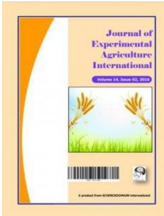

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Investigating the Optimal Day for Nitrogen Fertilization on Piatã palisadegrass and Quênia guineagrass after Defoliation

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Authors' contributions

This work was carried out in collaboration among all authors. Authors CEAC, JGA, CHAC and LVB designed and wrote the protocol. Authors DAF, ACDA, SFG, VGVD and LMBA conducted the experiment and wrote the first draft of the manuscript. Authors CEAC, DAF, LVB and JGA managed the analyses of the study. Authors DAF, ACDA, ABN and CEAC discussed the results, corrected and improved the writing of the manuscript. All authors read and approved the final manuscript.

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ABSTRACT

Considering that nitrogen is the main macronutrient limiting pasture productivity, the aim of this study was to investigate the most appropriate day for nitrogen fertilization of the grasses *Brachiaria brizantha* BRS Piatã and *Panicum maximum* BRS Quênia. The experiment was conducted in a

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greenhouse in the city of Rondonópolis, located in the state of Mato Grosso, Brazil, using a completely randomized design. The treatments consisted of five nitrogen fertilization periods: 0; 2; 4; 6 and 8 days after defoliation. The dry mass of the leaf blade (DMLB), dry mass of stem + sheath (DMSS), dry mass of residue (DMRES) and root dry mass (RDM) were evaluated. The non-structural carbohydrate of the grass roots was also quantified. The later nitrogen fertilization after defoliation reduced DMLB ($P < .01$) and DMSS ($P < .01$) of the BRS Piatã palisadegrass, and DMRES of both grasses ($P < .01$). Higher levels of water soluble carbohydrates were observed when nitrogen fertilization was performed on grass cutting (day 0). Nitrogen fertilization of the BRS Piatã palisadegrass close the time of defoliation is recommended. For Quênia guineagrass, nitrogen can be applied between the cutting day and the eighth day after defoliation. For the root system, there is a higher content of water soluble carbohydrates in the BRS Piatã palisadegrass and greater accumulation of starch in the BRS Quênia guineagrass.

Keywords: Brachiaria; nitrogen fertilization; non-structural carbohydrates; Panicum; Urochloa.

1. INTRODUCTION

In Brazil, pastures are the basis of ruminant production. These systems are predominantly extensive and degradation has been one of the greatest challenges for sustainability. One of the main reasons for the reduction in pasture productive potential is the inadequate nutritional management of the plants [1,2]. Therefore, the replacement of nutrients through fertilization is fundamental for recovery, maintenance and increase of forage productivity.

Nitrogen is the nutrient that most influences morphogenesis and tillering of grasses. Its application at the right dose provides greater production of photoassimilates and high growth rates, allowing a longer duration of tillering and tiller survival. In order to accelerate the formation and growth of new leaves, nitrogen fertilization can increase the re-growth vigor, generating an increase in pasture support capacity [3,4].

The forage grasses of *Brachiaria* (Syn. *Urochloa*) and *Panicum* genera represent the majority of Brazilian pastures. New cultivars of both genera have been released in recent years in the national market, aiming to diversify and intensify animal production. The grass *Brachiaria brizantha* cultivar BRS Piatã is a *Brachiaria* cultivar, released by Embrapa in 2007, which presents an easy establishment, fitness for integrated crop-livestock systems and good nutritive value, providing high meat production in well-managed pastures and fertile soil [5]. In 2017, the *Panicum maximum* cultivar BRS Quênia, with intermediate size, high yield and forage quality, was released with soft leaves, tender stems, high tillering and easy handling. In addition, the cultivar presents high resistance by antibiosis to pasture spittlebug. It has been recommended for areas with well drained soils, from medium to high fertility, especially for the Cerrado (Brazilian Savannah) and Amazon biomes [6].

The success of forage plants establishment depends, among other factors, on the adequate nitrogen fertilization strategies, which may be different between species and cultivars. This knowledge is relevant especially for newly released grasses, which lack information. In this sense, the aim of this work was to investigate the most appropriate day for nitrogen fertilization of the BRS Piatã palisadegrass and BRS Quênia guineagrass.

2. MATERIALS AND METHODS

The trial was conducted in a greenhouse at the Federal University of Rondonópolis (UFR), Mato Grosso state, Brazil, using a completely randomized design with ten replications. The treatments consisted of five nitrogen fertilization periods: 0; 2; 4; 6 and 8 days after defoliation, using *Brachiaria brizantha* cultivar BRS Piatã and *Panicum maximum* cultivar BRS Quênia.

In order to compose the experimental units, vases with a 5.5 dm^3 capacity containing four plants each were used. The utilized soil was the Red Oxisol (USDA soil taxonomy) of clayey texture (Table 1). After the soil was collected, base saturation was increased up to 60%, with incorporation of dolomitic limestone, with a relative total neutralization power of 86, maintaining humidity at 80% of the maximum water retention capacity in the soil [7].

Table 1. Chemical and particle size characterization of clay red oxisol from the Brazilian Savannah

pH	P	K	Ca+Mg	Al+H	CEC	V	m	OM	Sand	Silt	Clay
$CaCl_2$	mg dm^{-3}		cmol dm^{-3}			%		g kg^{-1}			
4.9	4.6	108	2.4	3.4	6.1	44	0.0	19.2	290	150	560

CEC: cation exchange capacity; OM: organic matter; V%: base saturation; m: aluminum saturation.

Phosphorus fertilization (P_2O_5) was performed 30 days after the limestone incorporation, with 300 mg dm^{-3} of single superphosphate, followed by the sowing.

The thinning was performed ten days after sowing, maintaining five plants per vase. Nitrogen and potassium fertilization, in the form of urea and potassium chloride, were applied with doses of 100 and 70 mg dm^{-3} for the BRS Piatã palisadegrass, and 200 and 70 mg dm^{-3} for BRS Quênia guineagrass, respectively [7,8,9].

The standardization cut was performed 30 days after sowing, with residue heights of 15 cm for the BRS Piatã palisadegrass and 25 cm for the BRS Quênia guineagrass. The height was measured from the soil to the curve of the forage canopy with a graduated ruler. Subsequently, the treatments were applied in an aqueous solution of urea, using 100 mg N dm^{-3} for the BRS Piatã palisadegrass and 200 mg N dm^{-3} for the BRS Quênia guineagrass. Twenty-five days after the standardization cut, the plants were cut at the pre-defined residue heights, starting the application of the treatments. After that, the interval between cuts occurred every 25 to 30 days, period necessary for the plants to reach the residue heights. In total, the BRS Piatã grass underwent four cuts and the BRS Quênia grass underwent five cuts. At every cut, the forage above the residue height was collected for morphological evaluation (leaf blades and stem + sheath). At the last cut, the residue and the root system were also collected for evaluation of mass production. Each parameter was obtained from the sum of the mass of all cuts for each grass [10].

The samples were collected and exposed to drying in a forced-air circulation oven at 55 ± 5ºC, for 72 hours, for later weighing. The evaluated parameters for all cut samples were: dry mass of the leaf blade (DMLB), dry mass of stem + sheath (DMSS), dry mass of residue (DMRES) and root dry mass (RDM). The results were subjected to linear and quadratic regression analysis at 5% probability.

The analysis of the non-structural carbohydrate (NSC) contents for the grass root system was performed at the Forage Laboratory and at the Plant Physiology Laboratory, Federal University of Mato Grosso (UFMT), Cuiabá, Mato Grosso state, Brazil, according to the methodology described by Passos [11].

The root samples of BRS Piatã palisadegrass and BRS Quênia guineagrass were washed and processed in a 1 mm mesh sieve. Afterwards, composite samples were composed and four laboratory replicates were defined. An aliquot of 0.5 g of roots was used in each sample.

The first step of the method consists in the alcoholic extraction of sugars with ethanol. Subsequently, the sediments from the alcoholic extraction are dried in order to extract the starch, using 0.5 mol L^{-1} NaOH as solvent.

The absorbance values of water soluble carbohydrates and starch were determined by using a spectrophotometer (Model Cirrus 80 - FEMTO), with wavelength of 625 nm.

A calibration curve with six glucose concentrations (0, 10, 20, 40, 80 and 150 μg) was used to obtain the concentrations of water soluble carbohydrates and starch contained in the root system of the grasses [11]:

$$x = (y - 0.00820) \div (0.00094)$$

Where: x is the concentration of water soluble carbohydrates and starch of the samples; y is the absorbance value. The results have been submitted to the Tukey test, to the level at 5% probability, to compare the grasses.

3. RESULTS AND DISCUSSION

There was a linear reduction of the DMLB in the BRS Piatã palisadegrass (P< .01) the later the

Table 2. Production and growth characteristics (g vase^{-1}) of BRS piatã palisadegrass and BRS quênia guineagrass fertilized on different days after defoliation

Variables	Days					Pr>F		CV (%)
	0	2	4	6	8	L	Q	
BRS piatã								
DMLB	74.43	58.86	59.03	47.39	45.28	<0.01	0.186	15.79
DMSS	35.15	16.13	19.75	15.37	16.02	<0.01	0.055	62.00
DMRES	26.59	24.52	24.47	22.35	20.06	<0.01	0.659	19.07
RDM	14.38	12.76	13.80	13.56	12.28	0.311	0.878	23.54
BRS quênia								
DMLB	77.7	73.9	74.0	75.8	75.9	0.736	0.119	6.57
DMRES	70.9	73.5	72.5	69.5	67.1	<0.01	<0.01	5.24
RDM	41.3	47.1	44.4	56.6	43.5	0.539	0.397	48.72

DMLB: dry mass of leaf blade; DMSS: dry mass of stem + sheath; DMRES: dry mass of the residue; RDM: root dry mass. CV: coefficient of variation

fertilization was performed (Table 2). This parameter ranged from 74.43 to 45.28 g vase^{-1} for the nitrogen fertilization performed at 0 and 8 days after defoliation, respectively. Increased leaf production provides better forage nutrient value, since it constitutes the most digestible vegetable component [12].

In addition, nitrogen application on the day of the BRS Piatã defoliation, by favoring the development of plants with more expanded leaves, indicates greater photosynthetic efficiency and higher amount of reserves before cutting. In addition to the high yield of the leaves, the grass presents an increase in protein concentration, better adaptation to grazing and satisfactory cut tolerance, allowing a more vigorous regrowth [13,14].

For BRS Quênia guineagrass, there was no effect of the fertilization moment on the DMLB (Table 2). The amount of leaf blades produced by the forage is fundamental to plant growth, since it constitutes the most photosynthetically active portion of the plant, besides being the major component selected by animals under grazing. Therefore, the ability of a grass to produce more accessible leaves, along with the provision of suitable conditions for this potential expression, may exert a great influence on animal production [15].

After defoliation, the speed with which the shoot is rebuilt and the rhythm of root system growth depends on a set of forage physiological mechanisms, such as photosynthetic capacity of the leaf tissue, plant organic reserves and nutrients absorption [16,17].

In this sense, the absence of effect of the nitrogen fertilization moment on the DMLB (Table 2) suggests that the BRS Quênia guineagrass has sufficient nitrogen reserves so that no foliage productive restriction occurs with later fertilization (up to the eighth day after defoliation), thus allowing a greater flexibility in the management of nitrogen fertilization on this grass.

A decreasing linear effect (P< .01) was observed for the DMSS the later the fertilization was performed for the BRS Piatã palisadegrass (Table 2). This component is important for forage production since stems and sheaths are the storage organs of organic substances in grasses, what may interfere with the ability of the grasses to regrowth [18]. For BRS Quênia guineagrass there was no production of this fraction above the residue height.

In terms of ruminant feeding, a lower stem proportion is desirable, meaning a greater number of green leaves and lower fiber content, with a consequently better nutritive value [19]. However, it is worth mentioning that the higher amount of DMSS obtained when the nitrogen fertilization was applied on the day of BRS Piatã defoliation is due to the higher grass production in this treatment, since more developed tillers require more robust structural organs to support the greater weight of the plant [20].

The DMRES was influenced by the nitrogen fertilization moment in the BRS

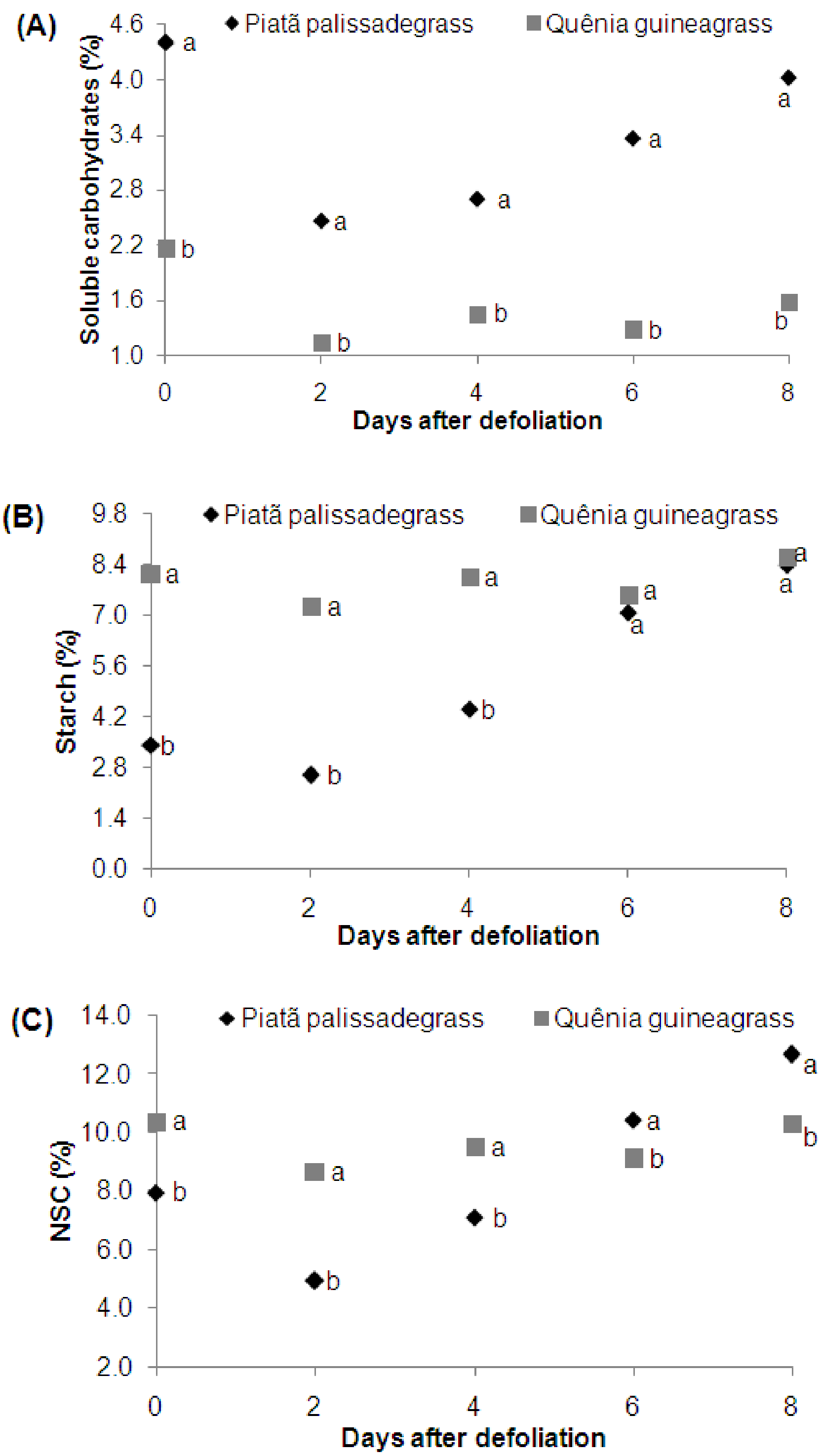

Fig. 1. Soluble carbohydrate (A), starch (B) and total non-structural carbohydrates (C; NSC) contents in roots of BRS piatã and BRS quênia grass with nitrogen fertilization performed at 0; 2; 4; 6 and 8 days after defoliation. Means followed by the same letter do not differ by Tukey's test at 5% probability

Quênia guineagrass ($P< .01$) (Table 2). Late nitrogen fertilization reduced the DMRES ($P< .01$) of the BRS Piatã palisadegrass, reaching higher values when the nutrient was applied in the first days after defoliation, suggesting that nitrogen fertilization during this period provided a better shoot development, with greater efficiency of nutrient absorption [21].

In a study with *Panicum maximum* cultivar Tanzania, Carvalho [22] obtained results that corroborate with those described in the present study, in which a reduction of DMRES was observed the later the nitrogen fertilization was performed. The residual forage mass is an important parameter for ensuring the vigor of the forage plant regrowth, interfering with the amount of accumulated dry mass and, consequently, in the utilization of the forage produced through reduction in the levels of losses by grazing [23].

Defoliation represents a stress for plants by reducing light interception, liquid photosynthesis of the canopy and the amount of organic compound reserves in the growth of the root system [24]. This reinforces the importance of defining management practices that provide better nutritional conditions for forage regrowth. In this sense, it was verified in the present work that the nitrogen fertilization performed in the first days after defoliation resulted in better recovery efficiency for the BRS Piatã palisadegrass and the BRS Quênia guineagrass.

In relation to RDM, no effect of the nitrogen fertilization moment was observed for the two evaluated grasses (Table 2). Therefore, the application of this nutrient until the eighth day after defoliation does not generate significant changes of this parameter. In a study on nitrogen fertilization in grasses of the genera *Brachiaria* and *Panicum*, Martuscello et al. [25] also did not find effect of nitrogen application in RDM. According to the authors, this result might be related to the strategy of dry mass partitioning by certain forage species, whose higher nitrogen contribution is related to a greater shoot dry mass accumulation instead of the root system.

According to Sarmento [17], the nitrogen fertilization effect on root growth is complex and depends on several factors, such as the source of the nutrient, the amount and location of the fertilizer, other soil, chemical and biological conditions, and the forage species used.

Regarding the water soluble carbohydrate contents of the root system, larger amounts of soluble carbohydrates were verified when nitrogen fertilization was performed at the cutting time on BRS Piatã palisade grass and BRS Quênia guineagrass. In the BRS Piatã cultivar there was a decrease of these compounds when the nutrient was applied on the second day after defoliation, followed by an increase until the eighth day. For the BRS Quênia guineagrass, soluble carbohydrate reduction was observed when nitrogen fertilization was performed at 2 and 6 days after defoliation (Fig. 1A).

The highest soluble carbohydrate content on the defoliation day probably occurred because nitrogen was applied when the plants were practically intact [26]. Therefore, nutrient supply, in addition to providing good dry mass production in the grasses (Table 2) also prevented a sudden drop in the reserve carbohydrates of the root system, what naturally occurs after grass defoliation [24,26,27,28].

This is possibly related to a better absorption of nitrogen, allowing adequate energy support to the plants for fast recovery without great losses in the levels of soluble carbohydrates in the roots. Although the base of the stem is commonly the primary structure of energy storage in grasses, Turner et al. [29] found significant relationships between the concentration of soluble carbohydrates in the roots and regrowth in the grass *Bromus willdenowii* Kunth.

The reduction of soluble carbohydrates, observed when nitrogen was applied on the second day after defoliation, with levels of 2.46 and 1.14% for the BRS Piatã palisadegrass and the BRS Quênia guineagrass, respectively, can be attributed to the decrease in the transport of assimilates to the roots in favor of the recovery of the leaf area, in order to reestablish the photosynthetic capacity of the plants [24].

This reflected in the grasses dry mass production, which was high in this moment of fertilization (Table 2), suggesting full recovery of the remaining plant area after defoliation and synthesis of new tissues. According to Bernal [30], under conditions that promote rapid growth, the reserve carbohydrate content may be reduced or remain at a low level, since most of the photoassimilates are retained as sucrose at the active growth points.

In a study performed with ryegrass, Schnyder and De Visser [28] observed a reduction in the soluble carbohydrate contents until the second day after cutting. According to the authors, although photosynthesis and nitrogen uptake are severely affected by defoliation, when meristems and leaf growth zones are preserved, respecting adequate height of residue as in the present study, there is foliar recovery and redistribution of the plant reserve compounds in later days.

The water soluble carbohydrate contents of the BRS Piatã palisadegrass roots presented an increasing effect when nitrogen fertilization was performed from the fourth to the eighth day after defoliation (Fig. 1A), suggesting that later application of this nutrient generated a higher accumulation of reserve sugars and smaller yields of DMLB, DMSS and DMRES (Table 2).

Under conditions of reduced grass growth due to lower nitrogen uptake, environmental stress or when the energy set in photosynthesis exceeds growth requirements, reserve compounds tend to accumulate. In tropical and subtropical grasses the most important nonstructural carbohydrate in the root system is normally starch, although sucrose may be a considerable reserve component during some periods of plant life [30].

It is possible that water soluble carbohydrates are not primary substrates for the biomass production of tillers after the first day after defoliation; however, they contribute indirectly to tiller growth by supplying energy to the plant shoot and preserving the reserves of the root system [28].

The BRS Piatã palisadegrass presented higher concentrations of soluble carbohydrates compared to the BRS Quênia guineagrass, at all evaluated times of nitrogen fertilization (Fig. 1A). Klimes and Klimesova [31] also found differences in the concentrations of reserve sugars in different grass species. According to the researchers, it is not clear whether these differences present ecological consequences, since the role of individual carbohydrate fractions in plants is still poorly understood.

On the other hand, higher levels of starch were observed in roots of the BRS Quênia guineagrass compared to cultivar BRS Piatã when nitrogen was applied at 0, 2 and 4 days after defoliation (Fig. 1B). Working with five species of Brazilian Savannah plants, Almeida et al. [32] also found distinct profiles of non-structural carbohydrates in different species, with a predominance of starch in plants of the genera *Trimezieae* and *Tigridieae*, and a higher amount of soluble carbohydrates in the genus *Sisyrinchieae*.

Likewise, Liu et al. [33] studying forest species, observed that some plants have a higher content of starch, whereas others present a higher concentration of soluble sugars. According to the authors, plants grown under similar environmental conditions may present different amounts and allocation of carbohydrates in relation to their life forms or ecological strategies.

Grass reserve compounds are used as energy source to initiate new growth until photosynthesis is enough to sustain plant respiration. The starch is immobile, being synthesized in the chloroplasts of the photosynthetic organs and in the amyloplasts of non photosynthetic organs. Sucrose, conversely, is mobile, synthesized in the cytosol of cells and discharged into the phloem, constituting the main substrate for plant respiration. Under stress conditions, amylaceous reserves can be partially converted into soluble sugars for use in the respiration process as an energy supply for growth resumption [34,35].

Therefore, the higher soluble carbohydrate contents at all times of nitrogen application observed in the BRS Piatã palisadegrass roots in the present study (Fig. 1A) indicate a higher demand of this forage for compounds that are readily available for plant growth. The BRS Quênia guineagrass, for accumulating higher levels of starch when nitrogen was applied at 0, 2 and 4 days after defoliation (Fig. 1B) without damaging plant dry matter production (Table 2), showed greater tolerance to defoliation stress.

Total non-structural carbohydrate levels of the roots are obtained from the sum of soluble and starch carbohydrate levels. With regard to this parameter, there was a decrease when nitrogen fertilization was performed on the second day after defoliation on both grasses in relation to the application of nitrogen on the cutting day. Afterwards, an increasing pattern was observed in the NSC contents when the fertilizer was applied from the fourth to the eighth day after defoliation of the BRS Piatã palisadegrass, and reduction of these compounds on the sixth day of fertilization in the BRS Quênia guineagrass (Fig. 1C).

In general, the application of nitrogen at low and moderate doses results in increased reserve carbohydrates, whereas moderate to high rates lead to decrease in these compounds [36]. However, little is known about the influence of the fertilizer application moment on the reserve components of forage grasses.

It is known that nitrogen is the main constituent of proteins that actively participate in the synthesis of the organic compounds that form the plant structure, being determinant in the speed of forage plants recovery [37,38]. Thus, the ready availability of photosynthesis assimilates may have allowed earlier transport to the roots or made them less dependent on organic reserves [24], when nitrogen fertilization was performed at defoliation (Fig. 1C). As a result, the plants presented good dry mass production during this evaluation period (Table 2).

When nitrogen fertilization was performed on the second day after defoliation, the lowest levels of NSC were obtained (Fig. 1C), but both grasses had good dry mass production means (Table 2) in this period. On the other hand, the later application of nitrogen generated a higher accumulation of reserve carbohydrates and lower dry mass production in the BRS Piatã palisadegrass.

This may indicate a better efficiency in the use of nitrogen fertilizers in the first stages of fertilization, since a greater quantity of organic reserves was used to compose new tissues [39]. Similarly, Gomide et al. [40] found a lower NSC accumulation in plants fertilized with nitrogen at the beginning of regrowth.

In addition, according to Buxton [41], larger root systems may benefit the nutrient absorption capacity of the forage, accumulating lower levels of reserve carbohydrates. This affirmation corroborates the results obtained in the present work, in which the nitrogen fertilization performed on the sixth day after the BRS Quênia guineagrass defoliation resulted in root systems containing the highest dry mass mean (Table 2), associated with a lower amount of reserve carbohydrates in relation to nitrogen at 4 days after cutting (Fig. 1C).

According to the author, there may be a root dependence on the supply of water and nutrients for carbohydrates to be used in plant growth processes. Thus, plants with smaller root systems may not fully absorb these essential growth factors, providing less forage yield and higher nonstructural carbohydrate content in the roots.

It should be noted that fertilization up to the fourth day after defoliation yielded higher NSC values in the BRS Quênia guineagrass compared to the BRS Piatã palisadegrass (Fig. 1C), and the application of nitrogen from the sixth to the eighth day resulted in accumulation of larger amounts of NSC in the BRS Piatã cultivar. Relating these results to the productive parameters (Table 2), the BRS Piatã palisadegrass accumulated higher levels of total non-structural carbohydrates under conditions in which there was less dry matter production of plant components, suggesting a survival strategy [36], prioritizing the maintenance of the reserves after defoliation stress when the nitrogen fertilization was performed later.

According to White [36], the effects of nitrogen fertilization on carbohydrate reserves in grasses are complex and variable. In this sense, the interaction of the plant with the environment and the balance between photosynthesis and respiration determine the alteration of carbohydrate reserves during plant growth.

The results obtained in the present study indicate that the BRS Piatã palisadegrass has a high initial requirement after defoliation, and it is recommended that nitrogen fertilization be performed close to the cutting time, in order to maximize the productive potential of the forage. For the BRS Quênia grass, the performing of studies using fertilizations in periods later than eight days after defoliation is recommended, so that it may be possible to identify when nitrogen fertilization will interfere with grass development.

Considering the important contribution of the reserve carbohydrates in forage recovery after defoliation, further studies are necessary in order to determine the dynamics of these compounds before different nitrogen fertilization periods. It should be noted that there is a large gap in the literature on the proportions of soluble carbohydrates and starch present in the roots and stem base of the main cultivated grasses. The generation of this information is relevant since the cultivars can present different levels, allocation and demand of these compounds, depending on their morphophysiological characteristics.

4. CONCLUSION

The BRS Piatã palisadegrass has a high initial requirement after defoliation, and it is recommended that nitrogen fertilization be performed close to the cutting moment. Nitrogen fertilization of the BRS Quênia guineagrass can be performed between the day of the defoliation and the eighth day after, without significant changes in its production. It is suggested to perform studies using later fertilizations. With regard to the non-structural carbohydrates in the roots, there is a higher content of soluble carbohydrates in the BRS Piatã palisadegrass, and a higher accumulation of starch in the BRS Quênia guineagrass.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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