

Full Length Research Paper

Effect of molybdenum and nitrogen rates on the growth and vegetative structure of *Megathyrsus maximus* (Syn. *Panicum maximum*)

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The objective of this study was to identify the rate of molybdenum that when associated with nitrogen fertilization, would provide the best responses on the vegetative structure of Mombasa grass. Forty days after planting, a uniform pruning was made when the established levels of molybdenum were applied and the experimental period started. The experimental design was a randomized block, with four replications (blocks), in a 5×6 factorial scheme, considering the combinations of treatments, the combinations of five levels of molybdenum (0, 50, 100, 150 and 200 g ha⁻¹ year⁻¹), in six evaluation ages (days 7, 14, 21, 28, 35 and 42 after pruning for uniformization) and four rates of nitrogen (0, 100, 250 and 500 of N. ha⁻¹ year⁻¹). The variables were subjected to variance analysis and adjustment in regression using orthogonal polynomials. Among the variables, an assessment of the number of leaves that were fully expanded (exposed ligula), in development or senescent in the main tillers (NFCE, NFEE and NFS, respectively) was carried out, as well as the total number of tillers per pot (NP) and the height of the vegetation (ALT). The rise in molybdenum supplies up to 200 g ha⁻¹ increases the production of vegetative biomass of mombasa grass in pastures, by using levels of 500 kg ha⁻¹ year⁻¹ of N, but there is no difference between the other doses of nitrogen (0, 100 and 250 ha⁻¹ year⁻¹).

Key words: Development, tropical grasses, intensive management of pastures, pots, biomass.

INTRODUCTION

Farmers are increasingly interested in new forage species with high yield potential to be implanted in intensive systems. In this context, the species

Megathyrsus maximus (Syn. *Panicum maximum*) stands out for its productivity and nutritional value. It is expected that higher Nitrogen concentrations promote an increase

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in the number of tillers in pastures with *Megathyrus maximus* (Syn. *Panicum maximum*) (Oliveira et al., 2012; Costa et al., 2016; Silveira, 2020).

The area occupied by this species corresponds to approximately 20% of all cultivated pastures in Brazil, supplying 30% of the forage seed market (Martuscello et al., 2007). Among the different cultivars, *Megathyrus maximus* (Syn. *Panicum maximum*) cv. Mombasa (mombaça grass) and cv. Tanzania (Tanzania grass) are prominent in the areas of cultivated pastures in the country (Cavalli, 2016; Silva, 2020).

Among the nutrients that ensure the success of similar species, nitrogen is undoubtedly the element that most influences the productivity of grasses. It is an element required by plants in greater quantity and generally makes up 20 to 40 g kg⁻¹ of dry matter in plant tissues (Taiz et al., 2017; Dupas et al., 2016; Galindo et al., 2018).

Nitrogen fertilization influences not only productivity, but also the quality of the product. Therefore, low availability of nitrogen to plants negatively affects the nutritional quality of forages, which limits the absorption of nutrients, and results in a product that does not meet the nutritional requirements of the animal (Braz et al., 2002, Cruz et al., 2021).

Therefore, one of the main limiting factors in the productivity of tropical forages in pastures is nitrogen deficiency, which results in a sharp drop in support capacity and in animal weight gain (Rocha et al., 2002). According to Fagundes et al. (2005) the supply of N normally does not meet the grasses demand, and their productivity is limited by the levels of this nutrient in the soil. Tillering is a structural characteristic strongly influenced by nutritional, environmental and management factors, which define the morphogenic characteristics that condition the morphogenic response of forage plants to management systems (Costa et al., 2018).

In order to increase the productivity of pasture forages, numerous studies have been carried out with the objective of evaluating the response of foragers to the application of nitrogen (Belarmino et al., 2003; Freitas et al., 2005). The results have shown that increased production of mombaça grass with the application of nitrogen occurs in a linear and increasing manner, reaching productions above 50 mg of DM ha⁻¹ year⁻¹.

It must be clarified, however, that the simple application of nitrogen does not mean that this nutrient will be effectively absorbed by the plants, because when nitrogen is applied on the soil, it may be absorbed by the plants, immobilized in the organic fraction, or lost to volatilization or leaching (Malhi et al., 2001; Lorenzini et al., 2012).

Inside the plants, nitrate-reductase catalyzes the biological reduction of NO₃⁻ to NO₂⁻, which is the first step towards the incorporation of nitrogen, in the form of NO₂⁻, into proteins (Cazetta and Villela, 2004). Nitrate-reductase is a flavoprotein that contains molybdenum as

a prosthetic group and whose synthesis is induced by the presence of molybdenum and NO₃⁻ in the medium (Boom, 2002). Thus, molybdenum plays an indispensable role in the assimilation of nitrate absorbed by plants, acting on the level of nitrate-reductase. Therefore, any deficiency in this nutrient can compromise the metabolism of nitrogen, as well as the plants response to nitrogen fertilization.

In intensive systems, in which nutrients such as molybdenum are extensively extracted from the soil, it must be clarified whether the addition of this micronutrient available to the plant in the soil will allow for an increase in the production of forage biomass. Thus, this study aimed to identify the molybdenum rate associated with nitrogen fertilization, would provide the best responses on the vegetative structure of Mombasa grass.

MATERIAL AND METHODS

The work was carried out in a greenhouse at the Federal Institute of Education, Science and Technology of Espírito Santo, *Campus Santa Teresa*, Espírito Santo, Brazil, located at latitude -19.808122 and longitude -40.684497 with an altitude of 128 m. The region is characterized according to the Köppen classification, type Aw, characteristic of humid tropical climates, with two well-defined seasons, that is, dry in winter and wet in summer. Average annual temperatures are around 24.35°C, with an average of 26.9°C for the hottest month of the year (February); and 21.8°C for the coldest month (June). Samples of Red-Yellow Latosol (LVA) were used, which were collected in the region from depths greater than 40 cm (sub-surface).

After collection, the soil was homogenized, air-dried and passed through a 2 mm sieve for chemical and physical characterization, which consisted of corrections and fertilization to chemical and physical fertilization according to the soil performed according to methodologies established by Ribeiro (1999) and Cantarutti et al. (1999). After their properties were analyzed, the soil samples were incubated with different levels of lime for a period of 90 days. Thus, during the study, a previous assessment (incubation period) was made necessary, with six rates of CaCO₃ (calcium carbonate) (0.824; 1.992; 3.160; 4.328; 5.492; 6.660 mg kg⁻¹), equivalent to elevations to 30, 40, 50, 60, 70 and 80% of soil base saturation. This procedure was carried out to determine the pH level resulting from the soil given the reaction with the different levels of CaCO₃.

Each application level of CaCO₃ was repeated four times to obtain an average for the post-incubation soil pH. For the calculation of CaCO₃ rates, the soil density (1.32 g cm⁻³) was verified. The soil samples were placed in PVC pipes of 150 mm, after being mixed with each respective rate of CaCO₃ and wetted daily for 90 days (February to May).

For the incubation, each sample treated with CaCO₃ received the same amount of water, equivalent to 100% of its field capacity of the studied soil. As a result of the incubation, it was determined that the most appropriate level of CaCO₃ is 4,328 mg kg⁻¹. This dosage was applied and mixed in total soil. After that, phosphate and micronutrient fertilizations were carried out, and the pots were filled.

Subsequently, the pots were planted with Mombaça grass and 10 days after germination, a thinning was performed to keep four plants per pot. A 40-day wait period of growth then followed, to allow for a uniform pruning to be carried out, in order to keep the plant height at a height of 30 cm above ground level in all pots. Thereafter, the recommended evaluations were initiated.

After this uniform pruning of the mombaça grass, molybdc fertilizations were carried out (referring to levels 0, 50, 100, 150 and 200 g ha⁻¹ year⁻¹ of molybdenum). A first nitrogen fertilization was also carried out immediately after pruning the forage in the pots to keep them uniform, maintaining a 30 cm plant height, followed by weekly nitrogen fertilizations, always one day before the evaluation period.

Climatological information (temperature and light) was collected from the Meteorological Station located at IFES - Campus Santa Teresa. During the evaluation period, soil moisture was maintained at 60% of the Total Pore Volume (VTP), recommended by Freire et al. (1980). A source of nitrogen used manually was a conventional granulator of urea [CO(NH₂)₂], applied manually.

The experimental design was a randomized block, with four replications (blocks), in a 5 x 6 factorial scheme, considering the combinations of treatments, the combinations of five levels of molybdenum (0, 50, 100, 150 and 200 g ha⁻¹ year⁻¹), in six evaluation ages (days 7, 14, 21, 28, 35 and 42 after pruning for uniformization) and four rates of nitrogen (0, 100, 250 and 500 of N ha⁻¹ year⁻¹).

The treatments were structured according to the combination of the main factors (levels of molybdenum and ages of evaluation), making a total of 30 treatments. The variables related to growth and vegetative structure was subjected to variance analysis considering the main factors and their interactions. In the different ages, the number of fully expanded green leaves (NFCE) or with exposed ligules, the number of expanding green leaves (NFNE) and the number of senescent leaves (NFS) in the main tillers were assessed. In addition, the number of total and senescent leaves that had fully expanded was counted every seven days. The amount of green leaves was obtained by subtracting senescent leaves of the total leaves of the plant, in two marked tillers per pot.

The height of the vegetation was obtained in a standardized manner using an acetate sheet placed above the pot. From there, the height was measured from the ground level to the acetate sheet.

All data were submitted to variance analysis. An ensuing regression analysis was carried out using the decomposition of freedom degrees of treatments in orthogonal polynomials method, with the System for Statistical and Genetic Analysis (SAEG 9.1). In this procedure, the type I (alpha) error of 5% was considered.

RESULTS

As a result of the variance analysis, for the NFCE variable, a significant effect ($P < 0.05$) was observed in the interaction between the molybdenum levels and post-uniformization age, shown by the F test ($P < 0.05$). With this, the five levels of molybdc fertilization and the six ages of post-uniformization assessment were compared together, evaluating the effects of post-uniformization ages within each pre-established level of molybdenum.

Thus, the number of live leaves per tiller (Figure 2), once they are constant for each species, constitutes an objective criterion in the definition of the pruning or grazing interval of forage plants, serving as a comparative standard on the effects of different interventions, such as the use of fertilizers (Fulkerson and Slack, 1995).

There was a significant isolated effect of molybdenum levels ($P < 0.05$) for the variables height of vegetation (ALT) and number of tillers per pot (NP). In the polynomial regression analysis, linear and quadratic

effects (Figure 3) were observed for ALT and NP, respectively.

In the assessment of the other variables, no significant effects ($P > 0.05$) were observed for the different levels of molybdenum. However, when evaluating the age factor alone, through the unfolding of degrees of freedom in orthogonal polynomials, a quadratic effect was observed (Figure 4B) for the number of senescent leaves (NFS) and the number of expanding leaves (NFNE; Figure 4A), with the increase in post-uniformization age. In the case of the ALT (Figure 4D) and NP (Figure 4C) variables, in relation to the post-uniformization ages, the equation that best fitted their behaviors was the one of increasing linear character ($P < 0.05$).

DISCUSSION

In the regression analysis (Figure 1), significant effects ($P < 0.05$) were observed in the quadratic models for the levels of 0 and 200 g ha⁻¹ of molybdenum, and first-degree linear effect for the levels of 50 and 150 g ha⁻¹ of molybdenum. For the 100 g ha⁻¹ level, no significant adjustment effect ($P > 0.05$) was observed.

This quadratic effect may indicate that these concentrations encourage greater performance of the Mombasa variety and provide greater accumulation of forage, since the accumulation of biomass in the pasture after defoliation is the result of the flow of elaboration of new leaf tissues (defined as primary production) and of the flow of senescence and decomposition of older leaf tissues (Simioni et al., 2014).

These authors also consider that with the appearance of new leaves and tillers in the pasture after defoliation, the competition for light, nutrients, water and other environmental factors increases, intensifying the process of senescence and death of leaves and older tillers.

This is because, when defoliation is very frequent, the greater availability of nutrients in the soil contributes to the marandu grass changing its form of growth from erect to prostrate, compared to the condition of lower availability of nutrients in the soil. Thus, the increase in the interval between cuts, or grazing, and the fertilization increase the removal of forage from the Marandu grass (Medica et al., 2017).

Therefore, the forage species may have reached the maximum number of live, fully expanded leaves per tiller at the levels of 50 and 150 g ha⁻¹, which demonstrates a balance between the appearance of leaves and their death. Quadratic response to the number of live, completely expanded leaves in the main tillers with increasing age was also observed by Gomide and Gomide (2000) with a maximum of 5.5 leaves at 25 days of age.

On the other hand, Santos et al. (2017) proved that in *Brachiaria brizantha* cv. Piatã nitrogen fertilization did not change the number of live and dead leaves ($P > 0.05$). These authors expected to verify an increase in the

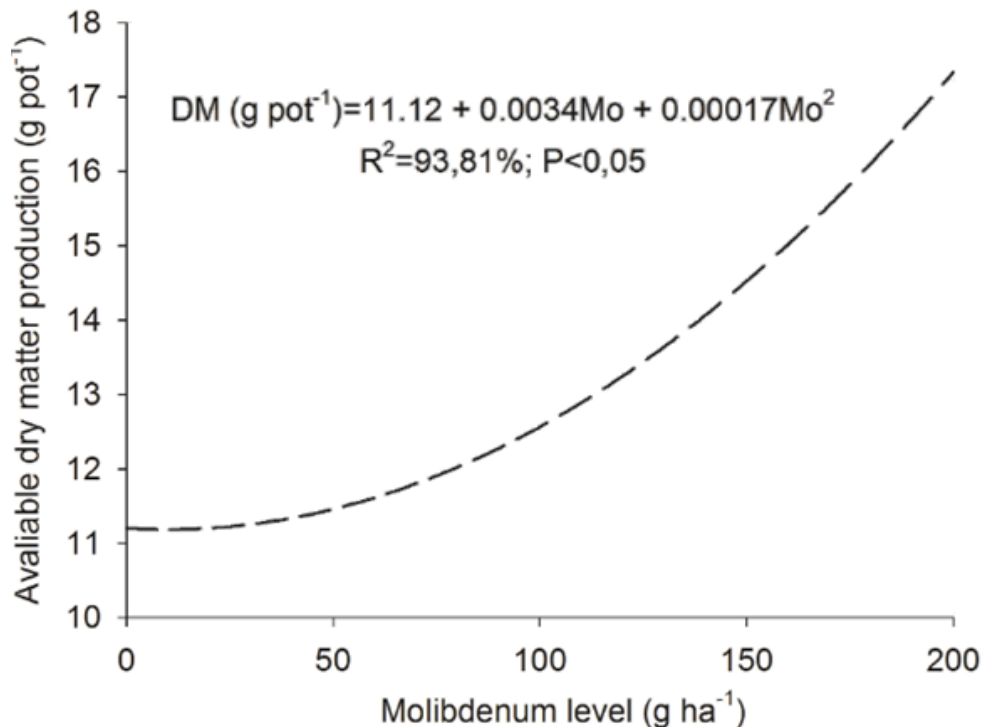


Figure 1. Production of biomass available above 30 cm from the ground level in the pots (MSDisp) with the different levels of Molybdenum.
Source: Author

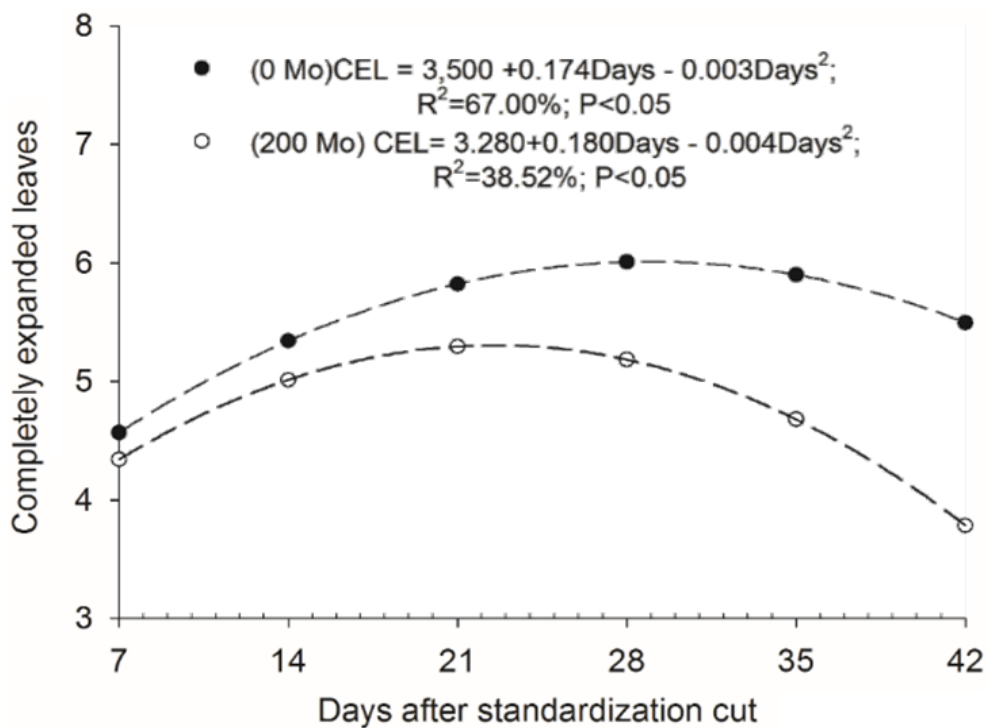


Figure 2. The effect of different levels of Molybdenum and of post-uniformization age on the number of fully expanded green leaves (NFCE), or with the exposed ligule of marked main tillers of Mombasa grass.
Source: Author

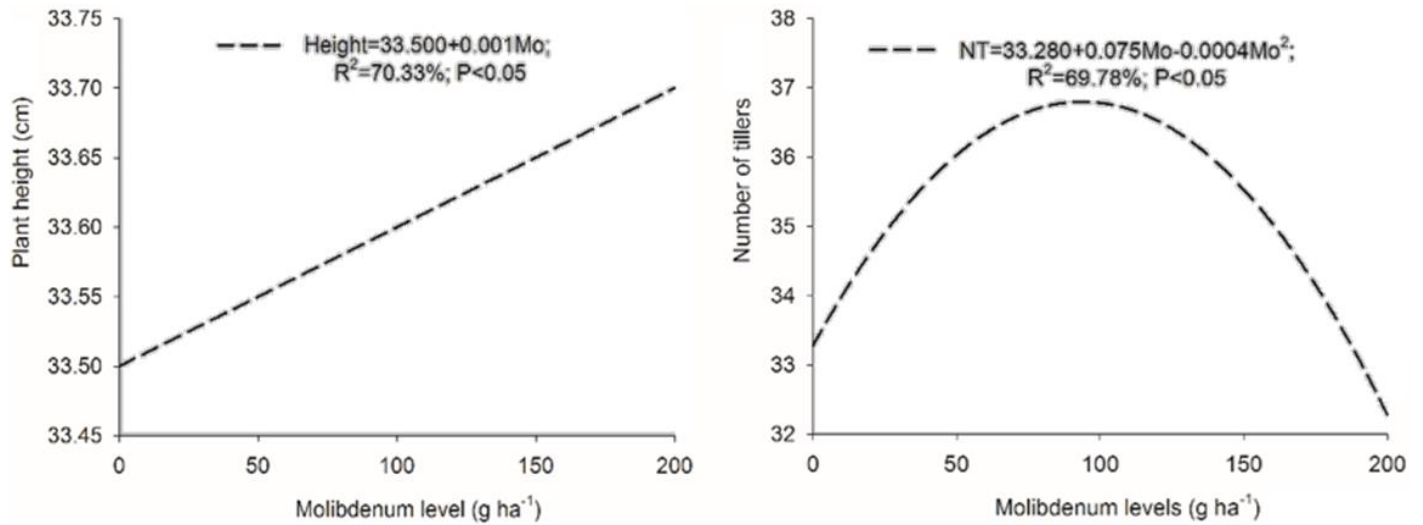


Figure 3. Isolated effect of Molybdenum levels on the vegetation height (ALT) and on the number of tillers (NP) of Mombasa grass. Source: Author

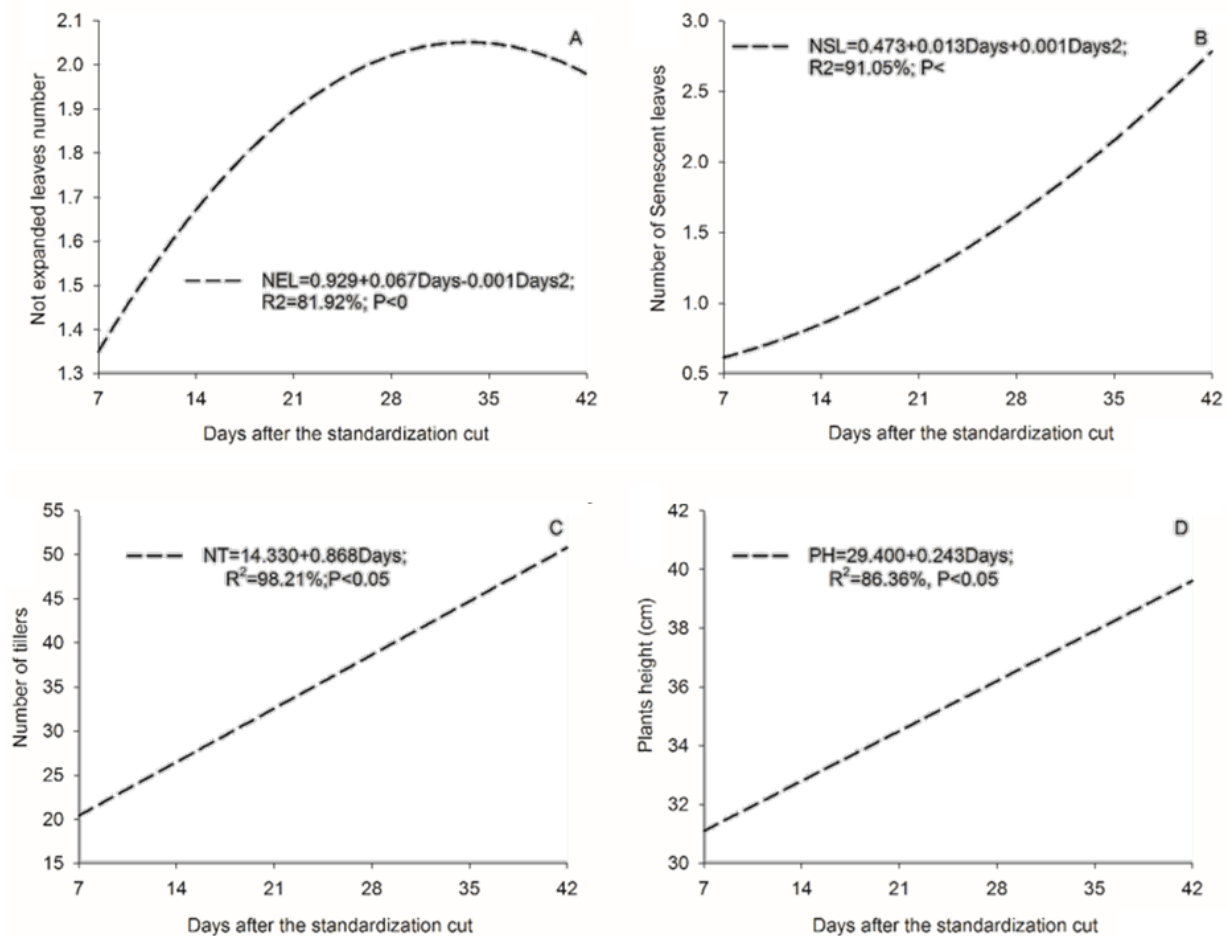


Figure 4. Isolated effect of the factor days post-uniformization of mombaça grass submitted to the different pre-established treatments on the variables: A) Number of expanding green leaves (NFNE); B) Number of senescent leaves (NFS); C) Number of tillers (NP) and D) Height of vegetation (ALT). Source: Author

number of dead leaves with nitrogen fertilization, considering that nitrogen stimulates the appearance of leaves in the tiller and, thus, those formed first would tend to die earlier, which would increase the number of dead leaves in the tiller. Possibly, the shedding of dead leaves in the Piata grass tiller may have been the factor responsible for the lack of nitrogen effect on this characteristic.

In this study, in addition to the similar behavior pattern for this variable, we have observed maximum values of 5.30 and 5.84 for the NFCE, at 23 and 28 days of age, for the levels of 0 and 200 g ha⁻¹ molybdenum. Also, the results indicate the effect of molybdenum in accelerating the growth of Mombasa grass in a parallel study, when a significant effect of molybdenum was observed in the highest biomass production due to the different applied levels of this nutrient ($P < 0.05$; $Y = 0.00017x^2 - 0.00335x + 11.12$; $R^2 = 93.81\%$).

For the other variables, the analysis of variance had no significant effect ($P > 0.05$) for the interaction between the molybdenum level and post-uniformization age, according to the F test. For this reason, the five levels of molybdenic fertilization and the six ages of post-uniformization assessment were compared independently.

The behavior patterns for both variables demonstrated a possible phenotypic modification of mombaça grass given the greater growth ($P < 0.05$) in plant height and the reductions ($P < 0.05$) in the number of tillers, the latter occurring from the level of 100g ha⁻¹.

These variables may have been influenced by the radiation pattern that reached the base of the plants, as the tillers grew. This increase in shading and the consequent increase in the interception of photosynthetically active radiation may have provided a differentiated lengthening of the plants and a reduction in their tillering rate.

As for the average number of tillers per plant, 9 to 12.5 were observed, a number close to those noticed by Gomide and Gomide (2000) of 9 to 14 tillers per plant. In spite of this, when the NT was related to age after uniformization, an increasing behavior was observed, without obtaining a maximum point for the variable (Figure 4), which indicated that other factors, such as the direct effect of molybdenum in increasing the size of the tillers to the detriment of their number may have occurred, given the increase in biomass levels with an increase in the days after uniformization in the parallel study ($P < 0.05$; $Y = 0.025x^2 + 1.021x + 24$, 73 ; $R^2 = 98.95\%$).

The trends shown for NFNE and NFS ($P < 0.05$) are also within normal behavior patterns for a tropical grass (Carneiro and Nascimento Júnior, 2007; Jank et al., 2010; Macedo et al., 2010; Dantas et al., 2020). It should be added that, with the evolution of the plant's growth, there are reductions in the appearance of leaves in the same tiller, culminating in the launching of inflorescence.

In addition, it has been previously discussed that the acceleration of the processes of death and leaf

senescence with the evolution of the plant's growth stage is a normal and intrinsic process.

Another important observation to be made, due to the curve pattern for the NFS, is its acceleration at ages between 21 and 28 days, a phase where the maximum values for the NFCE variable were obtained, which indicated the definition of the interval between pruning's or grazing. In general, in Brazil, areas that are cultivated with perennial pastures for years may present nutritional deficiencies and do not have a sufficient population of nutritive nutrients for the atmosphere in mosphere in the soil (Alves and Berti, 2022).

Crusciol et al. (2019) direct growth improving the effects of molybdenum application at different rates and a rhizobium inoculant on the characteristics of *Arachis pinto*, in a planting system after 20 years of use as pasture [*Urochloa brizantha* (Sin. *Brachiaria brizantha*)]. The different doses of molybdenum (0, 50, 100 g) (0, 50, 200 g⁻¹) verify that the nitrogenase activity and the specific nitrogenase activity were attested at 45 days a (DAE).

Conclusion

The scientific evidence observed in this study suggests that, in soils from which nutrients are intensively extracted, raising the molybdenum supply up to 200 g ha⁻¹ increases the production of vegetative biomass of mombaça grass in pastures, using levels of 500 kg ha⁻¹ year⁻¹ of N.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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