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ACID TOLERANCE OF PACIFIC NORTHWEST CONIFERS IN SOLUTION CULTURE:

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This report is a publication of the Regional Forest Nutrition Research Project, a cooperative program initiated in 1969 to provide forest managers with accurate growth data for managed stands of Douglas-fir and western hemlock in western Oregon and western Washington. Over 30 Pacific Northwest forest industry companies, state and federal agencies, and fertilizer manufacturers provide support and direction for the Project. The RFNRP Report Series is intended to enhance communication of forest fertilization research results within the RFNRP community. Prepared to meet internal RFNRP needs, reports in the series may be descriptions of work in progress as well as final statements of research results.

GENERAL SUMMARY

Conifers in western Washington occupy specific habitats which are defined by climatic and edaphic variables. One major site difference is the acidity or pH, which varies between sites but also between the forest floor and the mineral soil. In mineral soil low pH is accompanied by relatively high aluminum levels. High aluminum levels are known to be toxic to crop plants; however, the ability to Pacific Northwest conifers to tolerate aluminum is largely unknown. Since high aluminum and low pH occur simultaneously, it is difficult to distinguish the individual effects in soils. In these papers the individual effects of either high aluminum or low pH were investigated in solution culture.

The major findings of this research were:

1. Pacific Northwest conifers are quite tolerant of aluminum and low pH.

2. In contrast to western red cedar and Douglas-fir, western hemlock was relatively tolerant of low pH and intolerant of high aluminum.

3. The tolerance of western hemlock to low pH and the tolerance of western red cedar to low pH and high aluminum seems to be related to their ability to tolerate low tissue levels of Mg and Ca and to the ability of western red cedar to accumulate Ca.

From a management and ecological perspective, it appears that the growth of western hemlock in organic rich forest floor or downed woody material would protect it from high aluminum levels. The relatively greater tolerance of Douglas-fir for aluminum is consistent with its pioneering role and mineral soil rooting.

ACID TOLERANCE OF PACIFIC NORTHWEST CONIFERS IN SOLUTION CULTURE:

I. EFFECT OF HIGH ALUMINIUM CONCENTRATION AND SOLUTION ACIDITY

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SUMMARY. Seedlings of three Pacific northwest conifer species: Douglas-fir (*Pseudotsuga menziesii* (Mirb.) Franco), western hemlock (*Tsuga heterophylla* (Raf.) Sarg.), and western redcedar (*Thuja plicata* Donn) were grown in acid nutrient solutions with and without aluminium present. The acid treatments were paired so as to differentiate the effects of aluminium from the hydrogen ion concentration that a specific level of Al-cations induces via hydrolysis reactions. Relative to agronomic plants, all the conifers were found tolerant of the acid solutions and high levels of aluminium. Species differed in their relative tolerance to H and Al-cations. Douglas-fir and western redcedar both displayed similar or better growth in nutrient containing 175ppm Al than in solutions at the same pH (3.0) without aluminium. In contrast to the other species, western hemlock survived and thrived in acid solution of pH 3 while the presence of Al in acid solution adversely affected seedling root growth and tissue divalent cation concentrations, especially calcium and magnesium. Therefore, the ability of western hemlock to grow in acid conditions is postulated to be related to this species' physiological tolerance of excess H-cations in solution and low tissue requirements of Ca and Mg. This tolerance of H-cation concentrations found for western hemlock meant that the specific effects of high Al concentrations in solution could be differentiated in hemlock seedling growth and nutrition from those effects caused by increased H-concentration due to Al-hydrolysis.

KEY WORDS Acidity Al toxicity-tolerance H toxicity-tolerance Pacific Northwest *Pseudotsuga menziesii* Solution culture *Thuja plicata* *Tsuga heterophylla*

Introduction

Pacific Northwest conifers have high growth rates on acid soils along the coast of Washington and Oregon[12]. Western hemlock in particular seems to have a specific tolerance for acidic seed beds high in organic matter[22], and acidic soils[21]. The ability of these conifer species, and especially western hemlock, to attain high productivity[34] in an acid soil environment presented the impetus for this study.

The complex chemistry of acid soil and organic matter prompted initial investigations into the relationships of forest seedling growth in acid and Al-rich solution culture. The elimination of mineral and organic phases in hydroponic solution cultures (hereafter referred to as nutrient cultures) limits uncontrolled pH interactions to those between inorganic components of the nutrient solution and the plant root system. In such nutrient culture systems acid tolerance for any plant species would be, in part, an ability to tolerate high activities of H and/or Al-cations in solution. The activity and nature of the various Al-species in solution are dependent on solution pH via hydrolysis reactions of Al[19]. Therefore, the effect of Al on plant growth should not be considered in isolation of the pH regime. High Al concentrations in solution will also produce an acid pH[4,19].

H-ion activity and plant growth

It is difficult to discern a plant growth response due solely to H-ion presence because of the numerous chemical reactions affected by pH and the ability of plants to actively alter the pH in solutions adjacent to their roots[26,30]. Notwithstanding, Black[2] considered plants very tolerant of H-ion activities within the pH range of 4 to 8 and that only at very high H-ion activities ($pH<3$) was there any evidence of direct H-ion toxicity to plant growth. Excess H-ions in solution effect plant growth by two processes:[2,28]

1) Non-specific inhibition of root elongation, lateral branching, water absorption, and

2) Specific effects on root ion fluxes via H-ion competition with base cations for uptake, and H-ion damage to the ion-selective carrier in root membranes.

Calcium plays an important role in both these latter processes. It is required to substain cell membrane integrity plus facilitate the active uptake of otherwise competitive cations[15]. This "Viets" effect[20] of Ca can be demonstrated with other polyvalent cations (including Al)[3] and has been shown to alleviate the toxic effects of high H-ion activities[7,15,28]. At pH levels of <4 H-ions may out-compete Ca-ions, preventing their absorption, and even displacing Ca present in the root apoplast. Once Ca absorption is repressed, cell membranes lose integrity and the selective-ion carrier mechanism dysfunctions resulting in reduced base cation absorption and effluxes of cations[23,30]. Loss of root membrane integrity can also produce the "wilting" symptoms of low turgor-pressure observed with H-toxicity[2].

Al-ion activity and plant growth

The important role of Al in acid soil chemistry has been reviewed[4,19] as have. Aluminium effects on plant growth (predominantly horticultural and agronomic species)[8,9,16]. Three general processes (summarized from Foy[8]) by which Al affects plant growth are presented below:

a) Reduced divalent cation (especially Ca) uptake by plant roots due to the presence of excess Al in the rhizosphere or in the root apoplast.

b) Dysfunction of cell division in the root meristematic tissue due to penetration of Al into the root protoplasm and the production of abnormal root morphology.

c) Decreased anion (SO_4, PO_4 and Cl) adsorption by roots due to increased positive adsorption sites in the rhizosphere and root apoplast.

Aluminium activity is critical in these above processes because at low activities synergistic response with plant growth can occur. Aluminium is believed to facilitate monovalent cation (especially K uptake via the Viets effect[20]) and increased P sorption as hydroxy-Al-P complexes of low positive charge density have been proposed[13,24,35,36].

Forest Tree Growth in Acid Solutions

Sitka spruce seedlings grown in nutrient cultures produced optimum growth between pH 4-5 with either NH_4-N or NO_3-N source[17]. Depressed growth at low pH occurred at pH 3-5 for NO_3-N and at

<pH3 for NH_4-N. Root colour and morphology was adversely effected by decreasing pH.

A wide range of Al-tolerance was found between seedlings of a variety of northeastern conifers and hardwood species grown in Al-nutrient solutions[18,31]. Poplar species were as sensitive as certain agricultural crops[8], while Betula, Pinus and Quercus spp. were tolerant to high concentrations of Al in solution (80-160 ppm Al)[18]. Variation in paper birch (Betula papyrifera Marsh.) tolerance to nutrient solutions containing 120 ppm Al was significantly related to provenance[31]. Neither of these above studies attempted to account for the pH decrease in the Al treatments due to Al-hydrolysis.

Hypotheses

This review indicates there are still many unknowns concerning forest tree growth and nutrition in acid and Al-rich media. We proposed to examine some of these relationships for Pacific Northwest conifer seedlings by testing the following hypotheses:

a) That the greater ability of western hemlock to grow in acid media, compared to Douglas-fir and western redcedar, is due to western hemlock's physiological tolerance to high concentration of H and Al-cations in solution, and

b) That the effects of Al concentrations *per se* in solution on growth and nutrition of forest seedlings can be differentiated from the pH decrease due to the hydrolysis of the Al.

Materials and Methods

General Methods

Experimental equipment The nutrient culture experiments utilized large metal tanks; each capable of holding 20 l of hydroponic solution. The tank lids had twenty-eight 4cm holds on a 4 x 7 matrix. Into these holes were fitted large bottle corks, each containing a central hole within which individual tree seedlings were supported below their root collars. Two additional holes at either end of the lids allowed insertion of glass capillary tubes; angled at one end and connected to a compressed air source at the other end via rubber tubing. The interior of the metal tanks were coated with a bituminous paint and lined with plastic-sheeting to lessen any likelihood of solution contamination.

Nutrient solution composition Elemental ratios of nutrients within the hydroponic solutions were based on the "optimum" ratios of Ingestad[14] for various conifer seedlings. The solution N:K:P:Ca:Mg:S:Fe:Mn:B:Cu: Zn:Cl:Mo:Na ratio was 100:57.5:15.2:15.7:9.5:6.5:2.5:0.4:0.2:0.03:0.03: 2.05:0.007:-1.149, respectively. The differences in the chosen ratios to those of Ingestad[14] were due to parsimonious use of chemicals, simplification of the averaged concentration values for various tree species in terms of mM l^{-1} with a $NH:NO_3$ ratio of 2.5:4.8 to minimize solution pH decrease with nitrogen uptake yet allow a dual N-source. Iron was added separately as $FeSO_4$ to minimize precipitation in stock solutions. Solutions were renewed every 7 days.

Tissue chemical analysis Harvested plant tissue was dried at 70°C for 24 hours and weighted. Samples were then ground to pass through a 20-mesh screen on a Wiley Mill. Ground tissue samples were digested using two techniques: 1) H_2O_2 - H_2SO_4 - Li_2SO_4 digest[27] from which N, P, Ca, Mg, K, Fe and Mn could be determined; and 2) HNO_3 digest[11] from which Cu, Zn and Al could be determined. Total N and P were measured colormetrically on a Technicon Auto-analyzer II, while the other elements were analyzed on an IL 951 atomic absorption spectrometer. The United States National Bureau of Standards orchard leaves (SRM 1571) were used to check each analytical batch of samples.

Statistical analysis All Chi-squared tests and analysis of variance (ANOVA) of experimental data were made using SPSS (version 8)[25]. The *a priori* level of significance was set at α = 0.05. In the case of significant one-way ANOVAs, treatment means were ranked and compared for significant differences using Duncan's Multiple Range Test with α = 0.05 (also contained within SPSS).

Specific Methods

Treatments Three treatments were chosen:

1. [U]: unbuffered nutrient solution (pH=4.8),
2. [Al]: nutrient solution plus 175 ppm Al as $AlCl_3 \cdot 6H_2O$ which buffered the solution at pH 3.0, and
3. [H]: nutrient solution titrated with HCl to a pH equivalent to that of the [Al] solution (pH = 3.0).

That is, the [Al] and [H] treatments were designed to have the same pH, but differing in that one had Al. This concentration of Al had to be large enough to: a) approximate threshold toxic concentrations for seedlings of the three species, and b) cause a sufficient decrease in solution pH so that seedling growth in [H] treatment would be distinguished from that observed in unbuffered nutrient solutions.

Experimental design Douglas-fir, western hemlock, and western redcedar 2-0 plug seedlings (designated DFIR, WHEM and WRED, respectively) were obtain from Pack Forest Nursery, Le Grande, Washington (local seed source). Each tank was initially stocked with 14 individual seedlings of one species. The experimental design required 18 tanks (3 species x 3 treatments x 2 replications). Pretreatment growth in unbuffered nutrient solutions was maintained for 3 weeks to allow the seedlings to adapt to the nutrient solutions. This period was adequate for the seedlings to produce new foliage and primary roots. Any seedling mortalities during pretreatment were replaced.

pH control The pH of all three treatments decreased over a 7-day solution cycle so it was monitored daily. The [H] treatment pH was kept equivalent to that of the [Al] treatment by further addition of 1*M* HCl. This pH imbalance was infrequent because the pH change in [Al] and [H] treatments were comparable and small due to the buffering capacity of the solutions. The [U] treatment pH was not altered.

Harvest After 45 days of growth under treatments, all Douglas-fir and western redcedar seedlings were harvested. In contrast to these two species, western hemlock displayed no obvious foliage symptoms or differential shoot growth response across treatment even though its mortality was high in the [Al] treatment. Four western hemlock seedlings were harvested at random from each replicate treatment tank and the remainder were allowed to grow. These latter hemlock (designated WHEM2) were maintained for an extra 55 days and then harvested.

Measurements. At the start of treatment, seedling height was measured from root collar to tip of foliage. At both harvests final shoot height, stem diameter (1cm above the root collar), and root length were measured for all seedlings. Harvested seedlings were separated into root and shoot components with the latter being further separated into foliage and stem components for biomass determinations. In the case of western redcedar, lateral leaf-branches were separated from the main axial stem. These biomass components were dried, weighed and prepared for chemical analysis. Effect of treatments on root cation exchange capacity[5] (RCEC) was measured for each species.

Results

Mortality

The effect of treatment on seedling survival is shown in Fig. 1. Mortality of DFIR and WRED occurred only in acid treatments. Both [H] and [Al] treatments had equal DFIR mortalities, while WRED had higher mortality in [H] treatment. All three treatments caused WHEM mortality, but survival was highest in [H] treatment. Mortality in [Al] treatment was 17% greater than mortality in the unbuffered solutions and 25% greater than in solutions of the same pH but without Al present. An extra 55 days of growth for WHEM2 caused further mortalities, but only in [Al] treatment wherein 4 out of the remaining 11 seedlings died (36%). The null hypothesis that survival was similar across all treatments was rejected only for WHEM and WHEM2 using the Chi-squared Test.

Dimensional growth

One-way ANOVAs were performed on the following dimensional growth properties; absolute and relative height growth, final root length, final stem diameter, and root:shoot ratio across the three treatments for each species (including WHEM2). Treatment means were compared using Duncan's Multiple Range Test. The results of the ANOVA are summarized in Table 1. Figure 2a displays treatment means for relative height growth of the three species.

Acid treatment means for all DFIR dimensional growth properties measured after 45 days of growth were less than growth measured in unbuffered nutrient solutions. The presence of Al in the acid solutions further depressed growth as measured for all properties except final root length.

Western redcedar acid treatment growth was similar to these DFIR results with the exception of absolute and relative height growth which were lowest in [H] treatment. Root elongation was found to be independent of treatment for WRED and, therefore, root:shoot ratios were larger in the acid treatments (due to the lesser shoot growth in these treatments).

After 45 days of treatment the only WHEM dimensional growth properties having significant treatment response were root length (and, therefore, root:shoot ratio) and stem diameter. Only in [Al] treatment was root growth inhibited. The [H] treatment stem diameters were less than those of either [U] or [Al] treatment. An extra 55 days of growth for the western hemlock (WHEM2) did not produce any significant difference in height growth between treatments. Acid treatment root length and root:shoot ratio were, however, less than that in [U] treatment. The [Al] treatment had the largest decreases in these two root growth variables. Stem diameter was, however, significantly greater in [Al] treatment,

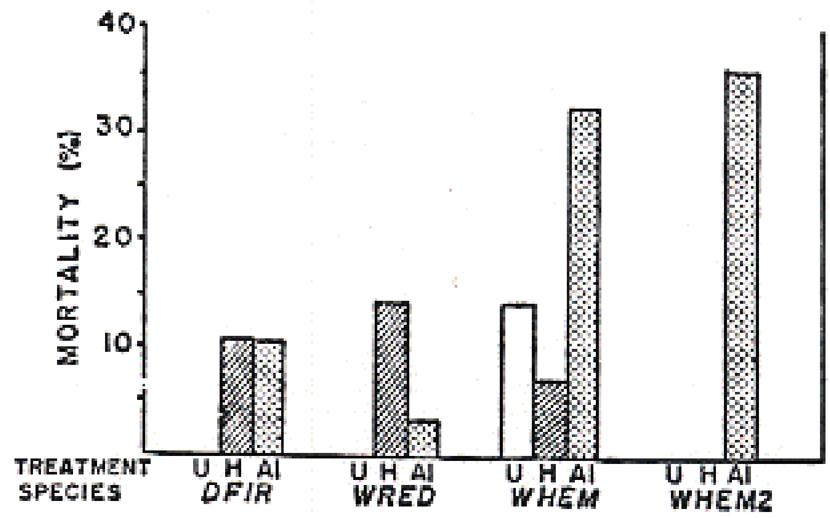

Fig. 1 Effect of treatments on mortality of Douglas-fir (DFIR), western redcedar (WRED), and western hemlock (WHEM, 1st harves; WHEM2, 2nd harvest). Data are number of dead seedlings as a percentage of the original 14 seedlings per species, except WHEM2 where at the beginning of the extra 55 days of growth there were 11 live seedlings.

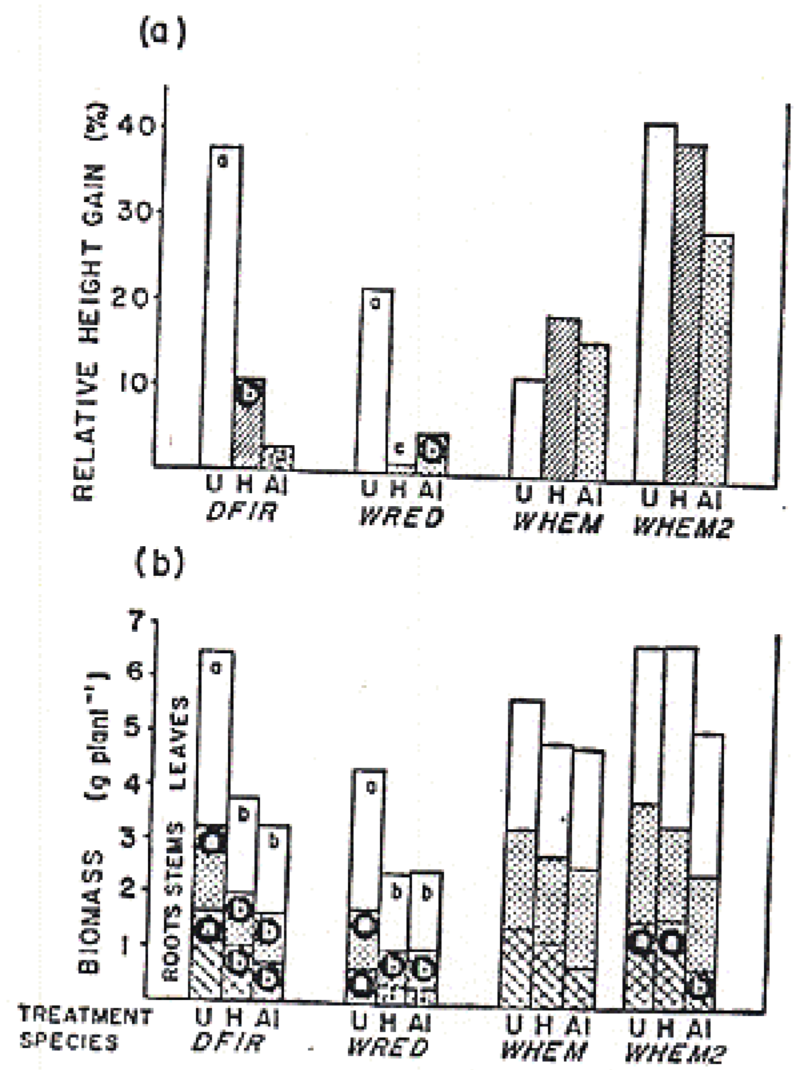

Fig. 2 Effect of treatments on a) relative height gain, and b) roof + stem + leaf biomass growth of Douglas-fir (DFIR), western redcedar (WRED), and the two harvests of western hemlock seedlings (WHEM and WHEM2). Significantly different treatment means within species by Duncan's Multiple Range Test are indicated by the letters (a,b,c).

Table 1. Effect of treatments on dimensional growth properties. Data are treatment means (upper line), standard errors (lower line in parenthesis), and sample sizes (lower line) for Douglas-fir (DFIR), western redcedar (WRED), and western hemlock (WHEM, last harvest; WHEM2, 2nd harvest). Within each species group, significantly different treatment means by Duncan's Multiple Range Test are indicated by differing letters (a,b,c), while lack of letters means ANOVA was not significant.

Dimensional Growth ANOVA	DFIR			WRED			WHEM			WHEM2		
	U	H	A1	U	H	A1	U	H	A1	U	H	A1
Absolute Height Growth(cm)	11.6 a (0.39) 28	3.0 b (0.45) 25	0.9 c (0.23) 24	7.1 a (0.51) 28	0.4 c (0.13) 24	1.6 b (0.13) 27	4.3 (0.80) 8	6.8 (1.46) 8	5.6 (0.99) 8	14.1 (1.38) 16	13.3 (0.81) 18	10.0 (0.70) 7
Relative Height Growth(%)	37.9 a (1.15) 28	10.8 b (1.64) 25	2.9 c (0.74) 24	21.7 a (1.62) 28	1.3 c (0.40) 24	4.9 b (0.41) 27	11.8 (2.08) 8	19.2 (3.90) 8	16.3 (2.94) 8	42.2 (4.77) 16	39.9 (2.43) 18	29.6 (2.45) 7
Final Root Length(cm)	44.0 a (1.40) 28	27.1 b (1.10) 25	23.9 b (0.63) 25	31.0 (1.68) 28	31.7 (1.94) 24	33.4 (1.70) 27	38.2 a (0.95) 8	36.1 a (1.98) 8	24.5 b (1.14) 8	49.0 a (1.34) 16	38.1 b (1.75) 18	26.3 c (1.68) 7
Final Stem Diam.(cm)	0.42 a (0.009)27	0.34 b (0.011)25	0.28 c (0.008(24	0.37 a (0.006)28	0.27 b (0.007)24	0.27 b (0.008)27	0.36 a (0.012)8	0.30 b (0.017)8	0.37 a (0.018)8	0.39 b (0.015)16	0.39 b (0.009)18	0.47 a (0.022)7
Root: Shoot Ratio	1.05 a (0.032)28	0.85 b (0.037)25	0.75 c (0.023)24	0.77 b (0.042)28	0.97 a (0.061)24	0.99 a (0.050)27	0.95 a (0.041)8	0.93 a (0.115)8	0.62 b (0.026)8	1.02 a (0.037)16	0.82 b (0.041)18	0.61 c (0.056)7

but this was related to a specific morphological change in [Al] treatment stems and not to a general stem volume increase.

Biomass growth

Table 2 summarizes the biomass growth data analysis while Fig. 2b displays mean leaf, stem, and root biomass components of each species for 3 treatments. Both DFIR and WRED had significantly depressed leaf, stem and root biomass in the acid treatments. The WHEM biomass was not significantly affected by treatment, but after an extra 55 days of growth WHEM2 root biomass in [Al] treatment was significantly less than that in [H] or [U] treatments.

Morphology

Initial effects of the acid treatments were observed in the roots of all three species except WHEM in [H] treatment where root morphology was indistinguishable from [U] treatment. After two weeks of treatment, DFIR roots in [Al] treatment had a brownish colour, stunted lateral roots, and mucillaginous root caps. The WRED roots in the same treatment were yellow-brown in colour with a slight swelling to the root tip. The [Al] treatment had its most marked effects in WHEM roots. Growth was noticeably depressed and prominent bulbous, club-like root tips were common. These symptoms generally increase in intensity until harvest. In [H] treatment DFIR roots had suppressed primary growth, brown discolouration, mucillaginous coatings, and swollen tips. The morphology of WRED roots in [H] treatment were effected more than those in [Al] treatment. Roots were coated in mucillage and discoloured with mottled bands of red or brown. Lateral roots were stunted.

Foliage symptoms were obvious in DFIR and WRED by the end of the third week of growth. The DFIR foliage from [Al] treatment developed a range of symptoms consisting of: 1) duller green colouration and loss of turgor, 2) necrosis of older foliage from leaf apex, and 3) red-purple discolouration and loss of turgor in recent flush stems and youngest flush leaves. In WRED, [Al] treatment produced a general purplish-brown colouration of the full foliage and scale-branches with some necrosis of the apical scale-leaves. Only after 55 extra days of growth under treatment did WHEM2 display obvious foliage symptoms, and then it was only for [Al] treatment plants. In this latter treatment, older leaves would become paler green and develop pale yellow mottled areas. This would progress into a colour zonation of the leaves: necrosis of the lower portion of the leaf which would gradate into a chlorotic zone with a normal green tip. Necrosis increased in the older leaves until they fell off the plant. Younger leaves also developed these symptoms just before the whole plant died.

Table 2. Effect of treatments on biomass component weights. Data are treatment means (upper line) and standard errors (lower line in parentheses) for Douglas-fir (DFIR), western redcedar (WRED), and western hemlock (WHEM, 1st harvest; WHEM2, 2nd harvest). Within each species group, significantly different treatment means by Duncan's Multiple Range Test are indicated by differing letters (a,b,c) while lack of letter means ANOVA was not significant. Treatment means are all for 2 replicates.

Biomass	DFIR			WRED			WHEM			WHEM2		
(g plant^{-1})	U	H	Al	U	H	Al	U	H	Al	U	H	Al
Leaf	3.24 a (0.35)	1.89 b 0.11)	1.64 b 0.09)	2.59 a 0.08)	1.42 b 0.07)	1.46 b 0.11)	1.74 0.13)	1.50 (0.33)	1.59 (0.03)	2.93 (0.46)	2.46 (0.45)	1.92 (0.05)
Stem	1.54 a (0.13)	1.01 b (0.04)	0.90 b (0.05)	1.12 a (0.04)	0.68 b (<0.01)	0.73 b (0.03)	1.34 (0.13)	1.18 (0.25)	1.34 (0.03)	2.25 (0.32)	1.74 (0.23)	1.77 (0.00)
Root	1.68 a (0.19)	1.05 b (0.08)	0.75 b (<0.01)	0.66 a (0.02)	0.36 b (0.01)	0.31 b (0.02)	1.07 (0.08)	0.88 (0.18)	0.54 (0.06)	1.62 a (0.18)	1.67 a (0.22)	0.75 b (0.01)

Foliage symptoms on species in [H] treatment were highly variable both within species and between species. No symptoms developed in WHEM or WHEM2. For DFIR and WRED, symptoms became particularly obvious after several consecutive days of very hot weather (June 15-22, 1982) during the third week of growth. This was especially the case for WRED whose foliage was slightly brownish in colour and lacking turgor before this period, but developed severe necrosis and wilting of upper scale-branches during and immediately after this period. By harvest, there had been some recovery in WRED. New green scale-leaves were evident at the base of the scale branches. Colouration varied in these scale-branches, progressing from green at the axil to purplish-brown to red to yellow to necrotic scales at the branch apices.

The high temperatures also adversely affected DFIR in [H] treatment, but this species displayed foliar symptoms prior to this period. At harvest there was a diverse range of foliage symptoms found on [H] treatment DFIR. They varied from: 1) necrotic older leaves, dull green new flush with low turgor, 2) pale to dull-green colouration of all the foliage, 3) pale green leaves with yellow or brown mottling (similar to aphid infestation damage), 4) similar to the latter symptoms, but with purplish-brown colouration of second flush leaves, to 5) purplish colouration of recent flush leaves, tan-brown older flush leaves to necrotic older leaves, and stems green up till flush tips there they became purplish and desiccated.

Chemical analysis

The effect of treatment on mean nutrient concentration of root and leaf components of the three species was determined by submitting the chemical analysis data for each element within species to one-way ANOVA and having the means compared by Duncan's Multiple Range Test (Tables 3 and 4). Both [Al] and [H] treatments significantly decreased various nutrient concentrations in different biomass components of the 3 species when compared to their respective [U] treatment values. Table 5 summarizes these data by detailing those elements with tissue concentrations in the acid treatment significantly less than that found for plants growing in [U] treatment. In some cases only the element concentrations in [Al] or [H] treatment are significantly less than the other two treatments, whereas other element levels were depressed in both acid treatments when compared to [U] treatment. Table 5 shows that these treatment patterns vary distinctly between biomass components and species.

Both acid treatments produced decreased levels of several elements in DFIR and WRED roots (Ca and Mg being the only two elements in common), while in the foliage the only significant decreases found were for Mg and Cu by [H] treatment in DFIR, K by [H] treatment and P by [Al] treatment in WRED, and Mn by both acid treatments in WRED.

Table 3. Effect of treatments on foliar chemistry. Data are treatment means for Douglas-fir (DFIR), western redcedar (WRED), and western hemlock (WHEM, 1st harvest; WHEM2, 2nd harvest). Within species significantly different means by Duncan's Multiple Range Test are indicated by differing letters (a,b,c), while no letters means the ANOVA was not significant. All treatment means are for 2 replicates.

	DFIR			WRED			WHEM			WHEM2		
	U	H	Al	U	H	Al	U	H	Al	U	H	Al
P (%)	0.54	0.57	0.55	0.40 a	0.37 a	0.30 b	0.41 a	0.36 ab	0.29 b	0.35 a	0.26 b	0.24 b
Ca (%)	0.20	0.14	0.18	0.56	0.54	0.46	0.24 a	0.26 a	0.16 b	0.24 a	0.28 a	0.16 b
Mg (%)	0.15	0.11 b	0.13 ab	0.19	0.18	0.16	0.15 a	0.15 a	0.10 b	0.16 a	0.15 a	0.10 b
K (%)	1.65	1.26	1.89	2.39 a	1.72 b	1.98 ab	1.34	1.27	1.30	1.47	1.43	1.38
Fe (ppm)	194	125	126	139	191	163	151	207	138	144	143	74
Mn (ppm)	130	88	104	102 a	76 b	72 b	184	164	108	192 a	168 a	100 b
Cu (ppm)	8.8 a	6.4 b	9.3 a	13.7	13.4	12.8	12.6	9.3	7.0	8.6 a	7.4 b	4.5 b
Al (ppm)	44 b	62 b	282 a	63 b	95 b	175 a	101 b	106 b	290 a	83 b	85 b	342 a

Table 4. Effect of treatments on root chemistry. Data are treatment means for Douglas-fir (DFIR), western redcedar (WRED), and western hemlock (WHEM, 1st harvest; WHEM2, 2nd harvest). Within species significantly different means by Duncan's Multiple Range Test are indicated by differing letters (a,b,c), while no letters means the ANNOVA was not significant. All Treatment means are for 2 replicates.

	DFIR			WRED			WHEM			WHEM2		
	U	H	Al	U	H	Al	U	H	Al	U	H	Al
P (%)	0.78 a	0.37 b	0.41 b	0.51 a	0.27 c	0.39 b	0.59	0.40	0.36	0.54 a	0.40 ab	0.33 b
Ca (%)	0.11 a	0.06 b	0.04 b	0.28 a	0.14 b	0.09 b	0.14 a	0.09 b	0.05 c	0.13 a	0.10 b	0.05 c
Mg (%)	0.07 a	0.04 b	0.02 c	0.06 a	0.04 b	0.02 b	0.07 a	0.05 b	0.01 c	0.06 a	0.04 b	<0.01 c
K (%)	1.58 a	0.40 b	0.15 b	1.32 a	0.20 b	0.27 b	1.13 a	0.75 ab	0.38 b	1.02 a	0.94 b	0.27 b
Fe (ppm)	701 a	710 a	136 b	996 a	662 b	107 c	1117 a	779 ab	69 b	1769 a	1280 ab	178 b
Mn (ppm)	43 a	17 b	12 b	31	20	16	85	41	22	70 a	40 b	14 c
Cu (ppm)	95.3 a	56.2 b	28.8 b	108.0 a	75.0 b	43.9 c	151.7 a	47.3 b	33.5 b	125.1 a	44.2 b	31.1 b
Al (ppm)	125 b	181 b	4056 a	210 b	242 b	3928 a	161 b	175 b	3562 a	128 b	123 b	3201 a

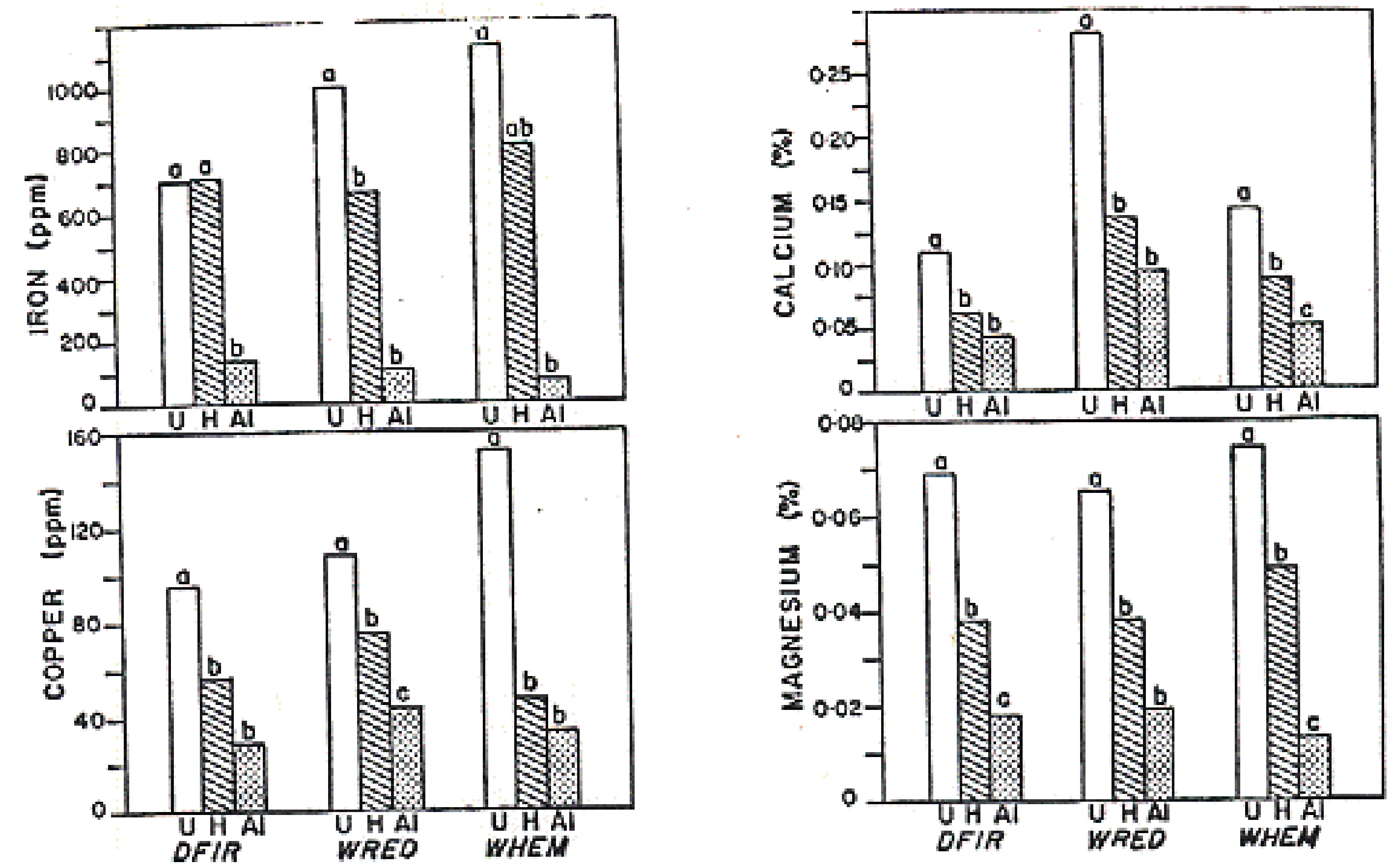

Fig. 3 Effect of treatments on root concentrations of Fe, Ca, Cu and Mg for Douglas-fir (DFIR), western redcedar (WRED), and western hemlock (WHEM). Significantly different treatment means within species by Duncan's Multiple Range Test are indicated by the letters (a,b,c).

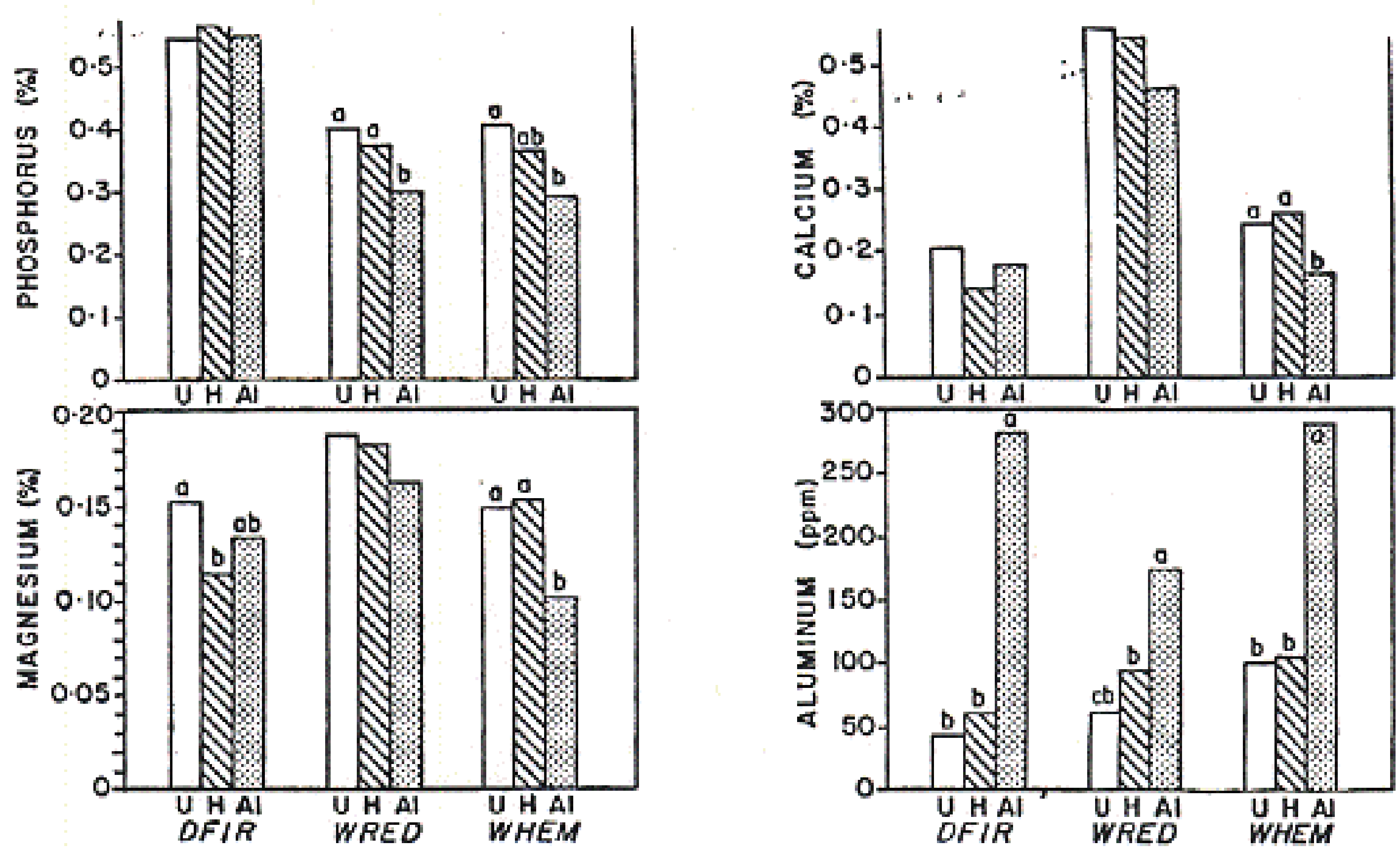

Fig. 4 Effect of treatments on foliar concentrations of P, Ca, Mg and Al for Douglas-fir (DFIR), western redcedar (WRED), and western hemlock (WHEM). Significantly different treatments within species by Duncan's Multiple Range Test are indicated by the letters (a,b,c).

In contrast, WHEM had foliar Ca, P and Mg significantly depressed in [Al] treatment. After an extra 55 days WHEM2 foliage had significantly less Ca, Mg, Mn and Cu in [Al] treatment only. The [H] treatment did not decrease any of the foliar elements analyzed for WHEM. For those elements with decreased WHEM-WHEM2 root concentrations in both acid treatments, the levels in [Al] treatment were either equivalent or less than those in [H] treatment.

Effects of high H and Al-cation concentrations on the nutrition of the tree seedlings is most obvious in the root nutrient content. Figure 3 compares treatment effects on root Ca, Mg, Fe and Cu concentrations. A similar pattern exists for all four divalent cations. Low solution pH decreases root concentrations of these cations (except Fe in DFIR roots) but the presence of Al in solution at the same pH further decreased root concentrations, sometimes to statistically significant lower levels.

Figure 4 displays comparative foliar levels of P, Ca and Mg and Al across species and treatment. Aluminum depressed foliar P in WRED and WHEM, but not in DFIR. Note that Douglas-fir's ability to take up luxury quantities of P was unaffected by acid treatment. In contrast to the other species, WRED acquired luxurious quantities of Ca independent of acid treatment. Only for WHEM in [Al] treatment were foliar Ca and Mg levels significantly decreased. Calcium and Mg in western hemlock biomass would seem to be particularly sensitive to the presence of Al in solution (Table 5, Fig. 3 and 4).

In [Al] treatments the highest biomass concentrations of Al occurred in the roots of all three species (3500 to 4100 ppm). Douglas-fir had high Al levels in leaves (282 ppm, Fig. 4) and stems (1053 ppm). While WHEM had similar foliar levels (290 ppm) to DFIR, this species differed by having the lowest stem concentrations (134 ppm). WRED had the lowest foliar Al levels (175 ppm) which coincide with this species high Ca levels. In comparison to the two other species, DFIR had the lowest Al concentrations in most of its biomass components for [U] and [H] treatments.

Root Cation Exchange Capacity

The [Al] treatment decreased RCEC for all species (Table 6). Compared to [U] treatment, [H] treatment increased RCEC for all species except DFIR. This latter species had the highest RCEC in [U] treatment.

Discussion

In comparison to unbuffered solutions, the acid [H] and [Al] treatments generally produced decreased biomass growth (Table 2) and root nutrient concentrations (Table 4) for Douglas-fir and western redcedar. It is difficult to then extract a definite

Table 5. Effect of the acid treatments on leaf and root component nutrient concentrations. Only relationships between significant treatment means by Duncan's Multiple Range Test are displayed.

	DEPRESSED BY [H] AND [Al]			DEPRESSED BY [H] ONLY	DEPRESSED BY [Al] ONLY
	H = Al	H > Al	H < Al		
DFIR					
Leaves				Mg,Cu	
Roots	P,Ca,K Mn,Cu	Mg			
WRED					
Leaves	Mn			K	P
Roots	Mg,Ca,K	Fe,Cu	P		N
WHEM					
Leaves					Ca,P,Mg
Roots	Cu	N,Ca,Mg			Fe,K
WHEM2					
Leaves					Ca,Mg,Mn,Cu
Roots	Cu	Ca,Mg, Mn,Zn			N,K

Table 6. Effect of treatments on Douglas-fir (DFIR), western redcedar (WRED), and western hemlock (WHEM) root cation exchange capacity. Data are treatment means ± standard deviations (2 replications).

Species	Root Cation Exchange Capacity (meq $100g^{-1}$)		
	[U]	[H]	[Al]
DFIR	38.0 ± 0	33.0 ± 2.1	20.0 ± 5.7
WRED	21.1 ± 1.8	34.0 ± 2.1	17.7 ± 1.1
WHEM	28.7 ± 1.8	37.0 ± 2.8	18.75 ± 1.1

effect of Al-cation per se in these two species for these properties beyond that which can be attributed to increased H-cation concentration the aluminum causes via hydrolysis reactions.

Dimensional growth properties were far more sensitive to treatment effects and for these properties the effect of Al in solution could be differentiated although it varied between species. Both Douglas-fir and western redcedar height growth was decreased in acid treatment, but for Douglas-fir the least growth was in the presence of Al, while for western redcedar Al in solution seems to partially alleviate the adverse effect of excess H-cations.

There were other differences between Douglas-fir and western redcedar in their respective response to the acid treatments. Western redcedar mortality was higher in [H] treatment and visual foliage symptoms of acid treatments were also distinctly different between [Al] and [H]. The purplish-brown discolouration of [Al] treatment foliage was probably due to P deficiency[33], which was not produced in Douglas-fir. The severe necrosis and discolouration of [H] treatment foliage seemed to be affected by several days of intense heat and possibly related to increased transpiration rates. Only K was found significantly less in [H] treatment foliage so there is also the possibility of direct H toxicity to western redcedar at high temperatures or high transpiration rates. The presence of Al in solution would seem to lessen this effect.

Western redcedar's ability to accumulate Ca in its biomass[33] is particularly interesting in the context of this experiment because Ca is one of the divalent cations whose absorption is inhibited by the presence of Al in solution. Foliar Ca levels in redcedar were almost double those of Douglas-fir or western hemlock (Fig. 3). Western redcedar grown in [Al] treatment had decreased foliar Ca levels, but this depression was not significant. Both acid treatments decreased root Ca, but [H] treatment levels were equivalent to concentrations for Douglas-fir and western hemlock [U] treatments. Western redcedar absorbed the least amount of Al into its foliage so there may be an interaction between cedar's ability to absorb excess Ca and exclude Al from its biomass.

In contrast to Douglas-fir and western redcedar, seedling growth of western hemlock in acidified [H] treatments (without Al) of pH 3.0 was either indistinguishable from, or superior to growth in unbuffered nutrient solutions.

Presence of Al in acid treatment solutions significantly decreased western hemlock survival, root biomass and length, foliar and root concentrations of Ca, Mg and Mn, and root:shoot ratio when [Al] and [H] treatments were compared. Hemlock roots in [Al] treatments also developed a distinctively abnormal morphology of stunted, club-like root tips with suppressed, bifurcated vascular bundles. Davies[6] observed such a root morphology in Ca-deficient *Pinus taeda*. Root Ca levels for western hemlock in [Al] treatment were significantly less than [H] or [U] treatments. Therefore, the deformed root tip morphology of western hemlock[29] was probably due to Al-induced Ca deficiency, rather than direct Al-toxic effects on root growth, although an Al-Ca interaction may exist. Surviving western hemlock displayed a general lack of differential response between treatments in shoot biomass or dimensional growth properties, although variability in these properties for western hemlock was high.

Western hemlock seedlings were, therefore, distinctly more tolerant than any of the other species to nutrient solutions of pH $\geq$ 3. This characteristic of western hemlock may be related to its ability to function at low tissue concentrations of Ca and Mg[32]. Acid solutions did reduce root concentrations of Ca and Mg, but this did not adversely affect seedling growth or uptake of these cations or other nutrients to the foliage.

Root cation exchange capacity of all three conifer seedlings generally decreased in solution containing Al. This process may be a simple blockage of exchange sites by Al or hydroxy-Al species, rather than a physiological adaption for Al-tolerance by which plants lower root CEC and thus decrease Al sorption in the root apoplast[8,9,10]. In fact, western hemlock and western redcedar roots in acid solutions without Al had higher RCEC than in any other treatment (Table 6). The significance of this RCEC increase is unknown.

Conclusions

Pacific Northwest forest species varied in their comparative response to two very acid nutrient solutions of the equivalent pH, but differing insofar as one contained Al at high concentrations. Growth and nutrition of Douglas-fir and western redcedar were adversely affected in both acid solutions. In contrast, Western hemlock's growth and nutrition in acid solutions of pH 3 was equivalent, and in some cases superior to that in untreated nutrient solution. But when the acid solutions contained Al at 175 ppm, western hemlock mortality increased and root growth and nutrient absorption was adversely affected. The specific effect of Al on western hemlock growth was discernible from effects caused by excess H-ion (low pH) because western hemlock was tolerant of nutrient solutions with high H-ion concentrations.

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ACID TOLERANCE OF PACIFIC NORTHWEST CONIFERS IN SOLUTION CULTURE:

II. EFFECT OF VARYING ALUMINIUM CONCENTRATION AT CONSTANT pH

P.J. RYAN, S.P. GESSEL and R.J. ZASOSKI

SUMMARY Seedlings of four Pacific Northwest conifer species; Douglas-fir (*Pseudotsuga menziesii*), western hemlock (*Tsuga heterophylla*), western redcedar (*Thuja plicata*), and Sitka spruce (*Picea sitchensis*) were grown in solution culture at six levels of aluminium concentration (0, 10, 25, 50, 75 and 100 ppm Al). The pH of these nutrient solutions was adjusted to be equivalent with that level induced by hydrolysis of the highest Al treatment (pH 3.5 of the 100 ppm Al treatment).

Divalent cation concentration in the roots of all four species decreased as Al activity increased. Potassium was the only nutrient that exhibited increased tissue concentrations as Al activity increased. Phosphorus and N in the seedlings had complex responses to increasing Al. Western hemlock and Sitka spruce root length was the main growth property found to be significantly affected by Al activity in the solutions.

Increasing Al concentrations of in these acid nutrient solutions did not significantly effect biomass growth of any of the four species. Western hemlock and western redcedar were especially tolerant of these acid-Al conditions. These results have been related to western hemlock's low tissue requirements of Ca and Mg and western redcedar's ability to accumulate high tissue levels of Ca even in the presence of excess H and Al-cations.

KEY WORDS Acidity Al toxicity-tolerance H toxicity-tolerance Pacific Northwest *Picea sitchensis* *Pseudotsuga menziesii* Solution culture *Thuja plicata* *Tsuga heterophylla*

Introduction.

Aluminium forms numerous monomeric and polymeric hydrolysis products, complexes and chelates in solution[9,21]. Critical to the predominance of any of these Al-species is solution pH, Al-activity, and solution ionic strength[9,14]. Therefore attributing observed plant tolerance or toxicity effects to total Al concentration in solution may be a gross simplification.

Facilitated plant absorption of monomeric and polymeric hydroxy-Al which have a lower net charge density than trivalent Al has been found as pH increased from 4.0 to 5.5[2,12]. Absorption of hydroxy-Al species with increased pH may not be deleterious to plant growth because of associations with P. The majority of reported "beneficial" effects of low solution Al levels (usually $<$ 20 ppm Al) has been attributed to facilitated uptake of Al-P complexes[2,12,13,22].

Aluminium toxicity has been directly related to Al-cation activity rather than total Al concentration[1,4]. Pavan and Bingham[15] and Paven *et al*[16] designed an elegant series of experiments which demonstrated that growth response of coffee in acid nutrient solutions and soils was best correlated to Al-cation activity rather than total Al or trivalent Al concentration. These authors used the Program GEOCHEM[19] to speciate Al in their solutions and found that toxicity symptoms in coffee were first exhibited at trivalent Al-cation activities of 1.2×10^{-5} $\underline{M}$ l^{-1} (solution culture) or 4.0×10^{-6} $\underline{M}$ l^{-1} (soil). These levels are much lower than the H-ion activities at which toxicity is first observed (e.g. 10^{-3}, Black[3]).

These experiments demonstrate that the interpretation of many Al-nutrient culture experiments depends upon the total Al concentration, composition of the nutrient solution, its ionic strength, and pH.

The previous nutrient culture experiment described in Ryan *et al*[18] utilized a high concentration of Al in solution which markedly decreased solution pH. Comparison was made of Douglas-fir, western hemlock and western redcedar growth and nutrition in this [Al] treated nutrient solution to; 1) growth in solutions of the same pH but without Al ([H] treatment), and 2) solutions whose pH were not adjusted ([U] treatment). Comparative differences in the adverse effects of [H] and [Al] treatments of Ryan *et al*[18] were not readily apparent in Douglas-fir and western redcedar growth while western hemlock maintained good growth in [H] treatment but was adversely effected by the presence of Al in solution. The following experiment was therefore designed to elucidate these species differences and to allow a more definitive examination of how increasing Al activity in solution at a constant low pH affected forest seedling growth.

Materials and Methods.

The experimental equipment, nutrient solution elemental ratios, tissue chemical analysis, and statistical analysis described in the general methods section of Ryan *et al*[18] were retained for this experiment.

Specific Methods

The design of this experiment differed from that described by Ryan *et al*[18] in three main areas;

1) Number and types of treatments.
2) Concentration of the nutrient solution.
3) Number of species and their allocation between the hydroponic tanks.

Treatments

Six treatment levels of aluminium (as $AlCl_3.6H_2O$) in solution were used. These concentrations were 0, 10,25, 50, 75 and 100 ppm Al, all of which were maintained at the pH of the 100 ppm Al treatment. Hydrolysis of this latter amount of $AlCl_3$ caused solution pH to decrease to approximately 3.5 at the start of a 7 day solution cycle. All of the other treatments (which contained less Al and therefore had higher initial solution pH) were titrated with 1M HCl till their pH were equivalent to that of the 100 ppm Al treatment. The nutrient solution used in this experiment had the same elemental ratios detailed in Ryan *et al*[18] but it was one third the total concentration. That is, the element ratios in solution were consistent with what Ingestad[8] presented for conifer growth but the total concentration was one third more dilute than that suggested by Ingestad[8] to produce "optimum" growth. Table 1 gives the treatment codes and compares their chemistry to that of the previous experiment[18].

Experimental design

Plug seedlings (1-0) of Douglas-fir (designated as DFIR), western redcedar (WRED), western hemlock (WHEM), and Sitka spruce (SSPR) were obtained from the Webster Nursery of the Department of

Table 1. Treatment solution codes, total Al, free trivalent Al-cation concentrations and activities, and solution ionic strengths of this experiment and that of Ryan *et al.*[18]

Treatment Code	Total Al (ppm)	*[Al^{3+}] ($m\underline{M}\ l^{-1}$)	*(Al Act.) ($m\underline{M}\ l^{-1}$)	*Ionic Strength ($m\underline{M}\ l^{-1}$)
1 [0AL]	0	0	0	1.46
2 [10AL]	10	0.33	0.19	3.40
3 [25AL]	25	0.84	0.39	6.48
4 [50AL]	50	1.68	0.62	11.52
5 [75AL]	75	2.52	0.77	16.68
6 [100AL]	100	3.29	0.88	21.44
[U] +	0	0	0	3.4
[H] +	0	0	0	4.5
[Al] +	175	5.66	1.04	38.3

* Ionic strengths, free cation concentrations, and activities were calculated using data from Program GEOCHEM (Sposito and Mattigod[19]) and the activity coefficient equation of Guntelberg (Stumm and Morgan[21]).

\+ Treatment solutions of Ryan *et al*[18].

Natural Resources, Scott Lake, Washington. Sitka spruce was the only species not used in the previous experiment[18]. Seven individual seedlings of each species were transferred to each tank (a total of 28 seedlings per tank). Three replications for each treatment resulted in 18 tanks being used in this experiment with 21 individual seedlings per species per treatment.

pH control

The pH of all six treatments decreased over a 7 day solution cycle (from 3.5 to 3.3) so it was monitored daily. The pH of the [100AL] treatment was maintained at equivalent levels in the other 6 treatments by small additions of 1M HCl. Since all four species were present in each tank, the pH decrease with should be appreciated as a combined species effect on net H-ion efflux into solution.

Harvest

After 98 days of growth under treatment the seedlings were harvested. Pretreatment interval, initial and harvested seedling measurements, and splitting of harvested biomass into leaf stem and root components were repeated as outlined in Ryan *et al*[18].

Results

Mortality

Seedling mortality was low in this experiment, being nonexistant for WRED and confined to [0AL] and [100AL] treatments for WHEM and SSPR. Single DFIR deaths (5% mortality) occurred in [10AL], [25AL] and [100AL] treatments while 3 seedlings (14%) died in [75AL] treatment. Douglas-fir mortality across treatments was the highest for any of the tree seedlings, followed by WHEM with 4 deaths (19%) in [100AL] treatment and 1 death in [0AL]. Sitka spruce had 3 deaths in [0AL] and 1 in [100AL] treatment. The null hypothesis that the number of surviving seedlings did not vary between treatment was rejected only for WHEM and SSPR at $\alpha = 0.05$ using the Chi-Squared Test.

Dimensional growth

One-way ANOVAs were performed for each species across the 6 treatments for data on absolute height gain, relative height gain, final stem diameter, final root length, and root:shoot ratio. Table 2 presents the treatment means, their standard errors, sample size and whether there are significant differences between means across treatments using Duncan's Multiple Range Test.

Table 2. Effect of Al concentration on selected growth properties of the conifer seedlings. Within species, significantly different treatment means by Duncans Multiple Range Test are indicated by differing letters (a, b or c) while the lack of letters indicates the ANOVA was not significant.

	TREATMENT					
SPECIES*	[0AL]	[10AL]	[25AL]	[50AL]	[75AL]	[100AL]
Final root length (cm)						
WHEM	49.5 a	55.8 a	55.3 a	49.5 a	41.3 b	37.4 b
DFIR	45.5	44.5	47.4	45.5	44.2	38.3
WRED	48.9	53.2	52.2	49.8	51.9	47.4
SSPR	49.9 bc	61.5 a	56.4 ab	46.0 c	48.8 bc	37.4 d
Final stem diameter (cm)						
WHEM	0.33	0.33	0.34	0.33	0.33	0.36
DFIR	0.44	0.40	0.42	0.41	0.43	0.46
WRED	0.38	0.40	0.41	0.39	0.41	0.42
SSPR	0.20c	0.23ab	0.23ab	0.21bc	0.24ab	0.25a
Absolute height gain (cm)						
WHEM	16.2	17.9	17.8	17.2	13.0	15.9
DFIR	4.1	1.8	1.5	2.8		0.8
WRED	11.1 ab	12.5 a	10.4 ab	11.8 a	11.4 ab	9.2 b
SSPR	0.3 a	-0.1 ab	0.3 a	-0.5 b	-0.2 ab	ab
Relative height gain (%)						
WHEM	82.3 a	93.4 a	91.2 a	83.7 a	59.5 b	82.2 a
DFIR	14.2 a	6.0 ab	4.4 ab	9.6 ab	b	3.0 b
WRED	39.3	43.9	37.4	39.6	37.7	32.5
SSPR	2.6	-0.1	2.8	-4.5	-0.4	-0.03
Root:shoot ratio						
WHEM	1.39b	1.49a	1.47a	1.29b	1.24bc	1.08c
DFIR	1.31	1.47	1.55	1.34	1.41	1.29
WRED	1.21	1.29	1.33	1.21	1.23	1.25
SSPR	4.35bc	5.46a	5.06ab	4.11bc	4.28bc	3.38c

* Western hemlock (WHEM), Douglas-fir (DFIR), Western redcedar (WRED), and Sitka Spruce (SSPR).

Only DFIR relative height gain treatment means were found significantly different with varying solution Al concentration. Relative height was highest in [0AL] treatment and generally decreased with increasing Al concentration. Compared to the [0AL] treatment, relative height gain in the [75AL] and [100AL] treatments was significantly decreased.

Western hemlock relative height gain was significantly different across treatments but this was due to the comparatively low mean of [75AL] treatment (59.5%). This value may seem anomalous in a comparison with [100AL] treatment of 82.2% but the high mortality is this latter treatment (4 out of 21 seedlings) should be considered. Most of the [100AL] mortalities were smaller seedlings so the relative height gain of the surviving seedlings was greater than those in [75AL] treatment which had small seedlings displaying foliar symptoms but no mortalities.

Major treatment effects were observed in final root length (Fig. 1a) and root:shoot ratio. Higher Al concentrations (treatments [75AL] and [100AL]) decreased WHEM root lengths. Figure 1a displays the trends of mean final root length over increasing treatment Al activity. A maximum value for both variables occurs between Al activities of 0 and 0.39 $\underline{m}M$ l^{-1} Al (0 and 20 ppm Al) in solution.

For WRED, absolute height gain in [10AL] and [50AL] treatment were significantly greater than that in [100AL] treatment. This relationship was however not repeated for relative height gain in WRED.

Sitka spruce dimensional growth results were similar to WHEM. The main treatment effects were found for final root length (Fig. 1a) and root:shoot ratio. These two properties were least in [100AL] and greatest in [10AL] treatment. Like WHEM, SSPR had trends indicating maximum values for these two properties between 0 and 0.39 $\underline{m}M$ l^{-1} Al. Absolute height gain was found significantly different across treatments but this was not considered experimentally meaningful for reasons which will be discussed later. Final stem diameter was significantly greater in [100AL] compared to [0AL] treatment (Table 2). Only SSPR stem diameter was significantly different across treatments, but a trend of increasing stem diameter with increasing Al concentration was evident for all species to varying degrees.

An important factor to remember when analyzing DFIR and SSPR growth data is that these plug seedlings had set bud at the Webster Nursery prior to the experiment. Having a predeterminant growth habit meant that there was little to no subsequent terminal shoot growth for either of these species. No bud flush occurred for SSPR over the 98 days of treatment growth even though root growth was prolific. Some lateral and terminal buds of DFIR did flush but relative height gain was minor (< 15%) and occurred only in treatments containing $\leq$ 50 ppm Al.

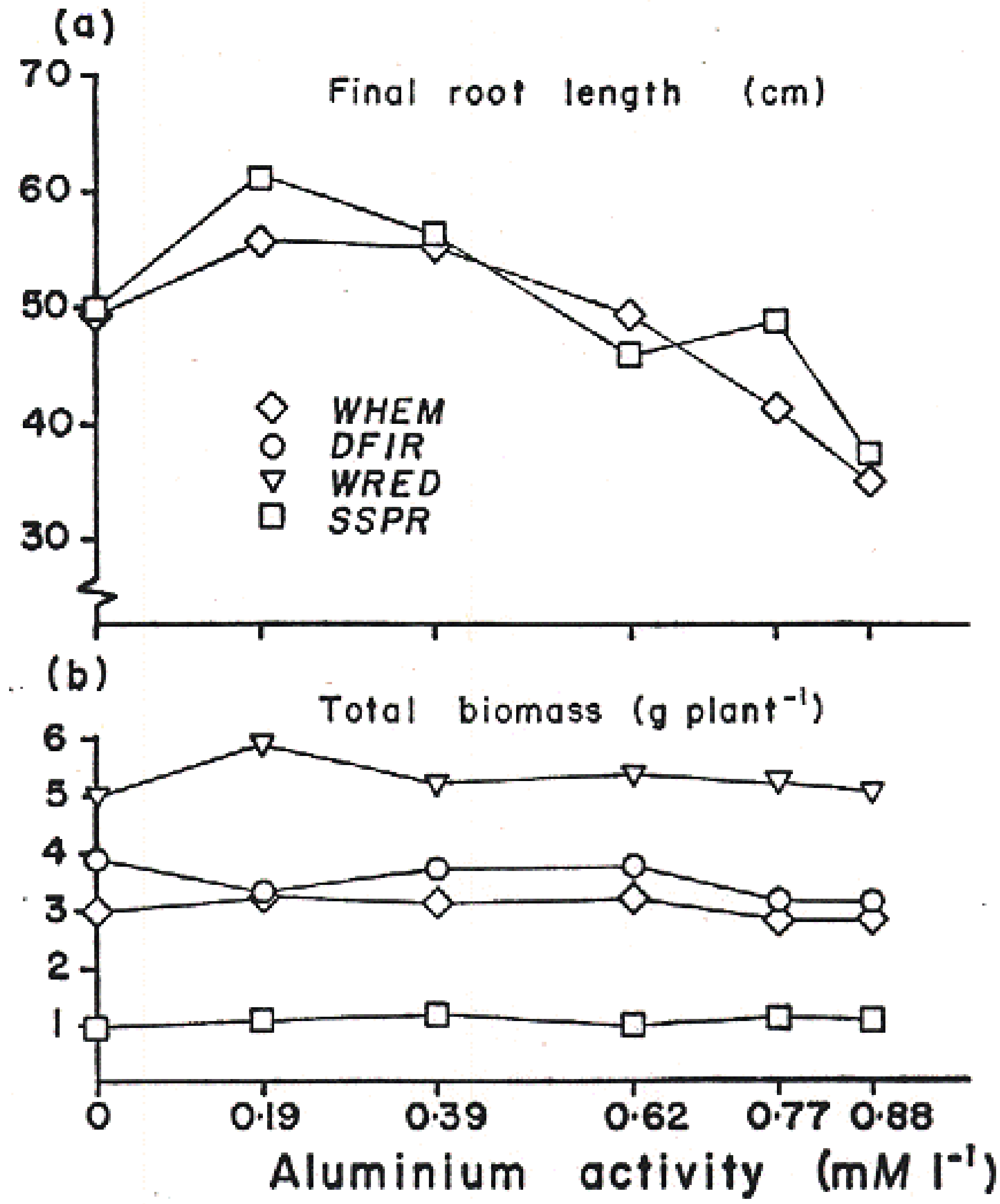

Fig. 1. Effect of treatment Al activities (from Table 1) on, 1) final root length of western hemlock and Sitka spruce seedlings, and b) total seedling biomass of all four conifer species.

Shoot growth for WHEM and WRED continued throughout this experiment with these seedlings having mean relative height gains of 30 to 95%. This lack of bud flush for DFIR and SSPR did not affect root growth. This is particularly noticeable in the comparison of root:shoot for WHEM and SSPR (Table 2) where SSPR had roots 4 to 5 times longer than its shoots while WHEM had roots only 1 to 1.5 times longer than their shoots. It is for the above reasons that the significant differences in SSPR absolute height growth (all less than 0.3 cm) are disregarded.

Biomass growth

No significant differences were found for any of the four species' biomass components across treatment. Figure 1b displays this evenness in total seedling biomass response to increasing solution Al concentration for all four forest species. Note that WRED had a high total biomass in [10AL] treatment.

Morphology

The general lack of terminal bud flush by SSPR or DFIR (although some lateral and terminal buds did burst for this latter species) contrasted with the good growth in WHEM and WRED seedlings. Treatment effects on foliage, stem and root morphology were less distinct in this experiment than that found in Ryan *et al*[18].

A diverse number of foliage symptoms were evident for one or two DFIR plants in treatments containing $\leq$ 50 ppm Al. In the [75AL] and [100AL] treatments, symptoms seen in the previous experiment associated with Al presence became evident. Purplish-brown colouration of stems and flush needles, yellow-green-brown mottling of needles and brown, jointed root morphology were typically observed.

No discernible treatment effects were evident in the growth of WHEM in treatments containing $\leq$ 50 ppm Al. For [75AL] treatment, smaller seedlings developed dull green to mottled yellow-green foliage. In some plants these symptoms progressed to a brown colouration and eventual necrosis of older leaves. Similar symptoms were apparent in [100AL] WHEM but these plants died earlier in the experiment and the remaining plants displayed fewer symptoms than [75AL] treatment at final harvest. No treatment effects on root morphology other than total root length were seen for WHEM.

Western redcedar grew well in all treatments without obvious leaf or root symptoms.

Treatment effects on SSPR morphology were variable. A common foliar symptom observed in [0AL], [50AL] and [100AL] treatments was a necrosis of old needles with some becoming purplish-brown in colouration. Root growth proceeded in SSPR even though buds did not flush. Roots in [OAL] treatment were brown in colouration while those in [100AL] treatment displayed some aberrant morphologies. Commonly, terminal root-tip growth had been suppressed and lateral roots had become dominant giving a forked "Y" appearance. In more severe cases where seedlings had foliage symptoms, their roots were shorter and had suppressed lateral roots forming "knobs". In other cases there was a similar "jointed" appearance as described for DFIR roots.

Chemical analysis

Leaf and root biomass components were analyzed for elemental concentrations. Treatment replicates for each species allowed statistical testing of the null hypothesis that increasing concentration of Al in an acid nutrient solution of constant pH did not affect nutrient concentrations of any of the four species leaf or root biomass components. One-way ANOVA tests were performed for each element analyzed across treatment.

In contrast to minor biomass and dimensional growth response to treatment, chemical analyses showed that root tissue concentrations of a large number of elements displayed significant treatment effects in all four species. Three generalized trends occurred when leaf or root nutrient concentrations were plotted as a function of treatment Al activity. These trends could be categorized as: 1) an antagonistic effect of Al, 2) a synergistic effect of Al, and 3) an interactive effect of Al. Species' element concentrations with significantly different treatment means have been classified under the above three categories and the results are displayed in Table 3. These trends are apparent for a number of other elements which are not displayed in Table 3 because of a large variability and lack of significance.

Figures 2 and 3 display a selection of these element-treatment response trends. Elemental concentration in leaf or root biomass is presented as a function of treatment Al activity (Table 1).

Table 3 and Fig. 2 show that the "antagonistic" trend response to increasing Al in solution was especially prominent for root Ca, Mn, Fe and Cu across all four species. Magnesium also displayed this antagonistic response in root concentrations for WRED. Foliar concentrations of Ca, Mg, Fe and Mn in WHEM had antagonistic responses to Al treatment but for the other three species only Ca for WRED and Mg for SSPR displayed similar trends in their respective leaf components. Both WRED and SSPR had high foliar Ca levels but only WRED had high levels in the roots (Fig. 2). Sitka spruce had the highest Mg concentrations in both leaf and root tissue of all four species. Root concentrations of Fe, Mn and Cu (Fig. 2) display very similar antagonist trends with SSPR having the highest values. In the case of Fe and Cu, concentration magnitude and its change with treatment appears strongly related to the respective species root-Al concentrations

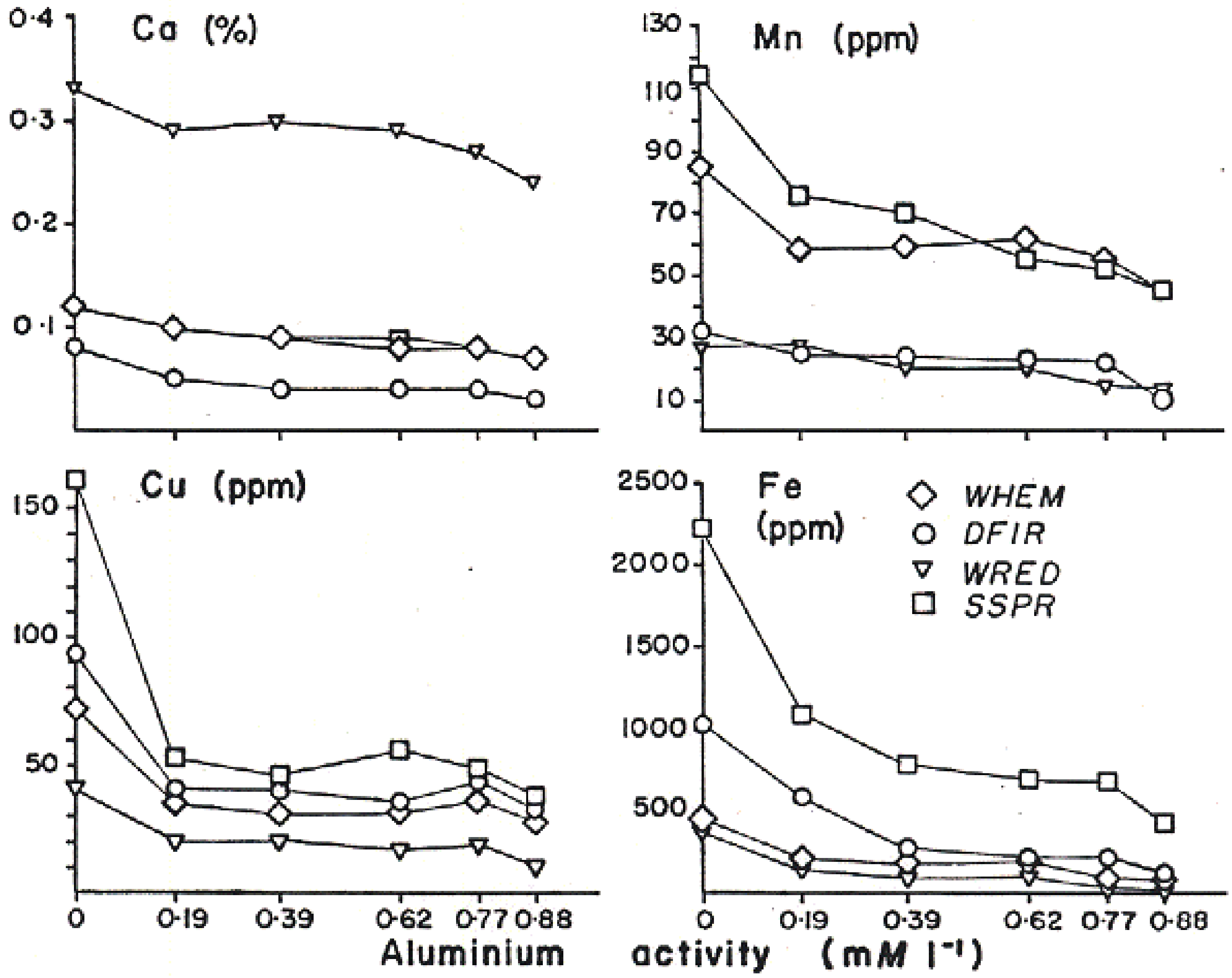

Fig. 2. Effect of treatment Al activities (from Table 1) on root concentrations of Ca, Mn, Cu and Fe for all four conifer species.

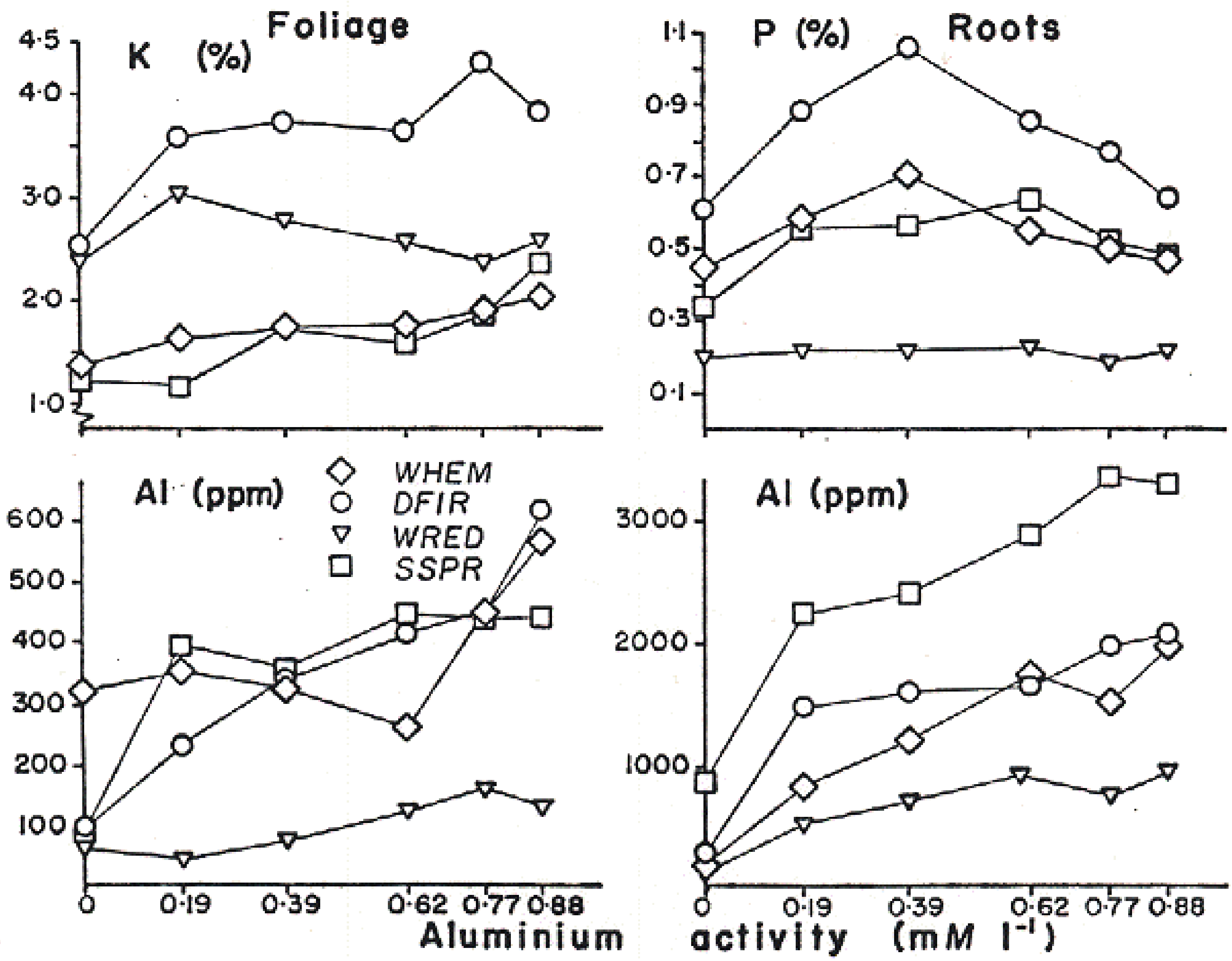

Fig. 3. Effect of treatment Al activities (from Table 1) on foliar K, Al, and root P and Al concentrations for all four conifer species.

Table 3. Classification of tissue element concentrations having significantly different treatment means into three types of trends with increasing solution Al.

	Treatment Trend Classification		
	Antagonistic	Synergistic	Interactive
Douglas-fir		Al	P
Leaves			
Roots	Ca, Fe, Mn, Cu		
Western hemlock			
Leaves	Ca, Mg, Fe, Mn	K	
Roots	Ca, Fe, Mn, Cu	Al	
Western redcedar			
Leaves	Ca		
Roots	Ca, Mg, Fe, Mn, Cu	Al	
Sitka spruce			
Leaves	Mg	K, Al	N
Roots	Ca, Fe, Mn, Cu	Al	P

(Fig. 3): the larger the initial level and sequential decline in root Fe or Cu concentration, the greater the amount of Al sorbed by the roots of any species.

A "synergistic" trend response would be expected in tissue Al concentrations with increasing Al in solution and this was found for all four species (Fig. 3). There were interesting differences between leaf and root components of the four species. Both DFIR and SSPR had significant positive Al trends in leaf tissue with treatment while only root tissue of WHEM, SSPR and WRED had significant positive treatment trends. Total Al concentrations were highest in SSPR roots and lowest in WRED leaves. Lowest root Al concentrations were also found in WRED while the highest foliar concentrations occurred in DFIR. The only other element displaying a significant synergistic response trend was K in the leaves of SSPR and WHEM (Fig. 3).

An "interactive" response trend (Table 3) was characterized by a tissue concentration maxima as Al-treatment level increased. Only P in the roots of DFIR and SSPR (Fig. 3) and foliar N on SSPR displayed such a trend. For P, all species except WRED displayed an "interactive" type treatment response. This trend was less obvious in root N levels.

An interesting relationship between leaf biomass and nutrient concentrations is apparent for WRED. Unlike the other species, WRED had an increase in leaf biomass in [10AL] treatment (Fig. 1b). Similarly, WRED was the only species which did not display a synergistic trend in leaf K concentration Al (Fig. 3). In fact K concentration was highest in [10AL] treatment. When foliar Ca:Al ratios and P:Al ratios were compared between species again WRED displayed maxima in [10AL] treatment. The most obvious causal factor of these concentration maxima in [10AL] treatment is Al absorption. WRED foliar Al in [10AL] treatment (45 ppm Al) was less than in [0AL] (64 pm Al) or any of the other Al treatments (> 75 ppm Al). Although root Al concentration increased with the initial addition of Al to the solutions, foliar levels decreased. This could be a dilution effect but the relationship between low solution levels of Al and increased K, Ca, P absorption and biomass growth would imply a synergistic effect of Al on WRED growth and nutrition in the presence of excess H-ions.

Discussion.

There was a general lack of response in root and shoot biomass, and shoot height growth across species to increasing Al in the treatment solutions. Terminal bud set in Douglas-fir and Sitka spruce accounted for part of this lack of shoot response but even western hemlock and western redcedar (which continued shoot growth throughout the experiment) displayed no shoot biomass response and only minor height growth response to treatments.
Root length of western hemlock and Sitka spruce was significantly

affected by treatment Al concentration but not root biomass. For both species, a maximum root length occurred in [10AL] treatment (Fig. 1a). A corresponding "interactive" treatment response (maximum values at low Al concentrations) in root concentration of P (and to a lesser extent N) was found for Douglas-fir, western hemlock and Sitka spruce (Fig. 3). Possible precipitation or increased uptake of P as an Al-P complex at low Al concentrations could not be tested with Program GEOCHEM[19] because this speciation program does not contain thermodynamic data for Al-P-OH complexation reactions.

Nutrient concentrations in seedling biomass were generally more responsive to treatment Al than any growth parameters. Most divalent cation concentrations in roots of all species displayed in "antagonistic" response to increasing Al in solution (Table 3). Only in western hemlock were these lower root concentrations also reflected in foliar concentrations.

In contrast, K concentrations in leaves of species except western redcedar increased with increased Al in solution (Fig. 3). The exception, western redcedar, had a maximum foliar K concentration in [10AL] treatment which coincides with a minimum foliar Al concentration (Fig. 3), maximum foliar Ca:Al and P:Al ratios, and maximum seedling biomass (Fig. 1b). It is therefore speculated that uptake of Al to leaves and K nutrition are interlinked. Root concentrations of Al also seems related to the affinity of root surfaces for the divalent metal cations Fe and Cu. The more Fe or Cu present in a species' root tissue in [0AL] treatment, the more Al that species' roots sorbed as solution Al concentration increased (Fig. 2). These results support the hypothesis of Hiatt *et al*[6] that aluminum and copper (and probably other transitional metal cations) compete for common binding sites in the root apoplast. Cation affinity for any root binding site may possibly be related to the ligand field stabilization energy of these divalent transition metal cations (Cu>Fe>Mn Zn)[5].

When the "extreme" acid treatments of Ryan *et al*[18] were replaced by this experiment's less severe "spectrum" of treatments (lower Al concentrations, lower ionic strengths, and higher pH), definitive effects of increasing Al in solution on species biomass and dimensional growth properties were difficult to elucidate over the 98 day experimental period. These acid treatments caused little seedling mortality and on the whole seedling growth across species was quite uniform. Even the high mortality of western hemlock in [Al] treatments of Ryan *et al*[18] was absent from these treatments except at the highest Al level [100AL] wherein 4 out of 21 seedlings died.

For both experiments, western hemlock root length was found to be the most sensitive seedling growth parameter to the presence and variation of Al activity (Sitka spruce root length also displayed a similar sensitivity in the second experiment). This relationship between Al concentration and western hemlock or Sitka spruce root elongation was not a simple linear correlation. At low nutrient solution pH the addition of Al at levels < 20 ppm Al were found to increase root length while at higher Al concentrations root length decreased. This phenomenon may be related to increase root P uptake at these low Al concentrations as Al-P complexes. This curvilinear response of root elongation to increasing Al concentration may explain the inconsistent results using root elongation as a "tolerance index" of forest species to Al[10,20].

Western redcedar displayed neither a root length effect nor a P uptake-relationship with increasing solution Al. Of the four species studied, western redcedar's mortality, biomass and dimensional growth were the least affected by treatment. None of the visual foliar symptoms of western redcedar in [H] or [Al] treatments of Ryan *et al*[18] were observed in this experiment.

Nutrient concentrations of root tissue were most sensitive to acid and acid plus Al treatment for all nutrient cultures. Root concentrations of divalent cations were reduced in [OAL] treatment but the presence of Al further decreased tissue concentrations of Ca, Mg, Fe, Cu and Mn. Not all species showed decreased foliar concentrations of these elements but as found in Ryan *et al*[18], western hemlock foliar levels of Ca, Mg, Fe and Mn were depressed once Al was present in acid solutions.

Stem diameters of western hemlock in Ryan *et al*[18] and Sitka spruce in this experiment were found to increase significantly in Al treatments. Stem biomass did not exhibit a similar increase although there were visible swellings on the lower stem, just above the root collar, in Douglas-fir, western hemlock, and Sitka spruce in both experiments.

Evidence presented would indicate that low levels of Al in solution (< 20 ppm) may alleviate some of the adverse effects of H-ion concentrations in acid solutions for most the forest species studied and especially for western redcedar. Phosphorus sorption for western hemlock, Douglas-fir and Sitka spruce roots was increased at low Al levels in the acid solutions. Possible increased adsorption of P (as Al-P complexes) could be related to significant increases in western hemlock and Sitka spruce root length. Western redcedar shoot growth and K absorption increased marginally at low solution levels of Al. This phenomenon was postulated to be an example of the "Viets" effect[11] with substituting for root Ca displaced by excess H-ions.[17]

In summary, western hemlock and western redcedar were found to be very tolerant of nutrient solutions of low pH which contained Al at activities up to 0.88 m$\underline{M}$ l^{-1}. Low levels of Al (activities < 0.39 m$\underline{M}$ l^{-1}) in acid solutions seem to improve western redcedar growth and nutrition above that found in acid solutions without Al present.

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