]pyrazine-5,8-diiumm dibromide is an herbicide belonging to the group of bipyridyls, which comprises of divalent cation herbicides that deflect the flow of electrons in PSI (Rodrigues and Almeida 2018). These electrons react with oxygen in the presence of light to form reactive oxygen species (ROS), such as $O2^{-\bullet}$ (superoxide anion), H_2O_2 (hydrogen peroxide), and $OH^{\bullet}$ (hydroxyl radical). The accumulation of ROS results in oxidative stress in plants due to lipid peroxidation and changes in membrane stability, which can trigger cell death (Hawkes 2014; Chang et al. 2016).

This herbicide is known to have greater growth control efficiency in eudicotyledons (Calderbank and Slade 1976), as these plants usually have a larger leaf area, which allows better exposure to the sprayed drops. The limitations to the use of diquat in grasses occur due to the leaf architecture, presence of trichomes, and mainly due to the presence of crystalline epicuticular wax, which interferes with leaf wetness and in the retention period of the drops, reducing control efficiency (De Ruiter et al. 1990; Machado et al. 2008). Furthermore, in eudicotyledons, meristems are exposed, whereas in grasses, during the vegetative phase, the apical meristem is usually positioned internally at the base of the stems, close to the soil surface, which makes control difficult (Costa et al. 2004). Another limiting factor is that in some Poaceae, such as sourgrass, there is the presence of starch-rich structures that allow plants to regrow (Machado et al. 2008).

Owing to the limited translocation, the action of diquat tends to be more efficient with a high application rate (>200 L of spray solutions ha^{-1}) and adjuvants, which allow the spreading of the spray drops, resulting in an increase in leaf cover and, consequently, herbicide absorption (Costa et al. 2014; Gitsopoulos et al. 2014). Translocation is also self-inhibited in applications under full sunlight, and the efficiency of the herbicide is restricted to plants in the initial growth stage or in sequential applications, after the application of systemic herbicides (Tahmasebi et al. 2018).

The rapid production of ROS in applications carried out in light suggests that night application can improve the efficiency of this herbicide. Pitelli et al. (2011) observed that night applications (9:00 p.m.) resulted in greater control of waterhyacinth (*Eichhornia crassipes* (Mart.) Solms) in relation to plants treated at 1:00 p.m. Its application in the night avoids the immediate damage caused by the accumulation of ROS and, thus, allows greater translocation of the herbicide, potentiating oxidative stress when exposed to light (Montgomery et al. 2017).

Although it is known that nocturnal application improves the efficiency of bipyridyls, there is no information on the control of sourgrass and the period of absence of light necessary for the translocation of the herbicide to occur before oxidative stress occurs. Thus, the aim of this study was to evaluate the effectiveness of diquat in sourgrass plants subjected to different periods of darkness after the application as well as to evaluate the photosynthetic activity and production of ROS.

2. Material and Methods

Two experiments were carried out during 2019, one in the field and the other in a greenhouse.

Characterization of the Experimental Area, Origin, and Production of Plant Material

Experiment I – Field

The experiment was conducted in plots with dimensions of 6.0 m in length and 4.0 m width (24 m^2), which is considered a useful area for evaluations of one m^2 in the central area of each plot. The soil in the experimental area was identified as dystrophic red latosol, consisting of 12% sand, 36% silt, and 52% clay; pH ($CaCl_2$) 4.8; 20.1 g dm^{-3} organic matter and 8.8 $cmol_c$ dm^{-3} cation exchange capacity. The area where the

experiment was conducted was fallow, with natural infestation of sourgrass resistant to glyphosate. During the experiment (October and November, 2019), the total rainfall was 79 mm, and the average air temperature was 25.4 °C (IDR Paraná 2019).

Experiment II – Greenhouse

The experiment was conducted in expanded polystyrene pots with a volume of 0.5 dm^{-3}, filled with sieved dystrophic red latosol, consisting of 30% sand, 25% silt, and 45% clay; pH ($CaCl_2$) 5.3; 20.1 g kg^{-1} organic matter and 9.9 $cmol_c$ dm^{-3} cation exchange capacity. Sourgrass seeds were collected in the same area in which the field experiment was conducted and sown in plastic trays containing a 10 cm layer of organic substrate. After germination, each seedling of sourgrass was transplanted, one per pot. The plants were irrigated by daily sprinkling from the transplant until the day before the treatments. After that, the soil was directly irrigated to avoid interference with the absorption of the herbicide through leaves.

Experimental Design and Treatments

In both experiments, the adopted design was completely randomized, with eight treatments and four repetitions. Diquat (Reglone®, 200 g a.i. L^{-1}, Syngenta) was used at a dose of 200 g a.i. ha^{-1}, with 0.5% (v/v) of aliphatic and aromatic hydrocarbon adjuvants (Joint'Oil®, Dow AgroSciences). The treatments consisted of different periods of darkness: 0, 1, 2, 3, 4, 5, and 6 h after application of spray solution, in addition to a control treatment without herbicide application. The dark incubation was achieved by covering the plants completely with boxes consisting of three layers of corrugated cardboard. In experiment I, which was conducted in the field, this coverage was performed on 1 m^2 in the central area of the plots.

Application of Treatments

In both experiments, the spray solution was applied when the plants were approximately 0.15 m high with 3 to 4 tillers. The spraying was performed with a CO_2 pressurized backpack sprayer equipped with a pre-orifice flat fan ADI 11002 spray nozzle 0.5 m apart and 0.5 m from the target surface. The working pressure used on the sprayer was 0.414 MPa, at a speed of 1.0 m s^{-1}, resulting in an application rate corresponding to 150 L of spray solution ha^{-1}. The spraying was performed at 11:00 a.m. under suitable weather conditions, with air temperature below 30 °C, relative air humidity above 55%, clear skies, without clouds.

Evaluations

In both experiments, visual growth inhibition evaluations were performed at 7 and 15 days after treatment (DAT). In each evaluation, scores were given on a percentage scale, where zero and 100 represent the absence of injury and death of the plants, respectively. At 15 DAT, the plants were collected and weighed to determine the shoot fresh weight (SFW). Subsequently, the samples were packed in paper bags and dried in an oven with forced air circulation at 60 °C until they reached constant mass, in order to determine the shoot dry weight (SDW). In experiment I, conducted in the field, the evaluations for determining SFW and SDW were carried out with three plants per experimental unit, randomly chosen in the useful area of the plots.

In the greenhouse experiment, photosystem II (PSII) activity and H_2O_2 accumulation were also evaluated. PSII activity was assessed by chlorophyll *a* fluorescence measured in intact leaves from the middle third of the plant early in the morning (at 7:00 am), 20 h after herbicide application, using an OS1p fluorometer (Opti-Sciences, Hudson, NH, USA). The maximum quantum yield of PSII was determined by estimating the F_v/F_m ratio in dark-adapted leaves for 15 min, where F_v is the variable fluorescence and F_m is the maximum fluorescence obtained after applying a saturating light pulse (Baker 2008). The effective quantum yield of PSII (YII) was detemined using the $\Delta F/F_m'$ ratio, where ΔF and F_m' are the variable chlorophyll and the maximum fluorescence, respectively, measured inlight-adapted leaves. The relative rate

of linear electron transport by PSII (rETR) was calculated as rETR = $\Delta F/F_m' \times PAR \times 0.5 \times 0.84$, where PAR is the photosynthetically active radiation, 0.5 is the light partition between the photosystems, and 0.84 is the leaf absorption coefficient (Baker 2008). Although diquat is a PSI inhibitor, the ROS produced as a result affects several components of chloroplasts (including membranes and photosynthetic pigments), which also leads to the inhibition of PSII activity (Schmitz-Eiberger and Noga 2001).

The evaluation of ROS accumulation was performed by determining the content of H_2O_2 in a medium third leaf per experimental unit, 7 h after diquat application. At the time of collection, the samples were individually placed in aluminum foil envelopes, immediately immersed in liquid nitrogen, and transferred to a biofreezer at -80°C until analysis. The leaves (0.1 g) were macerated in a crucible with liquid nitrogen and extracted with 1.2 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 for H_2O_2 measurement by reaction with 1 M potassium phosphate buffer (pH 7.5). Absorbance was measured using a microplate reader (Perkin Elmer, model Victor TM 3, Turku, Finland) at 390 nm, and H_2O_2 levels were calculated using a standard curve made with known concentrations of H_2O_2, and expressed in µmol g^{-1} of SFW (µmol g^{-1} FW) (Alexieva et al. 2001).

Statistical Analysis

In both experiments, the data collected were analyzed using descriptive statistics to study the central tendency, dispersion, and verification of the presence of outliers. The means of the evaluations were described by regression models ($p < 0.05$). Parameter estimators of the model to be used were defined, and then the adequacy of the model to describe the desired phenomenon was verified. The regression model was chosen according to the hypothesis test, the simulated envelope, the adjusted determination coefficient (R^2), Pearson's correlation coefficient (r), residual analysis, and the partial F test.

After the exploratory analysis, residual analyses were performed for the data distribution after the normality, homoscedasticity of variance, and the independence of error tests by Shapiro-Wilk, Bartlett, and Durbin Watson, respectively ($p < 0.05$). After accepting the assumptions, an analysis of variance (ANOVA) was performed. The control data without herbicide application were not considered in the regression analysis in order to meet the assumptions of ANOVA. Transformations of growth control data were performed at 7 ($\frac{x0,5-1}{0,5}$) and 15 DAT ($\frac{x0,5-1}{0,5}$) of the field experiment, in addition to the growth control data at 7 DAT ($\frac{x1,5-1}{1,5}$) and SDW ($\frac{x0,1-1}{0,1}$) from the greenhouse experiment. Pearson's correlation (r) ($p < 0.05$) was performed for the variables of Experiment II. Statistical analysis were performed using R software (R CORE TEAM 2020).

3. Results

Experiment I – Field

The adjusted linear regression model showed a positive association between the period of darkness after diquat application and the growth inhibition of sourgrass plants (Figures 1A and 1B). Thus, as the period of darkness increased after herbicide application, the growth inhibition levels increased at a rate of 12.5% and 14.4% every hour of darkness at 7 and 15 DAT, respectively. It took 6 h of darkness after diquat application for the growth inhibition to be total (100%), both at 7 DAT (Figure 1A) and at 15 DAT (Figure 1B). In treatments with periods of darkness less than 4 h, the plants recovered at 15 DAT, reducing the growth inhibition levels compared to the evaluation at 7 DAT. In plants kept in sunlight since application, the growth inhibition in the last evaluation was only 20%, demonstrating the inefficiency of this herbicide with sourgrass when applied under full light conditions.

Regarding the growth evaluations, there was a negative association between SFW or SDW and the period of darkness after diquat application (Figures 1C and 1D). Corroborating the growth inhibition data, the greatest reductions in SFW and SDW occurred in the treatment with the longest period of darkness after herbicide application, with reductions of 57% and 56%, respectively, in relation to the treatment without darkness.

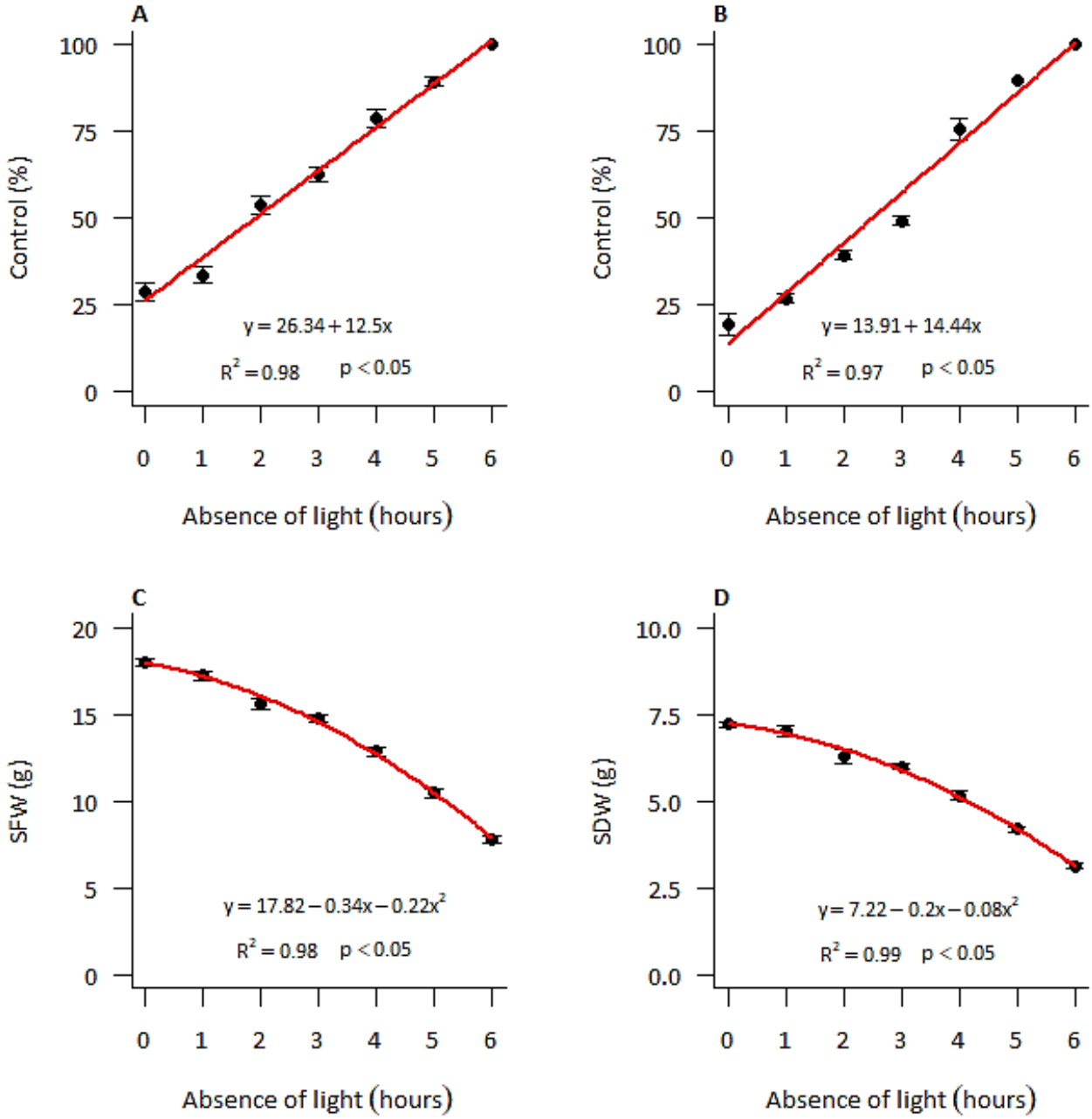

Figure 1. A - Growth inhibition (%) at 7 days after treatment (DAT) and B - 15 DAT, C - shoot fresh weight (SFW), and D - shoot dry weight (SDW) of sourgrass as a function of periods of darkness after the application of diquat in the field. The mean values used to construct the regression of the control variables at 7 and 15 DAT (A and B) were derived from the transformation of the data into $\frac{x^{0,5}-1}{0,5}$. The points indicate the means, and the vertical bars indicate the standard deviation.

Experiment II – Greenhouse

As observed in the field, the control of sourgrass plants with diquat under greenhouse conditions was positively associated with the period of darkness after herbicide application. When the plants were kept for 6 h in the dark after herbicide application, the growth inhibition was total at both 7 DAT (Figure 2A) and 15 DAT (Figures 2B and 3). When the plants were deprived of light for 5 h, the growth inhibition was approximately 90% in both evaluations, with plants displaying necrosis in practically all leaf tissue but maintaining a turgid and green stem, with the possibility of recovery (Figure 3). In the other treatments, with shorter periods of darkness, the growth inhibition was less than 80%, with recovery of the plants in the second evaluation, at 15 DAT (Figure 2B), compared to the evaluation at 7 DAT (Figure 2A). In treatment zero, in which plants were kept in sunlight after diquat application, the growth inhibition was 42% at 7 DAT, and it was reduced to 37% in the evaluation performed at 15 DAT, evidencing the low efficiency and plant

recovery when the application was carried out and the plants were kept in the light (Figures 2A, 2B, and 3). The reduction in the growth inhibition levels observed in these treatments was due to the occurrence of plant regrowth with the exposure of the green tissue from sourgrass plants (Figure 3). In these treatments, the injury caused by diquat occurred only in places where there was direct contact with the herbicide.

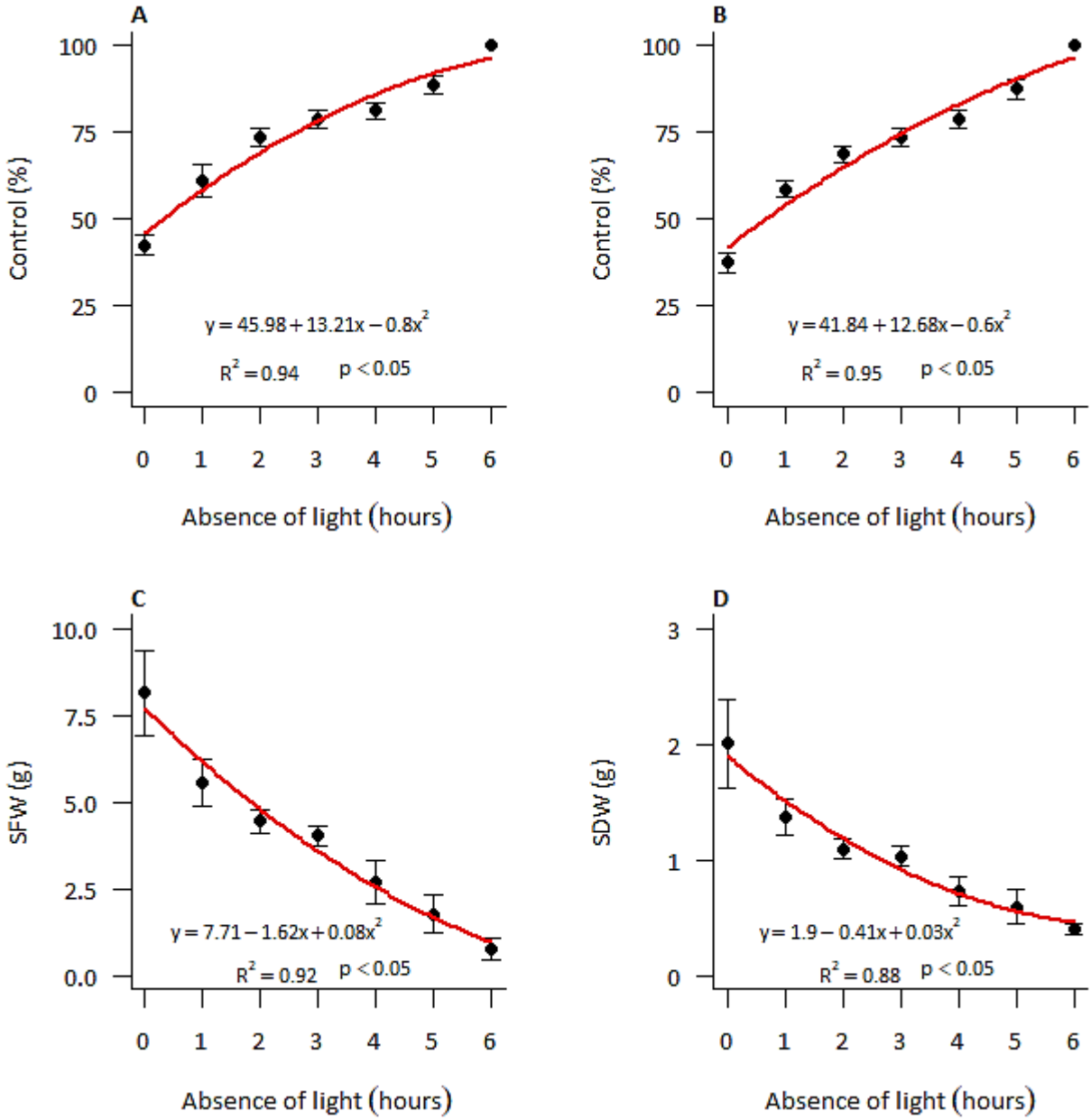

Figure 2. A - Growth inhibition (%) at 7 days after treatment (DAT) and B - 15 DAT, C - shoot fresh weight (SFW), and D - shoot dry weight (SDW) of sourgrass as a function of periods of darkness after the application of diquat in a greenhouse. The means used to construct the regression of the control variables at 7 DAT (A) and SDW (D) were derived from the transformation of the data into $\frac{x^{1,5}-1}{1,5}$ and $\frac{x^{0,1}-1}{0,1}$, respectively. The points indicate the mean values, and the vertical bars indicate the standard deviation.

As a result of the injury caused by diquat, there was interference in the accumulation of SFW and SDW. There was a negative association between the SFW or SDW of the sourgrass plants and the period of darkness after the application of diquat (Figures 2C and 2D). In the treatment with 6 h of darkness, these variables were reduced by 90% and 80%, respectively, compared to plants exposed to light immediately after application.

The evaluation of photosynthetic parameters indicated that the photosynthetic activity of diquat-treated sourgrass plants was reduced in response to light exclusion time, especially in treatments after 2 h of darkness (Figure 4). The F_v/F_m ratio decreased from 0.495 in plants that were exposed to light after herbicide application to less than 0.306 in treatments with 2 h or longer of darkness (Figure 4A). In the

control plants, without herbicide application, the F_v/F_m ratio was 0.77. The $\Delta F/F_m'$ values decreased from 0.47 to 0.09, compared to that of the treated plants that were not deprived of light with that of plants kept in the dark for 6 h (Figure 4B). In the control without herbicide, $\Delta F/F_m'$ was 0.54. As with the other photosynthetic parameters, rETR was also reduced as the period of darkness increased (Figure 4C). When plants were kept in light after diquat application, the rETR was 27.68 µmol m^{-2} s^{-1}, and only 5.08 µmol m^{-2} s^{-1} when plants were maintained for 6 h in the dark after diquat application. In the control treatment, the rETR was 34.55 µmol m^{-2} s^{-1}.

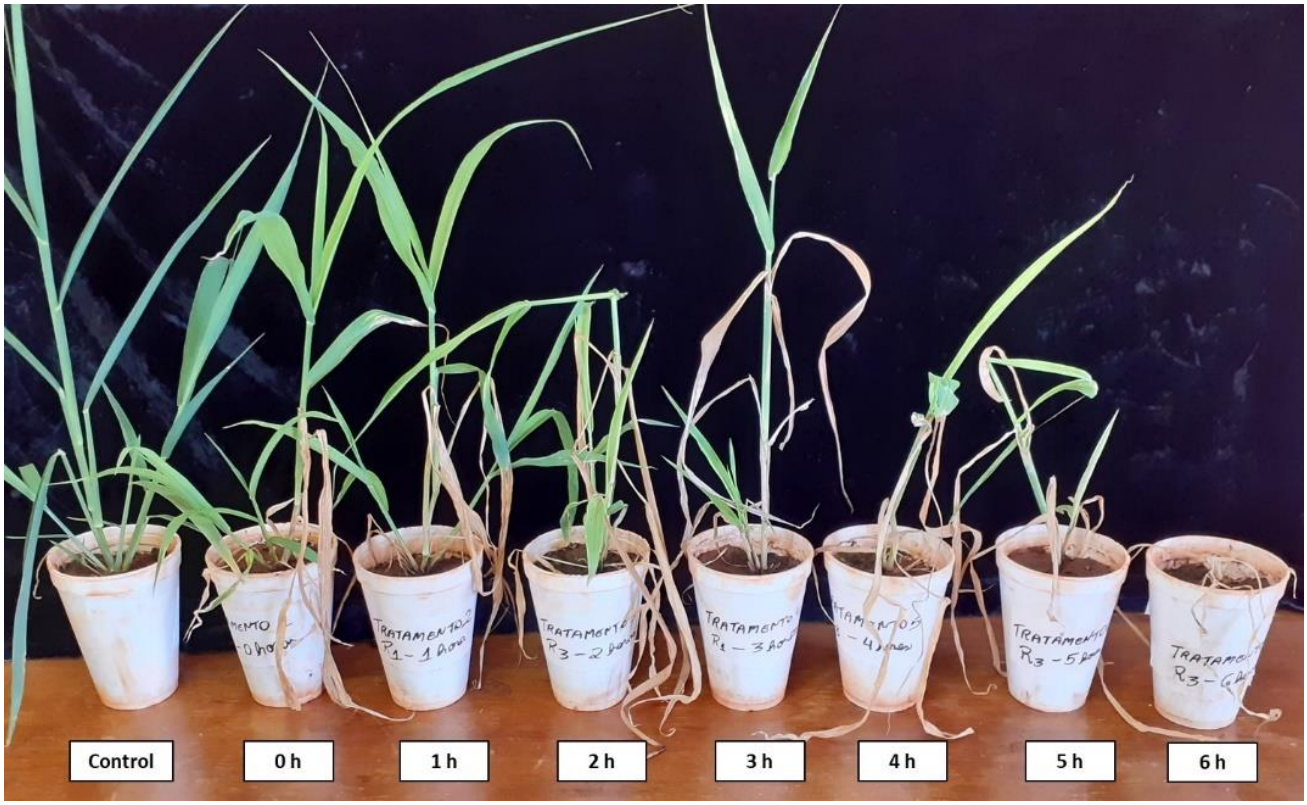

Figure 3. Sourgrass plants subjected to different periods of darkness (0, 1, 2, 3, 4, 5, and 6 h) after diquat application. The observations were recorded 15 days after diquat application. Control: refers to the control without herbicide application.

The results of H_2O_2 accumulation corroborated with the results for the photosynthetic parameters evaluated; that is, there was a reduction in the accumulation of this ROS with the increase in the period of darkness after diquat application (Figure 4D). In the treatment without light exclusion after application, the accumulation of H_2O_2 was approximately 7238.86 µmol g^{-1} FW. However, in plants that were kept in the dark for 6 h, the accumulation of H_2O_2 was approximately 2941.87 µmol g^{-1} FW, which was even lower than that in the control treatment without herbicide (3723.96 µmol g^{-1} FW), which remained in the light.

There was a positive correlation between the growth inhibition of sourgrass at 7 and 15 DAT (Figure 5). As expected, the growth inhibition in both evaluations was negatively correlated with the fresh and dry weights of sourgrass shoots. In addition, negative correlations were observed between the growth inhibition percentages at 7 or 15 DAT and the photosynthetic parameters of the sourgrass plants (F_v/F_m, $\Delta F/F_m'$, rETR, and leaf content of H_2O_2). All photosynthetic and mass variables correlated positively with each other.

4. Discussion

In both experiments, growth inhibition evaluations of sourgrass plants, both at 7 DAT (Figures 1A and 2A) and at 15 DAT (Figures 1B and 2B), indicated an increase in growth inhibition levels with an increase in the period of darkness after diquat application. There was a minimum requirement of 6 h of darkness after diquat application to obtain 100% plant mortality. The plants that remained for 5 h in the dark showed satisfactory control (> 80%); however, in treatments less than 4 h, the recovery of the plants was observed at 15 DAT, compared to the evaluation at 7 DAT.

Diquat is a fast-acting herbicide; however, the absence of light after application allows mobility inside the plants and reaches the growth points (Cobb and Reade 2010). This herbicide is characterized as hydrophilic (polar), passing through the plasma membrane mediated by the putrescine protein, which acts as a carrier for herbicide molecules which are structurally similar to natural compounds (Fujita and Shinozaki 2014). Its leaf absorption is fast, as the cuticle of the plant does not present a barrier to absorbtion (Bishop et al. 1987).

Because of its low log K_{ow} value (-4.26), diquat can be translocated either via xylem and ploem (Bromilow et al. 1990; Rodrigues and Almeida 2018). However, when the application is carried out under high light intensity, its immediate action damages the permeability of the membranes, restricting the damage to the area coming in contact with the sprayed drops (Brunharo and Hanson 2017). When plants were exposed to light from the moment of application, the damage is immediate, characterized by necrosis in places where there was direct contact with the herbicide, which resulted in inefficient control (Figure 3).

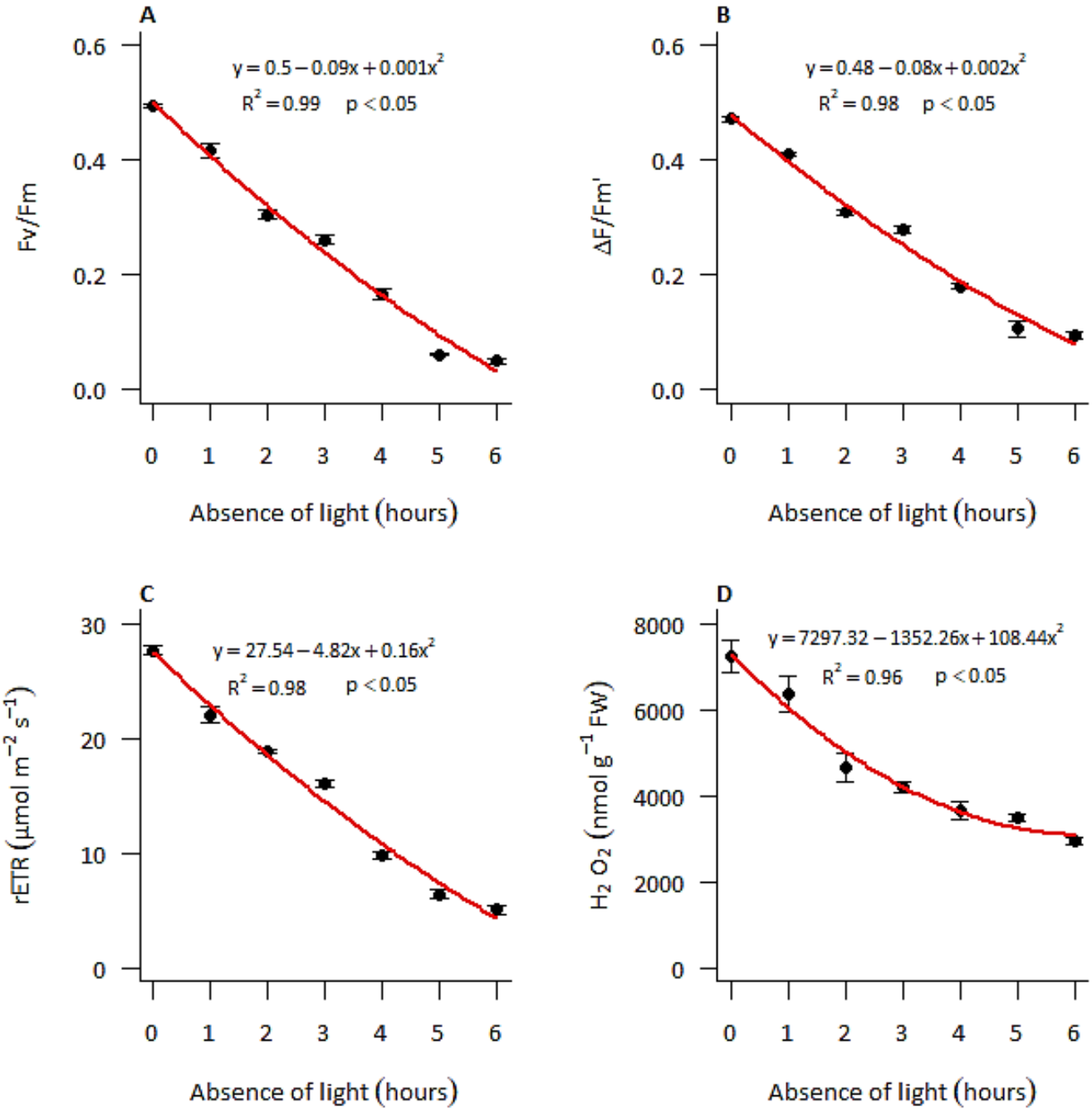

Figure 4. A - Maximum quantum yield of photosystem II (F_v/F_m); B - effective quantum yield of photosystem II ($\Delta F/F_m'$); C - relative rate of linear electron transport by photosystem II (rETR) and D - the accumulation of H_2O_2 in sourgrass plants as a function of periods of the darkness after the application of diquat in a greenhouse. The points indicate the mean values, and the vertical bars indicate the standard deviation.

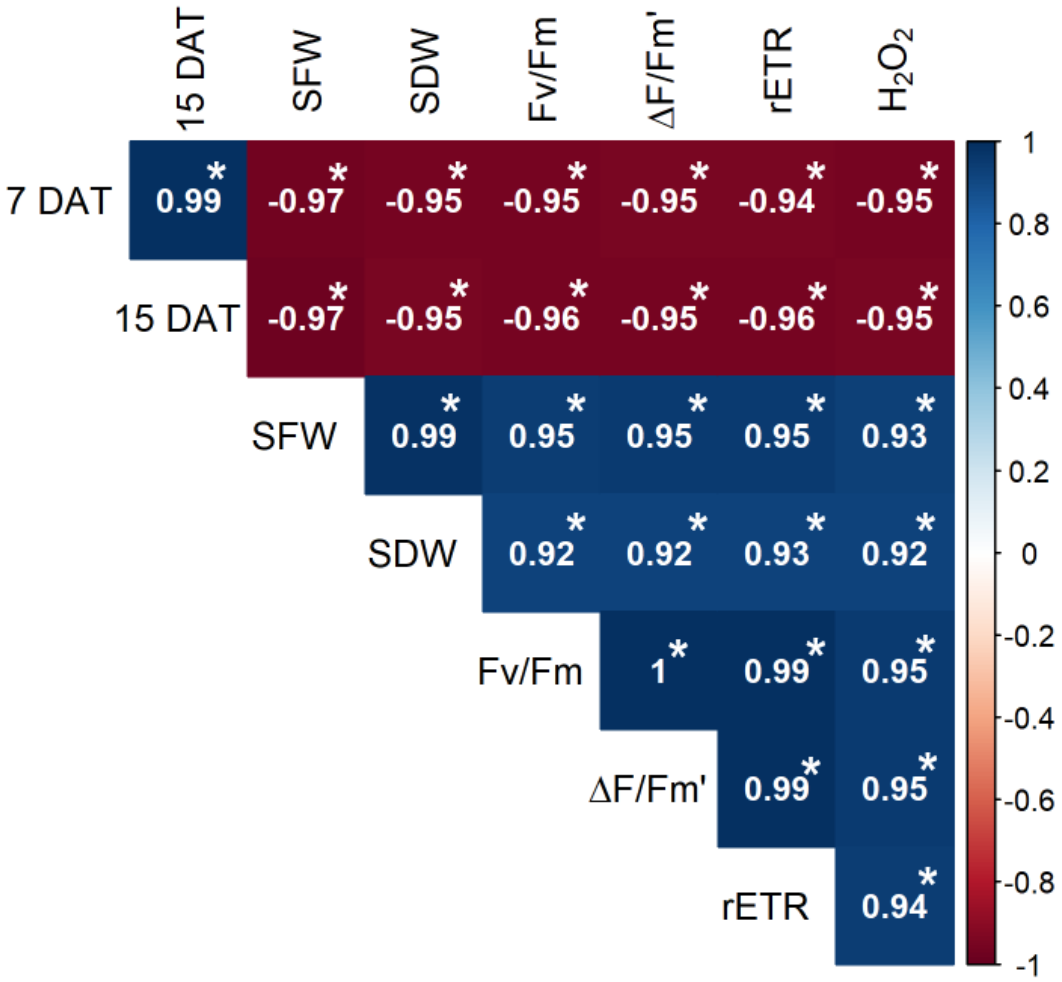

Figure 5. Pearson correlation of control variables (%) at 7 days after treatment (DAT) and at 15 DAT, shoot fresh weight (SFW), shoot dry weight (SDW), maximum quantum yield of photosystem II (F_v/F_m), effective quantum yield of photosystem II ($\Delta F/F_m'$), relative rate of linear electron transport by photosystem II (rETR), and leaf H_2O_2 concentration in corn plants as a function of darkness after diquat application in a greenhouse. * Significant ($p < 0.05$).

The increase in the period of darkness resulted in lower concentrations of H_2O_2 in the leaves, mainly in treatments with 5 and 6 h of darkness, with values lower than the control (Figure 4D). Diquat, like paraquat, belongs to the group of bipyridyls, which act on chloroplasts as an electron acceptor of ferredoxin in PSI (Brunharo and Hanson 2017). Therefore, they compete with $NADP^+$ for electrons, and as a consequence, ferredoxin donates electrons to O_2, forming species that are more reactive than molecular oxygen, such as H_2O_2, which affect phospholipid membranes by altering their integrity and interfering with protein activity (Hawkes 2014). The absence of light delays the immediate formation of ROS and thus preserves the stability of the conducting vessels, allowing the translocation of the herbicide, which enhances the growth control (Montgomery et al. 2017; Oliveira et al. 2022).

Plants that were not subjected to diquat application had a higher concentration of H_2O_2 compared to treatments with 5 and 6 h of darkness (Figure 4D), as they were exposed to light for a longer period, since the evaluation of the oxidative stress was carried out at the end of the day. In addition to the stress caused by the action of the herbicide, plants are also subjected to stress due to excess light, called photoinhibition, in which the energy produced cannot be dissipated, which results in the accumulation of ROS (Lima-Melo et al. 2019). The energy overload in the photosystems due to the high luminous intensity reduces photosynthesis (Blind et al. 2018). However, photosynthetic rate evaluations were carried out the day after the application (from 7:00 a.m.), enabling recovery of photosynthetic activity in plants without diquat application, characterized as dynamic photoinhibition (Guidi et al. 2019). The plants exposed to the herbicide, on the other hand, suffered permanent damage to their photosynthetic apparatus, which made it impossible to recover the F_v/F_m ratio (Figure 4A), $\Delta F/F_m'$ (Figure 4B), and rETR (Figure 4C). From chlorophyll

a fluorescence, it is possible to estimate the damage to PSII, as plants exposed to diquat have an impaired electron transport chain, which indirectly influences the activity of PSII (Brunharo and Hanson 2017).

The negative correlation between the data from the physiological evaluations and the results of plant growth inhibition shows that the increase in the period of darkness after the application of diquat contributes to the improvement in control efficiency (Figure 5). Moretti and Hanson (2016) observed that periods of darkness (4:00 p.m.), followed by periods of light, increased the translocation of bipyridyls in plants of *Conyza canadensis* (L.) Cronquist and *Conyza bonariensis* (L.) Cronquist. Similarly, Montgomery et al. (2017) observed that the use of bipyridyl herbicides at times with low light intensity has greater control of *C. canadensis* compared to full sunlight. When applied at 6:00 a.m. and 7:30 p.m., the growth inhibition was 66% and 96%, respectively, while at noon (12:00 p.m.), it resulted in only 25% growth inhibition.

In practice, the results of the present study suggest night applications of diquat, as long as there is a minimum of 6 h of darkness after application. In the early hours of the morning, after sunrise, the luminous intensity is generally low; therefore, the accumulation of ROS is also reduced, allowing greater mobility of the herbicide inside the plants and, thus, greater growth inhibition in relation to applications at noon. Therefore, the application of diquat in night has become a strategy to increase the efficiency of growth control and, consequently, avoid the regrowth of the sourgrass.

5. Conclusions

The herbicide diquat completely controls the growth of sourgrass plants at 3 to 4 tiller stage when kept in the dark for 6 h after application, preventing the occurrence of regrowth. The permanence in the dark of plants treated with diquat results in a reduction in photosynthetic activity and, consequently, less accumulation of ROS, which should allow the translocation of the herbicide to the meristems, causing the death of the weed.

Authors' Contributions: OLIVEIRA, G.M.P.: Conception and design, acquisition of data, analysis and interpretation of data, drafting the article, final approval; SILVA, M.A.A.: Critical review of important intellectual content, final approval; DALAZEN, G.: Conception and design, analysis and interpretation of data, drafting the article, critical review of important intellectual content and final approval. All authors have read and approved the final version of the manuscript.

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Ethics Approval: Not applicable.

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