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DOI: https://doi.org/10.1590/1807-1929/agriambi.v30n9e297627 ORIGINAL ARTICLE

Doses of imazethapyr + imazapic in the initial suppression of *Urochloa brizantha* cv. BRS Piatã[1]

Doses de imazethapyr + imazapic na supressão inicial de *Urochloa brizantha* cv. BRS Piatã

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HIGHLIGHTS:

The mixture of imazapic + imazethapyr has the potential to suppress Urochloa brizantha *cv. BRS Piatã.*
The forage crop with two tillers exhibited greater tolerance to herbicides than when the crop had only one tiller.
Field conditions conferred greater tolerance of BRS Piatã to imazethapyr + imazapic than greenhouse conditions.

ABSTRACT: Intercropping cereals with forage grasses can reduce grain yield due to forage competition, and herbicide-based suppression has emerged as a viable management strategy. This study aimed to evaluate the effects of the ready mix of imazethapyr + imazapic on the initial growth suppression of *Urochloa brizantha* cv. BRS Piatã. In a greenhouse experiment, four doses (0, 0.35, 0.70, and 1.4 L commercial product ha^{-1}) of the imazethapyr + imazapic ready mix (Zelone®, UPL, Campinas, SP, Brazil) were tested and applied at the first and second tiller stages of the forage. In a field experiment, eight doses (0, 0.0438, 0.0873, 0.175, 0.35, 0.70, 1.05, and 1.40 L commercial product ha^{-1}) were tested. In both trials, a randomized block design with four replications was used. The commercial dose of 1.40 L ha^{-1} caused greater phytotoxicity in the forage. In the greenhouse, physiologic and biometric variables were affected by the herbicide mixture. Except for the ambient CO_2 concentration, all variables showed a linearly decreasing behavior, especially when applied during the first tiller stage. Potential doses were determined as 0.35 L commercial product ha^{-1} when applied to plants with one tiller and 0.70 and 1.40 L commercial product ha^{-1} for two tillers. The forage showed greater tolerance when the application was made on plants with two tillers. Field conditions exhibited greater tolerance of BRS Piatã to imazethapyr + imazapic than observed under greenhouse conditions.

Key words: imidazolinone, tolerance to herbicide, forage yield

RESUMO: O consórcio de cereais com gramíneas forrageiras pode reduzir a produtividade de grãos devido à competição e a supressão com herbicidas tem surgido como uma estratégia de manejo viável. Objetivou-se neste estudo avaliar os efeitos da mistura pronta de imazetapir + imazapic na supressão do crescimento inicial de *Urochloa brizantha* cv. BRS Piatã. Em casa de vegetação, quatro doses (0, 0,35, 0,70 e 1,4 L de produto comercial ha^{-1}) da mistura pronta de imazetapir + imazapic (Zelone®, UPL, Campinas, Brasil) foram testadas e aplicadas nos estádios de primeiro e segundo perfilho da forrageira. Em campo, oito doses (0, 0,0438, 0,0873, 0,175, 0,35, 0,70, 1,05 e 1,40 L de produto comercial ha^{-1}) foram testadas. Em ambos os ensaios, utilizou-se um delineamento experimental em blocos casualizados, com quatro repetições. A dose comercial (1,40 L ha^{-1}) causou maior fitotoxicidade na forrageira. Em casa de vegetação, as variáveis fisiológicas e biométricas foram afetadas pela mistura de herbicidas. Com exceção da concentração de CO_2 ambiente, todas as outras variáveis apresentaram comportamento linear decrescente, especialmente quando as aplicações foram realizadas no primeiro perfilho. As doses potenciais foram determinadas em 0,35 L ha^{-1} do produto comercial quando aplicado em plantas com um perfilho e 0,70 e 1,40 L ha^{-1} do produto comercial para plantas com dois perfilhos. A forrageira apresentou maior tolerância quando a aplicação foi realizada em plantas com dois perfilhos. As condições de campo conferiram maior tolerância da BRS Piatã à mistura de imazetapir + imazapic do que a observada em casa de vegetação.

Palavras-chave: imidazolinona, tolerância a herbicida, rendimento de forragem

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Introduction

The genus Urochloa is prominent in pasture formation in Brazil, being the most cultivated forage due to its adaptability to more acidic and low-fertility soils. *Urochloa brizantha* is considered an excellent forage because of its high resistance to pests (especially pasture spittlebugs), positive response to fertilizer application, and high dry matter production. *U. brizantha* cv. BRS Piatã is a forage with good adaptation to medium fertility and poorly drained soils. It has greater leaf accumulation and finer stems than other species of the same genus, as well as productive precocity and high nutritional value (Lima et al., 2020; Ferreira Junior et al., 2025). Such characteristics make it attractive for integrated agricultural production systems.

Corn or sorghum is commonly intercropped in integrated systems with forages, especially in the second crop, when the forage biomass increases straw formation after cereal harvest to protect the soil. Thus, the soil remains covered during the off-season, serving as a conservation tool for no-till cultivation or for producing food for grazing animals (Sousa et al., 2021; Giagnocavo et al., 2022). On the other hand, intercropping can reduce grain yield due to forage competition, depending on the sowing method, plant density, and forage species. Interference reduction can be achieved through cultural practices, such as sowing the forage after cereal emergence or lower plant density (Gheno et al., 2021). Furthermore, low herbicide doses can suppress forage growth, making it a viable management option.

Forage sensitivity to herbicides is directly related to the dose used and the vegetative stage of the forage at the time of application, which determines the success or failure of the technique used. Therefore, the susceptibility of forages to herbicides that have the potential to be used in intercropping is essential to evaluate (Cabral et al., 2019). The forage should have some tolerance to the product, as the aim is to suppress it and not to compromise its potential due to the objective of straw or pasture formation. The development of sorghum plants tolerant to herbicides from the imidazolinone chemical group may be an alternative for managing intercropping between sorghum and Urochloa forage grasses.

Herbicides belonging to the imidazolinone chemical group inhibit the acetolactate synthase (ALS) enzyme, which is essential for the synthesis of branched-chain amino acids. Herbicides, such as imazethapyr and imazapic, are systemic, being absorbed and translocated from both leaves and roots, to inhibit amino acid synthesis, cell growth, and DNA synthesis (McCourt et al., 2006; Pandian et al., 2022; Rangel et al., 2022). The use of these herbicides in tolerant crops, such as sorghum, has become an efficient tool for forage suppression, enabling intercropping.

We hypothesized that the ready mix of imazethapyr + imazapic suppresses the initial growth of *U. brizantha* cv. BRS Piatã in a dose-dependent manner, with greater forage tolerance at more advanced phenological stages. Therefore, this study aimed to evaluate the effects of the ready mix of imazethapyr + imazapic on the initial growth suppression of *U. brizantha* cv. BRS Piatã.

Material and Methods

The experiments were conducted between February and September 2023 in a climate-controlled greenhouse and in a field in Rio Verde, which is located in the southwest of the state of Goiás, Brazil. The geographic coordinates of the location are 17° 49' 10.83" South latitude, 49° 12' 13.46" West longitude, with an altitude of 750 m. The regional climate is Aw, according to Köppen's classification, characterized by a mesothermal tropical savanna climate, with rain in summer and dry conditions in winter (Figure 1).

In the climate-controlled greenhouse experiment, the treatments were arranged in a randomized block design with four replications in a factorial scheme (4 × 2). The first factor consisted of four doses (0, 0.35, 0.70, and 1.40 L commercial product ha^{-1}) of the ready mix of imazapic + imazethapyr herbicides (Zelone®, UPL, Campinas, SP, Brazil), and the second factor consisted of two herbicide application times (when the plants had the first and second tillers).

The substrate used was soil from the 0–20 cm layer of a Rhodic Hapludox, according to the classification of Santos et al. (2018), with 49.3, 12.5, and 38.2% clay, silt, and sand, respectively. The soil had the following properties: pH $CaCl_2$ (1:2.5), 5.0; P Mehlich, 5.52 mg dm^{-3}; K, 132.80 mg dm^{-3}; S, 6.74 mg dm^{-3}; Ca, 6.25 $cmol_c$ dm^{-3}; Mg, 2.52 cmolc dm^{-3}; H + Al, 4.05 $cmol_c$ dm^{-3}; sum of bases, 9.11 $cmol_c$ dm^{-3}; CEC, 13.16 $cmol_c$ dm^{-3}; base saturation, 69.2%; and organic carbon, 2.41 dag kg^{-1}. The soil was mixed with organic compost in a 4:1 ratio (v/v) in a concrete mixer, and during homogenization, 5 g of the 08-20-18 formulation N-P_2O_5-K_2O, and 6 g of dolomitic limestone were added per volume of soil to each pot. The pots used were of 21 L, with an upper diameter of 28 cm, a bottom diameter of 21.5 cm, and height of 37 cm. After filling the pots, irrigation was carried out to maintain the soil moisture, and the pots were left to rest for 35 days.

The forage *U. brizantha* cv. BRS Piatã was sown on July 06, 2023, with 20 seeds placed per pot at a depth of 0.5 cm. Seedling emergence occurred up to July 17, 2023, and after emergence, two plants were maintained per pot. The spacing between pots was, on average, 40 × 40 cm. Drip irrigation was applied to the pots to standardize the volume of water applied to each pot. Hoagland's nutrient solution (Hoagland & Arnon, 1950) was applied once to the pots at a dose of 0.558 L per pot, only after the treatment application.

Applications of herbicide to plants occurred on July 26, 2023, at the first tiller stage and on August 09, 2023, at the second tiller stage. The imazapic + imazethapyr ready mix was applied using a CO_2-pressurized backpack sprayer, with a constant pressure of 2 bar, equipped with a bar of 4 TT110 02 tips spaced 0.5 m apart and a spray volume of 204 L ha^{-1}. In the first application, when the plants were in the first tiller stage, the climatic conditions were air temperature of 28.5 °C, relative humidity of 39%, and wind speed of 1.2 m s^{-1}. During the second application, the conditions showed an air temperature of 27.4 °C, relative humidity of 34.2%, and wind speed of 2.2 m s^{-1}. In all treatments, atrazine (Aclamado BR®, Ouro Fino, Brazil) was applied at a dose of 1500 g ha^{-1}.

Phytotoxicity evaluations were performed at 7, 14, 21, 28, and 35 days after application (DAA) of the imazapic + imazethapyr ready mix, with scores from 0 to 100, where 0 represented the absence of injury and 100 represented plant death (SBCPD, 1995). Phytotoxicity evaluations began on 02/08 and 16/08/2023 for applications made on plants with forage at the first and second tiller stages, respectively. On 23/08 (1st tiller) and 08/09/2023 (2nd tiller), physiological evaluations were carried out with an infrared gas analyzer (IRGA Li-6400 Licor) between 8 and 10 a.m., under constant photosynthetically active radiation (PAR) of 1000 µmol photons m^{-2} s^{-1}, CO_2 concentration of 415 µmol mol^{-1}, temperature of 25.3 °C, and relative humidity of 76%. The photosynthetic rate (A, µmol CO_2 m^{-2} s^{-1}); transpiration rate (E, mmol H_2O m^{-2} s^{-1}); stomatal conductance (gs, mol H_2O m^{-2} s^{-1}); intercellular (Ci, µmol mol^{-1}) and ambient CO_2 concentrations (Ca, µmol mol^{-1}), and their relationship; as well as their instantaneous (A/E, µmol CO_2 $mmol^{-1}$ H_2O) and intrinsic water use efficiencies (A/gs, µmol CO_2 mol^{-1} H_2O) were measured.

The forage was cut on September 18, 2023, 74 days after planting, and plant height, number of tillers, number of leaves, and dry masses of roots, culms, rhizomes, and leaves were evaluated. Plant height was measured in centimeters from the base of the plant to the apex of the last expanded leaf using a ruler. To obtain the dry mass variables of culms and leaves, plants were cut at the basal region and then placed in paper bags and dried in a forced-air circulation oven at 65 °C for 72 hours. After 72 hours, the samples were weighed on an analytical balance. The dry masses of rhizomes and roots were obtained after washing the plant material and subsequent separation of soil residues, sample conditioning, drying, and weighing.

Before starting the field experiment, soil samples were collected at a depth of 0–20 cm for chemical and physical analyses. The analysis of the Rhodic Hapludox found the following characteristics: pH 6.2 (SMP); K, 0.46 $cmol_c$ dm^{-3}; Ca, 4.64 $cmol_c$ dm^{-3}; Mg, 2.50 $cmol_c$ dm^{-3}; Al^{3+}, 0.04 $cmol_c$ dm^{-3}; H + Al, 4.5 $cmol_c$ dm^{-3}; CEC, 12.1 $cmol_c$ dm^{-3}; P (Melich), 13.1 mg dm^{-3}; organic matter, 3.62 dag kg^{-1}; base saturation, 62.8%; aluminum saturation, 0.5%; clay, 64.5; silt, 10.0; and sand, 25.5 dag kg^{-1}. Weeds in the area were chemically desiccated with glyphosate (1440 g ha^{-1}), and after 15 days, on 03/02/2023, soil preparation was done by plowing and then harrowing.

The experimental design employed was randomized blocks, with 8 treatments and 4 replications, composed of increasing doses of the imazapic + imazethapyr ready mix (0, 0.0438, 0.0873, 0.175, 0.35, 0.70, 1.05, and 1.40 L commercial product ha^{-1}) of the commercial formulation Zelone® (UPL, Brazil). Experimental plots consisted of eight 4-m-long rows and 1 m spacing between blocks. The observation area consisted of the two central rows, but without the 0.50 m borders on both sides. Sowing was carried out on 08/02/2023, using 8 kg ha^{-1} of pure viable seeds sown in rows spaced 0.50 m apart at a depth of 0.05 m. Sowing fertilization with N-P_2O_5-K_2O employed 150 kg ha^{-1} of the 04-28-16 formulation. In the total area, a dose of 1500 g a.i. ha^{-1} of atrazine (Aclamado BR®, Ouro Fino, Uberaba, MG, Brazil) was applied pre-emergence to control broadleaf weeds.

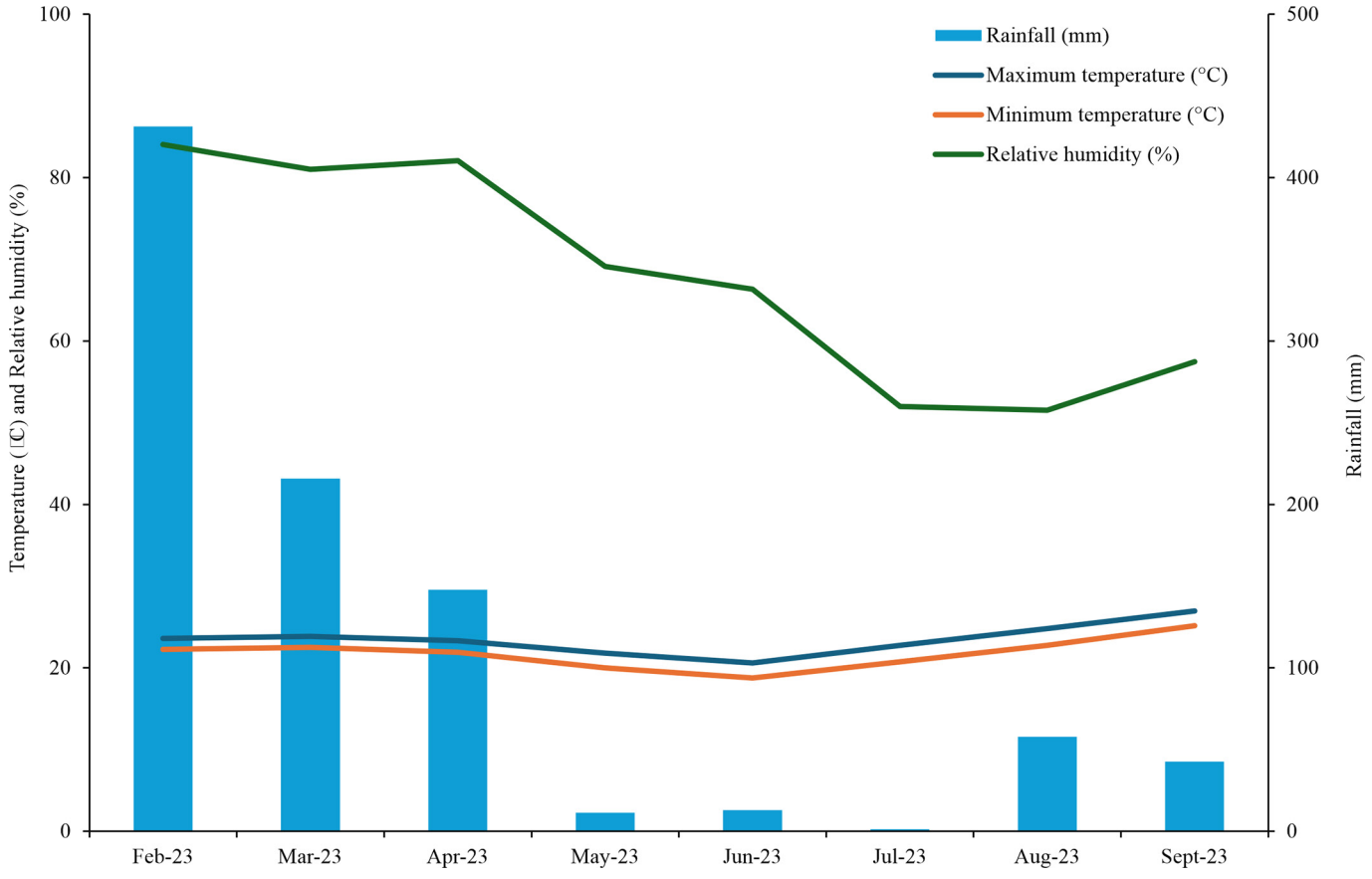


Figure 1. Maximum and minimum temperatures, relative humidity, and rainfall recorded during the experimental period

Treatments were applied on 04/03/2023 with a CO_2-pressurized sprayer, equipped with a bar of 4 TT11002 nozzles, spaced 0.50 m apart, positioned 0.50 m above the plant surface, with a spray volume of 200 L ha^{-1} and working pressure of 2 bar. The application was carried out in the morning, between 7:00 and 9:30 a.m., with a recorded air temperature of 27.2 °C, relative air humidity of 78%, wind speed of 1.0 m s^{-1}, and a moist soil surface. As cultural practices, 45 kg ha^{-1} of N in the form of urea was applied on 15/03/2023, and weeding was manually performed twice on 08/03 and 26/03/2023. The percentage of plant phytotoxicity was evaluated at 7, 14, 21, 28, and 35 DAA, establishing a percentage scale from 0 to 100.

On 19/04/2023, plant height was evaluated using a graduated ruler at three points in the plot, and the percentage of plant cover of the plot by the forage was estimated. The dry matter production of the forage was obtained by cutting the forage using a machete in an area of 2.0 m^2 of each experimental unit, at a height of 0.30 m. Subsequently, the fresh matter of the forage was measured, and a sample of about 0.50 kg was collected to determine the dry matter. This sample was separated into leaves and culms, placed in paper bags, and dried in a forced-air circulation chamber at 65 °C for 72 hours for subsequent dry matter determination. The leaf/culm ratio was calculated by dividing the dry matter values of the leaves and culms from 20 tillers randomly collected in each plot.

The number of clumps per m^2 and the number of tillers per clump were evaluated by putting a hollow 1 m^2 square within the cut area and counting the number of clumps and tillers. After the first plant collection, a standardization cut was performed across the entire experimental area using a manual brush cutter, and grass regrowth was evaluated. At 45 days after cutting, on 24/04/2023, 40 kg ha^{-1} of N in the form of urea was applied. On 09/10/2023, the second cut of the forage was performed, and height, plot plant cover, leaf/culm ratio, and forage production evaluations were carried out according to the procedures described in the first cut.

The data obtained were subjected to the Shapiro-Wilk test ($p \leq 0.05$) for normality analysis of the dataset and to Levene's test ($p \leq 0.05$) for equality of variances. In the climate-controlled greenhouse experiment, the results obtained were submitted to analysis of variance by the F-test ($p \leq 0.05$). When significant, the mean application times were submitted to Tukey's test ($p \leq 0.05$), and the mean herbicide doses to regression analysis. Phytotoxicity scores were submitted to response surface analysis as a function of ready mix herbicide doses and phytotoxicity evaluation periods.

In the field experiment, the results obtained were submitted to analysis of variance by the F-test ($p \leq 0.05$), and when significant, the means were adjusted to regression models. The models were selected based on their simplicity, biological significance, and coefficient of determination. Phytotoxicity scores were submitted to response surface analysis as a function of ready mix herbicide doses and phytotoxicity evaluation periods. Statistical analysis was performed using R version 4.0.4.

Results and Discussion

Analysis of variance revealed significant effects of herbicide doses, forage phenological stage (PSF), and days after herbicide application on the phytotoxicity of *U. brizantha* cv. BRS Piatã. Doses of the imazapic + imazethapyr mixture significantly affected phytotoxicity (Mean square = 11,300.10; $p \leq 0.05$ by the F-test), indicating greater foliar damage at high doses (Table 1). Phenological stage (PSF) was also relevant (Mean square = 6,438.90; $p \leq 0.05$ by the F-test), suggesting that plants in advanced stages respond differently to the herbicide. Interactions such as Doses × DAA and Doses × PSF ($p \leq 0.05$) point to variable responses over time and stages.

Figures 2A and B present the results of phytotoxicity evaluations when the ready mix herbicide was applied to plants at the first and second tiller stages, respectively. In plants with one tiller, an increase in dose resulted in greater phytotoxicity, with no tendency for symptom reduction over time at higher doses, peaking at 25 DAA. Plants with two tillers also displayed greater phytotoxicity at high doses; however, after 14 DAA, the symptoms decreased. In plants with one tiller, toxicity exceeded 60% at the highest dose, but with two tillers, the value was below 40%, indicating greater forage tolerance when the application was made at an advanced phenological stage.

The phytotoxicity symptoms observed, including leaf chlorosis, growth arrest, and necrosis, are characteristic of ALS-inhibiting herbicides, which block the biosynthesis of branched-chain amino acids (valine, leucine, and isoleucine), compromising meristematic activity and cell division (McCourt et al., 2006; Pandian et al., 2022). The more pronounced and persistent symptoms in plants with one tiller reflect the greater vulnerability of younger plants, which have a less developed root system and lower capacity for herbicide metabolization and dilution through growth. Cunha et al.

Table 1. Summary of the analysis of variance of phytotoxicity of *U. brizantha* cv. BRS Piatã forage treated with doses of the imazapic + imazethapyr ready mix evaluated at 7, 14, 21, 28, and 35 days after application (DAA)

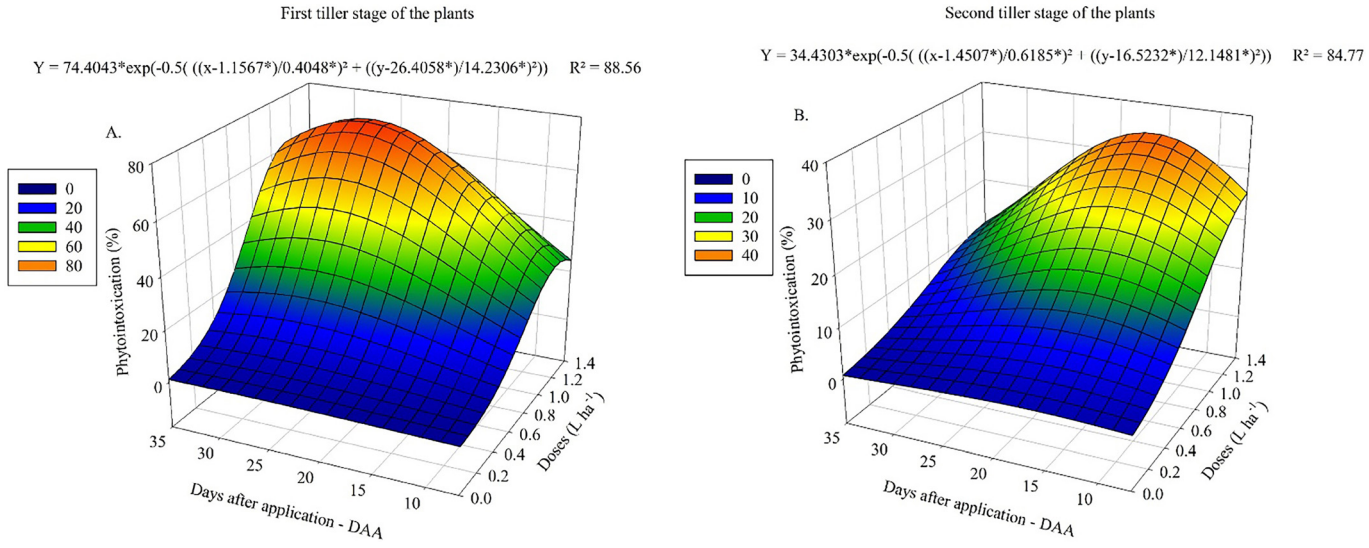
Source of variation	DF	Mean squares
		Phytotoxicity
Blocks	3	962.30*
Doses	3	11300.10*
DAA	4	352.28^{ns}
PSF	1	6438.90*
Doses × DAA	12	337.01*
Doses × PSF	3	1714.80*
DAA × PSF	4	517.87*
Doses × DAA × PSF	12	483.73*
Residual	117	157.01
CV (%)		72.88

DF - Degrees of freedom; CV - Coefficient of variation; DAA – Days after application; PSF - Phenological Stage of Forage; ns – Not significant ($p > 0.05$) by the F-test; * - Significant at $p \leq 0.05$ by the F-test

(2025) reported similar results, observing that increasing the dose increased phytotoxicity in BRS Ipyporã, but tolerance was greater when the application occurred in plants with two tillers. The authors emphasized that to avoid compromising forage potential, phytotoxicity symptoms should remain below 50% until 35 DAA. Thus, the imazethapyr + imazapic ready mix shows potential for suppressing *U. brizantha* cv. BRS Piatã, especially when applied at the second tiller stage of the plant.

The summary of the analysis of variance for the gas exchange variables of *U. brizantha* cv. BRS Piatã is presented in Table 2. A significant effect ($p \leq 0.05$) of the phenological stages of the forage (PSF) was observed on the photosynthetic rate (A); transpiration rate (E); stomatal conductance (gs); ambient (Ca) and intercellular (Ci) CO_2 concentrations; A/E ratio; and A/gs ratio. For the doses of the formulated mixture imazapic + imazethapyr, a significant effect on A and Ca was observed. The interaction Doses × PSF was significant for A, E, gs, and Ca, indicating that the response of the physiological variables to the herbicide doses depended on the phenological stage of the forage at the time of application. The variables Ci, Ci/Ca, A/E, and A/gs did not respond significantly to the interaction. The coefficients of variation (CV%) ranged from 0.86% for Ca to 27.96% for Ci, indicating good experimental precision for most of the variables evaluated.

Table 3 presents the physiological parameters evaluated. There was a significant interaction between dose and application time for photosynthetic rate (A), transpiration rate (E), stomatal conductance (gs), and ambient CO_2 concentration (Ca). For intercellular CO_2 concentration (Ci) and the Ci/Ca ratio, no significant effects of factors or their interactions were identified. The A/E and A/gs ratios were only influenced by application at different phenological stages, showing opposite behavior. A/E was higher when herbicides were applied at the first tiller stage, whereas A/gs was higher when applied at the second tiller stage of the forage. Comparing the stages within each dose, A, E, and gs showed higher values in plants with two tillers at all doses, while Ca was higher in plants with one tiller. Comparison of doses within each phenological stage indicated no difference between doses at the two-tiller stage, but a significant linear fit was observed for these variables at the first tiller stage. Except



* - Significant at $p \leq 0.05$ by the t-test

Figure 2. Phytotoxicity of *Urochloa brizantha* cv. BRS Piatã plants evaluated at 7, 14, 21, 28, and 35 days after application (DAA) (variable Y) of the doses (variable X) imazapic + imazethapyr ready mix applied at the first (A) and second tiller stages of the plants (B)

Table 2. Summary of the analysis of variance of photosynthetic rate (A), transpiration rate (E), stomatal conductance (gs), ambient (Ca), and intercellular (Ci) CO_2 concentrations, and Ci/Ca, A/E, and A/gs ratios of *Urochloa brizantha* cv. BRS Piatã forage treated with doses of the imazapic + imazethapyr ready mix at the first and second tiller stages (PSF) of the forage

SV	DF	Mean squares							
		A	E	gs	Ca	Ci	Ci/Ca	A/E	A/gs
Blocks	3	3.92^{ns}	0.07^{ns}	0.0002^{ns}	6.92^{ns}	552.41^{ns}	0.0037^{ns}	1.03ns	232.84^{ns}
Doses	3	52.38*	0.27^{ns}	0.0010^{ns}	90.68*	2606.75^{ns}	0.0165^{ns}	3.24^{ns}	766.95^{ns}
PSF	1	546.39*	15.04*	0.037*	1010.92*	2052.16^{ns}	0.0225^{ns}	28.53*	2864.86*
Doses × PSF	3	30.13*	0.88*	0.0035*	56.84*	622.73^{ns}	0.0054^{ns}	0.65^{ns}	409.47^{ns}
Residual	21	5.88	0.14	0.00055	10.68	1150.41	0.0078	1.31	457.89
CV (%)		15.77	20.72	22.88	0.86	27.96	27.67	13.12	13.88

SV – Source of variation; DF - Degrees of freedom; CV - Coefficient of variation; PSF - Phenological Stage of Forage; ns – Not significant ($p > 0.05$) by the F-test; * - Significant at $p \leq 0.05$ by the F-test

for Ca, which increased with dose, all other physiological parameters decreased with increasing dose.

The reductions in A, E, and gs of 6.2072 μmol CO_2 m^{-2} s^{-1}, 0.6761 mmol H_2O m^{-2} s^{-1}, and 0.0425 mol H_2O m^{-2} s^{-1} per liter of product applied, respectively, with R^2 values above 91%, reflect the direct impact of the herbicide mixture on plant gas exchange. Stomatal conductance governs the simultaneous diffusion of CO_2 into the leaf and water vapor out of it. Its reduction by ALS-inhibiting herbicides is associated with oxidative stress and disruption of guard cell metabolism (Farias et al., 2020). The consequent reduction in CO_2 availability limits the Calvin cycle, reducing photosynthetic assimilation (A) and, by extension, the transpiration rate (E) (Vieira et al., 2023). The increase in ambient CO_2 concentration (Ca) with increasing dose, despite the reduction in CO_2 uptake, is consistent with the simultaneous decrease in stomatal conductance and photosynthetic demand; as fewer stomata open and less CO_2 is fixed by the mesophyll, the external concentration rises around the leaf surface (McCourt et al., 2006).

The absence of significant differences in A, E, and gs among doses at the two-tiller stage indicates that plants at this phenological stage possessed sufficient metabolic capacity to buffer the herbicide effects. This tolerance may be attributed to a larger leaf area and more active root system at the two-tiller stage, enabling greater herbicide dilution through growth and more efficient metabolization of the imidazolinone molecules (Cunha et al., 2025). The A/gs ratio (intrinsic water use efficiency) was higher in plants treated at the two-tiller stage, suggesting that although these plants maintained adequate stomatal conductance, they used water more efficiently for carbon fixation – a physiological indicator of overall plant health under stress (Pandian et al., 2022).

The summary of the analysis of variance for the growth and biomass accumulation variables of *U. brizantha* cv. BRS Piatã is presented in Table 4. The analysis revealed a significant effect

Table 3. Photosynthetic rate (A), transpiration rate (E), stomatal conductance (gs), ambient (Ca) and intercellular (Ci) CO_2 concentrations, Ci/Ca ratio, A/E ratio, and A/gs ratio of *Urochloa brizantha* cv. BRS Piatã forage treated with doses of the imazapic + imazethapyr ready mix at the first and second tiller stages of the forage

Variables	Application Time	Doses (L ha^{-1})				Means	Model	R^2 %
		0	0.35	0.7	1.4			
A (μmol CO_2 m^{-2} s^{-1})	1st tiller	15.79 b	13.57 b	10.15 b	7.23 b	11.68	$\hat{Y} = 15.4855 - 6.2072 * x$	96.91
	2nd tiller	19.98 a	20.20 a	19.08 a	18.79 a	19.51	$\hat{Y} = \bar{Y} = 19.51$	-
E (mmol H_2O m^{-2} s^{-1})	1st tiller	1.75 b	1.28 b	1.06 b	0.75 b	1.21	$\hat{Y} = 1.6255 - 0.6761 * x$	92.23
	2nd tiller	2.47 a	2.43 a	2.38 a	2.87 a	2.54	$\hat{Y} = \bar{Y} = 2.54$	-
gs (mol H_2O m^{-2} s^{-1})	1st tiller	0.11 a	0.08 b	0.06 b	0.04 b	0.07	$\hat{Y} = 0.0987 - 0.0425 * x$	91.94
	2nd tiller	0.13 a	0.13 a	0.13 a	0.16 a	0.14	$\hat{Y} = \bar{Y} = 0.14$	-
Ca (μmol mol^{-1})	1st tiller	378.79 a	381.88 a	386.38 a	392.66 a	384.93	$\hat{Y} = 378.7745 + 10.0467 * x$	99.51
	2nd tiller	373.13 b	372.88 b	374.29 b	374.45 b	373.69	$\hat{Y} = \bar{Y} = 373.69$	-
Ci (μmol mol^{-1})	1st tiller	124.55^{ns}	90.03	108.50	130.05	113.28	$\hat{Y} = \bar{Y} = 113.28$	-
	2nd tiller	117.39^{ns}	117.05	120.56	162.19	129.30	$\hat{Y} = \bar{Y} = 129.30$	-
Ci/Ca	1st tiller	0.33^{ns}	0.24	0.28	0.33	0.29	$\hat{Y} = \bar{Y} = 0.29$	-
	2nd tiller	0.31^{ns}	0.31	0.32	0.43	0.35	$\hat{Y} = \bar{Y} = 0.35$	-
A/E (μmol CO_2 (mmol H_2O)$^{-1}$	1st tiller	9.25	10.56	9.89	9.16	9.71 a	$\hat{Y} = \bar{Y} = 9.71$	-
	2nd tiller	8.14	8.31	8.06	6.73	7.81 b	$\hat{Y} = \bar{Y} = 7.81$	-
A/gs (μmol CO_2 (mol H_2O)$^{-1}$	1st tiller	152.02	176.12	167.74	157.93	163.45 b	$\hat{Y} = \bar{Y} = 163.45$	-
	2nd tiller	151.80	151.91	150.66	124.41	144.70 a	$\hat{Y} = \bar{Y} = 144.70$	-

ns - Not significant by the F-test ($p > 0.05$). *Significant regression model by t-test ($p \leq 0.05$). Means followed by the same letters are statistically equal by Tukey's test at 5% significance

Table 4. Summary of the analysis of variance of plant height (PH), number of tillers per plant (NTP), number of leaves (NL), dry masses of leaves (DML), culms (DMC), rhizomes (DMRh), and roots (DMR) of *Urochloa brizantha* cv. BRS Piatã forage treated with doses of the imazapic + imazethapyr ready mix when the forage was at the 1st and 2nd tiller stages (PSF)

SV	DF	Mean squares						
		PH	NTP	NL	DML	DMC	DMRh	DMR
Blocks	3	516.03*	7.44^{ns}	681.00*	79.39^{ns}	50.31^{ns}	4.41*	14.08*
Doses	3	695.36*	7.71^{ns}	2304.60*	1014.64*	983.44*	18.06*	59.63*
PSF	1	520.03*	11.88^{ns}	1270.96*	219.81*	301.78*	2.73^{ns}	15.80*
Doses × PSF	3	106.78^{ns}	28.67*	1193.29*	185.16*	101.59^{ns}	7.56*	8.21*
Residual	21	72.29	6.52	232.47	42.05	40.10	1.31	1.85
CV (%)		8.96	24.07	19.81	24.91	26.94	32.68	25.30

SV – Source of variation; DF - Degrees of freedom; CV - Coefficient of variation; PSF - Phenological stage of forage; ns – Not significant at $p > 0.05$ by the F-test; * - Significant at $p \leq 0.05$ by the F-test

($p \leq 0.05$) of the doses of the formulated mixture imazapic + imazethapyr on plant height (PH), number of leaves (NL), dry masses of leaves (DML), stems (DMC) and rhizomes (DMRh), and roots (DMR), indicating that the application of herbicides compromised the morphological development and biomass accumulation of the forage. The number of tillers per plant (NTP) was not significantly influenced by the applied doses. The phenological stage (PSF) exerted a significant effect on PH, NL, DML, DMC, and DMR, showing that the timing of herbicide application is a determining factor for the intensity of the damage observed in the forage. The interaction between Doses × PSF was significant for NTP, NL, DML, DMRh, and DMR, demonstrating that the effect of doses on these variables varied according to the phenological stage the forage was in at the time of application. On the other hand, PH and DMC did not show a significant interaction, suggesting that the effect of doses on these characteristics was consistent regardless of the application stage.

Height and culm mass were greater when applications occurred at the second tiller stage and decreased linearly with increasing dose (Table 5). At the first tiller stage, all variables with significant interaction were reduced with increasing herbicide doses. At the second tiller stage, only leaf mass, rhizome mass, and root mass differed among doses, with decreasing linear behavior.

Forage height and culm mass decreased with increasing dose, with reductions per liter of herbicide of 15.54 cm and 18.0983 g per plant, with R^2 values of 99.28 and 95.20%, respectively. Reductions per liter of product for applications at the first and second tiller stages, for leaf mass were 23.7475 and 12.4245 g per plant, for rhizome mass 3.6210 and 1.1623 g per plant, and for root mass 5.1099 and 3.3230 g per plant, respectively. The number of tillers and leaves differed among doses only at the first tiller stage, with reductions of 3.7959 tillers and 41.4496 leaves per liter of commercial product applied.

Herbicides that inhibit the ALS enzyme reduce the synthesis of essential amino acids (valine, leucine, and isoleucine), which are critical for the biosynthesis of proteins, coenzymes, and secondary metabolites necessary for plant growth (McCourt et al., 2006; Mendes & Silva, 2023). The arrest of cell division and elongation in meristematic regions – particularly root tips and apical meristems – explains the disproportionate reductions observed in root and rhizome mass at the first tiller stage (Mendes & Silva, 2023). Roots are especially sensitive to ALS inhibitors because they depend on continuous meristematic activity and are directly exposed to the herbicide via soil absorption (Grzanka et al., 2024). The average 50% reduction in leaf, rhizome, and root masses at the second tiller stage compared to the first tiller stage, as well as the absence of differences in tiller and leaf numbers among doses at this stage, reinforces the greater developmental plasticity and metabolic resilience of BRS Piatã at more advanced phenological stages. At the first tiller stage, doses from 0.70 L ha^{-1} caused more than 60% reduction in biometric variables relative to the control, compromising forage yield potential and suggesting that applications at this stage must not exceed 0.35 L commercial product ha^{-1} (25% of the commercial dose) to preserve acceptable forage production (Gheno et al., 2021; Cunha et al., 2025).

The summary of the analysis of variance for the phytotoxicity of *U. brizantha* cv. BRS Piatã subjected to doses of the formulated mixture imazapic + imazethapyr is presented in Table 6. A significant effect ($p \leq 0.05$) was observed for all factors evaluated: herbicide doses, days after application (DAA), and the interaction Doses × DAA. The

Table 5. Plant height (PH), number of tillers per plant (NTP), number of leaves (NL), and dry masses of leaves (DML), culms (DMC), rhizomes (DMRh), and roots (DMR) of *Urochloa brizantha* cv. BRS Piatã forage treated with doses of the imazapic + imazethapyr ready mix at the first and second tiller stages of the forage

Variables	Application Time	Doses (L ha^{-1})				Means	Model	R^2 %
		0	0.35	0.70	1.40			
PH (cm)	1st tiller	102.75	99.50	85.25	76.00	90.88 b	$\hat{Y} = 104.425 - 15.54 * x$	99.28
	2nd tiller	106.50	99.50	99.50	90.25	98.94 a		
NTP	1st tiller	14.00 a	8.63 a	9.88 a	7.50 b	10.00	$\hat{Y} = 12.325 - 3.7959 * x$	63.94
	2nd tiller	9.75 b	12.13 a	11.50 a	11.50 a	11.22	$\hat{Y} = \bar{Y} = 11.22$	-
NL	1st tiller	108.25 a	67.75 b	63.25 a	43.33 b	70.65	$\hat{Y} = 96.0335 - 41.4496 * x$	82.83
	2nd tiller	85.00 b	97.50 a	81.00 a	69.50 a	83.25	$\hat{Y} = \bar{Y} = 83.25$	-
DML (g per plant)	1st tiller	45.13 a	22.95 a	17.03 b	8.53 b	23.41	$\hat{Y} = 37.9535 - 23.7475 * x$	82.37
	2nd tiller	36.00 a	31.85 a	28.18 a	18.58 a	28.65	$\hat{Y} = 36.26 - 12.4245 * x$	99.68
DMC (g per plant)	1st tiller	39.13	20.98	14.20	7.43	20.43 b	$\hat{Y} = 34.5892 - 18.0983 * x$	95.20
	2nd tiller	35.38	30.15	27.08	13.70	26.58 a		
DMRh (g per plant)	1st tiller	6.59 a	3.29 a	1.92 b	1.08 a	3.22	$\hat{Y} = 5.4384 - 3.6210 * x$	79.65
	2nd tiller	4.36 b	4.15 a	3.96 a	2.75 a	3.80	$\hat{Y} = 4.5161 - 1.1623 * x$	92.63
DMR (g per plant)	1st tiller	9.84 a	4.28 b	2.77 b	1.81 a	4.67	$\hat{Y} = 7.8036 - 5.1099 * x$	72.47
	2nd tiller	8.26 a	7.15 a	5.19 a	3.71 a	6.08	$\hat{Y} = 8.1142 - 3.323 * x$	93.11

ns - Not significant by F-test ($p > 0.05$). *Significant regression model by t-test ($p \leq 0.05$). Means followed by the same letters indicate no statistical differencesl by Tukey's test at 5% significance

significance of the interaction indicates that the expression of phytotoxicity symptoms in plants over time depended on the applied dose, i.e., the pattern of damage evolution varied according to the intensity of the herbicide treatment.

Figure 3 presents the phytotoxicity data, showing that increasing doses elevated symptoms; however, the values remained below 20% across all evaluation periods, indicating a slight herbicide effect and high forage tolerance under field conditions. According to Cruvinel et al. (2021), for initial suppression studies, phytotoxicity symptoms should remain below 60% until 21 DAA. The values observed in this study were well below this threshold, reinforcing the high tolerance of BRS Piatã under field conditions. The lower phytotoxicity in the field compared to the greenhouse may be attributed to several factors: the greater soil volume available for root exploration, which dilutes herbicide concentration per unit biomass; the higher organic matter content of the soil (3.62 dag kg^{-1}), which promotes greater herbicide sorption and reduces bioavailability; and the more vigorous plant development under field conditions, which favors more rapid metabolization and dilution of imidazolinone molecules through biomass accumulation (Gheno et al., 2021; Mendes & Silva, 2023).

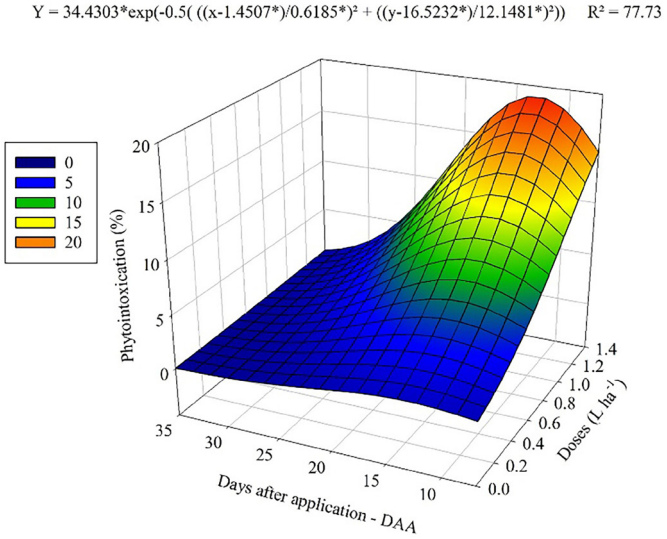
Table 6. Summary of the analysis of variance of phytotoxicity of *Urochloa brizantha* cv. BRS Piatã forage treated with doses of the imazapic + imazethapyr ready mix evaluated at 7, 14, 21, 28, and 35 days after application (DAA)

Source of variation	DF	Mean Square
		Phytotoxicity
Blocks	3	40.43*
Doses	7	271.52*
DAA	4	288.90*
Doses × DAA	28	50.21*
Residual	117	11.32
CV (%)		97.81

DF - Degrees of freedom; CV - Coefficient of variation; ns – Not significant ($p > 0.05$) by the F-test; * - Significant at $p \leq 0.05$ by the F-test

The summary of the analysis of variance for the production and canopy structure variables of *U. brizantha* cv. BRS Piatã evaluated in the first and second cuts is presented in Table 7. In the first cut, none of the evaluated variables—plant height (PH), soil cover area (SCAF), number of clumps (NC), number of tillers (NT), dry mass (DM), and leaf/stem ratio (LCR)—showed a significant effect of the doses of the formulated mixture imazapic + imazethapyr according to the F-test. Similarly, in the second cut, the doses also did not exert a significant influence on any of the analyzed variables. These results suggest that, under the evaluated conditions, the forage demonstrated recovery capacity throughout the production cycle, so that the phytotoxic effects initially observed did not translate into significant reductions in the production and canopy structure variables in the cuts performed.

Table 8 presents the biometric and productive characteristics of the forage in two cuttings, with no



* - Significant at $p \leq 0.05$ by the t-test

Figure 3. Phytotoxicity of *Urochloa brizantha* cv. BRS Piatã plants evaluated at 7, 14, 21, 28, and 35 days after application (DAA) (variable Y) as a function of imazapic + imazethapyr ready-mix doses (variable X)

Table 7. Summary of the analysis of variance of plant height (PH), soil covered area by forage (SCAF), number of clumps (NC), number of tillers (NT), dry mass (DM), and leaf/culm ratio (LCR) of *Urochloa brizantha* cv. BRS Piatã forage treated with doses of the imazapic + imazethapyr ready mix evaluated in the first and second cuts

SV	DF	Mean squares					
		PH	SCAF	NC	NT	DM	LCR
		First cut					
Blocks	3	73.08*	26.28^{ns}	0.70^{ns}	17348.91^{ns}	830319.44^{ns}	0.0069^{ns}
Doses	7	21.35^{ns}	11.70^{ns}	1.19^{ns}	49237.50^{ns}	772858.53^{ns}	0.065^{ns}
Residual	21	8.63	11.70	5.44	196463.05	692082.80	0.044
CV (%)		2.34	3.45	18.39	24.66	24.28	14.75
		Second cut					
Blocks	3	210.20*	1.75^{ns}	7.25^{ns}	1933325.91^{ns}	6424459.28*	0.085^{ns}
Doses	7	21.69^{ns}	3.64^{ns}	6.68^{ns}	1504158.28^{ns}	1693100.44^{ns}	0.073^{ns}
Residual	21	26.77	1.86	2.84	723711.70	1421737.37	0.103
CV (%)		5.24	1.37	16.06	26.79	24.87	17.38

SV – Source of variation; DF - Degrees of freedom; CV - Coefficient of variation; ns, * - Not significant and significant by the F-test, respectively

Table 8. Plant height (PH), soil covered area by forage (SCAF), number of clumps (NC), number of tillers (NT), dry mass (DM), and leaf/culm ratio (LCR) of *Urochloa brizantha* cv. BRS Piatã forage treated with doses of the imazapic + imazethapyr ready mix evaluated in the first and second cuts

Variables	Doses of imazapic + imazethapyr ready mix (L ha^{-1})							
	0.0	0.0438	0.0873	0.175	0.35	0.70	1.05	1.4
	First cut							
PH (cm)	128.50^{ns}	126.25	128.75	126.75	124.75	122.25	125.5	123.25
SCAF (%)	100.00^{ns}	100.00	98.75	99.50	99.50	100.00	100.00	95.00
NC (n m^{-2})	12.75^{ns}	12.25	12.00	12.75	13.00	13.75	12.25	12.75
NT (n m^{-2})	1798.75^{ns}	1702.00	1628.75	1938.25	1824.25	1863.5	1920.5	1705.00
DM (kg ha^{-1})	3718.89^{ns}	3862.67	3716.96	3483.98	3747.93	3121.62	3191.00	2567.80
LCR	1.65^{ns}	1.30	1.28	1.45	1.30	1.49	1.47	1.51
	Second cut							
PH (cm)	100.75^{ns}	94.75	99.50	100.50	96.75	101.00	100.25	97.00
SCAF (%)	100.00^{ns}	100.00	99.25	100.00	99.50	100.00	100.00	97.25
NC (n m^{-2})	9.75^{ns}	7.75	11.25	11.00	10.25	12.75	10.50	10.75
NT (n m^{-2})	1504.25^{ns}	1025.00	1770.50	1685.50	1484.25	2111.38	1612.13	1510.50
DM (kg ha^{-1})	4640.03^{ns}	4421.27	4294.55	5688.98	3741.40	5522.92	5081.25	4961.52
LCR	1.84^{ns}	2.10	1.90	1.68	1.82	1.70	1.80	1.95

ns –Not significant ($p > 0.05$) by F-test

significant effects of doses for any variable evaluated in either cut. This corroborates the phytotoxicity results, confirming the high tolerance of BRS Piatã to the imazethapyr + imazapic ready mix under field conditions. Dry matter production in the first cut ranged from 2,567.80 to 3,862.67 kg ha^{-1} and in the second cut from 3,741.40 to 5,688.98 kg ha^{-1}, without significant differences among doses. Doses that reduce dry mass by 20–60% compared to the control are considered effective for suppression (Gheno et al., 2021; Cunha et al., 2025); however, in the present study, no dose reached even the minimum 20% reduction threshold, even at 1.40 L ha^{-1}. Cunha et al. (2025) also indicated 1.40 L ha^{-1} as the dose with the greatest suppressive potential for BRS Ipyporã, noting that this corresponds to the commercial dose of the ready mix, which may simultaneously contribute to weed management in the intercropping system.

The absence of significant herbicide effects on plant height, soil cover, number of clumps, number of tillers, and leaf/culm ratio further underscores the robustness of BRS Piatã under field conditions. These results diverge from the greenhouse findings and highlight the importance of validating suppression studies under real field conditions before making management recommendations. The differences between environments may also reflect the application timing in the field experiment. Perhaps the treatments were applied at a more advanced phenological stage compared to the greenhouse first-tiller-stage applications, when tolerance is inherently lower. Oliveira et al. (2020) observed that BRS Piatã did not reduce sorghum grain yield in intercropping, suggesting that chemical suppression may not be indispensable in all systems. Nevertheless, the confirmed tolerance of BRS Piatã to imazapic + imazethapyr at the commercial dose reinforces the agronomic viability of using the labeled dose in intercropping with imidazolinone-tolerant sorghum, with the dual benefit of forage establishment and weed control. Further studies under field conditions, including different sowing dates, soil types, and intercropping arrangements, are recommended to consolidate the suppression dose recommendation.

Conclusions

1. The imazethapyr + imazapic ready mix suppressed the initial growth of *Urochloa brizantha* cv. BRS Piatã in a dose-dependent manner under greenhouse conditions. Greater forage tolerance was observed at the second tiller stage, with phytotoxicity remaining below 40% even at the highest dose.

2. At the first tiller stage, doses should not exceed 0.35 L commercial product ha^{-1} to preserve forage yield potential. At the second tiller stage, doses of 0.70 and 1.40 L commercial product ha^{-1} are viable without significant impairment of productive variables.

3. Under field conditions, BRS Piatã exhibited high tolerance to all tested doses, with phytotoxicity below 20% and no significant effects on productive variables. Field conditions conferred greater herbicide tolerance than observed under greenhouse conditions.

4. The tolerance of BRS Piatã to the commercial dose supports the agronomic viability of its use in intercropping with imidazolinone-tolerant sorghum. Additional field studies under different intercropping systems and edaphoclimatic conditions are needed to further verify the recommended suppression dose.

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analysis, Investigation, Methodology. Jardel L. Pereira: Supervision, Validation, Writing - review & editing. Adriano Jakelaitis: Supervision, Validation, Writing - review & editing.

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