EFFECT OF HYDROGEN ON SOYBEAN ROOT GROWTH IN A SUBSURFACE SOLUTION[1]

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ABSTRACT - A vertical split-root technique was used to evaluate the effects of pH levels in a subsurface solution compartment on root development of soybean (*Glycine max* (L.) Merr.). The surface root compartment (12 cm) was filled with limed and fertilized soil. Treatments in the solution compartment were seven pH levels (3.7, 4.0, 4.3, 4.6, 4.9, 5.2, and 5.5) with 2 µM Ca, 18 µM B, and 0.5 µM Zn. Even when root growth in the subsurface solution compartment was restricted by pH, there were no differences in aboveground dry matter production. The number of other roots extending from the soil into the solution compartment increased with time and, at day 12, decreased by 13.3 roots/pot with each unit increase in solution pH. Final main root length increased 26.6 cm/plant with each unit increase in solution pH, while lateral root length increased exponentially. Low pH (<4.3) inhibited lateral roots formation on main roots, and increased the number of other roots. At higher pH values the average length of laterals and number of other roots increased. At pH 3.7 other roots accounted for 88.3% of the total root length (TRL), main roots accounted for 11.7%, and lateral roots were not present. At pH 5.5 TRL was comprised by 15.1% from other roots, 54.9% from main roots, and 30.0% from lateral roots. Low pH reduced the length of laterals more than main roots.

Index terms: subsurface acidity, main root, lateral roots, root length.

EFEITO DO HIDROGÊNIO NA SOLUÇÃO SUBSUPERFICIAL NO CRESCIMENTO DE RAÍZES DE SOJA

RESUMO - Usou-se a divisão vertical das raízes para avaliar o efeito do H$^+$ na solução subsuperficial no crescimento de raízes de soja (*Glycine max* (L.) Merr.). O compartimento superior (12 cm) foi enchido com solo calcariado e adubado. Os tratamentos estavam na solução subsuperficial com os seguintes pHs (3,7, 4,0, 4,3, 4,6, 4,9, 5,2, e 5,5) com concentrações básicas de 2 µM Ca, 18 µM B, e 0,5 µM Zn. Mesmo quando o crescimento da raiz na subsuperfície foi afetado pelo pH, não houve diferenças na produção de matéria seca. O número de raízes vindas do solo através da membrana para a solução aumentou com o tempo e no 12° dia decresceu de 13,3 raízes/vaso com cada unidade de aumento de pH. O comprimento da raiz principal aumentou linearmente 26,6 cm/planta com o aumento de uma unidade do pH da solução, enquanto o das raízes laterais aumentou exponencialmente. Baixo pH (<4,3) inibiu a formação de raízes laterais na raiz principal e aumentou o número de outras raízes que cruzavam a membrana. O pH alto aumentou o comprimento médio das raízes laterais e de outras raízes. Em pH 3,7, outras raízes contribuíram com 88,3% e as raízes principais com 11,7% no comprimento radicular total (CRT) e não havia presença de raízes laterais. Em pH 5,5, a participação no CRT foi representado por 15,1% por outras raízes, 54,9% pelas raízes principais e 30,0% pelas raízes laterais. O comprimento das raízes laterais foi mais afetado pelo H+ do que o das raízes principais.

Termos para indexação: acidez subsuperficial, raiz principal, raízes laterais, comprimento radicular.

INTRODUCTION

In acid soils, shallow rooting and restricted plant access to available subsoil water are common problems. These problems occur despite appropriate liming and fertilization of surface soil layers. Adverse conditions to deep root growth have

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been attributed to subsoil acidity, infertility (Rios & Pearson, 1964; Lund, 1970; Adams & Moore, 1983)) and unfavorable physical conditions (Bowen, 1981). All of these conditions exist to some degree in highly weathered soils.

Poor plant growth in soils with pH < 5.0 has normally been associated with phytotoxic Al concentrations in the soil solution (Foy, 1984). In studies with soybean, Lund (1970) also indicated that a relatively high H+ concentration could be an important factor limiting plant growth in certain acid soils.

Many soils are acid in their native state with pH values around 4.0 (Kamprath, 1984). Soil pH values can be lowered when fertilizers are applied through displacement of Al from exchange sites and subsequent hydrolysis of the Al in solution (Kamprath, 1984). In these situations, root injury due to H+ concentration could be expected. At pH ≤ 5, H+ concentration can suppress uptake of both Ca and Mg and result in deficiencies of these elements, particularly in soils with very low cation exchange capacity (Kamprath, 1984). For non-acid soils pH can be lowered by continuous application of N fertilizers (Mason, 1980) and by high rates of atmospheric deposition of acidic compounds such as SO_2, NOx and NH_3 (Ulrich, 1983; Ulrich et al., 1980). Even under normal conditions, Williams (1980) observed a decrease in pH from 6 to 5 to a soil depth to 30 cm in a 50-year-old clover pasture. In some of these situations, root injury due to H+ can be expected.

At low pH values cell membranes leak and there are disturbances of the plasmalemma function by H^+ competition for negative charges at the plasmalemma. Integrity of the later is impaired if Ca^{++} is replaced by H^+ (Runge & Rode, 1991). Ionic displacement is probably responsible for the fact that H^+ increases the Ca requirement of membranes, sometimes resulting in a Ca deficiency (Lund, 1970). More recently, Noble et al. (1988) showed that soybean tap root growth was reduced at pH < 4.8. Wilkinson & Duncan (1989a, 1989b) showed that at pH < 5.5 sorghum juvenile root growth was inhibited through influences on IAA synthesis and/or transport. Optimum pH for root elongation in monocots (approximately pH 4) differs from dicots (pH 5 to 7) (Runge & Rode, 1991). Islam et al. (1980) also reported marked influence of solution pH on root weight among plant species with maximum root growth at the pH range of 5.5 to 6.5.

In acid soils with pH > 4.25 Howard & Adams (1965) considered, Al^{3+} and Mn^{2+} toxicities to be more important than H^+ toxicity in limiting the growth of cotton roots. Responses to H^+ toxicity depends, however, on plant species (monocots or dicots) (Edwards et al., 1976; Runge & Rode, 1991), and can vary within the same species (Blamey et al., 1982, 1987). Legumes are more susceptible (Islam et al., 1980; Foy, 1984) than grasses because high H^+ concentration affects rhizobia survival and multiplication in soils, root infection and nodule initiation (Andrew, 1976). Evans & Kamprath (1970) attributed reduced corn growth under greenhouse conditions in an organic soil with pH < 4.0 to H^+ toxicity.

Direct measures of how H^+ influences plant growth in acid soils are difficult to determine, because Al, Mn, and other mineral elements may also be soluble in toxic concentrations and the availabilities of essential elements, particularly Ca, Mg, P and Mo, may be suboptimal. Islam et al. (1980) and Blamey et al. (1987), pointed out that the direct effects of solution pH on growth are complex and difficult to separate from the indirect effects associated with changes in the solubility and availability of various elements. To overcome these difficulties, solution culture experiments have been used (Islam et al., 1980; Blamey et al., 1982, 1987).

The objective of this study was to determine the extent to which increasing H^+ concentrations in a subsurface solution compartment restricted root growth of soybean plants established in a limed and fertilized surface soil compartment.

MATERIAL AND METHODS

This study was conducted in a greenhouse at North Carolina State University, Raleigh, North Carolina. Plants were grown with a vertically split-root system as shown in Fig. 1.

PVC cylinders, 52 cm long and with 10 cm inside diameter, were divided into two compartments by a paraffin-petrolatum membrane. The surface compartment (12 cm) contained 1.3 kg of loamy sand taken from the Ap horizon of a Wagram (loamy, siliceous, thermic; Arenic

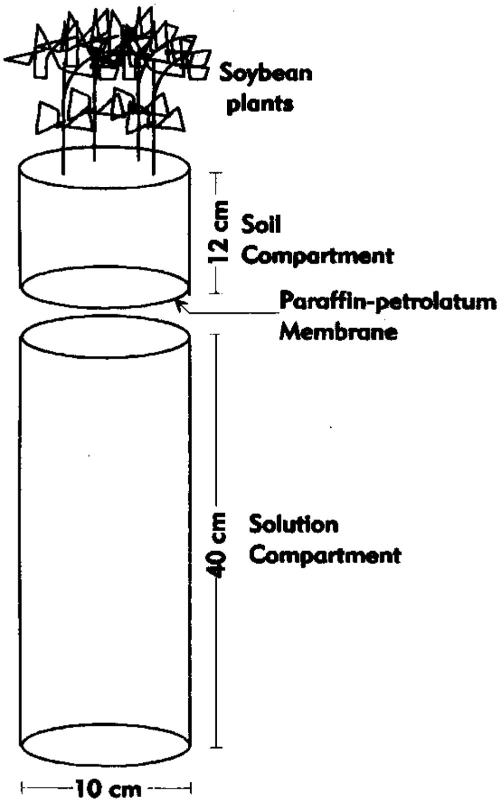

Fig. 1. Diagram of the experimental system for evaluating the effects of different acidity components on soybean root growth in a subsurface solution.

Kandiudult) collected from the Border Belt Tobacco Research Station at Whiteville, North Carolina. Selected chemical soil properties before liming and fertilization were pH 4.3, 0.81 cmol$_c$.kg^{-1} of Ca, 0.08 cmol$_c$.kg^{-1} of Mg, 0.05 cmol$_c$.kg^{-1} of K, 0.34 cmol$_c$.kg^{-1} of Al, 26.5 % Al saturation, 0.64 cmol$_c$.kg^{-1} of exchangeable acidity, and 20.5 mg.kg^{-1} of extractable P, and 0.9, 0.7, and 4.1 mg.kg^{-1} of Zn, Cu, and Mn, respectively. The soil was limed and incubated for two weeks to reach pH 5.5 with $CaCO_3$ and received 30 mg K.kg^{-1} before it was placed in the surface compartment. Prior to adding the soil the membrane was formed by dipping a single layer of attached cheesecloth for 10 seconds into a mixture of one part paraffin and two parts petrolatum heated to 80 °C.

The subsurface compartment contained 3.0 liters of continuously aerated solution. The bottom of the solution compartment was sealed with a PVC cap. The solution contained 2 mM Ca as $CaSO_4.H_2O$, 18.5 µM B as H_3BO_3 and 0.5 µM Zn as $ZnSO_4.7H_2O$. Solution treatments were seven pH levels (3.7, 4.0, 4.3, 4.6, 4.9, 5.2, and 5.5) which were adjusted daily by titration with either 0.05 N HCl or NaOH. There were three replications of each treatment in a randomized complete block design.

Soybean (*Glycine max* (L.) Merr.) cv. "Ransom" seeds were rinsed in 200 µM $CaSO_4.2H_2O$ and pregerminated in the dark for 2 days at 25 °C in Petri dishes containing filter paper moistened with the same Ca solution. Five seeds with a radicle length of 12±2mm were planted in the soil of each container which had been moistened to 80% of field capacity. This moisture was chosen to ensure that there was no water movement from the soil to the solution compartment during the experiment. The soil compartments were covered with clear polyethylene for two days, after which each container was thinned to four plants.

In the solution compartment length of the first root from each plant which crossed the membrane was measured daily for 12 consecutive days, beginning on the third day after planting which henceforth will be called day number one. On the sixth day and daily thereafter the number of other plant roots crossing the membrane were counted.

Plants were harvested on day 12 (15th day after planting). Aboveground plant height was measured and shoot dry weight was determined after drying for 24 hours at 70 °C in a forced-draft oven. Roots in the surface soil compartment were washed free of soil, length was measured by the line-intercept method (Tennant, 1975) and dry weight was determined after drying for 24 hours at 70 °C. In addition to daily root growth measurements in the solution compartment, length and number of laterals and other roots extending from the soil compartment were also measured at harvest. Data were analyzed using analysis of variance and regression procedures in the Statistical Analysis System (SAS Institute, Inc., 1985).

RESULTS AND DISCUSSION

Roots from soybean seedlings planted in the soil compartment crossed the membrane after three days (60 to 67 hours). The first root to cross the membrane was the main or primary root, which was easily identified throughout the experiment by its length, thickness, growth rate, and number of lateral roots. The secondary or lateral roots began to appear on the main root after four days of growth in the solution compartment. Other roots began to cross the membrane into the solution compartment on the fourth day and were counted daily from the sixth day forward. Maximum variation in solution pH over any 24-h period for each pH treatment was 3.7±0.03, 4.0±0.05, 4.3±0.07, 4.6±0.05, 4.9±0.07, 5.2±0.06, and 5.5±0.09.

Shoot growth and root growth in the soil compartment

Since there was adequate water and nutrient supply for plants in the soil compartment, pH treatments in the subsurface solution compartment did not influence aboveground dry matter production or root growth in the soil. Mean shoot heights for pH treatments 3.7 and 5.5 were 9.5 and 9.8 cm, and mean dry weights were 1.05 and 0.95 g/pot, respectively.

There was no difference among solution pH treatments in total length and dry weight of roots for the limed and fertilized surface soil compartment. For pH treatments of 3.7 and 5.5 total root length in the soil was 29.7 and 17.8 m/pot, and corresponding root dry weight was 1.14 and 0.67 g/pot, respectively. Although there was visually more root branching near the membrane in the soil compartment for the low pH treatment, differences among treatments were not significant.

Root growth in the solution compartment

Growth patterns over time:

There was a significant interaction between solution pH and time on soybean main root length in the subsurface compartment. There was no effect of solution pH on main root length for the first day after roots crossed the membrane into the solution compartment. Suthipradit & Alva (1986) evaluated soybean radicle growth in solutions with pH ranging from 4.0 to 5.5. The lack of differences in radicle length during the initial 72 hours of growth led these authors to suggest that germination or initial radicle growth were less sensitive to pH than root elongation.

Statistical analysis by time revealed that there were little or no differences ($P < 0.05$) in root length within three subsets of the pH treatments, namely low pH (<4.6), medium pH (4.6 and 4.9) and high pH (pH > 4.9). Treatments were, therefore, grouped accordingly and regression equations describing mean root length as a function of time were generated for each subset of pH treatments (Table 1).

Soybean main root length increased with both time and solution pH (Fig. 2). After the first day root length in the intermediate pH range was consistently lower than in the high pH treatments. Furthermore, differences in main root length between pH treatments increased with time. At day 12 mean root length for pH 4.6 and 4.9 was 59.1% of that measured in the high pH treatments (5.2 and 5.5). In solutions with low pH main root growth was small. During 12 days of exposure to solutions with pH < 4.6 average main root length increased by only 3.52 cm/plant. In

Table 1. Regression equations for prediction of main root length (cm/plant) with time of growth in a subsurface compartment with solutions adjusted to different pH levels.

pH range	Equations*	R^2
3.7 to 4.3	$Y= 0.66 + 0.62\text{day} - 0.033\text{day}^2$	0.96
4.6 and 4.9	$Y= 2.87 + 5.49\text{day} - 0.263\text{day}^2$	0.99
5.2 and 5.5	$Y= 6.10 + 8.87\text{day} - 0.041\text{day}^2$	0.99

* Regression coefficients are significant at $P < 0,05$.

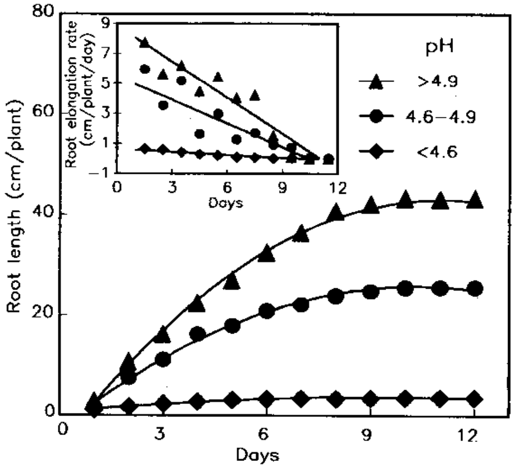

Fig. 2. Observed (symbols) and predicted (lines) soybean main root length and root elongation rate as a function of time of exposure to solutions with different pH levels.

solutions with pH < 4.6 main roots had visual symptoms of H^+ injury, namely stunted growth, brownish color and little lateral root development. Similar symptoms were observed by Islam et al. (1980) on wheat, maize and tomato roots at pH < 4.8 in a complete nutrient solution.

Other investigators have reported similar differences in root growth to variations of solution pH when the entire root system was contained in a single nutrient solution compartment. Wilkinson & Duncan (1989a, 1989b) observed H^+ damage to main root growth of sorghum upon reducing pH below 5.5. Noble et al. (1988) reported a 57% reduction in soybean tap root length when solution pH was decreased from 4.8 to 4.2, in the presence of 2.5 mM Ca. Manzi & Cartwright (1984) observed that differences in cowpea root length between solution of pH 4 and 5 increased with time. Root length differences increased from 20% at 12 days after planting to 84% at 63 days after planting.

Predicted root elongation rates (=dL/dt; where L=length and t=time) were calculated from regression equations in Table 1 and are compared in Fig. 2 with mean root elongation rates for each sampling interval. Root elongation rates in all treatments declined in a linear pattern with time. Initial elongation rates increased with solution pH and there was a 14 fold difference between the high and low pH treatments. Increasing solution pH prolonged the time of soybean main root elongation. Predicted root elongation rate achieved a value of zero on day 9 with low pH as opposed to day 11 with high pH treatments.

In addition to main root measurements, the number of other roots that crossed the membrane into the solution compartment was counted daily during the final six days of the experiment. In all treatments the number of roots extending from the soil into the solution compartment increased with time (Fig. 3). Solution treatments with low pH (3.7-4.3) tended to have a greater number of roots than treatments with high pH (5.2 and 5.5). These data support visual observations that in the soil compartment there was more root branching near the membrane separating compartments in the treatments with low pH. In low pH solutions impedance of main root growth by H^+ toxicity apparently stimulated root branching in the soil compartment, and a greater number of root extended into the subsurface solution. These results support observations by Zobel et al. (1992) and Zobel (1992) that plant roots are an integrated system and the quantity of one root component modifies the functions and quantities of other root components.

Characteristics of root components at harvest:

Total root length in the solution compartment at day 12 and contributions of main roots, laterals, and other roots which crossed the membrane are shown in Fig. 4 for each solution pH treatment. Main

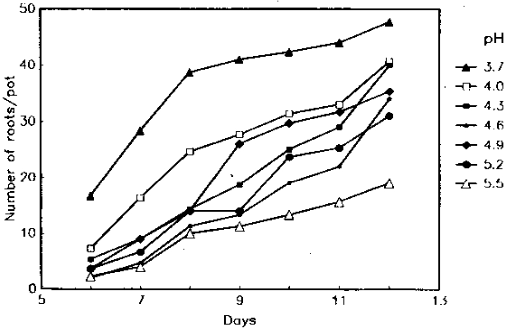

Fig. 3. Effect of subsurface solution pH on the number of roots crossing the membrane during the final six days of the experiment.

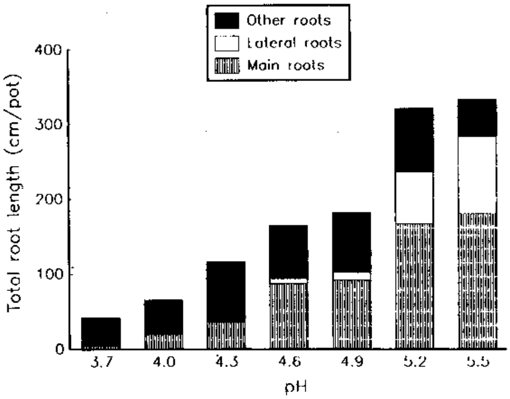

Fig. 4. Total root length in the solution compartment at 12 days as a function of root system components and solution pH.

root length increased at a linear rate of 26.6 cm/plant (r^2=0.985) with each unit increase in solution pH. These results are similar to the responses in sorghum root growth observed by Wilkinson & Duncan (1989a) across a solution pH range of 4.0 to 5.5 in the absence of toxic levels of Mn. Although soybean main root length increased from 1.23 to 5.12 cm/plant, between pH treatments of 3.7 and 4.0 no laterals developed on the main root. Between pH 4.3 and 5.5 lateral root length increased exponentially from 0.29 to 26 cm/plant and lateral root number increased from 2.92 to 118.8 per plant. Average lateral root length of each root on main root, therefore, increased from 0.10 at pH 4.3 to 0.22 cm at pH 5.5. Lateral root number increased with pH and lateral root density on main roots decreased from 6.3 roots/cm at pH 4.3 to 3.3 roots/cm at pH 5.5.

The number of other roots which crossed the membrane from the soil compartment decreased by 13.33 roots/pot with each unit increase in solution pH ($Y=97.287 - 13.33pH$, r^2=88**). Whereas the number of other roots decreased with increasing pH, their average length increased by 1.1 cm with each unit increase in solution pH ($Y=-2.931 + 1.10pH$, r^2=0.87**). Because of these opposing trends of pH on characteristics of other roots crossing the membrane, their contribution of total root length remained constant (mean=63.75 cm/pot) in all pH treatments (Fig. 4). Data in Fig. 4 also indicate that the contribution of individual root components to total root length in the solution compartment differed with pH treatments. At pH 3.7, for example, other roots crossing the membrane accounted for 88.3% of the total root length, and main roots accounted for 11.7%. At pH 5.5 total root length was comprised by 15.1% from other root length, 54.9% from main roots, and 30.0% from secondary roots. Results from this experiment indicate that limited soybean root growth in acid subsoils can be partially attributed to H^+ toxicity and the magnitude of the solution H^+ concentration will have different effects on the growth of individual root system components.

CONCLUSIONS

1. Hydrogen toxicity reduced main root growth and inhibited lateral roots formation on main roots, and increased the number of other roots that crossed the membrane from the soil into the solution compartment.
2. High H^+ concentration reduced the length of laterals more than main roots.
3. Restriction on soybean root growth in acid subsoils can be partially attributed to H toxicity.

REFERENCES

ADAMS, F.; MOORE, B.L. Chemical factors affecting root growth in subsoil horizons of Costal Plan Soils. **Soil Science Society of America Journal**, v.47, p.99--102, 1983.

ANDREW, C.S. Effect of calcium, pH and nitrogen on the growth and chemical composition of some tropical and temperate pasture legumes. I. Nodulation and growth. **Australian Journal of Agriculture Research**, v.27, p.611-623, 1976.

BLAMEY, F.P.C.; ASHER, C.J.; EDWARDS, D.G. Hydrogen and aluminum tolerance. **Plant and Soil**, v.99, p.31-37, 1987.

BLAMEY, F.P.C.; EDWARDS, D.J.; ASHER, C.J.; KIM, M.K. Response of sunflower to low pH. In: INTERNATIONAL PLANT NUTRITION COLLOQUIUM, 9., 1982, Warwick. **Proceedings**. Warwick: [s.n.], 1982. p.66-71.

BOWEN, H.D. Alleviation mechanical impedance. In: ARKIN, G.F.; TAYLOR, H.M. (Eds.). **Modifying the root environment to reduce crop stress**. St. Joseph: American Society of Agricultural Engineering, 1981. p.21-27. (ASAE Monograph, 4).

EDWARDS, D.G.; ASHER, C.J.; WILSON, G.L. Mineral nutrition of cassava and adaptation to low fertility conditions. In: SYMPOSIUM OF INTERNATIONAL SOCIETY OF TROPICAL ROOT CROPS, 4., 1976, Cali. **Proceedings**. Cali: CIAT, 1976. p.124-130.

EVANS, C.E.; KAMPRATH, E.J. Lime response as related to percent Al saturation, solution Al, and organic matter content. **Soil Science Society America Proceedings**, v.34, p.893-896, 1970.

FOY, C.D. Physiological effects of hydrogen, aluminum and manganese toxicities in acid soil. In: ADAMS, F. (Ed.). **Soil acidity and liming**. Madison: ASA/CSSA/SSSA, 1984. p.57-97. (Agronomy Monograph, 12).

HOWARD, D.D.; ADAMS, F. Calcium requirements for penetration of subsoils by primary cotton roots. **Soil Science Society America Proceedings**, v.29, p.558--562, 1965.

ISLAM, A.K.M.; EDWARDS, D.G.; ASHER, C.J. pH optima for crop growth:Results of a flowing solution culture experiment with six species. **Plant and Soil**, v.54, p.339-357, 1980.

KAMPRATH, E.J. Crop response to lime on soils in the tropics. In: ADAMS, F. (Ed.). **Soil acidity and liming**. Madison: ASA/CSSA/SSSA, 1984. p.349-367.

LUND, Z.F. The effect of calcium and its relation to several cations in soybean root growth. **Soil Science Society of America Journal**, v.34, p.456-459, 1970.

MASON, M.G. An investigation of reduction in wheat yields after use of a high level of ammonium sulphate for a number of years. **Australian Journal of Experimental Agriculture and Animal Husbandry**, v.20, p.210-219, 1980.

MANZI, J.M.; CARTWRIGHT, P.M. The effects of pH and Aluminum toxicity on the growth and symbiotic development of cowpeas (*Vignia unguiculata* (L.) Walp.). **Plant and Soil**, v.80, p.423-430, 1984.

NOBLE, A.D.; SUMNER, M.E.; ALVA, A.K. Suitability of the aluminon technique for measuring phytotoxic aluminum in solutions with varying sulfate concentrations. **Communications in Soil Science and Plant Analysis**, v.19, p.1495-1508, 1988.

RIOS, M.A.; PEARSON, R.W. The effect of some chemical environmental factors on cotton root behavior. **Soil Science Society of America Proceedings**, v.28, p.232-235, 1964.

RUNGE, M.; RODE, M.W. Effects of soil acidity on plant associations. In: ULRICH, B; SUMNER, M.E. (Eds.). **Soil Acidity**. New York: Springer-Verlag, 1991. p.181-202.

SAS Institute, Inc. **SAS user's guide**:*Statistics*. Cary, N.C.: SAS Inst., 1985.

SUTHIPRADIT, S.; ALVA, A.K. Aluminum and pH limitations for germination and radicle growth of soybean. **Journal of Plant Nutrition**, v.9, p.67-73, 1986.

TENNANT, D. A test of a modified line intersect method of estimating root length. **Journal of Ecology**. v.63, p.995-1001, 1975.

ULRICH, B. Soil acidity and its relation to acid deposition. In: ULRICH, B.; PANKRATH, J. (Eds.). **Effects of accumulation of air pollutants in forest ecosystems**. Bordrecht: Reidel Publishing, 1983. p.127-146.

ULRICH, B.; MAYER, R.; KHANNA, P.K. Chemical changes due to acid precipitation in loess derived soil in Central Europe. **Soil Science**, v.130, p.193--199, 1980.

WILKINSON, R.E.; DUNCAN, R.R. Sorghum seedlings root growth as influenced by H^+, and Ca^{++} and Mn^{++} concentrations. **Journal of Plant Nutrition**, v.12, p.1379-1394, 1989a.

WILKINSON, R.E.; DUNCAN, R.R. H^+, Ca^{++} and Mn^{++} influence on sorghum seedlings growth. **Journal of Plant Nutrition**, v.12, p.1395-1407, 1989b.

WILLIAMS, C.H. Soil acidification under clover pasture. **Australian Journal Experimental Agriculture and Animal Husbandry**, v.20, p.561-567, 1980.

ZOBEL, R.W. Soil environment constraints to root growth. **Advances in Soil Science**, 1° v.19, p.27-51, 1992.

ZOBEL, R.W.; L.V. KOCHIAN, L.V.; TOULEMONDE, T.G. Plant root systems. In: H.F. REETZ, H.F. (Ed.). **Proceedings of the Roots of Plant Nutrition**. Champaign: USDA/ARS, 1992. p.30-40.