POTASSIUM UPTAKE IN THREE PROVENANCES OF RED SPRUCE SEEDLINGS AT THREE AL^{3+} LEVELS [1/]

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ABSTRACT.--Three-week old red spruce (*Picea rubens* Sarg.) from Meransey Brook, New Brunswick and two Nova Scotia provenances were exposed to three aluminum ion (Al^{3+}) levels over seven potassium (K^+) levels in hydroponic culture. The two Nova Scotia provenances exhibited significantly greater ($P < 0.05$) potassium uptake capacity due to a diffusive component operative at high external K concentrations (> 1.0 mM). Uptake by Meransey Brook seedlings followed strict Michaelis Menten Kinetics. Patterns of K uptake in Meransey Brook material, when exposed to Al^{3+}, differed from patterns exhibited by seedlings from the other sources. A significant ($P < 0.05$) provenance by aluminum by exposure time interaction suggests varying aluminum tolerance may exist among these red spruce provenances.

Red spruce (*Picea rubens* Sarg.) is often a major component of the sub-alpine forest of the Appalachain Mountains as well as in New Brunswick and Nova Scotia. Recently Siccama (1981) and Siccama *et al.* (1982) reported data comparisons from 1965 and 1979 which suggest a reduction in number and in basal area of red spruce of all age classes in the Camels Hump region of the Green Mountains of Vermont and the Hubbard Brook Experimental forest in New Hampshire. Raynal *et al.* (1980) reported a similar reduction in red spruce growth since 1965 in the Adirondack Mountains of New York based on expected growth in response to prevailing environmental conditions, usually on upper elevation sites. This "spruce decline" manifests itself as death of branch tips and loss of foliage from all areas of the crown and occurs throughout all age classes. The precise causes of the decline are at present not known.

The occurrence of increased acidity of precipitation over wide areas of the northeastern United States raises questions about changes in heavy metal mobilization in forested ecosystems (Norton *et al.* 1980). Cronan

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(1980) reported increased aluminum leaching in spodic soils characteristic of high elevations in New Hampshire where the Al^{3+} ion is often the first or second most abundant cation in soil solution on a charge equivalent basis. High levels of aluminum were reported in phloem and cambium of shortleaf pine in polluted areas near Oak Ridge, Tennessee (Baes and McLaughlin 1984). These observations make study of aluminum effects on nutrient uptake in red spruce appropriate at this time.

Inhibition of root growth in the presence of aluminum has been well documented for many crop plants including pea (Matsumoto et al., 1982), wheat (Wallace et al., 1982), and coffee (Pavan et al.,1982). Characterization of AP effects on forest species have been less numerous. Ulrich et al. (1980) noted a decrease in the biomass of fine roots of Fagus as Al^{3+} concentration in the soil increased. A large degree of interspecific variation in aluminum tolerance between six tree genera was observed by McCormick and Steiner (1978). In their study, Populus hybrid root growth was sensitive to Al^{3+} levels as low 10 mg l^{-1} under hydroponic conditions. Hoyle (1971) found 81 mg Al^{3+} l^{-1} inhibitory to birch root development, dependent on macronutrient status of solution cultures. Aluminum in solution (5 mg l^{-1}) has been reported to reduce water movement through one-year-old red spruce seedlings with a significant aluminum concentration x osmotic potential interaction (Klein, in press).

The purpose of this study was to investigate seed source effects on potassium uptake at three aluminum levels. Seed source effects may be interpreted as potential genetic effects.

MATERIALS AND METHODS

Plant Material. Red spruce seed was supplied by the Maritimes Forest Research Centre[1] as seed lots from provenance collections originating at Meransey Brook, New Brunswick, Abraham's Lake, Nova Scotia, and a third Nova Scotian provenance of unknown origin. Three samples of 100 seed for each provenance were weighed to determine mean non-dessicated weights. Seed for uptake analysis was treated in 3% H_2O_2 for 12 hours prior to sowing in horticultural grade perlite. Seedlings were greenhouse grown under natural illumination for two weeks prior to experimentation during June-August 1983. Watering was carried out with 0.1 strength Ingestad's solution (ingestad 1959) at pH 5.3. Temperature and relative humidity fluctuated daily from 18 to 38 °C and 30 to 100% respectively.

Seedlings were moved to a large growth chamber with 24 h photoperiod and irradiance from combined fluorescent and incandescent sources yielding 112.7 + 5.6 microeinsteins m^{-2} s^{-1} PAR at seedling level. Temperature was maintained at 20 + 1 °C with a relative humidity of 78 ± 4%. This temperature has been shown to be the optimum for K^+ uptake in several forest species (Bledsoe and Rains, 1981). Seedlings were maintained under these conditions for one week. During this period acidity of the nutrient solution used to water seedlings was incrementally adjusted with HCl from pH 5.3 to 4.0.

Three-week-old seedlings were gently teased from the perlite and transferred to hydroponic culture in full-strength Ingestad's solution with phosphate adjusted to 0.032 mM. The hydroponic system consisted of 250 ml nalgene wide-mouth sample Jars taped with black electrical tape to exclude light. Root systems were suspended in nutrient solution in jars from lids in which 8 holes were drilled. Solutions were changed every 12 h and constantly aerated for 5 days prior to uptake experiments. Solution pH remained at 4.0 ± 0.1 units.

Potassium Uptake. Seedlings were assayed for K+ uptake using full Ingestad's solution modified to yield 7 potassium levels labelled with RbCi. Potassium concentrations were: 10, 5, I, .5, .1, .05, .01 mM. Uptake for each set of plants consisted of a 3 hour exposure to one potassium level. Following uptake, seedlings desorbed in Ingestad's solution with 0.05 mM DNP to remove non-metabolically adsorbed $^{8°}$Rb from root free space. One cm of each root apex was excised and subjected to liquid scintillation analysis. Units of uptake (u mol K absorbed g fresh wt^{-1} h^{-1}) were calculated for subsequent statistical analysis.

Aluminum Analyses. Following 24 h acclimation to hydroponic culture, seedlings were exposed to Ingestad's solution amended with 0, .037 and .148 mM $AlCl_3$ x 6H20 (0, I, 4 mg $Al^{3+}I^{-1}$).Exposures were initiated such that at the time of uptake experimentation, groups of seedlings were treated for 0, 24, and 72 h. All solutions were adjusted to and remained at pH 4.0 ± 0.1 units throughout the course of each experiment.

Aluminum solutions were analyzed colorimetrically for total aluminum by the Eriochrome Cyanine-R method (Am. Water Wks. Assn., 1980). Aluminum stock solutions were analysed colorimetrically and by atomic absorption spectrophotometry. Both methods yielded results in excellent agreement, and variation in solution aluminum levels was trivial.

Statistical Analysis. Velocity data were weighted by the reciprocal of the squared velocity (v^{-2}) (Borstlap, 1983) and fit by non-linear least-squares methods to the combined active and passive uptake model

$$v = V_{max}[S]/(K_m+[S]) + k_s[S].$$

This model has been previously shown to best describe potassium uptake in red spruce seedlings at potassium concentrations of 0.01 to 10.0 mM (Cumming et al. 1985, Cumming 1984). The first term of this model is the Michaelis- Menten term describing saturation kinetics. The second term, $k_s[S]$, accounts for concentration-dependent diffusive uptake which has been shown to contribute significantly to potassium accumulation in red spruce seedlings at solution concentrations greater than 1.0 mM (Cumming et al. 1985; Cumming 1984).

Comparison of treatment effects followed the methods of Neter and Wasserman (1974). According to this technique, full- and reduced-model weighted sums of squares for error (SSE(F) and SSE(R)), respectively are used to calculate an F-statistic involving the ratio of the difference between separate treatment SSE's and the SSE for the fit to the pooled data. Large values of F lead to a rejection of the null hypothesis, and paired t-tests are used to discern differences between the fitted parameters K_m and V_{max}.

Initial velocity data were also analysed as a thrice replicated factorial (provenance by aluminum by exposure) design, with potassium as a blocking factor, to detect possible interactions among aluminum and exposure treatments. Details of all procedures and analyses are available in Cumming (1984).

RESULTS AND DISCUSSION

Analysis of seed weight by completely random model ANOVA revealed that provenance seed weight differed markedly (Table I). Although the three sources were significantly different ($P < 0.05$), seed from Meransey Brook weighed less than half that of the other sources.

Table 1. Provenance seed weight.

provenance	weight[1]
Meransey Brook, New Brunswick	0.157 + 0.004[a]
Abraham's Lake, Nova Scotia	0.325 + 0.007b
Nova Scotia[2]	0.363 + 0.002[c]

[1] g (100 seed)$^{-1}$; means calculated from 31 replicates; means followed by the same letter are not significantly different at $P<0.05$ by LSD comparison.

[2] The third provenance is of unknown origin in Nova Scotia.

MacGillivray (1967) reported highly significant decrease in dry seed weight with increasingly northerly latitudes for red spruce with an average weight per 100 seed of 0.3117 g (calculated from his data). Provenances used in our study (Figure I) are located at the same latitude, thus we could not check for that trend.

For the experiment proper, the 3 provenances were considered random samples of red spruce genetic material and the provenance factor was intentionally confounded with replicates in order to save experimental material; use of provenance as a treatment factor would have required tripling the size of each run. Provenance effects are, therefore, confounded with replication effects. Replication effects, however, are equivalent to differences in time when uptake analyses were conducted. Since uptake was conducted on seedlings of the same age and under the same environmental conditions in a growth chamber, we believe replication effects to be small.

We were interested in the effect of provenance on potassium uptake and several analyses were undertaken, with caveat, including provenance as a treatment factor. Analysis of control data in a randomized complete block design with potassium concentration as blocks and provenance as the treatment factor indicated significant differences ($P < 0.05$) in the performance of seedlings from the three provenances (Table 2).

Table 2. Mean potassium uptake by seedlings of three red spruce provenances.

provenance	K+ influx'
Meransey Brook, New Brunswick	0.78 + 0.02[a]
Abraham's Lake, Nova Scotia	0.92 + 0.02[b]
Nova Scotia?	0.91 + 0.02[b]

[1] mol K g fresh wt^{-1} h^{-1} ; means and standard errors calculated from 3 replicates of 7 blocks; means followed by the same letter are not significantly different at $P < 0.05$.

[2] The third provenance is of unknown origin in Nova Scotia.

As noted earlier, the seed lots were considered random replicate samples of red spruce and data were pooled on an *a priori* basis. As model-fitting progressed, it became evident that the provenances did not behave similarly in their pattern of potassium uptake. F-tests were used to compare potassium uptake of provenances and fit to the model previously described. The results are summarized in Table 3.

Analysis of residuals generated by each model for each provenance indicated that while the Nova Scotia provenances exhibited uptake enhanced by a component that was concentration dependent above 1.0 mM KCI (Table 3), uptake in Meransey Brook, New Brunswick seedlings followed standard Michaelis-Menten kinetics. Figure 2 displays potassium uptake as a function of external KCI concentration. Uptake by the Nova Scotia provenances exhibits complex kinetics which may result from significant diffusive uptake at high external K+ concentrations. F-tests indicated that data for the latter two provenances could be pooled ($F = 0.44$) while uptake as a function of external K^+ concentration in Meransey Brook seedlings was significantly different from that of Abraham's Lake or the second Nova Scotia seed sources ($F = 9.62$ and 8.27, respectively). Demeritt and Hocker (1975) found seed weight positively influences size and weight of 1 and 2 year old white pine seedlings. Similar correlation between seed weight and seedling vigor has been reported for 3 month old chir pine seedlings (Chauhan and Raina, 1980). Seedlings from provenances with heavier seeds in our study exhibited greater potassium uptake which suggests that one advantage of greater seed weight is enhanced nutrient uptake.

Table 3. Comparison of model uptake parameters generated by red spruce provenances.[1]

provenance	Vmax[2]	Km[3]	k[4]
Meransey Brook, New Brunswick	1.128	0.09a	0.003[a]
Abraham's Lake, Nova Scotia	1.218	0.088	0.011[ab]
Nova Scotia	1.168	0.088	0.039[b]

1. Uptake measured in solutions ranging from 0.01 to 10.0 mM; parameter estimates calculated from 3 replicates; estimates followed by the same letter are not significantly different at $P < 0.05$.
2. Maximal rate of K+ absorption in μmol K g fresh wt^{-1} h^{-1}.
3. Michael is-Menten constant in pM KCI. Km is a relative measure of affinity for K+ ions.
4. Diffusional component in g fresh wt^{-1} h^{-1}.

Analysis of potassium uptake in a blocked 3 x 3 x 3 factorial (aluminum by exposure by provenance) arrangement indicated a significant ($P < 0.05$) three way interaction (Table 4). This indicates that uptake was dependent on all three treatment factors in a non-additive manner. While there were expected linear differences between provenances (replicates), the three way interaction highlights possible differences in seed source reaction to aluminum in solution and may be indicative of differential aluminum tolerances among provenances.

Table 4. ANOVA for potassium uptake as affected by aluminum, exposure, and provenance. External potassium concentration was previously shown not to interact with other factors and is treated as a blocking factor.

Source	df	F	P
mean	I		
potassium (block)	6	540.8	0.001
provenance	2	18.1	0.001
aluminum	2	19.4	0.001
exposure	2	1.8	0.162
provenance x aluminum	4	1.5	0.201
provenance x exposure	4	1.5	0.212
aluminum x exposure	4	4.2	0.003
provenance x aluminum x exposure	8	5.2	0.001
error	156		

Uptake at 1 mgl^{-1} Al in Meransey Brook material is similar in pattern to uptake at 4 mgl^{-1} Al in material from the other two seed sources (Figure 3). A peak in uptake at 24 h of aluminum exposure occurs followed by a decline at 72 h. The uptake peak at 24 h followed by reduction in uptake at 72 h may represent a repair response followed by incipient toxicity, however the short term exposure limits extrapolation. The reduction at 72 h was due to decrease in Vmax (Cumming *et* al. 1985) indicating a reduction in the number of absorption sites contributing to uptake. Both Abraham's Lake and Nova Scotia source material exhibit similar uptake patterns at 1 and 4 mgl^{-1} Al levels.

Plots of uptake for the three aluminum levels over time for each provenance illustrate that uptake by Meransey Brook seedlings for each aluminum treatment differed from patterns exhibited by the other two seed sources (Figure 3). Differential provenance response to aluminum in solution was reported by Steiner et al. (1980) for 13 provenances of paper birch. Provenances, but not families within provenances, differed in rate of root elongation and nutrient accumulation in the presence of aluminum. The authors noted that there were no consistent patterns in nutrient uptake among Al-tolerant and intolerant seed sources. Steiner Al. (1984) further reported high sensitivity of clonal hybrid poplars to Al at 3 ppm in solution culture. Hybrids derived at least in part from species in section *Tacamahaca* had significantly higher tolerance than those derived from species in section *Aegeiros*. The authors reviewed field performance of the clones and determined that *Tacamahaca* clones as a group have consistently performed better than *Aegeiros* clones on mildly to very acidic soils where AI levels would presumably be high. An observed variety by aluminum interaction for K+ uptake in potato lead Lee (1971) to suggest that aluminum tolerance in potato seedlings is due to differential cation fluxes. There are other reports of high level K^{+} uptake in response to aluminum in tolerant plants (Huett and Menary, 1980; Foy *et al*., 1978).

The literature on genetic variation in red spruce is not extensive. Significant variation in survival and height growth among provenances was reported by Morgenstern (1968). Provenance by plantation location interaction was significant in Morgenstern's study suggesting strong site effects on red spruce growth and survival. Site factors, which include l evels of Al + in certain soil types, may be important to growth and survival of this species. A factor which may also contribute to variation in red spruce response is introgression with black spruce (Morgenstern and Fowler 1969, MacGillivray 1967) although the level and location of introgression is generally not clear (c.f. Gordon 1976). Seed size seems to be an important factor in potassium uptake under our conditions. Levels of introgression at the provenances sources used in this study are unknown, but are presumed to be low.

CONCLUSION

Three provenances of red spruce seedlings exhibited significantly ($P < 0.05$) different rates of K+, uptake in hydroponic solutions. Differential nutrient uptake capacity may be related to seed size. Significant ($P < 0.05$) provenance by aluminum level by exposure time interactions were observed. These results suggest varying aluminum tolerance may exist among provenances of red spruce.

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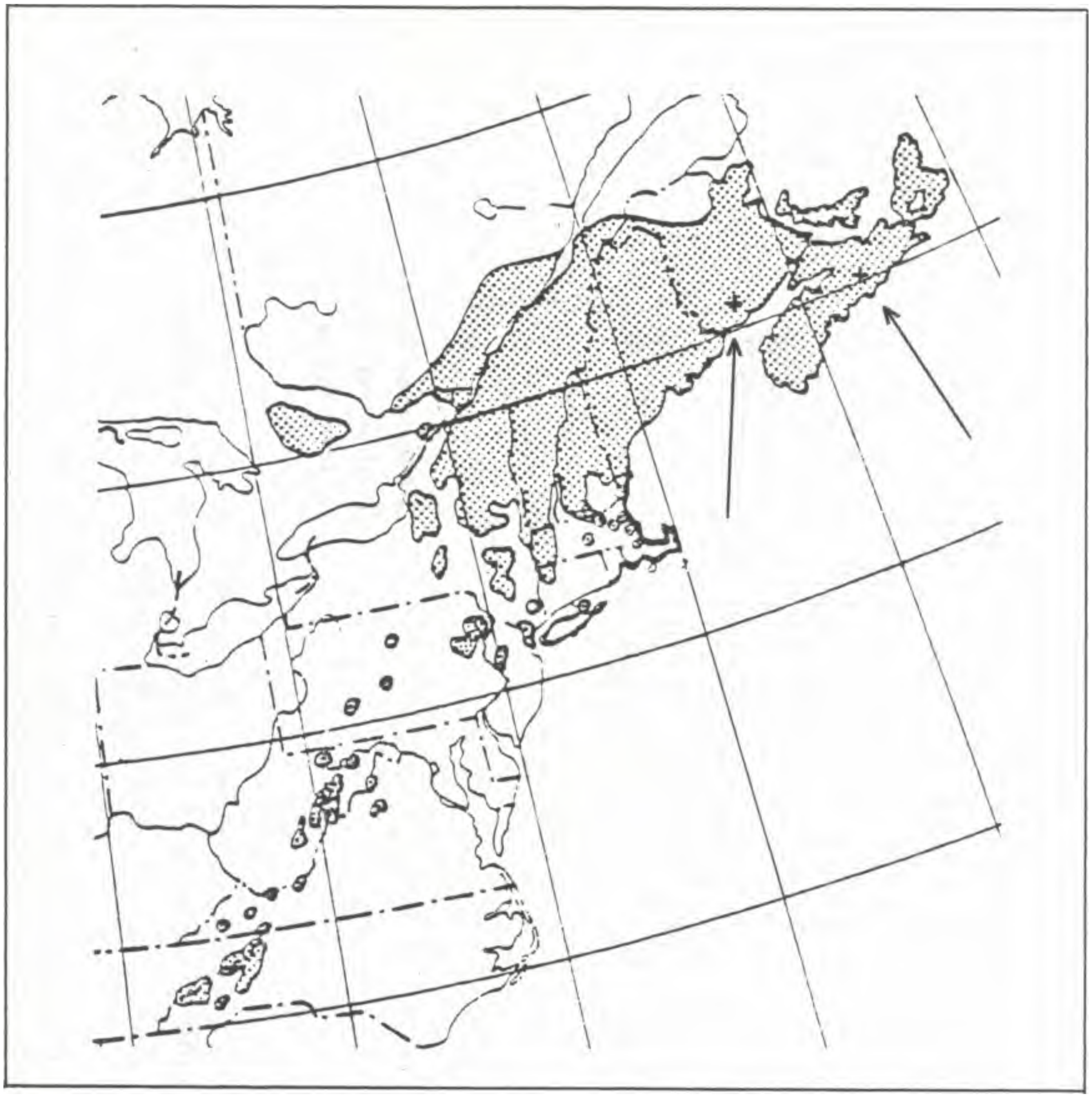

Figure 1. Range map of red spruce (*Picea rubens* Sarg.) showing locations (+) of Meransey Brook, New Brunswick and Abraham's Lake, Nova Scotia provenances. A third Nova Scotian provenance of unknown origin was utilized in the study.

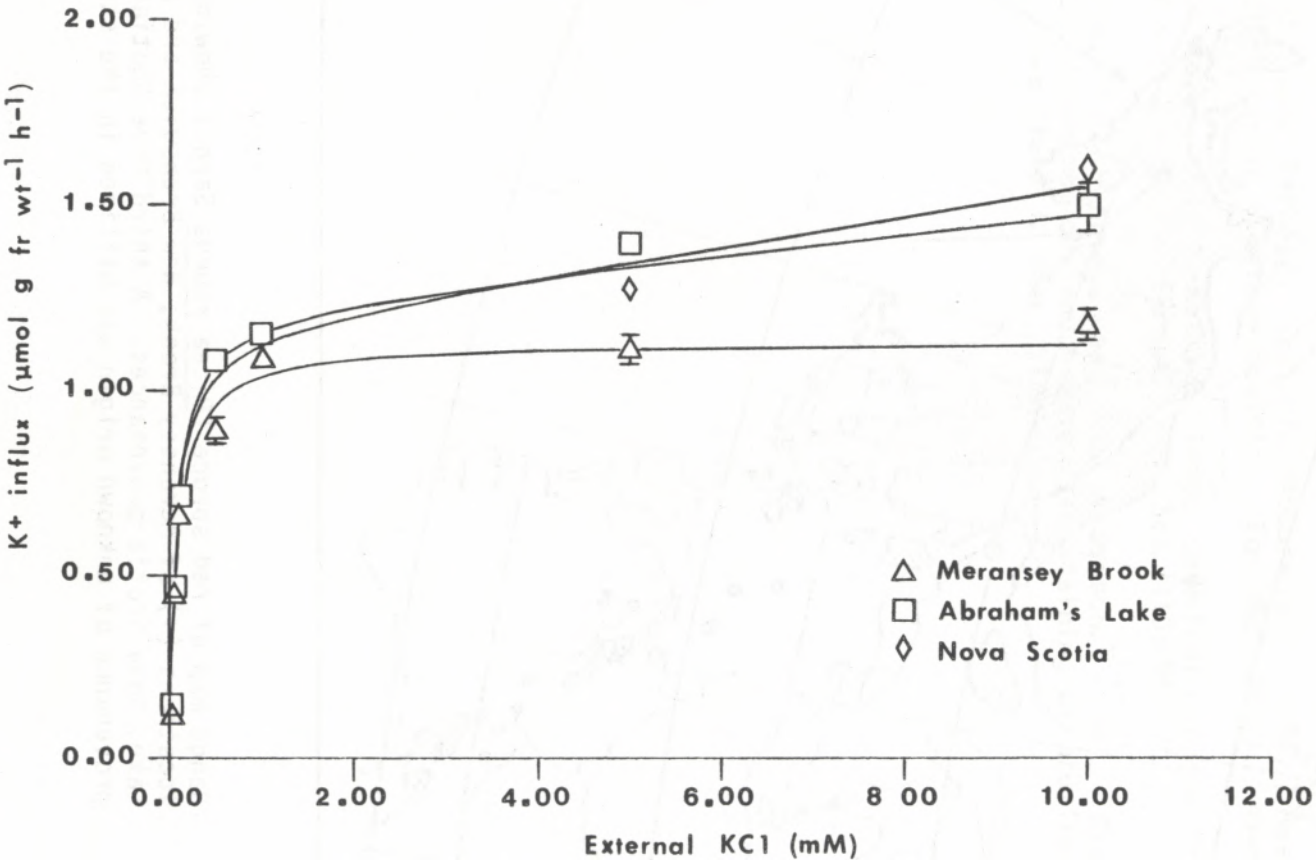

Figure 2. Potassium uptake as a function of external KCl concentration for three red spruce provenances. When error bars are not indicated, they are smaller than the symbols.

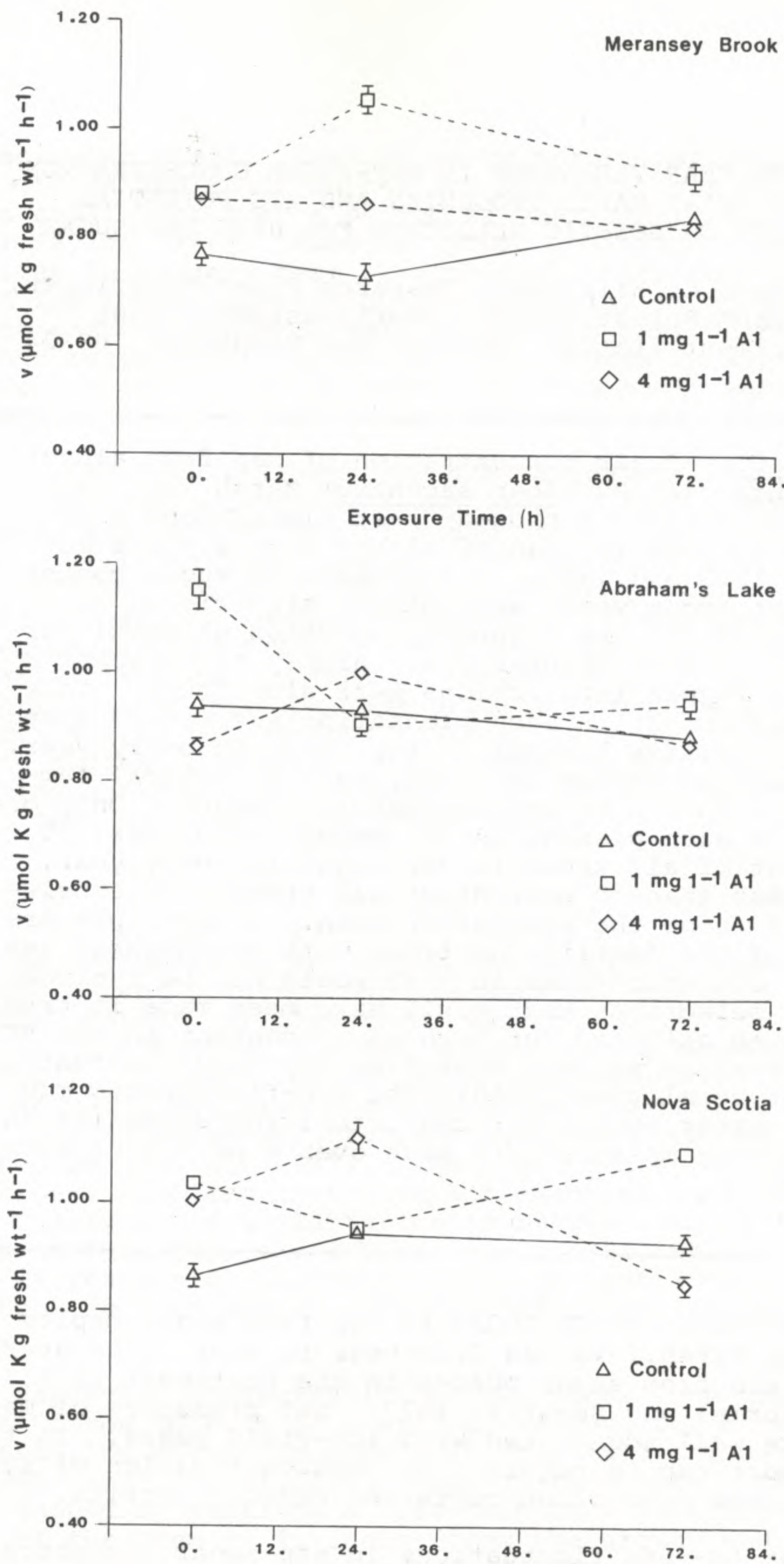

Figure 3. Potassium uptake response of three red spruce provenances to three levels of Al^{3+} at varying exposure times.