Tree Physiology 25, 245–250

Aluminum fractions in root tips of slash pine and loblolly pine families differing in Al resistance

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Received June 17, 2004; accepted August 22, 2004; published online December 1, 2004

Summary Aluminum (Al) distribution among several cellular fractions was investigated in root tips of seedlings of one Al-resistant and one Al-sensitive family of slash pine (*Pinus elliottii* Engelm.) and loblolly pine (*Pinus taeda* L.) grown in nutrient solution containing 100 µM $AlCl_3$ (pH 4) for 167 h. Aluminum present in 5-mm-long root tips was fractionated into cell-wall-labile (desorbed in 0.5 mM citric acid), cell-wall-bound (retained after filtering disrupted cells through 20-µm mesh) and symplasmic (filtrate following cell disruption) fractions. When averaged across both species, 12% of Al absorbed by root tips appeared in the symplasmic fraction and 88% in the apoplasmic fraction (55% as cell-wall-labile, and 33% as cell-wall-bound). On a fresh mass basis, total Al in root tips was lower in loblolly pine than in slash pine, lower in the Al-resistant slash pine family than in the Al-sensitive slash pine family, and lower in the Al-resistant families than in the Al-sensitive families across species. Although the data support the hypothesis that Al-resistant plants limit Al uptake to root apices, they do not exclude other mechanisms of Al resistance. Differential Al resistance between the species and between slash pine families may also be associated with the size of the total non-labile and cell-wall-labile Al fractions, respectively. We were unable to identify the basis for differential Al resistance in loblolly pine.

Keywords: aluminum tolerance, aluminum toxicity, cellular Al, genetic variation, Pinus elliottii, Pinus taeda.

Introduction

Aluminum (Al) toxicity limits crop production on up to 30% of arable soils worldwide (Campbell 1999). Although Al toxicity in agronomic species was documented almost a century ago (Hartwell and Pember 1918), interest in the impacts of Al toxicity in forest ecosystems has been triggered by recent concerns about the increase in anthropogenic soil acidification processes (Cronan and Schofield 1979, Ulrich et al. 1980, Godbold et al. 1988, de Vries et al. 1995, Likens et al. 1996). Tree resistance to Al toxicity is particularly important on naturally acidic soils and where anthropogenic acidifying inputs occur (Matzner et al. 1998, Hodson and Sangster 1999, Ahonen-Jonnarth et al. 2000, Fottová 2003). Rankings of Al resistance among tree species have been compiled based on growth responses from many studies (e.g., Schaedle et al. 1989, Table 1, and Raynal et al. 1990, Table 3). However, physiological differences among genotypes within a tree species have received less attention (Geburek and Scholz 1989, Wilkins and Hodson 1989, Raynal et al. 1990, Nowak and Friend 1995). A detailed understanding of these differences will aid in the design and management of full-sib family and clonal plantation forests established in areas where acid precipitation is frequent.

The predominant Al-resistance mechanism in plants is Al exclusion from root tips (Taylor 1987). This mechanism, mediated through organic acid exudation in response to Al stress, has been demonstrated in several crop (Miyasaka et al. 1991, Li et al. 2000, Piñeros et al. 2002, Shen et al. 2002, Yang et al. 2003) and tropical woody species (Nguyen et al. 2003), as well as in red spruce (*Picea rubens* Sarg.) cell suspension cultures (Minocha and Long 2004). The exuded organic acids chelate Al externally and prevent it from entering roots of Al-resistant genotypes.

Internal Al-resistance mechanisms have also been proposed; however, the extent of Al detoxification in various cell compartments remains unknown (Taylor et al. 2000). Information concerning Al distribution among root subcellular fractions could provide insight into internal Al-resistance mechanisms. Although intracellular measurements of Al in intact roots have been made (Lazof et al. 1994, 1996), the usefulness of the data is limited because the estimates were based on several assumptions with respect to potassium (K) content and Al:K ratios in roots (Lazof et al. 1996). Another approach is to measure Al uptake by individual cells in cell culture experiments. For example, in *Chara corallina* (charophyte algae) cells, accumulation of Al in the cell wall dominated total Al uptake, but transport across the plasma membrane and into vacuoles was also detected (Taylor et al. 2000). In several studies, indirect measurements of root Al apoplasmic and symplasmic fractions were based on kinetics of Al uptake or desorption, or both (Zhang and Taylor 1989, 1990, Tice et al. 1992, Archambault et al. 1996). Root apoplasmic Al pools,

which may vary by species, have been quantified by a variety of techniques. For example, Al bound in root mucilage has been analyzed (Archambault et al. 1996, Miyasaka and Hawes 2001). Another root apoplasmic fraction of interest is the Al tightly bound within the cell wall (Archambault et al. 1996, Schmohl and Horst 2000). Other researchers have focused on Al adsorbed on root cation exchange sites (e.g., Rufyikiri et al. 2002). There is evidence that some Al-sensitive genotypes have an increased root cation exchange capacity (Rufyikiri et al. 2002) or an increased abundance of free Al within the epidermis and internal root tissue (Campbell 1999).

To elucidate the physiological mechanism(s) underlying Al resistance in slash pine (*Pinus elliottii* Engelm.) and loblolly pine (*Pinus taeda* L.) , we first screened six full-sib families of each species for Al resistance based on seedling growth in the presence of Al in hydroponic culture (Nowak and Friend 1995). We then assessed the Al resistance of seedlings of the same genotypes in soil cultures with and without mycorrhizal inoculation (Nowak and Friend, unpublished data). In this study, we tested the basis for differential Al resistance in seedlings of these pine species by partitioning Al in root tips among several subcellular fractions. Our objectives were to: (1) separate Al present in root tips of Al-resistant and Al-sensitive full-sib slash pine and loblolly pine families into cell-wall-labile, cell-wall-bound and symplasmic Al fractions; and (2) evaluate the possible role of Al fractionation in Al resistance in these species. We hypothesized that seedlings of Al-resistant genotypes exclude Al from root tips and from root tip symplasm to a greater degree than seedlings of Al-sensitive genotypes.

Materials and methods

Plant material and pretreatment

Seedlings from two full-sib families each of loblolly pine and slash pine, identified as Al-resistant (LOB 4, and SLASH 14) and Al-sensitive (LOB 8, SLASH 17) (Nowak and Friend 1995) were germinated following standard stratification procedures (Bonner et al. 1974) and grown in Ray Leach tubes (Stuewe & Sons, Corvallis, OR) filled with silica sand in a greenhouse near Starkville, MS, between March 15 and July 13, 1995. Seedlings were watered with distilled water three times per week. After the first needles were about 1 cm long, seedlings were fertilized every other week with a complete nutrient solution (mM): 0.31 NH_4-N, 0.31 NO_3-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, 0.52 Cl and 0.16×10^{-3} Na (Nowak and Friend 1995). To control fungi and insects, 0.2% Benlate (benomyl) and 0.1% Sevin (carbaryl), respectively, were applied. Both pesticides were sprayed as solutions prepared in distilled deionized water (DDH_2O). Subsequently, seedlings were removed from the sand and grown in the same complete nutrient solution in the greenhouse from July 13 to September 10, 1995. Nutrient solutions were adjusted to pH 4 with 1 M NaOH or 1 M HCl when necessary, and were replaced every 2 weeks. Mean (± SD) nutrient solution pH was 3.99 ± 0.23 at 29.4 ± 1.8 °C. Mean minimum and maximum air temperatures in the greenhouse were 24 ± 2 °C and 37 ± 3 °C, respectively. On September 10, the seedlings were transferred to the laboratory and grown in the same nutrient solutions for another week at a mean pH of 3.88 ± 0.25, a mean solution temperature of 23.0 ± 0.5 °C and in a 14-h photoperiod, with supplemental light from a 400-W high-pressure sodium lamp (Day Brite, Tupelo, MS). Photosynthetically active photon flux (PPF) was between 200 (seedling crown bases) and 1400 μmol m^{-2} s^{-1} (just above seedling crowns) as measured with an LI-190SA quantum sensor (Li-Cor, Lincoln, NE).

Aluminum distribution in root tips

Five 6-month-old seedlings of each of the Al-resistant and Al-sensitive families of the two species were grown for 167 h in 35 l of aerated treatment nutrient solution (Nowak and Friend 1995) containing 100 μM $AlCl_3$. In pilot experiments with 100 μM $AlCl_3$ solutions and the same plant material, root tip Al concentrations peaked at 72 h, and then declined. Therefore a 167-h treatment was considered adequate to capture the Al concentration in the root tip after all potential physiological resistance mechanisms were expressed.

To limit the possibility of Al precipitation in the treatment solution, phosphate ion concentration was lowered 10-fold compared with the full nutrient solution previously described. The measured Al concentrations were between 98 (beginning of treatment) and 59 μM (end of treatment) at a mean pH of 4.01 ± 0.23 and a mean solution temperature of 23.2 ± 0.3 °C. The free Al^{3+} activity calculated with GEOCHEM-PC Version 2.0 computer software (Parker et al. 1995) ranged from 78 μM Al^{3+} at the beginning to 46 μM Al^{3+} at the end of the treatment.

Determination of the cell-wall-labile Al fraction in root tips was based on a method for apoplasmic, readily exchangeable, loosely bound Al (Zhang and Taylor 1989). For determination of cell-wall-bound Al and symplasmic Al, the methods of Zhang and Taylor (1990) were modified. At the end of the 167-h treatment, seedlings were placed in 35 l of DDH_2O at 23 °C during root sampling (5 h). The seedlings were withdrawn from DDH_2O one at a time (at random within each family), separated into shoot and roots, and rinsed in DDH_2O. Five to seven 5-mm-long white unsuberized root tips were excised with a new razor blade and put in a 30-ml polyethylene beaker. The beaker was weighed and the fresh mass of the root tips recorded, then 30 ml of cold (2 °C) 0.5 mM citric acid was added, and the contents stirred with a plastic rod. To desorb the Al from the root tips, the beaker was incubated for 7 h at 2 °C. Another five to seven root tips were excised and similarly treated. The remaining white root tips were excised, placed in a Pyrex vial, weighed to record fresh mass and incubated at 65 °C for 48 h.

After 7 h of desorption, the contents of each beaker were stirred and the citric acid was decanted through nylon mesh. (In pilot experiments, a 3–7 h incubation in 0.5 mM citric acid desorbed about 85% of the total Al from root tips on a dry mass basis.) Subsequently, the root tips were rinsed with 30 ml of DDH_2O. One set of desorbed root tips per seedling was placed in a Pyrex vial and incubated at 65 °C for 48 h. The other set of desorbed root tips from each seedling was processed in the same way, except that after the root tips were rinsed with

DDH_2O, they were cut into 1-mm segments on a Plexiglas plate with a new razor blade. The 1-mm segments were then transferred to a polyethylene vial and homogenized on ice in 3 ml of 0.1 M Hepes-Mes plus 0.3 M sucrose buffer (pH 7.84) with a Brinkmann PCU 11 homogenizer (Brinkmann Instruments, Westbury, NY) operating at maximum speed for six 10-s pulses. To disrupt plasma membranes, each sample was sonicated on ice with a Branson cell disruptor 185 (Branson Sonic Power, Danvury, CT) for three 5-s pulses at maximum power output for the microtip (cf. Tice et al. 1992). The sonicated material was filtered through a 20-µm nylon membrane (Magna Micron Separations, Westborough, MA) and the filtrate collected in a polyethylene vial and saved for Al analysis.

Chemical analysis

All glassware was acid washed with 4 M HNO_3, and polyethylene supplies were used whenever possible. All samples, except the sonicated root tip samples, were oven dried, digested and analyzed for Al by atomic absorption spectrometry. After the roots tips had been oven dried, the vials were covered with plastic screw tops and placed in a desiccator. When samples cooled to room temperature (23 °C), root tips were weighed to the nearest µg, placed in a covered crucible and ashed for 24 h in a muffle furnace at 500 °C. The ash was dissolved in 500 µl of trace-metal-grade HNO_3 and oxidized in 500 µl of 50% H_2O_2. The contents of each crucible were transferred to a Pyrex block digestion tube with two rinses (10 ml each) of DDH_2O, stirred with a vortex and transferred to a polyethylene vial. The samples were analyzed for Al (after dilution to below 50 ppb) with an atomic absorption Varian Spectra AA 20 Plus spectrometer (Varian Australia, Mulgrave, Victoria, Australia), with an automatic sampler and 17 mM $Mg(NO_3)_2$ as the sample modifier. For the sonicated samples, the filtered aliquot was analyzed for Al by atomic absorption spectrometry in the same manner after tenfold dilution.

Aluminum fraction measurements

Three Al fractions in root tips were measured by atomic absorption spectrometry and two Al fractions were calculated. The measured fractions were: (1) total Al concentration in the root tips before desorption; (2) total non-labile Al after a 7-h desorption in 0.5 mM citric acid; and (3) symplasmic Al in the filtrate after root tips were desorbed in citric acid, homogenized and sonicated. The two Al fractions calculated were: (1) cell-wall-bound Al, determined as the difference between the total non-labile and symplasmic fractions; and (2) cell-wall-labile Al, determined as the difference between the total Al in the root tips and the total non-labile fraction.

Statistical data analysis

The experiment was arranged in a completely randomized design, with each seedling treated as an experimental unit. Species and family means were compared by two-tailed *t*-tests in SAS Version 8e software (SAS, Cary, NC). After the fractionations, some samples were lost before they could be assayed for Al. As a result, some family means were based on fewer than five replications (Table 1). In cases where unequal vari-

Table 1. Percent symplasmic, apoplasmic (cell-wall-bound plus cell-wall-labile), total non-labile (remaining after desorption with 0.5 mM citric acid) and total Al in 5-mm-long root tips of loblolly pine and slash pine seedlings, and the Al-resistant family SLASH 14 compared with the Al-sensitive family SLASH 17, after a 167-h exposure to 100 µM $AlCl_3$ in nutrient solution. Probability values ($P > |t|$) are from two-tailed *t*-tests for species or family means. Percent values were arcsine-transformed before *t*-tests and then back-transformed before reporting.

Cellular Al fraction	Pine species				Slash pine families			
	Species	*n*	Percent of total Al in root tips	*P*	Family	*n*	Percent of total Al in root tips	*P*
Fresh mass basis								
Symplasmic	Loblolly pine	7	15.2	0.07	SLASH 14	2	9.9	0.64
	Slash pine	6	8.4		SLASH 17	4	7.6	
Apoplasmic	Loblolly pine	7	84.8	0.14	SLASH 14	2	90.1	0.81
	Slash pine	5	90.9		SLASH 17	3	91.5	
Cell-wall-bound	Loblolly pine	7	27.8	0.42	SLASH 14	2	57.9	0.38
	Slash pine	5	38.5		SLASH 17	3	26.3	
Cell-wall-labile	Loblolly pine	7	54.6	0.80	SLASH 14	2	31.2	0.32
	Slash pine	5	51.2		SLASH 17	3	64.5	
Total	Loblolly pine	7	100.0		SLASH 14	2	100.0	
	Slash pine	6	99.3		SLASH 17	4	100.0	
Dry mass basis								
Total non-labile	Loblolly pine	10	45.4	0.43	SLASH 14	5	59.5	0.55
	Slash pine	9	54.7		SLASH 17	4	48.8	
Cell-wall-labile	Loblolly pine	10	54.6	0.43	SLASH 14	5	40.5	0.55
	Slash pine	9	45.3		SLASH 17	4	51.2	
Total	Loblolly pine	10	100.0		SLASH 14	5	100.0	
	Slash pine	9	100.0		SLASH 17	4	100.0	

ances were detected, the Satterthwaite method was used instead of pooled variances for *t*-test comparisons. Aluminum concentrations expressed as percent of total were arcsine-transformed before *t*-tests, and then back-transformed before reporting. Differences were considered statistically significant when $P \leq 0.1$.

Results

Root tips of both pine species contained a small symplasmic Al fraction and a larger apoplasmic Al fraction. The symplasmic Al fraction averaged 6.4 mg kg^{-1} fresh mass (FM) (n = 13), or 11.8% of the total. In the root tip apoplasm, the cell-wall-labile Al fraction (27.8 mg kg_{FM}^{-1}, or 53.2%) was nearly twice the cell-wall-bound Al fraction (16.7 mg kg_{FM}^{-1}, or 32.1%). Together, the Al fractions totaled 54.5 mg kg_{FM}^{-1}, and were almost equally divided between cell-wall-labile and total non-labile Al. Also, on a dry mass basis (DM), the cell-wall-labile (385.5 mg kg_{DM}^{-1}) and total non-labile (305.5 mg kg_{DM}^{-1}) Al fractions were similarly divided between the desorbed and not-desorbed Al (50.2 and 49.8%, respectively). Mean total Al concentration was 691.0 mg kg_{DM}^{-1} across species (n = 19).

Species differed in percent symplasmic (Table 1), non-labile (Figure 1c) and total root tip Al accumulation (Figure 1a). In relative terms, almost twice as much symplasmic Al was found in loblolly pine (15.2%) as in slash pine (8.4%). Slash pine root tips accumulated significantly more Al (fresh mass), and had a larger non-labile Al fraction (dry mass) than loblolly pine. Across species, the Al-sensitive families accumulated more total Al (59.9 mg kg_{FM}^{-1}, n = 8) than the Al-resistant families (45.8 mg kg_{FM}^{-1}, n = 5, P = 0.07).

Within species, significant differences were detected only between the slash pine families, and only on a fresh mass basis. Namely, the Al-sensitive family SLASH 17 accumulated more Al in root tips than the Al-resistant family SLASH 14, and the cell-wall labile Al fraction was 2.7 times greater in SLASH 17 than in SLASH 14 (Figure 1b).

Discussion

The major site of Al accumulation in root apices of slash pine and loblolly pine seedlings was the cell wall. Of the total Al in root tips, only 12% was in the symplasmic fraction, whereas 88% of all Al was associated with the apoplasm (more than half as cell-wall-labile, and about one third as cell-wall-bound). Schaedle et al. (1986) removed up to 30% of Al from loblolly pine roots by rinsing them with a 0.1 M $CaCl_2$ solution, and Zhang and Taylor (1989) reported a similar labile Al fraction (40%) in excised wheat roots that was removed with a 30-min desorption in citric acid. We did not differentiate mucilage-bound Al, but believe it may be accounted for in the cell-wall-bound fraction, as this fraction is not removable by citric acid desorption (Archambault et al. 1996). Cell-wall-bound Al was 16.7 mg kg_{FM}^{-1} in our study, which is similar to Al concentrations (~15 mg kg_{FM}^{-1}) in purified cell wall material isolated from excised roots of an Al-resistant wheat cultivar after desorption in citric acid (Zhang and Taylor 1990, Figure 4A). This provides credence to our protocol, as we did not attempt to isolate the cell wall material, relying instead on Al fractionation methods. In our study, symplasmic Al (6.4 mg kg_{FM}^{-1}) was 3.4 times higher than intracellular Al concentrations reported for intact soybean (*Glycine max* cv. Essex) root tips (Lazof et

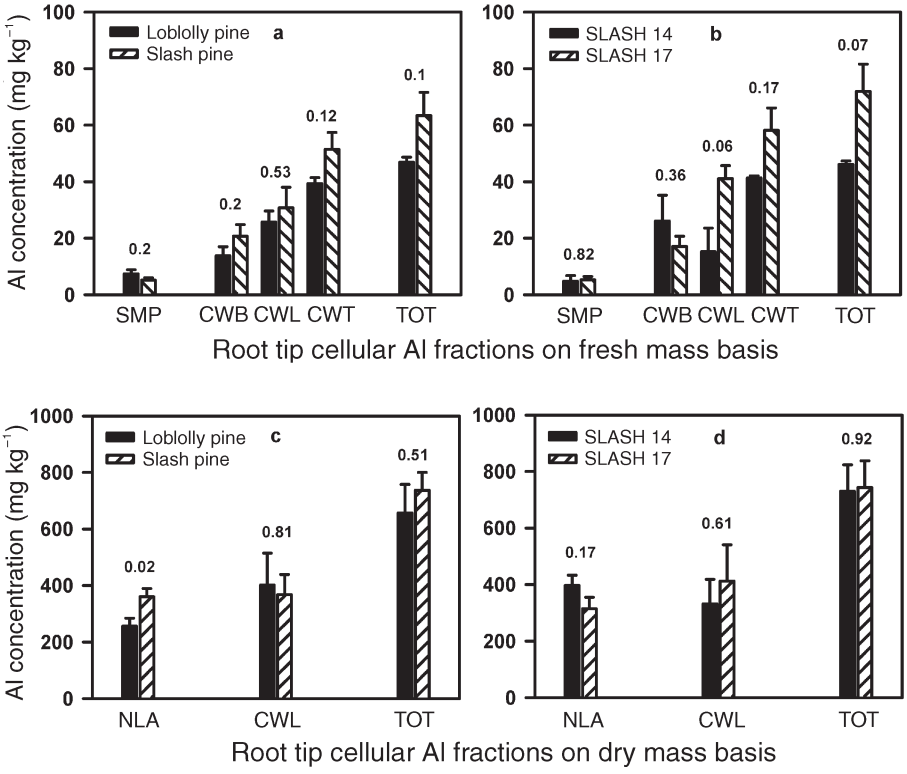

Figure 1. Symplasmic (SMP), cell-wall-bound (CWB), cell-wall-labile (CWL), apoplasmic (cell-wall-total (CWT)), total non-labile (remaining after desorption with 0.5 mM citric acid (NLA)) and total (TOT) Al in 5-mm-long root tips of loblolly pine and slash pine seedlings (a, c), and in the Al-resistant family SLASH 14 and the Al-sensitive family SLASH 17 (b, d), after a 167-h exposure to 100 µM $AlCl_3$ in nutrient solution. Values are species and family means and bars denote 1 SE of the mean. Probability values above error bars ($P > |t|$) are from two-tailed *t*-tests for respective pairs of means.

al. 1994). This result is consistent with the fact that our calculated initial Al^{3+} activity (78 μM) was 2.1 times higher than that reported by Lazof et al. (1994), and root Al uptake is generally concentration-dependent (Schaedle et al. 1989). Although our results are in the same order of magnitude as those of Lazof et al. (1994), they differ markedly from Tice et al. (1992) and Taylor et al. (2000), who reported symplasmic Al to be 63%, and less than 0.5% of total Al uptake, respectively. Such large differences may reflect differences among species, experimental conditions, or detection techniques (Taylor et al. 2000).

The results support our hypothesis that Al resistance in slash pine is associated with Al exclusion from root tips. First, the Al-resistant SLASH 14 family had lower total Al concentrations in root tips than the Al-sensitive SLASH 17 family. Second, loblolly pine, which was previously identified as more Al resistant than slash pine based on stem volume growth responses of the same full-sib families (Nowak and Friend 1995), accumulated less Al in root tips than slash pine. However, our second hypothesis that Al-resistant loblolly pine and slash pine genotypes exclude Al from root tip symplasm was not supported. The results showed no difference in symplasmic Al concentrations between the Al-resistant and Al-sensitive genotypes; instead, a lower cell-wall-labile Al fraction was recorded in the Al-resistant family SLASH 14 compared with the Al-sensitive family SLASH 17 (Figure 1b), and the total non-labile Al fraction was lower in loblolly pine than in slash pine (Figure 1c).

The hypothesis that Al-resistant genotypes excluded Al from root entry was also supported by comparisons across species, with both Al-resistant families accumulating a mean of 45.8 mg kg_{FM}^{-1}, whereas Al-sensitive families averaged 59.9 mg kg_{FM}^{-1} total Al in root tips ($P = 0.07$). There are similar examples of Al exclusion from root tips in woody plants (Ofei-Manu et al. 2001) and agronomic species (Ryan et al. 1997, Ma et al. 2002, Tang et al. 2002), and there is mounting evidence that organic acid exudation in response to Al stress complexes and detoxifies Al externally (e.g., Miyasaka et al. 1991, Piñeros et al. 2002, Shen et al. 2002, Nguyen et al. 2003) thereby preventing its entry into roots. Although we did not measure organic acid exudation, we observed lower Al concentrations in 5-mm-long root tips of both pine species after 168 h compared with 72 h of exposure to 71 μM Al^{3+} in nutrient solution (Nowak and Friend, unpublished data), suggesting the existence of an Al-induced mechanism that excluded Al uptake from newly developing root tips. These results are consistent with efflux of chelating compounds limiting Al entry into the root tips after 72 h of exposure to Al.

Aluminum resistance appears to be a complex multigenic trait in many plant species (Tang et al. 2002, Samac and Tesfaye 2003) including the genotypes used in our study (Kubisiak et al. 2000). In addition to the external resistance mechanism involving organic acid exudation and chelation, internal resistance mechanisms have also been documented (see review by Barceló and Poschenrieder 2002). For example, internal protoplasmic detoxification mechanism(s) might explain the lack of differences in symplasmic Al concentrations between the Al-resistant and Al-sensitive genotypes in our study, except when expressed on a percent basis for the two species. Lower total non-labile Al concentration (dry mass basis) in loblolly pine compared with slash pine may be associated with higher Al resistance, because immobilized Al (regardless of the site) should be less toxic than free and unbound Al. This suggestion is indirectly supported by the finding of higher cell-wall-labile Al in the Al-sensitive SLASH 17 than in the Al-resistant SLASH 14 family, and is consistent with the documented abundance of free Al in roots of Al-sensitive clones of alfalfa (*Medicago sativa* L.) (Campbell 1999), as well as with the high root cation exchange capacity reported for the most Al-sensitive banana (*Musa* spp.) cultivar (Rufyikiri et al. 2002).

In summary, we fractionated Al in root tips of Al-resistant and Al-sensitive full-sib families of slash pine and loblolly pine and found that, on average, 12% of the Al was associated with the symplasmic compartment, whereas 88% was in the cell walls (up to 55% was easily removable by desorption, and about 33% was tightly bound). Exclusion of Al from root entry appeared to be the main Al-resistance mechanism operating in Al-resistant genotypes in both species. Aluminum exclusion was demonstrated for the Al-resistant slash pine family, but the evidence that a similar resistance mechanism operates in loblolly pine was less conclusive.

Acknowledgments

Financial support for this study was provided by the National Aeronautics and Space Administration (NASA) Stennis Space Center under NASA contract NAS13-330, and by the Mississippi Agricultural and Forestry Experiment Station, Stress Physiology Project MIS-0601. Thanks are extended to Mr. John Hendrickson of Scott Paper Co. for providing seeds and technical support. Special thanks to Dr. Dawn Luthe for making specialized equipment available, and Mrs. Juanita A. Mobley and Mr. Douglas A. Crawford for assistance with chemical analyses. The assistance and support of Drs. William G. Cibula, Gregory A. Carter of NASA Stennis Space Center and John D. Hodges of Mississippi State University is gratefully acknowledged. We thank our reviewers Drs. David L. Wright, Peter C. Andersen, Timothy A. Martin and Shibu Jose, whose comments helped to improve the original manuscript and Dr. Cheryl Mackowiak for assistance with chemical speciation analyses. This article appears as Journal Series Paper No. R-10202 of the Florida Agricultural Experiment Station.

References

Ahonen-Jonnarth, U., P.A.W. Van Hees, U.S. Lundstrom and R.D. Finlay. 2000. Organic acids produced by mycorrhizal *Pinus sylvestris* exposed to elevated aluminum and heavy metal concentrations. New Phytol. 146:557–567.

Archambault, D.J., G. Zhang and G.J. Taylor. 1996. Accumulation of Al in root mucilage of an Al-resistant and Al-sensitive cultivar of wheat. Plant Physiol. 112:1471–1478.

Barceló, J. and C. Poschenrieder. 2002. Fast root growth responses, root exudates, and internal detoxification as clues to the mechanism of aluminum toxicity and resistance: a review. Environ. Exp. Bot. 48:75–92.

Bonner, F.T., B.F. McLemore and J.P. Barnett. 1974. Presowing treatment of seed to speed germination. *In* Seeds of Woody Plants in the United States. Agriculture Handbook No. 450. Ed. C.S. Schopmeyer. USDA For. Serv., Washington, DC, pp 126–135.

Campbell, T.A. 1999. Magnetic resonance imaging of absorbed aluminum in alfalfa roots. J. Plant Nutr. 22:827–834.

Cronan, C.S. and C.L. Schofield. 1979. Aluminum leaching response to acid precipitation: effects on high-elevation watersheds in the Northeast. Science 204:304–306.

de Vries, W., E.E.J.M. Leeters and C.M.A. Hendriks. 1995. Effects of acid deposition on Dutch forest ecosystems. Water Air Soil Pollut. 85:1063–1068.

Fottová, D. 2003. Trends in sulphur and nitrogen deposition fluxes in the Geomon Network, Czech Republic, between 1994 and 2000. Water Air Soil Pollut. 150:73–87.

Geburek, T. and F. Scholz. 1989. Response of *Picea abies* (L.), Karst. provenances to aluminum in hydroponics. *In* Genetic Effects of Air Pollutants in Forest Tree Populations. Eds. F. Scholz, H-R. Gregorius and D. Rudin. Springer-Verlag, Berlin, pp 55–65.

Godbold, D.L., E. Fritz and A. Hüttermann. 1988. Aluminum toxicity and forest decline. Ecology 85:3888–3892.

Hartwell, B.L. and F.R. Pember. 1918. The presence of aluminum as a reason for the difference in the effect of so called acid soils on barley and rye. Soil Sci. 6:259–277.

Hodson, M.J. and A.G. Sangster. 1999. Aluminum/silicon interactions in conifers. J. Inorg. Biochem. 76:89–98.

Kubisiak, T.L., C.D. Nelson, J. Nowak and A.L. Friend. 2000. Genetic linkage mapping of genomic regions conferring tolerance to high aluminum in slash pine. J. Sust. For. 10:69–78.

Lazof, D.B., J.G. Goldsmith, T.W. Rufty and R.W. Linton. 1994. Rapid uptake of aluminum into cells of intact soybean root tips. A microanalytical study using secondary ion mass spectrometry. Plant Physiol. 106:1107–1114.

Lazof, D.B., J.G. Goldsmith, T.W. Rufty and R.W. Linton. 1996. The early entry of Al into cells of intact soybean roots. A comparison of three developmental root regions using secondary ion mass spectrometry imaging. Plant Physiol. 112:1289–1300.

Li, X.F., J.F. Ma and H. Matsumoto. 2000. Pattern of aluminum-induced secretion of organic acids differs between rye and wheat. Plant Physiol. 123:1537–1543.

Likens, G.E., C.T. Driscoll and D.C. Buso. 1996. Long-term effects of acid rain: response and recovery of a forest ecosystem. Science 272:244–246.

Ma, J.F., R. Shen, Z. Zhao, M. Wissuwa, Y. Takeuchi, T. Ebitani and M. Yano. 2002. Response of rice to Al stress and identification of quantitative trait loci for Al tolerance. Plant Cell Physiol. 43: 652–659.

Matzner, E., M. Pijpers, W. Holland and B. Manderscheid. 1998. Aluminum in soil solutions of forest soils: influence of water flow and soil aluminum pools. Soil Sci. Soc. Am. J. 62:445–454.

Minocha, R. and S. Long. 2004. Effects of aluminum on organic acid metabolism and secretion by red spruce cell suspension cultures and the reversal of Al effects on growth and polyamine metabolism by exogenous organic acids. Tree Physiol. 24:55–64.

Miyasaka, S.C., J.G. Buta, R.K. Howell and C.D. Foy. 1991. Mechanism of aluminum tolerance in snapbeans. Root exudation of citric acid. Plant Physiol. 96:737–743.

Miyasaka, S.C. and M.C. Hawes. 2001. Possible role of root border cells in detection and avoidance of aluminum toxicity. Plant Physiol. 125:1978–1987.

Nguyen, N.T., K. Nakabayashi, J. Thompson and K. Fujita. 2003. Role of exudation of organic acids and phosphate in aluminum tolerance of four tropical woody species. Tree Physiol. 23: 1041–1050.

Nowak, J. and A.L. Friend. 1995. Aluminum sensitivity of loblolly pine and slash pine seedlings grown in solution culture. Tree Physiol. 15:605–609.

Ofei-Manu, P., T. Wagatsuma, S. Ishikawa and K. Tawaraya. 2001. The plasma membrane strength of the root-tip cells and root phenolic compounds are correlated with Al tolerance in several common woody plants. Soil Sci. Plant Nutr. 47:359–375.

Parker, D.R., W.A. Norvell and R.L. Chaney. 1995. GEOCHEM-PC: A chemical speciation program for IBM and compatible personal computers. *In* Chemical Equilibrium and Reaction Models. Eds. R.H. Loeppert et al. SSSA Spec. Publ. 42, ASA and SSSA, Madison, WI, pp 253–269.

Piñeros, M.A., J.V. Magalhaes, V.M. Carvalho Alves and L.V. Kochian. 2002. The physiology and biophysics of an aluminum tolerance mechanism based on root citrate exudation in maize. Plant Physiol. 129:1194–1206.

Raynal, D.J., J.D. Joslin, F.C. Thornton, M. Schaedle and G.S. Henderson. 1990. Sensitivity of tree seedlings to aluminum: III. Red spruce and loblolly pine. J. Environ. Qual. 19:180–187.

Rufyikiri, G., J.E. Dufey, R. Achard and B. Delvaux. 2002. Cation exchange capacity and aluminum–calcium–magnesium binding in roots of bananas cultivated in soils and in nutrient solutions. Commun. Soil Sci. Plant Anal. 33:991–1009.

Ryan, P.R., R.J. Reid and F.A. Smith. 1997. Direct evaluation of the Ca^{2+}-displacement hypothesis for Al toxicity. Plant Physiol. 113: 1351–1357.

Samac, D.A. and M. Tesfaye. 2003. Plant improvement for tolerance to aluminum in acid soils—a review. Plant Cell Tissue Organ Cult. 75:189–207.

Schaedle, M., F.C. Thornton and D.J. Raynal. 1986. Non-metabolic binding of aluminum to roots of loblolly pine and honeylocust. J. Plant Nutr. 9:1227–1238.

Schaedle, M., F.C. Thornton, D.J. Raynal and H.B. Tepper. 1989. Response of tree seedlings to aluminum. Tree Physiol. 5:337–356.

Schmohl, N. and W.J. Horst. 2000. Cell wall pectin content modulates aluminum sensitivity of *Zea mays* (L.) cells grown in suspension culture. Plant Cell Environ. 23:735–742.

Shen, H., X-L. Yan, X-R. Wang and S-L. Zheng. 2002. Exudation of citrate in common bean in response to aluminum stress. J. Plant Nutr. 25:1921–1932.

Tang, Y., D.F. Garvin, L.V. Kochian, M.E. Sorrells and B.F. Carver. 2002. Physiological genetics of aluminum tolerance in the wheat cultivar Atlas 66. Crop Sci. 42:1541–1546.

Taylor, G.J. 1987. Exclusion of metals from the symplasm: a possible mechanism of metal tolerance in higher plants. J. Plant Nutr. 10: 1213–1222.

Taylor, G.J., J.L. McDonald-Stephens, D.B. Hunter, P.M. Bertsch, D. Elmore, Z. Rengel and R.J. Reid. 2000. Direct measurement of aluminum uptake and distribution in single cells of *Chara corallina*. Plant Physiol. 123:987–996.

Tice, K.R., D.R. Parker and D.A. DeMason. 1992. Operationally defined apoplastic and symplastic aluminum fractions in root tips of aluminum-intoxicated wheat. Plant Physiol. 100:309–318.

Ulrich, B., R. Mayer and P.K. Khanna. 1980. Chemical changes due to acid precipitation in a loess-derived soil in central Europe. Soil Sci. 130:193–199.

Wilkins, D.A. and M.J. Hodson. 1989. The effects of aluminum and *Paxilus involutus* Fr. on the growth of Norway spruce (*Picea abies* (L.) Karst.). New Phytol. 113:225–232.

Yang, Z-M., J. Wang, S.-H. Wang and L-L. Xu. 2003. Salicylic acid-induced aluminum tolerance by modulation of citrate efflux from roots of *Cassia tora* L. Planta 217:168–174.

Zhang, G. and G.J. Taylor. 1989. Kinetics of aluminum uptake by excised roots of aluminum-tolerant and aluminum-sensitive cultivars of *Triticum aestivum* L. Plant Physiol. 91:1094–1099.

Zhang, G. and G.J. Taylor. 1990. Kinetics of aluminum uptake in *Triticum aestivum* L. Identity of the linear phase of aluminum uptake by excised roots of aluminum-tolerant and aluminum-sensitive cultivars. Plant Physiol. 94:577–584.