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Predicting Initial Tall Fescue Root Growth Response to Calcium/Aluminum Solution Concentrations

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Abstract: A study was conducted to quantify effects of soluble aluminum (Al) and gypsum ($CaSO_4$) on initial root growth of three varieties of tall fescue (*Festuca arundinacea*). Experiments were performed in a growth chamber using hydroponic solutions containing Al from 0 to74 μM in combination with $CaSO_4$ at 0 to10 mM. Seedlings were grown for 7 d, harvested, air dried, scanned, and weighed for treatment comparisons. Significant differences in root length existed between varieties in Al-only solutions at low Al concentrations. All varieties showed reduced root growth at concentrations greater than 37 μM Al. Increased calcium (Ca^{2+}) and sulfate (SO_4^{2-}) at given concentrations of Al resulted in greater root growth. Relative root growth increased approximately 30% to >80% at 37 μM Al as $CaSO_4$ increased from 2.5 to 10 mM. A simple logistic model adequately described the effects of Al and $CaSO_4$ on root growth (r^2 = 0.86, 0.95, and 0.96 for the three varieties).

Keywords: Aluminum toxicity, $CaSO_4$, simple logistic model, tall fescue, turfgrass roots

INTRODUCTION

Aluminum toxicity is a common occurrence on acidic soils that limits root growth and reduces water and nutrient uptake, particularly from

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subsoils where conventional liming treatments are impractical (Foy and Fleming 1978; Sumner and Yamada 2002). Field studies have demonstrated that amendment with gypsum ($CaSO_4 \cdot 2H_2O$) promotes root growth into subsoils for a number of field crops and have suggested mechanisms for this effect that include reduced exchangeable aluminum (Al), reduced Al activity in soil solution, and greater calcium (Ca) availability (Shainberg et al. 1989).

Studies of Al toxicity often focus on plant species differences in tolerance to Al but do not address the interactions of Al with other ions in solution that may affect root health (Kim et al. 2001; Liu 2005). The presence of Ca is known to affect root growth response in Al-containing hydroponic solutions (Pintro and Taylor 2004; Ryan and Kochian 1993; Noble, Fey, and Sumner 1988; Alva et al. 1986a, 1986b). Mechanistically, elongating root tips require adequate Ca supplies, whereas Al binds to root surfaces and inhibits root development and function. Thus, models of root growth in mixed Ca/Al solutions, such as soil solutions from acidic soils, must take into account the interactive effects of these ions in describing root development (Ryan and Kochian 1993). Concentrations of Al measured in water extractions from acidic soils range from 0 to 223 μM (Carvalho and van Raij 1997; Shainberg et al. 1989; Sumner et al. 1986; Adams, Pearson, and Doss 1967).

The logistic model is a regression approach used to model sigmoidally distributed data. It has been used to model soil mesofauna growth response to pesticide residuals (Folker-Hansen, Krogh, and Holmstrup 1996), crop response to environmental factors such as ambient temperature and soil moisture (Panozzo and Eagles 1999), and optimal rates of nitrogen application to forage grasses under various conditions (Overman and Brock 2003a, 2003b).

Given that few mechanistic studies of Ca/Al interactions have been conducted with grass species, tall fescue (*Festuca arundinacea*, Schreb.) was used as the assay plant to pursue the following experimental objectives: 1) determine seedling root response to various Al and Ca concentrations in solution, 2) use the logistic model to quantify the interactive effects of Ca and Al on root growth, and 3) employ the model to identify levels of Ca that may ameliorate toxic Al effects at levels found in field soils.

MATERIALS AND METHODS

Hydroponic Experiments

Hydroponic studies were conducted utilizing an incomplete factorial design with varying Ca and Al levels and three varieties of turf-type tall fescue. The tall fescue varieties selected ('Rendition,' 'Dynasty,' and 'K31') were chosen on the basis of their frequent utilization in the southeastern United States. Square polyethylene containers (approx. 11 × 11 × 8 cm deep) were fitted with a 156-cm^2 stainless steel wire mesh to suspend seed at the top of the pot and were covered with a layer of cheesecloth. Each pot received 1.60 g of a single seed variety. After hand distributing the seed on the cheesecloth, pots were filled with deionized (DI) water to the point that the cheesecloth became saturated and randomly arranged in a growth chamber (25 °C) maintained under continuous light intensity of 95 $W\,m^{-2}$. Water levels were maintained daily using DI water. When roots in all pots became visible through the wire mesh (approx. 5d), the DI water was replaced with an appropriate hydroponic solution.

In initial experiments investigating varietal root sensitivity to Al (without added Ca), fescue varieties were grown in $AlCl_3$ solutions ranging from 0 to 74.1 μM as treatment levels. All solutions were stabilized at pH 4.5 by slowly adding 0.01 M hydrochloric acid (HCl) under constant stirring. Three replicates of each treatment and variety were grown for 7 d, after which roots were harvested by clipping at the contact with the steel mesh support. Roots were dried and weighed to the nearest 0.1 mg.

In subsequent experiments, tall fescue was grown in solutions containing levels of Al from aluminum chloride ($AlCl_3 \cdot 6H_2O$; 0 to 70 μM) and/or $CaSO_4$ (0 to 10,000 μM). One to three experimental replicates were assigned to each treatment combination to maximize the range of Al and $CaSO_4$ levels evaluated. The combined Al and $CaSO_4$ solutions were formulated by first diluting saturated 14 mM $CaSO_4$ solution with DI water to the appropriate molarity, then slowly adding 6.67 mM Al from a buret as the solution was stirred, followed by addition of 0.01 M HCl from a buret under constant stirring until a pH of 4.5 was reached. No other nutrients were added to the solution. Plants were grown and harvested after 7 d, as in the initial experiments.

Root Measurement Evaluation

In addition to root mass measurements, selected subsamples of dried roots were placed on a 300-dpi flatbed scanner with a black background and scanned to obtain root surface area values. ARC INFO software (Esri, Inc.; Redlands, Calif.), calibrated using known areas of white monofilament line, was used to quantify white pixels from the scanner images. Examination of root samples under 40 × magnification showed a uniform root width of approximately 0.3 mm, and this value was used to estimate root length from the root area measurements.

Statistical Analysis

To assess interactions between tall fescue varieties and Al factors in the model, root response to Al was analyzed by the analysis of varience (ANOVA) procedure (SAS Institute 1991). Contrast analyses were evaluated, assuming optimal root growth occurred at Al = 0 level for each of the three varieties. Dunnett's method was used to assess significant differences in root response to added Al.

A simple, three-parameter logistic model predicted effects of $CaSO_4$ concentrations on root growth across Al levels for the three varieties independently; the form and parameters of the model are shown in Figure 1 and Table 1. Where Al is absent (Al = 0), the equation $(\mathbf{\Phi}_{1i} - \boldsymbol{\beta}_i \times \gamma_i^{Ca(k)}) \times \mathbf{D}_1$ models the initial curve and horizontal asymptote of the predicted Al = 0 growth curve for each variety. Where Al is present, the remaining portion of the equation models the predicted growth curves for each variety as a function of $CaSO_4$ and Al. The $\mathbf{D}_1$ and $\mathbf{D}_2$ act as toggle switches to activate or deactivate appropriate portions of the equation, depending on the presence or absence of Al in solution. Optimal levels of root growth were estimated by interpreting the $\mathbf{\Phi}_1$ parameter of the model for each tall fescue variety. These estimates were interpreted as the

$$y_{ijkl} = (\Phi_l - \beta * \gamma^{CaSO_4(k)}) * D_l \left(\frac{\Phi_{1j}}{1 + \exp\left(\frac{\Phi_{2j} - CaSO_{4(k)}}{0.721(\Phi_{3j} - \Phi_{2j})} \right)} \right) * D_2 + \varepsilon_{jkl}$$

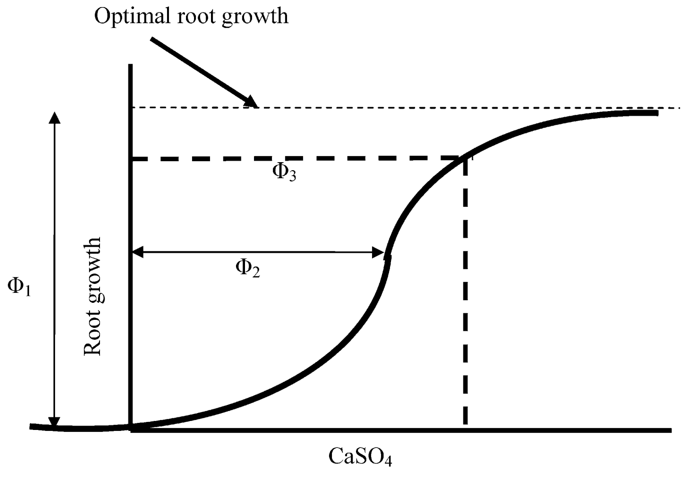

Figure 1. Simple logistic model showing the parameters Φ_1, the horizontal asymptote as $x \rightarrow \infty$, Φ_2 the value of x for which $y = \Phi_1 * 0.5$, and Φ_3, the value of x for which $y = \Phi_1 * 0.8$.

Table 1. Definitions of symbols in a modified simple logistic model used to characterize tall fescue root growth in response to Al and $CaSO_4$ in solution and to predict $CaSO_4$ levels necessary to overcome Al toxicity

Symbol	*Definition*
i	1 to 3 fescue varieties
j	1 to $m_{(i)}$ levels of aluminum
k	1 to 6 levels of calcium
l	1 to $n_{(i)}$ replicates of each variety of turf
$m_{(i)}$	Where $m_{(1)} = 8$ for 'Rendition', $m_{(2)} = 5$ for 'K31', and $m_{(3)} = 5$ for 'Dynasty'
$n_{(i)}$	Where $n_{(1)} = 90$ for 'Rendition', $n_{(2)} = 48$ for 'K31', and $n_{(3)} = 48$ for 'Dynasty'
Φ_{1ij}	A parameter determining the horizontal asymptote of the predicted growth curves for each variety of fescue at each level of aluminum
Φ_{2ij}	A parameter determining the value of calcium at which ½ of the horizontal asymptote is achieved for each variety of fescue at each level of aluminum
Φ_{3ij}	A parameter determining the value of calcium at which 0.80 of the horizontal asymptote is achieved for each variety of fescue at each level of aluminum
y_{ijkl}	The mean predicted root mass (mg) for the i-th fescue variety, measured at the j-th level of aluminum, at the k-th level of calcium.
β_i	A parameter for the aluminum = 0 curve for the i-th fescue variety determining the initial intercept of that curve
γ_i	A parameter for the aluminum = 0 curve for the i-th fescue variety
$Ca_{(k)}$	The value of the k-th level of calcium
D_1	Dummy variable coded 1 if Al = 0, 0 otherwise
D_2	Dummy variable coded 1 if Al > 0, 0 otherwise
ε_{ijkl}	Residual error assumed to be $N(0,\sigma^2)$

optimal root growth of a given variety at a given level of Al. Predicted values of $CaSO_4$ that would achieve 80% of optimal root growth were obtained by interpreting the $\mathbf{\Phi}_3$ parameter for each tall fescue variety.

RESULTS AND DISCUSSION

Root Measurement Evaluation

Root growth at low Al was very rapid, and roots reached the bottom of the containers at the end of the growth period. Root mass was highly correlated with the calculated root length based on root scanning, resulting in the relationship

$$\text{Length(m)} = 152.33 \times \text{Mass(g)} - 0.34$$

with an $r^2 = 0.91$ (n = 36) (Figure 2). As observed root diameter remained unchanged across treatment levels, root mass was used to determine root growth response to the treatments (Figure 3).

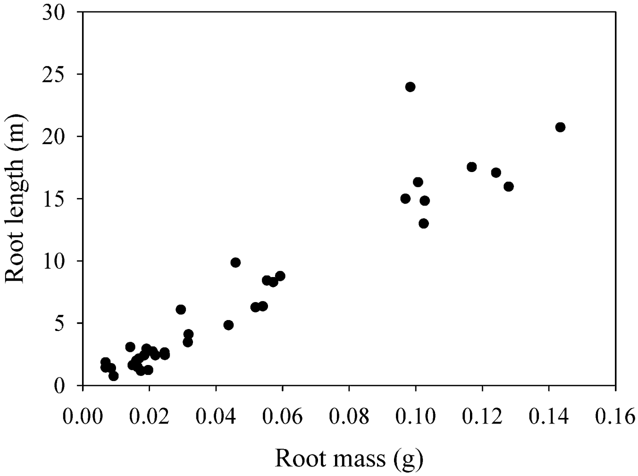

Figure 2. Comparison of calculated root length to root mass of tall fescue seedlings grown in hydroponic solution containing various levels of $CaSO_4$ and Al.

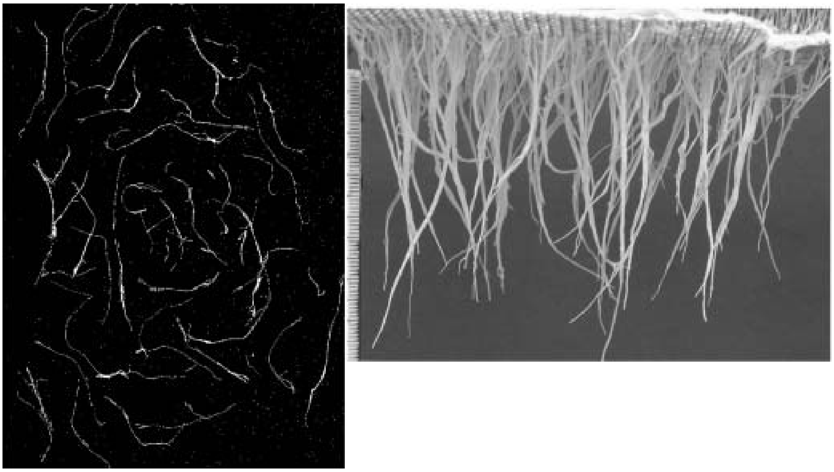

Figure 3. Root scan for digital analysis of root surface area using ARC INFO (left) and fescue root growth in 0 Al and 10,000 µM $CaSO_4$ (right), showing uniformity of root diameters in solution.

Root Response to Aluminum

All three fescue varieties demonstrated similar sensitivity to increasing levels of Al in solution (Figure 4). Initial results of the ANOVA showed tall fescue root mass to be significantly affected by interactions between Al concentration and cultivar. Contrast analysis showed significant reduction in root mass in all varieties at 7.4 μM Al. Despite initial assumptions that turf varieties would differ greatly in Al sensitivity, all varieties showed less than 15% relative root growth at solution concentrations of 37.0 μM Al (1 mg L^{-1}).

Root Response to Ca and Al in Solution

The addition of $CaSO_4$ to Al-containing solutions significantly impacted root growth. Increasing $CaSO_4$ was directly related to relative root growth for all three varieties at 7.4 μM Al and greater (Figure 5). Greater $CaSO_4$ levels resulted in significantly greater relative root growth, particularly at concentrations greater than 14.8 μM Al. For example, at 37.0 μM Al (1 mg L^{-1}), a concentration of 2500 μM $CaSO_4$ increased relative root growth from less than 15% to about 30%. At 5000 μM $CaSO_4$, relative root growth at the same level of Al increased 60 to 70% relative to the untreated, and at 10,000 μM $CaSO_4$ (about 70% saturated) relative root growth was 80% of optimal. Significant differences were observed between varieties at reduced levels of $CaSO_4$ and greater levels

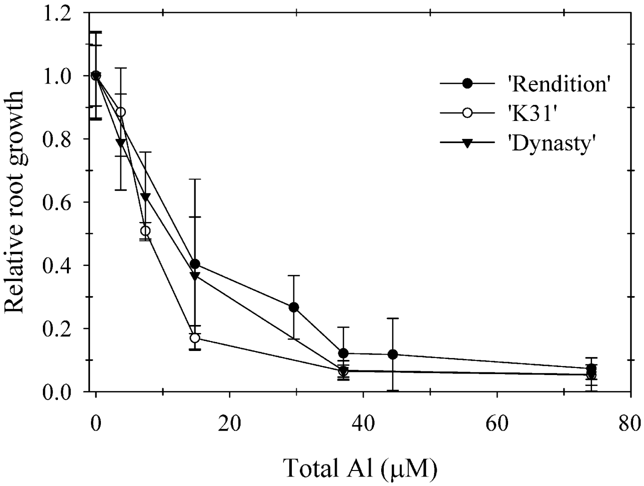

Figure 4. Relative tall fescue root growth sensitivity to total Al in an acidic (pH 4.5) hydroponic solution. Error bars indicate standard deviation of mean values.

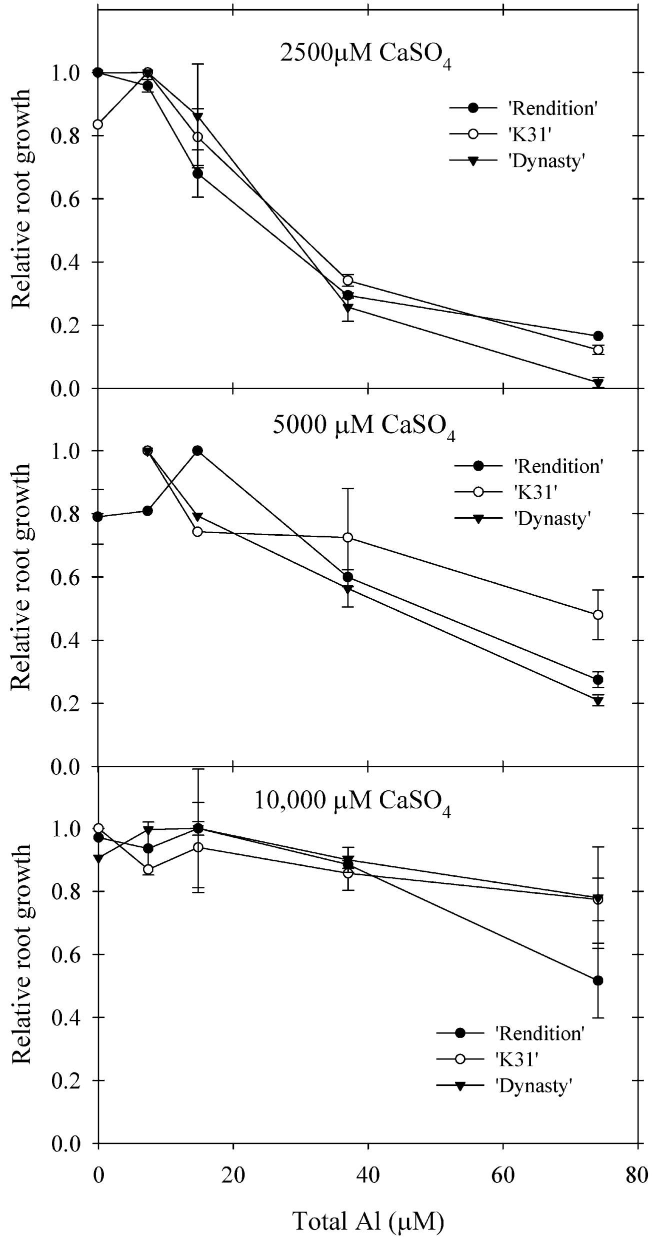

Figure 5. Effect of increasing $CaSO_4$ in solution on relative root growth in the presence of Al. Error bars indicate standard deviation of mean values.

of Al. The 'K31' variety showed significantly more relative root growth than the other varieties at 37.0 μM Al at 2500 and 5000, but not 10,000, μM $CaSO_4$. At combined concentrations of 2500 μM $CaSO_4$ and the highest Al treatment level (74.1 μM Al), root growth of 'Rendition' was greater than 'K31,' which was greater than 'Dynasty.' When $CaSO_4$ was increased to 5000 μM, root growth of 'K31' was significantly greater than that of 'Rendition' and 'Dynasty,' but all three showed greater relative root growth compared to the lesser $CaSO_4$ level. At 10,000 μM $CaSO_4$ and the greatest Al level, 'Dynasty' demonstrated the greatest root growth, increasing from roughly 20% to 80% relative root growth. From this it can be inferred 'Dynasty' is more sensitive to Ca-to-Al ratios than the other cultivars tested. Huang, Grunes, and Kochian (1992) reported Ca uptake by an Al-sensitive cultivar of wheat (*Triticum aestivum* L.Thell) was more inhibited by Al than a tolerant cultivar.

The factorial ANOVA analysis demonstrated no significant interaction of Al by $CaSO_4$ by variety. A significant interaction did occur between $CaSO_4$ and Al, probably due to the effect of ionic strength on Al speciation (Noble, Fey, and Sumner 1988; Alva et al. 1986a). Although there was a significant interaction between Al and variety ($P > F = 0.0228$), no such interaction occurred between $CaSO_4$ and variety ($P > F = 0.9338$), suggesting much less root sensitivity to changes in Ca solution concentration than Al. This observed response appears applicable to the soil environment where Ca uptake occurs by mass flow and Ca concentrations are typically orders of magnitude greater than Al in soil solution.

Simple Logistic Model

The reparameterized simple logistic model demonstrated slightly varying levels of fitness for each of the three varieties (Table 2). The model fit statistic was interpreted as the ratio of the variation explained by the model over the total variation in the data. Residual plots suggested no violations of assumptions for the model. Overall goodness of fit was satisfactory for the model to the data, with $r^2 = 0.86$, 0.95, and 0.96 for 'Rendition,' 'K31,' and 'Dynasty,' respectively.

Predicted optimal levels of root growth were calculated for each level of Al based on the $\mathbf{\Phi}_1$ parameter for each variety and are reported in

Table 2. Model fit statistics for the logistic model of each tall fescue variety

Variety	Model (DF)	Error (DF)	SSE	MSE	Root MSE	Model fit statistic
'Rendition'	19	62	22525.8	363.3	19.1	0.8589
'K31'	12	31	2084.2	67.2	8.2	0.9495
'Dynasty'	12	31	2828.9	91.3	9.6	0.9586

Table 3. There were significant differences in optimal root mass for each variety, indicating differences in overall vigor between cultivars. At some levels of $CaSO_4$, the varieties had greater optimal root growth in small concentrations of Al than in Al = 0. This phenomenon has been previously reported (Pintro and Taylor 2004; Ryan and Kochian 1993; Noble, Fey, and Sumner 1988) where parameter estimates were generated to define the molar concentration of $CaSO_4$ necessary to achieve 80% of optimal root growth for each variety.

Graphic displays of selected model-fit exercises are presented in Figure 6. In the absence of Al, an initial response to added $CaSO_4$ is observed. As Al concentration increased, recovery to 80% of optimal root growth occurred at greater concentrations of $CaSO_4$. This observed response enhanced the sigmoidal shape of the curve and shifted it to the right. At the greatest level of Al (74.1 µM), root growth does not approach the 80% level until the highest concentration of $CaSO_4$ is present (10,000 µM).

Model parameters suggest 'Rendition' required more $CaSO_4$ in solution to achieve 80% root growth recovery than the other varieties at all Al levels tested (Table 3). 'Rendition' also had the greatest predicted optimal level of root growth, suggesting vigorous rooting varieties may require greater levels of Ca. 'K31' required greater levels of $CaSO_4$ than 'Dynasty' at low Al; however, at greater levels of Al, the opposite was true. Although Al activity in soil solution is difficult to measure, most acidic soils in the southeastern United States contain soil-solution Al concentrations in the lower range of Al levels tested in this experiment (Shainberg et al. 1989; Sumner et al. 1986).

Gypsum ($CaSO_4 \cdot 2H_2O$) offers the potential to ameliorate subsoil acidity under established turfgrasses, including tall fescue. Gypsum is more soluble than limestone (2.41 g L^{-1} at 25 °C for $CaSO_4 \cdot 2H_2O$ versus 0.0153 g L^{-1} at 25 °C for $CaCO_3$) and demonstrates greater mobility through the soil profile with rainfall and irrigation water. Extensive research on soil chemical changes induced by surface applications of

Table 3. Predicted optimal levels and 80% levels of fescue root growth (mg), and $CaSO_4$ estimates (µM) for 80% of optimal root growth by level of aluminum and tall fescue variety

Variety	Root mass (mg)		Al (µM)				
	Predicted optimal level	80% of optimal level	3.7	7.4	14.8	37.0	74.1
'Rendition'	119.9	95.9	739	1438	3710	6285	8938
'K31'	89.8	71.8	nd[a]	731	1581	4328	6076
'Dynasty'	101.2	81.0	nd	470	1430	5504	7153

[a] nd, not determined.

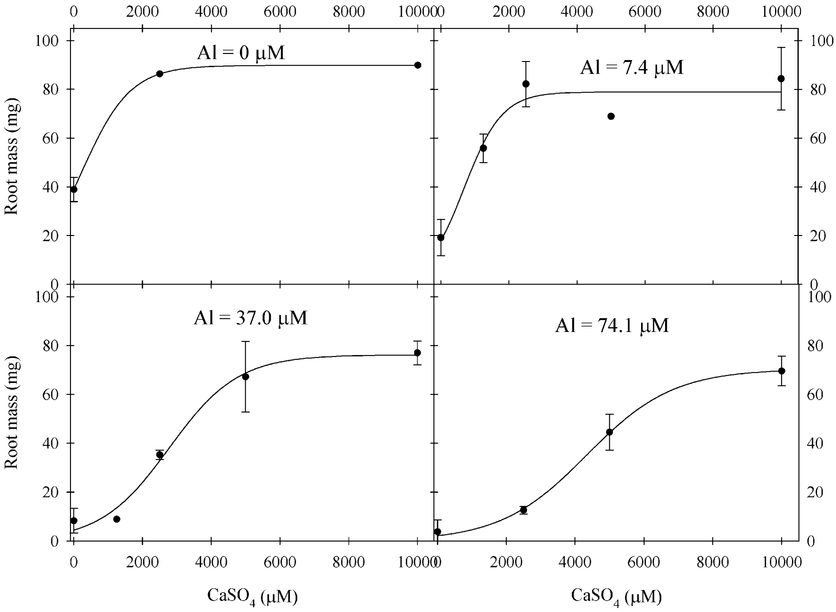

Figure 6. An example of fitting the simple logistic model: K31 root response to increasing levels of $CaSO_4$ at various fixed levels of Al. Error bars indicate standard deviation of mean values.

gypsum show improved crop yields (Ritchey, Belesky, and Halvorson 2004; Sumner 1995; Shainberg et al. 1989). The simple logistic model fit to this hydroponic investigation of tall fescue indicates gypsum may elicit a positive root growth response in the field by reducing Al activity in soil solution. At 37 μM Al in solution, approximately 5000 μM $CaSO_4$ (~1/3 saturated $CaSO_4$ solution) was required to maintain relative tall fescue root growth at 80% of the optimal level (Table 3). These values reside within the range of Al and $CaSO_4$ soil solution concentrations reported in several studies of acidic Ultisols and Oxisols treated with gypsum (Shainberg et al. 1989).

CONCLUSIONS

Acidic soils predominate in many parts of the world, and research results indicate gypsum can be an effective ameliorant of soil and subsoil acidity (Shainberg et al. 1989; Sumner 1995; Farina, Channon, and Thibaud 2000). A current limitation to the use of gypsum to effectively ameliorate soil acidity is accurately determining the quantity of material necessary for application. Although this study does not account for exchangeable and reserve soil acidity, the statistical modeling approach does estimate the quantity of gypsum required to reduce toxicity of Al in soil solution under acidic conditions. Some varieties of tall fescue are more vigorous in rooting than others, yet require more $CaSO_4$ to ameliorate the effects of Al toxicity. As Al in solution increases, more $CaSO_4$ is required to maintain root growth. $CaSO_4$ levels required to overcome Al are orders of magnitude higher than Al, suggesting severe sensitivity to slight increases in soil solution Al. These simple logistic models describe the relationship between $CaSO_4$ and tall fescue root growth at a given Al soil solution concentration and may serve as a valuable component of a more sophisticated model that predicts potential root growth from traditionally measured exchangeable/reserve acidity values.

REFERENCES

Adams, F., R. W. Pearson, and B. D. Doss. 1967. Relative effects of acid subsoils on cotton yields in field experiments and on cotton roots in growth-chamber experiments. *Agron. J.* 59:453–456.

Alva, A. K., D. G. Edwards, C. J. Asher, and F. P. C. Blamey. 1986a. Effects of phosphorus/aluminum molar ratio and calcium concentration on plant response to aluminum toxicity. *Soil Sci. Soc. Am. J.* 50:133–137.

Alva, A. K., D. G. Edwards, C. J. Asher, and F. P. C. Blamey. 1986b. Relationship between root length of soybean and calculated activities of aluminum monomers in nutrient solution. *Soil Sci. Soc. Am. J.* 50:959–962.

Carvalho, M. C. S., and B. van Raij. 1997. Calcium sulphate, phosphogypsum, and calcium carbonate in the amelioration of acid subsoils for root growth. *Plant and Soil* 192:37–48.

Farina, M. P. W., P. Channon, and G. R. Thibaud. 2000. A comparison of strategies for ameliorating subsoil acidity, I: Long-term growth effects. *Soil Sci. Soc. Am. J.* 64:646–651.

Folker-Hansen, P., P. H. Krogh, and M. Holmstrup. 1996. Effect of dimethoate on body growth of representatives of the soil living mesofauna. *Ecotox. Environ. Safety* 33:207–216.

Foy, C. D., and A. L. Fleming. 1978. The physiology of crop tolerance to excess available aluminum and manganese in acid soils. In *Crop tolerance to suboptimal land conditions* (Spec. Pub. 32), ed. G. A. Jung, 301–328. Madison, Wisc.: ASA.

Huang, J. W., D. L. Grunes, and L. V. Kochian. 1992. Aluminum effects on the kinetics of calcium uptake into the cells of wheat root apex: Quantitation of calcium fluxes using a calcium selective vibrating microelectrode. *Planta* 188:414–421.

Kim, B. Y., A. C. Baier, D. J. Somers, and J. P. Gustafson. 2001. Aluminum tolerance in triticale, wheat, and rye. *Euphytica* 120:329–337.

Liu, H. 2005. Aluminum resistance among seeded bermudagrasses. *HortScience* 40:221–223.

Noble, A. D., M. V. Fey, and M. E. Sumner. 1988. Calcium–aluminum balance and the growth of soybean roots in nutrient solutions. *Soil Sci. Soc. Am. J.* 52:1651–1656.

Overman, A. R., and K. H. Brock. 2003a. Model comparison of coastal bermudagrass and Pensacola bahiagrass response to applied nitrogen. *Commu. Soil Sci. Plant Anal.* 34:2163–2176.

Overman, A. R., and K. H. Brock. 2003b. Model analysis of corn response to applied nitrogen and tillage. *Commun. Soil Sci. Plant Anal.* 34:2177–2191.

Panozzo, J. F., and H. A. Eagles. 1999. Rate and duration of grain filling and grain nitrogen accumulation of wheat cultivars grown in different environments. *Aust. J. Agric. Res.* 50:1007–1015.

Pintro, J. C., and G. J. Taylor. 2004. Effects of aluminum toxicity on wheat plants cultivated under conditions of varying ionic strength. *J. Plant Nutri.* 27:907–919.

Ritchey, K. D., D. P. Belesky, and J. J. Halvorson. 2004. Soil properties and clover establishment six years after surface application of calcium-rich by-products. *Agron. J.* 96:1531–1539.

Ryan, P. R., and L. V. Kochian. 1993. Interaction between aluminum toxicity and calcium uptake at the root apex in near-isogenic lines of wheat (*Triticum aestivum* L.) differing in aluminum tolerance. *Plant Physiol.* 102:975–982.

SAS Institute. 1991. *SAS/STAT users' guide*, ver. 7, 4th ed. Cary, N.C.: SAS Institute.

Shainberg, I., M. E. Sumner, W. P. Miller, M. P. W. Farina, M.A. Pavan, and M.V. Fey. 1989. Use of gypsum on soils: A review. In *Advances in soil science*, vol. 9, ed. B. A. Stewart, 1–111. New York: Springer-Verlag.

Sumner, M. E. 1995. Amelioration of subsoil acidity with minimum disturbance. In *Advances in soil science*, ed. N. S. Jayawardane and B. A. Stewart, 147–185. Boca Raton, Fl.: CRC Press.

Sumner, M. E., H. Shahandeh, J. Bouton, and J. E. Hammel. 1986. Amelioration of an acid soil profile through deep liming and surface application of gypsum. *Soil Sci. Soc. Am. J.* 50:1254–1258.

Sumner, M. E., and T. Yamada. 2002. Farming with acidity. *Commun. Soil Sci. Plant Anal.* 33:2467–2496.