Plant and Soil **191:** 109–122, 1997.

Relationships among foliar chemistry, foliar polyamines, and soil chemistry in red spruce trees growing across the northeastern United States

Rakesh Minocha[1], Walter C. Shortle[1], Gregory B. Lawrence[2], Mark B. David[3] and Subhash C. Minocha[4]

[1]*USDA Forest Service, Northeastern Forest Experiment Station, PO Box 640, Durham, NH 03824, USA**, [2]*US Geological Survey, 425 Jordan Road, Troy, NY 12180, USA,* [3]*University of Illinois, W-503 Turner Hall, 1102 South Goodwin Avenue, Urbana, IL 61801, USA and* [4]*Department of Plant Biology, University of New Hampshire, Durham, NH 03824, USA*

Received 12 September 1996. Accepted in revised form 14 March 1997

Key words: aluminium, calcium, magnesium, putrescine, spermidine

Abstract

Forest trees are constantly exposed to various types of natural and anthropogenic stressors. A major long-term goal of our research is to develop a set of early physiological and biochemical markers of stress in trees before the appearance of visual symptoms. Six red spruce (*Picea rubens* Sarg.) stands from the northeastern United States were selected for collection of soil and foliage samples. All of the chosen sites had soil solution pH values below 4.0 in the Oa horizon but varied in their geochemistry. Some of these sites were apparently under some form of environmental stress as indicated by a large number of dead and dying red spruce trees. Samples of soil and needles (from apparently healthy red spruce trees) were collected from these sites four times during a two-year period. The needles were analyzed for perchloric acid-soluble polyamines and exchangeable inorganic ions. Soil and soil solution samples from the Oa and B horizons were analyzed for their exchange chemistry. The data showed a strong positive correlation between Ca and Mg concentrations in the needles and in the Oa horizon of the soil. However, needles from trees growing on relatively Ca-rich soils with a low exchangeable Al concentration and a low Al:Ca soil solution ratio had significantly lower concentrations of putrescine and spermidine than those growing on Ca-poor soils with a high exchangeable Al concentration and a high Al:Ca soil solution in the Oa horizon. The magnitude of this change was several fold higher for putrescine concentrations than for spermidine concentrations. Neither putrescine nor spermidine were correlated with soil solution Ca, Mg, and Al concentrations in the B horizon. The putrescine concentrations of the needles always correlated significantly with exchangeable Al (r^2=0.73, $p \leq 0.05$) and soil solution Al:Ca ratios (r^2=0.91, $p \leq 0.01$) of the Oa horizon. This suggests that in conjunction with soil chemistry, putrescine and/or spermidine may be used as a potential early indicator of Al stress before the appearance of visual symptoms in red spruce trees.

Introduction

The adverse effects of acidic deposition on soil productivity, due to the solubilization of Al and leaching of bases, are of major concern to forest land managers because such processes may impact growth over large areas. Lawrence et al. (1995) have proposed that Al is mobilized in the mineral soil by acidic deposition and transported into the forest floor in a reactive form by biocycling and water movement. Here the reactive Al competes for Ca exchange sites, increasing its leaching into the mineral soil and eventually into the stream waters and thus reducing Ca availability to the roots (Shortle and Smith, 1988). Early detection of changes in soil chemistry and resulting changes in physiological and biochemical processes in plants, before the appearance of visible symptoms, may be a key to successful ecological risk assessment and abatement. One such

* FAX No.: +16038687604. E-mail: rminocha@hopper.unh.edu

ecological indicator of aluminum stress is the ratio of Al:Ca (or Ca:Al) in soil solutions or plant tissue (Cronan and Grigal, 1995; Shortle and Smith, 1988).

Monomeric Al is an important toxic species to plants, both in the rhizosphere and within the plant symplasm (Kochian, 1995). Aluminum interferes with cation uptake and can cause damage to plant cells by interacting with sensitive macromolecules (Haug, 1984; Sucoff et al., 1990). The major symptoms of Al toxicity are inhibition of root growth, cell division, DNA synthesis, needle biomass, and seedling height (McQuattie and Schier, 1990; Minocha et al., 1992). Aliphatic polyamines (putrescine, spermidine, and spermine) play an important role in the growth and development of all living organisms. Among the many suggested roles for polyamines, their participation in stress responses has become an active area of research. Cellular polyamine content is highly regulated under normal growth conditions. A variety of stimuli such as high concentrations of ozone, acid, pathogen infections, SO_2 fumigation, high salt, and mineral nutrient deficiency (e.g. Ca, Mg deficiency) lead to an accumulation of one or more of the polyamines (Flores, 1991; Galston, 1989). Studies with cell cultures of red spruce (*Picea rubens* Sarg.) showed that treatment with Al caused an increase in cellular putrescine concentrations and a decrease in the concentrations of cellular inorganic cations such as Ca, Mg, Mn, and K. This increase in putrescine was initially accompanied by an increase in the concentration of its biosynthetic enzyme, arginine decarboxylase. A small increase was also observed in cellular spermidine concentrations but it was not always dose dependent with respect to Al (Minocha et al., 1996). It has been suggested (Shortle and Smith, 1988) that Al also causes a reduction in the uptake of Ca and Mg in the roots thus causing Al induced Ca deficiency in the trees. This deficiency may be the cause for induction of biosynthesis and/or accumulation (by interconversion) of putrescine and spermidine in the foliage tissue. It is not clear if the changes in polyamine metabolism are the primary response to this stress or are caused indirectly via the effects of stress on other physiological processes through signal transduction.

There is a scarcity of literature on the direct effects of soil Al on the physiology of mature forest trees. This makes it difficult to compare the results of controlled environment studies on Al toxicity using cell cultures and/or seedlings grown in hydroponic cultures with those of the field situation. Red spruce cell suspension cultures maintained in growth media containing Al may behave in a manner similar to fine root tips in soil and soil solutions containing Al because both are in direct contact with Al. Therefore, the tissue culture studies may provide insights into the effects of toxic elements such as Al on fine root systems and thus the whole plants. The present study is an attempt to try to extend the results of the laboratory studies to field conditions. Based upon our results with Al effects on red spruce cell cultures (Minocha et al., 1996), we hypothesize that changes in the concentrations of putrescine in the foliage of mature red spruce trees may be used as a potential nonspecific indicator of stress in these trees. The objectives of the present study were: i) to measure foliar polyamine concentrations in mature red spruce trees growing under a range of soil conditions; and ii) to determine relationships among foliar chemistry (including polyamines) and soil and soil solution chemistry at different sites.

Materials and methods

Description of sites

Six sites from the northeastern United States were selected for collection of soil and needle samples, as part of a larger collaborative effort funded under the USDA Forest Service, Global Change Program. The sites are located at Howland, ME, Bear Brook (Lead Mountain), ME, Kossuth, ME, Crawford Notch, NH, Groton, VT, and Big Moose, NY (Figure 1). The six sites range in elevation from 80 m at Howland, ME to 810 m at Crawford Notch, NH (Table 1). Annual precipitation at these sites is typically between 90-140 cm, of which one third falls as snow. The growing season extends from May to late September at the low elevation sites and becomes shorter as elevation increases. Bedrock mineralogy and forest floor thickness varies among sites (Table 1). These sites were selected to reflect a range of variability in environmental conditions, soil chemistry, and stand health. Details on the site descriptions as well as collection and analyses of soils from these sites have been published (David and Lawrence, 1996; Lawrence et al., 1997).

At the beginning of the study, seventy-two healthy red spruce trees were selected randomly at each site and tagged but only a subset of these trees were used for this study. The trees varied in age from 96 to 175 years (years to pith at d.b.h.), with the average for all the trees from all sites being 128 years. The tree diameter (d.b.h.) ranged from 26.2 to 62.4 cm with an

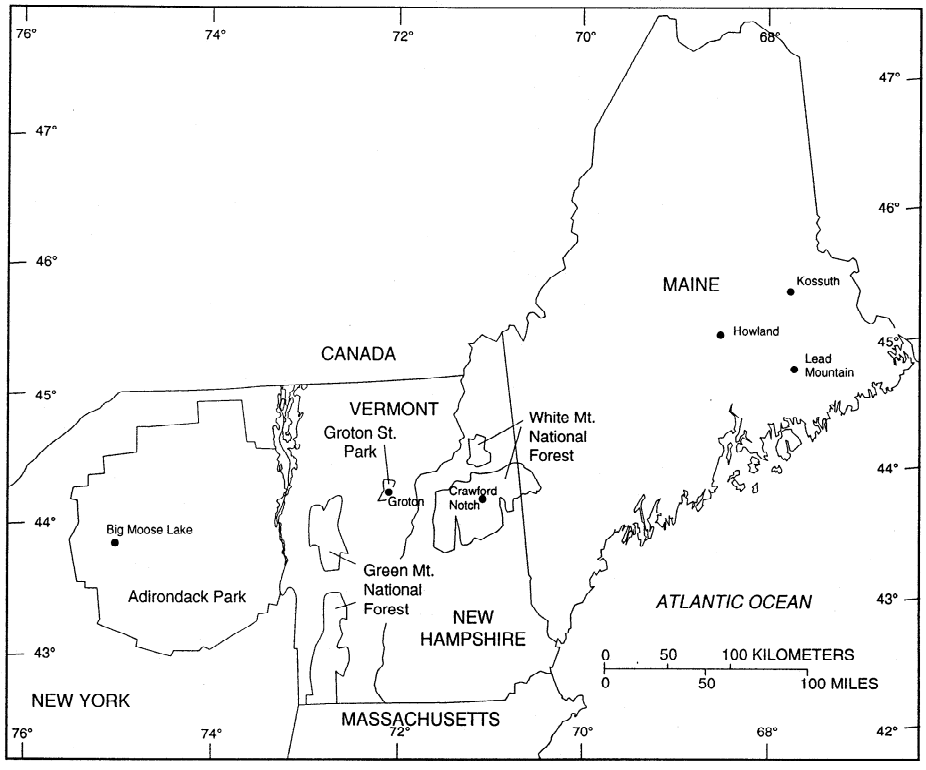

Figure 1. Geographic location of the six red spruce sites sampled from 1992 to 1994.

Table 1. Site characteristics of the red spruce stands sampled in the northeastern United States. Modified from David and Lawrence (1996) and Lawrence et al. (1997)

Site name	Site ID code	Elevation (m)	Forest floor thickness (cm)	Oa horizon soil solution pH	Upper B horizon soil solution pH	Bedrock	Other influences on parent material
Groton	GT	510	9.0	3.3	4.8	Granite	Calcareous metapelite
Howland	HW	80	12.6	3.3	4.5	Pelite and sandstone	Possibly calcareous sandstone
Kossuth	KS	150	9.0	3.4	4.6	Pelite	Sandstone and thyolite
Crawford Notch	CR	810	13.7	3.3	4.5	Metapelite and quartzite	Mixed granitic rocks
Bear Brook	BB	395	12.0	3.8	4.4	Sulfidic/carbonaceous	Granite and calcareous sandstone
Big Moose Lake	MO	550	23.9	3.4	4.0	Granite gneiss	Mixed metasedimentary

average of 35.3 cm. Visual observations at each site indicated that at Howland (HW), Kossuth (KS), Bear Brook (BB), and Groton (GT) there was no dieback or unusual mortality of trees although the crown condition of the trees was variable. However, the stands at Big Moose (MO) and Crawford Notch (CR) were experiencing dieback, although it was less advanced at Big Moose (Lawrence et al., 1997).

Soil samples were collected in mid June and October of 1992 and 1993. The foliar samples from healthy trees were collected in June or July and October of 1993 and 1994 for all sites except for Kossuth and Big Moose, which were sampled only once a year in June of 1993 and October of 1994. Due to small sample size per collection and large built-in genetic variation of the foliar samples within each site, the data from 4 collections was pooled to increase the sample size per site for analysis.

Collection and analyses of needle samples

At each sampling time, needle samples were collected from south side facing branches from ten different trees out of the 72 flagged healthy trees. In a few cases, because of the difficulty in reaching branches of tall trees with a pole pruner, untagged trees in the vicinity of tagged trees in the same stand were used for needle samples. Healthy current-year and one-year-old needles were collected from freshly cut branches in the field and immediately placed in individual preweighed microfuge tubes containing 1 mL of 5% perchloric acid. The tubes were kept on ice during transportation to the laboratory and were stored at $-20\,^{\circ}$C until they were processed.

The samples were weighed, frozen and thawed ($3\times$) and centrifuged at 14,000 rpm in a microfuge for 10 min. The details of the freeze-thawing procedure are described in Minocha and Shortle (1993) and Minocha et al. (1994). The freeze-thawing method was chosen for this study to extract the soluble fraction of ions and polyamines. This method extracted between 40-50% of the total acid extractable inorganic ions from green needles of mature trees (data not presented). The above supernatant was used directly for polyamine analysis without further dilution as well as for inorganic-ion analysis after proper dilution with distilled, deionized water (final concentration of perchloric acid 0.01 or 0.02 *N*) by the procedures described below. The diluted fractions were analyzed for inorganic ion content with a Beckman Spectrospan V ARL DCP (Direct Current Plasma Emission Spectrometer, Beckman Instruments, Inc., Fullerton, CA) using the Environmental Protection Agency's method number 66-AE0029 (1986). For quantitation of polyamines, heptanediamine was added as an internal standard to aliquots of the above extracts prior to dansylation. Fifty or one hundred μL of the extract were dansylated according to the procedure described in Minocha et al. (1990). Dansylated polyamines were separated by reversed phase HPLC (Perkin-Elmer Corp., Norwalk, CT) using a gradient of acetonitrile and heptanesulfonate, and quantified by a fluorescence detector (Minocha et al., 1990).

Collection and analyses of soil samples

At each sampling time, 3 clusters of three trees each were selected from the 72 tagged trees. Soil samples were collected from the Oa horizon and upper 10 cm of the B horizon near each of the nine selected trees and combined for each cluster by horizon. Thus a total of 12 pooled samples from each of the Oa and B horizons were collected at each site over the four sampling periods. Soil samples were extracted with 1 *M* ammonium chloride and the extracts were analyzed for exchangeable Ca and Mg according to Blume et al. (1990). Exchangeable Al was determined by extraction with 1 *M* potassium chloride (Thomas, 1982). To obtain soil solution, each soil sample was placed in a sealed cylinder. An artificial throughfall solution, chemically similar to Howland throughfall, was added to attain a moisture content that approximated field capacity. The soil solution was then expelled by positive air pressure. This procedure for expulsion of soil solution was developed, thoroughly evaluated and compared with lysimetry by Lawrence and David (1996) in order to enable us to do large scale regional sampling. This method was used instead of soil lysimeters because it: (i) does not require prior installation of field equipment; (ii) is not dependent on soil moisture conditions at the time of sampling; and (iii) eliminates problems due to soil disturbance caused by lysimeter installation and sample degradation due to long-term storage in underground lysimeter collection containers. The soil solution was analyzed for aqueous Al species and other ions according to the procedures of Driscoll (1984) and Lawrence et al. (1995).

Statistical methods

Linear regression analyses were performed to establish the strength and significance of the relationship between two different variables (n=6) using Excel 5.0 for Windows (Microsoft Corporation). Data for each variable (e.g., foliar or soil Ca, Mg and Al) were analyzed as a series of one-way analysis of variance (ANOVA) to determine whether statistically significant site differences occurred between sites for each individual variable. When F values for one-way ANOVA were significant, differences in site means were tested by Tukey's multiple comparisons test. ANOVA and Tukey's tests were performed with Systat for Windows, version 5.0 (Systat Inc., Evanston, II).

Results

Foliar chemistry

In general, current-year needles had a significantly lower concentration of exchangeable Ca, Mg, Mn, and Al as compared to one-year-old needles. The reverse

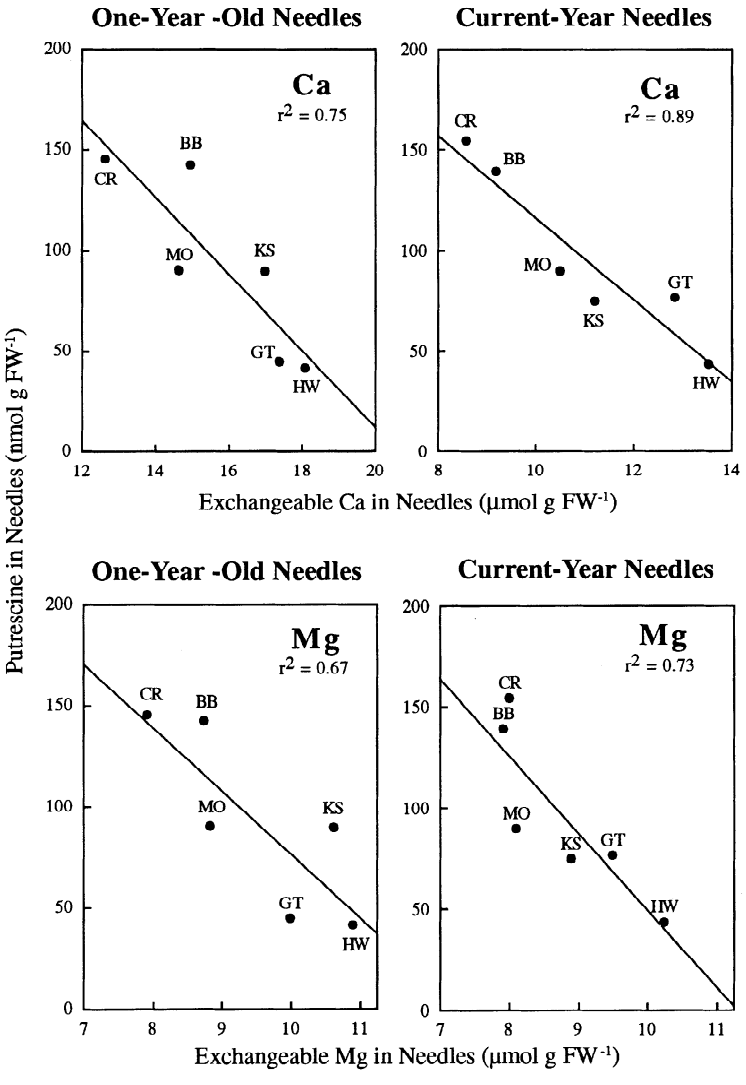

Figure 2. Correlations between foliar soluble putrescine, exchangeable Ca and Mg of red spruce trees growing at the six sites. Data are means ±SE (n=40 except for Kossuth, ME, and Big Moose, NY, for which n=20). The abbreviations used for site names are: Groton (GT), Howland (HW), Kossuth (KS), Big Moose (MO), Crawford Notch (CR) and Bear Brook (BB).

was true for foliar exchangeable P. Putrescine and spermidine concentrations were slightly higher for current-year needles but the differences were not statistically significant from one-year-old needles ($p \leq 0.05$; Table 2). Exchangeable K concentrations were similar for both age groups of needles. With few exceptions, needles of both age groups from trees at Groton and/or Howland had significantly higher mean concentrations of exchangeable Ca and Mg and lower concentrations of Al, putrescine, and spermidine compared to those from Crawford Notch and/or Bear Brook ($p \leq 0.05$). Mean foliar putrescine and spermidine concentrations were inversely correlated with mean foliar Ca and Mg concentrations for both age groups of needles from different sites (Table 3, Figure 2). There were no significant correlations of foliar putrescine, spermidine, Ca, and Mg with foliar Al (Table 3), K, P, and Mn. The variation in the concentrations of spermidine in foliage from different sites was much less (1 to 1.5 fold) as compared to that for putrescine (1 to 3.5 fold). Spermine was barely detectable in these samples. Therefore, detailed data on this polyamine were not analyzed.

Table 2. Exchangeable cations in current-year and one-year-old needles of red spruce. Data presented are means ±SE for n=40 except for Kossuth, ME, and Big Moose, NY, for which n=20

Variables	Groton, VT	Howland, ME	Kossuth, ME	Big Moose, NY	Crawford Notch, NH	Bear Brook, ME
Exchangeable cations in current-year needles (μmol (g FW)$^{-1}$)						
Putrescine*	77.04±8.81	43.63±4.93	74.98±11.59	90.05±8.25	154.87±19.99	139.24±12.46
Spermidine*	86.94±5.89	83.62±4.41	95.39±5.66	109.98±9.56	119.33±7.39	116.98±5.35
Ca	12.84±0.74	13.52±0.75	11.21±0.77	10.50±0.77	8.58±0.55	9.18±0.59
Mg	9.49±0.43	10.24±0.65	8.89±0.77	8.09±0.56	8.01±0.55	7.91±0.46
Mn	2.72±0.18	3.88±0.26	10.66±1.24	2.62±0.31	2.79±0.19	4.61±0.41
K	28.66±1.37	31.59±1.78	21.05±1.02	27.95±0.96	28.96±2.28	30.14±1.66
Al	0.31±0.03	0.31±0.03	0.32±0.02	0.16±0.02	0.21±0.02	0.31±0.03
P	6.54±0.42	6.35±0.46	6.60±0.46	2.21±0.34	5.42±0.45	4.30±0.38
Exchangeable cations in one-year-old needles (μmol (g FW)$^{-1}$)						
Putrescine*	44.67±4.38	41.59±4.44	89.67±10.22	90.69±11.60	145.61±14.61	142.57±22.95
Spermidine*	73.20±5.23	71.43±3.45	90.21±4.04	108.59±12.53	124.48±9.26	106.73±4.28
Ca	17.38±0.82	18.06±1.15	16.99±0.89	14.64±0.95	12.62±0.74	14.96±0.92
Mg	9.98±0.42	10.89±0.68	10.62±0.67	8.82±0.84	7.91±0.42	8.74±0.53
Mn	3.59±0.24	5.74±0.42	17.22±0.97	3.80±0.57	4.12±0.28	7.53±0.86
K	28.02±1.36	33.22±2.38	25.85±0.91	28.68±1.35	27.71±0.89	31.46±2.20
Al	0.31±0.02	0.32±0.02	0.41±0.03	0.21±0.02	0.23±0.01	0.41±0.04
P	5.6±0.33	5.74±0.55	7.62±0.72	1.76±0.35	4.32±0.28	3.61±0.26

* Putrescine and spermidine concentrations given as nmol (gFW)$^{-1}$.

Correlations between foliar and soil chemistry

All field sites sampled had a soil pH (measured in 0.01 *M* $CaCl_2$) between 2.56 to 3.11 for the Oa horizon and 3.58 to 4.46 for the B horizon. While no correlation was observed between exchangeable H and exchangeable Al for the Oa horizon, there was a strong positive correlation between these variables in the upper 10 cm of the B horizon (r^2 = 0.85; $p \leq 0.01$). Calcium, Mg and Al concentrations in the Oa horizon of soil were significantly different among sites. However, no distinct patterns for site differences emerged after running Tukey's multiple comparison test for means except for the following: Howland and/or Groton sites had significantly higher mean concentrations of exchangeable Ca, Mg, and lower mean concentrations of Al as compared to Crawford Notch and/or Bear Brook for the Oa horizon (Table 4). Similar trends were also found for soil chemistry of the upper 10 cm of the B horizon except for Ca, where there were no differences among site means (Table 4).

There was a significant correlation between mean exchangeable Ca, Mg, Al, and the Al:Ca charge ratio in the Oa horizon of soil and the foliar mean Ca and Mg concentrations in the current-year needles but no such correlation was seen with one-year-old needles (Table 3, Figure 3). The correlations of foliar Ca and Mg with soil exchangeable Al, however, were much stronger as compared to those with the Al:Ca charge ratio in the Oa horizon. With one exception, there was no correlation between foliar Ca and Mg and soil Ca in the upper 10 cm of the B horizon for both age groups of needles. However, there was a significant correlation of foliar Ca and/or Mg with soil Al for this horizon (Table 3). The sites with higher exchangeable soil Ca, Mg, and lower Al had higher foliar Ca and Mg for both age groups of needles (Tables 2 and 4).

There was a significant inverse correlation between: (i) foliar putrescine and exchangeable Ca and Mg in the soil of the Oa horizon; and (ii) foliar putrescine and exchangeable Mg in the upper 10 cm of B horizon for one-year-old needles. Putrescine content for this age group of needles had a significant positive correlation with the Al and molar Al:Ca ratios in the soil of the Oa horizon (Table 3, Figure 3). This is opposite of the results with foliar Ca and Mg where the significant correlations with soil were seen in only current-year needles (Table 3, Figure 3). No significant correlation was observed between putrescine and exchangeable Ca and Al in the B horizon for both age

Table 3. Correlation coefficients among various soil and foliar chemical measurements (n=6). Mean data for needle samples are computed based on n=40 for all sites except for Kossuth, ME, and Big Moose, NY, for which n=20). Mean data for soil samples are computed based on n=12 (36 samples collected individually and combined into 12 samples for analysis) for all sites

	Current-year needles				One-year-old needles			
	Putrescine	Spermidine	Exchangeable Ca	Exchangeable Mg	Putrescine	Spermidine	Exchangeable Ca	Exchangeable Mg
Exchangeable cations	r^2	r^2	r^2	r^2	r^2	r^2	r^2	r^2
Needles								
Ca	-0.89**	-0.96**			-0.75**	-0.95**		
Mg	-0.73**	-0.92**	+0.90**		-0.67**	-0.82**	+0.93**	
Al	0.22	0.32	0.20	0.23	0.00	0.13	0.24	0.24
Soil: Oa Horizon								
Ca	0.36	-0.65**	+0.68**	+0.68**	-0.69**	0.64*	0.42	0.28
Mg	0.43	-0.66**	+0.63*	+0.67**	-0.69**	0.49	0.33	0.31
Al	+0.59*	+0.80**	-0.70**	-0.68**	+0.73**	-0.61*	0.52	-0.55*
Soil: B Horizon								
Ca	0.29	0.39	0.24	0.47	0.17	0.26	0.32	+0.54*
Mg	0.39	-0.63*	+0.55*	0.51	-0.62*	0.47	0.35	0.33
Al	0.26	+0.64*	-0.57*	-0.54*	0.49	+0.61*	-0.56*	0.40
Cations in soil solution								
Oa Horizon								
Ca	0.41	-0.77**	+0.70**	+0.73**	-0.67**	-0.76**	+0.59*	0.46
Mg	0.35	-0.73**	+0.61*	+0.66**	-0.55**	-0.74**	+0.61*	0.50
Al	0.16	0.27	0.23	0.43	0.10	0.35	0.39	0.41
B Horizon								
Ca	0.04	0.01	0.02	0.04	0.05	0.01	0.03	0.00
Mg	0.06	0.06	0.05	0.05	0.01	0.17	0.28	0.24
Al	0.05	0.29	0.16	0.29	0.08	0.34	0.32	0.30

*$p \leq 0.1$; **$p \leq 0.05$.

groups of needles. The mineral soil had much lower concentrations of exchangeable Ca and Mg compared to that present in the Oa horizon. Foliar putrescine was also not correlated with soil or soil solution H in either the Oa or the upper 10 cm of the B horizons (data not presented). In summary, the sites with higher soil exchangeable Ca, Mg, and lower soil exchangeable Al concentrations had lower foliar putrescine concentrations and those with lower soil exchangeable Ca, Mg, and higher soil exchangeable Al concentrations had higher foliar putrescine concentrations for both age groups of needles (Tables 2 and 4). Correlations similar to putrescine and soil exchange chemistry were also observed between spermidine and soil exchange chemistry but for both age groups of needles (Table 3).

Correlations between foliar and soil solution chemistry

All of the sites had a higher soil solution pH for both the Oa and B horizons than soil pH values described above (Table 1). Whereas no correlation was observed between soil solution H concentrations and soil solution Al concentrations for the Oa horizon, there was a strong positive correlation between these variables in the upper 10 cm of the B horizon ($r^2 = 0.95$; $p \leq 0.001$). ANOVA indicated that with the single exception of Ca in the upper 10 cm of the B horizon, there were significant differences among sites for all the soil solution variables examined for both the Oa and B horizons. However, with few exceptions, no distinct patterns for site differences emerged after running Tukey's multi-

Table 4. Comparison of soil and soil solution chemistry. Data presented are means ± SE for *n*=12. At each site, 36 samples were collected individually and combined into 12 samples for analysis. For details see Materials and methods section

Variables	Groton, VT	Howland, ME	Kossuth, ME	Big Moose, NY	Crawford Notch, NH	Bear Brook, ME
Exchangeable cations in soil ($cmol_c\ kg^{-1}$)						
Oa Horizon						
Ca	13.68±1.17	11.41±1.03	6.28±1.00	6.42±0.82	6.77±1.20	5.33±1.01
Mg	2.88±0.43	2.65±0.18	2.15±0.19	1.98±0.32	2.14±0.40	1.36±0.18
Al	3.55±0.35	4.68±0.49	5.05±0.93	7.52±0.53	7.53±0.68	9.98±1.36
Al:Ca	0.3±0.05	0.46±0.07	2.1±1.03	1.76±0.47	1.78±0.05	4.75±2.04
B Horizon						
Ca	0.32±0.18	0.19±0.04	0.19±0.05	0.40±0.06	0.30±0.03	0.37±0.04
Mg	0.05±0.01	0.08±0.01	0.08±0.01	0.10±0.01	0.09±0.01	0.12±0.01
Al	1.64±0.28	4.09±0.63	6.45±0.60	9.94±0.46	8.03±0.72	7.25±0.31
Cations in soil solution ($\mu mol\ L^{-1}$)						
Oa Horizon						
Ca	198.99±43.26	138.93±25.58	87.54±24.54	22.06±2.71	44.94±11.86	29.68±3.63
Mg	76.06±10.83	54.93±6.86	48.5±6.63	15.18±3.66	27.35±3.41	27.50±3.57
Al	15.61±1.83	26.57±5.95	18.60±3.81	7.44±0.79	13.47±1.85	19.85±3.58
Monomeric Al:Ca	0.14±0.05	0.24±0.05	0.53±0.18	0.41±0.6	0.85±0.28	1.04±0.04
B Horizon						
Ca	5.80±0.79	4.63±1.28	5.38±1.24	4.51±1.20	3.77±0.89	8.12±0.84
Mg	12.12±0.86	13.97±2.03	14.03±1.01	11.09±1.65	9.82±1.12	15.86±1.01
Al	13.77±3.13	20.56±3.87	16.03±2.46	43.35±4.84	26.65±3.03	22.65±1.87

ple comparison test for means. The mean soil solution Ca and Mg concentrations were significantly higher at Groton and Howland as compared to Crawford Notch and Bear Brook. No differences were observed for Ca and Mg chemistry of the upper 10 cm of the B horizon (Table 4).

Exchangeable Ca and Mg in the current-year needles and soil solution Ca, Mg, and total monomeric Al:Ca molar ratios were correlated (Table 3, Figure 4). No correlation was found between foliar Ca and Mg of both needle age groups and soil solution Al of the Oa horizon. Foliar Ca had a significant positive correlation with soil solution Ca and Mg in the one-year-old needles as well. There was no correlation between soil solution Ca, Mg, and Al concentrations in the upper 10 cm of the B horizon with foliar Ca and Mg concentrations (Table 3). Foliar Al concentrations, however, were correlated with soil solution Al concentrations in the upper 10 cm of the B horizon for current-year needles ($r^2 = 0.61$; $p \leq 0.07$). Similar to soil exchange chemistry, sites with higher soil solution Ca and Mg and lower Al concentration also had higher foliar Ca and Mg for both age groups of needles (Tables 2 and 4).

Putrescine in one-year-old needles had a significant inverse correlation with soil solution Ca and Mg of the Oa horizon. Putrescine was not related to soil solution Al in the Oa horizon or the soil solution chemistry of the upper 10 cm of the B horizon for both age groups of needles (Table 3). This is the reverse of soil Al results where foliar putrescine, Ca, and Mg significantly correlated with soil Al. However, foliar putrescine showed a strong correlation with soil solution Al:Ca ratios ($r^2 = 0.91$; $p \leq 0.001$, Figure 4). Similar to the exchangeable soil chemistry, soil solution of the upper 10 cm of the B horizon had much lower concentrations of Ca and Mg as compared to that present in the soil solution of the Oa horizon and foliar putrescine was not correlated with these concentrations. Putrescine from both age groups of needles was not correlated with H concentrations in the soil solutions of the Oa or B horizons (data not presented). In summary, the sites with higher soil solution Ca, Mg, and lower soil solution Al:Ca ratios had lower foliar putrescine concentrations and

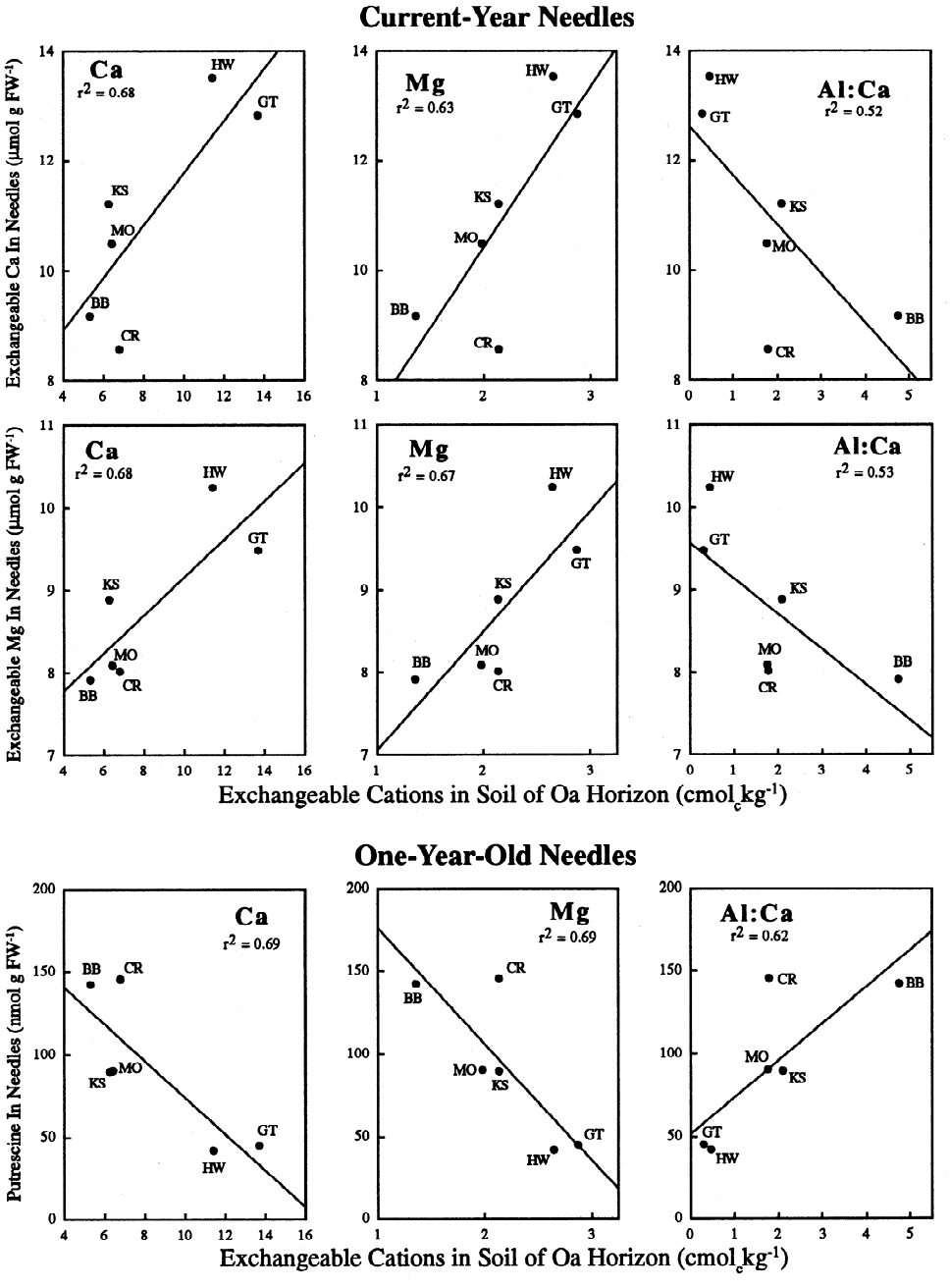

Figure 3. Correlations of foliar soluble putrescine and exchangeable Ca and Mg of red spruce with exchangeable soil chemistry at the six sites. Al:Ca is expressed here as a charge ratio. Foliar data are means ±SE (*n*=40 except for Kossuth, ME, and Big Moose, NY, for which *n*=20). Data for soil samples are given as means ±SE (*n*=12). The abbreviations used for site names are: Groton (GT); Howland (HW); Kossuth (KS); Big Moose (MO); Crawford Notch (CR); Bear Brook (BB)

those with lower soil solution Ca, Mg, and higher soil solution Al:Ca ratio had higher foliar putrescine concentrations for both age groups of needles (Tables 2 and 4). Correlations similar to putrescine and soil solution chemistry were also observed between spermidine and soil solution chemistry but for both age group needles (Table 3).

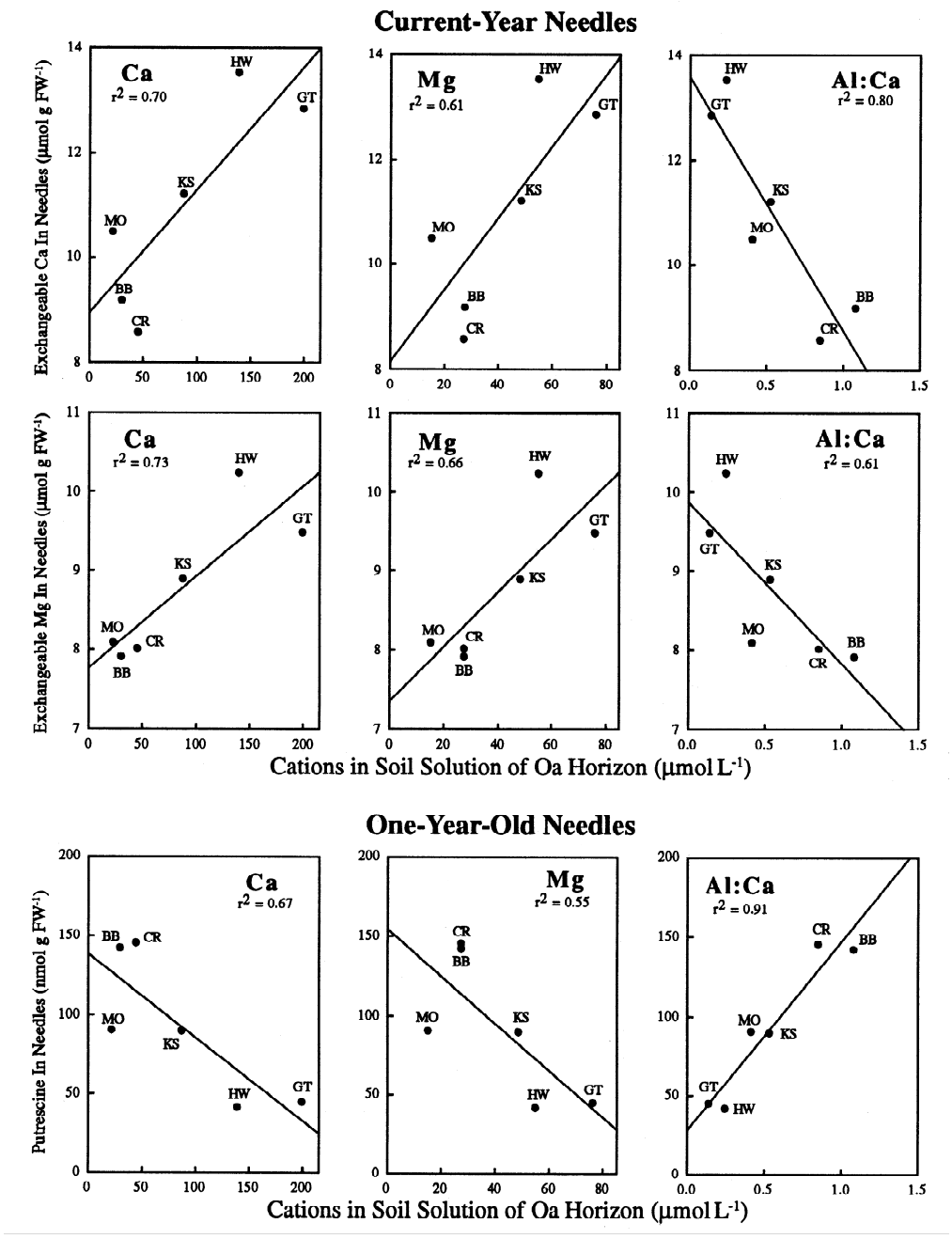

Figure 4. Correlations of foliar soluble putrescine and exchangeable Ca and Mg of red spruce with soil solution chemistry at the six sites. Al:Ca is expressed here as a molar ratio of total monomeric Al:Ca. Foliar data are means ±SE (n=40 except for Kossuth, ME, and Big Moose, NY, for which n=20). Data for soil samples are given as means + SE (n=12). The abbreviations used for site names are: Groton (GT); Howland (HW); Kossuth (KS); Big Moose (MO); Crawford Notch (CR); Bear Brook (BB).

Discussion

A direct or indirect interaction of some type between Al and Ca has been suggested in Al toxicity since several earlier studies have shown that field symptoms of severe Al toxicity resemble the symptoms of Ca deficiency (Delhaize and Ryan, 1995). Calcium ions alleviate toxic effects of Al and various heavy met-

als (Baker and Proctor, 1990; Foy, 1988). Aluminium often reduces cellular Mg and Ca concentrations in cell cultures, needles, and roots of red spruce and pine seedlings (Asp et al., 1988; Jentschke et al., 1991; Minocha et al., 1996; Ohno et al., 1988; Schier et al., 1990; Schroder et al., 1988). The effects of Al on K, however, vary with plant species (Cummings et al., 1985; Godbold et al., 1988; Schier et al., 1990). In the present study we have attempted to correlate the presence of varying concentrations of Al in the soil and/or soil solution with foliar chemistry and physiology. Furthermore, it is envisioned that comparison of the inferences drawn from this field study with those conducted under controlled environment conditions will help us evaluate the usefulness of the latter for providing insight into the effects of exposure to Al in nature on the physiology and health of mature trees. Similar to the situation with the cell culture and seedling studies, the concentrations of exchangeable Ca and Mg in the current-year needles of mature red spruce trees were inversely correlated with the soil Al and soil solution Al:Ca ratios of the Oa horizon (Table 3, Figures 3, 4).

A variety of biological and chemical stress factors induce the accumulation of putrescine in plants (Dohmen et al., 1990; Flores, 1991; Santerre et al., 1990). Red spruce cell cultures treated with Al showed a dose dependent increase in putrescine concentration and a decrease in Ca and Mg concentrations. This increase in putrescine was observed as early as 4h following Al treatment (Minocha et al., 1996). The putrescine concentration was found to be inversely related with cellular Ca and Mg in Al-treated cell cultures of *Catharanthus roseus* as well (Minocha et al., 1992; Zhou et al., 1995). Similarly, in the present study an increase in foliar putrescine concentration in one-year-old needles was associated with a decrease in foliar as well as soil Ca and Mg concentrations and an increase in the Oa horizon soil and soil solution Al or Al:Ca ratios for both age groups of needles (Table 3). Spermidine concentrations are, in general, tightly regulated by the cell and do not vary too much in magnitude. Nevertheless, strong correlations were observed among foliar spermidine and foliar Ca and Mg as well as soil parameters. Under conditions of inorganic ion deficiency, accumulation of putrescine (a divalent organic cation) may play an important role in the survival of plants. A possible reason for this may be that polyamines are synthesized within the cells and their cellular concentrations can be controlled not only by new synthesis based on the cell's physiological needs but also by conjugation, degradation, and sequestration via enzymatic means. In contrast, even if the inorganic ions undergo recompartmentalization in response to a stress stimulus, their overall cellular concentrations are controlled by the ability of the cells to uptake these ions from their external environment (Minocha et al., 1996). Thus polyamines can potentially substitute for inorganic cations in certain functions when the latter are in limited supply.

The lack of significant correlations ($p \geq 0.05$) of foliar chemistry with soil or soil solution chemistry in the upper 10 cm of the B horizon may be due to the fact that fine roots of red spruce trees grow primarily in the Oa horizon (Joslin and Wolfe, 1992). The higher Al concentrations and Al:Ca ratios in the B horizon negatively affect or inhibit Ca uptake and root growth in this horizon. As a consequence, the soil chemistry of the Oa horizon may be most important in affecting the health of a tree. Lawrence et al. (1995) proposed that acidic deposition induces mobilization of Al in the mineral soil. This reactive, mobilized form of Al is transported into the forest floor through biocycling and water movement where it competitively binds to Ca binding sites causing leaching of Ca into the B horizon and eventually into the stream waters. The addition of H to the Oa horizon via acidic deposition could not enhance further dissolution of mineral Al in this layer since the solution pH values are already less than 4 (Lawrence et al., 1995). This hypothesis is supported by our data in that there was no correlation between H and Al in the soil and soil solution of the Oa horizon but there was a significant correlation between these two factors in the B horizon. We believe that a similar explanation may be valid for the loss of soil Mg as well. The reduced storage of exchangeable Ca and Mg in the Oa horizon causes a reduction of Ca and Mg uptake into the fine roots (Shortle and Smith, 1988) and foliage (present data). This may, in turn, cause the induction and/or accumulation of putrescine and to a lesser extent spermidine in the foliage. Furthermore, this explains why foliar putrescine concentrations correlate both with foliar and Oa horizon Ca and Mg concentrations.

Although putrescine concentrations in the cells may increase in response to several factors (e.g., ozone, K deficiency, high salt, and SO_2 fumigation), in the present study the foliar putrescine concentrations did not show any significant correlations with soil and soil solution K of the Oa horizon and SO_4 concentrations in the soil solution (data not presented). If there were any random changes in putrescine concentrations, e.g. due to pathogen infections in the needles, they were

too few and insignificant within each site so as not to affect the overall pattern observed for putrescine concentrations across sites in relation to Al concentrations. The increase in putrescine in one-year-old needles of mature red spruce trees was correlated with an increase in the soil exchangeable Al and an increase in the soil solution Al:Ca ratio of the Oa horizon of the forest floor at all six sites. In general, the concentration of putrescine in one-year-old needles was significantly higher in the needles of red spruce growing in soils containing high Al concentrations and Al:Ca ratios, such as Big Moose, NY, Crawford Notch, NH and Bear Brook, ME. These data further suggest that all parts of a tree need not be in direct contact with high concentrations of Al to show a stress response. At present, we can not explain why in the case of soil solution chemistry, the correlations of foliar chemistry are stronger and significant with total monomeric Al:Ca molar ratios but in case of soil exchange chemistry the correlations of foliar chemistry are stronger with Al instead of Al:Ca charge ratios.

The Al:Ca ratio of the soil solution is one of several tools that can be used in assessing and predicting forest health (Cronan and Grigal, 1995). Several studies have reported that an Al:Ca molar ratio of greater than 1.0 in the soil solution or growth medium has inhibitory effects on growth (Kruger and Sucoff, 1989; Schier, 1985; Schulze, 1989; Stienen and Bauch, 1988). On the basis of a critical review of the literature on Al stress, Cronan and Grigal (1995) estimated that there may be a 50, 75, and 100% risk of adverse impacts on tree growth or nutrition with molar Al:Ca ratios of 1, 2, and 5, respectively. Although these numbers have been developed using studies involving soil solution ratios, it remains to be seen if a similar situation holds when one considers the charge ratios of exchangeable Al:Ca in soil. The molar ratio of Al:Ca, rather than absolute amounts of these ions in the root tips of spruce, has been suggested as being important in determining the extent of Al toxicity (Schroder et al., 1988; Shortle and Smith, 1988). In the present study, although a strong positive correlation was observed between total monomeric Al:Ca molar ratio in soil solution of the Oa horizon and foliar putrescine, the concentrations of the latter were not always correlated with visual site damage.

Even though the sites varied in stand health, all needle samples were taken from healthy trees and no necrotic needles were included. Whereas high putrescine concentrations coincided with visual site damage at Big Moose, NY and Crawford Notch, NH, no such correlation was seen for Bear Brook, ME. The trees at Bear Brook site may be under early stages of stress. The trees at this site may already be experiencing adverse growth such as stunted root and stem growth with no visual damage yet apparent. Exposure to an adverse biotic or abiotic stress is known to cause early biochemical responses in various tissues (Flores, 1991). It can thus be postulated that at this stage the trees appear healthy, but are actually more vulnerable to damage by other adverse environmental conditions than the trees growing under healthy conditions. If this stress were to continue for long periods of time, the trees may start showing visual symptoms. The sites chosen for our study may vary in their progression stage towards a visually unhealthy stand. This may explain why putrescine and spermidine correlate with soil and soil solution Al and Al:Ca but not always with visual site damage. Our goal has been to develop early indicators of stress before the appearance of visual symptoms. If an indicator only correlates with visual symptoms, its relevance to early site remediation is negligible. Thus putrescine and/or spermidine may be a potential nonspecific early indicator of stress. This marker may even be used as an indicator of a specific stress, e.g. exposure to Al, when used in conjunction with other factors such as soil chemistry. Our results are supported by other studies on Norway spruce (*Picea abies*) which show that cellular putrescine concentrations and putrescine/spermidine ratios are higher in needles collected from trees growing in polluted areas (Tenter and Wild, 1991; Villanueva and Santerre, 1989).

We conclude that the inferences drawn from our field study on the physiological and biological effects of exposure to various concentrations of Al on the foliar tissue of mature red spruce trees are similar to those drawn from our controlled experimental study with cell cultures (Minocha et al., 1996). This indicates that the cell culture studies are a valuable and reliable tool for gaining insights into the effects of certain elements on trees. We also demonstrate here that an increase in foliar putrescine and/or spermidine concentrations in response to direct or indirect stress imposed on red spruce trees by Al exposure may possibly be used as an early warning tool for assessing and predicting tree health before the appearance of visual symptoms.

Acknowledgements

The authors thank Kenneth R Dudzik, Daniel J Coughlin Jr and Stephanie Long for their superb technical assistance and Tracey Taylor-Lupien for assistance in preparing the manuscript for publication.

References

Asp H, Bengtsson B and Jensén P 1988 Growth and cation uptake in spruce (*Picea abies* Karst.) grown in sand culture with various aluminum contents. Plant Soil 111, 127–133.

Baker A J M and Proctor J 1990 The influence of cadmium, copper, lead, and zinc on the distribution and evolution of metallophytes in the British Isles. Plant Syst. Evol. 173, 91–108.

Blume L J, Schumacher B A, Schaffer P W, Cappo K A, Papp M L, Van Remortel R D, Coffey D S, Johnson M G and Chaloud D J 1990 Handbook of methods for acid deposition studies laboratory analyses for soil chemistry. Publ. EPA/6OO/4-90/023. Environmental Protection Agency, Environmental Monitoring Systems Laboratory, Las Vegas, NV.

Cronan C S and Grigal D F 1995 Use of calcium/aluminum ratios as indicators of stress in forest ecosystems. J. Environ. Qual. 24, 209–226.

Cummings J K, Eckert R T and Evans L S 1985 Effects of aluminum on uptake by red spruce seedlings. Can. J. Bot. 63, 1099–1103.

David M B and Lawrence G B 1996 Soil and soil solution chemistry under red spruce stands across the northeastern US. Soil Sci. 161, 314–328.

Delhaize E and Ryan P R 1995 Aluminum toxicity and tolerance in plants. Plant Physiol. 107, 315–321.

Dohmen G P, Koppers A and Langebartels C 1990 Biochemical response of Norway spruce (*Picea abies* (L.) Karst.) towards 14-month exposure to ozone and acid mist: effects on amino acid, glutathione and polyamine titers. Environ. Pollut. 64, 375–383.

Driscoll C T 1984 A procedure for the fractionation of aqueous aluminum in dilute acidic waters. Int. J. Environ. Anal. Chem. 16, 267–283.

Flores H E 1991 Changes in polyamine metabolism in response to abiotic stress. *In* Biochemistry and Physiology of Polyamines in Plants. Eds. R D Slocum and H E Flores. pp 213–228. CRC Press, Boca Raton, FL.

Foy C D 1988 Plant adaptation to acidic, aluminium-toxic soils. Commun. Soil Sci. Plant Anal. 19, 959–987.

Galston A W 1989 Polyamines and plant response to stress. *In* The Physiology of Polyamines II. Eds. U Bachrach and Y M Heimer. pp 99–106. CRC Press, Boca Raton, FL.

Godbold D L, Fritz E and Huttermann A 1988 Aluminum toxicity and forest decline. Proc. Nat. Acad. Sci. USA 85, 3888–3892.

Haug A 1984 Molecular aspects of aluminum toxicity. Crit. Rev. Plant Sci. 345–373.

Jentschke G, Schlegel H and Godbold D L 1991 The effect of aluminum on uptake and distribution of magnesium and calcium in roots of mycorrhizal Norway spruce seedlings. Physiol. Plant. 82, 266–270.

Joslin J D and Wolfe M H 1992 Red spruce soil solution chemistry and root distribution across a cloud water deposition gradient. Can. J. For. Res. 22, 893–904.

Kochain L V 1995 Cellular mechanisms of aluminum toxicity and resistance in plants. Annu. Rev. Plant Physiol. Plant Mol. Biol. 46, 237–260.

Kruger E and Sucoff E 1989 Aluminum and hydraulic conductivity of *Quercus rubra* L. in response to Al and Ca. J. Exp. Bot. 40, 659–665.

Lawrence G B and David M B 1996 Chemical evaluation of soil-solution in acid forest soils. Soil Sci. 161, 298–313.

Lawrence G B, David M B and Shortle W C 1995 A new mechanism for calcium loss in forest-floor soils. Nature 378, 162–165.

Lawrence G B, David M B, Bailey S W and Shortle W C 1997 Assessment of calcium status in soils of red spruce forests in the northeastern United States. Biogeochemistry (*In press*).

Lawrence G B, Lincoln T H, Horan-Ross D, Olson M L and Waldron L A 1995 Analytical Methods of the US Geological Survey, New York District Water Analysis Laboratory US Geological Survey. Open-File Report. 95-416, Troy, N.Y.

McQuattie C J and Schier G A 1990 Response of red spruce seedlings to aluminum toxicity in nutrient solution: Alterations in root anatomy. Can. J. For. Res. 20, 1001–1011.

Minocha R, Minocha S C, Long S L and Shortle W C 1992 Effects of aluminum on DNA synthesis, cellular polyamines, polyamine biosynthetic enzymes and inorganic ions in cell suspension cultures of a woody plant, *Catharanthus roseus*. Physiol. Plant. 85, 417–424

Minocha R and Shortle W C 1993 Fast, safe, and reliable methods for extraction of major inorganic cations from small quantities of woody plant tissues. Can. J. For. Res. 23, 1645–1654.

Minocha R, Shortle W C, Long S L and Minocha S C 1994 A rapid and reliable procedure for extraction of cellular polyamines and inorganic ions from plant tissues. Plant Growth Regul. 13, 187–193.

Minocha R, Shortle W C, Coughlin D J and Minocha S C 1996 Effects of Al on growth, polyamine metabolism, and inorganic ions in suspension cultures of red spruce (*Picea rubens*). Can. J. For. Res. 26, 550–559.

Minocha S C, Minocha R and Robie C A 1990 High-performance liquid chromatographic method for the determination of dansyl-polyamines. J. Chromatogr. 511, 177–183.

Ohno T, Sucoff E I, Earich M S, Bloom P R, Buschena C A and Dixon R K 1988 Growth and nutrient content of red spruce seedlings in soil amended with aluminum. J. Environ. Qual. 17, 666–672.

Santerre A, Markiewicz M and Villanueva V R 1990 Effect of acid rain on polyamines in *Picea*. Phytochemistry 29, 1767–1769.

Schier G A 1985 Response of red spruce and balsam fir seedlings to aluminum toxicity in nutrient solutions. Can. J. For. Res. 15, 29–33.

Schier G A, McQuattie C J and Jensen K F 1990 Effect of ozone and aluminum on pitch pine (*Pinus rigida*) seedlings: Growth and nutrition relations. Can. J. For. Res 20, 1714–1719.

Schroder W H, Bauch J and Endeward R 1988 Microbeam analysis of Ca exchange and uptake in the fine roots of spruce: Influence of pH and aluminum. Trees 2, 96–103.

Schulze E D 1989 Air pollution and forest decline in a spruce (*Picea abies*). Science 244, 776–781.

Shortle W C and Smith K T 1988 Aluminum-induced calcium deficiency syndrome in declining red spruce. Science 240, 1017–1018.

Stienen H and Bauch J 1988 Element content in tissues of spruce seedlings from hydroponic cultures simulating acidification and deacidification. Plant Soil 106, 231–238.

Sucoff E, Thornton F and Joslin J D 1990 Sensitivity of tree seedlings to aluminum. I. Honeylocust. J. Environ. Qual. 19, 163–171.

Thomas G W 1982 Exchangeable cations. *In* Methods of Soil Analysis, part 2; 2nd ed. Eds. A L Page et al. pp 161–164. Agronomy 9, ASA, Madison, WI.

Tenter M and Wild A 1991 Investigations on the polyamine content of spruce needles nutrition: Aluminum. Prog. Bot. 53, 63–67.

Villanueva V R and Santerre A 1989 On the mechanism of adaptive metabolism of healthy resistant trees from forest polluted areas. Water Air Soil Pollut. 48, 59–75.

Zhou X, Minocha R and Minocha S C 1995 Physiological responses of suspension cultures of *Catharanthus roseus* to aluminum: Changes in polyamines and inorganic ions. J. Plant Physiol. 145, 277–284.

Section editor: R F Huettl