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Phytoremediation of Brazilian Cerrado soil to reduce herbicide persistence using tropical grasses

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Abstract

Brazil has extensive areas covered in pastures with a large infestation of invasive plants. Picloram is one of the most widely used herbicides due to its residual properties in the soil. Phytoremediation is a promising technique for soil decontamination carried out by plants. The objective of this study was to investigate phytoremediation in soil from the Brazilian Cerrado biome through the cultivation of phytoremediating tropical grasses to reduce herbicide persistence. The experimental design was entirely randomized in a 4x6 factorial arrangement, corresponding to four cultivation systems (uncultivated soil, cultivated with *Brachiaria decumbens*, cultivated with *Sorghum bicolor* and cultivated with *Pennisetum glaucum*), and six doses (0, 2, 4, 8, 16, and 32 g i.a. L-1 ha-1) of the herbicide picloram (triethanolamine salt). The herbicide applied 48 hours before emergence (pre-emergence), resulting in 24 treatments with 4 replications. The established period for the phytoremediation species was 90 days after emergence. After 24 hours, soybean (*Glycine max*) was sown as a bioindicator species for the presence of picloram. Phytotoxicity, Falker® chlorophyll index, plant height and dry biomass of the aerial part and dry biomass of the roots of the phytoremediating species and the bioindicator species were evaluated and the data was submitted to analysis of variance. When significant, they were subjected to regression analysis for herbicide doses and Scott-Knott test for crop types, both with an error level of 5% probability. The tropical forage grasses showed resistance to the herbicide picloram with positive effects on phytoremediation in the analyzed variables in the bioindicators when applied at doses ranging from 2, 4 and 8 g i.a. L-1 ha-1. All the phytoremediating grasses had the potential to reduce the persistence of picloram in the soil when applied at doses within the commercial recommendation, even over a short period of 90 days.

Keywords: bioremediation, picloram, pastures, weeds, herbicide.

Introduction

Certain spontaneous plant species directly compete with cultivated plants for resources such as water, light, and nutrients, leading to reduced production and increased operational costs. Therefore, there is a need to adopt control methods to prevent losses and ensure productivity in the area (Silva et al., 2003; Costa et al., 2018).
The most effective and popular method for controlling spontaneous plants in crops is the application of herbicides. Its popularity is highlighted by its high operational efficiency, as it requires minimal labor and investment compared to other control methods (Pellegrini et al., 2007; Idziak et al., 2022).
However, like any other technique, the use of herbicides also has limitations. When the application technology is not used correctly, its efficiency is compromised. Poorly executed applications can have toxic effects on humans and animals, lead to weed resistance over time, increase the seed bank of uncontrolled species, and cause residual effects that damage subsequently planted crops (De Oliveira JR. et al., 2011; Nieweglowski Filho et al., 2014).
Synthetic auxin herbicides have been widely used for weed control in pastures, mainly because they are effective against dicotyledonous plants and have long persistence in the soil, which is an undesirable characteristic as it prevents the use of the area for subsequent agricultural crops affected by the product (Santos et al., 2006; Franceschi, 2017).
In this scenario, phytoremediation emerges as one of the alternatives for the decontamination of polluted areas, employing physiological processes promoted by plants and their associated microbiota. This technique achieves soil decontamination by rapidly removing toxic compounds, such as herbicides, with low associated costs, ease of

implementation, and non-invasiveness (Cunningham et al., 1996; Pires et al., 2005; Morina and Moreno, 2022).

To identify plant species with phytoremediation potential, one alternative is to select plants that have a certain resistance to herbicides and/or that colonize contaminated areas (Silva et al., 2019).

The objective of this study was to investigate phytoremediation in soil from the Brazilian Cerrado biome through the cultivation of phytoremediating tropical grasses to reduce herbicide persistence.

Results and Discussion

Developmental characteristics of tropical phytoremediation grasses

The analysis of variance for the plant height of the phytoremediating species (*B. decumbens*, *S. bicolor*, and *P. glaucum*) did not show any statistically significant difference for the interaction between the crop factors and herbicide doses, according to the F test at a 5% probability level. The mean heights over the periods of 15, 60, 75, and 90 days after emergence (DAE) were 31.14 cm, 98.78 cm, 102.09 cm, and 102.64 cm, respectively.

When analyzing the isolated effect of the crop factor (Figure 1), a significant difference was observed among the treatments when comparing the means using the Scott-Knott test at a 0.05% probability level. According to Silva et al. (2021), for a plant to be considered a good remediator, it should also have the ability to develop in the presence of the contaminant and survive without reducing its growth rate.

P. glaucum plants showed the highest mean heights (36.47 cm, 92.23 cm, 112.80 cm, 119.27 cm, 125.30 cm, and 127.15 cm) in all evaluated periods, respectively. At 15 DAE, *S. bicolor* showed the highest mean height (36.09 cm), but at 75 DAE and 90 DAE, the means were lower than the other crops (82.08 cm and 82.43 cm). *B. decumbens* had the lowest values in the first four evaluations at 15, 30, 45, and 60 DAE (20.85 cm, 75.23 cm, 81.55 cm, 93.21 cm). At 75 and 90 DAE (98.88 cm and 98.33 cm), the values were higher than those of *S. bicolor* and lower than those of *P. glaucum*. One of the modes of action of picloram is through hormonal activity, thus the response in plant height is similar to that of natural plant auxin (IAA). However, excessive doses cause alterations that promote physiological disturbances, especially in the younger and aerial parts of the plants, leading to abnormal elongations at random points on the stem (Brito et al., 2021).

A decreasing statistical difference among the treatments was observed over the evaluated period, when analyzing the variable based on the doses used (Figure 2), with the height values adjusted to the quadratic regression model. It can be inferred that the effect of herbicides on phytoremediating species is more evident in the early days after emergence, resulting in slower plant growth. However, as time passes and the species establish themselves, the difference in growth gradually decreases, indicating that the effects of herbicides tend to diminish over time.

The lowest height values were found in all periods when the dose of 32 g a.i. L^{-1} ha^{-1} was used, demonstrating that even the phytoremediating species had their size reduced with increasing doses. Madalão et al. (2013), evaluated the susceptibility of plant species with phytoremediation potential to the herbicide sulfentrazone, and observed that the reduction in plant height with increasing herbicide doses was more gradual, indicating a less intense effect on plant architecture.

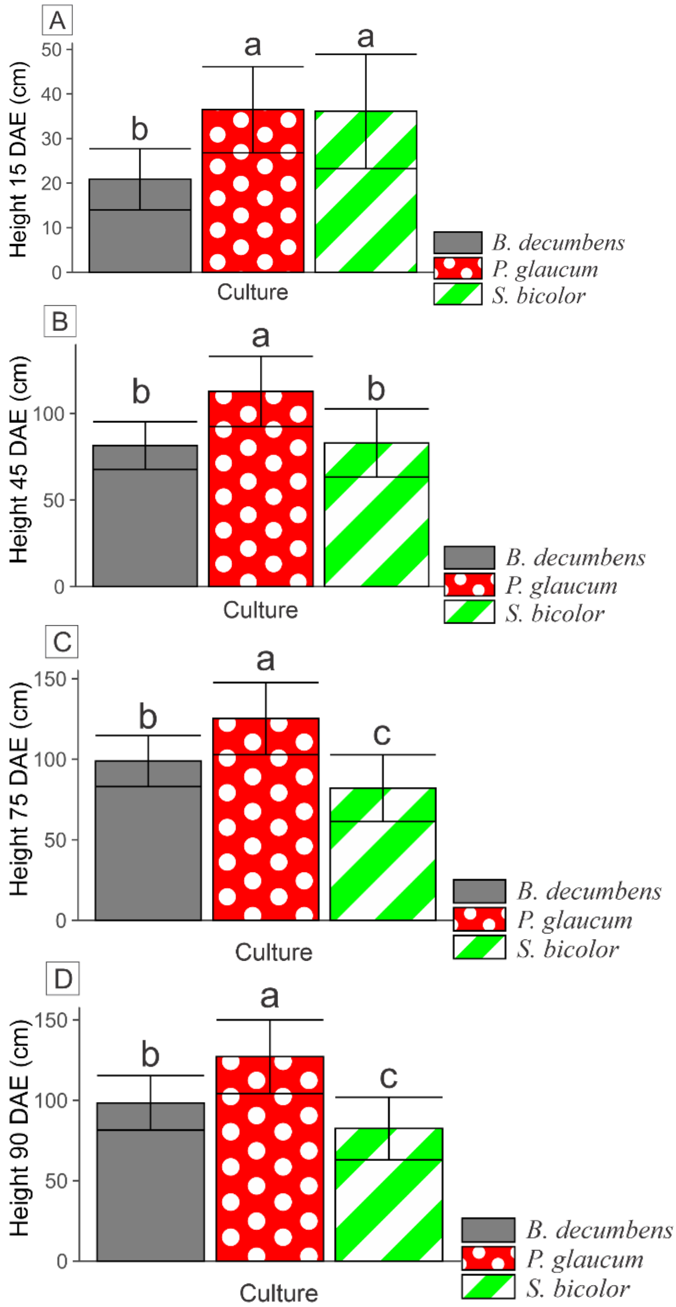

Figure 1. Plant height of phytoremediation species in DAE: (A) 15; (B) 45; (C) 75; (D) 90. Values followed by the same letter do not differ statistically according to Scott-Knott p>0.05.

The Falker Chlorophyll Index of the phytoremediating species did not show any statistically significant difference for the interaction between the crop factors and herbicide doses, according to the F test at a 5% probability level, with the following means: 26.10, 29.10, 24.59, 19.95, 20.92, according to the evaluated periods (15, 60, 75, and 90 days after emergence (DAE)). However, a statistical difference was observed among the crops when compared separately using the Scott-Knott test at a 5% probability level. The highest values of the Falker Chlorophyll Index were found for *B. decumbens* in all evaluated periods (27.06, 28.56, 23.15, 24.85), followed by *S. bicolor* (28.21, 22.90, 21.09, 21.56). *P. glaucum* recorded the lowest results in all evaluations (23.04, 22.31, 15.62, 16.33) (Figure 3).

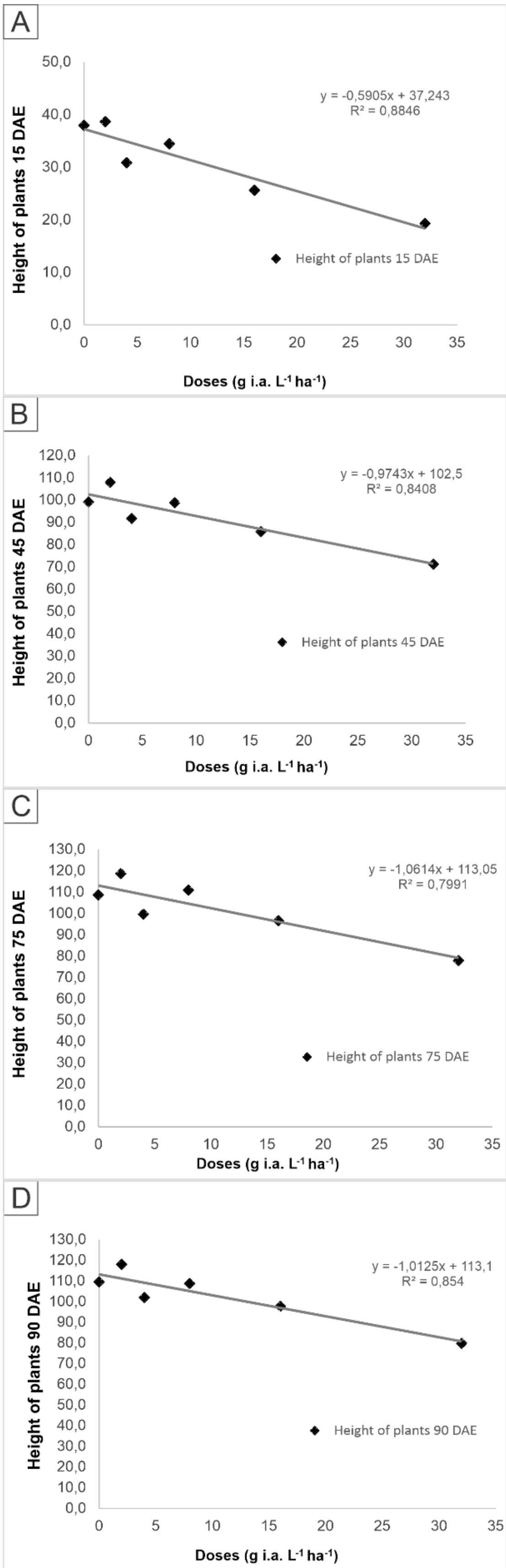

Figure 2. Plant height of phytoremediation species in relation to herbicide doses in DAE: (A) 15; (B) 45; (C) 75; (D) 90.

Significance was found with adjustment to the quadratic regression model in all evaluated periods, when analyzing the isolated factor of herbicide doses (Figure 4), except for the

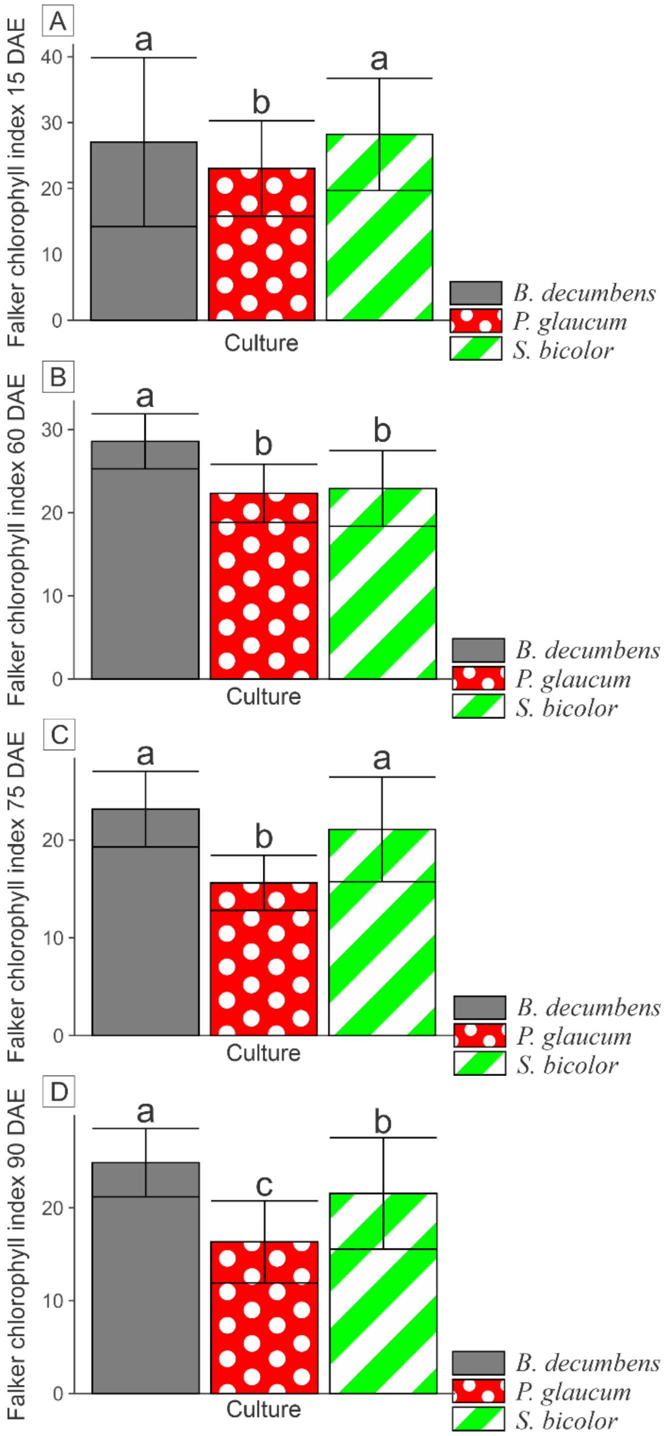

Figure 3. Falker chlorophyll index of phytoremediation species in DAE: (A) 15; (B) 60; (C) 75; (D) 90. Values followed by the same letter do not differ statistically according to Scott-Knott p>0.05.

results obtained at 60 DAE, where there was no statistical difference. It was found that the dose of 32 g a.i. L^{-1} ha^{-1} resulted in the lowest chlorophyll content.

As observed by Vendrame et al. (2015), auxin herbicides induce stomatal closure and can cause disturbances in photosynthetic activity and respiration, as these processes depend on various factors such as the constant flow of CO_2 and O_2 (Zhao; Wang, 2010; Franco et al., 2015). This alteration, reducing photosynthetic activity, leads to a significant decrease in chlorophyll content in leaves, and this effect can be observed even in plants considered tolerant

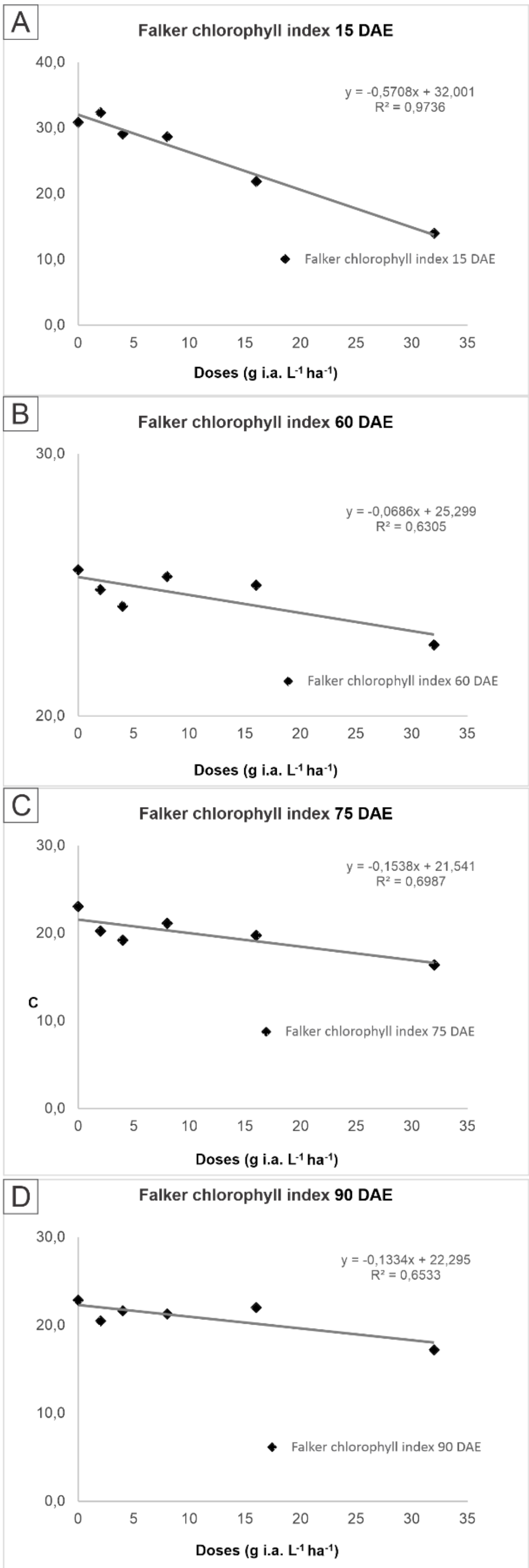

Figure 4. Falker chlorophyll index of phytoremediation species in relation to doses in DAE: (A) 15; (B) 60; (C) 75; (D) 90.

(Vendrame et al., 2015). Thus, any effect caused by auxin-mimicking herbicides that leads to reduced water absorption or translocation can affect stomatal conductance, reducing the photosynthetic rate (Belo et al., 2011).

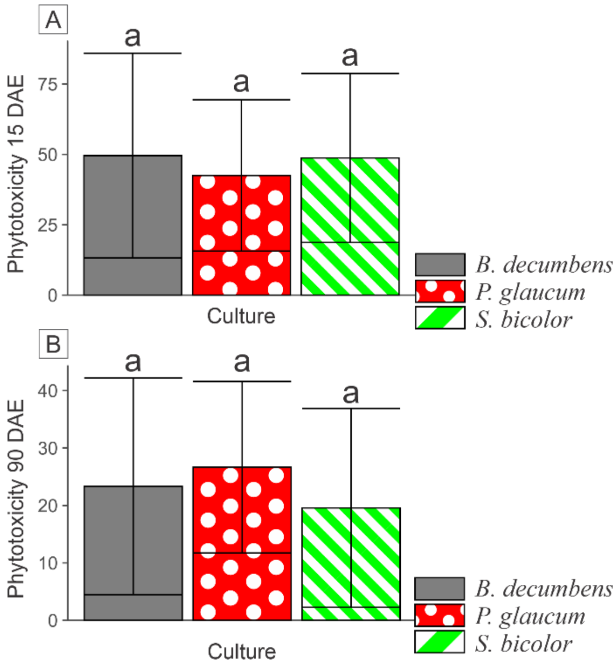

Figure 5. Analysis of phytotoxic effects on phytoremediation species in DAE: (A) 15; (B) 90. Values followed by the same letter do not differ statistically according to Scott-Knott p>0.05.

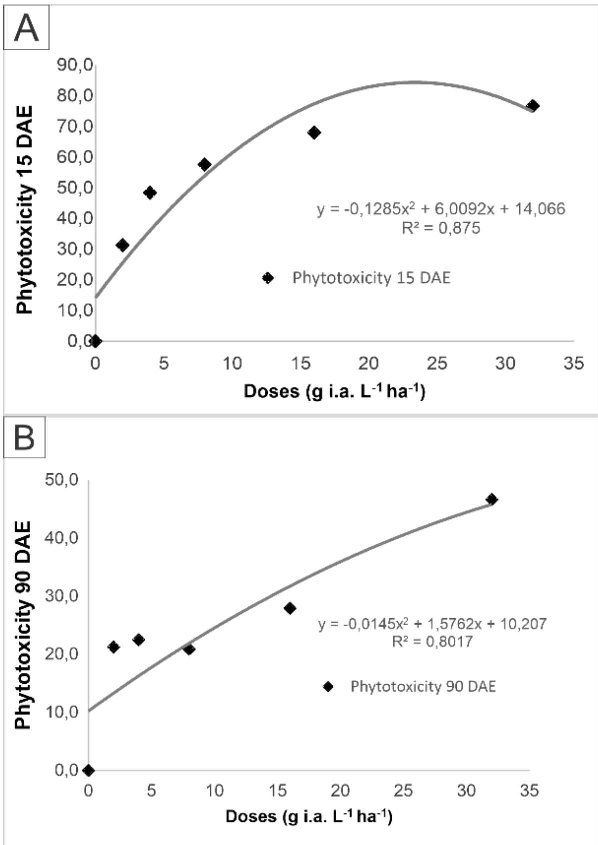

Figure 6. Analysis of phytotoxic effects on phytoremediation species in relation to doses in DAE: (A) 15; (B) 90.

In the visual analysis of phytotoxicity in phytoremediating species, a statistical difference was observed for the interaction between the crop factors and herbicide doses only at 45 DAE. As illustrated in Figure 5, all crops showed mean values statistically equal to each other and lower than 50% in all evaluations, when analyzing the isolated effects,

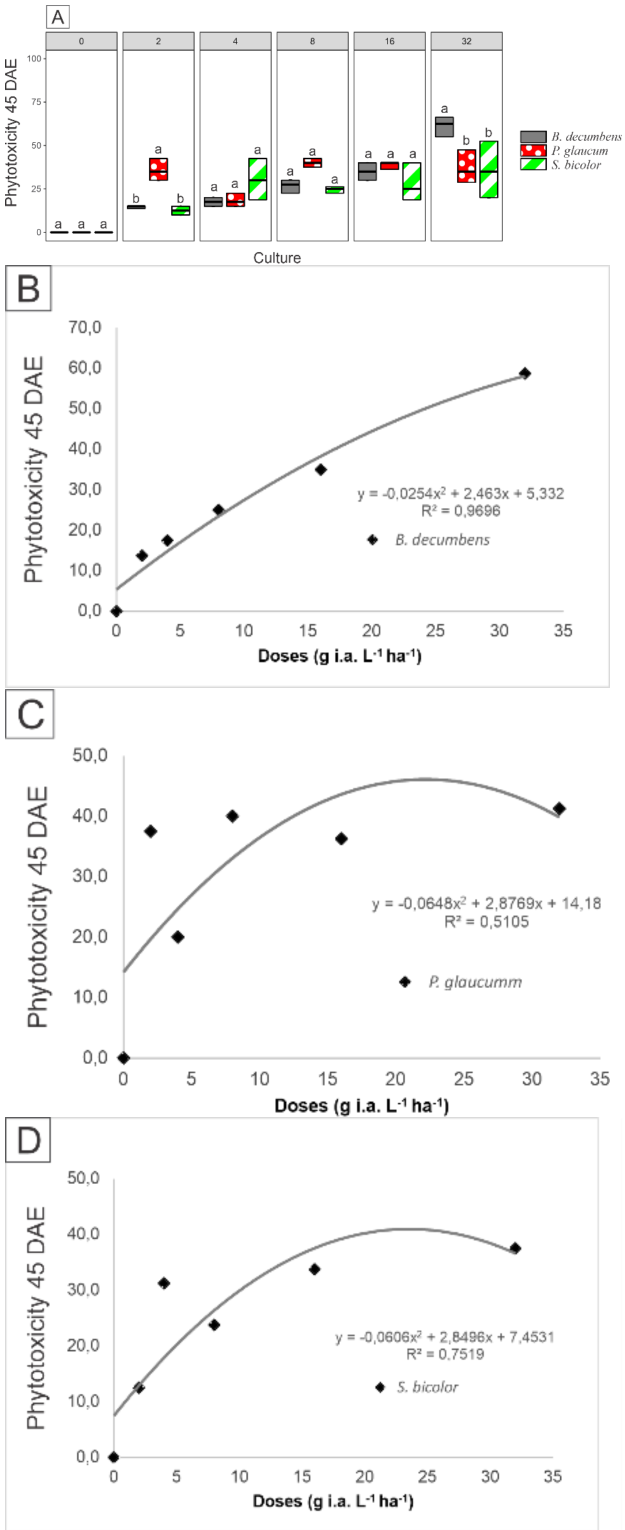

Figure 7. Analysis of phytotoxic effects on phytoremediation species in relation to the (A) crops and doses of (B) *B. decumbens*, (C) *P. glaucumm*, (D) *S. bicolor*. Means followed by the same letter do not differ statistically according to Scott-Knott p>0.05.

demonstrating that the evaluated species have the same type of tolerance to the herbicide. According to Timossi et al. (2020) and Inoue et al. (2003), picloram is considered an herbicide with a high potential for phytotoxicity, due to its long persistence and low sorption coefficient. However, Carmo et al. (2008a), when working with grass species (Tanzania, Mombaça, Cover Crop, Guinea grass, and corn),

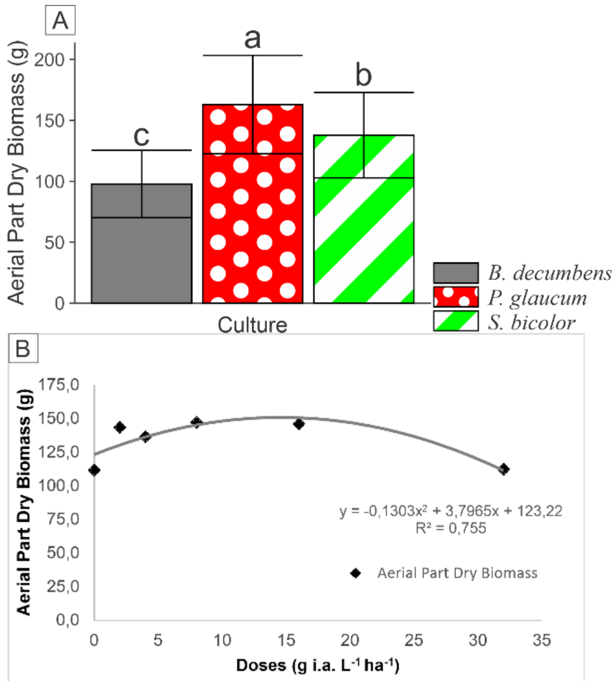

Figure 8. (A) Dry aboveground biomass; (B) Dry aboveground biomass in relation to doses. Values followed by the same letter do not differ statistically according to Scott-Knott p>0.05.

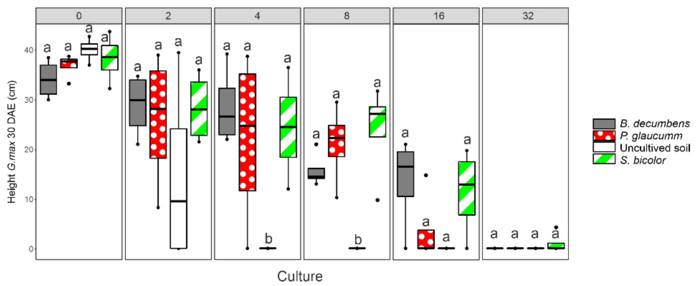

Figure 9. Plant height of *G. max* in relation to the cultivated crops at 30 DAE. Values followed by the same letter do not differ statistically according to Scott-Knott p>0.05.

found that these cultivars showed extremely high tolerance to picloram, with almost imperceptible symptoms when 80 g/ha of the herbicide was applied.

When analyzing the factor of herbicide doses, after adjusting the results to the quadratic regression model, it was observed that the dose of 32 g a.i. L^{-1} ha^{-1}, among the applied doses, caused the highest phytotoxicity indices in all evaluated periods (Figure 6).

At 45 DAE (Figure 7), an interaction between the evaluated treatments is observed. When breaking down the doses according to the crops, the results were adjusted to the quadratic regression model. When breaking down the relationship of the crops within the dose level, a significant difference was observed only when the dose of 2 g a.i. L^{-1} ha^{-1} was applied, where *P. glaucum* showed the highest phytotoxicity indices compared to the other crops. At the dose of 32 g a.i. L^{-1} ha^{-1}, *B. decumbens* showed the highest symptoms of intoxication.

From 60 DAE onwards, all herbicide doses showed levels of symptoms perceived by the plants lower than 50%, demonstrating the resistance that the species have to the herbicide. The accumulation of fresh and dry biomass in the aboveground part of the phytoremediating species did not show any statistical difference in the analysis of variance for

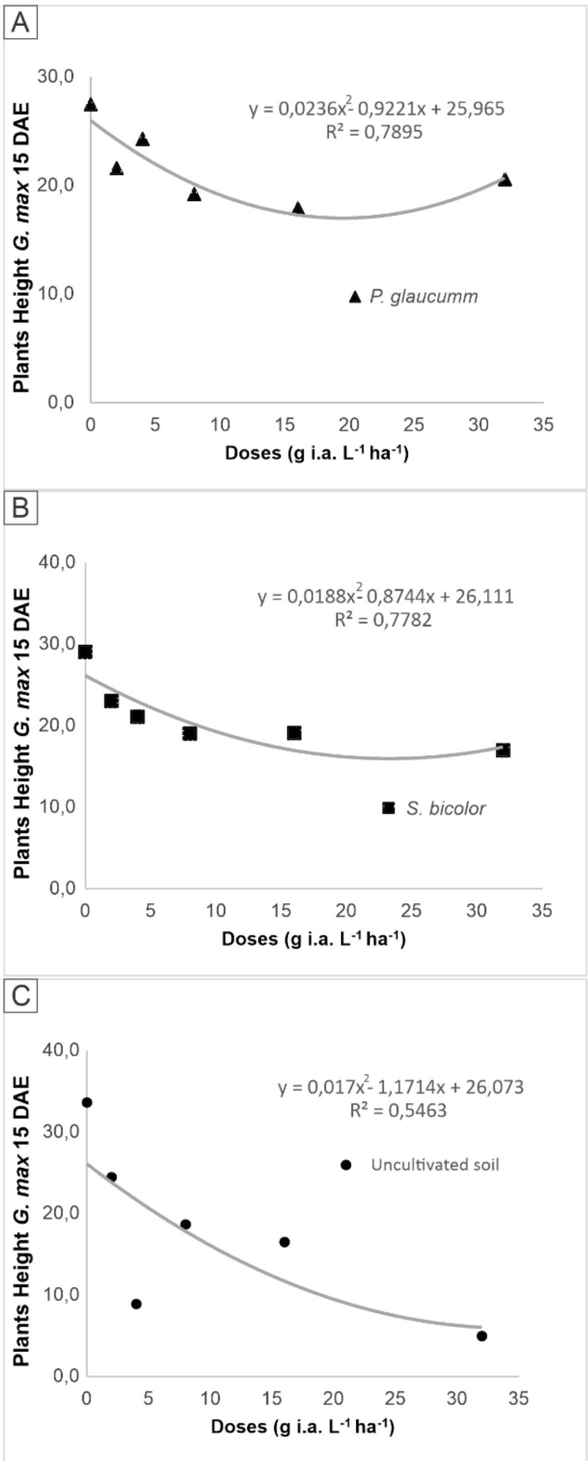

Figure 10. Plant height of *G. max* at 15 DAE in relation to herbicide doses with soil cultivated with (A) *P. glaucumm*, (B) Sorghum, (C) Uncultivated.

the interaction between the crop factors and herbicide doses according to the F test at a 5% probability level. However, a statistical difference was observed among the crops when separately analyzed using the Scott-Knott test at a 5% significance level.

As illustrated in Figure 8, *P. glaucum* had the highest increase in fresh and dry matter, followed by *S. bicolor,* and finally, *B. decumbens*. The results of the increase in biomass due to herbicide doses were adjusted to the quadratic model, where it was observed that the lowest accumulation occurred when the dose of 32 g a.i. L^{-1} ha^{-1} was applied.

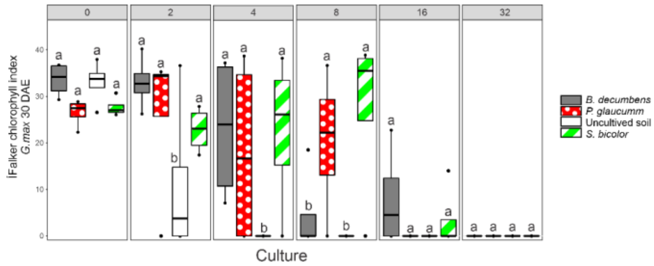

Figure 11. Falker relative chlorophyll index of *G. max* at 30 DAE in relation to soil cultivation. Values followed by the same letter do not differ statistically according to Scott-Knott p>0.05.

According to Pires et al. (2005), in the search for species with phytoremediation potential, it is important to involve species of agronomic interest, especially for their easy control and contribution to the production of biomass.

Developmental characteristics of the bioindicator species

The plant height of the bioindicator species (*Glycine max*) showed a statistical difference in its analysis of variance for the interaction between the cultivated crop factors and herbicide doses according to the F test at a 5% probability level in both evaluated periods (15 DAE and 30 DAE). In all evaluated periods (15 DAE and 30 DAE), soils cultivated with grasses obtained equivalent means when compared within the herbicide dose level according to the Scott-Knott test at a significance level of 0.05 (Figure 9).

It was observed that the control group had the highest height value dose of 0 when compared to the other crops at 15 DAE. This is due to being the first crop to be cultivated in the soil with all nutrients still available. It is worth noting that there was a positive effect on soils cultivated with Phyto-remediating species as they provided higher means compared to the uncultivated control group even when the dose of 4 g a.i. L^{-1} ha^{-1} and 32 g a.i. L^{-1} ha^{-1} were used. At 30 DAE, this behavior was only similar for doses of 4 and 8 g a.i. L^{-1} ha^{-1}, in the data from the uncultivated treatment occurred to plants death caused by herbicide toxicity.

However, these results were still lower than the height observed in the untreated control group, suggesting that even after cultivating these species, the presence of picloram in the soil was still noticeable. According to Procópio et al. (2008), in their study on phytoremediation of *E. coracana* in picloram-contaminated soil, they found a tendency of stabilization in phytoremediation efficiency as the density of remediating plants increased, based on the analysis of plant height.

The height results analyzed independently in relation to herbicide doses were adjusted to the quadratic regression model for all crops at 15 DAE (Figure 10). All crops had minimum height points at doses different from the maximum applied dose (32 g a.i. L^{-1} ha^{-1}). This can be explained by one of the effects caused by picloram, which is cell elongation, promoting etiolation and slow plant death.

It can be observed that the minimum height points, especially when doses of 16 and 32 g a.i. L^{-1} ha^{-1} applied, were very close to 0, as few *G. max* plants remained alive, demonstrating that the evaluated species were not able to remediate the soil at high picloram dosages, during the cultivation period. There were no more living plants in the uncultivated treatments when doses above 4 g a.i. L^{-1} ha^{-1} were applied, demonstrating the sensitivity of *G. max* to the herbicide.

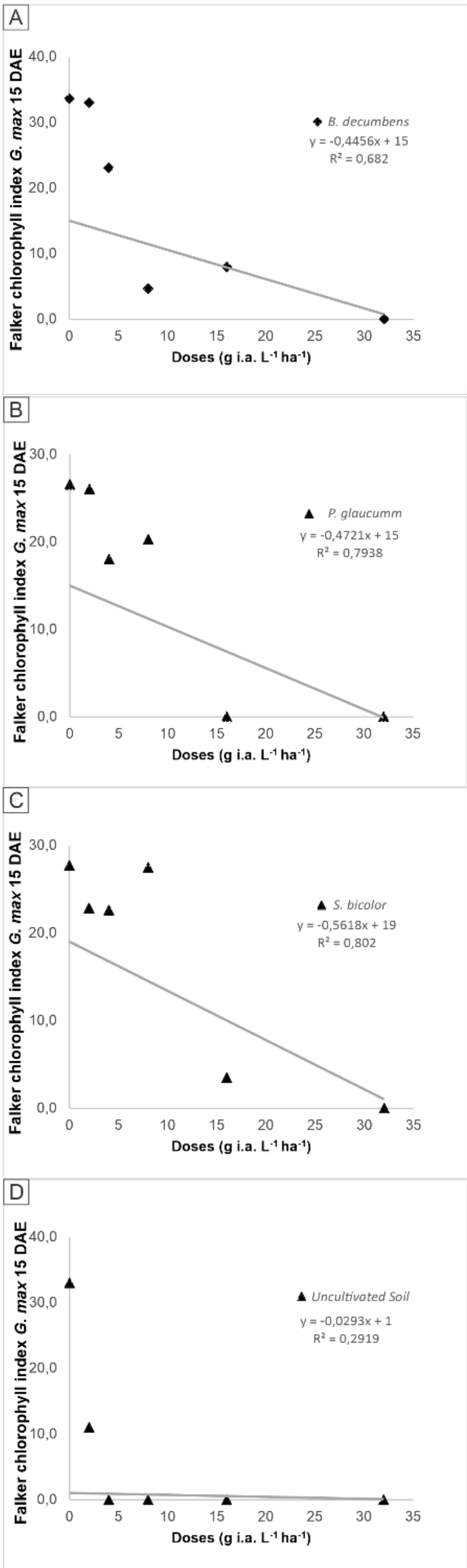

Figure 12. Falker relative chlorophyll index of *G. max* at 30 DAE in relation to doses with soil cultivated with (A) *B. decumbens*, (B) *P. glaucumm*, (C) *S. bicolor*, (D) Uncultivated.

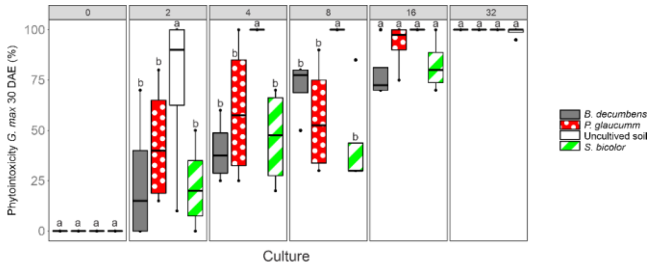

Figure 13. Visual analysis of phytotoxicity on *G. max* in relation to the cultivated crops at 30 DAE. Values followed by the same letter do not differ statistically according to Scott-Knott p>0.05.

These results are consistent with Pires et al. (2005), in their study on phytoremediation of soils contaminated with Tebuthiuron using green manure species, where they found that plant height and dry aboveground biomass of black oats decreased with increasing doses of tebuthiuron for the seven evaluated species. Franco et al. (2015), evaluated the physiological characteristics of common bean cultivated in soils after picloram phytoremediation, and concluded that even after remediation by *B. decumbens* plants, subdoses of picloram affected the physiological characteristics of common beans.

Sequentially cultivated *G. max* plants in the treated soils showed better values compared to uncultivated soil, as the symptoms of herbicide intoxication, expressed by the plants exposed to untreated herbicide, affecting the development of stems and leaves. Regarding the herbicide dose factor, the data were adjusted to a decreasing linear regression model, where interference in the herbicide's action on the crop was observed. The results gradually decreased with increasing picloram dosage, with values close to zero at a dose of 32 g a.i. L^{-1} ha^{-1}.

At 30 DAE (Figure 11 and 12), a statistical difference was observed for the interaction between soil cultivation and herbicide doses through the analysis of variance. The crops were compared using the Scott-Knott test within each dose level. The doses were analyzed separately with the results adjusted to the decreasing linear regression model.

The uncultivated control at the lowest herbicide dose, a statistically significant difference was already identified compared to the herbicide-free soil. Thus, the cultivation of phytoremediation species demonstrated actions that remediated the phytotoxic action of picloram regarding chlorophyll content. In the doses of 2, 4, and 8 g a.i. L^{-1} ha^{-1}, the cultivated treatments achieved superior results compared to the uncultivated control, except for the soil cultivated with *S. bicolor* at a dose of 8 g a.i. L^{-1} ha^{-1}. Starting from the dose of 16 g a.i. L^{-1} ha^{-1}, all treatments had values close to zero due to the death of *G. max* plants. Some authors observed a reduction in photosynthetic rate and stomatal conductance when analyzing the effect of auxinic herbicides on mustard (Khan et al., 2002).

The visual phytotoxicity analysis of bioindicator species showed a statistical difference for the interaction between soil cultivation and herbicide doses in the analysis of variance using the F-test at a 5% probability level at 15 and 30 DAE. The cultivation systems were compared using the Scott-Knott test at a 5% probability level at 30 DAE within each dose level (Figure 13). The doses were adjusted to the quadratic regression model at 30 DAE according to each cultivation system (Figure 14).

At 15 DAE (Days After Emergence), it was already possible to visually observe the effect of picloram on *G. max* cultivation,

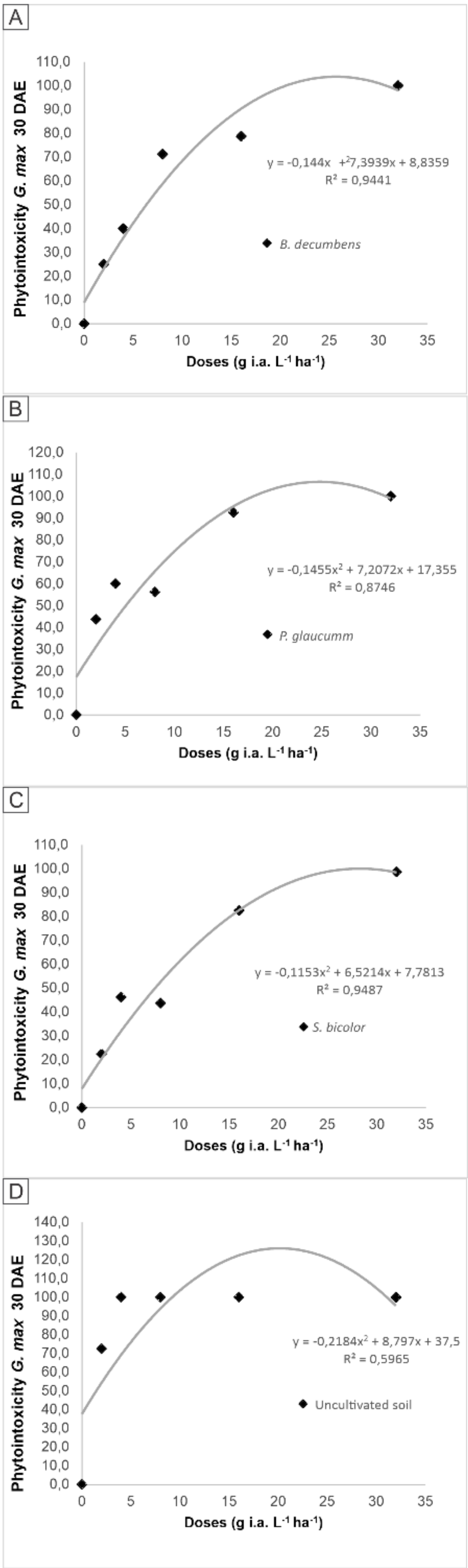

Figure 14. Analysis of phytotoxicity at 30 DAE of *G. max* in relation to herbicide doses with soil cultivated with (A) *B. decumbens*, (B) *P. glaucumm*, (C) *S. bicolor*, (D) Uncultivated.

where there was complete plant death (100% on the visual scale) in pots treated with the herbicide and in untreated pots. For the other treatments, there was a reduction in the observed intoxication effects in plants compared to the control, with equivalent means among the cultivated crops.

At 30 DAE, starting from a dose of 2 g a.i. L^{-1} ha^{-1}, there was a gradual increase in plant injuries with increasing applied dosage. This result was also observed by Assis et al. (2010a) and Assis et al. (2010b), who found high phytotoxicity in *G. max* plants cultivated after the action of *Eleusine coracana*, regardless of the water replacement level applied to the soil. This is highlighting the plant's high sensitivity to the product even at low doses.

Nevertheless, the work performed by the phytoremediation species to mitigate the presence of the picloram herbicide and reduce its assimilation by the sensitive crop is evident. In soils planted with *S. bicolor* and *P. glaucum*, few symptoms were observed in *G. max* crops when the dose of 2 g a.i. L^{-1} ha^{-1}, which corresponds to the commercially used dose, was applied, with visual phytotoxicity means of approximately 25% and 35%, respectively.

In pots treated with doses of 4, 8, and 16 g a.i. L^{-1} ha^{-1} and with soils cultivated with these species, moderate to severe injury symptoms were observed in *G. max* plants but did not cause total plant death. *B. decumbens* showed a similar behavior, but the average phytotoxicity rate was below 40% at doses of 2 and 4 g a.i. L^{-1} ha^{-1}. Carmo et al. (2008c) observed similar effects in the average toxicity of *G. max* plants, with a 43.75% reduction in symptoms, a level that often does not lead to yield losses. At 30 DAE, a similar response was observed among the phytoremediation species in reducing the effects of picloram on the bioindicator crop. However, greater damage to *G. max* plants was observed due to longer exposure to the product in the soil, especially in treatments with higher doses.

In pots treated with *S. bicolor* and *P. glaucum*, the mean phytotoxicity was below 50% for doses of 2, 4, and 8 g a.i. L^{-1} ha^{-1}. Nevertheless, a significant reduction in symptoms perceived by *G. max* was observed in treatments with prior cultivation of plants until the application of the 8 g a.i. L^{-1} ha^{-1} dose, demonstrating the phytoremediation action of the evaluated plant species.

Assis et al. (2010a) reported that the improvement in phytoremediation is significant only when soil contamination with the herbicide is not very high. It is worth noting that, for all evaluated doses, the pots cultivated with phytoremediation species presented lower mean intoxication than the untreated soil control. Figure 15 shows the plant structure of *G. max* according to each dose and cultivation.

There was no statistical difference observed for the interaction between crop factors and herbicide doses in the analysis of variance for the accumulation of aboveground and root biomass of the bioindicator species at the F-test level of 5%. There was no statistical difference between the treatments for the fresh and dry biomass variables of the aboveground part when analyzing the isolated effect of the crops (Figure 16), tested using Scott-Knott test at a 5% probability level. However, it was possible to verify a reduction of up to 30% in biomass accumulation in the pots that did not have cultivation. The mean values were 5.358 g for fresh aboveground biomass, 1.466 g for dry aboveground biomass, and 0.809 g for dry root biomass.

When examining the doses (Figure 17), a decrease in the average plant biomass was observed in the treatments where the herbicide was administered, with adjustments made to a

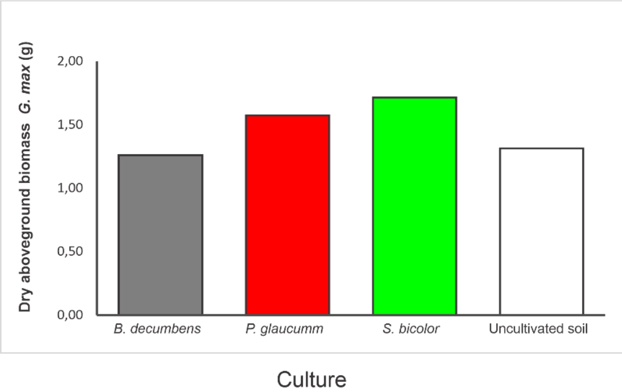

Figure 15. Dry aboveground biomass of *G. max* in relation to the crops. Values followed by the same letter do not differ statistically according to Scott-Knott p>0.05.

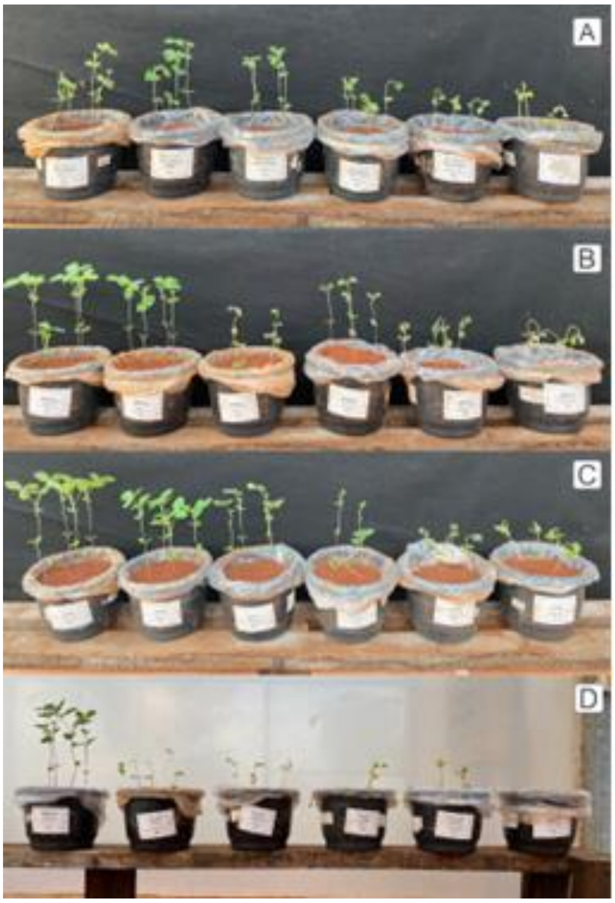

Figure 16. Plant structure of soybean according to herbicide doses and separated by soil treatment cultivated with (A) *B. decumbens*, (B) *P. glaucumm*, (C) *S. bicolor*, (D) Uncultivated.

quadratic regression model. Doses of 16 and 32 g a.i. L^{-1} ha^{-1} yielded the lowest results, with values close to zero, mainly due to the scarcity of decomposed plant material. The other doses also resulted in lower biomass accumulation compared to the untreated control.

These results are consistent with Assis et al. (2010a), who found that the phytoremediation with *E. coracana* plants, and Tanzanian grass plants (Assis et al., 2010b), was not efficient in ensuring complete accumulation of green and dry biomass by *G. max* plants, especially at higher doses. Similarly, Timossi et al. (2020) observed a reduction in the values of fresh and dry biomass of *G. max* with increasing doses of the auxinic

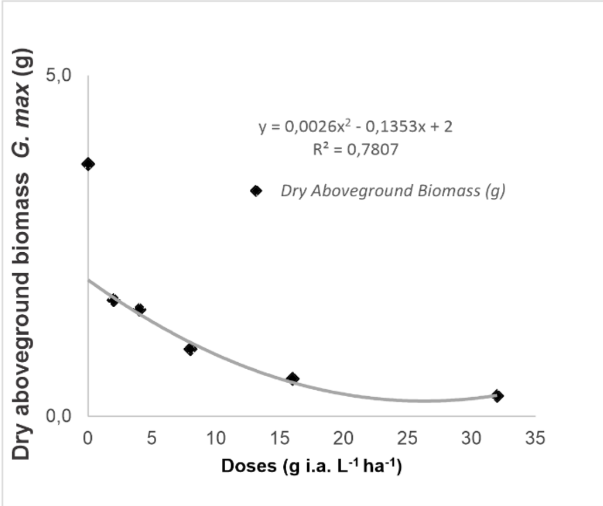

Figure 17. Dry aboveground biomass of *G. max* in relation to herbicide doses.

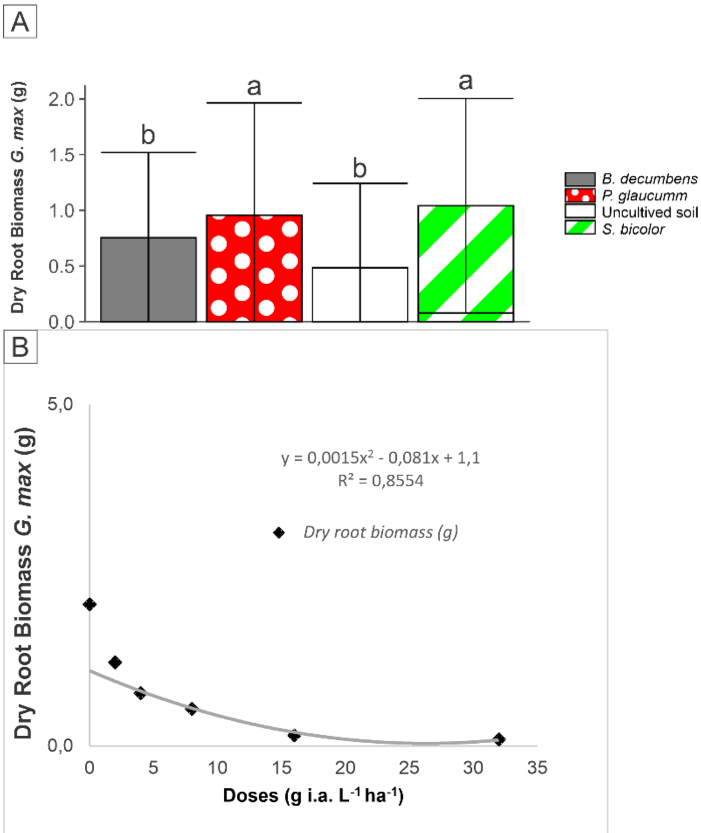

Figure 18. Dry root biomass of *G. max* in (A) relation to the crops and (B) relation to herbicide doses. Values followed by the same letter do not differ statistically according to Scott-Knott p>0.05.

herbicide picloram, when evaluating the residual effects of auxinic herbicides.

The highest values were found in *S. bicolor* and *P. glaucum* plants, analyzing the crop factor separately for the variable of dry root biomass, using the Scott-Knott test at 5% probability level (Figure 18).

The results of herbicide dose factor identified a reduction in root formation from the dose of 2 g a.i. L^{-1} ha^{-1}. These results were adjusted to a quadratic regression model. In soils treated with doses of 4 and 8 g a.i. L^{-1} ha^{-1}, a smaller increase in root biomass was observed, but it was still higher than the

doses of 16 and 32 g a.i. L^{-1} ha^{-1}, where almost no material was found for analysis, with values close to zero.
Franco et al. (2014), analyzed the phytoremediation of picloram-contaminated soils by *Urochloa brizantha*. Carmo et al. (2008b), also worked with different cultivation periods of *Panicum maximum*. These authors obtained satisfactory results in the accumulation of dry biomass in bioindicator plants as the phytoremediation species had a longer presence.

Materials and Methods

The experiment was conducted in a greenhouse at the Federal University of Rondonópolis, with geographical coordinates 16°27'48" South latitude, 54°34'45" West longitude, and an altitude of 284m, with an average temperature of 28°C, and Aw climate classification.

Experimental design

A completely randomized design was used in a 4x6 factorial scheme, corresponding to four cropping systems (uncultivated soil, soil cultivated with *B. decumbens*, soil cultivated with *Sorghum bicolor*, soil cultivated with *Pennisetum glaucum*) and six doses of picloram (triethanolamine salt), applied pre-emergence (0, 2, 4, 8, 16, and 32 g a.i. L^{-1} ha^{-1}), with the soil at 60% of its pot capacity (Bonfim-Silva, 2011), in four replications.

Conduction of study

The soil was collected from a Cerrado fragment area located within the Federal University of Rondonópolis, classified as dystrophic Red Latosol (Santos et al., 2013), at a depth of 0-20 cm. It was sieved through a 4 mm mesh and dried in the shade. A composite sample was taken for chemical and particle size analysis, for liming and fertilization procedures. The Liming were carried out to achieve 60% of base saturation, following the methodology described by Bomfim-Silva et al. (2011). Then, the soil was allocated to bags and after 5 dm^3 pots. Phosphorus (P_2O_5) and potassium (K_2O) fertilization were applied to all treatments before transplanting. Nitrogen fertilization was applied after transplanting the plants when they reached an average height of 10 cm, using urea as a nitrogen source.

Spraying and plant assessments

The product used was picloram, which is selective for controlling dicotyledonous arboreal, shrubby, and sub-shrubby in pasture areas, in the form of a soluble concentrate. Spraying was done following regulations for the application of agricultural pesticides (Regulatory Standard NR 31), in the morning outdoors. The pressurized sprayer used CO_2, and the spraying boom was equipped with six Magno 11002 AD flat fan nozzles, spaced 0.5m apart and positioned 0.5m above the soil. The travel speed was 4 km/h with a spray solution consumption of 200 L/ha. The equipment was operated at a constant pressure of 2 bar.
The soil was moistened 12 hours before spraying until it reached 60% of pot capacity. After 24 hours of spraying, 300 mL of water was added to each experimental unit to allow movement of the herbicide in the soil profile. To ensure that the herbicide's effect did not influence the plant germination process, the sowing of all phytoremediation species was done in trays 7 days before transplanting. This process occurred 48 hours after spraying, and seven plants were transplanted per pot.
The soil moisture was maintained at 80% of pot capacity throughout the experimental period. To achieve this value, the amount of evaporated water was determined using the gravimetric method (Bonfim-Silva, 2011). The duration of the phytoremediation species' growth phase was 90 days after transplanting the plants. The aboveground part was cut at the base of the stem at ground level and placed in paper bags. For root collection, the soil was sieved and placed in new plastic bags in the respective pots. The roots were washed, dried, and stored in paper bags. The variables were evaluated at 15, 30, 45, 60, 75, and 90 days after emergence during the phytoremediation species' growth phase.
After cutting the phytoremediation species, the pots were standardized to maintain the same weight among all treatments. Subsequently, the bioindicator species for the presence of picloram (*Glycine max* cultivar Monsoy 6101), previously sown in trays, were transplanted, with four plants per pot. The duration of the bioindicator phase was 40 days after plant emergence and the evaluation at 15 and 30 days after emergence. In both phases, phytotoxicity, plant height, Falker chlorophyll index, fresh aboveground and root biomass were analyzed. Phytotoxicity was assessed according to an adapted scale of 0 to 100% intoxication (EWRC, 1964) and the model proposed by the Brazilian Weed Science Society (SBCPD, 1995), where 0% corresponds to no intoxication and 100% to plant death. In this scale, the score was assigned based on the symptoms of intoxication compared to the herbicide-free control.

Statistical analysis

The results were subjected to analysis of variance using the F-test ($P \leq 0.05$), and when significant, regression analysis was performed for herbicide doses, and the Scott-Knott test was used for crop types, at a 5% probability level for mean comparison. The tests were performed using the free software R Statistical 4.4.3® (Wickham, 2009; R Core Team, 2018).

Conclusions

The tropical phytoremediation grasses *B. decumbens*, *P. glaucum*, and *S. bicolor* exhibited resistance to the picloram herbicide without severe symptoms of intoxication or impairment of physiological characteristics. The phytoremediation grasses demonstrated potential in attenuating the effects of picloram on the sensitive crop (*G. max*) at the applied doses according to commercial recommendations, even within a short period of 90 days.

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