weedcontrol journal

RESEARCH ARTICLE

Addition of ammonium sulfate to glyphosate in the desiccation of *Urochloa brizantha* cv. BRS "Piatã"

Adição de sulfato de amônio ao glifosato na dessecação de *Urochloa brizantha* cv. BRS "Piatã"

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Abstract: **Background**: Glyphosate is the most widely used herbicide in Brazilian agriculture, and management techniques that increase its efficacy are important tools for the production system.
Objective: Evaluate the effect of adding the nitrogen adjuvant ammonium sulfate [$(NH_4)_2$ SO_4] to glyphosate on the control of *Urochloa brizantha* BRS Piatã and establish the best [$(NH_4)_2$ SO_4] dose to mix with the herbicide.
Methods: Two greenhouse experiments were conducted. In the first, six glyphosate doses (0; 33.75; 67.5; 135; 270; 540 g a.e. ha^{-1}) were used to determine C_{80} (glyphosate dose that achieved 80% control). This dose was used as a reference for the second experiment in a mixture with four [$(NH_4)_2$ SO_4] doses (0; 6.67; 13.33; and 20 g L^{-1} of solution) to determine the best nitrogen dose. Both experiments assessed BRS Piatã grass control at 7, 14, and 21 days after treatment (DAT) and shoot length and dry weight at 21 DAT.
Results: The C_{80} of glyphosate was established at 270 g a.e ha^{-1}. The visual scale indicated that adding [$(NH_4)_2$ SO_4] accelerated the glyphosate-related symptoms but did not change plant fresh and dry weight and shoot length. Maximum glyphosate efficacy was obtained by adding ammonium sulfate at 16.2 g L^{-1} of solution.
Conclusions: Ammonium sulfate accelerates the glyphosate action in the desiccation of *Urochloa brizantha* BRS Piatã.

Resumo: **Introdução**: O glifosato é o herbicida mais utilizado na agricultura nacional, e técnicas de manejo que aumentam a sua eficiência são importantes ferramentas para o sistema de produção.
Objetivo: Avaliar o efeito da adição de adjuvante nitrogenado sulfato de amônio (SAM) ao glifosato sobre o controle de *Urochloa brizantha* BRS Piatã e definir a melhor dose de SAM a ser misturada com o herbicida.
Métodos: Dois experimentos foram conduzidos em casa de vegetação. No primeiro foram avaliadas seis doses de glifosato (0; 33,75; 67,5; 135; 270; 540 g e.a. ha^{-1}) para determinação da C80 (dose de glifosato que causou 80% de controle). Essa dose foi utilizada como referência para o segundo experimento, em mistura com quatro doses de SAM (0; 6,67; 13,33; 20 g L^{-1} de calda) para definir a melhor dose do nitrogenado. Ambos os experimentos avaliaram o controle do capim aos 7, 14 e 21 dias após o tratamento (DAT), e o comprimento e a massa seca da parte aérea aos 21 DAT.
Resultados: A C_{80} de glifosato foi estabelecida em 270 g e.a. ha^{-1}. A escala visual indicou que a adição de SAM antecipou os sintomas do causados pelo glifosato, porém não alterou a massa fresca e seca e o comprimento de parte aérea das plantas. A máxima eficácia do glifosato foi obtida pela adição de sulfato de amônio na concentração de 16,2 g L^{-1} de calda.
Conclusão: O sulfato de amônio acelera a ação do glifosato na dessecação de *Urochloa brizantha* BRS Piatã.

Keywords: brachiaria, herbicide, weeds, no-till, sustainability.

Palavras-chave: braquiária, herbicida, plantas daninhas, plantio direto, sustentabilidade.

Journal Information:
ISSN: 2763-8332
Website: https://www.weedcontroljournal.org/
Jornal da Sociedade Brasileira da Ciência das Plantas Daninhas

Como citar: Firmani JF, Gasque GH, Takahashi CN, Firmano LA, Amorim EA, Bovo RF, Dalazen G, Stol-Moreira R. Addition of ammonium sulfate to glyphosate in the desiccation of *Urochloa brizantha* cv. BRS "Piatã". Weed Control J. 2024;23:e202400857.
https://doi.org/10.7824/wcj.2024;23:00857

Aprovado por:
Editor-Chefe: Cristiano Piasecki

Conflitos de interesse: Os autores declaram não haver conflito de interesses em relação à publicação deste manuscrito.

Recebido: Março 1, 2024
Aprovado: Novembro 26, 2024

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1. Introduction

Glyphosate is currently one of the most widely used herbicides in Brazilian agriculture. It is a systemic, post-emergent broad-spectrum herbicide (Oliveira JR. et al., 2011). No-till farming and Roundup Ready technology favor the increasing use of this product. The leaves absorb glyphosate via a two-phase process: initial cuticle penetration followed by slow symplastic absorption. Process duration is strongly influenced by plant species, age and physiological condition, environmental conditions, and active ingredient concentration in the application medium (Monquero et al., 2004; Barroso and Murata, 2021).

Knowing the physiological processes linked to the herbicide and species tolerance is essential to understanding strategies to enhance weed management (Bastiani et al., 2021). Glyphosate inhibits the enzyme 5-enolpyruvylshikimate-3-phosphate synthase (EPSPS), which is responsible for the reaction that catalyzes the bond between shikimate 3-phosphate (S3P) and phosphoenolpyruvate (PEP). In susceptible plants, glyphosate binds to the EPSPS-S3P complex, forming an inactive EPSPS-S3P-glyphosate complex. After inhibition, shikimate accumulates, and aromatic amino acid synthesis is disrupted (Barroso and Murata, 2021). This interrupts tissue development and some metabolic pathways related to plant hormone production, causing slow plant death and tissue degradation (four to 20 days) (Yamada and Castro, 2007).

Strategies that increase herbicide efficiency involve adapting to new circumstances and rational product use. The use of adjuvants to improve herbicide performance is well known. Several authors added nitrogen sources to the glyphosate solution, especially the nitrogen fertilizers ammonium sulfate and urea (Salisbury et al., 1991; Fleck et al., 1997; Young et al., 2003; Carvalho et al., 2008; 2009a, b; 2010; 2011; Leite et al., 2014; Bastiani et al., 2021)

There are indications that nitrogen fertilizers have an influence on the dynamics of the herbicide solution (Fleck et al., 1997; Carvalho et al., 2009a), in the way of application drop deposition on the leaf of plants (Masclsaac et al., 1991), and actions on plant physiology. Some authors report that apoplast acidification favors herbicide translocation, and changes membrane potential, facilitating herbicide entry into the cell, urea through the cuticle, and ammonium sulfate via cell absorption (Carvalho et al., 2008; 2010; Young et al., 2003).

However, there are still variations in nitrogen source concentrations, with reports of antagonistic effects at higher-than-ideal concentrations (Carvalho et al., 2009b). Moreover, ammonium sulfate has exhibited better results in some weed species (Carvalho et al., 2009b, 2010). In *Sorghum halepense* and *Alternanthera tenella*, species less and more susceptible to glyphosate, respectively, adding a nitrogen source accelerated plant death only for *S. halepense* (Carvalho et al., 2010). Adding urea and ammonium sulfate and simultaneously using both sources reduced glyphosate C_{50} in *Digitaria insularis* (Carvalho et al., 2009b).

In addition to weed control, the species used as cover crop or pasture in an integrated system with livestock should always be properly desiccated before seeding crops, such as soybean, for maximum grain yield (Werner et al., 2023). In this respect, dose-response curve greenhouse experiments were conducted to determine the effect of adding ammonium sulfate to the glyphosate solution applied to *Urochloa brizantha* cv. BRS Piatã.

2. Material and Methods

Two greenhouse experiments were carried out using *Urochloa brizantha* cv. BRS Piatã grass seeds planted in 800 mL pots (10 cm high and 14 cm in diameter) filled with substrate consisting of clay soil and sand previously sterilized in an air oven at a ratio of 1:1. Ten seeds were sown in each pot. These plants were thinned after three fully expanded leaves emerged, maintaining two plants per pot.

A completely randomized design was used, with four repetitions in both experiments. In the first experiment, six glyphosate doses were assessed (Crucial, 540 g a.e. L^{-1}, SL, Sumitomo Chemical), varying from 0 to 540 g a.e. ha^{-1} (Table 1). The aim was to determine the value of C_{80}, the dose that achieves 80% control in *U. brizantha* Piatã grass. This dose was used to conduct the second experiment, which four ammonium sulfate [$(NH_4)_2\ SO_4$] doses in g L^{-1} of the solution were evaluated, applied together with the glyphosate C80 dose determined in experiment 1. Table 2 presents the treatments analyzed in the second experiment

Table 1. Glyphosate doses assessed in experiment 1 to establish C_{80} to control *Urochloa brizantha* cv. BRS Piatã.

Nº	Treatment	Dose (g a.e. ha^{-1})	Dose (mL P. C. ha^{-1})
1	Control	-	-
2	Glyphosate	33.75	60
3	Glyphosate	67.5	120
4	Glyphosate	135	250
5	Glyphosate	270	500
6	Glyphosate	540	1000

Table 2. Ammonium sulfate [(NH4)2 SO4] doses assessed in a mixture with glyphosate to control *Urochloa brizantha* cv. BRS Piatã

Nº	Treatment	Ammonium sulfate		Glyphosate	
		(g L^{-1})	(Kg ha^{-1})	(g a.e. ha^{-1})	(mL P. C. ha^{-1})
1	Glyphosate	0	0	270	500
2	Ammonium sulfate + Glyphosate	6.67	1	270	500
3	Ammonium sulfate + Glyphosate	13.33	2	270	500
4	Ammonium sulfate + Glyphosate	20	3	270	500

Treatments were applied when the plants exhibited five or six fully expanded leaves. For this, a CO_2-pressurized backpack sprayer was used, equipped with a boom containing two TJ110.02 nozzles spaced 0.5 m apart, maintained 0.5 m above the target surface during application, at a pressure of 0.2 MPa and speed of 1 m s^{-1}, resulting in a spray volume of 150 L ha^{-1}. After application, the plants were deprived of water for 24 hours to ensure adequate leaf absorption of the treatments.

In both experiments, control was visually assessed at seven, 14, and 21 days after treatment (DAT), following the method of Frans et al. (1986), where the repetitions are scored between 0 and 100, with 0 representing no symptoms and 100 plant death. At 21 DAT, shoot length and fresh weight were measured on a semi-analytical balance (SAUTER RC 2013), and shoot dry weight after oven drying at 65 ºC for 72 hours.

The data were submitted for analysis of variance

(ANOVA) using the RStudio statistical package. In experiment 1, a complementary analysis was conducted, fitting the data to the sigmoid logistic regression equation $y = a/[1 + (x/x0)^b]$, where "y" is the control percentage, "x" is the herbicide dose, "a" is the difference between the maximum and minimum points of the curve, "x0" is the dose that provides a 50% response of the variable (C50), and "b" the slope of the curve at x0. To estimate C_{80}, the "y" value of the equation was replaced by 80 (in relation to 80% control). For experiment 2, complementary analysis was conducted, fitting the data to the quadratic linear equation, where the y-axis consisted of the parameters assessed and the x-axis of ammonium sulfate concentrations. The resulting equation was used to calculate the vertex of the parabola and the concentration of the nitrogen fertilizer, providing greater control in the mixture with glyphosate. Due to better model fit, the regression at seven DAT was used to calculate the best concentration.

3. Results and Discussion

Assessment at 14 DAT demonstrated, via the regression curve, that at the largest glyphosate dose (540 g a. e. ha^{-1}), BRS Piatã plants received the highest control scores, reaching approximately 90% (Figure 1A). At lower doses, control levels were less than 80%. However, the assessment carried out at 21 DAT, at doses of 135 and 270 g a.e. ha^{-1}, plants exhibited satisfactory control, equivalent to 91 and 97.5%, respectively (Figure 1B). Similarly to what occurred at 14 DAT, at 21 DAT, a progressive increase in herbicide doses resulted in greater injury, reaching 100% at the highest dose assessed.

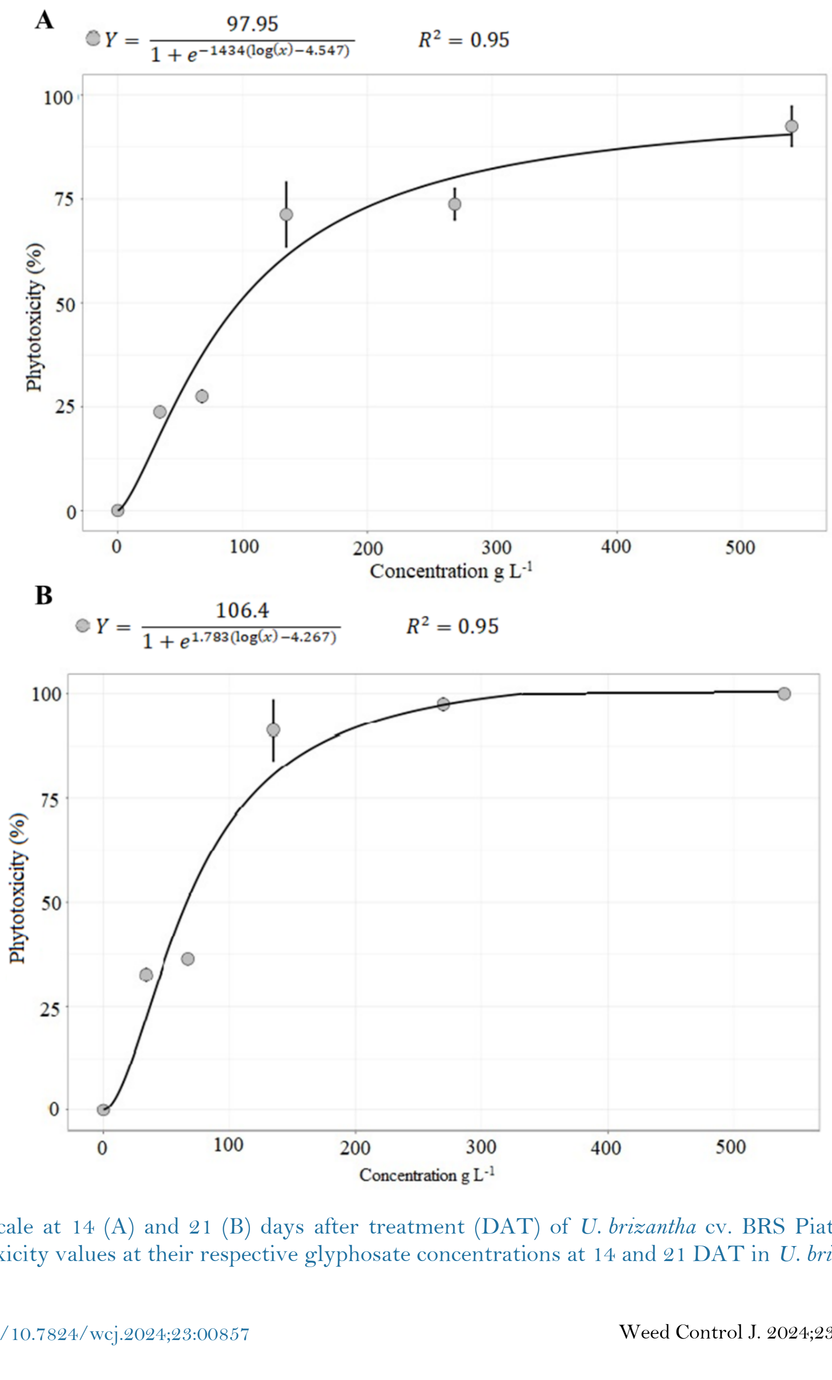

Figure 1. Control visual scale at 14 (A) and 21 (B) days after treatment (DAT) of *U. brizantha* cv. BRS Piatã. The graphs represent the regression of mean phytotoxicity values at their respective glyphosate concentrations at 14 and 21 DAT in *U. brizantha* cv. BRS Piatã.

Shoot length and dry weight declined at all the glyphosate doses, with greater effects at higher doses (Figure 2). It is important to note that even small doses resulted in smaller and lower-weight plants, demonstrating the effect of the herbicide on growth, possibly due to interference in the glyphosate mechanism of action in the treated plants, primarily in the metabolism of the aromatic amino acids tyrosine, phenylamine and tryptophan (Yamada and Castro 2007; Helander et al., 2012). Moraes (2016) described the hormetic effect of glyphosate in *B. decumbens*, observing a beneficial effect at a sub dose of 11.25 g a.e. ha^{-1}. However, at the concentrations used in the present study, this effect was not observed from a dose of 33.75 g a.e. ha^{-1} onwards. These results confirm that higher herbicide doses result in better plant control, as reported by Lacerda and Victoria Filho (2004). Moraes et al. (2017) obtained similar results, where an increase in dose decreased *U. decumbens* growth.

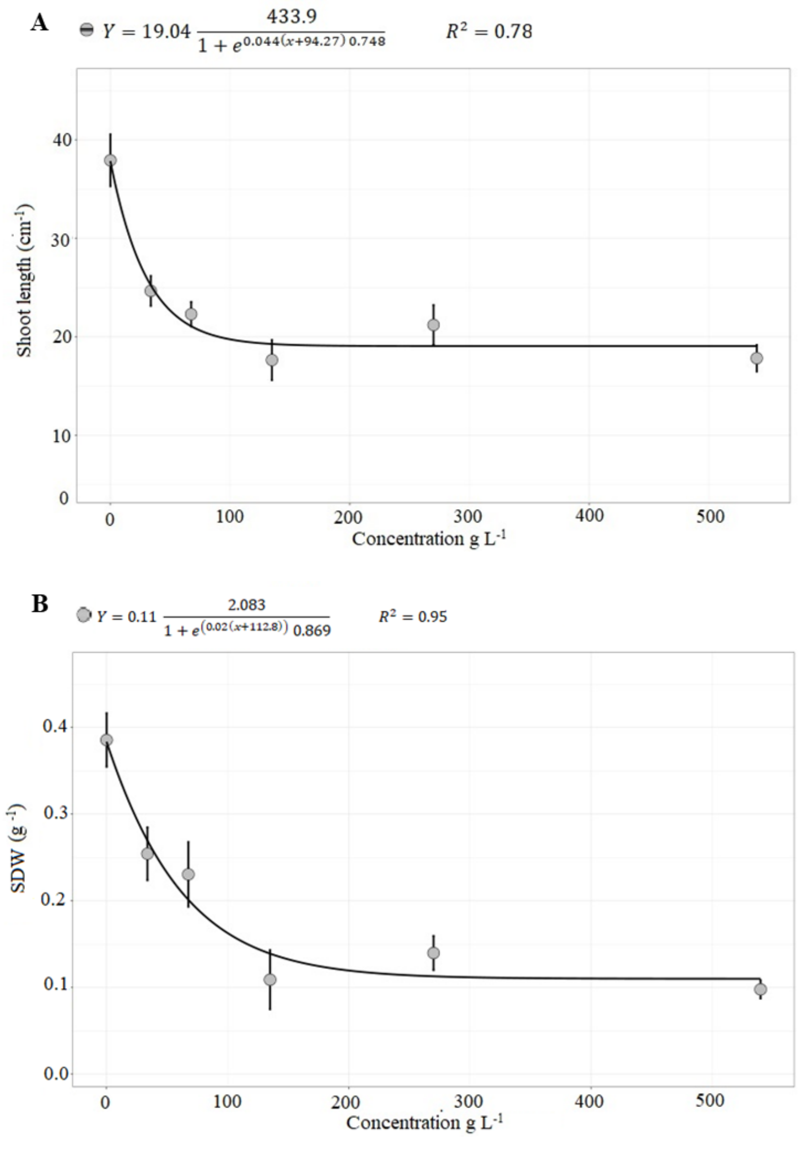

Figure 2. Mean shoot length (A) and dry weight (B) at 21 days after treatment (DAT) at the respective glyphosate concentrations in *U. brizantha* cv. BRS Piatã.

After fitting the dose-response curve, it was possible to determine the C_{80} value of glyphosate for *U. brizantha* cv. BRS Piatã at 14 DAT, which was 270.0 g a.e. ha^{-1} (Figure 1A). This value shows that the dose needed to obtain 80% control of the species also resulted in the same percentage decrease in SDW. As such, this dose was used in a mixture with [$(NH_4)_2$ SO_4] in the second experiment. In an experiment with *U. decumbens*, a species belonging to the same genus as that used here, Moraes et al. (2017) studied the dose-response curve of glyphosate for the species and found that the 45 g a.e. ha^{-1} dose was enough to obtain an

80% decline in plant biomass. Brighenti et al. (2011) used linear regression to determine the C_{50} of three brachiaria species: *U. ruziziensis*, *U. decumbens* and *U. brizantha*. The C_{50} values varied with each species, being smaller for the first two. The C_{50} of *U. brizantha* was 769.0 g a.e. ha^{-1}, but the developmental stage of the plants at application was more advanced compared to the present study, given that it occurred 45 days after the uniformity cut. This demonstrates that plant species, developmental stage, and age are important management factors, given that smaller plants require lower herbicide doses.

Adding ammonium sulfate to the glyphosate solution accelerated phytotoxicity symptoms since the visual scale at seven and 14 DAT revealed a difference between treatments. Linear regression for the visual phytotoxicity scale at seven DAT (Figure 3) shows that adding ammonium sulfate increases glyphosate damage. At seven DAT, [$NH_4)_2SO_4$] concentrations (6.67, 13.33, 20 g L^{-1}) resulted in control levels of 47.5, 57.5, and 47.5 %, respectively, while at 14 DAT the levels were 75, 96.2 and 93.7 %. The isolated application of glyphosate resulted in controls of only 27 and 73% at seven and 14 DAT, respectively.

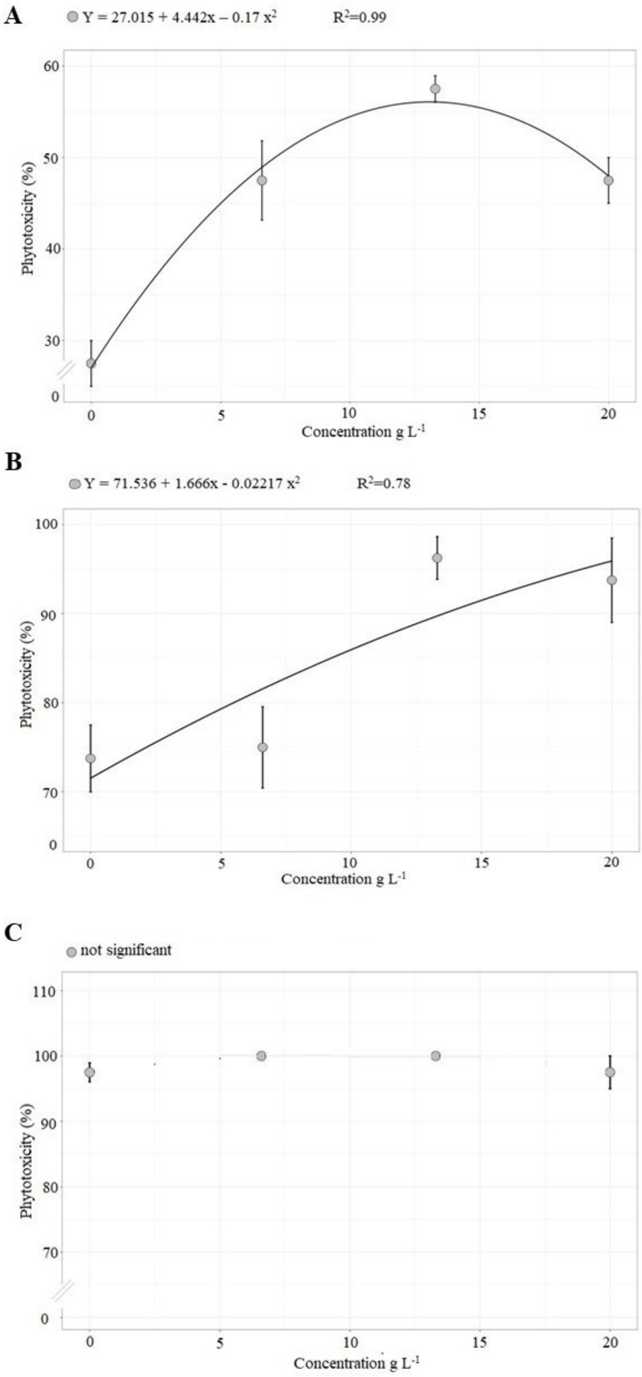

Figure 3. Quadratic regression curve for [$(NH_4)_2$ SO_4] concentrations in a mixture with glyphosate and mean control visual scale scores seven (A) 14 (B) and 21 (C) days after treatments (DAT).

Regression also showed a slight decline in the response of the y-axis variable, possibly indicating the onset of antagonism. However, more studies with higher concentrations are needed to confirm this effect. The ammonium sulfate concentration curve showed the best result was 16.2 g L^{-1}. Assessment at 21 DAT revealed no difference between treatments, demonstrating no increase in control at the end of the experiment but an intensification of symptoms in the first evaluations.

Corroborating the results obtained here, Carvalho et al. (2008) conducted a field experiment with different weed species using different [$(NH_4)_2$ SO_4] concentrations (2.5; 5.0; 10.0; and 20.0 g L^{-1}) and a dose of 360 g a.e. ha^{-1} of glyphosate and compared it with a single dose of 720 g a.e. ha^{-1}. The glyphosate combinations with concentrations of 2.5 and 10 g L^{-1} of [$(NH_4)_2$ SO_4] provided control similar to that of the highest single herbicide dose. This study indicates that species requiring higher herbicide doses exhibit a smaller response to the addition of [$(NH_4)_2$ SO_4] (Carvalho et al., 2011).

The non-response at higher herbicide doses was not observed in the present study. This is partly because the C_{80} for the species was established at a lower concentration than the maximum used in experiment one, because the application conditions did not require higher concentrations to obtain 80% control.

In this study, [$(NH_4)_2$ SO_4] accelerated the herbicide action without changing the biomass accumulation of the plants, a finding also reported by Fleck et al. (1997) in *Avena strigosa*. This may be because [$(NH_4)_2$ SO_4] helped in glyphosate translocation through the plant, as observed by Bastiani et al. (2021), using molecular radioactive tracer techniques. The ideal [$(NH_4)_2$ SO_4] concentration was lower than the maximum used, indicating a negative effect at higher-than-ideal concentrations. Carvalho et al. (2008) found a similar result, given that control was greater at low than at high concentrations, similar to that reported by Salisbury et al. (1991), who observed that higher-than-ideal [$(NH_4)_2$ SO_4] concentrations are undesirable, leading to an antagonistic effect. This finding was obtained in the present study because the ideal [$(NH_4)_2$ SO_4] concentration was lower than the maximum used, with the visual scale curve declining at the maximum concentration (Figure 3).

4. Conclusions

The C_{80} of glyphosate for *U. brizantha* cv. BRS Piatã was established at 270 g a.e. ha^{-1}. Ammonium sulfate [$(NH_4)_2$ SO_4] accelerated herbicide symptoms but did not change biomass accumulation at the end of the experiment. The ideal [$(NH_4)_2$ SO_4] concentration for the species under study and the conditions described in the experiment were determined at 16.2 g L^{-1}.

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