Aluminum sensitivity of loblolly pine and slash pine seedlings grown in solution culture

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Summary  To probe variation in Al sensitivity of two co-occurring pine species, seedlings from six full-sib families of loblolly pine (Pinus taeda L.) and slash pine (Pinus elliottii Engelm.) were grown in solution culture containing 4.4 mM (high-Al) or 0.01 mM (low-Al) AlCl₃ at pH 4 for 58 days. On average, both pine species had 41% less total dry weight in the high-Al treatment than in the low-Al treatment. Stem volume growth of slash pine was more sensitive to the high-Al treatment than that of loblolly pine. In both species, the high-Al treatment inhibited root dry weight more than shoot dry weight. Within-species variation in Al sensitivity among families was greater in loblolly pine (24 to 52% inhibition of seedling dry weight) than in slash pine (35 to 47% inhibition of seedling dry weight). Foliar Al concentration was positively correlated with Al sensitivity in slash pine but not in loblolly pine; however, in both species, the concentration of Al in roots was 20-fold greater than in foliage.

Keywords: aluminum tolerance, genetic variation, Pinus elliottii, Pinus taeda.

Introduction

Aluminum toxicity is a common agronomic problem (Foy 1984) and has also been implicated in many cases of forest decline (e.g., Shipley et al. 1992, Schlegel et al. 1992, Bengtsson et al. 1994). Although many tree species have been classified according to their Al sensitivity (Hutchinson et al. 1986, Schaedle et al. 1989, Kelly et al. 1990, Raynal et al. 1990, Sucoff et al. 1990, Hentschel et al. 1993), the extent of intraspecific variation in Al sensitivity of trees has not been fully explored. Geburek and Scholz (1992) found differences in shoot and root growth among six Norway spruce (Picea abies Karst.) provenances in response to Al and SO₄ treatment. Genetic variation in loblolly pine (Pinus taeda L.) appears to exist for many environmental stresses (Qiu et al. 1990, McLaughlin et al. 1994, Taylor 1994), but there have been few conclusive studies on intraspecific variation in Al sensitivity of southern pine species. For example, Raynal et al. (1990) reported differences in sensitivity between two seed sources of loblolly pine exposed to 1.5 mM Al, whereas Williams (1982) observed no differences in five half-sib slash pine (Pinus elliottii Engelm.) families grown in solutions containing up to 2.2 mM Al. The objective of this experiment was to evaluate the variation in Al sensitivity of six full-sib loblolly pine and six full-sib slash pine families in solution culture.

Materials and methods

Plant material and pretreatment

Loblolly pine and slash pine seedlings were grown in sand from seeds supplied by Scott Paper Co. (Mobile, Alabama, USA). Six full-sib families were provided for each species. In both species, families had no parents in common, and parents were from the Gulf Coastal Plain Province of southeastern USA. Seedlings were fertilized with nutrient solution once per week, beginning 2 weeks after germination. The nutrient solution contained (mM): 0.31 NH₄-N, 0.31 NO₃-N, 0.08 P, 0.21 K, 0.20 Ca, 0.08 Mg, 4.0 × 10⁻³ Fe (as EDTA), 4.0 × 10⁻³ B, 0.40 × 10⁻³ Mn, 0.40 × 10⁻³ Zn, 0.08 × 10⁻³ Cu, 0.08 × 10⁻³ Mo, 0.08 × 10⁻³ Co, 0.08 S, 0.53 Cl, and 0.16 × 10⁻³ Na. The experiment was conducted in a greenhouse near Starkville, Mississippi, USA, between February 16 and August 4, 1992. During the study, greenhouse air temperatures were generally between 15 and 35 °C, average nutrient solution temperature was 27.2 ± 0.8 °C (mean ± standard deviation), and photosynthetic photon flux density (PPFD) was up to 1000 µmol m⁻² s⁻¹ in full sun.

Experimental treatments and measurements

Ten weeks after germination, seedlings were transferred from silica sand to six styrofoam chests containing 25 l of aerated nutrient solution and acclimated for 2 weeks. After acclimation, the phosphate ion concentration was lowered 10-fold in the nutrient solution to avoid precipitation of aluminum–phosphate complexes, and aluminum in the form of AlCl₃ was added to provide a low-Al (0.01 mM) and a high-Al (4.4 mM) treatment. Aluminum in the nutrient solutions was monitored by atomic absorption spectrophotometry (Isaac and Kerber 1971). The pHs of the high-Al and low-Al treatment solutions were 3.93 ± 0.07 (mean ± standard deviation) and 3.96 ± 0.22, respectively. The solution pH was adjusted to a target value of 4 with 1 N HCl or 1 N NaOH when necessary. All solutions
were replaced every 2 weeks. To estimate the activity of Al\(^{3+}\) ions in the high-Al and low-Al treatments, ion concentrations for the solutions were entered in a spreadsheet-based program (“SPECIES,” Barak 1990), and the chemical equilibrium calculated. At pH 4, the nutrient solutions yielded 86% (low-Al treatment) and 94% (high-Al treatment) of all aluminum species in Al\(^{3+}\) form. Chests were shifted by position on the greenhouse bench at regular intervals to ensure equal exposure of each chest to any environmental microsites that existed within the greenhouse. The two Al treatments were replicated three times (i.e., three chests per Al treatment). Each family of loblolly pine and slash pine was represented by two seedlings in each chest (i.e., six seedlings per treatment for each family and 144 seedlings total). Seedlings were placed at random in each chest. Seedling heights and root collar diameters were measured before the Al treatments began and every 11–13 days thereafter (except for one interval of 24 days).

The experiment was terminated after 58 days of exposure to Al. Needles, stems, fine roots (< 2 mm diameter) and coarse roots (≥ 2 mm diameter) were separated and rinsed in deionized water. Subsamples of fine roots (10–20% by fresh weight) from each seedling were stored in 20% methanol solution and measured for length with a digital image analysis system (DIAS II, Decagon Devices Inc., Pullman, Washington, USA; Harris and Campbell 1989). Total fine root length was calculated from subsample length and its proportion of total fine root dry weight. All tissue types were oven dried at 70 °C for 48 h and weighed. Needles and fine roots were analyzed for Al by atomic absorption spectrophotometry (Isaac and Kerber 1971).

**Statistical analysis**

Data were subjected to ANOVA using the Statistical Analysis System software (SAS Institute Inc., Cary, NC). The analysis was a modified split-plot design with replication within treatment substituting for block. Type III mean square for replication within treatment was used as an error term to test significance of the treatment effects. Type III mean square for replication within treatment × plant type (where plant type refers to species or family effects) was used as an error term to test significance of plant type and treatment × plant type interactive effects. Modified models were used to test for treatment effects within a species, and for plant type effects within a treatment. In the latter case, separation of family means was by Duncan’s Multiple Range Test, with Type III mean square for replication × family used as an error term (α = 0.1). A paired comparisons t-test was used to test the hypotheses that the mean differences between root and shoot inhibitions were significantly different from zero. To allow for growth comparisons of families and tissue types, normalized variables were created as \( I = (1 - H/L) \times 100 \), where \( I \) = percent inhibition in the high-Al treatment relative to that in the low-Al treatment, \( H \) = individual value of the variable observed in the high-Al treatment, and \( L \) = mean value for the variable observed in the low-Al treatment.

**Results**

In both species, the high-Al treatment caused large inhibitions of biomass components relative to the low-Al treatment (\( P < 0.01 \)). Root dry weight was affected more by the high-Al treatment than shoot (stem plus needles) dry weight (\( P < 0.01 \)) for both species. On average, loblolly pine shoot dry weight was 25% less and root dry weight was 62% less in the high-Al treatment than in the low-Al treatment. The corresponding values for slash pine were 32 and 56%, respectively, indicating greater root sensitivity to Al in loblolly pine and greater shoot sensitivity to Al in slash pine. In both species, whole-seeding dry weight was 41% less in the high-Al treatment than in the low-Al treatment.

Aluminum-induced inhibition of stem volume \( (d^2 \times h) \) growth occurred earlier and to a greater extent in slash pine seedlings than in loblolly pine seedlings. Two weeks after exposure to the high-Al treatment, stem volume growth of slash pine was inhibited 12% (\( P = 0.06 \)), whereas there were no significant treatment effects (\( P = 0.97 \)) for loblolly pine. After 8 weeks in the high-Al treatment, stem volume growth of slash pine was inhibited 31% (\( P < 0.01 \)), whereas stem volume growth was inhibited only 17% (\( P = 0.08 \)) in loblolly pine.

The extent of Al-induced inhibition of total dry weight, fine root dry weight and fine root length varied more among the loblolly pine families than among the slash pine families (Table 1). Inhibition of whole-seedling dry weight varied between 24 and 52% in loblolly pine but only between 35 and 47% in slash pine. Similarly, the range of variation in inhibition of fine root dry weight and fine root length was less for slash pine than for loblolly pine. In both species, resistant and sensitive families could be identified based on Al-induced inhibition of biomass or fine root length. Specifically, there was one resistant (Family 4) and two sensitive (Families 8 and 10) loblolly pine families, and one resistant (Family 13) and one sensitive (Family 17) slash pine family. Other families of both species showed intermediate sensitivity to Al with respect to the measured parameters (Table 1).

At the end of the experiment, loblolly pine needles had significantly higher Al concentrations (0.29 ± 0.02 g kg\(^{-1}\), mean ± standard error) than slash pine needles (0.17 ± 0.01 g kg\(^{-1}\)), whereas slash pine roots had significantly higher Al concentrations (0.97 ± 0.02 g kg\(^{-1}\)) than loblolly pine roots (0.87 ± 0.03 g kg\(^{-1}\)), but only in the low-Al treatment (\( P < 0.01 \)). Neither needle nor root Al concentrations differed by species in the high-Al treatment (\( P \geq 0.47 \)); across-species average Al concentrations were 0.51 ± 0.03 g kg\(^{-1}\) for needles and 11.79 ± 0.38 g kg\(^{-1}\) for roots. At harvest, needle Al concentrations did not differ by family in the low-Al treatment for either of the species (\( P > 0.45 \)) (Figure 1). In the high-Al treatment, however, needle Al concentrations were affected by family for both species (\( P = 0.02 \) for loblolly pine, \( P = 0.12 \) for slash pine). The average Al sensitivity (i.e., % inhibition for whole-seeding dry weight, Table 1) of the slash pine families was closely correlated (\( r = 0.96 \)) with average needle Al concentration in the high-Al treatment (Figure 1b); this trend was not present for the loblolly pine families (Figure 1a). Root
Al concentrations of families grown in the high-Al treatment were weakly correlated with Al sensitivity for loblolly pine ($r = 0.80, P = 0.06$) but not for slash pine ($P = 0.63$).

### Discussion

Both loblolly pine and slash pine were quite resistant to Al$^{3+}$ in solution (cf. Schaedle et al. 1989, Raynal et al. 1990). We observed that slash pine shoot growth was more sensitive to Al than loblolly pine shoot growth. Both species exhibited similar root sensitivity to Al, whereas Williams (1982) found that root growth in slash pine but not in loblolly pine was inhibited by exposure to 0.55 mM Al in solution. In both species, roots were more sensitive to the high-Al treatment than shoots, which is a common characteristic of aluminum toxicity in plants (Taylor 1988a).

We found more variation in the growth responses of loblolly pine seedlings to Al than has previously been reported. For instance, loblolly pine Family 4, with only 29% inhibition of fine root length in the high-Al treatment (4.4 mM), was more resistant to Al than any seed source from three other loblolly pine studies, all of which exhibited, on average, 30% inhibition of root elongation in response to 3.3 mM Al (Raynal et al. 1990). Similar inhibition (35%) was reported by Tepper et al. (1989) for tap root elongation of loblolly pine seedlings grown in the presence of only 1.5 mM Al. At the other extreme, our most Al-sensitive loblolly pine families (Families 8 and 10) showed more than 60% inhibition of fine root length and biomass when exposed to 4.4 mM Al for 58 days (Table 1), which is considerably more inhibition than would be predicted from the results reported by Raynal et al. (1990).

Intraspecific variation in Al sensitivity was less in slash pine than in loblolly pine. The relatively narrow range of variation in slash pine is consistent with the findings of Williams (1982) who reported no statistically significant differences in root growth responses among slash pine families after 2 weeks of exposure to 2.2 mM Al in solution. In Williams’s study, root length inhibition only varied from 22 to 30% (calculated from Williams 1982) for five open-pollinated families.

The finding that Al sensitivity was correlated with foliar Al concentration in the slash pine families but not in the loblolly pine families (Figure 1) suggests that different mechanisms of Al resistance are operating in the two species. One possibility is that variation among slash pine families reflects the ability of resistant individuals to restrict the movement of Al from roots to foliage, whereas resistance in loblolly pine is not associated with this trait. It is worth noting that, in both species, 20 times more aluminum accumulated in the root system than in the foliage. This is consistent with previous studies of loblolly pine (Raynal et al. 1990) and suggests that

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1 Percent decrease in growth variable in the high-Al treatment relative to that in the low-Al treatment, based on the average of I as defined in the text.
2 Probability > $F$ for Al treatment effect.
root exclusion may underlie the general phenomenon. Such root-based mechanisms also appear to be important for Al resistance in many crop species (Taylor 1988b, Tice et al. 1992).

In summary, shoot growth of slash pine was more sensitive to high concentrations of available Al than shoot growth of loblolly pine, and among the six full-sib families of each species studied, loblolly pine exhibited greater intraspecific variation in Al sensitivity than slash pine.

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References


