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Glyphosate in the production and forage quality of marandu grass

Glifosato na produção e qualidade de forragem de capim marandu

Luciane da Cunha Codognoto[1]*; Thassiane Telles Conde[2]; Katia Luciene Maltoni[3]; Glaucia Amorim Faria[4]

Highlights

An underdose of glyphosate alters specific biological characteristics in plants.
Glyphosate underdose increases biomass and crude protein production of marandu grass.
Food quality and functional variables were optimized by glyphosate underdose.

Abstract

The drift of the herbicide glyphosate, used for desiccating crops or controlling weeds, can result in growth-promoting or non-target plant development effects. Thus, it is possible to use the compound to increase the biomass of forage through the hormone effect. The objective of this study was to evaluate the effects of sublethal doses of glyphosate on the production of Urochloa brizantha cv. Marandu (Marandu grass) and its nutritional quality in ruminants. The design used was completely randomized, with five replications in a factorial scheme. The treatments used were as follows: control (without glyphosate application) and four sublethal doses of glyphosate (4, 10, 14, and 20 g ha^{-1} of the acid equivalent). The monthly collections consisted of collecting the plant material (0.20 m) from the surface, comprising of leaves and pseudocolmos (stem and leaf sheath) to determine the dry matter production and forage chemical-bromatological parameters. The results showed that leaf/stem ratio, neutral detergent fiber, and acid detergent fiber were affected exclusively by the harvest factor. The hormone effect of the herbicide occurs in the production of dry matter and lignin in the evaluated subdoses. For crude protein, there was an interaction between the factor doses and harvest, due to the effect of glyphosate and the management applied to the harvests, showing that the sublethal doses of glyphosate promoted the production of dry matter and the food quality of Marandu grass.

Key words: Harvest. Herbicid. Underdoses. *Urochloa brizantha.*

[1] Agronomic Engineer, Federal Institute of Rondônia, IFRO, Ariquemes, RO, Brasil. E-mail: luciane.codognoto@ifro.edu.br
[2] Profa Dra, Department of Education, IFRO/DE, Ariquemes, RO, Brasil. E-mail: thassiane.conde@ifro.edu.br
[3] Profa Dra, Phytosanitary, Rural and Soil Engineering Department, Paulista State University "Júlio de Mesquita Filho", UNESP/DEFERS, Ilha Solteira, SP, Brasil. E-mail: katia.maltoni@unesp.br
[4] Profa Dra, Mathematics Department, Paulista State University "Júlio de Mesquita Filho", UNESP/DMAT, Ilha Solteira, SP, Brasil. E-mail: glaucia.a.faria@unesp.br
* Author for correspondence

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Resumo

A deriva do herbicida glifosato, usado para dessecação de culturas ou no controle de plantas daninhas, pode resultar em efeitos promotores de crescimento ou desenvolvimento de plantas não alvo. Assim, surge a possibilidade de utilização do composto para incremento da biomassa de forrageiras, por efeito hormese. O objetivo deste trabalho foi avaliar os efeitos da aplicação de doses subletais de glifosato na produção de *Urochloa brizantha* cv. Marandu (capim Marandu) e sobre a qualidade nutricional para ruminantes. O delineamento utilizado foi o inteiramente casualizado com cinco repetições, em esquema fatorial. Os tratamentos utilizados foram: controle (sem aplicação de glifosato) e quatro doses subletais de glifosato (4, 10, 14 e 20 g ha^{-1} do equivalente ácido). As coletas mensais consistiram do recolhimento do material vegetal a 0,20 m da superfície, compreendendo folhas e pseudocolmos (colmo e bainha foliar) para determinação da produção de matéria seca e de parâmetros químico-bromatológicos da forragem. Os resultados mostraram que relação folha/colmo, fibra em detergente neutro e fibra em detergente ácido sofreram efeitos exclusivamente do fator corte. O efeito hormese do herbicida ocorre na produção de matéria seca e lignina nas subdoses avaliadas. Para proteína bruta houve interação entre os fatores doses e cortes, por efeito do glifosato e do manejo aplicado nos cortes, mostrando que as doses subletais do glifosato promovem a produção de matéria seca e a qualidade alimentar do capim Marandu.

Palavras-chave: Colheita. Herbicida. Subdoses. *Urochloa brizantha.*

Introduction

In agriculture, herbicides administered for the chemical control of weed plants in crops of commercial interest are essential for production. As a consequence, the hormone phenomenon can occur, which consists of a stimulatory or toxic biological response in plants, depending on the administration of a minimum or abundant dose of a certain compound (Gomes et al., 2017).

Glyphosate is the most widely used herbicides worldwide; it has systemic and non-selective action, capable of withering a wide variety of plant species (Brito, Tropaldi, Carbonari, & Velini, 2017). The universal symptoms to the application of the herbicide are leaf chlorosis, followed by necrosis, characterizing the effect of photosynthesis, especially the route of shikimic acid and physiological processes. However, there are records of plants exposed to drift levels with a hormone effect on plant height, leaf area, aerial part mass, seed yield, and total biomass (Kappes, Arf, Arf, Gitti, & Ferreira, 2012; Carbonari et al., 2014; Nascentes et al., 2017).

Brazil has the largest commercial bovine herd, and in addition, is the largest exporter and producer of meat annually, estimated at 10.3 million tons (United States Department of Agriculture [USDA], 2020). The cultivation of pastures for animal feed is fundamental to the success of Brazilian livestock, in economic terms, due to the low cost of production, based on the exploitation of the natural fertility of the soils or its correction. In this case, *Urochloa brizantha* cv. Marandu, introduced in 1984, is predominant in extensive livestock production systems, especially because of its adaptation to natural soils that have low to medium fertility.

Studies of the effects of hormones in *Brachiaria* grass on productive parameters and food quality have shown promising results. However, these results are restricted to immediate or unique evaluation, since the effectiveness or longevity of the herbicidal effect on plants is limited (Brito et al., 2017). Thus, the objective of this study was to evaluate the occurrence of hormones, through consecutive applications of very low doses of glyphosate, on the production and chemical-bromatological composition of *U. brizantha* cv. Marandu.

Material and Methods

The experiment was installed in a greenhouse at the Federal Institute of Education, Science, and Technology of Rondônia (IFRO), Campus Ariquemes, from September 2018 to April 2019. The experimental design was completely randomized (CRD), in a factorial scheme, with plots subdivided over time and five repetitions, totaling 100 experimental units. The main factors were organized into five levels (D): control and four sublethal doses of glyphosate. The subsets of the experiment were the harvest (H), which constituted the data collection, on four successive occasions. Only the root dry mass variable was analyzed in CRD, as there were no repetitions over time.

Urochloa Brizantha cv. Marandu (Marandu grass) seeds were obtained from a commercial lot sample (2017/2018) with a cultural value of 55%. The seedlings were contained in a styrofoam tray with 200 cells (one seedling per cell, conical in shape and with a volume of 15.6 mL,) until the 21st day after sowing (DAS) and, on 06/10/2018, the transplant to pots was performed. Each pot (7 dm^3) represented an experimental unit and received three seedlings in an area of 0.0314 m^2.

The substrate used, both for sowing and for conducting the experiment, consisted of homogenization of gully soil, washed sand, and organic compost in the following proportions: 60, 26, and 14%, respectively. Fertility and substrate texture analyses were performed and the results showed: pH, in water = 6.75; organic carbon = 1.66 dag kg^{-1}; P and K$_{(Mehlich\ 1)}$ = 1869.4 and 111 mg dm^{-3}, respectively; Ca^{2+}, Mg^{2+}, and Al$^{3+}_{(KCl\ 1\ mol\ L^{-1})}$ = 5.83, 2.08, and 0.0 cmol$_c$ dm^{-3}, respectively; base saturation= 88.7%; and, clayey texture (520, 40, and 440 g kg^{-1} clay, silt, and sand, respectively). The substrate in the pots was accommodated considering an average density of 1.1 kg m^{-3} and maintaining humidity at roughly 60% of the field capacity.

After 52 DAS, on, 11/06/2018, a uniform cut of forage was carried out in all experimental plots, from the stratum above 0.20 m from the surface level (Dias, 2012), beginning the trial period. Nitrogen (urea) and potassium (potassium chloride) fertilization was carried out at three different times (12/06/2018, 01/05/2019, and 04/04/2019), totaling 50 kg ha^{-1} N and 40 kg ha^{-1} K$_2$O (Cantarutti; Alvarez; & Ribeiro, 1999).

On the seventh day after each harvest, treatments/levels (D) were applied: control and sublethal doses of glyphosate, equivalent to 4, 10, 14, and 20 g ha^{-1} of the acid equivalent (a.e.). The commercial product Shadow (356 g a.e. L^{-1}) was applied with a compression sprayer, pressure of 2 bar, with a bar adapted with a spray tip (DG11002 VS), providing a spray volume of 100 L ha^{-1}. Said products recommend a grace period of/reentry into pastures after two days.

The harvests of the experimental units occurred on the 21st day after application of the sublethal doses of glyphosate: 1st harvest (84 DAS), 2nd harvest (112 DAS), 3rd harvest

(142 DAS), and 4th harvest (172 DAS). Aerial plant production was measured as the total weight of green forage harvested in pots, 0.20 m from the surface. In addition, the material was separated into leaf blades and pride stock (stem + sheath).

Fresh mass of leaves and pride stock, and volume of roots harvested at the end of the experimental period were weighed and dried in a forced ventilation oven at 65 °C until a constant mass was obtained. The dry mass of forage and roots, per pot, was determined in an oven at 105 °C for 24 h.

Forage fractions were crushed in a Willey knife mill with 1 mm mesh for the determination of crude protein (CP) by the Kjeldahl method; neutral and acid detergent fibers (NDF and ADF) by the Ankom® Filter Bag Technique method; and lignin (LIG) was oxidized to potassium permanganate (Detmann et al., 2012).

With the aid of SISVAR software (Ferreira, 2019), the data were subjected to analysis of variance by the F test, and when significant, the effect of sublethal doses of glyphosate (D) and the interaction between factors were evaluated by regression, and the effects of the harvest (H) was compared with the Scott-Knott means test, at the level of 5% significance.

Results and Discussion

The analysis of variance indicated the significance of the underdoses of glyphosate for dry matter (DM) and LIG production in Marandu grass. The periodic harvest significantly influenced DM, leaf/stem ratio (L/S), NDF, ADF, and LIG. The interactions showed significant effects on the CP content, which was an indication of the interdependence of factors D and H (Table 1).

Table 1
Analysis of variance of dry mass production (DM), leaf/stem ratio (L/S), crude protein (CP), neutral detergent fiber (NDF), acid detergent fiber (ADF), and grass lignin (LIG) in Marandu grass due to underdoses of glyphosate (D), and harvest (H)

Variables	Dose (D)	Harvest (H)	D x H	CV (1)	Mean
	------------------ Valor of F -----------------			-- (%) --	----------
DM (g vase^{-1})	9,537**	1112,469**	0,376^{ns}	7,09	9,59
L/S	1,414^{ns}	20,387**	1,284^{ns}	37,91	4,24
CP (% DM)	16,822**	10,797**	17,709**	7,43	12,05
NDF (% DM)	1,702^{ns}	5,190*	1,823^{ns}	5,24	63,01
ADF (% DM)	1,701^{ns}	8,436**	1,663^{ns}	8,39	30,88
LIG (% DM)	31,279**	8,123**	1,447^{ns}	26,96	2,44

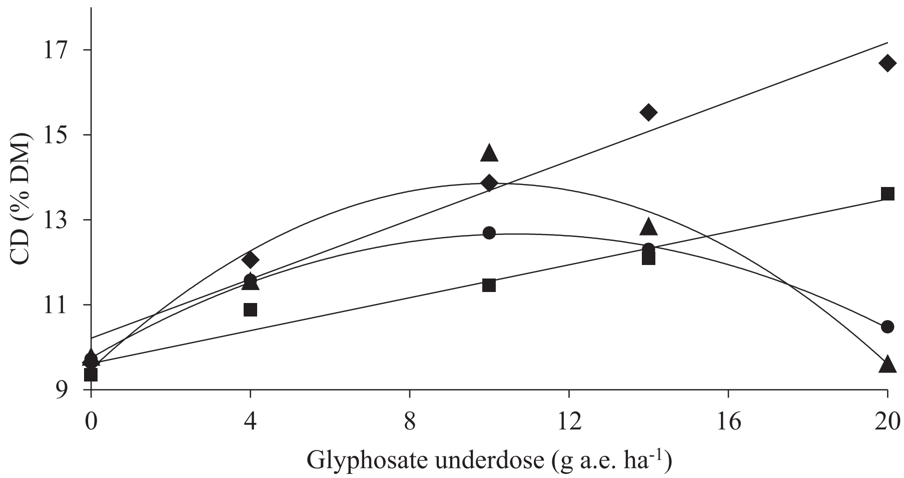
(1) Coefficient of variation. **, * and ns, significant to 1%, 5% and non-significant, respectively, by the Test F.

The split of glyphosate and the witnessed subdose between the harvests characterized the superiority of the 1st harvest at doses of 10, 14, and 20 g a.e. ha^{-1} of the compound (Table 2). CP includes substances with chemical similarities, such as nucleic acids, amines, carbohydrates, and lipids replaced by nitrogenous radicals and nonprotein amino acids (Detmann et al., 2012). The average CP content recorded (Table 1) describes the enrichment of free amino acids, suggesting that proteolytic activities are influenced by herbicide treatment, implying a hormone effect.

Glyphosate underdoses showed different effects on CP production (Figure 1). There was an increasing linear performance for the 1st and 2nd harvest with maximum CP content at the subdose of glyphosate acid equivalent (a.e.), 20 g ha^{-1}, obtaining an increase in relation to the control equivalent to 81, 52 and 45.56%, respectively, indicating the hormone effect. However, for the 3rd harvest, the behavior was adjusted to the quadratic model and decreased from the estimated doses of 10.24 and 10.82 g a.e. ha^{-1} of glyphosate. At this level, the increase in CP on the control corresponds to 4.60 and 11.32%, respectively. From then on, there was a depressive effect on CP production.

When assessing disturbances in the metabolism of amino acids associated with inhibition of photosynthesis in glyphosate-resistant and susceptible soybean genotypes, Vivancos et al. (2011) found that nitrogen-rich amino acids were triggered by changes in proteins involved in photosynthesis and photorespiration. Thus, the nutritive value of forage, especially CP, can be altered by the rational and controlled use of sublethal doses of glyphosate.

The LIG values showed significant reductions, characterizing linear behavior inversely proportional to glyphosate doses (Figure 2B). The limit dose (20 g a.e. ha^{-1} of glyphosate) resulted in a reduction equivalent to 49.85% of the LIG content in relation to the control treatment. Zobiole, Bonini, Oliveira, Kremer and Ferrarese (2010) observed that with an increase in glyphosate doses, there was damage to the LIG content in herbicide-resistant soy.



1st harvest (◆): $\hat{y} = +0,3695x + 9,9773$; $R^2 = 0,9877$
2nd harvest (■): $\hat{y} = +0,1834x + 9,6420$; $R^2 = 0,9323$
3rd harvest (▲): $\hat{y} = -0,0460x^2 + 0,9413x + 9,3651$; $R^2 = 0,9376$
4th harvest (●): $\hat{y} = -0,0268x^2 + 0,5799x + 9.6904$; $R^2 = 0,9902$

Figure 1. Crude protein (CP) in Marandu grass exposed to underdoses of glyphosate, in four evaluation harvests.

Table 2
Crude protein from Marandu grass submitted to underdoses of glyphosate and control, according to evaluation harvest

Harvest	Glyphosate underdoses (g a.e. ha^{-1})				
	0	4	10	14	20
1st	9,63 a(1)	11,86 a	13,86 a	15,52 a	17,48 a
2nd	9,35 a	10,87 a	11,46 c	12,09 b	13,61 b
3rd	9,79 a	11,57 a	14,59 a	12,85 b	9,62 c
4th	9,72 a	11,58 a	12,69 b	12,30 b	10,48 c

(1) Averages followed by the same letter in the column do not differ statistically (p> 0.05) by the Scott-Knott test.

There was a potential indication for the use of management to improve productivity, since the treatment (sublethal dose of glyphosate) had a stimulating effect on the DM of the forage, as evidenced by positive and linear behavior, according to the adjusted mathematical model (Figure 2A). The 20 g a.e. ha^{-1} dose of glyphosate provided an increase in DM equivalent to 9.20%, compared to the control treatment. Among the evaluative harvests, the DM obtained in the 1st harvest differed significantly from the subsequent cuts, with lower production masses (Table 2). Therefore, for hormone evaluation, consecutive applications characterize the depressive effect on forage yield.

LIG is a highly complex phenolic molecule, and in the feeding of ruminants, it interferes with nutritional quality, as it compromises the digestibility of forage nutrients and, consequently, animal productivity in pastures (Berchielli, Pires, & Oliveira, 2011). The LIG content obtained for the studied doses, including the control treatment, was lower than that obtained by Meschede, Velini, Carbonari and Moraes (2011) when evaluating the effect of glyphosate in *Urochloa decumbens* pasture, established 8 years ago, registering 24.54, and 26.11%, at 30 and 60 days after application, respectively. However, the authors found a depressive effect (about 40%) in the levels of LIG at higher doses (36, 72, 180, and 360 g a.e. ha^{-1} of glyphosate) in relation to the control.

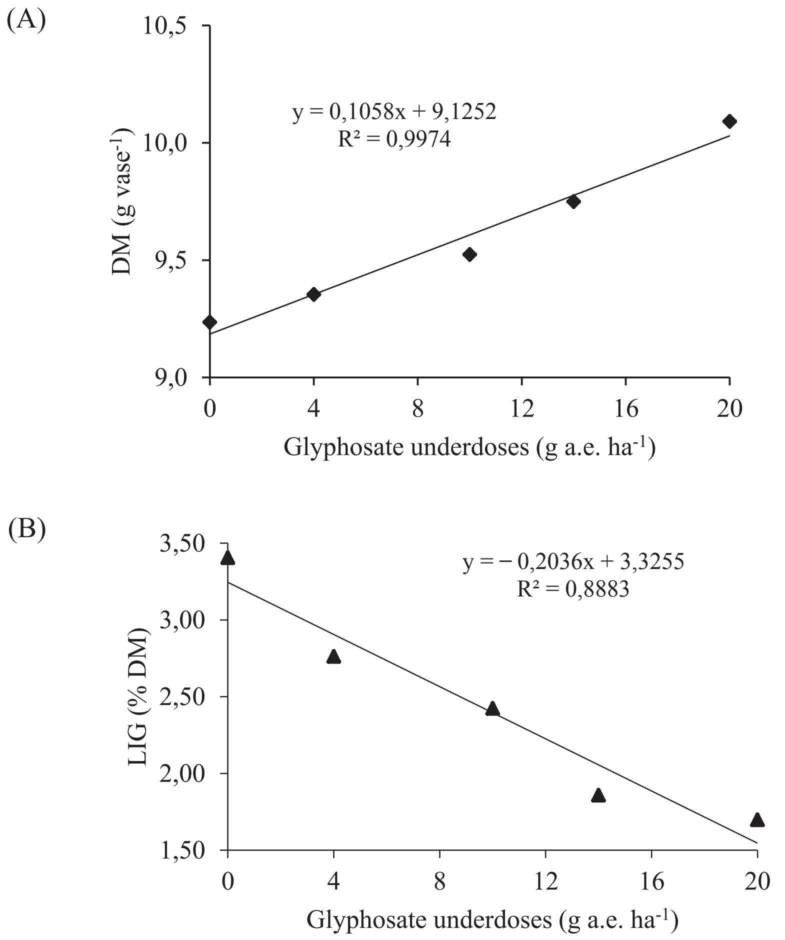


Figure 2. Production of dry matter (A) and lignin (B) of Marandu grass as a function of exposure to glyphosate underdoses.

Glyphosate has been proposed as a growth regulator in some crops, such as Crotalaria and upland rice, or to eliminate the lodging of rice plants (Gitti et al., 2011; Kappes et al., 2012). However, this practice resulted in reduced production. In addition to the functions inherent to plant physiology, lignification is an adaptive mechanism for stability and tolerance to abiotic and biotic stresses (Taiz & Zeiger, 2013). The literature reports that there is a threshold between the concentrations of the herbicide used for this purpose, where higher doses of glyphosate do not alter the levels of LIG, since there is herbicidal action and this does not regulate growth or LIG content (Meschede et al., 2011).

In the temporal evaluation, the 1st harvest presented a significantly higher LIG content than the other evaluations (Table 3). Successive applications have characterized the behavior of plant growth regulation, since the inhibition of the enzyme 5-enolpyruvylchiquimate-3-phosphate synthase (EPSPs) affects the production of LIG, interfering with carbon metabolism, depressing lignification, and prioritizing non-structural carbohydrate metabolism.

Table 3
Production of dry matter (DM), neutral detergent fiber (NDF), acid detergent fiber (ADF), lignin (LIG), and leaf/stem ratio (L/S) of Marandu grass in four evaluative harvest

Harvest	DM	LIG	NDF	ADF	L/S
	-- (g vase^{-1}) --	------------------	(% DM)	------------------	------
1st	16,17 a[(1)]	2,98 a	63,68 a	31,60 a	2,71 c
2nd	7,77 c	2,15 b	61,29 b	29,50 b	3,87 b
3rd	5,86 d	2,37 b	62,38 b	30,08 b	4,56 a
4th	8,50 b	2,23 b	64,77 a	32,88 a	5,09 a

(1) Averages followed by the same letter in the column do not differ statistically (p> 0.05) according to the Scott–Knott test.

According to Gitti et al. (2011), carbon not assimilated in the lignification process, may have been responsible for the increase in DM, as shown in Figures 2A and 2B. The production of LIG is controlled by phenylalanine, a key product of the chiquimate pathway (Zobiole et al., 2010) and, therefore, highly affected by glyphosate (Silva, Duke, Dayan, & Velini, 2015). The reduction in the LIG content is an important physiological aspect, since this biopolymer integrates the indigestible fraction of the plants used in the feeding of ruminants, characterizing a gain in food quality (Berchielli et al., 2011).

There was an effect of factor H, revealing a difference in the levels of NDF and ADF in the 1st and 4th harvest over the intermediate (Table 3). The levels of NDF obtained did not limit the consumption of forage. The limit of 65% NDF in DM in tropical forages, with a defoliation interval of 30 days, characterizes good nutritional value of forage, favoring animal performance and productivity in pastures (Alencar et al., 2010; Hanisch, Balbinot, & Vogt, 2017).

The AFD levels obtained in this study (Table 3) positively relate to the digestibility of forage, indicating low levels of lignified components, favoring digestibility and the use of ingested forage (Oliveira, Bonfim-Silva, Silveira, & Monteiro, 2010). AFD in forages with values of approximately 30% are considered ideal for animal consumption (Miranda et al., 2018). Meschede et al. (2011) tested the effect of glyphosate on chemical-bromatological variables of *U. decumbens,* with the initial dose of glyphosate being 32 g a.e. ha^{-1} and a control treatment (without application), obtaining AFD equivalent to 40.50 and 42.50%, respectively, and they did not register a hormone effect.

The L/S ratio was not affected by glyphosate doses (Table 1). However, there was an effect of factor H. Apical dominance was not influenced by chemical action, but by the management used. The growth of the grass, especially the L/S ratio, in response to defoliation, is due to the remaining height of the canopy (0.20 m), which positively influences the root reserves, and the maintenance of fertilizer and substrate moisture, which directly interferes with the production and quality of forage by stimulating the growth of tissues with high levels of CP, along with low levels of NDF and LIG (Lopes, Cândido, Pompeu, Silva, & Bezerra, 2011; Fontes et al., 2014).

Furthermore, mineral nutrition can lead to resistance to glyphosate, as plants that have high concentrations of some cationic nutrients can significantly reduce the phytotoxic effects of the substance through the formation of poorly soluble complexes (Su, Ozturk, Cakmak, & Budak, 2009). However, low doses of glyphosate alter the responses of plants to subsequent treatments with the herbicide (Silva et al., 2015). Thus, based on the data obtained for L/S ratio in the evaluated harvests (Table 2), it was found that the evaluated management is important for the productive, structural, and morphogenic characteristics of the forage canopy.

The underdoses of glyphosate had an effect on the dry mass of Marandu grass root (Table 4), and the subdose of 4 g a.e. ha^{-1} had a hormonal effect (Figure 3). In foliar applications, the herbicide is quickly absorbed and transported to plant tissues with a high metabolic rate, such as sprouts and roots. Roots are important sinks for glyphosate, which leads to the releasing of the compound in the soil, which is later adsorbed to the soil particles and either degraded by microorganisms, or absorbed by roots of adjacent/subsequent plants (Gomes et al., 2014). However, as the cultivation took place in pots in the current study, there was a volumetric limitation for dispersion/dissipation of the glyphosate exuded by the root system, which may result in a depressive/cumulative effect to stabilize the forage root system. Vitti et al. (2019) evaluated the translocation and root exudation of glyphosate using *U. brizantha* and its transport in sugarcane and citrus and found insufficient exudates to harm the dry mass of agricultural crops. However, competitive superiority of plants occurs due to hormone effects, resulting in productive stimulus, especially initial growth speed (Barbosa et al., 2017). Plant organs with high rates of metabolism and growth are important sinks for the product (Duke, 2011). Thus, the glyphosate hormone has a significant effect on the competitive performance of plants and influences their management.

Table 4
Analysis of variance for dry root mass of Marandu grass exposed to glyphosate underdoses

Variation factor	Degrees of freedom	Value of F	CV[(1)] (%)	Mean ($g\ vase^{-1}$)	Number of observations
Doses	4	14,250**	11,42	7,77	25

(1) Coefficient of variation. ** significant at 1%, by Test F.

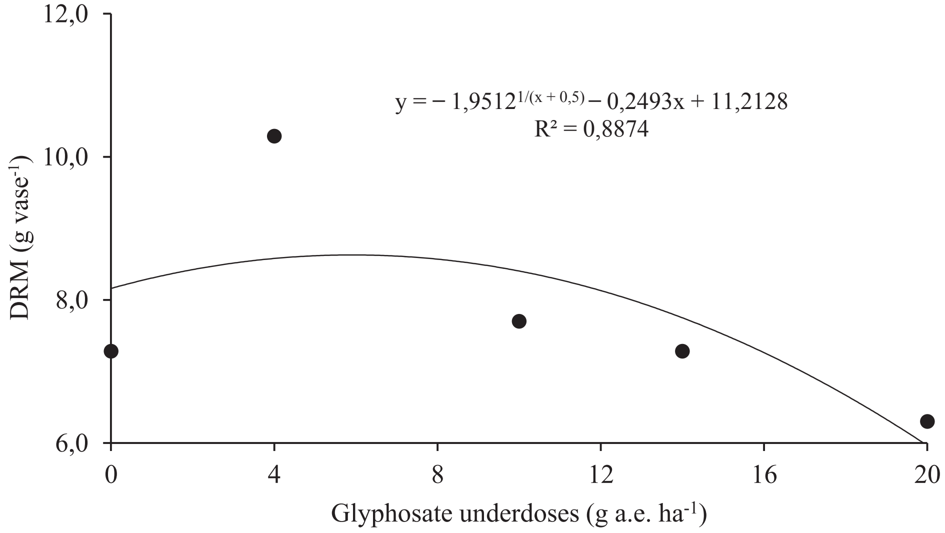


Figure 3. Production of dry root mass (DRM) of Marandu grass subjected to underdoses of glyphosate.

Conclusion

Glyphosate underdoses responded hormonally to the production and food quality of Marandu grass.

The maximum increase in DM production occurred due to the subdose of the glyphosate acid equivalent, 20 g ha^{-1}, representing 9.20% in relation to the treatment without application of the herbicide.

There was a glyphosate hormone effect for CP, with interaction of factors (underdoses and harvest), characterized by positive linear growth in the initial cuts, and positive growth in the last two harvests, until the subdose of the glyphosate acid equivalent was 10 g ha^{-1}.

For LIG, there was a surprising linear effect, culminating in the subdose of the glyphosate acid equivalent, 20 g ha^{-1}, in relation to the treatment without application of the herbicide, with an approximate loss of 50%.

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