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Evaluation of sulfur source and dose on the nutritional state and production of piatã forage

Avaliação de fonte e dose de enxofre no estado nutricional e produção de forragem do piatã

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Abstract

Sulfur deficiency in soils has become a worldwide concern for agricultural production. This study aimed to evaluate the concequence of variable sulfur source and dosing on the nutritional status and production of Piatã forage in a dystrophic Ultisol. The experiment was arranged in a completely randomized block design with four replications. The 4 x 5 factorial treatments consisted of four sulfur sources (elemental sulfur pastilles [ESPA], gypsum [GY], gypsite [GI], and elemental sulfur powder [ESPO]) and five sulfur doses (0, 50, 100, 150. and 200 mg kg^{-1}). The sulfur applications resulted in similar maximum shoot dry mass (SDM) production (16.66 to 17.69 g pot^{-1}) with all sources. However, Piatã grass achieved maximum production in the treatments with 112 mg kg^{-1} of GI, 118 mg kg^{-1} of GY and ESPA, and 146 mg kg^{-1} of ESPO. The number of tillers, the leaf:stem ratio, and SPAD increased with increasing sulfur dose. The greatest increments of root dry mass (RDM) and of the tiller number were obtained with the ESPO source. Macronutrient concentrations in shoot dry mass tissue were in the order K > N > Ca > P > S > Mg. Low sulfur supply resulted in decreased Piatã grass growth with all sulfur sources tested. Differential responses were found with applications of different sulfur sources for SDM, RDM, and tillers number. The sources did not alter the leaf:stem ratio or SPAD index.

Key words: Fertilizer. Plant nutrition. Pastures. *Urochloa brizantha.*

Resumo

A deficiência de enxofre nos solos tem se tornado uma preocupação mundial para a produção agrícola. Este estudo teve como objetivo avaliar a conceituação da variável fonte e dose de enxofre no estado nutricional e produção de forragem do Piatã em um Argissolo Vermelho distrófico. O experimento foi arranjado em blocos ao acaso com quatro repetições. Os tratamentos foram dispostos em esquema

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fatorial 4 x 5, sendo constítuído de quatro fontes de enxofre (enxofre elementar pastilhado [ESPA], gesso [GY], gibsita [GI] e enxofre elementar em pó [ESPO]) e cinco doses de enxofre (0, 50, 100, 150 e 200 mg kg^{-1}). As aplicações de enxofre resultaram em uma produção similar de massa seca da parte aérea (SDM) (16,66 a 17,69 g $vaso^{-1}$) com todas as fontes. No entanto, o capim Piatã alcançou a produção máxima nos tratamentos com 112 mg kg^{-1} para GI; 118 mg kg^{-1} para GY e ESPA; e 146 mg kg^{-1} de ESPO. O número de perfilhos, a relação folha: haste e SPAD aumentaram com o aumento das doses de enxofre. Os maiores incrementos de massa seca da raiz (RDM) e do número de perfilhos foram obtidos com a fonte ESPO. As concentrações de macronutrientes na parte aérea foram na ordem de K > N > Ca > P > S > Mg. A baixa oferta de enxofre resultou na diminuição do crescimento do capim Piatã com todas as fontes de enxofre testadas. Respostas diferenciais foram encontradas com as diferentes aplicações de fontes de enxofre para SDM, RDM e número de perfilhos. As fontes não alteraram a relação folha: haste ou índice SPAD.

Palavras-chave: Fertilizantes. Nutrição de plantas. Pastagem. *Urochloa brizantha.*

Introduction

Livestock occupies a prominent position in the world economy, especially due to increasing global consumption and international agroindustry demand (SOARES et al., 2016). Brazil is responsible for one fifth of the international meat trade and exports to over 180 countries (PAULA et al., 2016).

The total area occupied by pastures in Brazil is 173 million hectares (DIAS-FILHO, 2014). *Urochloa* species are the most abundant pasture species because they are considered productive with a rapid leaf accumulation and are tolerant of poorly drained soils and resistant to spittlebugs (PIMENTA, 2009). Among the species in the genus *Urochloa*, the cultivar Piatã has favorable management characteristics, such as low stem elongation rate, producing greater leaf blade mass relative to stem, continued forage accumulation during the dry period, lower seasonality of production, and good performance under moderate to high soil fertility (EUCLIDES et al., 2009).

Sulfur deficiency in soils induced by intensive agricultural and use of highly concentrated fertilizers has initiated worldwide interest in this nutient, particularly in regions with sandy soils containing low organic matter that receive high rainfall (SALVAGIOTTI; MIRALLES, 2008; SUN et al., 2017). Sulfur deficiency substantially reductes biomass production of forage plants and their quality because sulfur is associated with the production of essential cellular components, such as sulfur-containing amino acids, vitamins, acetyl-CoA, and components of photosynthetic and nitrogen metabolism (CAPALDI et al., 2015; DING et al., 2016).

Modern agricultural production requires efficient fertilizer management practices that seek to enhance the sustainability of the productive system. Adequate fertilizer rates, appropriate sources, efficient application methods, and application in the correct period are important considerations (FAGERIA; BALIGAR, 2005). Sulfur applicated may interact with soil paticules and results in greater or lesser fertilization efficiency and nutrient uptake. Fertilizers that provide sulfur in the form of sulfate are readily absorbed, but the elemental form of the sulfur requires oxidation prior to absorbtion, which may be favourable for a balanced and continuous supply during the the cropping cycle (DEGRYSE et al., 2018; GRANT et al., 2012).

In this study we aimed to evaluate the nutritional status and forage production of Piatã grass (*Urochloa brizantha*) in response to the source type and dose of sulfur in a dystrophic Ultisol.

Material and Methods

Experimental materials, soil, and plant

The experiment was conducted under greenhouse conditions at São Paulo State University (Unesp), College of Agricultural and Technological Sciences (21°29′ LS and 51° 2′ LW; 396 m altitude), São Paulo State, Brazil, using the forage grass *Urochloa brizantha* (*syn. Brachiaria brizantha*) cv. Piatã. The soil was a dystrophic Ultisol with the following chemical properties: pH ($CaCl_2$) = 4.6; organic matter = 17.0 g kg^{-1}; P (resin) = 6.0 mg kg^{-1}; K = 1.2 mmol kg^{-1}; Ca = 6.0 mmolc kg^{-1}; Mg = 3.0 mmol kg^{-1}; S (SO_4^{-2}) = 7.0 mg kg^{-1}; potential acidity hydrogen + aluminum (H + Al) = 21.0 mmol kg^{-1}; Al = 3.0 mmol kg^{-1}; sum of bases (Σ K, Ca, Mg) = 10.2 mmol kg^{-1}; cation exchange capacity (Σ K, Ca, Mg, H + Al) = 31.2 mmol kg^{-1}; base saturation [(Σ K, Ca, Mg)/(Σ K, Ca, Mg, H + Al)] × 100 (V) = 32.7 %. Chemical properties were determined as described by Raij et al. (2001): P, K, Ca, and Mg determinations were performed using the ion exchange resin method, S was extracted with $Ca(K_2PO_4)_2$ solution, pH was in $CaCl_2$, organic matter was by colorimetry, H + Al was with SMP buffer, and Al was by KCl. Cu, Fe, Mn, and Zn were determined as extracts in DTPA TEA at pH 7.3. The soil was sampled from 0-20 cm depth for a composite sample. Before filling the pots, the soil was air dried and sieved (4.0 mm).

Experimental design

The experimental design was completely randomized and arranged in a 5x4 factorial arrangement that included five doses of S applied to soil (0, 50, 100, 150, and 200 mg kg^{-1}) and four sources of S. These S sources were elemental sulphur pastilles (ESPA, 90.0 % S) with size of 1-4 mm, gypsum (GY) residue from the manufacture of phosphate fertilizers (24 % Ca and 17 % S), ground natural gypsite (GI) rock (24 % Ca and 17 % S) with, and elemental sulphur powder (ESPO, 95 % S), with particles < 0.1 mm.

Experimental condition and procedures

Soil base saturation (V) was increased to 70 % (RAIJ et al., 1996) by adding calcium carbonate ($CaCO_3$) and magnesium carbonate ($MgCO_3$) p.a. reagents with the aim of obtaining soil Ca:Mg ratio of 3:1. The soil plus carbonate salts were then incubated for 30 days in the pots to allow for reaction and moisture was maintained at 80 % of field capacity. At the end of the incubation period, the soil was air-dried for seven days. The soil of each pot (4.0 kg^{-1}) was placed in plastic trays where the treatments and fertilizers were applied (in mg kg^{-1}): 300 of N [urea (NH_2CO)], 200 of P [calcium phosphate $Ca_3(PO_4)_2$], 150 of K [potassium chloride (KCl)], 0.5 of B (boric acid (H_3BO_3)], 0.05 of Co [cobalt chloride ($CoCl_2$)], 1.0 of Cu [copper sulfate ($CuSO_4$)], 0.05 of Mo [molybdic acid (H_2MoO_4)], 0.05 of Ni [nickel sulfate ($NiSO_4$)], 5 of Mn [manganese sulfate ($MnSO_4 \times 5H_2O$)], and 2.0 of Zn [zinc sulfate ($ZnSO_4 \times 7H_2O$)] (MALAVOLTA et al., 1997). Final soil density was 1.3 g cm^{-3}.

Four days after fertilizer application and irrigation of each pot, Piatã grass was sown and the seeds were evenly covered with a thin soil layer. Thinning to four plants per pot was performed after two weeks. Irrigation was carried out manually with deionized water to maintaining soil moisture at 80 % of field capacity.

Parameters evaluated

Production of dry mass and tillering

The Piatã grass was cut 5.0 cm above the soil surface 50 days after germination. Shoots were separated into leaves and stems + sheaths and oven dryed with forced air circulation adjusted to a constant temperature of 65 ± 2 °C until constant weight to determine shoots dry mass (SDM, expressed in g pot^{1}). The leaf:stem+sheaths ratio was obtained as the quotient between the dry mass of the leaf blades and the dry mass of stems + sheaths. The roots of the plants of each pot were collected and washed in a 2 mm screen and dried as described for shoots to determine the root dry mass (RDM, expressed in g pot^{1}). A tiller count was performed immediately before each cut and expressed as number pot^{-1}.

SPAD and Macronutrients

The SPAD index was evaluated in the middle third of newly expanded leaves and expressed in relative units using the Minolta Co. chlorophyll meter (MINOLTA, 1989). The mean of 10 SPAD readings per pot was evaluated.

Shoots dry mass was ground in a Wiley mill and the total concentrations of nitrogen (N), phosphorus (P), potassium (K), calcium (Ca), magnesium (Mg), and Sulphur (S) were determined as described by Malavolta et al. (1997). To determine N, the samples were digested in sulphuric acid (H_2SO_4), followed by distillation and titration by the semimicro-Kjeldahl method. The concentrations of P, K, Ca, Mg, and S were determined by digesting the samples in nitric-perchloric acid (65 % HNO_3 and 70 % $HClO_4$). Atomic absorption spectrophotometry was used to determine the concentrations of Ca and Mg, and the concentration of K by flame photometry. The concentration of P was obtained by colorimetry and the concentration of S was obtained by barium chloride ($BaCl_2$) turbidimetry. Macronutrient concentrations wer expressed as g kg^{-1}.

The relationship between the N and S (N:S) leaf concentrations was also calculated as a function of the S doses.

Data analysis

For each measured variable, an analysis of variance was performed considering the randomized block design in a factorial scheme with four replications. The factors were: four sources and five doses of sulfur, as previously indicated. Quantitative factor (dose of sulfur) were submitted to regression analysis and the factor qualitative (source of S) were submitted to a Tukey test ($p < 0.05$). All statistical analysis was performed with routines developed in R version 3.5.0 (R CORE TEAM, 2018).

Results

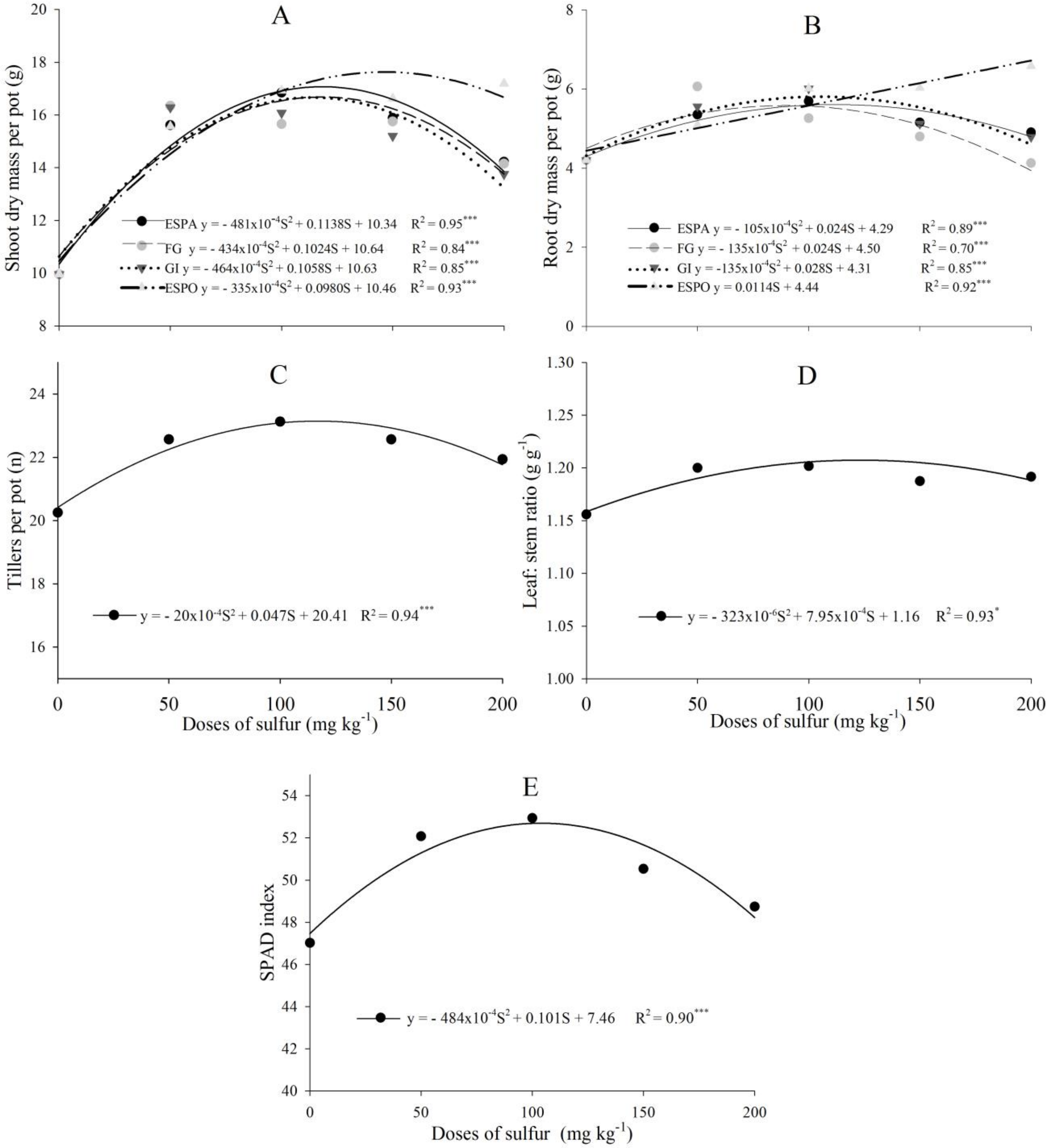
Biomass Production

Piatã grass SDM and RDM production were influenced by the interaction of sulfur dose and source in the soil. The SDM and RDM had a quadratic response to all S sources (Figure 1A and 1B) except for the ESPO source, which in the case of RDM was adjusted in the linear model.

Shoots dry mass was similar among the studied sources, ranging from 16.66 g pot^{-1} to 17.69 g pot^{-1}. However, the dose required for maximum production varied, with values of 112 mg kg^{-1} for GI, 118 mg kg^{-1} for GY and ESPA, and 146 mg kg^{-1} for ESPO.

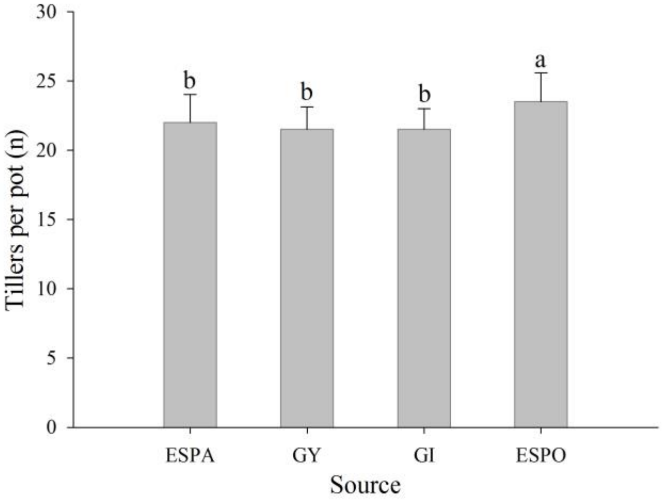
The number of tillers, the leaf:stem ratio, and the SPAD index were not influenced by interaction between sulfur dose and source (Figure 1C, 1D, and 1E). The variations in the responses of these variables were adjusted to the quadratic model as a function of sulfur dose. Maximum values of dry matter production for tillers (23.16 g pot^{-1}), for leaf blade (1.21 g pot^{-1}), and SPAD index (52.70) were obtained with the doses of 117 mg kg^{-1}, 123 mg kg^{-1}, and 104 mg kg^{-1}, respectively.

Figure 1. Shoot dry mass per pot (A), root dry mass per pot (B), tillers per pot (C), leaf:stem ratio (D), and SPAD index (E) of Piatã grass in response to the source (A and B) and dose (A-E) of sulfur. ESPA is elemental sulfur pastilles, GY is gypsum, GI is gypsite, and ESPO is elemental sulfur powder. $^{*}p < 0.05$; $^{**}p < 0.01$; $^{***}p < 0.001$.



An isolated effect of sulfur source was observed for the tillers. When ESPO was applied as the source, tiller number increased by 7.5 % relative to the mean of the ESPA, GY, and GI sources, which did not differ among one another (Figure 2).

Figure 2. Tillers per pot of Piatã grass fertilized with different sources of sulfur. ESPA is elemental sulfur pastilles, GY is gypsum, GI is gypsite, and ESPO is elemental sulfur powder. Bars are means and error bars are standard deviation (n = 4). Bars with the same letter do not differ significantly according to a Tukey test ($p < 0.05$).



Nutritional status

The interaction of sulfur doses and source was significant for nitrogen, potassium, sulfur, and the N:S ratio (Figure 3A, 3C, 3E, and 3F). Nitrogen and sulfur presented similar behavior. The ESPO, GY, and GI sources increased the concentration of N and S until the doses of 140 mg kg^{-1}. The increase in N concentration was on average 17 % in relation to control, respectively for ESPO, GY, and GI. For sulfur the increment was 96, 68, 75 % in relation to control, respectively for ESPO, GY, and GI.

Sulfur supplied in the pastilles elemental form (ESPA) induced a linear response, reaching increments of 10 % for N and 63 % for S in relation to control.

Potassium concentrations and N:S ratios showed a tendency to decrease with increasing sulfur doses. The reduction of potassium concentration occurred differently with different sulfur sources. The ESPO and GY sources were fit with a quadratic model and the K concentration was reduced 60 and 26 % at sulfur doses of 125 and 155 mg kg^{-1}, respectively. Potassium concentrations were analyzed with a linear model in the treatments with GI, ESPA, and GI for S sources, with a reduction of 18 and 59 %, respectively.

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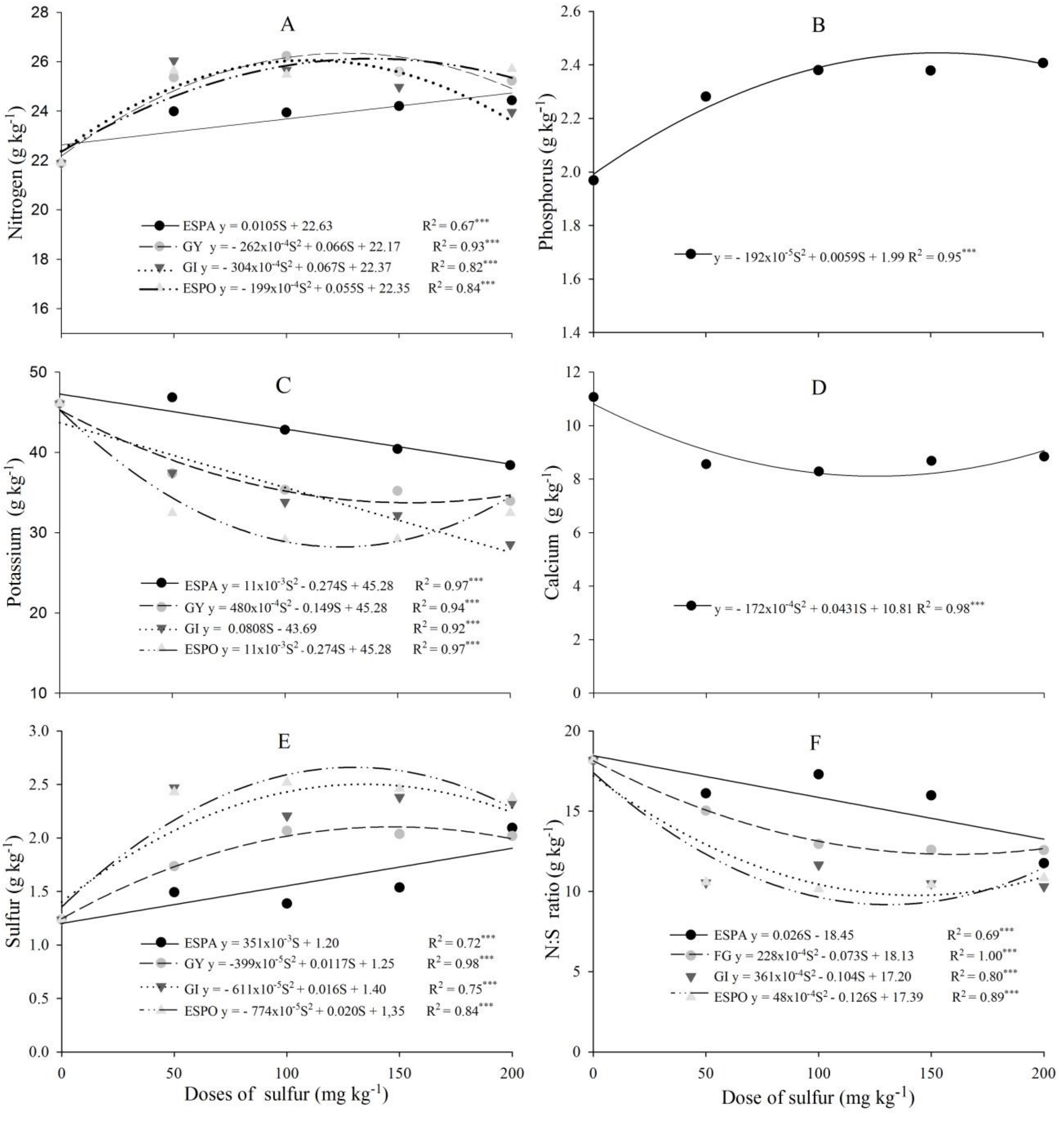
The N:S ratios associated with maximum Piatã grass shoot dry mass were 15.5:1 in the ESPA treatment, 13:1 in the gypsum treatment, 10:1 for the GI treatment, and 9.5:1 in the ESPO treatment.

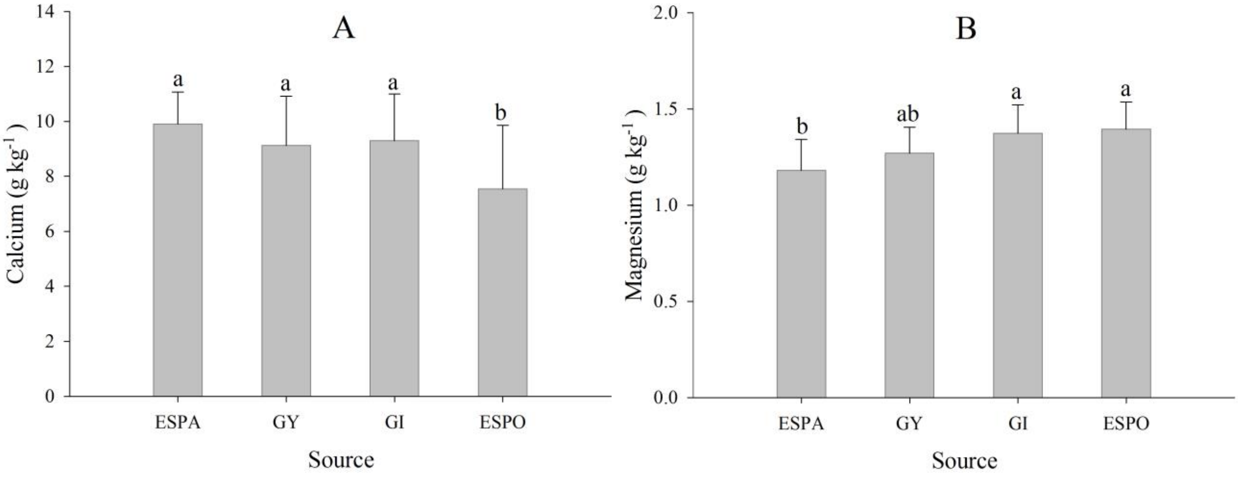
An isolated effect sulfur dose was observed for calcium and phosphorus concentrations (Figure 3B and 3D). Sulfur fertilization increased the P concentration up to the sulfur dose of 154 mg kg^{-1} and reduced the Ca concentration up to the sulfur dose of 125 mg kg^{-1}.

Figure 3. Nitrogen (A), phosphorus (B), potassium (C), calcium (D), and sulfur (E) concentrations, and N:S ratio (F) in shoot dry mass of Piatã grass in response to source and dose of sulfur. ESPA is elemental sulfur pastilles, GY is gypsum, GI is gypsite, and ESPO is elemental sulfur powder. $^{*}p < 0.05$; $^{**}p < 0.01$; $^{***}p < 0.001$.



The isolated effect of sulfur source was observed in SDM Ca and Mg concentrations (Figure 4A and 4B). EPSO reduced shoot calcium concentrations, while Ca in the shoots of plants in other source treatments did not differ. The lowest magnesium concentration was observed when the ESPA was applied and plants in this treatment had lower Mg than those in the GI and ESPO treatments and did not differ from the GY source.

Figure 4. Calcium (A) and magnesium (B) concentrations in forage of Piatã grass fertilized with different sources of sulfur. ESPA is elemental sulfur pastilles, GY is gypsum, GI is gypsite, and ESPO is elemental sulfur powder. Bars are means and error bars are standard deviations (n = 4). Bars with the same letter do not differ significantly according to a Tukey test ($p < 0.05$).



Discussion

Forage production

Sulfur deficiency in the control treatment (without S addition) limited the growth and development of Piatã grass (Figure 1). Addition of sulfur through fertilization optimized Piatã grass production, with maximum production achieved with similar doses (approximately 115 mg kg^{-1}) of gypsite (GI), gypsum (GY), and elemental sulphur pastilles (ESPA) sulfur sources, while maximum production was achieved with the elemental sulfur powder (ESPO) source at a dose of 150 mg kg^{-1}.

Piatã grass responded similarly to other forage species. The incremental effect observed here was also seen in Marandu grass by Lavres Júnior et al. (2008) and in Tanzania grass by Custódio et al. (2005) and Schmidt and Monteiro (2015). Increased soil sulfate availability results in greater production of dry mass of fodder and roots. The increase in production is due to the essential nature of sulfur containing proteins. Sulfur compounds have catalytic, regulatory, and structural functions, including functioning in physiological processes dependent on the Fe-S cluster-containing that include the metabolism of nitrogen and sulfur, photosynthesis, plant hormone synthesis, and gene expression regulation (CAPALDI et al., 2015; DING et al., 2016).

Maximum shoot and root dry mass production with the ESPO source was observed at greater sulfur doses relative to the other sources, especially the case for root production (35 % additional S was required when supplied as ESPO). Grant et al. (2012) and Sun et al. (2017) also reported this variation in production due to the physical and chemical form of sulfur fertilizer. Elemental sulfur is only taken up by plants after microbial oxidation. However, the effectiveness of elemental sulfur is affected by temperature, moisture, pH, and soil organic matter content due to their influence on the oxidation rate (BRAHIM et al., 2017; DEGRYSE et al., 2018).

Tillering is an important factor in pasture production and yield stability, and tillering was positively influenced by sulfur fertilization (Figure 1C). These results are in accordance with De Bona and Monteiro (2010b) who observed the greatest tillering increases when N was associated with S during cultivation of Marandu grass under nitrogen and sulphur fertilization. Schmidt and Monteiro (2015) reported that the number of tillers in Tanzania grass increased sharply with increasing S availability, reaching 120 and 170 % more tillers per pot at the first and second cut, respectively. The effects of sulfur app1lication on the three primary vegetative parameters evaluated (SDM, RDM, and tiller number; Figures 1A, 1B and 1C, respectively) are fundamental for optimizing the productivity of Piatã grass and assuring fast growth of the plants after cuts or grazes (HEINRICHS et al., 2013; SCHMIDT; MONTEIRO, 2015).

The increase of the leaf:stem ratio increments the supply of quality forage and the voluntary consumption by the animals, due to the greater number of leaves available, which has better nutritional content and palatability in relation to the stem. An increase in the proportion of leaves after sulfur application is associated with the protein synthesis, increasing growth, leaf expansion, and dry mass production. In addition, sulfur application reduces the time to appearance of new leaves (DE BONA; MONTEIRO, 2010b; SALVAGIOTTI; MIRALLES, 2008; SCHMIDT; MONTEIRO, 2015).

The SPAD index, besides indicating leaf chlorophyll concentrations, is also useful for quantifying the nutritional state of plants, mainly in relation to nitrogen and sulfur, which are linked to carbon metabolism. Increasing SPAD index is related to actions performed by sulfur-containing molecules during photosynthesis (PANDEY et al., 2009) and is associated with improving efficiency of the ferredoxin-thioredoxin system, which regulates the functioning of enzymes involved in carbon fixation in addition to several other chloroplast funtions (CRUSCIOL et al., 2013; LAVRES JÚNIOR et al., 2008; TAIZ et al., 2017).

Nutritional status of grass

Nitrogen concentrations in Piatã grass increased with sulfur fertilization (Figure 3A). This synergism may be associated with increased production of the S-containing amino acids cysteine and methionine, which are important for determining protein tertiary structure and protein synthesis, generating a greater demand for N in forage culture (CAPALDI et al., 2015). Moreover, sulfur favors the activity of the enzymes nitrate reductase and glutamine synthetase, which are part of the metabolic pathway of nitrogen uptake into amino acids (DE BONA; MONTEIRO, 2010a; GENG et al., 2016; SALVAGIOTTI; MIRALLES, 2008).

The phosphorus concentration in shoot dry matter (SDM) varied similarly to nitrogen (Figure 3B). However, there were no differences among sulfur sources. These results highlight the importance of these nutrients in metabolic processes associated with synthesis, allowing for greater growth of shoots and biomass production (CUSTÓDIO et al., 2005; CRUSCIOL et al., 2013). The increase in phosphorus concentration at higher doses of sulfur may be related to greater growth of the root system that favors exploration of a larger volume of soil and absorption of phosphorus since the transport of this nutrient in the soil occurs by diffusion (FAGERIA; MOREIRA, 2011).

Potassium and calcium concentrations were reduced in response to sulfur fertilization (Figure 3C and 3D). Possibly, greater Piatã grass biomass accumulation resulted in the dilution effect of these nutrients, since the oxidation process of elemental sulfur releases protons, acidifying the soil and reducing K and Ca availability (ARAUJO et al., 2015). The result of sulfur fertilization on shoot potassium and calcium concentrations are conflicting in the literature. Results obtained in this study agree with those observed by Custódio et al. (2005). In contrast, Heinrichs et al. (2013) found no significant effects of sulfur fertilization on K or Ca concentrations.

Plants had sulfur levels very near to the critical limit of deficiency in the absence of sulfur fertilization. Low sulfur concentrations are aggravated by successive cultivation, and without adequate replacement of this nutrient, progressive depletion of soil organic sulfur will occur (DEGRYSE et al., 2018; SCHMIDT; MONTEIRO, 2015). With increasing sulfur dosing there was an increase in shoot dry matter sulfur concentrationin. The maximum concentration were obtained with sulfur doses of 134 mg kg^{-1}, 135 mg kg^{-1}, 146 mg kg^{-1}, and 200 mg kg^{-1} for the sources GI, ESPO, GY, and ESPA, respectively.

Leaf S content in Pietã grass plants was lowest when the ESPA source was used. Sulfur content in leaves relative to dosing concentrations with ESPA was best fit with a linear function indicating that the doses tested were not sufficiently high to determine a maximum point. Further, this response reveals the importance of fertilizer granulometry to the availability of this nutrient. The largest fertilizer particles sizes have reduced surface area in contact with the soil surface, reduced reaction rate and nutrient liberation, and ultimately reduced nutrient absorption throughout the cropping cycle (DEGRYSE et al., 2018).

In the absence of sulfur fertilization, the N:S ratio in shoot dry matter of Piatã grass was high, approximatly 18:1, which indicates a slight metabolic imbalance (Figure 3F). In treatments with the highest SDM, the N:S ratio ranged from 10:1 to 15:1. According to De Bona and Monteiro (2010b), an N:S ratio of 14:1 indicates an adequate nutritional status for plants in the Poaceae family (Gramineae). Crusciol et al. (2013) report that the insufficient sulfur fertilization results in an imbalance of N:S and P:S ratios. A high N:S ratio leads to accumulation of nitrogen in non-protein form, mainly N-NO_3^- (nitrate) and soluble organic nitrogen (KUMAR; SINGH, 1980), which results in reduced the plant growth and lower nutritional quality of the pastures.

Conclusions

Low soil sulfur supply reduced the growth of Piatã grass (*Urochloa brizantha*) regardless of the sulfur source.

Shoot and root dry matter production varied with the source and dose of sulfur, however, these did not influence the leaf:stem ratio or SPAD index.

Piatã grass reached maximum production with sulfur doses of 112 mg kg^{-1} for GI, 118 mg kg^{-1} for GY and ESPA, and 146 mg kg^{-1} for ESPO.

The greatest RDM and tiller number increments were obtained with the ESPO sulfur source.

Macronutrient concentrations in shoot dry mass tissue were in the order K > N > Ca > P > S > Mg

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