

Glyphosate induced hormesis in *Urochloa* cultivars with sequential application

Hormese induzida por glifosato em cultivares de *Urochloa* com aplicação sequencial

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Abstract

Glyphosate is the most widely used herbicide and is responsible for a significant technical/productive advance in world agriculture. Glyphosate drift after its application to control weeds in agricultural fields can stimulate growth and/or productive performance in non-target plants, located adjacent to the treatment, characterizing the hormesis effect. However, the hormesis effect of glyphosate may be different depending on the plant species, its stage of development, and the applied dose. Considering the stimulus of forage biomass production for animal feed, this study aimed to assess the hormesis effect by successive applications of low glyphosate doses to cultivars of the genus *Urochloa*. The shoot and root productive responses of three grass cultivars (*Urochloa brizantha* cv. Marandu, *U. brizantha* cv. Piatã, and *U. ruziziensis* cv. Ruziziensis) were assessed in pots through leaf applications of subdoses of the acid equivalent (ae) of glyphosate (5.40, 10.80, 21.60, 43.20, and 86.40 g ae ha⁻¹) and a control (no glyphosate application). Four sequential harvests, conducted with a frequency of 21 days in a completely randomized design and a 6 x 3 x 4 factorial arrangement, with three replications, were assessed. Doses equal to or higher than 43.20 g ae ha⁻¹ of glyphosate, applied in two sequential applications impaired the biomass production of the assessed forages. Ruziziensis was the most susceptible cultivar to the phytotoxic effect. Sequential applications of the subdoses 5.40 and 10.80 g ae ha⁻¹ of glyphosate characterized the hormesis effect, promoting the shoot and root biomass production of the forage plants *U. brizantha* cv. Marandu, *U. brizantha* cv. Piatã, and *U. ruziziensis* cv. Ruziziensis.

Keywords: Drift; acid equivalent; forage; hormesis; *Urochloa*.

Resumo

Após a aplicação do herbicida glifosato para controle de plantas daninhas em cultivo agrícola, constata-se que a deriva do produto pode estimular crescimento e/ou rendimento produtivo em plantas não-alvo, localizadas nas adjacências do tratamento, caracterizando efeito hormese. Entretanto, os efeitos horméticos por glifosato podem ser diferentes para uma determinada espécie vegetal, o que depende da dose e do estágio de desenvolvimento da planta. Pressupondo estímulo de produção de biomassa de forragem para alimentação animal, o trabalho teve por objetivo avaliar o efeito hormese por aplicações sucessivas de subdose de glifosato em cultivares do gênero *Urochloa*. Foram avaliadas as respostas produtivas aérea e radicular em três cultivares de gramíneas (*Urochloa brizantha* cv. Marandu, *U. brizantha* cv. Piatã e *U. ruziziensis* cv. Ruziziensis) por aplicação foliar das subdoses do equivalente ácido (e.a.) de glifosato (5,40; 10,80; 21,60; 43,20; e, 86,40 g e.a. ha⁻¹) e controle. Foram avaliadas quatro colheitas sequenciais, realizadas com frequência de 21 dias, em esquema fatorial 6 x 3 x 4, com três repetições, em delineamento inteiramente casualizado. Dose igual e superior a 43,2 g e.a. ha⁻¹ de glifosato, em duas aplicações sequenciais, prejudicaram a produção de biomassa das forrageiras avaliadas. Dentre os cultivares, Ruziziensis foi mais susceptível ao efeito fitotóxico. Aplicações sequenciais das subdoses 5,40 e 10,80 g e.a. ha⁻¹ de glifosato, caracterizaram efeito hormético, promovendo a produção de biomassa aérea e radicular das forrageiras Marandu, Piatã e Ruziziensis.

Palavras-chave: deriva; equivalente ácido; forragem; hormese; *Urochloa*

1. Introduction

Glyphosate is the most widely used phytosanitary product among agricultural pesticides and is responsible for one of the greatest advances in world agriculture⁽¹⁾. Glyphosate is a systemic and non-selective herbicide, used in different crops for pre-planting desiccation, weed control

in post-emergence, and genetically modified crops (such as soybean, corn, and cotton). It is also used in the pre-harvest of soybean as a desiccant, in fallow areas, and as a sugarcane ripener, among other non-agricultural purposes^(2,3).

Weed management with herbicide application is fundamental for agriculture, prioritizing the rapid and vigorous initial development of the crop of productive

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interest⁽⁵⁾. Drift caused by application errors or the action of wind in dragging the glyphosate mist to other areas may cause an unintended stimulatory effect when low doses are absorbed by the plant⁽⁶⁾. Drift to non-target crops can lead to a productive stimulus or qualitative (as reported in protein⁽⁷⁾, lignin^(8, 9), and sugars⁽¹⁰⁾) and quantitative (e.g. in height⁽¹¹⁾ and leaf and root lengths⁽¹²⁾) changes in the plants, characterizing the effect called hormesis.

The application of underdoses of a product is the most used way to quantify its drift effect. Few studies have assessed the hormesis effect on forage grasses. The effects of underdoses of glyphosate are not yet very practical for technological implementation due to limited study material. However, a possible induction of plant growth has been identified with the application of underdoses of glyphosate in various plant species. The fact that low doses per area are required to obtain a response, the affordable price of these products, and the availability of different active ingredients have contributed to the recommendation of glyphosate as a stimulant for plant growth and/or development⁽¹¹⁻¹³⁾.

Forage biomass production for animal feed significantly alters the carrying capacity of the pasture, which is influenced by soil fertility and forage management, as well as climate conditions, while the nutritional value interferes with the animal's weight gain and depends, mainly, on the plant age, plant species, and grazing height⁽¹⁴⁾. Pastures play a fundamental role in Brazilian cattle raising, as they are the most economical and practical way of producing and offering food to animals, with low production costs.

Research into the effects of hormesis is necessary for its implementation. Therefore, this study aimed to assess forage biomass production after the sequential application of glyphosate underdoses to grasses of the genus *Urochloa* (synonym *Brachiaria*).

2. Material and methods

An experiment with grasses of the genus *Urochloa* was conducted from March to August 2019 in pots in a greenhouse at the Federal Institute of Education, Science, and Technology of Rondônia (IFRO), Ariquemes Campus, Brazil.

The experiment was laid out in a completely randomized design with a 6 x 3 x 4 factorial arrangement, involving three replications. The glyphosate levels factor was limited to five underdoses based on recommendations by Silveira et al.⁽¹⁵⁾, according to whom doses higher than 90 g ha⁻¹ of the acid equivalent (ae) of glyphosate allow effective control of the initial growth of the main forage crops. Thus, the main plots consisted of leaf application of the herbicide glyphosate at the commercial concentration of 360 g L⁻¹ at five underdoses (5.40, 10.80, 21.60, 43.20, and 86.40 g ae ha⁻¹) and a control (without herbicide), in three cultivars (*Urochloa brizantha* cv. Marandu, *U. brizantha* cv.

Piatã, and *U. ruzizensis* cv. Ruzizensis). The measurements were repeated over time, with a 21-day collection frequency, totaling four sequential assessments.

The seeds of the cultivars were obtained from a commercial lot sample (2018/2019 harvest) and sown on March 17, 2019. The substrate consisted of the homogenization of ravine soil (Oxisol), washed sand, and organic material derived from compost at the proportions of 60, 26, and 14%, respectively. The substrate fertility and texture were analyzed and the following results were obtained: pH (in water) = 7.6; organic carbon = 1.0 dag kg⁻¹; P and K (Mehlich-1) = 440 and 55 mg dm⁻³, respectively; Ca²⁺, Mg²⁺, and Al³⁺ (1 mol L⁻¹ KCl) = 5.0, 1.2, and 0.0 cmol_c dm⁻³, respectively; Mn and Fe = 104.9 and 133 mg dm⁻³; base saturation = 100; and clay, silt, and sand = 353, 77, and 580 g kg⁻¹, respectively (a sandy clay loam textured soil). The experimental units consisted of pots with a capacity of 7 dm³ (surface area of 0.0314 m²). The substrate was arranged considering an average density of 1.1 kg dm⁻³ and a moisture maintenance of around 60% of the field capacity by gravimetry.

Thinning was performed 15 days after sowing (DAS), leaving four forage plants per pot. A forage uniformity cut was performed in all experimental plots at 55 DAS (March 11, 2019) by harvesting the shoots, with a defoliation intensity of 20 cm from the substrate surface level⁽¹⁶⁾, aiming to obtain a portion of the forage with residual leaf area and basal and lateral buds for regrowth⁽¹⁷⁾. Fertilization with nitrogen (urea) and potassium (potassium chloride) was split at the time of the uniformity cut and the first three assessment cuts, totaling 50 kg N ha⁻¹ and 40 kg K₂O ha⁻¹⁽¹⁸⁾. Glyphosate (Nortox SL[®], 360 g ae L⁻¹) underdoses were applied to the experimental plots on the 7th day after the uniformity cut and the first three cuts (Figure 1). The applications were performed using a manual CO₂ compression sprayer, providing a spray solution volume of 100 L ha⁻¹, corresponding to 3.2 mL pot⁻¹ to completely reach the forage canopy.

The assessments were performed at 76 (1st cut), 97 (2nd cut), 118 (3rd cut), and 138 DAS (4th cut) by harvesting the total green forage mass from the pots at 20 cm from the surface. Dry mass production (DMP) was determined by drying the samples of shoot fresh mass, consisting of leaves and pseudostems, in a forced-air ventilation oven at 55 °C for 72 h, following the INCT-CA G-001/2 method⁽¹⁹⁾.

Root dry mass (RDM) was obtained from a single measurement carried out in the 4th assessment cut. The plants were taken from the pots and their roots were washed over sieves in running water, followed by drying in a forced-air circulation oven at 55 °C until constant weight. In this case, the analysis was carried out in a completely randomized design, consisting of glyphosate and control doses, with three replications, totaling 54 experimental units.

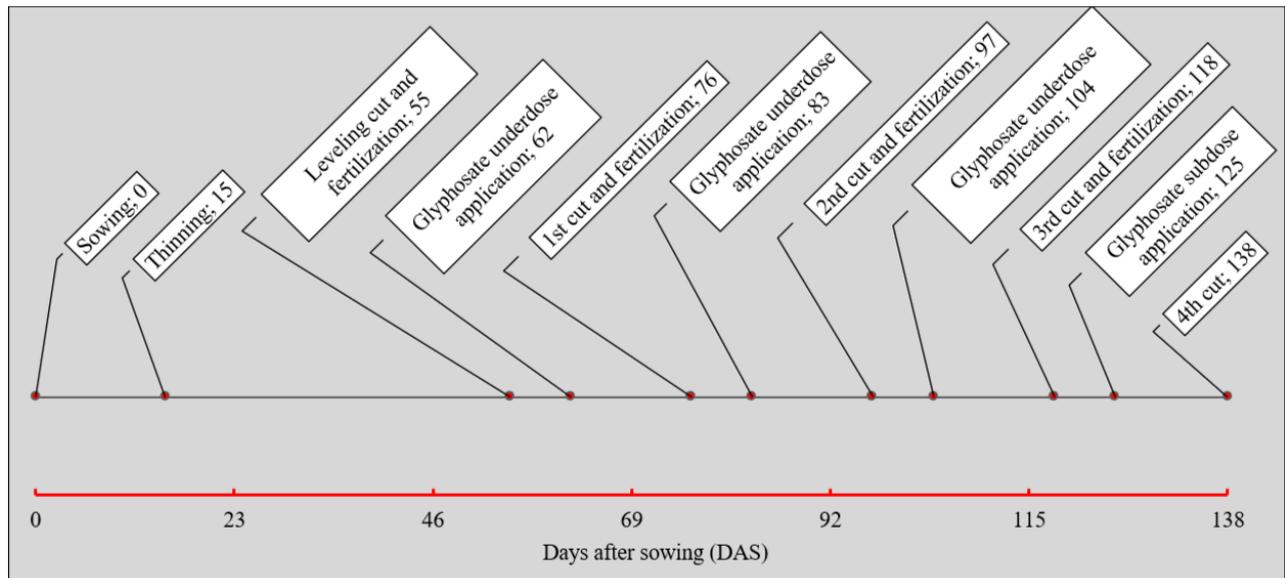


Figure 1. Experiment schedule.

The data were subjected to analysis of variance and F-test to detect differences between factor levels. Once a significant effect was found, the means of the quantitative factors were subjected to regression analysis, in which the goodness of fit of the models was checked based on the p-value of the regression deviation (not significant). The selected polynomial regression models were based on the highest coefficients of determination (R^2) among the significant regressions by the F-test. The means for the qualitative factors were compared by Tukey's test at the 5% level of significance, using SISVAR software⁽²⁰⁾.

3. Results and discussion

The F-test of analysis of variance (Table 1) identified a significant interaction for doses (D), *Urochloa* cultivars (B), and cuts (C), characterizing interdependence between the factors. The decomposition of the quantitative variable (D) revealed significance only for cultivar *U. brizantha* cv. Marandu (Table 1), characterizing polynomial behavior. The models (2nd- and 3rd-order linear and polynomial models) tested for the other cultivars (*U. brizantha* cv. Piatã and *U. ruziziensis* cv. Ruziziensis) did not fit the data (Table 1), considering the assessment of specific mathematical models⁽²¹⁾.

The 1st and 2nd cuts fitted the quadratic polynomial model for cv. Marandu (Figure 1). In the 1st cut, the glyphosate dose of 86.40 g ae ha⁻¹ provided maximum forage production (1.76 g pot⁻¹), which is higher than the control treatment and equivalent to 11.82%. However, the dose of 86.40 g ae ha⁻¹ reduced the biomass by 35.22% in the 2nd cut, with a production of 1.14 g pot⁻¹. Moraes et al.⁽²²⁾ conducted an assessment at 21 days after application (DAA) of a less

concentrated spray solution volume (200 L ha⁻¹) and found that a dose between 30 and 62 g ae ha⁻¹ of the herbicide reduced the growth of *U. decumbens* by 50%, without characterizing symptoms of phytotoxicity. Carbonari et al.⁽²³⁾ assessed the effects of the application of different underdoses of glyphosate on the growth of sugarcane (*Saccharum officinarum*) and observed that the application of doses higher than 72 g ae ha⁻¹ significantly compromised biomass production.

The literature has encountered challenges in specifying a precise dosage for hormesis, typically offering only ranges due to the influence of environmental factors on application efficacy and the interaction between the environment and the morphological and phenological traits of plants⁽²⁴⁾. Nascentes et al.⁽²⁵⁾ assessed the production of Marandu grass and found maximum forage production with the underdose of 12.62 g ae ha⁻¹ at 30 DAA, obtaining 2,862.2 kg ha⁻¹ of DM (dry mass), with an increase equivalent to 21.8% relative to the control. In sugarcane, Nascentes et al.⁽²⁶⁾ showed a hormesis effect in the range of 5 and 9 g ae ha⁻¹ of glyphosate. The lower underdoses (5.40 and 10.80 g ae ha⁻¹) led to higher production (Figure 2), showcasing the hormesis effect.

The glyphosate doses of 5.40 and 10.80 g ae ha⁻¹ provided maximum forage production in cv. Marandu (Figure 2), especially in the 3rd and 4th cuts, and the data fitted the cubic polynomial model (Table 1 and Figure 2). However, herbicide doses equal to or higher than 43.20 g ae ha⁻¹ in these cuts induced stress to the grass stubble (Figure 2), as the low photosynthetic efficiency of the remaining leaves provided no forage growth, impairing shoot biomass production. In this situation, roots and the base of the stem, present in the mass of residues, redirect organic reserves

Table 1. F-values calculated from analysis of variance, regression analysis, and significance for dry mass production of three *Urochloa* cultivars (B) treated with low glyphosate doses (D) in four assessment cuts (C)

Factor	Doses (D)	Cultivars (B)	Cuts (C)	D × B	B × C	D × C	D × B × C
F-value	410.357**	30.962**	94.766**	6.253**	5.862**	94.281**	4.210**
DF ⁽¹⁾	5	2	3	10	6	15	30
	Coefficient of variation: 15.93%			Mean: 1.37 g pot ⁻¹			
Regression	1st cut	2nd cut	3rd cut	4th cut			
<i>Urochloa brizantha</i> cv. Marandu							
Linear	4.846*	51.947**		229.976**			424.150**
Quadratic	24.596**	56.907**		36.872**			17.799**
Cubic	0.237 ^{ns}	2.368 ^{ns}		11.980**			139.197**
Deviation	2.123 ^{ns}	0.923 ^{ns}		0.466 ^{ns}			0.608 ^{ns}
<i>Urochloa brizantha</i> cv. Piatã							
Linear	0.760 ^{ns}	54.423**		261.818**			637.119**
Quadratic	41.497**	71.668**		33.916**			42.853**
Cubic	6.669*	7.099**		28.647**			176.724**
Deviation	7.791**	13.249**		6.950**			16.320**
<i>Urochloa ruziziensis</i> cv. Ruziziensis							
Linear	0.201 ^{ns}	60.695**		147.080**			432.866**
Quadratic	82.817**	72.062**		33.368**			70.024**
Cubic	13.541**	33.947**		7.804**			63.981**
Deviation	14.068**	3.272*		12.895**			57.678**

** , * , and ns, significant at 1%, 5%, and not significant, respectively, by the F-test. ⁽¹⁾ Degree of freedom.

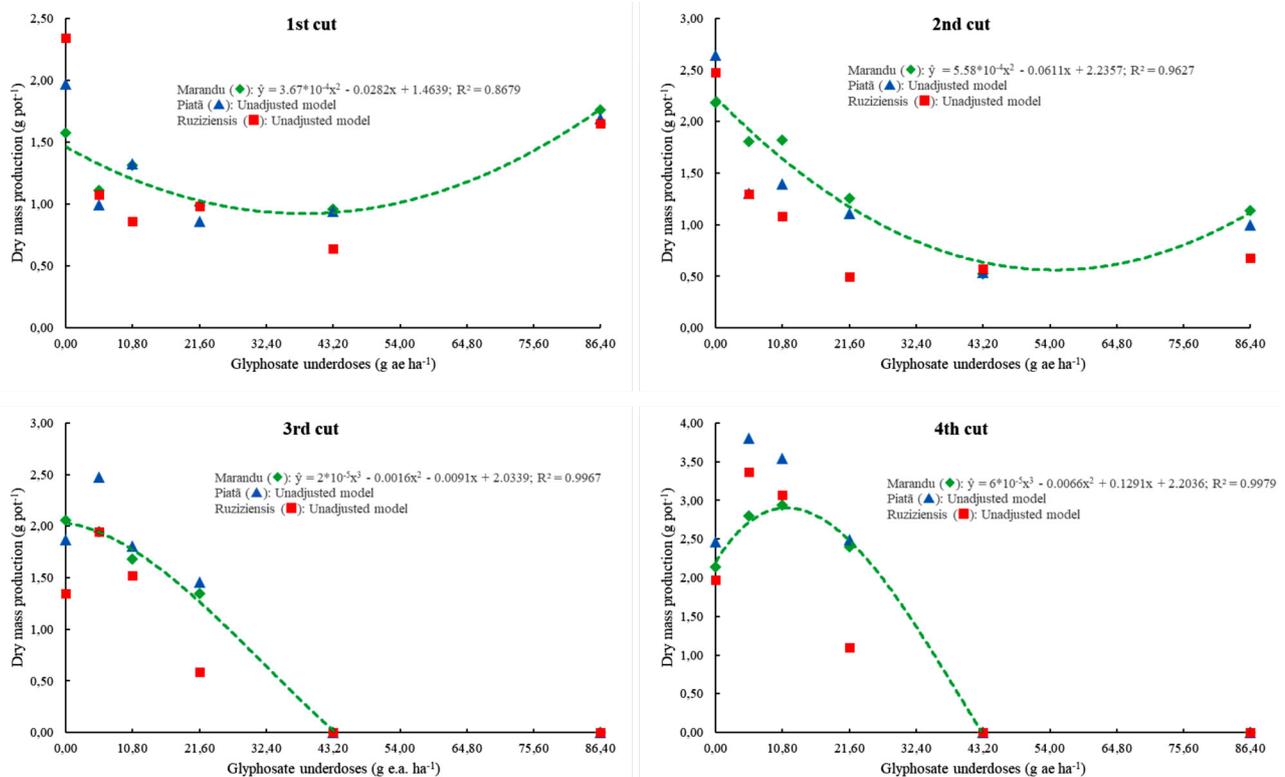


Figure 2. Regression equation for dry mass production of three *Urochloa* cultivars treated with low glyphosate doses in four assessment cuts.

Table 2. Means of dry mass production of three *Urochloa* cultivars treated with low glyphosate doses in four evaluation cuts

Cut	Glyphosate underdose (g ae ha ⁻¹)					
	0.0	5.4	10.8	21.6	43.2	86.4
Dry mass production (g pot⁻¹)						
<i>Urochloa brizantha</i> cv. Marandu						
1°	1.57 abB ⁽¹⁾	1.11 bcC	1.31 abcC	0.99 cB	0.96 cA	1.76 aA
2°	2.18 aA	1.81 aB	1.82 aB	1.25 bB	0.53 cA	1.14 bB
3°	2.06 aA	1.94 aB	1.68 abAB	1.34 bB	0.00 cB	0.00 cC
4°	2.14 cA	2.80 abA	2.94 aA	2.40 bcA	0.00 dB	0.00 dC
Dry mass production (g pot⁻¹)						
<i>Urochloa brizantha</i> cv. Piatã						
1°	1.97 aB	0.99 cC	1.32 bcC	0.86 cC	0.94 cA	1.69 abA
2°	2.65 aA	1.31 bC	1.40 bBC	1.11 bBC	0.54 cA	1.00 bcB
3°	1.87 bB	2.47 aB	1.81 bB	1.46 bB	0.00 cB	0.00 cC
4°	2.46 bA	3.80 aA	3.55 aA	2.49 bA	0.00 cB	0.00 cC
Dry mass production (g pot⁻¹)						
<i>Urochloa ruziziensis</i> cv. Ruziziensis						
1°	2.34 aAB	1.08 cC	0.86 cC	0.98 cAB	0.64 cA	1.65 bA
2°	2.47 aA	1.30 bC	1.08 bcBC	0.49 dC	0.57 cdA	0.68 cdB
3°	1.34 bC	1.94 aB	1.52 abB	0.58cBC	0.00 dB	0.00 dC
4°	1.97 bB	3.37 aA	3.07 aA	1.10 cA	0.00 cB	0.00cC

Means followed by the same lowercase letter in the row and uppercase letter in the column do not differ from each other ($p < 0.05$) by Tukey's test.

(carbohydrates/proteins) to replenish the forage. This characterized a negative energy balance, as the photosynthesis of the remaining leaves is lower than respiration and energy is expended for the synthesis of new leaves⁽²⁷⁾.

Varieties Piatã and Ruziziensis did not fit a mathematical model (Table 1), and spraying with underdoses of glyphosate equal to or higher than 43.20 g ae ha⁻¹ had an herbicidal effect in the last two cuts, as there was no forage production above the stubble (Figure 2). Other authors have identified the hormesis effect of glyphosate in plants⁽¹¹⁻²⁸⁻²⁹⁾. However, the studies were restricted to a single application. The hormesis effect extends for 40⁽³⁰⁾ or up to 60 days⁽²⁶⁾ for doses up to 60 g ae ha⁻¹, characterizing induced plant growth by the underdose action of glyphosate, with the potential to be used in forage management⁽³¹⁾. Thus, the 21-day frequency between glyphosate applications in this experiment may not have been adequate for hormesis expression.

Intentional sequential applications of underdoses of glyphosate did not have a beneficial effect on forage production in the experimental period (four cuts), particularly underdoses equal to or higher than 43.20 g ae ha⁻¹ of glyphosate (Figure 2). In this case, the persistent stimulus of hormesis does not lead to productive benefits due to the continuous plant stress. The constant disturbance significantly interferes with the biological mechanisms that ensure the compensatory adaptive processes of re-establishment of the cellular and physiological functions of plant tissues, which characterize adaptive homeostasis⁽³²⁻³³⁾. Defoliation followed by hormesis stress results in energy and oxidative exhaustion by toxin disturbance to the plant.

Therefore, frequent applications of a low glyphosate dose do not result in an adaptive response, as forage plants are routinely harvested in animal production.

Among the evaluated proportions, the glyphosate underdose of 43.20 g ae ha⁻¹ led to a reduction in dry mass production (DMP) (Table 2). In the 3rd and 4th cuts, no regrowth of forage plants was observed above the stubble in the evaluated cultivars at glyphosate underdoses of 43.20 and 86.40 g ae ha⁻¹, differing significantly from the lowest underdoses (5.40, 10.80, and 21.60 g ae ha⁻¹ of glyphosate).

Lower underdoses showed maximum DMP in the 4th cut of the tested forages, especially in the 1st assessment cut (Table 2). The proportional productive increment of the 4th cut over the initial one corresponded to 2.5, 3.83, and 3.13 times for cv. Marandu, 2.24, 2.67, and 3.57 times for cv. Piatã, and 2.43, 2.89, and 1.12 times for cv. Ruziziensis at glyphosate underdoses of 5.40, 10.80, and 21.60 g ae ha⁻¹, respectively. These underdoses promoted vegetative growth, with the stress resulting in the hormesis effect. The DMP observed for the control treatment in the 4th cut relative to the 1st cut corresponded to more moderate (1.36 and 1.25 for cvs. Marandu and Piatã, respectively) or depressor increments (0.84 for cv. Ruziziensis) (Table 2). Moraes et al.⁽²²⁾ used a less concentrated spray solution volume (200 L ha⁻¹) and found that the dose of 62 g ae ha⁻¹ reduced the growth of *U. decumbens* plants by 50%, also allowing forage production.

Ruziziensis was more susceptible to phytotoxicity by glyphosate for the assessed underdoses, which agrees with the analysis of variance (Table 1). Brighenti et al.⁽³⁴⁾ observed variability among *Urochloa* species regarding susceptibility to glyphosate, with *U. ruziziensis* being the

most susceptible and allowing savings of 12 to 16% of the used dose for control (360 g ae ha⁻¹)⁽³⁵⁾. Silveira et al.⁽¹⁵⁾ reported the need for studies assessing the control of *Urochloa* forages at the initial stages of development for doses lower than 90 g ae ha⁻¹ of glyphosate. Matias et al.⁽³⁶⁾ found that underdoses higher than 58 g ae ha⁻¹ characterized potential for use in the suppression of cv. Ruziziensis, provided that the applications are conducted at the initial stages of development, with up to five to seven tillers.

Herbicide underdoses can elicit a different susceptibility response in grasses. Thus, underdoses equal to or higher than 43.20 g ae ha⁻¹ of glyphosate in up to two sequential applications were effective in the productive suppression of Marandu, Piatã, and Ruziziensis grasses (Table 2), paralyzing the growth of the forage canopy, extinguishing potential DMP for animal feed. Therefore, glyphosate doses lower than 90 g ae ha⁻¹ cannot produce symptoms of yellowing, leaf necrosis, and, consequently, plant death⁽³⁷⁾.

Root dry mass (RDM) production was affected by the interaction between underdoses of glyphosate (D) and *Urochloa* cultivars (B) (Table 3). The interactions showed significant effects, as an indication of the mutual dependence of factors D and B.

Table 3. F-values calculated from analysis of variance, regression analysis, and significance for root dry mass (RDM) in three *Urochloa* cultivars (B) treated with low glyphosate doses (D)

Factor	Doses (D)	Cultivars (B)	D x B
F-value	21.343**	56.438**	4.997**
DF ⁽¹⁾	5	2	10
Coefficient of variation: 16.39%		Mean: 4.06 g pot ⁻¹	
Regression	Marandu	Piatã	Ruziziensis
Linear	7.855**	15.908**	59.912**
Quadratic	0.053 ^{ns}	1.663 ^{ns}	0.776 ^{ns}
Cubic	0.049 ^{ns}	44.707**	1.925 ^{ns}
Deviation	4.801*	1.936 ^{ns}	5.183*

** , * , and ns, significant at 1%, 5%, and not significant, respectively, by the F-test.
⁽¹⁾ Degree of freedom.

The decomposition of the quantitative variable revealed no significance for cultivars Marandu and Ruziziensis (Table 1), and the RDM data did not fit the tested models. Only cv. Piatã fit a 3rd-order polynomial behavior, whose mathematical model expressed 94.15% of the obtained data (Figure 3), with maximum RDM achieved at glyphosate underdoses between 10.8 and 21.6 g ae ha⁻¹.

The plants showed no shoot production at the highest doses (43.20 and 86.40 g ae ha⁻¹), with RDM characterizing the ability of plants to resprout from the basal and lateral buds⁽¹⁷⁾, as the treatments received irrigation and fertilization in accordance with the others. Also, the pot volume (7 dm³) and forage cutting height at

0.20 m promoted the development of the root system until the beginning of the experimental stage, providing carbohydrate reserves for plant regrowth⁽³⁸⁾.

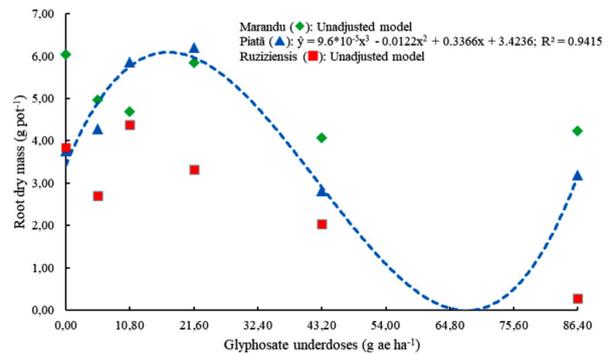


Figure 3. Regression equation for root dry mass of three *Urochloa* cultivars treated with low glyphosate doses.

The cultivar influenced the effectiveness of the herbicide in the different evaluated underdoses. Cultivar Ruziziensis showed lower RDM, significantly differing from cultivars Piatã and Marandu (Table 4). Root production is an indication of the ability to reconstitute the crop after reestablishing plant homeostasis.

Table 4. Mean root dry mass of three *Urochloa* cultivars treated with low glyphosate doses.

Glyphosate underdose (g ae ha ⁻¹)	Root dry mass (g pot ⁻¹)		
	Marandu	Piatã	Ruziziensis
0.0	6.03 a ⁽¹⁾	3.84 b	3.77 b
5.4	4.96 a	4.29 a	2.70 b
10.8	4.68 ab	5.86 a	4.38 b
21.6	5.85 a	6.20 a	3.33 b
43.2	4.62 a	2.82 b	2.04 b
86.4	4.24 a	3.19 a	0.28 b

Means followed by the same letter in the row do not differ from each other (p<0.05) by Tukey's test.

Glyphosate is rapidly translocated from leaves to meristematic and reserve/storage tissues⁽³⁹⁾. Therefore, the effect of doses varies according to the forage species⁽³⁴⁾, plant age/stage⁽¹⁵⁾, pot volume⁽³⁸⁾, and spray solution concentration⁽³⁷⁾.

4. Conclusion

Sequential applications with underdoses of 5.40 and 10.80 g ae ha⁻¹ of glyphosate promoted forage biomass production in *U. brizantha* cv. Marandu, *U. brizantha* cv. Piatã, and *U. ruziziensis* cv. Ruziziensis. A glyphosate underdose equal to or higher than 43.2 g ae ha⁻¹ in two sequential applications impairs the shoot biomass

production of the forage plants *U. brizantha* cv. Marandu, *U. brizantha* cv. Piatã, and *U. ruziziensis* cv. Ruziziensis. *Urochloa ruziziensis* cv. Ruziziensis was more susceptible to glyphosate phytotoxicity, which affects root dry mass production and limits the potential for reestablishment of forage cultivation.

Conflict of interests

The authors declare that there is no conflict of interest.

Author contributions

Conceptualization: Codognoto L.L. *Investigation*: Codognoto L.L. and Conde T.T. *Data curation*: Faria G.A. *Supervision*: Maltoni K.L. *Writing (original draft)*: Codognoto L.L. *Writing (revision and editing)*: Codognoto L.L., Conde TT, Faria G.A and Maltoni K.L.

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