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Growth, nutrition and production of dry matter of Kikuyu Grass (*Brachiaria humidicula*) as a function of Mn-fertilizer

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Abstract

Manganese (Mn) is important to increase forage crop yields. However, there is little information regarding the adequate Mn-fertilizer rates for *Brachiaria humidicula* species. The objective of this research was to evaluate the effect of manganese on growth, nutrition and yield of *Brachiaria humidicula*. The study was carried out in a green house in a randomized block design with five rates of manganese (0, 15, 30, 60 and 120 mg dm^{-3}) and four replicates. Were evaluated plant height, leaf area, relative chlorophyll index, dry matter production, manganese accumulation and content, besides absorption efficiencies and transport and use of manganese (Mn). *Brachiaria humidicula* showed high tolerance to this nutrient, because the application of only 120 mg dm^{-3} to the soil was phytotoxic, showing symptoms such as brown spots and leaf tip curling. Manganese applied at a dose of 120 mg dm^{-3} reduced aerial part biomass yield by 25% and promoted lower efficiency of use of this nutrient by the forage by 49%. However, even with the initial content of manganese in the soil considered sufficient to meet nutritional demands to achieving high yields, the application of 60 mg dm^{-3} of manganese to the soil is recommended.

Keywords: Micronutrients; pastures; phytotoxicity; fertilization; plant nutrition.
Abbreviations: AB_{ef}_Absorption efficiency; DM_Dry matter; LA_Leaf area; Mn_Manganese; RCI_Relative chlorophyll index; TR_{ef}_Transport efficiency; UT_{ef}_Utilization efficiency.

Introduction

The genus *Brachiaria* (Syn. *Urochloa*) covers fodder plants grown in large areas of the world livestock. Among these stands *Brachiaria humidicula* (Vilela et al., 2007), characterized by having decumbent habit, fast rooting and vigorous growth (Martins et al., 2013). *Brachiaria humidicula* has expanded largely in the South American humid tropics because of its high ability to adapt to naturally acid and low fertility soils (Martins et al., 2013). However, fertilization is recommended in either single crop or in consortium with forage legumes systems aimed at high yields (Vilela et al., 2007). The big challenge is that the rates of some nutrients have not yet been properly established for *Brachiaria humidicula*, especially manganese, the second most required micronutrient by this species (Malavolta, 2006). Still, in some areas preventive applications have been recommended without prior knowledge of the level of Mn present in the soil and its nutritional requirement for the crop of interest, making the supply insufficient or excessive (Schmidt et al., 2013), and causing nutritional imbalances.

Mn is essential for plant growth and development, acting as cofactor for enzyme activation in processes such as photosynthesis, biosynthesis of lipids and oxidative stress (Malavolta, 2006; Prado, 2008; Marschner, 2012; Socha and Guerinot, 2014; Yasuor et al., 2015). An essential-transition heavy metal, its low level can cause nutritional deficiencies, and high levels can cause phytotoxicity (Ducic and Polle, 2005). Several factors can reduce the soil manganese content availability, such as pH higher than 6.5 (Ducic and Polle, 2005) and high levels of soil organic matter (Prado, 2008). Besides that, favorable conditions for increased bioavailability of nutrients in the soil as well as low oxygen supply (reduced atmosphere), acid pH (<5.0) or excess fertilization can cause Mn toxicity (Malavolta, 2006; Dechen and Nachtigall, 2007; Marschner, 2012; Millaleo et al., 2013; Saidi et al., 2014). Research in tropical regions have reported the importance of manganese in forage, such as *Panicum maximum* (Mingotte et al., 2011; Sylvestre et al., 2012) and *Brachiaria brizantha* (Puga et al., 2011; Guirra et al., 2011). However, the relationship between nutritional status and production of *Brachiaria humidicula* species has not been investigated yet. Studies with livestock have also reported the importance of adding adequate levels of micronutrients to the diet of cattle. Diets deficient in Mn may interfere with fertility (Carvalho et al., 2010), while its excess can cause poisoning and affect the reproductive performance (Reis et al., 2014). Given the importance of establishing appropriate levels of manganese in forage species used in livestock worldwide, highlighting *Brachiaria* (Syn. *Urochloa*) genus, the objective of this study was to evaluate the effect of

manganese fertilization on growth, nutrition and production of *Brachiaria humidicula*.

Results and Discussion

Plant height, leaf area and relative chlorophyll index

The application of manganese to the soil affected plant height at the first and second cut (Table 1). There was quadratic adjustment, being 22.91 cm the maximum height obtained at the first cut with 68.79 mg dm^{-3} Mn, and 17.41 cm the maximum obtained with the rate of 63.91 mg dm^{-3} Mn at the second cut, respectively (Fig 1). Increases in forage height were approximately 77 and 35% with the rates of 69 and 64 mg dm^{-3} Mn in the first and second cut, respectively. The highest Mn levels were observed in the plant growth sites, since Mn is concentrated mainly in meristematic tissues (Vitti et al., 2006). As the redistribution of Mn by the phloem is limited (Prado, 2008; Marschner, 2012, Schmidt et al., 2013), the responses of the forage to Mn applications occurs primarily in plant height. Manganese rates did not affect leaf area at the first cut, averaging 64.65 cm^2 (Fig 2) but affected leaf area at the second cut, reaching 121.57 cm^2 with 51.70 mg dm^{-3}, an increase of approximately 17% compared to the initial value of 103.93 cm^2 (Fig 2). The increase in Mn uptake due to higher availability of the nutrient in the soil increases the synthesis of nonstructural carbohydrates (Marschner, 2012) and, consequently, the synthesis of lignin, resulting in perennial leaves (Doncheva et al., 2009) and increased leaf area. However, excess of Mn is detrimental to plants and may reduce leaf biomass (Marschner, 2012; Saidi et al., 2014) by chlorophyll degradation (Papadakis et al., 2007) with consequent low carboxylation efficiency (Millaleo et al., 2013). Manganese rates affected relative chlorophyll index (RCI) at the first and second cuts, with quadratic adjustment (Fig 3). The highest RCI values obtained were 30.69 and 34.12 µg cm^{-2} using 62.36 and 51.84 mg dm^{-3} Mn at the first and second cuts, respectively (Fig 3). Manganese interferes with the structure of the thylakoids in the chloroplasts (Vitti et al., 2006) increasing plant cells photosynthetic performance (Schmidt et al., 2013) and, as a result, increasing RCI, as observed in this study. However, the provision of 120 mg dm^{-3} of manganese reduced RCI in 11.92 and 29.94%, when compared to the control at the first and second cuts, respectively (Fig 3). The reduction in RCI occurs because high Mn reduces the chlorophyll biosynthesis (Wang et al., 2009) and induces reactions with oxygen, which can damage cellular macromolecules (Papadakis et al., 2007) and affect the operation of photosystem II in the photosynthesis photochemical phase (Millaleo et al., 2013).

Mn content in roots and aerial part

There was an increase in the Mn content in leaves and roots with increasing supply of this nutrient to the soil (Table 2). The nutrient content was 517, 676 and 381 mg kg^{-1} in the aerial part at the first and second cuts, and in the roots at the second cut, respectively, at the highest rate (120 mg dm^{-3}) (Fig 4). Increases in manganese contents in plants were approximately 97, 91 and 105% in the aerial part at the first and second cuts, and in roots at the second cut regarding the initial content of 263, 354 and 186 mg kg^{-1}, respectively.
Research results on *Brachiaria brizantha* plants also showed higher concentrations of Mn at the second production cut (Guirra et al., 2011). This fact can be attributed to the greater root growth at the second cut from the first, enabling exploration of greater volume of soil and favoring higher absorption of nutrients at the second cut (Sylvestre et al., 2012), especially those nutrients that make contact between ion and root via root interception, such as Mn (Prado, 2008). Passive absorption of Mn may occur due to high concentrations of this nutrient (Dechen and Nachtigall, 2007), flowing free in the xylem (Yasuor et al., 2015) up to the aerial part. Some papers claim that increments of Mn in the aerial part from the first to the second cut may occur due to decreased pH caused by nitrogen topdressing (Sylvestre et al., 2012) with ammonia-based sources (Ducic and Polle, 2005). This decrease in pH is due to the ammonium nitrate transformation process (Cantarella et al., 2007), which raises the Mn concentration in the soil solution (Dechenhohle and Nachtigall, 2007). It is noteworthy that in this study 150 mg dm^{-3} of N as urea were applied at sowing (100 mg dm^{-3}) and the rest (50 mg dm^{-3}) 30 days after.

Dry matter production

Mn affected dry matter (DM) production at the first and second cut (Table 3). There was a quadratic adjustment for aerial part DM production. Dry matter from aerial parts obtained at the first and second cut was 4.78 and 4.30 g $plant^{-1}$ using 69.67 and 62.50 mg dm^{-3} Mn, respectively (Fig 5). Similarly, the highest production of DM by roots and aerial part and total DM in the second cut were 2.59 and 9.07 g $plant^{-1}$ with the use of 67.25 and 66.08 mg dm^{-3} of Mn, respectively (Fig 5). These increases in DM in all variables represent increments of approximately 44, 37, 54 and 41% in the aerial part at the first and second cuts, in roots at the second cut, and in the total DM production in the aerial part over the production without the application of manganese to the soil, respectively. Increases in DM production of aerial parts, roots and total DM can be attributed to increases in the atmospheric CO_2 assimilation capacity and consequent increases in the photosynthetic rate due to higher absorption of Mn by the forage (Malavolta, 2006; Millaleo et al., 2013).
At the first cut, plant height values were positively correlated with dry matter production of aerial part and total dry matter ($r = 0.76^{**}$ and $r = 0.75^{**}$), respectively. While at the second cut leaf area was positively correlated with aerial part dry matter production and total dry matter ($r = 0.64^{**}$ and $r = 0.63^{**}$), respectively. At the second cut relative chlorophyll index was positively correlated with root DM and aerial part total DM ($r = 0.70^{**}$ and $r = 0.60^{**}$), respectively. In a similar study, Puga et al. (2011) evaluated the effect of Mn fertilizer rates on the production of *Brachiaria brizantha* and observed that the application of 120 mg dm^{-3} also favored Mn accumulation in the leaves, without losses in DM production due to toxicity. However, in the present study the same rate promoted decreases of 28, 22, 40, and 25% in DM accumulation in the aerial part, roots, and in aerial part total DM at the first and second cut, respectively, in relation to the rates that promoted maximum production. The decrease in DM production by Mn excess occurs due to higher chlorophyll degradation (Papadakis et al., 2007; Wang et al., 2009), because the lower the chlorophyll biosynthesis the lower the net photosynthesis and the carboxylation efficiency (Millaleo et al., 2013), and consequently, there is a decrease in the biosynthesis of carbohydrates (Mingotte et al., 2011), affecting root growth and dry matter yield (Malavolta, 2006; Marschner, 2012; Saidi et al., 2014).

Mn accumulation in roots and aerial part

Mn applications affected the accumulation of this nutrient in aerial parts in the first cut, and in aerial parts and roots in the

Table 1. Height, leaf area (LA) and relative chlorophyll index (RCI) of *Brachiaria humidicula*, according to the application of manganese in the soil.

Rates of Manganese	First cut			Second cut		
	Height	LA	RCI	Height	LA	RCI
mg dm^{-3}	cm	cm^2	µg cm^{-2}	cm	cm^2	µg cm^{-2}
0	13.55	56.01	26.48	13.73	95.50	28.98
15	15.84	67.61	28.32	14.34	117.13	29.78
30	19.95	73.72	29.22	14.71	133.17	33.43
60	22.95	67.03	31.07	18.47	108.11	34.39
120	17.04	57.38	27.66	13.07	92.90	24.09
F test	7.20**	0.51^{ns}	1.43^{ns}	6.02**	4.92**	3.74*
C.V. (%)	15.28	32.60	10.13	11.55	13.62	14.03
^{1}LR	3.38^{ns}	0.12^{ns}	0.19^{ns}	0.02^{ns}	2.98^{ns}	3.52^{ns}
^{2}QR	24.64**	1.34^{ns}	5.41*	18.15**	8.04*	10.87**

$^{(1)}$Linear regression; $^{(2)}$Quadratic Regression; $^{n.s.}$, *, ** – not significant at the 5%; significant at the 5% and significant at the 1% level probability by the F test, respectively.

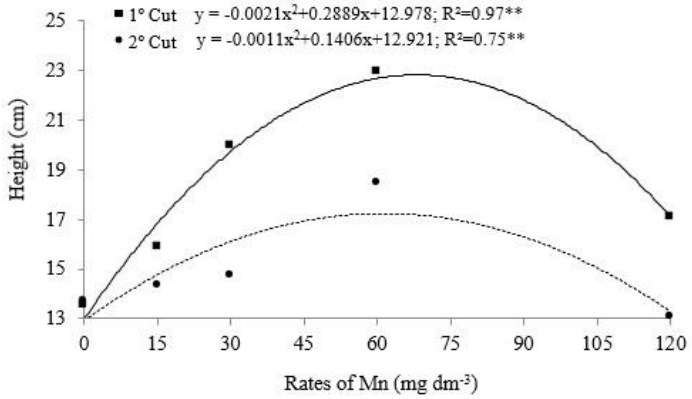

Fig 1. Height (cm) of *Brachiaria humidicula* in the first and second cut forage, according to the application of manganese in the soil. ** - significant at the 1% level probability by the F test.

Table 2. Manganese content in plants of *Brachiaria humidicula* in the aerial part at the first and second cuts and the second cut roots, according to the application of manganese in the soil.

Rates of	First cut	Second cut	
Manganese	Aerial Part	Aerial Part	Roots
mg dm^{-3}	---------- mg kg^{-1}----------		
0	247	328	173
15	294	391	211
30	330	462	238
60	416	531	303
120	502	662	370
F test	15.75**	4.49*	15.79**
C.V. (%)	13.42	25.73	15.16
^{1}LR	61.65**	17.46**	61.35**
^{2}QR	0.83^{ns}	0.42^{ns}	1.70^{ns}

$^{(1)}$Linear regression; $^{(2)}$Quadratic Regression; $^{n.s.}$, *, ** – not significant at the 5%; significant at the 5% and significant at the 1% level probability by the F test, respectively.

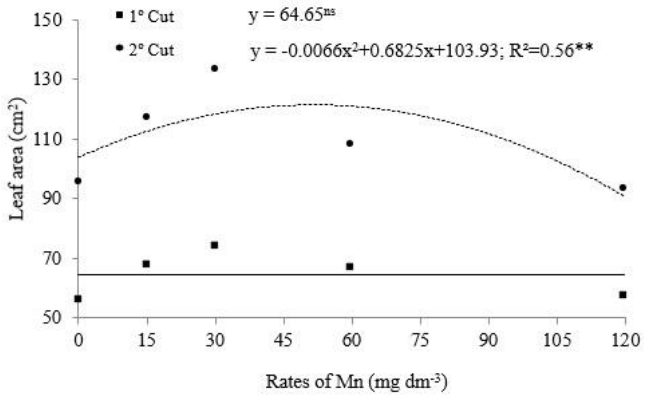

Fig 2. Leaf area of *Brachiaria humidicula* in the first and second cut forage, according to the application of manganese in the soil. ^{ns}, ** - not significant at the 5%; significant at the 1% level probability by the F test, respectively.

Table 3. Dry matter production in plants of *Brachiaria humidicula* in the aerial part at the first and second cuts, in the roots of the second cut and total aerial parts forage, according to the application of manganese in the soil.

Rates of Manganese	First cut	Second cut		Total
	Dry matter Aerial Part	Dry matter Aerial Part	Dry matter roots	Dry matter Aerial Part
mg dm^{-3}	---------- g plant^{-1} ----------			
0	3.22	3.00	1.67	6.22
15	3.75	3.60	1.90	7.35
30	4.77	4.35	2.57	9.12
60	4.27	4.02	2.30	8.29
120	3.42	3.32	1.55	6.74
F test	15.67**	14.45**	11.72**	19.31**
C.V. (%)	8.24	7.74	12.55	7.05
^{1}LR	0.29^{ns}	0.01^{ns}	2.89^{ns}	0.07^{ns}
^{2}QR	47.43**	46.55**	35.82**	60.44**

$^{(1)}$Linear regression; $^{(2)}$Quadratic Regression; $^{n.s.}$, *, ** – not significant at the 5%; significant at the 5% and significant at the 1% level probability by the F test, respectively.

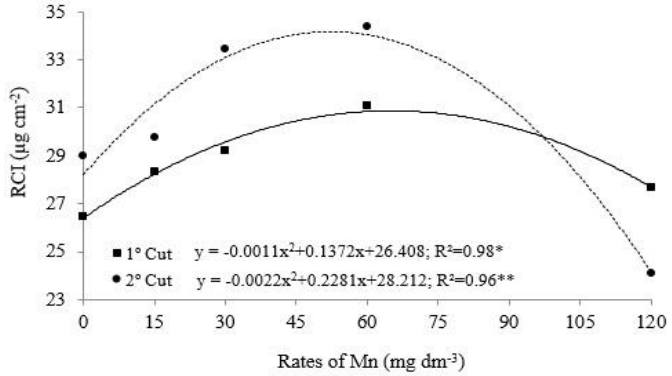

Fig 3. Relative chlorophyll index (RCI) of *Brachiaria humidicula* in the first and second cut forage, according to the application of manganese in the soil. *, ** - significant at the 1% and significant at the 5% level of probability by the F test, respectively.

Table 4. Manganese accumulation in plants of *Brachiaria humidicula* in the aerial part at the first and second cuts, in the roots of the second cut and total aerial part forage, according to the application of manganese in the soil.

Rates of Manganese	First cut	Second cut		Total
	Aerial Part	Aerial Part	Roots	Aerial Part
mg dm^{-3}	---------- mg plant^{-1} ----------			
0	0.86	0.98	0.29	1.84
15	1.10	1.39	0.40	2.49
30	1.57	2.02	0.61	3.59
60	1.76	2.14	0.71	3.90
120	1.71	2.18	0.57	3.89
F Test	25.05**	5.25**	8.35**	11.87**
C.V. (%)	11.39	26.74	22.61	17.20
^{1}LR	61.26**	12.96**	11.85**	29.29**
^{2}QR	35.57**	6.80*	20.43**	16.06**

$^{(1)}$Linear regression; $^{(2)}$Quadratic Regression; $^{n.s.}$, *, ** – not significant at the 5%; significant at the 5% and significant at the 1% level probability by the F test, respectively.

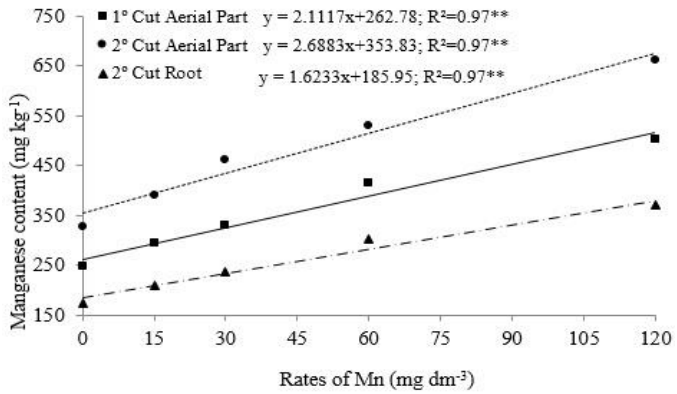

Fig 4. Manganese content in plants of *Brachiaria humidicula* in the aerial part at the first and second cuts, and second cut roots, according to the application of manganese in the soil. ** - significant at the 1% level probability by the F test.

Table 4. Manganese accumulation in plants of *Brachiaria humidicula* in the aerial part at the first and second cuts, in the roots of the second cut and total aerial part forage, according to the application of manganese in the soil.

Rates of Manganese	First cut	Second cut		Total
	Aerial Part	Aerial Part	Roots	Aerial Part
mg dm^{-3}		---------- mg plant^{-1} ----------		
0	0.86	0.98	0.29	1.84
15	1.10	1.39	0.40	2.49
30	1.57	2.02	0.61	3.59
60	1.76	2.14	0.71	3.90
120	1.71	2.18	0.57	3.89
F Test	25.05**	5.25**	8.35**	11.87**
C.V. (%)	11.39	26.74	22.61	17.20
^{1}LR	61.26**	12.96**	11.85**	29.29**
^{2}QR	35.57**	6.80*	20.43**	16.06**

$^{(1)}$Linear regression; $^{(2)}$Quadratic Regression; $^{n.s.}$, *, ** – not significant at the 5%; significant at the 5% and significant at the 1% level probability by the F test, respectively.

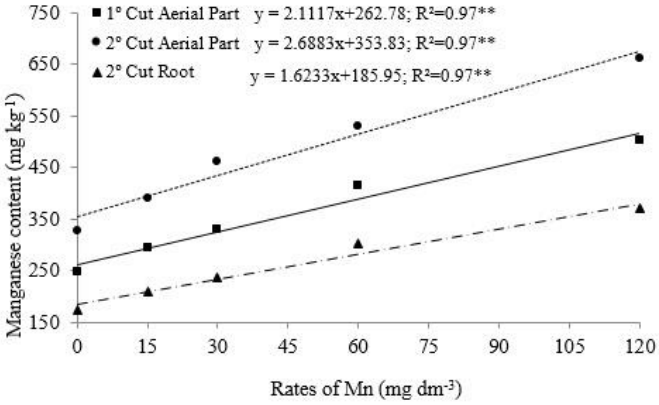

Fig 4. Manganese content in plants of *Brachiaria humidicula* in the aerial part at the first and second cuts, and second cut roots, according to the application of manganese in the soil. ** - significant at the 1% level probability by the F test.

second cut (Table 4). Note that after the regression analysis, there was a quadratic adjustment, and the maximum accumulation of manganese in the aerial part was 2.39 and 2.31 mg plant^{-1}, using 120 and 80.50 mg dm^{-3} Mn, at the first and second cut, respectively (Fig 6). Similarly, the maximum accumulation of Mn in the roots in the second cut and in the total dry matter of the aerial part were 0.75 and 4.58 mg plant^{-1}, using 76.88 and 95.17 mg Mn dm^{-3}, respectively (Fig 6). This increase in manganese accumulation observed in all evaluations represents an increase of approximately 83, 128, 171 and 146% for the aerial part at the first and second cut, for the roots in the second cut and for the total Mn accumulation in the aerial part over the production, without the application of manganese to the soil, respectively. Note also that the accumulation of Mn in the roots, at the first and second cut were positively correlated with the Mn contents in the roots at the first and second cut (r = 0.72**, r = 0.89** and r = 0.82**), respectively.

Efficiency indices: Absorption, transport and utilization

Transport efficiency (TR_{ef}), which measures the amount of nutrient transported in relation to what was absorbed was not affected by manganese (Table 5) and averaged 85.7%. However, absorption efficiency (AB_{ef}) and utilization efficiency (UT_{ef}) were affected, presenting linear adjustments at the 1% level of probability with the application of manganese (Table 5). Note that there was an increase of 128% in TR_{ef} compared to the control treatment at the highest rate, reaching 2.89 mg g^{-1} (Fig 7). However, there was 49% reduction in the manganese UT_{ef}, as a function of the application of manganese (Fig 7) reaching 16.73 mg g^{-1} at the highest rate. AB_{ef} and UT_{ef} showed negative correlation (r = -0.86**), namely, the disadvantage of an increase in the efficiency of the other, and vice versa. However, there was a positive correlation of AB_{ef} with the levels of Mn in roots and aerial part at the first and second cut (r = 0.72** r = 0.84** r = 0.88**), respectively. The increase in Mn AB_{ef} in the soil by *Brachiaria humidicula* plants demonstrate the occurrence of conditions favorable for the absorption of this nutrient from the soil solution, such as reduced organic matter content and low soil pH (Ducic and Poplle, 2005; Dechen and Nachtigall, 2007; Prado, 2008; Marschner, 2012). Note, though, that Mn UT_{ef} for the forage was negatively correlated with the Mn content in the roots and aerial part at the first and second cut (r = -0.58**, r = -0.78** and r = -0.84**), respectively. The reduction of manganese UT_{ef} by plants may be associated with a plant defense mechanism due to the excess of Mn in the soil solution (Hernandes et al., 2010). Several plant Mn excess detoxification mechanisms are based on the reduction of the free metal concentration in the cells cytoplasm, such as storage in the vacuoles (Peiter et al., 2007) and restriction of Mn uptake by roots, reducing the ascent through the xylem by Mn sequestration by the apoplast (Ducic and Poplle, 2005; Socha and Guerinot, 2014). Still, there may be segregation of Mn together with water and minerals through hydathodes (Peiter et al., 2007). However, this could not be confirmed in this study because the highest contents and accumulations of manganese occurred in the aerial part of *Brachiaria humidicula* compared to the roots (Figs 4 and 6). Symptoms of Mn deficiency were not observed without the application of Mn-

Table 5. Absorption efficiency, transport efficiency and utilization efficiency of manganese in plants of *Brachiaria humidicula*, according to the application of manganese in the soil.

Rates of Manganese	Absorption efficiency	Transport efficiency	Utilization efficiency
mg dm^{-3}	mg g^{-1}	%	mg g^{-1}
0	1.27	86.12	29.54
15	1.52	85.90	30.19
30	1.65	85.09	33.12
60	2.03	84.27	24.71
120	2.91	87.10	15.63
F test	17.21**	0.28^{ns}	8.54**
C.V. (%)	16.39	4.69	17.61
^{1}LR	68.60**	0.13^{ns}	28.54**
^{2}QR	0.11^{ns}	0.93^{ns}	2.44^{ns}

$^{(1)}$Linear regression; $^{(2)}$Quadratic Regression; $^{n.s.}$, *, ** – not significant at the 5%; significant at the 5% and significant at the 1% level probability by the F test, respectively.

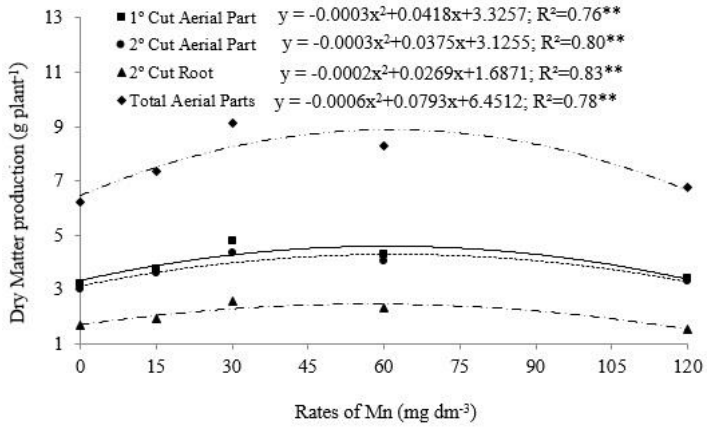

Fig 5. Dry matter production in plants of *Brachiaria humidicula* in the aerial part at the first and second cuts, in the roots of the second cut and total aerial part forage, according to the application of manganese in the soil. ** - significant at the 1% level probability by the F test.

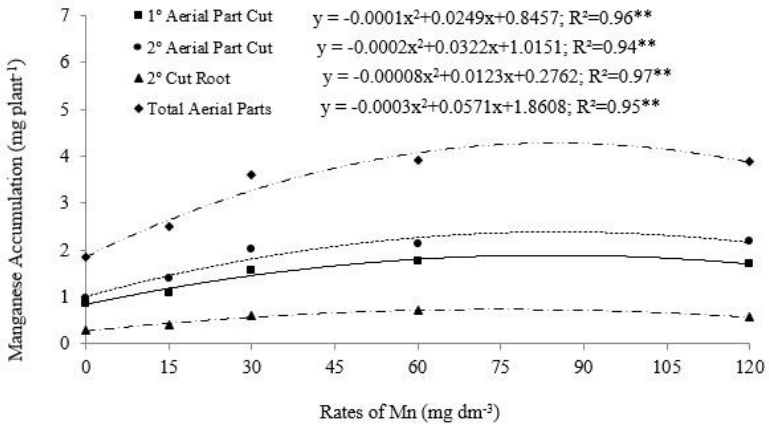

Fig 6. Manganese accumulation in plants of *Brachiaria humidicula* in the aerial part at the first and second cuts, in the roots of the second cut and total aerial part forage, according to the application of manganese in the soil. ** - significant at the 1% level probability by the F test.

fertilizer, which could be related to the initial amount present in the soil (44 mg dm^{-3}) considered adequate, according to Raij (2011). For this author, Mn contents in the soil greater than 5.0 mg dm^{-3} are high; therefore, sufficient to meet the demand of the crop. Still, Marschner (2012) states that foliar Mn contents (> 30 mg kg^{-1}) are adequate. In this study, the leaf contents obtained were 247 mg kg^{-1} in the absence of Mn. At the first cut, when the rate applied was 120 mg dm^{-3}, a reduction in plant growth was observed, noted earlier in plant height and in leaf area, without toxicity symptoms. However, at the second cut, serious nutritional disorder symptoms were observed, such as brown spot, low plant growth (Socha and Guerinot, 2014) and leaf tip curling (Malavolta, 2006; Prado, 2008), as well as severe reduction in growth and biomass (Marschner, 2012). The presence of visual symptoms of toxicity demonstrates that plants have already suffered some damage at cellular and tissue levels, showing low tolerance to Mn excess, an indication that this plant species has a low efficiency defense mechanism for the metabolic control of high levels of Mn. As information on proper Mn supply is scarce, specifically for *Brachiaria humidicula*, we can assume that the rate of 120 mg dm^{-3} is

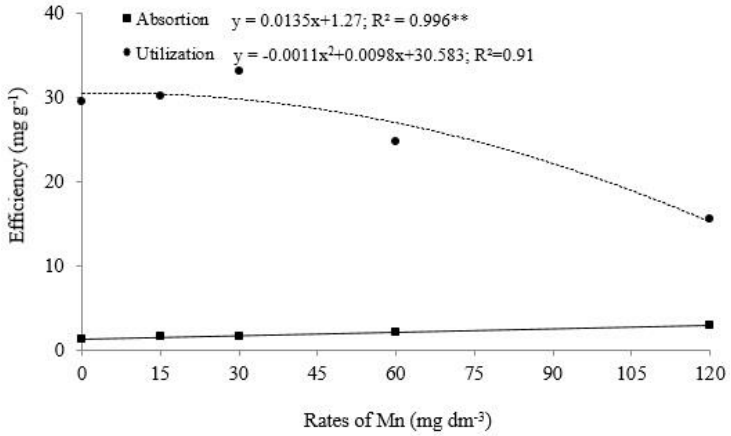

Fig 7. Absorption efficiency and utilization of manganese in plants of *Brachiaria humidicula*, according to the application of manganese in the soil. ** - significant at the 1% level probability by the F test.

extremely toxic to this plant species under this study conditions. Therefore, one should be careful with soil management and excessive Mn fertilization, because the high absorption of this element can promote phytotoxicity, reducing growth and yield.

Materials and Methods

Growing conditions

This research was conducted in a greenhouse at Federal University of Goiás, State of Goiás, Brazil, coordinates: 16° 35" latitude south and 49° 21' longitude west, at approximately 730 m of altitude and 1,600 mm average annual rainfall. The climate is Aw (mega thermal) or tropical savannah, with dry winters and rainy summers, according to Köppen classification. The soil analysis showed the following properties: pH = 5.0; Organic matter = 2.0 g dm^{-3}; P = 5.5 mg dm^{-3}; K = 60 mg dm^{-3}; Ca = 2.7 $mmol_c$ dm^{-3}; Mg = 0.5 $mmol_c$ dm^{-3}; B = 0.21 mg dm^{-3}; Cu = 2.8 mg dm^{-3}; Fe = 82 mg dm^{-3}; Mn = 44 mg dm^{-3}; Zn = 4.6 mg dm^{-3}; H+Al = 1.8 $mmol_c$ dm^{-3}; CEC = 5.2 $mmol_c$ dm^{-3}; Base saturation (%) = 65.1%, with 432 g kg^{-1} of clay.

Treatments and experimental design

Treatments were 0 (control), 15, 30, 60 and 120 mg dm^{-3} of Mn as manganese sulfate (35.5% Mn), arranged in an entirely randomized bloc design, with four replicates. Each experimental unit consisted of one 4 dm^3 pot, filled with 3.5 dm^3 of a clayey dystrophic red Oxisol (Embrapa, 2013), drawn from the topsoil layer (0-0,2m deep).

Treatments application and analysis

Liming was performed on August 2, 2014, using calcined lime (CaO = 58.5%; MgO = 9%; NP = 127%; RPTN = 99.4%), to reach base saturation (V%) equal to 80%, while maintaining the moist soil mass at 60% retention capacity, and incubated for 30 days. After the incubation period a fertilizer solution was applied to the soil with the following rates of micronutrients: 1.5 mg dm^{-3} of Cu ($CuSO_4.5H_2O$ p.a.); 0.8 mg dm^{-3} of B (H_3BO_3 p.a.); 0.15 mg dm^{-3} of Mo ($NaMoO_4.2H_2O$ p.a.); 4.0 mg dm^{-3} of Fe [Fe_2 $(SO_4)_3$. $4H_2O$ p.a.] and 5.0 mg dm^{-3} of Zn ($ZnSO_4$ p.a.) (Mesquita et al., 2004). The following rates of macronutrients were also applied: 305 mg dm^{-3} of P as single superphosphate (Mesquita et al., 2004); 150 mg dm^{-3} of N as urea applied at sowing (100 mg dm^{-3} of N) and the remaining (50 mg dm^{-3} of N) at 30 days after, according to Mesquita et al. (2004); and 200 mg dm^{-3} of K (KCl p.a.) (Bonfim et al., 2004). Treatments (Mn rates) were applied to the soil surface and incorporated 10 cm deep at seedling emergence.

Traits measured and nutritional indices

Sowing has held on September 4, 2014, and thinning performed 10 days after emergence, leaving five plants per pot and irrigation done with deionized water by the weighing method, keeping soil moisture content at 60% retention capacity. Plants were evaluated daily for symptoms of nutritional disorder. Two cuts were performed: the first at 60 days after sowing (DAS) and the second at 94 DAS. At 60 DAS and 94 DAS the plant´s height was recorded by measuring the largest tiller from the base to the last leaf insertion, and the relative chlorophyll index (RCI) obtained from five leaves per experimental unit with the help of the OPTI-Sciences® device, model CM-200, and leaf area with the LI-3100 Area Meter device. Plant tissue samples were washed with a 0.1% detergent solution, a 0.3% acid solution and distilled water, and dried in oven at 65°C for 48 hours for aerial part and root dry mass determinations (second cut, only). The manganese contents from aerial part and root plant tissues were determined following methodology described by Battaglia et al. (1983).

From the dry matter and content of nutrients in plants data were performed the calculation of the nutritional indices comprising absorption efficiency (AB_{ef}), translocation efficiency/transport (TR_{ef}) and efficiency of use of nutrients for conversion to dry matter (UT_{ef}) (Prado, 2008). The calculation of these indices is below:

$$AB_{ef} = \frac{total\ nutrient\ conten\ in\ plant}{\text{root dry matter}}$$

(Swiader et al., 1994).

$$TR_{ef} = \frac{nutrient\ conten\ in\ paerial\ part}{total\ nutrient\ conten\ in\ the\ plant}$$

(Li et al., 1991).

$$UT_{ef} = \frac{(total\ dry\ matter\ produced)^2}{total\ nutrient\ conten\ in\ the\ plant}$$

(Siddiqi and Glass, 1981).

Statistical analysis

Results were subjected to the analysis of variance using software Sisvar Inc., Brazil (Ferreira, 2008) and to polynomial regression analysis. Linear and quadratic

mathematical models were tested to select the one that provided the best data adjustment, based on the magnitude of the regression coefficients significance at 5% probability by the t test. The maximum points were calculated by deriving the significant equations. Variables were correlated by the Pearson linear correlation test (Sigma-plot In., USA), considering the correlation significances ($p \leq 0.01$ and 0.05).

Conclusions

Brachiaria humidicula has high tolerance to manganese, because with the application of only 120 mg dm^{-3} of the micronutrient to the soil, plants presented phytotoxicity symptoms such as brown spots and curling of the tips leaves. Manganese application at a dose of 120 mg dm^{-3} decreases aerial part biomass yield by 25% and decreases the efficiency use of this nutrient by 49%. However, even with the initial content of manganese in the soil considered sufficient to meet nutritional demands aiming at high yields, the application of up to 60 mg dm^{-3} of manganese to the soil is recommended.

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