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Tolerance of *Eucalyptus* and *Pinus* seedlings to exchangeable Aluminium

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ABSTRACT: Tolerance to acid soil is an important trait of *Eucalyptus* and *Pinus* species introduced into commercial forestry plantations in tropical ecosystems. The objective of this study was to evaluate the effect of the exchangeable Al on growth of the seedlings of two species and one hybrid of *Eucalyptus* and two species of *Pinus*. We also wanted to identify the role of the root cation exchangeable capacity (CEC) on Al tolerance. The experiment was conducted under controlled conditions in a greenhouse. The soil used was collected from the top layer of a red yellow Oxisol, from the Brazilian savanna region. The treatments consisted of different rates of P and the presence or absence of liming with $CaCO_3$ and $MgCO_3$. The species of *Eucalyptus* and *Pinus* showed no growth differences between the treatments. For all species, a higher density of fine roots was found in treatments with lower levels of bases and a higher Al exchangeable concentration. The *Pinus* species had a higher root CEC than the *Eucalyptus* species, which had a lower leaf Al concentration. Al concentration in the fine roots was 50 fold greater than in the leaves of all species. Similar to Al, concentrations of Fe, Cu and Zn in the roots were significantly higher than in the leaves in all species. In contrast, higher Mn concentration was found in the leaf tissue. This may lead species of *Eucalyptus* and *Pinus* to be more susceptible to Mn than to Al toxicity.

Keywords: Al uptake, root CEC, root growth, liming

Introduction

Al toxicity limits crop productivity in acidic soils, and acidic soils account for approximately 60 % of tropical soils (Silva et al., 2004). In general, the toxic effect of Al on plant has been associated with a reduction in the development and functioning of root system. Under these conditions, the roots explore a smaller volume of soil thus sensitive plants have low efficiency of nutrient uptake and high susceptibility to drought effects. Soil acidity often induces multiple stresses on crops, such as enhanced Al and H rhizotoxicity, and base cations deficiencies of Ca and Mg, for example, and phosphate (Kochian et al., 2004; Kinraide et al., 2004; Marschner, 1991; Cristancho et al., 2014).

Eucalyptus and *Pinus* species are considered tolerant to Al toxicity (Silva et al., 2004; Gonçalves et al., 2013), showing good productivity rates in acidic soils and do not respond to pH correction by liming (Vale et al., 1996; Silva et al., 2010; Paes et al., 2013; Rodrigues et al., 2016). However, little is known about the mechanisms of Al tolerance in these species.

Several studies provide strong evidence that part of the tolerance mechanism against Al toxicity is found in the roots, preventing Al uptake (Silva et al., 2004; Tahara et al., 2008; Ikka et al., 2013). According to Barros et al. (1990), Al tolerance varies according to species and depends on the plant variety and physiological state, probably because no single tolerance mechanism exists. Al complexation in the rhizosphere by low molecular weight organic acids has been identified as a major tolerance mechanism in *Eucalyptus* species (Silva et al., 2004). However, roots CEC and Al-phosphate precipita-

tion may also be involved in the tolerance mechanism (Mugai et al., 2000).

Nambiar et al. (1982) carried out a thorough literature review and highlighted the importance of root setting and function in forest planting management practices and forest improvement programs, mainly in terms of adaptation to adverse conditions of climate and soil. The authors concluded that most forest species have great genetic variability and high heritability in terms of root system attributes and can be altered genetically to produce plants that are more tolerant to environmental adversities. Therefore, resistance to soil acidity is an important target trait for fast-growing *Eucalyptus* clones.

The objective of this study was to evaluate the effect of exchangeable Al on seedlings shoots and roots growth of two species and one hybrid of *Eucalyptus* (*E. grandis*, *E. urophylla* and *E. grandis* × *E. urophylla*) and two species of *Pinus* (*P. oocarpa* and *P. caribaea* var. *hondurensis*). We also wanted to identify the role of the root CEC on Al tolerance.

Materials and Methods

Study site and treatments

This study was conducted in a greenhouse at the municipality of Piracicaba, São Paulo State (22°42′30″ S and 47°38′30″ W; 546 m above sea level). The soil used was collected from the 0-20 cm layer of a red yellow Oxisol, dystrophic, median texture (240 g kg^{-1} clay, 60 g kg^{-1} silt and 700 g kg^{-1} of sand), from the Brazilian savanna (Cerrado) region. The soil pH was 3.9 (0.01 mol L^{-1} CaCl$_2$), organic matter (OM) 36 g kg^{-1}, available P 7 mg kg^{-1} (resin method), exchangeable Ca 4.6 mmol$_c$ kg^{-1},

exchangeable Mg 3.3 mmol$_c$ kg^{-1} and exchangeable K 0.4 mmol$_c$ kg^{-1}, available S 50 mg kg^{-1} and exchangeable Al 20 mmol$_c$ kg^{-1}. The concentration of Fe, Cu, Mn and Zn extracted by DTPA (Lindsay and Norvell, 1978) were 53, 2.3, 1.4 and 0.4 mg kg^{-1}, respectively.

Two experiments were carried out in a randomized block design. The first experiment was a 3 × 4 factorial, where 3 was the *Eucalyptus* species and 4 the fertilization. The second experiment was a 2 × 4 factorial, where 2 was the *Pinus* species and 4 was the fertilization levels, both with 3 blocks. Each replication consisted of a pot where two plants were cultivated.

The species of *Eucalyptus* used were *E. grandis* Hill ex Maiden, *E. grandis* × *urophylla* hybrid and *E. urophylla* S.T. Blake. The *Pinus* species were *P. oocarpa* and *P. caribaea* var. hondurensis. Fertilization levels were: 1) BF-250P: basic fertilization (50 mg kg^{-1} of N, 100 mg kg^{-1} of K and 45 mg kg^{-1} of S) plus 250 mg kg^{-1} P; 2) CC-250P: basic fertilization plus 250 kg^{-1} mg P and CaCO$_3$ and MgCO$_3$; 3) CS-250P: basic fertilization plus 250 mg kg^{-1} P, CaSO$_4$ and MgCl$_2$; and 4) CS-125P: basic fertilization plus 125 mg kg^{-1} P, CaSO$_4$ and MgCl$_2$. Ca and Mg were applied at the stoichiometric ratio of 4:1 enough to raise the available Ca + Mg to 13 mmol$_c$ kg^{-1} soil. The sources of N, P, K and S were ammonium sulfate, monophosphate sodium and potassium chloride, respectively. The soil chemical attributes 300 days after treatment application are presented in Table 1.

Installation and conducting the experiment

Base fertilization comprised of P, CaCO$_3$, MgCO$_3$, CaSO$_4$ and MgCl$_2$ was mixed with the soil, wrapped in polyethylene pots (up to 4 kg of soil), irrigated to obtain humidity near field capacity and incubated for a period of 30 days.

Seeds of *Eucalyptus* and *Pinus* were germinated and grown in washed sand until they reached 5 cm in height. Then, the seedlings were transplanted to pots containing the treatments previously mentioned.

At 60, 120 and 180 days after transplanting (DAT), top dressing fertilization was applied in all treatments. At 60 and 120 DAT, 25 mg kg^{-1} of N and 22.4 mg kg^{-1} of S was applied. At 180 DAT, 50 mg kg^{-1} of N and 45 mg kg^{-1} of S was applied. At 60 DAT, a micronutrient solution was also applied (4 mg of Zn, 1 mg of B, 1.5 mg of Fe, 1 mg of Mn, 1 mg of Cu and 0.1 mg of Mo per kg of soil). Soil moisture was kept near field capacity throughout the experiment, using distilled water.

Assessment

At 210 DAT, growth assessments were done for *Eucalyptus* species and at 300 DAT for *Pinus* species. The heights were measured from soil level until the insertion point of the youngest leaf. The stem diameters were measured at the soil level for each plant. Plants were harvested and sectioned into stem, branch, leaf and root. After drying to constant weight in an oven at 65 °C, the material was weighed and grounded to determine the amounts of nutrients. To-

Table 1 – Soil chemical attributes[1] of samples after 300 days of treatment application.

Treatment	pH	P	S- SO$_4^{2-}$	Ca	Al	Fe	Zn
		— mg kg^{-1} —		— mmol$_c$ kg^{-1} —		— mg kg^{-1} —	
E. grandis							
BF-250P	3.9 b[2]	36 c	33 c	7 b	17 a	52 a	6.5 ab
CC-250P	5.1 a	55 b	9 d	30 a	1 b	27 b	10.4 a
CS-250P	3.9 b	78 a	250 a	23 a	13 a	64 a	2.6 b
CS-125P	3.9 b	40 c	81 b	12 b	17 a	48 a	2.6 b
E. urophylla							
BF-250P	4.0 b	27 b	21 c	7 b	17 a	56 a	13.0 a
CC-250P	4.9 a	40 b	5 d	26 a	1 b	38 b	11.7 a
CS-250P	4.1 b	68 a	120 a	14 b	13 a	59 a	2.6 b
CS-125P	4.0 b	30 b	77 b	12 b	16 a	57 a	2.6 b
E. grandis × *urophylla*							
BF-250P	3.9 b	39 b	21 c	7 b	13 a	39 b	11.7 a
CC-250P	4.9 a	53 a	8 c	25 a	1 b	531 a	6.5 b
CS-250P	3.9 b	61 a	122 a	13 b	17 a	59 a	5.2 b
CS-125P	3.9 b	38 b	81 b	9 b	16 a	52 a	2.6 b
Pinus oocarpa							
BF-250P	3.5 b	199 a	30 c	3 c	36 a	69 a	1.7
CC-250P	5.0 a	127 b	10 d	30 a	3 b	39 b	0.9
CS-250P	4.0 b	134 b	104 a	21 b	27 a	60 a	1.6
CS-125P	4.0 b	68 c	90 b	27 a	27 a	48 b	1.7
Pinus caribaea var. *honduresis*							
BF-250P	3.7 b	164 a	29 c	3 c	34 a	70 a	1.6
CC-250P	5.1 a	120 b	10 d	34 a	3 b	36 c	1.4
CS-250P	3.9 b	165 a	111 a	21 b	26 a	68 a	2.5
CS-125P	4.0 b	68 c	91 b	33 a	25 a	52 b	2.2

[1]Analysis carried out according to the methods described by van Raij et al. (2001); [2]Averages in the same column followed by the same letter do not differ at 5 % significance by the Tukey test.

tal N was determined after sulfuric acid digestion by the micro Kjeldahl method. After nitric perchloric digestion, P was determined by colorimetry; S by turbidimetric; K by flame photometry, and Ca, Mg, Fe, Mn, Cu and Zn by atomic absorption (Malavolta et al., 1989).

The roots were separated manually from the soil with aid of sieves (0.053 mm) and washed with distilled water. Color and flexibility were used as criteria for separation of live and dead roots. White and flexible roots were considered live and dark roots were considered dead (Laclau et al., 2013). Roots were classified into fine (active nutrient uptake) and thick roots (> 3 mm in diameter). Fine roots were separated into two classes: a) root diameter less than 1 mm; b) roots with a diameter between 1 and 3 mm. According to Lyford (1975), roots with a diameter smaller than 3 mm are considered fine roots, because they generally do not show thickening of secondary xylem and have developed phloem, which is very permeable to water and nutrient uptake. Root length was estimated by the SIARCS system (Integrated System for Roots Analysis and Land Cover), which is the processing and analysis of digital images.

The cation exchange capacity (CEC in mmol$_c$ kg^{-1}) of fine roots (> 1 mm diameter) was determined according to the method described by Crooke (1964). The

method consists in saturating root CEC with H⁺ ions using HCl 0.1 mol L^{-1}, then removing acid excess with distilled water. The H⁺ ions adsorbed on CEC of roots were removed with a solution of KCl 1 mol L^{-1} at pH 7. The amount of H⁺ adsorbed is determined by titration with KOH 0.1 mol L^{-1}.

Data analysis

Before the analysis, the data were tested for normality (Shapiro-Wilk test) and homoscedasticity (Box-Cox text). When necessary, the data were corrected to attend the assumptions. The F-test was applied to each experiment using fertilization level and block as variation of source species and, when significant ($p < 0.05$), the Tukey test at 5 % of probability was performed. Statistical analyses were performed using the PROC GLM in the software SAS University Edition.

Results

Growth

The species of *Eucalyptus* and *Pinus* showed no shoot growth differences between treatments ($p > 0.1$). However, differences in DM production of fine roots

(< 1 mm) were found. Greater DM production of fine roots (< 1 mm) in BF-250P and CS-125P treatments was found for all species of *Eucalyptus* and *Pinus*. No difference was found in the production of root DM between CC-250P and CS-250P treatments (Table 2).

There were differences in the density of fine roots between treatments for all species. Treatment BF-250P resulted in higher density of roots to all species. It did not differentiate from treatment CS-125P in the *Pinus* species and in the hybrid *E. grandis × urophylla*. The lowest density of roots occurred in treatment CC-250P, which did not differ from treatment CS-250P for the *Pinus* species. Density difference between the roots of treatments CC-250P and CS-250P occurred for the *Eucalyptus* species, where the lowest density was found for treatment CC-250P (Table 3).

Fine root density was higher in *E. grandis* than in *E. urophylla*, which was higher than hybrid *E. grandis × urophylla*. No differences in fine root density were found in *Pinus* species (Table 3). At 300 DAT, these species had lower density than fine roots of the *Eucalyptus* species at 210 DAT. In general, in all species of *Eucalyptus* and *Pinus* has more than 95 % of the length of fine roots belonged to root class < 1 mm (data do not shown).

Table 2 – Growth in height (H), stem diameter (SD) and dry matter production (Lf = Leaf, B = branch, S = stem, PA = aerial part, R = root, TR = Total root, PT = plant total) of shoots and roots of different species of *Eucalyptus* and *Pinus* in the treatments[1].

Treatment	H	SD	Lf	B	S	PA	R < 1 mm	R > 1 mm	TR	PT
	cm	mm				g seedling^{-1}				
E. grandis										
BF-250P	51.3 B	6.0 B	7.9 B	1.6 B	2.2 B	12 B	6.8 aB	0.6 B	7.4 aB	19.4 B
CC-250P	54.0 B	6.0 B	8.4 B	1.8 B	2.5 B	13 B	5.0 bB	0.6 B	5.6 bB	18.6 B
CS-250P	56.0 B	6.8 B	8.5 B	1.9 B	2.7 B	13 B	5.1 bB	0.6 B	5.7 bB	18.1 B
CS-125P	51.6 B	6.6 B	8.0 B	1.7 B	2.2 B	12 B	6.7 aB	0.7 B	7.4 aB	19.4 B
E. urophylla										
BF-250P	57.5 B	6.7 B	8.6 B	2.0 B	2.4 B	13 B	7.4 aB	0.7 B	8.1 aB	21 B
CC-250P	59.0 B	7.1 B	8.6 B	2.0 B	2.8 B	13 B	5.9 bB	0.8A	6.7 bB	20 B
CS-250P	60.0 B	7.0 B	8.6 B	2.3 B	3.1 B	14 B	6.0 bB	0.8A	6.8 bB	21 B
CS-125P	58.5 B	6.4 B	8.1 B	1.9 B	2.8 B	13 B	7.5 aB	0.8A	8.3 aB	21 B
E.grandis × urophylla										
BF-250P	72.5 A	9.8 A	11 A	2.9 A	3.3 A	19 A	9.0 aA	0.9 A	9.9 aA	29 A
CC-250P	70.2 A	9.0 A	12 A	3.0 A	3.7 A	21 A	8.1 bA	0.8 A	8.9 bA	30 A
CS-250P	76.0 A	9.1 A	11 A	3.1 A	3.7 A	20 A	8.0 bA	0.9 A	8.9 bA	29 A
CS-125P	74.0 A	8.4 A	11 A	2.8 A	3.4 A	20 A	9.1 aA	0.9 A	10 aA	30 A
Pinus oocarpa										
BF-250P	41.0	4.8	10.2	-	1.59	11.8	4.9 a	0.4	5.5 a	17.3
CC-250P	42.0	5.0	10.5	-	1.88	12.4	3.9 b	0.3	4.3 b	19.7
CS-250P	44.0	6.2	11.8	-	1.80	13.6	4.0 b	0.4	4.5 b	18.1
CS-125P	43.0	5.0	12.0	-	2.00	12.4	5.2 a	0.3	5.6 a	17.6
Pinus caribaea var. hondurensis										
BF-250P	46.0	5.2	10	-	1.55	11.6	5.0 a	0.3	5.4 a	17.0
CC-250P	49.0	5.0	9.9	-	1.21	11.1	4.0 b	0.2	4.3 b	15.4
CS-250P	49.0	5.6	11	-	1.30	12.3	4.1 b	0.4	4.6 b	16.9
CS-125P	48.0	5.5	11	-	1.55	12.6	5.6 a	0.3	6.0 a	18.6

[1]Averages in the same column followed by the same letter do not differ at 5 % significance by the Tukey test. Difference between treatments (lowercase) and species (capital letters).

Table 3 – Density of fine roots (< 1 mm) in the different species and treatment[1].

Species	Density of thin roots			
	BF-250P	CC-250P	CS-125P	CS-250P
	——————— cm cm⁻³ ———————			
E.grandis	0.20 aA	0.12 dA	0.18 bA	0.15 cA
E. urophylla	0.18 aB	0.12 dA	0.17 bB	0.14 cB
E.urophylla vs. E.grandis	0.15 aC	0.11 cB	0.15 aC	0.12 bC
Mean	0.18	0.12	0.17	0.14
Pinus oocarpa	0.10 a	0.07 b	0.09 a	0.07 b
Pinus caribaea var. *hondurensis*	0.11 a	0.07 b	0.09 a	0.07 b
Mean	0.11	0.07	0.09	0.07

[1]Means with the same letter do not differ at 5 % significance by the Tukey test. Difference between treatments (lowercase) and species (capital letters).

Nutrients concentration

Al concentration in the roots of *Eucalyptus* and *Pinus* seedlings was higher in plants under treatment BF-250P (Table 4). In all treatments, *E. urophylla* showed lower Al concentration in the roots than the other species of *Eucalyptus*, which did not show any differences. In the aerial part, although there were no differences between species of *Eucalyptus*, in general, the hybrid *E. grandis × urophylla* tended to have lower Al concentration, followed by *E. urophylla*. At 300 DAT, the *Pinus* species showed higher Al concentration in roots and shoots than the *Eucalyptus* species did at 210 DAT (Table 4).

The hybrid *E. grandis × urophylla* and *E. urophylla* showed no differences in Ca and Mg levels in the root in treatments BF-250P and CC-250P, despite differences in the concentrations of exchangeable cations of these nutrients in the soil (Table 1). This effect was not observed for *E. grandis*, which had the lowest concentrations of these nutrients in treatment BF- 250P than in CC-250P. *E. urophylla* showed no differences in the treatments. The *Pinus* species had lower Ca concentration in roots in treatment BF-250P. However, no differences were observed in Ca concentration in the shoots in the treatments. *E. grandis* showed higher P concentration in roots in treatments with higher exchangeable Al in the soil (Table 4). There were no differences in P concentration in the roots of *E. urophylla* and the hybrid *E. grandis × urophylla*. In general, the *Eucalyptus* species showed no differences in P concentration in shoots of seedlings. Proportionally, no differences in P accumulation in roots and shoots were observed in seedlings of *Eucalyptus* and *Pinus* (Table 4).

Similar to that observations for Al, concentrations of Fe, Cu and Zn in the roots were much higher than in leaf tissues of all species, in proportion to the quantities available in the soil (Table 4). In contrast, higher concentrations and Mn accumulation were observed in leaf tissues.

Cation exchange capacity (CEC) of fine roots

The *Eucalyptus* species presented a distinct behavior of root CEC. The hybrid *E. grandis × urophylla*

showed higher CEC in treatment BF-250P. For *E. urophylla*, the lowest CEC value occurred in treatment CC-250P, while in *E. grandis*, there was no significant difference in CEC values in the treatments. The *Pinus* species did not present any difference in the root CEC, however, they showed higher root CEC at 300 DAT than *Eucalyptus* species did at 210 DAT (Table 5).

Discussion

Plant development and nutrient concentration

The high level of exchangeable Al in the soil (Table 1) did not influence the growth of species of *Eucalyptus* and *Pinus*, indicating that both are tolerant to this element (Table 2). Al did not impair uptake and translocation of P and S (evidenced by leaf concentration) for any of the species (Table 4). This result suggests a possible adaptation of these species, since the typical pattern of P distribution in plants stressed by Al is accumulation in the roots and decrease in shoot organs (Chen et al., 2012). Assuming that the negative effects of exchangeable Al on P uptake were small, tolerance to Al toxicity should be related to increased efficiency of Al uptake through the expansion of fine roots length, improving also the ability of P uptake in Al overflow condition. We found improvement in thin root density under high Al availability, however, no difference was found in the treatments at different P fertilizer application levels (Table 3).

Except for the hybrid *E. grandis × urophylla*, there was no difference in Al concentration in plant leaves between treatments BF-250P (17 mg kg⁻¹ of exchangeable Al) and CC-250P (1 mg kg⁻¹ exchangeable Al). In this hybrid, there was small difference in Al concentration, which was higher in treatment BF-250P. This result supports the hypothesis that the mechanism involved in Al tolerance by these species is found in the roots, avoiding uptake and/or translocation of Al (Silva et al., 2004; Ikka et al., 2013).

The high root concentration of Fe, Cu and Zn in relation to leaf, suggests that many of these elements were adsorbed to root CEC, limiting their entry into the symplast, as also found for Al. The opposite was found for Mn (Table 4). This predisposes species of *Eucalyptus* and *Pinus* to greater susceptibility to Mn than to Al toxicity.

Al tolerance mechanisms

As noted by other authors (Taylor, 1988; Kochian, 1995; Silva et al., 2004; Ikka et al., 2013), exclusionary mechanisms control tolerance to high soil Al concentrations in the species considered in this study. It means that Al reaches the root surface and/or root apoplast, but its entry into the symplast is limited.

Al concentration in plant roots was about 30-fold higher than that found in the leaves under low Al availability conditions and about 50-fold higher under high Al availability conditions. This compartmentalization of Al might be associated to Al adsorption in fine root

Table 4 – Chemical composition[1] of the plant components of *Eucalyptus* and *Pinus* species.

Treatment	N	P	K	Ca	Mg	S	Fe	Zn	Cu	Mn	Al
			g kg⁻¹						mg kg⁻¹		
						E. grandis					
						Leaf					
BF-250P	7.3	1.6	6.1 aA	1.7 c	0.7 bB	1.7	220 A	29 bB	10 aA	563 bA	210
CC-250P	6.9	1.3	3.4 bA	4.6 b	2.1 aA	1.7	200 A	13 cA	7 bA	110 cA	218
CS-250P	7.0	1.1	5.6 aA	6.0 a	2.1 aA	1.4	177 B	46 aA	6 bA	887 aA	258
CS-125P	6.7	1.2	5.6 aA	6.4 a	2.2 aA	1.0	168 B	34 bA	6 bA	673 bA	270
						Root					
BF-250P	5.9 B	1.6 b	3.6	1.6 cB	0.9 b	2.2	15308 aA	117 aB	25 aA	159 aA	10455 aAA
CC-250P	5.5 B	1.0 c	4.3	3.3 bA	1.4 b	2.0	7149 bB	135 aA	17 bA	85 bA	6602 bA
CS-250P	5.6 B	2.7 a	3.9	2.6 bA	1.4 b	2.4	14364 aA	167 bB	27 aA	94 bA	7525 bA
CS-125P	5.5 B	1.8 b	3.7	4.6 aA	2.1 a	2.0	8437 bAB	92 cB	21 aA	69 bA	6704 bA
						E. urophylla					
						Leaf					
BF-250P	7.5	1.6	6.6 aA	1.9 b	0.8 bB	1.6	137 bB	43 aA	10 aA	399 bB	190 b
CC-250P	6.4	1.4	2.7 cA	5.0 a	2.3 aA	1.0	224 aA	13 bA	3 cB	177 cA	209 b
CS-250P	6.7	1.5	5.1 bAB	6.7 a	2.6 aA	1.5	234 aA	42 aA	4 cB	761 aA	204 b
CS-125P	7.3	1.3	5.0 bA	6.2 a	2.4 aA	1.3	272 aA	46 aA	6 bA	738 aA	282 a
						Root					
BF-250P	7.4 aA	2.1 a	4.0	2.7 bA	1.2	2.5 a	10214 aB	120 bB	26 aA	72 bB	8158 aB
CC-250P	7.3 aA	1.3 b	3.6	2.8 bA	1.0	1.3 b	3937 cC	129 bA	16 bB	66 bA	5592 bA
CS-250P	5.6 bB	1.9 a	4.3	3.3 bA	1.3	1.4 b	3712 cC	280 aA	18 bB	56 bA	4822 bB
CS-125P	7.2 aA	1.4 b	4.2	4.1 aA	1.9	1.9 b	7885 bB	151 bA	18 bA	90 aA	5565 bA
						E. grandis × urophylla					
						Leaf					
BF-250P	7.8	1.8 a	7.2 aA	3.2 a	1.5 A	1.0 a	245 aA	22 abA	4 bB	362 bB	243 a
CC-250P	7.4	1.2 a	3.9 bA	4.6 a	2.3 A	0.6 a	203 abA	19 bA	4 bB	270 cB	181 b
CS-250P	7.3	1.5 a	4.5 bB	5.2 a	2.3 A	2.0 b	186 bB	36 aA	7 aA	762 aAA	188 b
CS-125P	7.1	0.9 a	4.0 bB	6.7 b	2.6 A	1.2 a	178 bB	33 aA	4 bB	732 aA	268 a
						Root					
BF-250P	7.4 aA	1.5	3.8	1.7 bB	0.8 b	1.7	12754 aB	227 aA	28 A	112 aA	10784 aA
CC-250P	7.3 aA	2.1	3.3	1.7 bB	0.7 b	1.5	12692 aA	167 bA	26 A	103 aA	6116 bA
CS-250P	6.9 aA	1.8	3.5	3.0 aA	1.7 a	2.4	7275 cB	153 bB	25 A	67 bA	6568 bA
CS-125P	5.4 bB	1.1	4.5	3.3 aA	1.4 a	1.8	9268 bA	123 bB	21 A	67 bA	6550 bA
						Pinus oocarpa					
						Needle					
BF-250P	8.3	1.2	6.7	2.1	0.9	1.1	204 A	24	5	302 a	365
CC-250P	8.0	1.0	5.9	2.0	1.2	1.5	235 A	19	5	69 b	287
CS-250P	8.8	1.1	6.1	2.3	1.4	1.5	221 A	23	5	315 a	343
CS-125P	7.6	1.0	7.7	2.3	1.2	2.0	294 A	23	4	385 a	311
						Root					
BF-250P	6.6 B	1.3	3.6	1.7 b	0.8	1.2	5905	42 a	15	64 bB	11295 a
CC-250P	6.2 B	0.9	3.3	3.0 a	1.2	1.3	6379	23 b	14	37 cA	7243 b
CS-250P	6.0 B	0.8	4.4	2.8 a	0.8	1.6	5245	40 a	14	91 aB	7294 b
CS-125P	6.0 B	1.0	3.5	2.6 a	1.9	1.5	6300	52 a	16	69 bB	7270 b
						P. caribaea var. hondurensis					
						Needle					
BF-250P	8.2	1.1	5.8	2.1	1.3	1.3	150 B	20	4	354 a	305
CC-250P	8.2	1.1	5.8	2.2	1.3	1.3	150 B	20	4	72 b	272
CS-250P	8.9	1.1	7.2	2.3	1.3	1.7	108 B	28	4	373 a	360
CS-125P	7.3	1.0	6.0	2.9	1.3	2.0	132 B	27	4	395 a	367
						Root					
BF-250P	8.0 A	1.4	4.6	1.2 b	0.6	1.5	5263	42 a	15	103 aA	11159 a
CC-250P	7.4 A	1.0	3.0	2.7 a	1.1	1.2	5944	22 b	13	33 bA	7396 b
CS-250P	8.2 A	0.9	5.3	3.3 a	0.9	1.9	4815	57 a	17	163 aA	7517 b
CS-125P	7.7 A	0.8	6.1	3.2 a	0.9	1.6	4231	41 a	14	108 aA	7555 b

[1]Averages in the same column followed by the same letter do not differ at 5 % significance by the Tukey Test. Difference between treatments (lowercase) and species (capital letters).

Table 5 – Cation exchange capacity of fine roots (< 1 mm) for the different species and treatment[1].

Species	Cation exchange capacity of fine roots			
	BF-250P	CC-250P	CS-125P	CS-250P
	mmol$_c$ kg^{-1}			
E.grandis	312	339 A	351 A	310 A
E. urophylla	320 a	282 bB	343 aA	317 aA
E. grandis × urophylla	313 a	247 bC	275 bB	252 bB
Mean	315	289	323	293
Pinus oocarpa	390	369	394	356
P. caribaea var. *hondurensis*	375	356	390	360
Mean	383	363	392	358

[1]Means with the same letter do not differ at 5 % significance by the Tukey test. Difference between treatments (lowercase) and species (capital letters).

cation exchange complex or to chelates formation with proteins and organic acids (Silva et al., 2004; Ikka et al., 2013).

Exudation of organic acids with low molecular weight may also be involved, which promote Al complexation in the rhizosphere and/or apoplast (Silva et al., 2004).

Chen et al. (2012) suggested that phosphate supply to substrates growth tends to have a protective effect against plant injury caused by Al, because Al-phosphate precipitates on the fine root surface or in the apoplast, inhibiting Al entry into symplast. This mechanism played some role especially to *Eucalyptus* species. In these species, despite higher P availability in CS-250P (high Al availability), a trend to lower leaf P concentration and higher root P concentration was found when compared with CC-250P (low Al availability). We also found higher leaf Al concentration when low P fertilizer was applied (Table 4).

There was higher CEC in *E. urophylla* roots in treatments under greater soil Al availability (Table 5). This is contrary to our initial hypothesis, because reduction of root CEC is an Al exclusion mechanism that reduces Al diffusion to the surface (Vale et al., 1996). This increase in root CEC can be a strategy to increase plant Ca uptake and reduce Ca:Al ratio in the roots (Marschner, 1991). According to Abdala et al. (1998), a higher root CEC reduces NH$_4^+$ uptake, resulting in lower proton extrusion and higher rhizosphere pH. This may increase solubilization of P forms precipitated with Al and Fe.

The anions flux through micro pores of the cell wall to uptake plasmalemma sites is hampered by repulsion of fixed negative charges. Al neutralizes fixed negative charges, facilitating P flux. These may be mechanisms to raise the P uptake by the eucalypt plant. These mechanisms have possibly evolved along with ecological adaptation of several *Eucalyptus* species to dystrophic soils of Australia (Specht, 1986).

Silva et al. (2004) reported that root exposure of seedlings of six *Eucalyptus* species (*E. globulus* Labill., *E. urophylla* S.T. Blake, *E. dunnii* Maiden, *E. saligna* Sm.,

E. cloeziana F.J. Muell and *E. grandis* w. Hill ex Maiden) to exchangeable Al increased by more than 200 % the malic acid concentration in root tips, as well as a small increase in the citric acid concentration. On the other hand, Ikka et al. (2013) found that malate concentration in *E. camaldulensis* roots did not or only slightly increased under increasing Al availability, suggesting that *E. camaldulensis* detoxifies Al by forming Al-citrate complexes, which is achieved through Al-induced citrate accumulation in the root tips via the suppression of the citrate decomposition pathway. These findings suggest that *Eucalyptus* species might accumulate different types of organic acids in their roots in response to exchangeable Al.

In addition to these reported tolerance mechanisms to Al, *Eucalyptus* and *Pinus* are able to form association with soil microorganisms that may synergistically promote or stimulate Al tolerance to plant host (van Scholl et al., 2005; Arriagada et al., 2007; Smith and Read, 2008; Seguel et al., 2013). Once the substrate used was not sterilized, such association, especially with mycorrhizal fungi, may have played some role in Al tolerance; however, this was not assessed in our study.

Conclusion

Al availability in the soil at a level of up to 28 mmol$_c$ kg^{-1} had no effect on the growth of *Eucalyptus* and *Pinus* species. Both species avoided Al uptake rather than a detoxification mechanism in the leaves, once Al concentration in the root was 50-fold greater than in the leaves under high Al availability in the soil. Contradicting our initial hypothesis, root CEC had no influence on Al tolerance. The P supply played some role in Al tolerance only to *Eucalyptus* species. Foliar Mn concentration increases correlatively to soil Mn availability, indicating that the species of both genera have no mechanisms to avoid high Mn uptake. This suggest that these species are more susceptible to Mn than to Al toxicity.

Authors' Contributions

Conceptualization: Gonçalves, J.L.M.; Gomes, S.S. Data acquisition: Gomes, S.S. Data Analysis: Gomes, S.S.; Rocha, J.H.T. Design of Methodology: Gomes, S.S.; Gonçalves, J.L.M. Writing and editing: Rocha, J.H.T.; Gomes, S.S.; Gonçalves, J.L.M.; Menegale, M.L.C.

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