Acidic Deposition, Cation Mobilization, and Biochemical Indicators of Stress in Healthy Red Spruce

Walter C. Shortle, Kevin T. Smith,* Rakesh Minocha, Gregory B. Lawrence, and Mark B. David

ABSTRACT

Dendrochemical and biochemical markers link stress in apparently healthy red spruce trees (*Picea rubens*) to acidic deposition. Acidic deposition to spruce forests of the northeastern USA increased sharply during the 1960s. Previous reports related visible damage of trees at high elevations to root and soil processes. In this report, dendrochemical and foliar biochemical markers indicate perturbations in biological processes in healthy red spruce trees across the northeastern USA. Previous research on the dendrochemistry of red spruce stemwood indicated that under uniform environmental conditions, stemwood concentrations of Ca and Mg decreased with increasing radial distance from the pith. For nine forest locations, frequency analysis shows that 28 and 52% of samples of red spruce stemwood formed in the 1960s are enriched in Ca and Mg, respectively, relative to wood formed prior to and after the 1960s. This enrichment in trees throughout the northeastern USA may be interpretable as a signal of increased availability of essential cations in forest soils. Such a temporary increase in the availability of Ca and Mg could be caused by cation mobilization, a consequence of increased acidic deposition. During cation mobilization, essential Ca and Mg as well as potentially harmful Al become more available for interaction with binding sites in the soil and absorbing roots. As conditions which favor cation mobilization continue, Ca and Mg can be leached or displaced from the soil. A measure of the interaction between Ca and Al is the Al/Ca binding ratio (molar charge ratio of exchangeable Al to exchangeable Ca). As the Al/Ca binding ratio in the root zone increased from 0.3 to 1.9, the foliar concentration of the biochemical stress marker putrescine also increased from 45 to 145 nm g^{-1}. The correlation of the putrescine concentration to the Al/Ca binding ratio (adj. $r^2 = 0.68$, $P < 0.027$) suggests that foliar stress may be linked to soil chemistry.

ACID rain was proposed as a serious environmental problem in forests of the northeastern USA in the early 1970s following marked increases in precipitation acidity (Likens and Bormann, 1974). Within a few years, the mobilization of harmful Al in high elevation watersheds of the northeastern USA was associated with the atmospheric deposition of nitric and sulfuric acid (Cronan and Schofield, 1979). This mobilization of Al was described as a possible cause for the deterioration of forest canopies, and death of red spruce trees in these same watersheds (Hertel, 1988).

Acidic deposition mobilizes Ca and Mg which are essential for root development and stem growth, as well as potentially harmful Al (Johnson and Fernandez, 1992). At first, mobilization would enhance the availability of Ca and Mg for root uptake. As conditions which favor cation mobilization continue, Ca and Mg can be depleted from forest-floor soils by leaching and displacement (Johnson and Fernandez, 1992; Lawrence et al., 1995). The supply of available Ca and Mg in the forest floor is limited by storage (Johnson and Fernandez, 1992), low rates of mineral weathering (Bailey et al., 1996; Johnson and Fernandez, 1992), and reductions in the atmospheric deposition of bases (Likens et al., 1996). Mobilized Al can also bind to fine root tips of red spruce, further limiting uptake of Ca and Mg (Shortle and Smith, 1988; Smith et al., 1995). Growth reductions in red spruce have been documented at all elevations throughout the northeastern USA and have been variously attributed to stressors such as acidic deposition and the reduced uptake of Ca and Mg as well as to natural growth patterns (Shortle and Smith, 1988; Johnson et al., 1995; Reams and Van Duesen, 1995). A previous investigation of stemwood sampled from 20 red spruce trees detected an anomalous enrichment of Ca and Mg that was interpreted as signal of cation mobilization in the soil (Shortle et al., 1995; Smith et al., 1996).

Considerable recent attention has been directed towards the identification and utilization of biochemical stress markers in forest trees. One promising source of such markers may be changes in the concentration of intracellular aliphatic amines such as the diamine putrescine and/or the polyamines spermine and spermidine. Putrescine, spermidine, and spermine have been implicated in the regulation of cellular pH, membrane stability, somatic embryogenesis, light-induced growth

W.C. Shortle, K.T. Smith, and R. Minocha, USDA Forest Service, Northeastern Forest Exp. Stn., P.O. Box 640, Durham, NH 03824; G.B. Lawrence, U.S. Geological Survey, 425 Jordan Road, Troy, NY 12180; M.B. David, Univ. of Illinois, W-503 Turner Hall, 1102 South Goodwin Avenue, Urbana, IL 61801.This research was funded primarily through the Northern Global Change Research Program of the U.S. Forest Service. Received 31 May 1996.*Corresponding author (kts@hopper.unh.edu).

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responses, and senescence. Cellular concentrations of these amines are highly regulated and one or more of them may increase in response to a variety of stimuli including Ca and Mg deprivation, low pH, low temperature, osmotic shock (Flores, 1991) and Al exposure (Minocha et al., 1992, 1996). In most cases, the increase in concentration is the result of de novo synthesis (Flores, 1991). Putrescine was a marker of Al-induced stress in tissue cultures of red spruce and other woody plants and was accompanied by blocked DNA synthesis, suppression of mitosis, ionic imbalances, impaired viability, and reduced biomass (Minocha et al., 1992, 1996).

This report addresses two questions: (i) Are radial trends in stemwood Ca and Mg enrichment consistent with inferred temporal trends in the soil chemistry of red spruce stands in the northeastern USA? (ii) Are concentrations of the stress marker putrescine in apparently healthy foliage related to Al/Ca ratios in the soil?

MATERIALS AND METHODS

Dendrochemistry

Increment cores were collected in 1992 to 1993 at 12 locations dominated by red spruce in the northeastern USA. The soil and soil solution chemistry of the forest floor and upper mineral soil associated with the cored trees has been described (David and Lawrence, 1996; Lawrence et al., 1995). Red spruce trees selected for coring were dominant or codominant in canopy position, 30 to 60 cm in diameter at breast height, and free of obvious external cracks, decay, and pests. At eight locations 36 trees and at four locations 18 trees were bored. Two increment cores (12-mm diam.) were removed from each stem at approximately 1.4 m aboveground. The growth ring series of the 720 cores were crossdated. One core from each of 148 trees from nine locations (Table 1) contained records of adequate length (nine decades) and quality for cation analysis. Care was taken to exclude cores that had periods of severely suppressed growth or wood altered by infection and tree response to infection (Smith and Shortle, 1996). To determine the effect of wood maturation on dendrochemistry, the sapwood/ heartwood boundary was marked on 40 of the cores used in cation analysis, with the cores distributed across the sample locations.

Cores were mounted in grooved wooden blocks, sanded, and crossdated. Decadal boundaries were marked on each core mount. Decadal samples were drilled from each mounted core using a Ti-coated drill bit, 6.4 mm in diameter. For each decade, 25 mg of drill shavings were placed in a 15-mL acid-washed glass test tube. To each tube was added 6 mL of 0.01 *N* HCl. Sample tubes were incubated overnight at −20°C, thawed to room temperature, and refrozen for a total of three freeze-thaw cycles. After filtration, decadal extracts were analyzed by direct-coupled plasma atomic emission spectroscopy (DCP-AES) (Minocha and Shortle, 1993; Shortle et al., 1995).

The mean enrichment frequency (the percent frequency of a decade containing a ≥5% increase in Ca or Mg relative to the previous decade) for each decade and location was calculated. Enrichment of ≥5% was selected as a conservative measure that rejected small, potentially spurious increases due to inherent variability in sample processing and analysis. The mean enrichment frequency in red spruce is itself a conservative measure, due to the natural trend of decreasing cation exchange capacity with increasing radial distance from the pith (Momoshima and Bondietti, 1990). Consequently, under uniform environmental conditions, Ca and Mg concentrations would tend to decrease and the frequency of enrichment would be quite low. Ranked decadal enrichment frequencies were subjected to Kruskall-Wallis analysis of variance ($N = 9$). Each location was considered as a replicate and each replicate mean was derived from analysis of 8 to 20 trees. Following the determination of a significant decadal effect, Dunnett's test was used to determine whether the decade with the highest enrichment frequency was unique among the decades tested ($P < 0.05$).

Foliar Putrescine

Six of the 12 forest locations were selected on the basis of soil chemistry to represent a gradient of Al stress (David and Lawrence, 1996; Lawrence et al., 1995; Table 1). The two extreme sites with the lowest and highest Al/Ca ratios (Sleepers River and Mount Abraham, Vermont, respectively) were not selected because of the unavailability of a sufficient number of mature, healthy red spruce trees in the forest canopy. Most locations were sampled in June and October of 1993 and July and October of 1994. Foliage at the Kossuth and Big Moose Lake locations were sampled in June 1993 and October 1994. For each location and sampling, healthy, sun-lit branches were pruned from 10 trees in the vicinity of the soil sampling pits. From each tree, 1-yr-old needles were placed in pre-weighed microfuge tubes containing 1 mL of 5% perchloric acid. The tubes were kept chilled during sample collection and then stored at −20°C until processed (Minocha et al., 1994). The sample tubes were weighed, frozen, and thawed for a total of three freeze-thaw cycles. The thawed sample tubes were centrifuged at 14 000 rpm in a microfuge for 10 min. Supernatants were frozen at −20°C until analysis. Heptanediamine was added as an internal standard to aliquots of the extracts that were then dansylated (Minocha et al., 1990). Dansylated polyamines were separated by reversed phase HPLC using a gradient of acetonitrile and heptane sulfonate. Polyamines were quantified with a fluorescence detector (Mi-

Table 1. Forest locations, Al/Ca binding ratios in the forest floor, and sampling details.

Location	Al/Ca	No. of trees cored	Sampled for dendrochemistry	Sampled for foliar putrescine
Sleepers River, Vermont	0.2	18		
Groton, Vermont	0.3	36	+	+
Howland, Maine	0.4	36	+	+
Bartlett, New Hampshire	0.5	36	+	
Kossuth, Maine	0.8	36	+	+
Hubbard Brook, New Hampshire	0.8	18	+	
Whiteface Mountain, New York	0.8	18	+	
Crawford Notch, New Hampshire	1.1	36	+	+
Big Moose Lake, New York	1.2	36	+	+
Bear Brook, Maine	1.9	36		+
Cone Pond, New Hampshire	5.2	36	+	
Mt. Abraham, Vermont	5.6	18		

nocha et al., 1990). Total polyamine concentration was determined for each sampling time. For each location, the foliar putrescine concentration was averaged across all four sampling times. Linear regression analysis was used to establish the strength and significance of the relationships between foliar putrescine concentration, Al/Ca binding ratios (molar charge ratio of exchangeable Al to exchangeable Ca), and cation concentration in soil solutions and associated solids.

RESULTS AND DISCUSSION

The binding of divalent Ca and Mg to ion exchange sites in the wood cell wall system follows the characteristics of a Donnan equilibrium (Momoshima and Bondietti, 1990). In brief, the amount of Ca and Mg in wood is a function of pH, the peak concentration of Ca and Mg in the surrounding sap, and the number of binding sites available for exchange. Under uniform soil and sap conditions, Ca and Mg concentrations tend to decrease with increasing radial distance from the pith, due to decreasing numbers of available binding sites (Momoshima and Bondietti, 1990).

Dendrochemical analysis indicated that stemwood formed in mature red spruce trees in the 1960s was more frequently enriched in Ca (Fig. 1A) and Mg (Fig. 1B) than at any other time over the past eight decades ($P < 0.05$). The enrichment frequency of 28% for Ca and 52% for Mg in wood formed from 1961 to 1970 is remarkable because the enrichment indicates a perturbation in the equilibrium between soil chemistry, sap chemistry, and cation binding sites in stemwood. The observed Ca and Mg enrichment is also remarkable because of its regional nature.

What internal or external factors could cause the enrichment in Ca and Mg for wood formed in the 1960s? Internal infection processes and the tree response to injury and infection can greatly alter Mg and Ca concentration in the wood of living trees. However, the careful screening of both candidate trees and cores argue against that explanation. In some tree species, especially those (unlike red spruce) which contain a highly colored heartwood, heartwood formation can affect wood chemistry. Simply because of tree age and the maturation process of red spruce, 31 of the 40 (78%) marked sapwood/heartwood boundaries occurred in wood formed in 1961 to 1970. However, the sapwood/heartwood boundary coincided with peak Ca enrichment in only 22 of the 40 cores (55%). We suggest that if Ca enrichment was a constitutive feature of heartwood formation in red spruce, essentially all, rather than the observed 55%, of the peak Ca enrichment would coincide with the sapwood/heartwood boundary.

The pattern of stemwood enrichment in Ca and Mg is consistent with the hypothesis that Ca and Mg were mobilized in the soil as acidic deposition increased, and transported in unusually high concentrations to stemwood via root uptake and sap transport (Shortle et al., 1995). This temporary increase in binding of Ca and Mg in wood formed in 1961 to 1970 coincided with (i) increased SO_2 and NO_x emissions (NAPAP, 1993; Fig. 1C), (ii) increased deposition of SO_4 and NO_3 in the Greenland ice sheet (Mayewski et al., 1986; Fig. 1D), (iii) decreased pH of lake water in the Adirondacks (Charles, 1984; Fig. 1E), and (iv) reduced concentrations of Ca and Mg in the forest floor (Shortle and Bondietti, 1992; Fig. 1F).

If reductions in the concentration of Ca and Mg from the forest floor were due to exchange of H for Ca, either from plant growth (Johnson and Anderson, 1994) or from acidic deposition, then the concentration of exchangeable Ca and Mg ($[Ca + Mg]_{ex}$) would be negatively correlated with the concentration of exchangeable H ($[H]_{ex}$). Across our 12 study locations, soil analysis indicated that $[Ca + Mg]_{ex}$ was not significantly related to $[H]_{ex}$ for untransformed or $\log_e$ transformed data from the forest floor ($P = 0.12$) or mineral soil ($P = 0.70$) (Fig. 2A). However, $\log_e [Ca + Mg]_{ex}$ was significantly and negatively correlated to $\log_e [Al]_{ex}$ in the forest floor, the principle rooting zone of red spruce (Fig. 2B, $P < 0.001$). Lawrence et al. (1995) have attributed this negative correlation between exchangeable Ca and exchangeable Al in the forest floor to increased translocation of Al from the mineral soil, where acidic deposition has increased Al dissolution. Acid-extractable Al in the forest floor increased over the past two decades at the Hubbard Brook Experimental Forest, and ratios of Al to Ca in mineral soil solutions (but not forest floor solutions) were strongly correlated with exchangeable Al content in the forest floor. Mineral dissolution of Al in the forest floor was ruled out as a cause for this relation because mineral Al concentrations were unrelated to exchangeable Al concentrations (Lawrence et al., 1995). Leaching of Ca by SO_4^{2-} has directly contributed to depletion of forest floor Ca, but mobilization of Al in the mineral soil has also indirectly contributed to this process by (i) reducing uptake of Ca from the mineral soil, (ii) providing a supply of reactive Al that exchanges with Ca in the forest floor, enabling the leaching of Ca from the forest floor, and (iii) increasing Al saturation so that the number of exchange sites available for adsorbing added Ca is reduced.

The foliar concentration of the stress marker putrescine was significantly correlated ($P < 0.02$) to the Al/Ca binding ratio of the forest floor (Fig. 2C). This indicates that even healthy-appearing trees are stressed due to adverse Al/Ca ratios. Regression analysis indicates that $\log_e$ Al/Ca binding ratio was strongly related with the $\log_e$ Al/Ca ratio in the soil solution of both the forest floor and the mineral soil (Fig. 2D, $P < 0.001$). Nonwoody absorbing root tips in the forest floor appear to be binding Al in the same manner as the surrounding soil, at the expense of Ca and Mg (Fig. 2D, and Shortle and Bondietti, 1992; Smith et al., 1995). The Al/Ca ratio in the soil solution (expressed as the reciprocal Ca/Al ratio) was described as an ecological indicator of approximate thresholds beyond which the risk of forest damage increased due to Al stress and nutrient imbalances (Cronan and Grigal 1995). The risk of forest damage was assessed at 50% when the soil solution Al/Ca ratio was 2 and at 100% when the ratio was 5. At all locations, Al/Ca ratios of mineral soil solutions exceeded the 50% risk level and half of the locations were above the 100% risk level. Most forest floor solutions

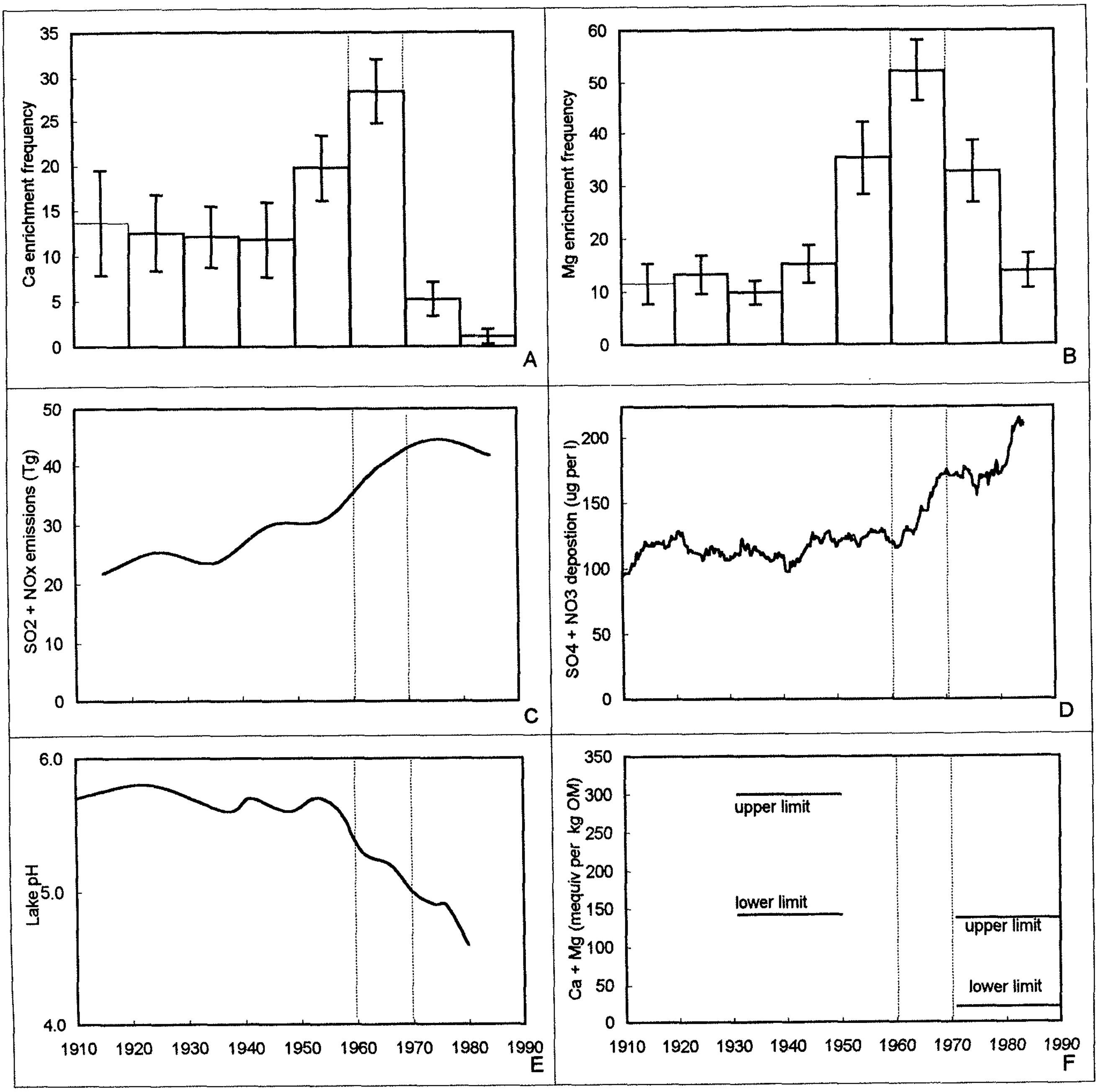

Fig. 1. Dendrochemical markers of cation mobilization and coincident environmental changes. The vertical, dashed lines mark the years 1960 and 1970. (*A*) Percent frequency of Ca enrichment by decade. For example, the 1961–1970 bar indicates that the mean frequency of Ca enrichment relative to the preceeding decade was 28% across all sample locations (± SE). (*B*) Enrichment frequency for Mg was calculated as for Ca in (*A*). (*C*) Combined emissions of SO_2 and NO_X for the USA (NAPAP, 1993). (*D*) Combined deposition of nonmarine SO_4 and NO_3 in ice core 20D collected from south-central Greenland (Mayewski et al., 1986). (*E*) Water pH at Big Moose Lake in the southwest Adirondacks of New York. Acidity was estimated through stratigraphy of diatoms in sediments (Charles, 1984). (*F*) Historical range of concentration of Ca and Mg in the forest floor of the northeastern United States according to available reports (Shortle and Bondietti, 1992).

ratios were below the 50% risk level and trees appeared generally healthy. The forest floor solution ratio was above the 50% risk level at only one location, Mt. Abraham, Vermont, where one-half of the canopy spruce were dead or dying. Because we sampled the most healthy portions of the red spruce forest at each location, the area we investigated at Whiteface Mountain, New York did not have a particularly high Al/Ca binding ratio (0.8) in the forest floor. This particular area contained neither evidence of unusual mortality or current tree decline.

These results support and expand upon the model that Robert Hartig proposed in 1897 to relate sulfate deposition to damage ("smoke injury") of spruce-fir forests (Hartig, 1897). Hartig stated that essential base cations would be lost from the root zone of trees subjected to high inputs of sulfate. We suggest that mobilization of Al has accelerated the loss of essential bases.

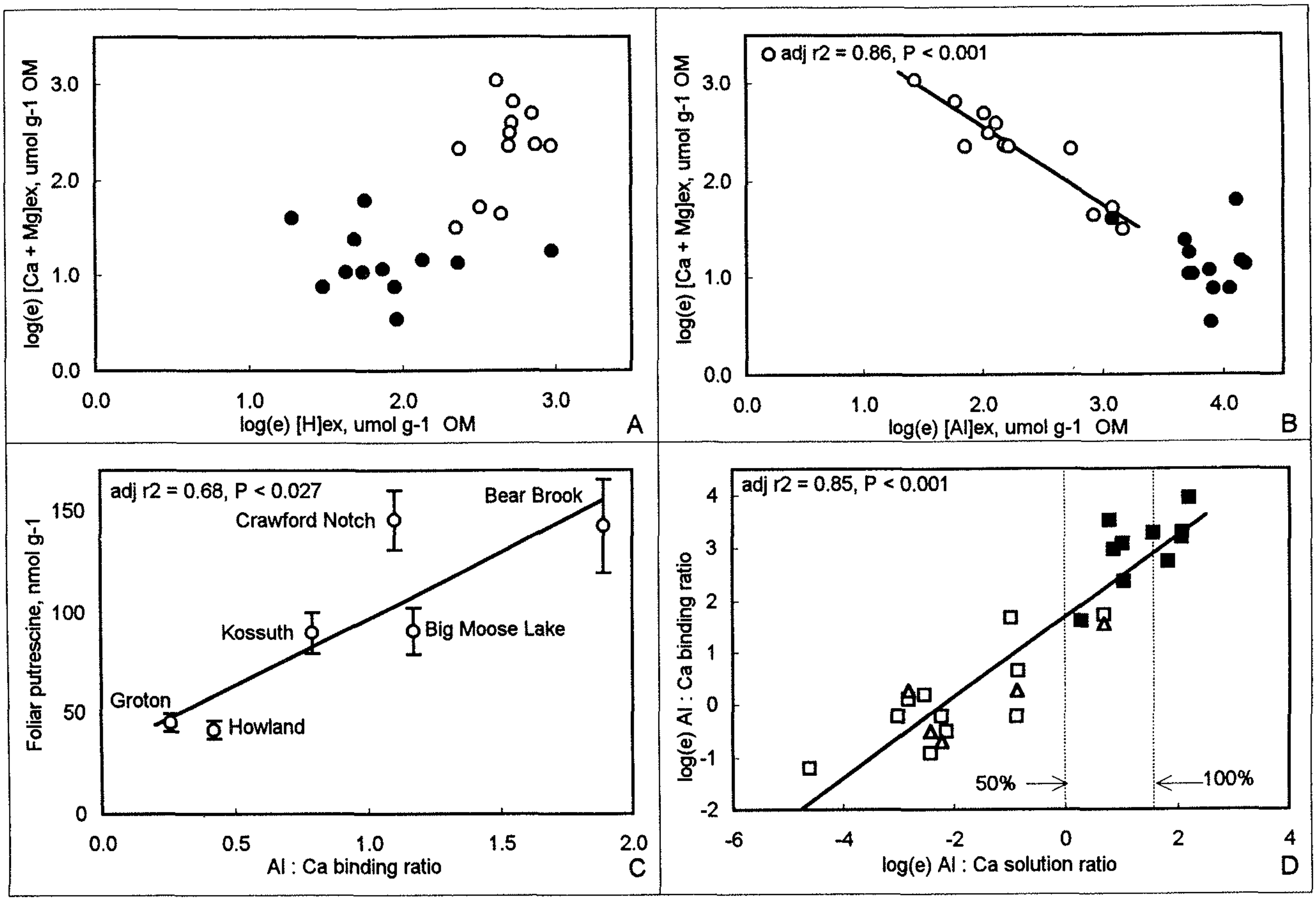

Fig. 2. Relationships among major cations in forest soils and soil solutions (David and Lawrence, 1996; Lawrence et al., 1995) and the stress marker, putrescine. (*A*) Regression analysis indicates no significant relationship between $\log_e$ [Ca + Mg]$_{ex}$ to $\log_e$ [H]$_{ex}$ either in mineral soil (closed circles, $P < 0.70$) or forest floor (open circles, $P < 0.12$). (*B*) Regression analysis indicates a significant relationship between $\log_e$ [Ca + Mg]$_{ex}$ and $\log_e$ [Al]$_{ex}$ in the forest floor (open circles, $P < 0.001$) but no significant relationship in mineral soil (closed circles, $P < 0.36$). (*C*) Regression analysis indicates that concentrations of putrescine in red spruce foliage are significantly related to the Al/Ca binding ratio (molar charge ratio of exchangeable Al to exchangeable Ca) of the forest floor (open circles, $P < 0.027$). (*D*) Regression analysis indicates a significant relationship between $\log_e$ Al/Ca ratio in the forest floor soil solution to the $\log_e$ Al/Ca binding ratio in the forest floor (open squares) and fine root tips (triangles), and between $\log_e$ Al/Ca ratio in the mineral soil solution to the $\log_e$ Al/Ca binding ratio in mineral soil (closed squares, $P < 0.001$). Vertical, dotted lines indicate the suggested 50 and 100% risk level of forest damage due to adverse Al/Ca ratios in the soil solution (Cronan and Grigal, 1995).

We make this suggestion because of the evidence of a general mobilization of base cations in the mid-20th Century and the dynamics of Al behavior as described above. As Hartig described, the loss of essential bases would suppress root growth. Roots not able to grow into new areas of the forest soil would eventually starve and die, making the below-ground parts of trees susceptible to attack by facultative pathogens. Aboveground parts would respond to the loss of roots by shedding needles. Most trees would survive but grow poorly, be more frequently infected by facultative pathogens and insects, and be less tolerant of abiotic stressors such as drought.

In conclusion, the patterns of the dendrochemical marker of Ca and Mg enrichment in red spruce stemwood is inconsistent with a stable chemical environment in forest soil for the 20th Century in the northeastern USA. The enrichment in wood formed in the 1960s is consistent with mobilization of base cations in the mid-20th Century. This hypothetical mobilization coincides with increases in the atmospheric emission and deposition of nitrates and sulfates. The biochemical marker, foliar putrescine concentration, indicated that even apparently healthy trees are under stress due to adverse Al/Ca ratios in the forest floor. The hypothetical mobilization of Al, which would result in greater Al/Ca ratios, would be enhanced by acidic deposition (Cronan and Goldstein 1989; Driscoll et al. 1984; Lawrence and Fernandez 1991). Spruce stands most vulnerable to multiple stressors, such as those at high elevations, have already been damaged. We anticipate that red spruce and other tree species growing under less harsh conditions will become stressed and more vulnerable with continued depletion of essential base cations and mobilization of Al.

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