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BIOMASS PRODUCTION, B ACCUMULATION AND Ca/B RATIO IN *Eucalyptus* UNDER VARIOUS CONDITIONS OF WATER AVAILABILITY AND B DOSES

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ABSTRACT: Soils destined for forest crops are often limiting with respect to boron (B) and in several eucalyptus crops boron deficiency has been found to be a common occurrence. The objective of this study is to evaluate biomass production, B accumulation and Ca/B ratio in eucalyptus cultivated under different conditions of soil, water availability and doses of B. To that end, an experiment was conducted using a completely randomized design with a 5 x 2 x 2 factorial arrangement consisting of five doses of B (0.00, 0.25, 0.75, 2.25 and 6.25 mg kg^{-1}), two types of soil, namely a Dark Red Latosol (LE) and a Red-Yellow Latosol (LV), and two water tension levels (-0.033 and -0.010 MPa), with four replicates. After plants were grown, determinations were made of Ca and B contents as well as Ca/B ratios in different portions of the plants. Accumulation of B was obtained from the ratio of its contents to dry biomass in each plant portion. It was found that, under natural conditions, both Dark Red Latosol and Red-Yellow Latosol fail to meet the nutritional requirements for B in eucalyptus, potentially being limiting to that species. Overall, soil moisture influenced dry biomass production and accumulation of B only in the case of the more sandy texture soil (Red-Yellow Latosol), denoting that this factor is conditional on soil texture. Ca/B ratio results showed that application of B to the soil without a suitable supply of Ca can lead to an imbalance between these nutrients, with possible implications for plant growth and nutrition.

Key words: Micronutrient, synergism, water tension.

PRODUÇÃO, ACÚMULO DE B E RELAÇÃO Ca/B EM EUCALIPTO SOB DISPONIBILIDADE DE B E ÁGUA

RESUMO: *Os solos destinados aos plantios florestais são geralmente limitantes em boro (B) e, em vários plantios de eucalipto a deficiência desse elemento tem sido comum. Objetivou-se, no presente trabalho, avaliar a produção de biomassa, acúmulo de B e relação Ca/B em eucalipto cultivado em diferentes solos, disponibilidade de água e doses de B. Para tanto, foi conduzido um experimento utilizando o delineamento experimental inteiramente casualizado, em esquema fatorial 5 x 2 x 2, sendo cinco doses de B (0,00; 0,25; 0,75; 2,25 e 6,25 mg kg^{-1}); dois tipos de solos: Latossolo Vermelho Escuro (LE) e Latossolo Vermelho Amarelo (LV) e duas tensões hídricas (-0,033 e -0,010 MPa), com quatro repetições. Após o cultivo das plantas, determinaram os teores de Ca e B, em seguida, as relações Ca/B nas diversas partes das plantas. O acúmulo de B foi obtido pela relação entre seus teores com a produção de biomassa seca das respectivas partes. Verificou-se que tanto o LE quanto o LV, nas suas condições naturais, não atendem às exigências nutricionais de B para o eucalipto, o que pode tornar limitante para a espécie. De maneira geral, a umidade do solo interferiu na produção de biomassa seca e no acúmulo de B somente no solo mais arenoso (LV), o que mostra um efeito dependente da textura do solo. A relação Ca/B mostrou que a aplicação de B ao solo sem o devido suprimento de Ca pode levar a um desequilíbrio entre esses nutrientes, com possíveis implicações no crescimento das plantas.*

Palavras-chave: Micronutriente, sinergismo, tensão hídrica.

1 INTRODUCTION

In several parts of the world, the insufficiency of boron (B) is one of the most common deficiencies among micronutrients (SAKYA et al., 2002). In Brazil, particularly in cerrado areas, where virtually every crop of *Eucalyptus* sp is non-native, B deficiency is a common occurrence, with shoot dieback being one of the most characteristic symptoms of B deficiency (SILVEIRA et al., 2004).

It is known that the main transport mechanism of B in the soil solution is through mass flow (FAQUIN, 2005). Accordingly, soil moisture conditions exert great influence on the availability of this nutrient for plants. This fact becomes particularly important in areas susceptible to

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long periods of water deficit, as is the case with cerrado regions. According to Sakya et al. (2002), water stress reduces B uptake by plant roots and also its consequent translocation to the shoots, promoting an increasing plant requirement for this nutrient.

Another important aspect relating to B nutrition is its interaction with Ca, noting that Ca/B ratio has been cited in literature for assessing deficiency, sufficiency and toxicity levels of B in plants (KANWAL et al., 2008; ROSOLEM; LEITE, 2007).

Therefore, the objective of this work is to evaluate biomass production, accumulation and critical levels of B, and Ca/B ratio in *Eucalyptus citriodora* cultivated under different conditions of soil, water availability and doses of B.

2 MATERIAL AND METHODS

The experiment was conducted using samples of clayey texture Dark Red Latosol (LE) originally from Curvelo, MG - Brazil, and medium texture Red-Yellow Latosol (LV) originally from the city of Itumirim, MG - Brazil. For each soil, samples were collected from the depth layer 0-20 cm and, once air-dried, they were sieved through a 2-mm mesh in preparation for the chemical and physical analyzes (Table 1).

Seeds of *Eucalyptus citriodora* were used in this study, as provided by Cia. Agrícola e Florestal Santa Bárbara (batch no. 167), as this species is sensitive to B deficiency and suitable for subtropical areas with severe seasonal droughts (GOLFARI, 1975).

Treatments were applied in a completely randomized design with a 5 x 2 x 2 factorial arrangement, consisting of five doses of B (0.00, 0.25, 0.75, 2.25 and 6.25 mg kg^{-1}, supplied in the form of boric acid – H_3BO_3), two soil types, namely Dark Red Latosol (LE) and Red-Yellow Latosol (LV), and two water tension levels (-0,033 MPa and -0,010 MPa), obtained by plotting the moisture curve characteristic of each soil and as described by (FERREIRA; MARCOS, 1983). Four replicates were used, and each experimental plot consisted of one plant per pot, to a total of 80 experimental plots. The plants were cultivated in pots containing 3 dm^3 of soil, receiving basic fertilization of macronutrients and micronutrients as follows, per dm^3: 50 mg of N, 240 mg of P, 100 mg of K, 20 mg of S, 3 mg of Mn, 1.33 mg of Cu, 5 mg of Fe and 5 mg of Zn, according to the methodology proposed by (MALAVOLTA, 1980).

Table 1 – Chemical, physical and mineral characteristics of Dark Red Latosol (LE) and Red-Yellow Latosol (LV) before applying each treatment.

Tabela 1 – *Características química, física e mineralógica do Latossolo Vermelho Escuro (LE) e Latossolo Vermelho Amarelo (LV) antes da aplicação dos tratamentos.*

Characteristic	LE	LV
pH in water	4.9	5.1
P (mg dm^{-3})	1.0	1.0
K^+ (mg dm^{-3})	22.0	30.0
Ca^{2+} (cmol$_c$ dm^{-3})	0.4	0.3
Mg^{2+} (cmol$_c$ dm^{-3})	0.1	0.1
Al^{3+} (cmol$_c$ dm^{-3})	0.5	0.4
H+Al (cmol$_c$ dm^{-3})	4.0	1.5
SB (cmol$_c$ dm^{-3})	0.6	0.5
SOM (g dm^{-3})	2.7	1.3
S (mg dm^{-3})	0.66	6.9
B (mg dm^{-3})	0.14	0.10
Sand (g kg^{-1})	360	720
Silt (g kg^{-1})	180	120
Clay (g kg^{-1})	460	160

P and K – Extractant Mehlich I; Ca, Mg and Al – Extractant KCl 1 mol L^{-1}; H+Al – Extractant SMP; SB – sum of bases; SOM – oxidation $Na_2Cr_2O_7$ 0.67 mol L^{-1} + H_2SO_4 5 mol L^{-1} (EMPRESA BRASILEIRA DE PESQUISA AGROPECUÁRIA - EMBRAPA, 1999).

Next, the soil samples remained incubated in each relevant pot for 15 days, with moisture level corresponding to a tension of -0.010 MPa. Following that period, 10 seeds of *Eucalyptus citriodora* were sown in each pot, while soil moisture was kept at -0.010 MPa. At this stage, the pots were protected from sunlight using a 50% *sombrite* shading net until thinning time, which occurred 35 days after sowing, leaving one plant per pot.

Only at thinning stage was the water tension -0.033 MPa applied to the relevant pots. It consisted of suspending irrigation until the above moisture level was reached and ensuring it was kept until the end of the experiment, by daily weighing the pot-soil-plant aggregate. Topdressing of nitrogen was applied 60 days after seed sowing, every 10 days at a dosage of 80 mg dm^{-3}. Fertilization with S was applied concurrently with N, in two applications of 22.86 mg dm^{-3}, together with the second and fourth topdressings of nitrogen. Throughout the experiment, soil moisture corresponding to tensions -0.033 MPa and -0.010 MPa were strictly controlled by daily weighing of the pot-soil-plant aggregate, restoring with demineralized water any volume lost through evapotranspiration.

Plants were each subjected to its relevant water tension for 95 days, then harvested, all of which lasted 140 days from planting to harvest. After harvest, plants were separated into young leaves, intermediate leaves and older leaves, branches and roots, and each portion was placed in a forced-air oven (65-70°C) to constant weight, after which the dry matter of each portion was obtained. The plant material was ground separately using a Willey mill and submitted to analysis of B and Ca. Ca levels in the tissues were determined after nitric-perchloric digestion, by using atomic absorption spectrophotometry (MALAVOLTA et al., 1997), and B levels were determined after dry digestion, by using a spectrophotometer set at 555 ηm, according to the methodology described by Jackson (1970). These values then derived Ca/B ratios in the various plant portions. Accumulation of B in the tissues of each plant portion was obtained by the relevant ratio of B content to dry biomass.

Data were subjected to analysis of variance by the F-test ($p \leq 0.05$) using software SISVAR® (FERREIRA, 2000). If significant, a polynomial regression would be performed for the factor 'doses of B' and means would be compared (Tukey, $p \leq 0.05$) for the factors 'soil type' and 'water tension'.

3 RESULTS AND DISCUSSION

Biomass production of shoots and roots in *Eucalyptus citriodora* was found to be significantly affected by doses of B, soil types and water tensions. Regarding these variables, only the soil *X* water tension interaction was found to be significant. Figure 1 shows that the effect of doses of B on dry biomass production of shoots and roots had a square root fit, indicating a marked increase in biomass when using small quantities of B, regardless of the soil type and water tension being applied. Similar results were found by Sakya et al. (2002) for dry biomass production of roots and shoots in *Eucalyptus globulus* subjected to increasing concentrations of B.

The results found in this study for dry biomass production in *Eucalyptus citriodora* as a function of B doses are probably due to the low availability of B in soils used under natural conditions (Table 1). Based on interpretations by Abreu et al. (2007), before being applied,

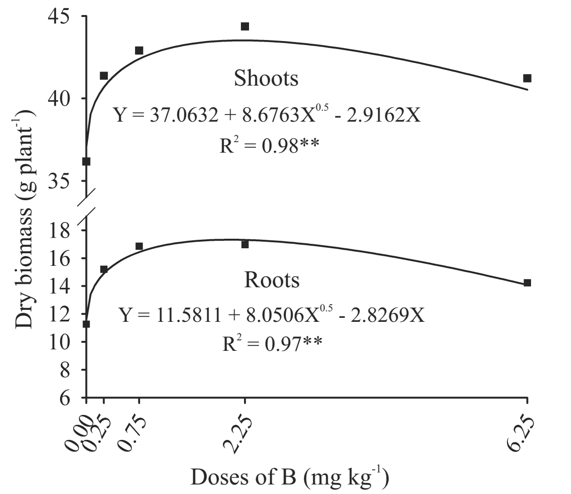

Figure 1 – Dry biomass production of shoots and roots in *Eucalyptus citriodora* 140 days after seed planting, depending on B doses. **Significant at 1%.

Figura 1 *– Produção de biomassa seca da parte aérea e raiz em Eucalyptus citriodora aos 140 dias após o plantio, em função das doses de B. ** Significativo a 1%.*

B contents in these soils are rated from low to very low. Maximum dry biomass production was obtained when doses 2.03 and 2.21 mg kg^{-1} of B were used, for shoots and roots respectively. For doses above 2.25 mg kg^{-1}, however, a reduction was noted in biomass production, indicating a possible toxic effect of B.

The effect of soil and moisture interactions on production of dry biomass in eucalyptus is shown in Figure 2. Shoots and roots were found to have similar behaviors as a function of soil and water tension combinations, regardless of the doses of B applied. Overall, for these variables, the highest values were found in LV soil, in conditions of more water availability (tension of -0.010 MPa).

As for accumulation of B in different parts of the plant, a significant effect was found of the interaction between soils and doses of B (Figure 3). In the roots, an effect was also found of the interaction between doses of B and water tensions (Figure 3 d).

Regardless of the water tension being applied, a greater accumulation of B in young leaves occurred in LV soil (Figure 3 a). In older leaves, a reverse pattern was noted, in which a greater accumulation of B occurred in LE soil (Figure 3 b). In roots, LV soil surpassed LE soil but only at doses above 1.50 mg kg^{-1} (Figure 3 c). The accumulation of a nutrient is a function of its concentration

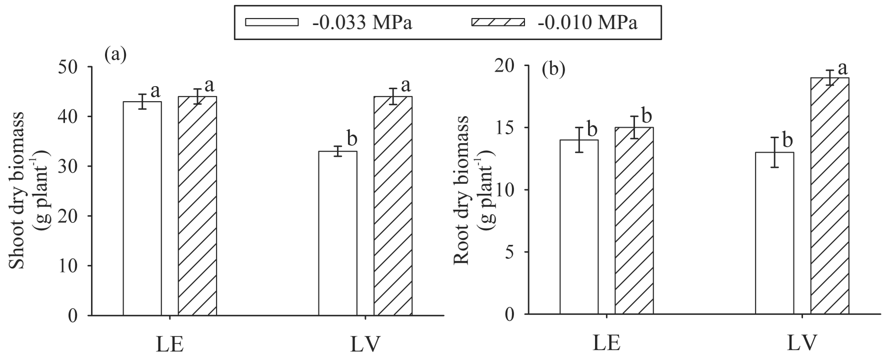

Figure 2 – Dry biomass production of shoots (a) and roots (b) of *Eucalyptus citriodora* 140 days after planting, grown in two Latosols (LE and LV) and subjected to two water tensions (-0.033 and -0.010 MPa). Means followed by the same letter do not differ for each variable (Tukey, 5%).

Figura 2 *– Produção de biomassa seca da parte aérea (a) e raiz (b) do Eucalyptus citriodora aos 140 dias após o plantio, cultivado em dois Latossolos (LE e LV) e submetido a duas tensões hídricas (-0,033 e -0,010 MPa). Médias seguidas de letras iguais, para cada variável, não diferem entre si (Tukey, 5%).*

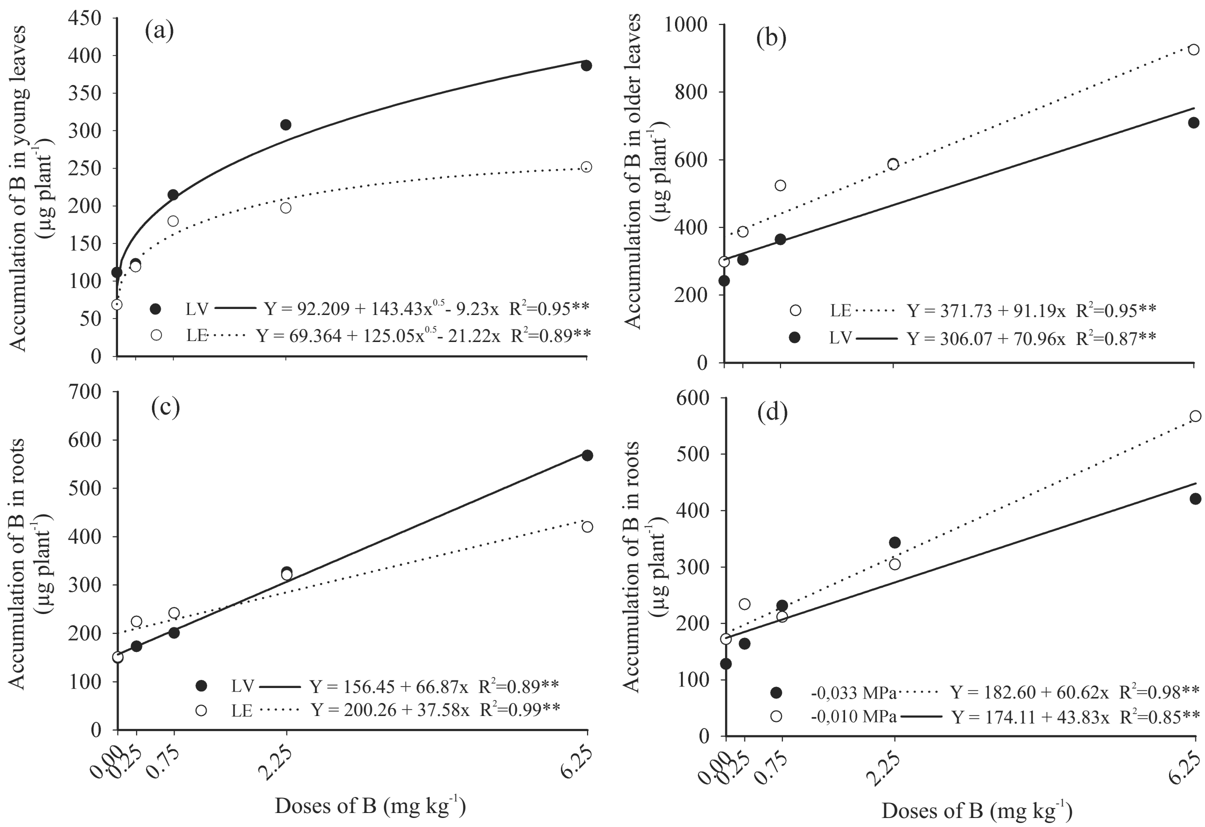

Figure 3 – Accumulation of B in young leaves (a) and in older leaves (b) as well as in roots (c and d) of *Eucalyptus citriodora* grown in two Latosols (LV and LE) subjected to two water tensions (-0.033 and -0.010 MPa), as a function of doses of B applied.

Figura 3 *– Acúmulo de B nas folhas novas (a) e velhas (b) e nas raízes (c e d) do Eucalyptus citriodora cultivado em dois Latossolos (LV e LE) sob duas tensões hídricas (-0,033 e -0,010 MPa), em função das doses de B aplicadas.*

in plant tissues and of dry biomass produced. Therefore, the fact that a greater accumulation of B in the case of young leaves occurred in LV soil is possibly due to greater absorption of B in this soil, which increased B levels in these leaves, since transport of B occurs via xylem through the transpiration stream (FAQUIN, 2005). In older leaves, the fact that a greater accumulation of B occurred in LE soil was due to B being virtually immobile in the phloem, which favored increased B levels in these leaves and, consequently, greater accumulation of B.

An analysis of the interaction soils x water tensions revealed that in conditions of less water availability, the LV soil promoted less accumulation of B in branches, unlike the LE soil (Figure 4). As for other plant portions, no interaction was found between soil types and water tensions regarding effect on accumulation of B. Despite no significance being found, in both soils and more specifically in LV soil, greater accumulation of B was found under conditions of more water availability. Accordingly, because the main contact mechanism between B and the root is through mass flow, where water availability is low, B absorption is impaired (EPSTEIN; BLOOM, 2006). In the case of the LV soil, in addition to this effect, it being a sandy soil, under unsaturated conditions its hydraulic conductivity is reduced more drastically than in clayey soils (LIBARDI, 2005), which reflects in lower supply of B to plants, reducing its concentration and, consequently, its accumulation. Besides concentration, the other component of B accumulation, which is dry biomass production, was overall reduced under conditions of low water availability (Figure 2).

Given the importance of Ca and B as components of cell wall compounds, the correlation between these two nutrients has been explored in scientific literature (ROSOLEM; LEITE, 2007), and the correlation of these components in leaves has been used as a diagnosis index of B toxicity as well as a nutritional indicator of this micronutrient (GUPTA, 1979). In this study, the Ca/B ratio was affected by different doses of B, water availability, soil types and by the triple interaction between these factors, only for intermediate leaves (Figure 5). In this case, with each water tension being applied, to both soils, the Ca/B ratio decreased with increasing doses of B, fitting to the square root model (Figure 5). These results agree with the findings of Rosolem and Leite (2007) and Silveira et al. (2000), who also reported a decreasing Ca/B ratio with increasing concentration of B in the soil.

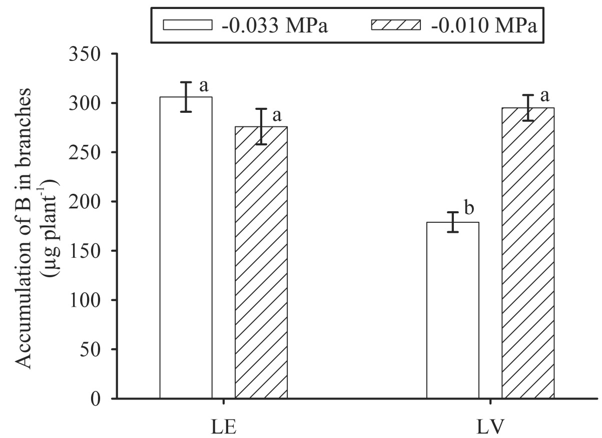

Figure 4 – Accumulation of B in branches of *Eucalyptus citriodora*, as a function of soil types and water tensions. Means followed by the same letter do not differ (Tukey, 5%).

***Figura 4** – Acúmulo de B nos ramos do Eucalyptus citriodora, em função dos tipos de solos e tensões hídricas. Médias seguidas de letras iguais não diferem entre si (Tukey, 5%).*

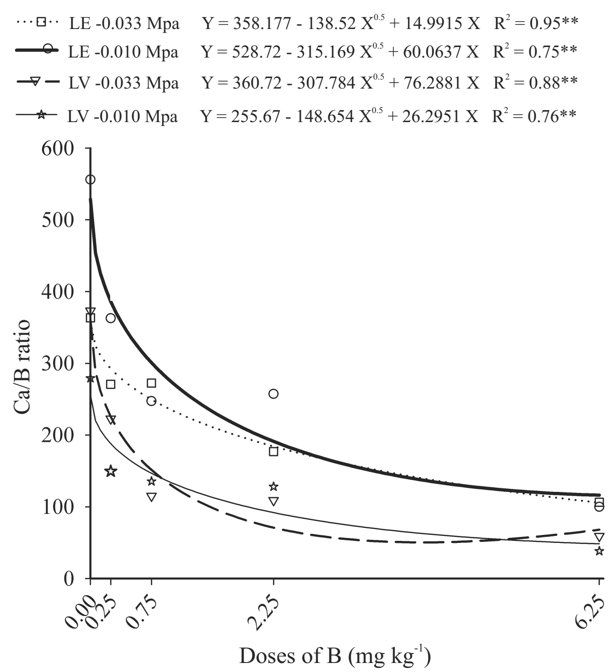

Figure 5 – Ca/B ratio in intermediate leaves of *E. citriodora*, as a function of doses of B applied, as grown in two Latosols (LV and LE), under two water tensions (-0.033 and -0.010 MPa).

***Figura 5** – Relação Ca/B das folhas medianas do E. citriodora, em função das doses de B aplicadas, cultivado em dois Latossolos (LV e LE), sob duas tensões hídricas (-0,033 e -0,010 MPa).*

However, as the concentration of B rose in the soil, the decrease in Ca/B ratio gradually became less pronounced. It appears that the increased concentration of B had a synergistic effect on absorption of Ca. This synergistic interaction of B with Ca was also reported by Yamagishi and Yamamoto (1994) with soybean and by Kanwal et al. (2008) with studies of Ca/B ratio in corn. From another standpoint, a considerable increase in the concentration of Ca in the soil solution has an antagonistic effect on the absorption of B, probably due to formation of low-solubility compounds, such as Ca metaborate (GOLAKIYA; PATEL, 1988; KANWAL et al., 2008). Drake et al. (1941) found in tobacco plants that the best combination regarding supply of B and Ca to plants were associated with Ca/B ratios of around 340:1, while the ratio 500:1 was associated with B deficiency. In this study, it was found that the Ca/B ratio in intermediate leaves of *Eucalyptus citriodora* tended to be close to 500:1 only if grown in LE soil at -0.010 MPa and if subjected to low doses of B (0.0 and 0.25 mg kg^{-1}). Overall, in the case of intermediate leaves, lower values of Ca/B ratio were observed in the LV soil, probably due to its lower hydraulic conductivity under unsaturated conditions, in comparison with the LE soil, resulting in less mass flow and thus reducing more sharply Ca availability relative to B availability in eucalyptus, since Ca flow to the inside of the plant occurs in greater proportion than B flow does.

4 CONCLUSIONS

Both Dark Red Latosol (LE) and Red-Yellow Latosol (LV) soils, in natural conditions, failed to meet the nutritional requirements for B of eucalyptus, which can be limiting to that species.

Soil moisture affected biomass production and accumulation of B in eucalyptus but only in the sandy soil (LV), which denotes a soil texture-dependent effect.

Results of Ca/B ratio revealed that application of B to the soil without a suitable supply of Ca can lead to an imbalance between these nutrients, with possible implications for plant growth and nutrition.

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