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Agronomic performance of Marandu grass treated with plant growth biostimulants in the Amazon biome

[*Desempenho agronômico de capim-marandu tratado com bioestimulantes no bioma Amazônia*]

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ABSTRACT

The aim was to evaluate the effect of different doses of two biostimulants on the productivity and canopy structure of *Urochloa brizantha* cv. Marandu grass in the establishment fase. The study was conducted in Universidade Federal Rural da Amazônia, Parauapebas, Pará, Brazil. One module of 35 plots of 25m² were established. A completely randomized experimental design was used, with seven treatments and five replicates. The treatments included a control, 0.5, 1, and 2kg/ha of biostimulant A (BIOST.A); and 0.25, 0.5, and 1L/ha of biostimulant B (BIOST.B). Three collections were performed. The data for application of the two biostimulants were analyzed separately, using the Dummy variable method and regression analysis. The application of 2kg/ha BIOST.A resulted in increases of 842kg/ha in the forage mass. The application of BIOST.B on Marandu grass resulted in a linear increase in stem mass. The application of 2kg/ha BIOST.A in the establishment of Marandu grass result in higher growth rates, forage accumulation, and stem proportion in the canopy.

Keywords: auxin, cytokinin, gibberellin, forage stimulation, *Urochloa brizantha*

RESUMO

Objetivou-se avaliar o efeito de diferentes doses de dois bioestimulantes sobre a produtividade e a estrutura do dossel do capim Urochloa brizantha cv. Marandu na fase de estabelecimento. O estudo foi realizado na Universidade Federal Rural da Amazônia, Parauapebas, Pará, Brasil. Um módulo de 35 parcelas de 25m² foi estabelecido. Utilizou-se delineamento experimental inteiramente ao acaso, composto de sete tratamentos e cinco repetições cada. Os tratamentos incluídos no controle foram: 0,5, 1 e 2kg/ha de bioestimulante A (BIOST.A); 0,25; 0,5 e 1L/ha de bioestimulante B (BIOST.B). Foram realizadas três coletas. Os dados para a aplicação dos dois bioestimulantes foram analisados separadamente, utilizando-se organização por método variável Dummy e análise de regressão. A aplicação de 2kg/ha BIOST.A resultou em incrementos de 842kg/ha na massa de forragem. A aplicação do BIOST.B na grama marandu resultou em aumento linear na produção de massa do caule. A aplicação de 2kg/ha BIOST.A no estabelecimento de erva de marandu resultou em maiores taxas de crescimento, acumulação de forragem e proporção de caule no dossel.

Palavras-chave: auxina, citocinina, giberelina, estimulação forrageira, Urochloa brizantha

INTRODUCTION

Several technologies are available for use in agricultural crops, of which plant biostimulants stand out. Biostimulants are mixes of plant growth regulators and other biochemical compounds such as amino acids, nutrients, and vitamins (Silva *et al.*, 2012). Some of the commercial products available in Brazil are registered as mixed mineral foliar fertilizers.

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These fertilizers include plant extracts, biologically active plant hormones, and macro- and micronutrients in their composition. These products act through the plant hormones in their composition by stimulating cell division and elongation in growth centers such as apices, developing leaves, and roots. Biostimulants aim at increasing the plant production potential, have been used increasingly in modern agriculture, and are widely used in countries such as the USA, Spain, Chile, Mexico, and Italy (Silva *et al*, 2012).

Because their use recommendations are aimed at fruit and vegetable crops (Castro & Vieira, 2001; Cato, 2006; Silva *et al*.2013a; Silva *et al*., 2013b; Lunelli *et al*., 2015), a lack of knowledge exists about the biostimulation effects of these products on tropical forage plants. In this context, plant biostimulants are therefore a promising alternative for forage production since, as with other crops, they could have beneficial effects on forage plants by stimulating forage mass production per unit of area, decreasing rest periods, and/or improving plant responses to fertilization. Regarding animal production, these effects would result in increased forage and, consequently, higher support capacity in addition to a greater number of grazing cycles per year without compromising the defoliation. To achieve this, the possible effects of biostimulant treatment on tropical forage species growth and canopy characteristics need to be quantified, and the amount of biostimulants that results in desirable effects in pastures needs to be determined.

When it comes to the implantation phase of pasture production systems, the use of technologies designed to provide productive advantages in a practical and low cost way are of great importance. In this context, the objective of the present study was to quantify the effect of foliar spraying of different doses of biostimulants in a herbicide set in the weed control phase in the establishment of *Urochloa brizantha* cv. Marandu on the parameters of plant productivity and canopy structure.

MATERIALS AND METHODS

The study was conducted in the experimental forage crops field of the Universidade Federal Rural da Amazônia, Parauapebas, state of Pará (PA), Brazil, at latitude 06°04'16.4"South, longitude 049°49'8.3"West, and 270M of altitude. The relief at the experimental site was classified as mildly hilly, with a bed of Precambrian granitic rock and metasediments (Brasil, 1974). The soil is predominantly red-yellow Argisol (Sistema…, 2006). The experimental area was previously used for animal husbandry and had not been used for approximately 2 years at the time of soil preparation.

According to the Koppen, (1948) classification, the region’s climate is Aw, i.e., tropical, with a dry season from May to October, and a very pronounced wet season, with torrential rains from November through April. Monthly data on the rainfall and the maximum, minimum, and average temperatures were collected throughout the experiment at the Meteorological Station of the Federal Rural University of Amazon, Parauapebas Campus, located approximately 500m from the experimental site (Figure 1).

On 4 December 2013, two modules, one for each species, of 35 plots of 25m² (5.0 x 5.0m) were established. After harrowing and application of establishment fertilization, sowing was performed using pure viable seeds, with a sowing rate of 2kg/ha. The soil characteristics at the 0- to 0.20-M soil layer before the beginning of the experiment were pH in H_20 5.3, 7mg/dm^3 P, 0.2cmol$_c$/dm^3 K, and 5.8cmol$_c$/dm^3 Ca+Mg. Based on the recommendations for fertilizer use in the state of Minas Gerais (5th approximation; Cantarutti *et al*., 1999), for clay-loam soils (16 to 35% clay) and forage species of medium technological level, 60kg P_2O_5 /ha was applied at the moment of sowing, followed by 60kg K_2O /ha and 112.5kg N /ha split into two applications (at sowing and after the first harvest). The fertilizers used were simple superphosphate (phosphorus source), potassium chloride (potassium source), and ammonium sulfate (nitrogen source).

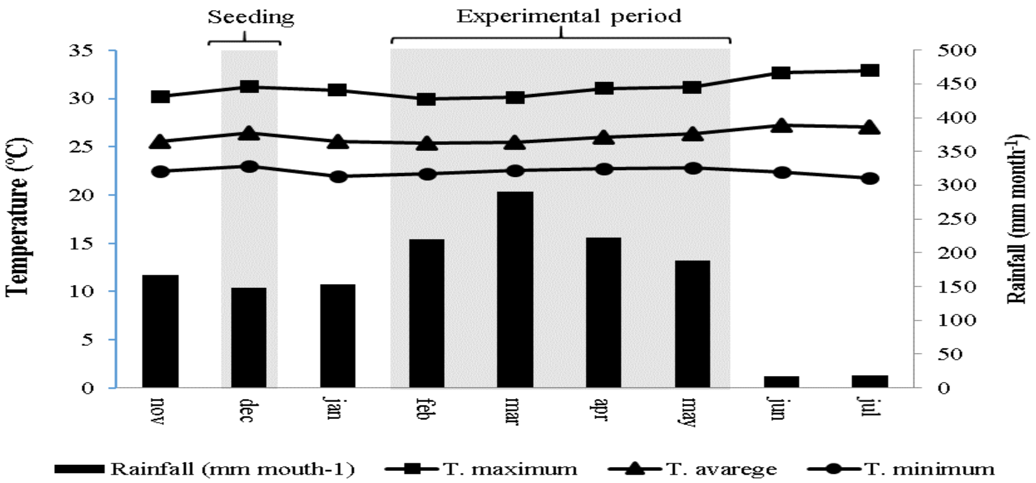

Figure 1. Accumulated rainfall per mouth, temperature (T.) maximum, average and minimum recorded during the period from November 2013 to June 2014.

Seven treatments were applied to each species in a completely randomized experimental design, with five replicates per treatment. Each experimental plot corresponded to one replicate, with 35 plots. The treatments tested are described in Table 1. A selective herbicide composed of picloram (64g/L) and 2,4-dichlorophenoxyacetic acid (240g/L) was applied to all plots at 3L/ha for control of dicotyledonous weeds.

Table 1. Bioestimulants and doses used in the composition of the treatments imposed on the species *Urochloa brizantha* cv. Marandu

Treatment	Auxin doses sprayed
Control (dose 0)	0µg/ha
BIOEST.A*	
0.5kg/ha	200µg/ha
1.0kg/ha	400µg/ha
2.0kg/ha	800µg/ha
BIOEST.B†	
0.25L/ha	8.05µg/ha
0.50L/ha	16.10µg/ha
1.00L/ha	32.20µg/ha

* BIOEST.A: Auxinic complex: 400ppm; Total nitrogen (N): 9%; Available phosphorus (P2O5): 45%; Potassium (K2O): 11%; Magnesium (Mg): 0.60% e Sulfur (S): 0.80%. † BIOEST.B: Gibberellins: 32.2ppm; Indole acetic acid: 32.2ppm, Zeatin: 83.2ppm, Magnesium (Mg): 0.14%; Sulfur (S): 0.44%; Boron (B): 0.30%; Iron (Fe): 0.49%; Manganese (Mn): 0.12% e Zinc (Zn): 0.37%.

Biostimulant A (BIOST.A) is composed of 400mg/kg auxinic complex and 9, 45, 11, 0.60, and 0.80 percent of total nitrogen (N), available phosphorus (P_2O_5), potassium (K_2O), magnesium (Mg) and sulfur (S) respectively. Biostimulant B (BIOST.B) is based on plant extracts with hormonal activity, and these extracts make up 788g/kg of its solution. BIOST.B is composed 32.2mg/kg of gibberellins, 32.2mg/kg of indole acetic acid, 83.2mg/kg of zeatin and 0. 14, 0.44, 0.30, 0.49, 0.12, and 0.37 percent of magnesium (Mg), sulfur (S), boron (B), iron (Fe), manganese (Mn) and zinc (Zn) respectively.

Application was performed using a CO_2 pressurized backpack sprayer with a 2-m boom and XR 110.02 spray nozzles calibrated to deliver 185L/ha at a constant pressure. The biostimulants were applied in a single spraying 60 days after sowing (2/3/2014). Three collections were performed: 29 (3/4/2014; period 1), 58 (4/2/2014; period 2), and 98 (5/13/2014;

period 3) days after biostimulant application. At each collection time, the samples were collected for evaluation of forage grass morphological characteristics and productivity.

Plant growth was monitored by measuring the canopy height before the collect. Three imaginary lines were set, and canopy height was measured at three points along the lines, in the two extremes and the middle of the plot, using a ruler graduated in centimeters and allowing a 1-m border. Tiller density was measured by counting the number of tillers in $1m^2$ of each plot, using a quadrat placed at canopy mid-height.

The Accumulation was measured by collecting and weighing three forage grass samples in each plot, consisting of all the grass present within a $3m^2$ (1 x 3m) quadrat, cut at the residual height adopted for Marandu palisade grass: 0.12m from soil. The quadrats were placed at points that were representative of the average canopy height. Following sampling, the plots were cut to the residual height adopted for each species. In the laboratory, the samples were homogenized, subsampled, and used to estimate the dry matter content (DM). The measured accumulation values were converted into kg/ha. The Forage mass was calculated by adding all accumulations occurred during the experimental.

An aliquot was removed from each plot and separated into leaf blade, stem (stem + leaf sheath), senescence, and reproductive stems (stems with inflorescence). The sub-samples were then placed in an air circulation oven heated at 55°C for 72 hours. The proportion of each shoot component was expressed as the percentage dry weight relative to the total dry weight (sum of all shoot components). The leaf: stem ratio was calculated by dividing the leaf percentage by the stem percentage for each plot. The mass production for each botanical composition was calculated by multiplying the percentage of DM each component for the Forage mass production per hectare of the respective plot. The accumulation rate was estimated by dividing the accumulation during each cut period by the duration of the respective rest time.

The data were subjected to a variance analysis using the F-Test, using the Statistical Analysis System software (SAS…, 2015). The data for application of the two biostimulants were analyzed separately, using the Dummy variable method and regression analysis. The goodness of fit of linear and quadratic models was tested, at P <0.05 for error type I, considering the control treatment as dose zero for both biostimulants.

RESULTS

The response of forage mass, leaf mass, stem mass, reproductive stem mass, and accumulation rate to BIOST.A application on Marandu palisade grass was best fitted by a quadratic equation (P <0.05; Table 2). The Forage mass production increased 842kg/ha with application of 2kg/ha BIOST.A and decreased 1063 and 1277kg/ha with 0.5 and 1.0kg/ha BIOST.A, respectively, compared to the control treatment (0kg/ha biostimulant). When the growth rate was analyzed per period, the application of 2kg/ha BIOST.A only resulted in higher growth rates during the second period after spraying (Figure 2).

During this period, accumulation and accumulation rates increased linearly with increasing BIOST.A doses (P= 0.026). This was only observed for the second period, as no significant differences between treatments were observed for the third period (P >0.05; Figure 2). Leaf mass production increased 269.7kg/ha with 2kg/ha BIOST.A compared to the control treatment. A similar variation was observed for stem mass and reproductive stem production, which increased 387 and 182kg/ha, respectively.

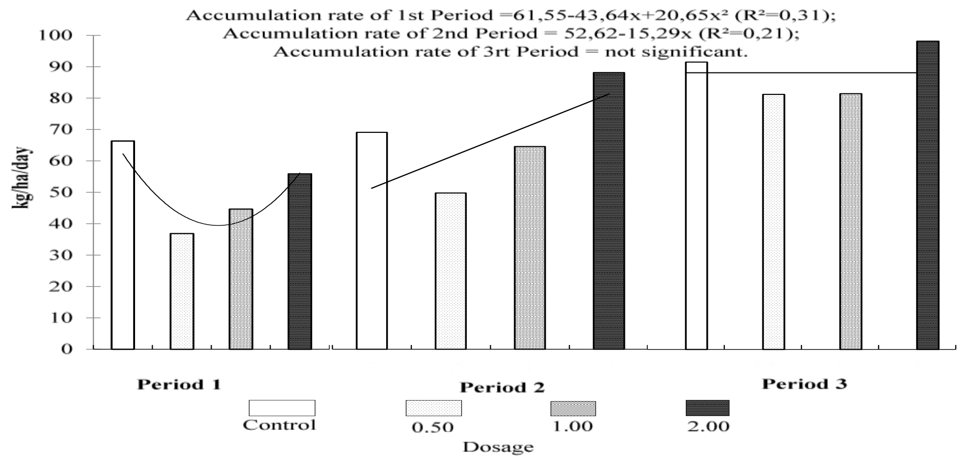

Figure 2. Observed and estimated accumulation rate of Marandu palisade grass submitted to different dosages of bioestimulant A (BIOEST.A) during the collection periods.

The variations in the botanical composition of Marandu palisade grass were best fitted by quadratic equations ($P < 0.05$). Application of BIOST.A in doses higher than 0.5kg/ha increased the proportion of stems and reproductive stems in the canopy. The variation in the average canopy height at collection was best fitted by a quadratic equation, with variation lowest at 0.7kg/ha BIOST.A and highest at 2kg/ha BIOST.A (Table 2).

Table 2. Mean values, standard error of the mean (SEM) and p-value of the productive and botanical composition of *Urochloa brizantha* cv. Marandu submitted to different dosages of Bioestimulant A (BIOEST.A)

Item*	Dosage, kg/ha				SEM	*p- Value*	
	0	0.5	1	2		L	Q
Productivity, kg of forage mass/ha							
Forage mass†	7538	5622	6349	7998	293	0.086	0.004
Accumulation of 1st Period†	2059	1157	1373	1686	123	0.998	0.004
Accumulation of 2nd Period †	2189	1595	2027	2720	131	0.011	0.069
Accumulation of 3rd Period	3289	2869	2948	3591	119	0.345	0.069
Leaf mass†	5427	4374	4738	5474	151	0.217	0.020
Stem mass†	1949	1074	1481	2175	148	0.128	0.004
Senescence mass	127	148	122	123	9	0.795	0.693
Reproductive stem mass †	33.4	25.6	7.7	224.9	17	0.001	0.026
Accumulation rate, kg /ha/day†	75.0	55.9	63.3	80.4	3	0.068	0.004
Botanical composition							
Canopy height, cm†	29.3	22.8	28.0	34.1	1	0.029	0.049
Tillers/m²	741.0	735.2	775.0	790.2	20	0.301	0.841
Leaf, %†	71.9	77.7	74.4	70.3	1	0.081	0.010
Stem, %†	25.7	19.2	23.5	25.8	1	0.306	0.029
Senescence, %	1.9	2.5	1.9	1.7	0.2	0.778	0.312
Reproductive stem, %†	0.5	0.5	0.2	2.3	0.2	0.009	0.050
Leaf: stem ratio†	2.8	4.1	3.3	2.8	0.2	0.246	0.016

* Forage mass accumulated between zero at 98 days after the application; Accumulation of 1st Period (zero at 29 days after the application); Accumulation of 2nd Period (30 at 58 days after the application); Accumulation of 3rd Period (59 at 98 days after the application). † $Y_{Forage\ mass} = 7223.84-2976.03x+1698.49x^2$ (R^2= 0.32); $Y_{Accumulation\ of\ 1st\ Period} = 1919.52-1353.34x+625.96x^2$ (R^2= 0.31); $Y_{Accumulation\ of\ 2nd\ Period} = 1708.03+435.70x$ (R^2= 0.19); $Y_{Leaf\ mass} = 5240.99-1578.61x+856.73x^2$ (R^2 = 0.22); $Y_{Stem\ mass} = 1824.11-1305.16x+749.37x^2$ (R^2 = 0.34); $Y_{Reproductive\ stem\ mass} = 39.80-124.08x+107.66x^2$ (R^2 = 0.42); $Y_{Accumulation\ rate} = 71.87-29.77x+17.18x^2$ (R^2 = 0.33); $Y_{Canopy\ height} = 28.11-7.59x+5.38x^2$ (R^2 = 0.24); $Y_{Leaf} = 72.83+7.76x-4.59x^2$ (R^2 = 0.28); $Y_{Stem} = 24.66-7.33x+4.04x^2$ (R^2 = 0.22); $Y_{Reproductive\ stem} = 0.67-1.34x+1.06x^2$ (R^2 = 0.34); $Y_{Leaf:\ stem\ ratio} = 3.05+1.40x-0.78x^2$ (R^2 = 0.25). L-Linear; Q-Quadratic.

Regarding the effect of BIOST.B on Marandu palisade grass, no significant differences in forage mass for the whole experimental period were observed between treatments. However, when each experimental period was analyzed separately, the accumulation was significantly fitted by a quadratic equation for period 1 and by a linear equation for periods 2 and 3 (Table 3). BIOST.B significantly affected stem mass ($P < 0.05$), which increased linearly with increasing doses of BIOST.B. The application of 1L/ha BIOST.B resulted in an increase of 704kg/ha compared to the control treatment (Table 3).

Table 3. Mean values, standard error of the mean (SEM) and p-value of the productive and botanical composition of *Urochloa brizantha* cv. Marandu submitted to different dosages of Bioestimulant B (BIOEST.B)

Item*	Dosage, L/ha				SEM	*p- Value*	
	0	0.25	0.5	1		L	Q
Productivity, kg of forage mass/ha							
Forage mass	7538	6382	6713	7494	293	0.120	0.108
Accumulation of 1st Period †	2059	1303	1248	1331	123	0.255	0.022
Accumulation of 2nd Period †	2189	1722	1986	2477	131	0.042	0.161
Accumulation of 3rd Period †	3289	3356	3478	3685	119	0.044	0.997
Leaf mass	5427	4606	4769	5168	151	0.426	0.091
Stem mass †	1949	1685	1795	2179	148	0.025	0.312
Senescence mass	127.6	81.6	149.3	142.6	9	0.613	0.708
Reproductive stem mass	33.4	9.0	0.0	3.9	17	0.795	0.587
Accumulation rate, kg/ha/day	75.0	63.4	66.9	75.2	3	0.101	0.101
Botanical composition							
Canopy height, cm	29.3	27.2	28.0	30.4	1	0.116	0.542
Tillers/m²	741.0	736.8	623.0	707.6	20	0.469	0.300
Leaf, %	71.9	74.4	74.0	71.6	1	0.105	0.220
Stem, %	25.7	24.0	23.8	26.6	1	0.077	0.331
Senescence, %	1.9	1.3	2.2	1.8	0.2	0.665	0.886
Reproductive stem, %	0.5	0.4	0.0	0.0	0.2	0.801	0.590
Leaf: stem ratio	2.8	3.3	3.2	2.7	0.2	0.071	0.314

* Forage mass accumulated between zero at 98 days after the application; Accumulation of 1st Period (zero at 29 days after the application); Accumulation of 2nd Period (30 at 58 days after the application); Accumulation of 3rd Period (59 at 98 days after the application). †$Y_{\text{Accumulation of 1st Period}}$ = 1919,52-2509,50x+1937,82x² (R^2=0,31); $Y_{\text{Accumulation of 2nd Period}}$ = 1708,03+689,12x (R^2=0,19); $Y_{\text{Accumulation of 3rd Period}}$ = 3073,83+678,75x (R^2=0,11); Y_{Steam} = 1472,29+704,05x (R^2=0,15). L-Linear; Q-Quadratic.

Application of 1 L/ha BIOST.B only resulted in significantly accumulation rate during period 2 ($P < 0.05$). For period 2, accumulation and yield, represented by the daily production, increased linearly with increasing doses of BIOST.B. This trend was observed until period 3, when no differences in accumulations rates were observed between treatments (Figure 3).

DISCUSSION

The observed increase in the forage mass accumulation rate with doses higher than 1.8kg/ha BIOST.A can therefore be attributed to stimulation by the sprayed auxin. Previous studies evaluating the effects of exogenous auxin sources on other plant species also observed increased productivity (Cato, 2006; Silva *et al.*, 2010; Silva *et al.*, 2013b and Lunelli *et al.*, 2015).

Although auxin acts in synergism with cytokinin to stimulate cell division, these hormones have an antagonist effect on the control of branch and root initiation in tissue culture (Scoog & Miller, 1957). Higher auxin:cytokinin ratios therefore induce root differentiation, whereas lower ratios increase the development of stem buds (shoot) (Kerbauy, 2013). Therefore, if (i) the endogenous levels of cytokinins in the plant at treatment application are similar and if (ii) BIOST.A is an exogenous auxin source and (iii) increasing doses of BIOST.A are applied, an increase in the auxin:cytokinin ratio in the plant is assumed to occur. Furthermore, higher doses of BIOST.A can be assumed to result in higher initial root mass production per tiller, due to higher auxin:cytokinin ratios. This was confirmed by the separate analysis of the accumulation rate for period 1 (Figure 2), when higher shoot growth rates were observed without biostimulant application (Treatment with no bioestimulant), whereas growth rates increased with increasing doses of BIOST.A during period 2 (Figure 2). The improvement of the soil nutritional status resulting from the fertilization applied during period 2, together with the assumption of higher root growth in response to stimulation by exogenous auxin, explains the higher growth rate observed during this period because plants with more developed roots respond better to fertilization (Valadão *et al.*, 2015). This results in higher growth rates and, consequently, higher accumulation of forage mass. The higher forage mass observed at the end of the experiment with the highest dose of BIOST.A can therefore be attributed to this effect, as the response to BIOST.A application was best immediately following fertilization (period 2), and no response was observed between periods 2 and 3 because no fertilization was applied (Figure 2). This effect occurred in all treatments, but the adopted fertilization management only resulted in higher productivity for treatment 2kg/ha BIOST.A at the end of the experiment. Considering the observed responses and the hypotheses proposed, further studies evaluating the use of BIOST.A with different fertilization levels and types are needed.

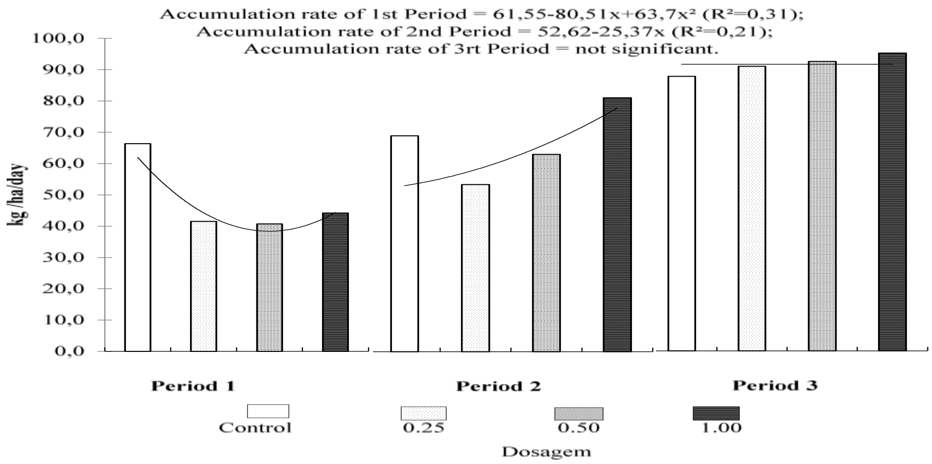

Figure 3. Observed and estimated accumulation rate of Marandu palisade grass submitted to different dosages of bioestimulant B (BIOEST.B) during the collection periods.

In addition, increasing the doses of BIOST.A was hypothesized to result in immediate and constant increases in forage mass production in Marandu palisade grass. However, doses lower than 1.8kg/ha BIOST.A resulted in lower forage mass than for the control treatment. This may have resulted from plant stress following BIOST.A application, which was indicated by the lower averages observed during period 1 in plots receiving applications of the biostimulant. Acording to Ljung *et al*. (2002), increasing auxin concentrations and activity in plant tissues may change plant growth and lead to plant death. The auxin source is extremely important in this effect because natural auxins such as indole acetic acid (IAA) are usually quickly inactivated by conjugation and degradation by several pathways in plants, whereas synthetic auxins act for longer and are more effective than IAA (Grossmann, 2003). Plant spraying with synthetic auxins may cause stress because the auxin active transport, which is mediated by protein transporters, and auxin conjugation and degradation, require energy (Taiz and Zeiger, 2013). The simultaneous use of two exogenous auxin sources (herbicide and BIOST.A) may have therefore been responsible for the putative stress due to the increased energy demand to plants, resulting in the decreased accumulation rate observed for period 1 (Figure 2). Considering that the amount of herbicide applied was the same for all treatments and that only the BIOST.A dose varied, the effect observed for forage mass, accumulation rate, leaf, stem and reproductive stem mass may be inferred to result from the increased doses of BIOST.A. The effect observed with lower doses of BIOST.A for period 1 may be explained by the supply of nutrients present in the product. These nutrients, in the case of stress caused by BIOST.A doses higher than 1kg/ha, may have met the increased plant nutrient needs, resulting in the increased production rates (Figure 2).

The lower average canopy height observed with intermediate doses of BIOST.A and the higher average canopy height with 2kg/ha BIOST.A were in agreement with the observed forage mass. Dim *et al*. (2015) evaluated the effect of different sward heights on total forage dry mass in Piatã grass and observed increasing total forage mass with increasing canopy height in the beginning of the rainy season. Therefore, the application of an exogenous auxin source (BIOST.A) can be inferred to result in higher accumulation rates, as the average interval between collections was the same for all treatments.

The high canopy height, which reflected higher accumulation, in the plots treated with BIOST.A were associated with high stem proportion in the canopy and therefore high stem mass. As the canopy height increases, leaves begin competing for the top of the canopy to meet their photosynthetic needs, resulting in shading (Fontes *et al*., 2014). One of the main plant responses to shading is stem elongation followed by leaf elongation, which is an attempt to expose leaves to higher light intensity and, consequently, higher red/far red ratios (Deregibus *et al*., 1983; Martuscello *et al*., 2009).

The decreased leaf proportion, together with the increased stem proportion at the canopy observed with doses higher than 1kg/ha BIOST.A, did not result in lower estimated leaf production per hectare. The application of 2kg/ha BIOST.A therefore resulted in higher estimated leaf production per hectare. Sales *et al*. (2014) hypothesized that the leaf emergence rate is widely determined by the leaf elongation rate and leaf sheath length, which affect the emergence of the leaf, and changes in one of these two factors affect the leaf emergence rate. Higher canopy height followed by higher stem proportion resulted in lower leaf proportion in the canopy but not in lower estimated leaf production. This could be explained by the plants competing for light presenting higher specific leaf area, i.e., bigger leaves, but lower weight (Martuscello *et al*., 2009).

A possible explanation for the lower leaf proportion and higher stem production observed in the canopy of Marandu palisade grass treated with doses higher than 1kg/ha BIOST.A is that auxin stimulates cell growth and proliferation as it moves through the plant (Grebe, 2005). Because BIOST.A was applied following cutting and the stem density was higher closer to the ground, spray liquid absorption by stems was likely higher, which resulted in higher stimulation. This is in accordance with the acid growth theory, which describes how auxin induces cell elongation; that is, auxin activity causes proton extrusion into the cell wall, resulting in cell wall acidification and loosening, thereby allowing cell expansion (Grebe, 2005).

The analysis of the canopy height for the treatment with 2kg/ha BIOST.A suggested that higher leaf:stem ratios and more leaves could probably be obtained, as the canopy height before cutting was 0.293 to 0.341 M (Table 2) and therefore higher than 0.25M, which corresponds to 95% light interception (Giacomini *et al*., 2009).

BIOST.B increased the stem proportion resulting from the action of its components, especially the gibberellin, which increases stem growth in higher plants (Taiz & Zeiger, 2013). Silva *et al*. (2010) observed that application of Stimulate® (which has similar active compounds to BIOST.B) increased stem production in sugarcane shoots up to 35% compared to the control treatment. Regarding pasture management, lower canopy stem proportions are ideal because the stems possess higher lignin contents and are therefore less digestible and nutritious than leaves (Silva *et al*., 2013a). Future studies may be performed to evaluate tissue morphogenesis and flow and to determine the canopy height that corresponds to 95% light interception in Marandu palisade grass treated with high doses of BIOST.B.

The accumulation rates during the different experimental periods with BIOST.B treatment were similar to those observed with BIOST.A. accumulation rates were higher for period 2 than for the remaining periods, and no significant differences between treatments were observed in period 3 (Figure 2 and 3). The same explanations found for the responses to BIOST.A application in Marandu palisade grass may therefore be applied to the responses to BIOST.B. However, BIOST.B affected the accumulated DM production during the three periods, although this effect was not significant to the average of the three periods. This was likely due to the severity of the stress caused by its application, which resulted in lower accumulation rates than the control treatment during period 1. Another possible explanation would be the different product doses and composition, since BIOST.A contains higher amounts of auxin (Table 1), which resulted in higher accumulation rates in period 1, especially for the highest BIOST.A dose tested (Figure 2 and 3).

CONCLUSION

The use of 2kg/ha BIOST.A, supplying 800µg auxin/ha, in the establishment of Marandu grass promote higher growth rates, forage accumulation, and canopy stem proportion.

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