Aluminum toxicity in roots of legume seedlings assessed by topological analysis

Simone Meredith Scheffer-Basso[1*] and Bruna Carla Prior[2]

[1]Programa de Pós-graduação em Agronomia, Faculdade de Agronomia e Medicina Veterinária, Universidade de Passo Fundo, BR-185, 99052-900, Rio Grande do Sul, Passo Fundo, Brazil. [2]Curso de Agronomia, Faculdade de Agronomia e Medicina Veterinária, Universidade de Passo Fundo, Passo Fundo, Rio Grande do Sul, Brazil. *Author for correspondence. E-mail: sbasso@upf.br

ABSTRACT. The effect of aluminum (Al) on roots has been evaluated based on linear and weight measurements, but how this element influences the branching configuration of the root system remains unknown. The objective of this study was to assess aluminum toxicity in the roots of 21-day old seedlings from three forage legumes using topological analysis: *Adesmia latifolia, Trifolium repens* and *T. pratense*. The legumes were grown in dystrophic Red Latosol that had either been treated or not treated with dolomitic lime. This resulted in two Al saturation and pH treatments: (a) Al= 0%, pH= 6.2; and (b) Al= 16%, pH= 4.8. The following attributes were determined: number of first-order roots, external links (magnitude), total links in the longest unique path (altitude), total links in the primary root (primary root altitude), total exterior path length, total links, internal links, branching points and proportion of branching in the primary root. The topological variables were significantly reduced by Al for all of the legume species. There was a more randomized or dichotomous branching configuration in the seedlings grown in acid soil and *A. latifolia* was the most plastic of the species.

Keywords: *Adesmia*, altitude, *Lotus*, magnitude, path length, *Trifolium*.

Toxicidade do alumínio em raízes de plântulas de leguminosas forrageiras avaliada por análise topológica

RESUMO. O efeito do alumínio (Al) em raízes tem sido avaliado por medidas lineares e de peso, mas ainda não se conhece a influência desse elemento na configuração da ramificação do sistema radicial. Este estudo teve como objetivo avaliar a toxicidade do alumínio em plântulas de 21 dias de idade de três leguminosas forrageiras, *Adesmia latifolia, Trifolium repens* and *T. pratense*, por meio de análise topológica. As plantas foram cultivadas em Latossolo vermelho distrófico tratado ou não com calcário dolomítico, o que resultou em dois níveis de saturação de Al e pH: (a) Al= 0%, pH= 6,2; (b) Al= 16%, pH= 4,8. Foram determinados: número de raízes de primeira ordem, segmentos externos (magnitude), segmentos do caminho mais longo (altitude), total de segmentos da raiz primária (altitude da raiz primária), segmentos totais, segmentos internos, pontos de ramificação e pontos de ramificação, caminho total percorrido total, proporção de ramificações na raiz primária e comprimento da raiz primária. As variáveis topológicas foram reduzidas significativamente pelo Al, independente de leguminosa. Na presença de alumínio o sistema radicial mostrou configuração de ramificação mais aleatória ou dicotômica e *A. latifolia* foi a leguminosa com maior plasticidade.

Palavras-chave: *Adesmia*, altitude, *Lotus*, magnitude, caminho percorrido, *Trifolium*.

Introduction

The aluminum (Al) tolerance of plants is commonly evaluated during the seedling stage, which might be more critical than their later stages of growth (MERIÑO-GERGICHEVICH et al., 2010; VOIGT; MOSJIDIS, 2002). Al toxicity is associated with severe changes in root morphology. Briefly, it results in curved, swollen, cracked, brownish, stubby and stiff root apices (VARDAR et al., 2006). The criteria most often utilized for evaluating Al toxicity are measurements of the growth, number, color and branching pattern of the root. However, other aspects are also examined, such as the root topology, which is an important component of the entire root system architecture (BERNTSON, 1997). To the best of our knowledge, there has been no published studies on root topology in response to Al toxicity, and knowledge of root morphology and architecture would be very useful both for efforts to breed plants for nutrient efficiency (CRUSH et al., 2005) and for ecological studies on the adaptation of species to acid soils.

Architectural analysis of the roots might be approached in terms of the distribution of the

branches within the system (topology), the lengths and diameters of the internodes or links, or the two angles of branching; these three factors combined can be defined as the geometry of the system (FITTER, 1987). The earliest method for performing topology studies was proposed by Werner and Smart (1973); these studies evaluated drainage networks and were based on the concept of topologic path length or the number of links between the network outlet and a junction or source. Fitter (1987) adapted this method to root systems, proposing that under conditions in which the acquisition of soil resources limits growth, the topology of the roots could be termed 'herringbone', which was defined as a main axis and its primary lateral roots only. An extreme version of this type was termed 'dichotomous' or randomized branching, in which all external links formed new branches (Figure 1). The author proposed a topological index to characterize the branching structure of root system. Using this index, branching of root systems is possible to describe as a herringbone (theoretical maximum index value 1) or dichotomous type (index value approaches zero), or between these two extremes, and thus relate growth strategy of root system – i.e. exploration versus exploitation – to the explicit value (KALLIOKOSKI et al., 2008). Teruel et al. (2001) indicated that the topological index efficiently detected structural changes in soybean (*Glycine max* (L.) Merrill) root systems while they were growing under low soil phosphorus (P) concentrations.
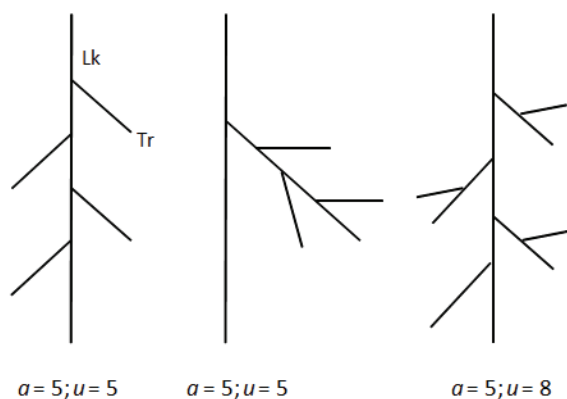
Figure 1. Diagram of branching configuration of system root with herringbone pattern relative to primary root (1a) and lateral root (1b) and randomized branching (1c). a = number of links (Lk) of the greater path length; u = number of the tip roots (Tr = external links).

This approach has not been used for evaluating the responses of plants to Al or to acid soils, and investigating these aspects is important for studying processes of adaptation or tolerance to these specific conditions. In this paper, the responses of three

forage legumes to Al are described and assessed by topological analysis. The legumes are wide-leaf adesmia (*Adesmia latifolia* (Spreng.) Vog.), white clover (*Trifolium repens* L.) and red clover (*T. pratense* L.). Wide-leaf adesmia is a non-domesticated and stoloniferous plant that is native to South America. It grows on river banks, in humid areas and even in flooded places. It tolerates sea water, flooded soils and marshes with a pH of 6-7, providing excellent quality forage in lowland areas (ARTUCIO; BEYHAUT, 1998). White clover and red clover are the most important legumes in temperate climate pastures, whose pH should be in the range of 6.0 to 7.0 (CQFS, 2004). Both legumes have the same life-history (perennial) and life form (herbaceous).

The objective of this study was to evaluate the effects of Al on the root topology of these forage legumes to answer the following questions: (1) Does Al affect the topological variables of seedling roots?; (2) Does Al change the topological configuration of the root system?; (3) Do the species vary in their responses to Al?

Material and methods

This experiment was conducted in Passo Fundo, Rio Grande do Sul State (28° 15' S, 52° 24' W and 687 m above sea level), in March 2011, in a greenhouse covered with transparent plastic film and protected laterally by a transparent plastic net without temperature or radiation controls. The minimum, maximum and mean temperatures during this month were 16.4, 27.0 and 21.0ºC, respectively. The 1000-seed weight was determined for the legume species (Sp): wide-leaf adesmia = 2.86 g, white clover = 0.64 g and red clover = 0.70 g. The seeds of wide-leaf adesmia were scarified with hot water at 70ºC for 60 s before planting because their seed coats are otherwise impermeable to water.

The seedlings were cultivated in soil that was classified as a typical dystrophic Red Latosol (EMBRAPA, 2005) using two Al saturation levels (0 and 16%) in a completely randomized design with eight replications. The Al levels were achieved by altering the supply of lime: in the control treatment (0%), dolomitic lime was applied at the recommended rate (CQFS, 2004) required to increase the pH to 6.0; in the 16% treatment, dolomitic lime was applied at 20% of this rate. No fertilizer or inoculant was applied to the soil. The soil was watered at pot capacity, and after two months, the soil analysis detected variations in both the pH and the Al levels (Table 1); at this point, the experiment was started.

Table 1. Physicochemical attributes of dystrophic Red Latosol with total and partial liming.

Attributes	Soil	
	Partial liming (20%)	Total liming (100%)
Clay (%)	65.0	65.0
pH H_2O	4.8	6.2
P (mg dm^{-3})	6.8	7.4
K (mg dm^{-3})	80.0	80.0
Organic matter (%)	2.8	2.3
Al (cmol dm^{-3})	0.9	0.0
Ca (cmol dm^{-3})	3.7	8.0
Mg (cmol dm^{-3})	0.9	0.5
Base saturation (%)	44.0	83.0
Aluminum saturation (%)	16.0	0.0

Polystyrene pots (21 cm height x 10 cm diameter) were filled with 4 dm^3 of treated soil and sand in a 3:1 ratio, followed by watering and seeding. Approximately one week after the seedlings emerged, they were thinned to one plant per pot. The plants were grown without water restriction. The harvest occurred 21 days after seedling emergence; the roots were immediately immersed in water to remove the substrate and the length of the primary root was measured. Each plant was placed on wet cotton cloth inside a plastic bag and maintained in a refrigerator until the end of their evaluation, which was performed two days later. The 21-day growth period was selected based on the research of Glimskär (2000) and Arredondo and Johnson (2009).

The roots were spread on a pane of glass with no overlapping root intersections, and each seedling root system was illustrated using free hand drawings for a total of 64 drawings. All measurements were taken from these drawings as follows: (1) the number of first-order roots (F_R); (2) the number of external links or root tips (μ = magnitude); (3) the number of links in the longest unique path, from the base link to the exterior link (a = altitude); (4) the number of links on the primary root, from the base link to the exterior link (a_{PR} = primary root altitude); (5) the total exterior path length (P_e = sum of the links along all possible unique paths from the base link to all exterior links); and (6) the length of the links from the primary root. Based on these data, the following variables were calculated according to Werner and Smart (1973): (1) the number of links in the root system ($Lk = 2(\mu)-1$); (2) the number of internal links ($Lk_I = Lk - \mu$); (3) the branching points ($BP = \mu-1$); (4) $a_{min} = (\log_2(u -1) + 2]$; (5); $Pe_{max} = \frac{1}{2}(u^2 + 3u - 2)$; and (6) $Pe_{min} = u (a_{min} + 1) - 2^{amin-1}$. The proportion of the branching on the primary root (F_R/BP) relative to the entire root system was based on Paula and Pausas (2011). The response index by the legumes to Al was calculated using the difference between the two means corresponding to the Al treatments divided by the maximum value of each variable.

The branching pattern, whether herringbone or randomized, was verified using the followings indices: (1) altitude-slope (a-slope): slope of regression of $Log_{10}(a)$ on $Log_{10}(\mu)$ (FITTER; STICKLAND, 1991); (2) $Log_{10}(a)/Log_{10}(u)$, which was proposed by Glimskär (2000) as an alternative to the first index because it is independent of plant size; and (3) $Log10(a) – Log10(a_{min})/Log10(a_{max}) – Log10(a_{min})$, which according to Danjon et al. (2004) allows for comparisons between root systems of various sizes. A new index was proposed in this study and was calculated using the ratio of $Log_{10}(a_{PR})$ to $Log_{10}(u)$ to verify the relative positions of branching in the root systems relative to the primary root because the indices mentioned above do not indicate whether the herringbone pattern forms along the primary root or a lateral root (Figure 1). For all of the indices, values near 1.0 (maximum value) indicate a herringbone branching pattern, while lower values indicate randomized branching. The plasticity of the legumes was verified by the difference in the a-slope indices during the Al treatment.

All of the topological variables were Log_{10} transformed prior to the analysis to meet assumptions of normality and they were analyzed as a factorial analysis of variance (Sp x Al) with a completely randomized design. When the sources of variation were significant, the means were compared by the Tukey test. The response index was submitted to analysis of variance to evaluate the differences between the species, and compared using the Tukey test. The a-slope index was examined by graphical presentation (GLIMSKÄR, 2000).

Results and discussion

The toxicity caused by Al is thought to be one of the most significant limiting factors in the growth and development of plants in acid soils. Plant roots become thinner and darker in color, resulting in a lower efficiency of water and nutrient absorption, and this effect is more pronounced in seedlings than in adult plants (MERIÑO-GERGICHEVICH et al., 2010). Symptoms of Al toxicity are not commonly quantified, except for root length, and the effects of Al toxicity are determined based on visual evaluation alone. In this study, the effects of Al were quantified using topological variables in addition to the length of the primary root. The stress levels from acidity or Al were determined to be low to intermediate based on Voigt and Mosjidis (2002), which indicated a pH of 5.0 to 5.2 (Al saturation level from 6 to 14%) to be low stress and a pH of 4.6 to 4.7 (Al saturation level from 32 to 39%) to be intermediate stress.

Significant differences were observed between Al treatments, regardless of the legumes tested, although the interactions between the factors were not significant. In acid soil, all root attributes were reduced (p ≤ 0.05), except for the link lengths on the primary root (Table 2). The primary roots of the seedlings grown in acid soil were shorter and had lower altitudes due to the decreased branching. The first-order branches had an average reduction of 65% in the legumes grown in acid soil. The classic symptoms of Al toxicity are inhibition of root growth and reduction in root penetration and branching, which were observed in this study. Pavlovkin et al. (2009) and Janke et al. (2010) also observed reduction in the elongation of the roots of birdsfoot trefoil (*Lotus corniculatus* L.).

Table 2. Means and standard deviations of the root attributes of 21-day old seedlings from three forage legumes grown in soil with distinct aluminum saturation, in the average of the species.

Root attributes	Aluminum saturation	
	0%	16%
Primary root altitude (nº)	19.19 ± 6.12	7.57 ± 3.43 B
Root system altitude (nº)	19.19 ± 6.12	8.52 ± 3.60 B
Root system magnitude (nº)	20.67 ± 7.08	10.32 ± 4.44 B
Primary root length (cm)	11.46 ± 3.09	4.48 ± 2.41
Internode length of primary root (cm)	0.64 ± 0.19	0.66 ± 0.31 NS
First-order roots (nº)	18.19 ± 6.12	6.57 ± 3.43
Path length (nº)	248.19 ± 144.03	70.43 ± 47.33 B
Maximum path length (nº)	278.33 ± 169.17	83.29 ± 59.59 B
Minimum path length (nº)	114.33 ± 49.89	47.71 ± 26.13
Total links (nº)	40.33 ± 14.16	19.76 ± 8.87
Internal links (nº)	19.67 ± 7.08	9.38 ± 4.44
Branching in the primary root (%)	94.48 ± 6.59	73.12 ± 24.69

Different letters in the row indicate significant differences between treatments by Tukey test at p ≤ 0.05 with no-transformation values.

The reduction in branching was verified by the measured values for internal links, branching points and magnitude (Table 2). In acid soil, the decrease (50%) in magnitude (root tips or external links) revealed the indirect effect of Al on the absorption process because the root tips are the parts of the plant with the highest water and nutrient absorptive capacity (PAULA; PAUSAS, 2011). Exposure to Al causes stunting of the primary root and inhibition of lateral root formation, and the root tips become stubby due to the inhibition of both cell division and cell elongation (SAMAC; TESFAYE, 2003). Intensively branched roots, which have abundant root tips, are highly efficient at transporting water because of the small overall distance between the tips and the root crown (FITTER, 1986).

The branches were located almost totally along the primary root in the seedlings grown in the soil without Al (Table 2), which is consistent with the finding that at the seedling stage, the most significant allocation of branches occurs along the main axis (PAULA; PAUSAS, 2011). However, this pattern was altered when the plants were cultivated

in acid soil, where 27% of the internal links were located outside of the primary root, revealing the inhibited growth of the primary root. The comparison between the altitudes of the root system and of the primary root also indicates variations in the locations of root branching. In the absence of Al, the root system and the primary root exhibited the same altitude, revealing that in this condition, there was a marked development of the main axis, resulting in a herringbone pattern along this root. The opposite effect was observed in the presence of Al, where the altitude of the primary root was smaller than the root system altitude, which represents a more randomized branching pattern.

The observed path length, as well as the maximum and minimum possible values for observed magnitude, was reduced in the plants grown in acid soil (Table 2). The number of links along all possible paths from the base link to all exterior links (FITTER; STICKLAND, 1991) represents the paths taken by nutrients from their absorption to the root base. The similarity of Pe to Pe_{max} reveals a more herringbone pattern, which was specifically observed in the absence of Al, where the differences were smaller compared with the acid soil. In this branching configuration, the path length is greater when compared with the randomized pattern, which provides a more efficient structure for soil exploration. On the other hand, a randomized branching root system is more efficient for nutrient transport because of the shorter path length. On rangelands, where lime and fertilizers are superficially applied (KAMINSKI et al., 2005), it is possible that species with randomly branching root systems could have advantages because of the greater efficiency of this root configuration in exploiting a restricted soil volume. In places with frequent droughts, selecting species with herringbone root systems would be desirable.

Although the Sp x Al was not significant (p ≥ 0.05), the Al-response index of the legumes showed significant differences for the main topological variables (Table 3). Wide-leaf adesmia and red clover had the extreme values, with more significant changes in the root traits in the former, suggesting differences in Al tolerance. Although the occurrence of wide-leaf adesmia in the south of Brazil might indicate a greater tolerance to acid soil relative to red and white clovers, there is not enough information on their degree of Al tolerance. Unfortunately, there are no references to the pH in the locations where this species occurs in southern Brazil, but Artucio and Beyhaut (1998) indicated that this species inhabits marshy areas, with a pH of 6-7. This pH is the recommended pH for white and red clovers, which might explain the similar Al

response of the three species based on the topological variables measured in this study.

Table 3. Aluminum-response index of 21-day old seedlings from three forage legumes considering the topological variables.

Topological variables	Legumes		
	A. latifolia	T. pratense	T. repens
Root system altitude	0.39 A	0.22 B	0.30 AB
Root system magnitude	0.36 A	0.18 B	0.21 AB
Path length	0.36 A	0.19 B	0.24 AB
Number of total links	0.31 A	0.15 B	0.17 B
Number of internal links	0.41 A	0.20 B	0.25 AB

Different letters in the row indicate significant differences between treatments by Tukey test at p ≤ 0.05 with no-transformation values. Aluminum-response index = difference between the two means corresponding to Al treatments divided by the maximum value for each species.

Among the indices calculated to verify the distribution of the branches within the root systems, the only one that showed a significant difference between the Al treatments was the one based on the altitude of the primary root (Table 4). This index indicated that seedlings grown in acid soil showed a small (0.95) but significant (p ≤ 0.05) deviation from the herringbone branching pattern (1.0). In contrast, in the absence of Al, the index revealed a markedly herringbone pattern, with a value (0.99) near the theoretical maximum (1.0), indicating that the lateral roots were located on the primary root. The other indices, which also include the root system altitude instead of the primary root value, were in agreement, showing no differences in root topology between Al treatments. This result is in contrast to that reported by Berntson (1997), in which the indices used to describe the root topology were poorly correlated with one another.
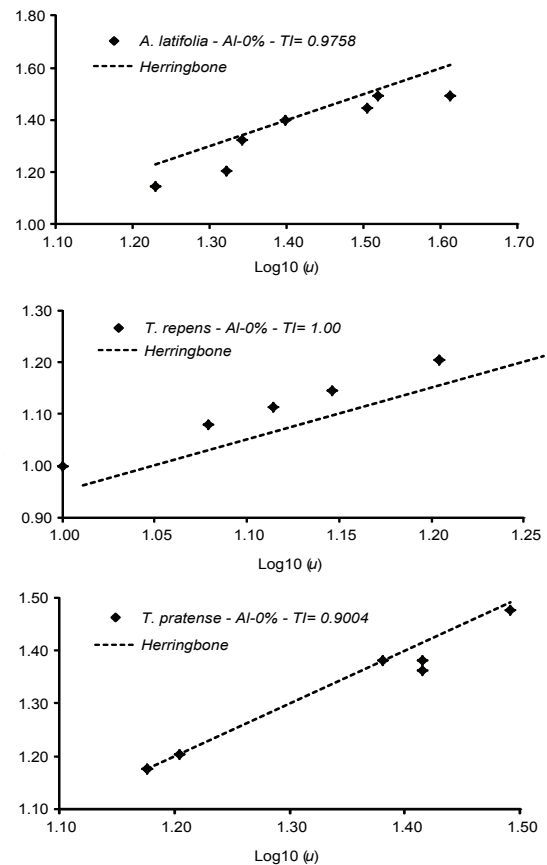
Table 4. Topological index of 21-day old seedlings from three forage legumes grown in soil with distinct aluminum saturation, in the average of species.

Index	Al saturation	
	0%	16%
Alternative topological index (GLIMSKÄR, 2000) $[\mathrm{Log}_{10}(a)/\mathrm{Log}_{10}(u)]$	0.989 ± 0.01^{ns}	0.971 ± 0.02^{ns}
Relative topological index (DANJON et al., 2004) $[\mathrm{Log}10(a) - \mathrm{Log}10\,(a_{min})/\mathrm{Log}10\,(a_{max}) - \mathrm{Log}10\,(a_{min})]$	0.984 ± 0.02^{ns}	0.917 ± 0.08^{ns}
Primary root index $[\mathrm{Log}_{10}(a_{PR})/\mathrm{Log}_{10}(u)]$	0.988 ± 0.01 A	0.955 ± 0.04 B

Different letters in the row indicate significant differences between treatments by Tukey test at p ≤ 0.05. ^{ns}= no significant.

The branching pattern, which was verified by the slope of $\mathrm{Log}_{10}(a)$ on $\mathrm{Log}_{10}(u)$ and is routinely used to illustrate changes in root architecture (WU et al., 2005), showed very high values for the legumes grown in the absence of Al, with values between 0.8942 (red clover) and 1.0 (white clover). This indicated a marked herringbone configuration (Figure 2). Fitter and Stickland (1991) found a slope of 0.8111 to be common to several dicots, which was considered a very high value, implying a near-

herringbone branching pattern. In the seedlings of dicots, this branching pattern is common as documented by Larkin et al. (1995) in alfalfa (*Medicago sativa* L.) because it is necessary for initial penetration, exploration and anchorage by the roots. However, as the plants grew to 3-4 weeks of age, the root branches became more prolific, and the root systems shifted gradually from a herringbone pattern towards a more highly branched pattern.

Figure 2. Relationship between logarithm of altitude (*a*) and logarithm of magnitude (*u*) for the root system of 21-day old seedlings of *Adesmia latifolia, Trifolium pratense* and *T. repens* grown in aluminum absence. TI = topological index. Dotted line indicates a hypothetic herringbone configuration (*a = u;* TI = 1.0).

In the present study, in the acid soil, the slopes were smaller (Figure 3) and showed a more random branching pattern, especially in wide-leaf adesmia, with a value of 0.5935. This slope was smaller when compared with the slope found by Wu et al. (2005) for *Fraxinus mandshurica* Rupr., which was grown using a different phosphate concentration. Their research determined that the decrease in the slopes, from 0.9823 to 0.8514, indicated that the seedlings with low phosphate availability (0.05 mmol L^{-1}) changed from their original herringbone pattern to random branching when phosphate availability increased (1.00 mmol L^{-1}). Figures 2 and 3 also illustrate the

differences among the magnitude values according to different soil treatments. In the presence of Al, the magnitude varied between 0.4771 (wide-leaf adesmia) and 1.3892 (red clover), with smaller values and higher amplitudes between the various legumes. In the absence of Al, the magnitude was higher, but the amplitude lower, varying from 0.9542 (white clover) to 1.6128 (red clover).
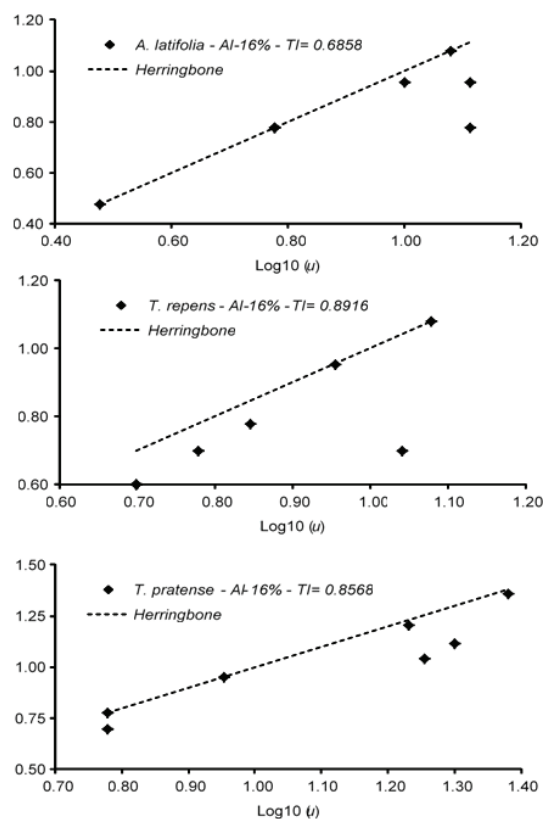
Figure 3. Relationship between logarithm of altitude (a) and logarithm of magnitude (u) for the root system of 21-day old seedlings of *Adesmia latifolia, Trifolium pratense* and *T. repens* grown in aluminum presence. TI = topological index. Dotted line indicates a hypothetic herringbone configuration ($a = u$; TI = 1.0).

The topological variation in wide-leaf adesmia at the two Al levels showed the species' greater plasticity based on the difference between the slopes in the two soil conditions (0.29), compared with the white (0.10) and red clovers (0.04); this result was consistent with the Al-response index (Table 3). In white clover, Fitter and Stickland (1992) also indicated an apparent lack of response to soil nutrient variations, with the pH between 4.6 and 7.7 and water-soluble P between 2.9 and 11.3 $\mu g\ g^{-1}$. For the wide-leaf adesmia, considering its occurrence in natural pastures in southern Brazil where acid soil is the normal condition, whether the more topological plasticity of wide-leaf adesmia is an adaptive trait for acid soil could be determined in a future study is would?

The only reference to the effects of Al on root topology was a statement that Al toxicity can increase the topological index of red clover (FITTER; STICKLAND, 1992), but this result was not published. The lack of information about the effects of Al on this aspect of root architecture limits the comparison and discussion of the present results. However, Fitter (1986) and Fitter and Stickland (1991) documented a significant variation in the topological index of red clover plants under water restriction and low nutrient conditions, indicating a herringbone pattern. In white clover, Fitter and Stickland (1992) indicated that the root system became more herringbone shaped in moist soil, and Crush et al. (2005) observed indices between 0.93 and 0.95 when this legume was grown in sand and nutrient solutions, respectively, indicating that root media had little effect on the branching configuration. Larkin et al. (1995) verified that the seedling roots of alfalfa exhibited this branching pattern when infected by *Pythium* spp., compared with those in un-infected soil, suggesting that the root system was maintained at a more juvenile or immature stage under stressful conditions. Glimskär (2000) indicated that nitrogen limitation led to more herringbone-like root systems in *Polygala vulgaris* L. and *Crepis praemorsa* (L.). F.L. Teruel et al. (2001) verified that soybeans cultivated in a solution with low phosphorus concentrations showed a more herringbone-like configuration. Echeverria et al. (2008), evaluating the effects of salinity on the root system of the *Lotus glaber* Mill (Fabaceae) genotypes, verified a more herringbone-like architecture in the sensitive genotype.

The use of topological analysis for the selection of or breeding for Al tolerance needs to be evaluated using several genotypes within each species, as well as using species of known tolerance and sensitivity to this element. For forage legumes, it is suggested that alfalfa (Al sensitive) and big trefoil (*Lotus pedunculatus* L.; syn.: *L. uliginosus* Schkuhr) (Al tolerant). The main limitation of this method is in the labor needed for drawing the root systems, which prevents the use of a larger number of replicates. However, it is believed that this technique may be improved by staining the roots to enhance the contrast when digitizing of the roots.

Conclusion

The well-known effects of aluminum on the exploration of soil resources by plants based on its deleterious effects on root elongation and on the

exploitation process itself based on its reduction of absorption points are confirmed in this study using topological analysis. The results confirm the validity of this methodology for describing the external effects of Al on root systems in addition to those described in the literature. Independent of indices or variables, the effect of Al on root systems is illustrated by a more randomized branching configuration.

Acknowledgements

References

ARREDONDO, J. T; JOHNSON, D. A. Root responses to short-lived of soil nutrients and shoot defoliation in seedlings of three rangeland grasses. **Rangeland Ecology and Management**, v. 62, n. 5, p. 470-479, 2009.

ARTUCIO, P. I.; BEYHAUT, R. **Las leguminosas en Uruguay y regiones vecinas**. Parte 1: Papilionoideae. Montevideo: Hemisferio Sur, 1998.

BERNTSON, G. M. Topological scaling and plant root system architecture: development and functional hierarchies. **New Phytologist**, v. 135, n. 4, p. 621-634, 1997.

CQFS-Comissão de Química e Fertilidade do Solo. **Manual de adubação e de calagem para os Estados do Rio Grande do Sul e de Santa Catarina**. 10. ed. Porto Alegre: SBCS; Núcleo Regional Sul/UFRGS, 2004.

CRUSH, J. R.; CARE, D. A.; GOURDIN, A.; WOODFIELD, D. R. Root growth media effects on root morphology and architecture in white clover. **New Zealand Journal of Agricultural Research**, v. 48, n. 2, p. 255-263, 2005.

DANJON, F.; BERTHIER, S.; GOUSKOU, K. Root system topological and fractal branching analysis in *P. pinaster*. In: GODIN, C.; HANAN, J.; KURTH, W.; LACOINTE, A.; TAKENAKA, A.; PRUSINKIEWICS, P.; JON, T.; BERERIDGE, C.; ANDRIEU, B. (Ed.). **International Workshop on Functional-Structural Plant Models**. Montepellier: UMR AMAP, 2004. p. 75-78.

ECHEVERRIA, M.; SCAMBATO, A. A.; SANNAZZARO, A. I.; MAIALE, S.; RUIZ, O. A.; MENÉNDEZ, A. B. Phenotypic plasticity with respect to salt stress response by *Lotus glaber*: the role of its AM fungal and rhizobial symbionts. **Mycorrhiza**, v. 18, n. 6-7, p. 317-329, 2008.

EMBRAPA-Empresa Brasileira de Pesquisa Agropecuária. **Sistema Brasileiro de classificação de solos**. Rio de Janeiro: Embrapa-Solos, 2005.

FITTER, A. H. An architectural approach to the comparative ecology of plant root systems. **New Phytologist**, v. 106, Supplement, p. 61-77, 1987.

FITTER, A. H. The topology and geometry of plant root systems: influence of watering rate on root system topology in *Trifolium pratense*. **Annals of Botany**, v. 58, n. 1, p. 91-101, 1986.

FITTER, A. H.; STICKLAND, T. R. Architectural analysis of plant root systems. II. Influence of nutrient supply on architecture in contrasting plant species. **New Phytologist**, v. 118, n. 3, p. 375-389, 1991.

FITTER, A. H.; STICKLAND, T. R. Architectural analysis of plant root systems. III. Studies on plants under field conditions. **New Phytologist**, v. 121, n. 2, p. 243-248, 1992.

GLIMSKÄR, A. Estimates of root system topology of five plants species grown at steady-state nutrition. **Plant and Soil**, v. 227, n. 1, p. 249-256, 2000.

JANKE, A.; DALL'AGNOL, M.; SANTOS, A. M.; BISSANI, C. A. Seleção de populações de *Lotus corniculatus* L. com maior tolerância ao alumínio em solução nutritiva. **Revista Brasileira de Zootecnia**, v. 39, n. 11, p. 2366-2370, 2010.

KALLIOKOSKI, T.; NYGREN, P.; SIEVÄNEN, R. Coarse root architecture of three boreal tree species growing in mixed stands. **Silva Fennica**, v. 42, n. 2, p. 189-210, 2008.

KAMINSKI, J.; SANTOS, D. E.; GATIBONI, L. C.; BRUNETTO, G.; SILVA, L. S. Eficiência da calagem superficial e incorporada precedendo o sistema plantio direto em um argissolo sob pastagem natural. **Revista Brasileira de Ciência do Solo**, v. 29, n. 4, p. 573-580, 2005.

LARKIN, R. P.; ENGHISH, J. T.; MIHAIL, J. D. Effects of infection by *Pythium* spp. on root system morphology of alfalfa seedlings. **The American Phytopathological Society**, v. 85, n. 4, p. 430-435, 1995.

MERIÑO-GERGICHEVICH, M.; ALBERDI, A. G.; IVANOV, A. G.; REYES-DIAZ, M. Al^{3+}- Ca^{2+} interaction in plants growing in acid soils: Al-phytotoxicity response to calcareous amendments. **Journal of Soil Science and Plant Nutrition**, v. 10, n. 3, p. 217-243, 2010.

PAULA, S.; PAUSAS, J. G. Root traits explain different foraging strategies between resprouting life histories. **Oecologia**, v. 165, n. 2, p. 321-331, 2011.

PAVLOVKIN, J.; PAL'OVE-BALANG, P.; KOLAROVIC, L.; ZELINOVÁ, V. Growth and functional responses of different cultivars of *Lotus corniculatus* to aluminum and low pH stress. **Journal of Plant Physiology**, v. 166, n. 4, p. 1479-1487, 2009.

SAMAC, D. A.; TESFAYE, M. Plant improvement for tolerance to aluminum in acid soils – a review. **Plant Cell, Tissue and Organ Culture**, v. 75, n. 3, p. 189-207, 2003.

TERUEL, D. A.; DOURADO-NETO, D.; HOPMANS, J. W. Alterações estruturais do sistema radicular de soja em resposta à disponibilidade de fósforo no solo. **Scientia Agricola**, v. 58, n. 1, p. 55-60, 2001.

VARDAR, F.; ARICAN, E.; GOZUKIRMIZI, N. Effects of the aluminum on *in vitro* root growth and seed

germination of tobacco (*Nicotiana tabacum* L.). **Advances in Food Science**, v. 28, n. 1, p. 85-88, 2006.

VOIGT, P. W.; MOSJIDIS, J. A. Acid-soil resistance of forage legumes as assessed by a soil-on-agar method. **Crop Science**, v. 42, n. 5, p. 1631-1639, 2002.

WERNER, C.; SMART, J. S. Some new methods of topologic classification of channel networks. **Geographical Analysis**, v. 5, n. 4, p. 271-295, 1973.

WU, C.; WEI, X.; SUN, H.; WANG, Z. Phosphate availability alters lateral root anatomy and root architecture of *Fraxinus mandshurica* Rupr. seedlings. **Journal of Integrative Plant Biology**, v. 47, n. 3, p. 292-301, 2005.