

# Vitality and chemistry of roots of red spruce in forest floors of stands with a gradient of soil Al/Ca ratios in the northeastern United States

**Philip M. Wargo, Kristiina Vogt, Daniel Vogt, Quintaniay Holifield, Joel Tilley, Gregory Lawrence, and Mark David**

**Abstract:** Number of living root tips per branch, percent dead roots, percent mycorrhizae and mycorrhizal morphotype, response of woody roots to wounding and colonization by fungi, and concentrations of starch, soluble sugars, phenols, percent C and N and C/N ratio, and Al, Ca, Fe, K, Mg, Mn, and P were measured for 2 consecutive years in roots of red spruce (*Picea rubens* Sarg.) in stands in the northeastern United States (nine in 1993 and two additional in 1994) dominated by red spruce and with a gradient of forest floor exchangeable Al/Ca ratios. Root vitality was measured for nonwoody and coarse woody roots; chemical variables were measured for nonwoody (<1 mm), fine woody (1 to <2 mm), and coarse woody (2 to <5 mm) roots. There were significant differences among sites for all variables, particularly in 1993, although few were related to the Al/Ca ratio gradient. Percent mycorrhizae decreased, while some morphotypes increased or decreased as the Al/Ca ratio increased. In nonwoody roots, N increased as the Al/Ca ratio increased. Most sampled trees appeared to be in good or fair health, suggesting that an adverse response of these root variables to high Al concentrations may be apparent only after a significant change in crown health.

**Résumé :** Le nombre d'apex racinaires par branche, le pourcentage de racines mortes, le pourcentage de mycorhizes et leur morphotype, la réaction des racines ligneuses aux blessures et leur colonisation par les champignons, la concentration d'amidon, de sucres solubles et de composés phénoliques, le pourcentage de C et N et le rapport C/N ainsi que le contenu en Al, Ca, Fe, K, Mg, Mn et P ont été mesurés durant 2 années consécutives dans les racines d'épinette rouge (*Picea rubens* Sarg.). Les racines provenaient de peuplements (neuf en 1993 et deux de plus en 1994) du Nord-Est des États-Unis dominés par l'épinette rouge et représentant un gradient du rapport Al/Ca échangeables dans la couverture morte. La vitalité des racines a été mesurée pour les racines non ligneuses et les grosses racines ligneuses; les variables chimiques ont été mesurées pour les racines non ligneuses (<1 mm), les racines fines ligneuses (1 à <2 mm) et les grosses racines ligneuses (2 à <5 mm). Il y avait des différences significatives entre les sites pour toutes les variables, particulièrement en 1993, quoique peu de ces différences soient reliées au gradient du rapport Al/Ca. Le pourcentage de mycorhizes diminuait tandis que certains morphotypes augmentaient ou diminuaient avec l'augmentation du rapport Al/Ca. Dans les racines non ligneuses, N augmentait avec l'augmentation du rapport Al/Ca. La santé de la plupart des arbres échantillonnés variait de bonne à passable, ce qui indique qu'une réaction négative de ces variables racinaires à de fortes concentrations de Al peut n'être apparente seulement après que la santé de la cime ait été substantiellement altérée.

[Traduit par la Rédaction]

## Introduction

In the early 1980s, an unexplained increase in dieback and decline of red spruce (*Picea rubens* Sarg.) was documented

Received 25 April 2002. Accepted 31 October 2002.

Published on the NRC Research Press Web site at  
<http://cjfr.nrc.ca> on 19 March 2003.

**P.M. Wargo<sup>1</sup> and Q. Holifield.** USDA Forest Service, Northeastern Research Station, Hamden, CT 06514, U.S.A.

**K. Vogt and D. Vogt.** College of Forest Resources, University of Washington, Seattle, WA 98195-2100, U.S.A.

**J. Tilley.** University of Vermont, Burlington, VT 05405, U.S.A.

**G. Lawrence.** U.S. Geological Survey, Troy, NY 12180-8349, U.S.A.

**M. David.** University of Illinois, NRES, Urbana, IL 61801, U.S.A.

<sup>1</sup>Corresponding author (e-mail: pwargo@fs.fed.us).

in mountainous regions of the northeastern United States (Siccama et al. 1982; Scott et al. 1984). Subsequent studies reported that both reduced growth and extensive mortality occurred throughout the natural range of this species (Hornbeck and Smith 1985; Weiss et al. 1985; McLaughlin et al. 1987).

Evaluation of radial growth patterns showed that the recent reduction in radial growth in red spruce was not related to climate (Cook et al. 1987; Johnson et al. 1988; Cook and Johnson 1989a, 1989b; Smith et al. 1999). Based on evidence of marked increases in acidity of precipitation in the early 1970s (Likens and Bormann 1974) and increases in Al concentration in high-elevation watershed streams in the northeast (Cronan and Schofield 1979), Al mobilization was suggested as a possible cause for the anomalous growth reductions in and death of canopy-dominant red spruce in these forests (Hertel 1988).

Ulrich (1983) proposed that the adverse effects of acidic deposition on forest ecosystems would be reduced levels of

base cations in soil solutions, particularly Ca, resulting in low Ca/Al ratios. Subsequent studies in conifer forests in Germany linked changes in the molar ratio of Al/Ca in the forest floor to the death of fine roots, reduced tree growth, deteriorating crowns, and increased tree mortality (Stienen et al. 1984; Bauch et al. 1985a, 1985b; Bauch and Schroeder 1982). In these studies, when Al/Ca ratios in the cortex of fine roots were  $>1$ , growth was reduced or tree mortality resulted that coincided with the loss of root integrity as levels of root Ca decreased. Radioisotope studies also verified reduced uptake of Ca by fine roots when equimolar concentrations of  $\text{Al}^{3+}$  and  $\text{Ca}^{2+}$  were provided to roots (Schroeder et al. 1988).

Root physiology and pathology are affected indirectly by acidic deposition primarily by two mechanisms: (i) a change in soil chemistry that directly modifies root morphology, longevity, and turnover rates, mycorrhizal status of the root, and root chemistry, metabolism, and ultimately the ability to take up nutrients (Bloomfield et al. 1996) and (ii) a reduction in the total amount of C fixed by trees in response to the effects generated by changes in soil chemicals. Total-tree C sequestration is reduced due to (i) reduced nutrient uptake, (ii) reduced photosynthetic rates related to reduced  $\text{CO}_2$  assimilation (Ellsworth and Liu 1994) and reduced foliar area associated with dieback phenomena, e.g., winter injury in red spruce (DeHayes et al. 1999; Schaberg et al. 2000), and (iii) increased root mortality (Wargo et al. 1993). A change in C allocation reduces available carbohydrate required to maintain existing roots and mycorrhizae, replace dead and dying roots, and provide defense chemicals needed to protect roots against pathogenic fungi and other microorganisms (Bloomfield et al. 1996).

This study was established to determine whether changes in vitality and chemistry of red spruce roots were related to forest floor cation chemistry, especially the exchangeable Al/Ca ratio. If these correlations exist, the forest floor Al/Ca ratio would be an effective "early-warning" indicator of the changing health status of red spruce and its potential susceptibility to pathogens and (or) insect pests. The northeastern United States is an ideal location to test these links because this region has experienced varying levels of reduced growth and increased mortality of red spruce putatively related to acid deposition. Stands were selected to provide a gradient of concentrations of soil elements and soil Al/Ca ratios of the Oa horizon that varied from 0.3 to 7.1 (Lawrence et al. 1995). This range of ratios (the inverse of the Ca/Al ratio) is related (David and Lawrence 1996) but not identical to the range of soil solution molar ratios that Cronan and Grigal (1995) have suggested places trees in a <50–100% risk of being adversely affected by Al in the soil. The root vitality assessments also focused on measuring the changing susceptibility of fine roots of red spruce to root pathogens and the chemical indicators that reflect a change in susceptibility to pathogens.

## Materials and methods

In forests dominated by red spruce, plots were established at nine sites in 1993 and two additional sites in 1994 in New York (two), Vermont (one), New Hampshire (four), and Maine (four) (Table 1). Concurrent research at these sites

had (i) documented changes in Al to Ca relationships as indicated by changes in Ca concentration bound in stemwood over time (Shortle et al. 1997), (ii) characterized the soil and soil solution chemistry, and (iii) identified the existence of a range of exchangeable Al/Ca ratios in the Oa horizon of the forest floor (Lawrence et al. 1995, 1997; David and Lawrence 1996) (Table 1). Soils are classified as Spodosols at all 11 sites.

## Nonwoody root vitality and pathology

### Tree selection

Dominant or codominant red spruce that were 30–60 cm diameter at breast height and had trunks free of obvious external cracks, decay, and pests were identified and then selected at random at each location (Shortle et al. 1997). Trees were grouped into nearest neighbor clusters of three trees each, and root samples were taken from three trees in each of three clusters (nine trees per plot) at each of nine sites in 1993 and 11 (original nine plus two additional) sites in 1994. This was the same sampling procedure that was used in the companion soil studies (David and Lawrence 1996). When sampled, crown condition of each tree was rated by two individuals; three rankings were used based on amount of dieback and (or) necrotic needles (current year) in the upper two thirds of the crown: 1, good crowns with  $\leq 10\%$  dieback and (or) necrosis; 2, fair crowns with 11–49% dieback and (or) necrosis; and 3, poor crowns with  $>50\%$  dieback and (or) necrosis. Each crown quadrant (northeast, southeast, southwest, and northwest) was rated separately and an average rating for each tree was calculated from the four quadrants. Trees that were obviously dying were not included in the sample. Nine different trees were sampled each year; sampling roots from different trees in the second year (1994) prevented the disturbance of root sampling in the first year (1993) from influencing the root sample collected in the second year. Tree roots were sampled during the first 3 weeks of June of each year. In 1993, we sampled stands from east to west; in 1994, we reversed the direction because of phenologic differences noticed in 1993.

### Root sampling

Major buttress roots (first order) on each tree were assigned a number; three were chosen randomly for sampling. If it was impossible to excavate smaller roots from these roots, the next nearest buttress root in a clockwise direction was selected. The root was excavated and followed away from the root stem base by carefully removing the forest floor with handtools until a branch root (second order) was encountered (see Wargo et al. 1993). This second-order root was excavated until a healthy third-order branch was uncovered. From this third-order branch, three healthy fourth-order woody root systems with fifth- and sixth-order woody roots and their nonwoody branches (roots  $<1$  mm in diameter) were extracted carefully from the forest floor (Oe and Oa but predominantly from the Oe horizon). No roots from the mineral soil were sampled intentionally. Roots with adhering soil particles were placed in plastic bags on ice in coolers until transported to the laboratory where they were stored at  $\sim 3^\circ\text{C}$  until processed. Roots were processed for vitality and chemical analyses within 6 weeks after sample

**Table 1.** Site locations and sample years for plots in mature red spruce dominated stands in the northeastern United States ordered by Al/Ca ratio in the Oa horizon of the forest floor.

| Location   | Approximate elevation (m) | Sampling year(s) | Al/Ca ratio <sup>a</sup> |                         |
|--|---------------------------|------------------|--------------------------|-------------------------|
|  |                           |                  | Oa horizon <sup>b</sup>  | Oe horizon <sup>c</sup> |
| Groton, Vermont (GR)                             | 520                       | 1993, 1994       | 0.3                      | 0.04                    |
| Howland, Maine (HO)                              | 60                        | 1993, 1994       | 0.4                      | 0.06                    |
| Bartlett, New Hampshire (BR)                     | 525                       | 1993, 1994       | 0.5                      | 0.11                    |
| Kossuth, Maine (KO)                              | 100                       | 1993, 1994       | 0.8                      | 0.09                    |
| Hubbard Brook, New Hampshire (HB)                | 755                       | 1994             | 0.8                      | 0.12                    |
| Whiteface Mountain, New York (WF)                | 950                       | 1993, 1994       | 0.8                      | 0.09                    |
| Crawford Notch, New Hampshire (CR)               | 670                       | 1993, 1994       | 1.1*                     | 0.15                    |
| Big Moose Lake, New York (BM)                    | 550                       | 1993, 1994       | 1.2*                     | 0.15                    |
| Bear Brook, Maine (BB)                           | 400                       | 1993, 1994       | 1.9**                    | 0.23                    |
| Cone Pond, New Hampshire (CP)                    | 610                       | 1993, 1994       | 5.2***                   | 0.52                    |
| Bear Brook, Maine (BF) (fertilized) <sup>d</sup> | 400                       | 1994             | 7.1***                   | 0.52                    |

<sup>a</sup>Al/Ca ratios related potentially to the classes of risk of adverse impact on tree growth and nutrition based on Al/Ca ratios in the soil solution: \*, 50:50; \*\*, 75; and \*\*\*, 100% risk (Cronan and Grigal 1995).

<sup>b</sup>From Lawrence et al. (1995).

<sup>c</sup>Calculated from concentrations of Al and Ca estimated from equations derived from Oe–Oa relationships at three sites used in this study.

<sup>d</sup>Adjacent to the Bear Brook site but received 1800 equiv.  $(\text{NH}_4)_2\text{SO}_4 \cdot \text{ha}^{-1} \cdot \text{year}^{-1}$  since 1989 (Rustad et al. 1996).

collection in June of each year. Time in storage was approximately equal for each site (5–6 weeks).

In the laboratory, root systems were placed in trays with water to loosen and remove soil debris. Three fifth- or sixth-order woody roots with fine nonwoody branches attached were selected randomly from each fourth-order root beginning at the distal end of the root. A fourth branch (fifth or sixth order) was removed from one of the fourth-order roots to yield 10 root systems per buttress root or 30 fine-root system samples (10 root systems  $\times$  3 buttress roots) per tree. The selected fine-root systems were placed in clean trays with water and washed gently with soft-bristled brushes and running water to clean debris from the root system.

#### Nonwoody root vitality and mycorrhizal types

A nonwoody root system with the most branches (estimated visually) was removed from each of the 10 fine woody branches and examined with a dissecting microscope (10–45 $\times$ ) to count total number of living branches, living tips (mycorrhizal and nonmycorrhizal separately), and dead tips per system. Turgidity, texture, and color were used to distinguish living from dead root tips. The number of living root tips per branch and the percentage of dead and mycorrhizal tips were calculated for each of the 10 nonwoody roots; an average was generated for each buttress root on each tree. Mycorrhizal tips were classified according to morphological type using criteria described in Glenn et al. (1991) and Wargo et al. (1993). We observed nine morphotypes, seven of which had been previously described by Glenn et al. (1991) and Wargo et al. (1993) and two new ones, Type 8: creamy tan translucent mantle (swollen, translucent with darker inner core characteristic of roots growing in decayed wood) and Type 9: golden tan mantle (distinct with fuzzy surface). The frequency of each morphotype on

each tree was determined by the number of times that it occurred on the nonwoody root systems in 30 samples.

#### Nonwoody root pathology

During the 1993 processing of root samples, three additional nonwoody root systems selected randomly from each collection from a buttress root were used to isolate potential fine-root pathogens. Dead root tips or small sections of putative necrotic roots were removed from the root system with a scalpel and tweezers, surface sterilized in 1% NaOCl for 10 min, rinsed three times with sterile distilled water, blotted on sterilized toweling, and placed in Petri dishes on 2% malt agar with rose bengal ( $35 \text{ mg} \cdot \text{L}^{-1}$ ), pentachloronitrobenzene ( $100 \text{ mg} \cdot \text{L}^{-1}$ ), or streptomycin ( $100 \text{ mg} \cdot \text{L}^{-1}$ ) supplements. A maximum of six tips or root pieces were placed on each plate per medium and incubated in the dark at  $\sim 22^\circ\text{C}$ . Incubated root tips and pieces were examined after 1 week for fungal growth; fungi with similar morphological characteristics were recorded and subcultured for possible identification.

#### Root chemistry

##### Processing

The remaining roots were cleaned of soil and plant debris and separated (using a handheld sliding micrometer) into three diameter classes: <1 mm (nonwoody), 1 to <2 mm (fine woody), and 2 to <5 mm (coarse woody). Roots were combined by diameter class to yield one sample for each size class per tree, rinsed thoroughly with deionized water, and dried at  $70^\circ\text{C}$ . Prior to analysis, roots were ground to a fine powder with a Kleco pulverizer (Kinetic Laboratory Equipment Co., Visalia, Calif.) and then analyzed for carbohydrates, phenols, and C, N, Al, Ca, Fe, K, Mg, Mn, and P concentrations by diameter class.

### Carbohydrates and phenols

Soluble carbohydrates and phenolic constituents were extracted from ~0.100 g of ground tissue with 8 mL of 50% methanol in centrifuge tubes in an 80°C water bath for 60 min. Tubes were shaken after 30 min and returned to the water bath for an additional 30 min. The tubes were shaken again and centrifuged for 20 min at 15 000 rpm. Supernatants were saved for carbohydrate and phenol analyses; the pellet was saved for starch analysis.

Total soluble carbohydrate ( $\text{mg}\cdot\text{g}^{-1}$  dry mass) was determined colorimetrically using the phenol – sulfuric acid method (Dubois et al. 1956). Total phenols ( $\text{mg}\cdot\text{g}^{-1}$  dry mass) in the extracts were determined colorimetrically using the Folin–Denis test (Rosenblatt and Peluso 1941). Total oxidized phenol was estimated by absorbance of the methanol extract (diluted 1:10) at 450 nm and expressed as absorbance units per gram dry mass of extracted tissue (Walter and Purcell 1980). Starch concentration ( $\text{mg}\cdot\text{g}^{-1}$  dry mass) in the residual root tissue was determined by gelatinization and enzyme digestion of starch to glucose and by measurement of glucose with glucose oxidase – peroxidase reagent (Wargo et al. 1993). For all organic constituent analyses, a composite sample of red spruce root tissue was used as a standard to maintain consistency of extraction and analyses.

### Element analyses

Subsamples of roots were analyzed for C and N with a LECO CHN-600 elemental combustion analyzer (Bremner 1996). Commercial rye flour was used as a standard (Alpha Resources AR-2020). Readings were recorded as percent C or N per gram dry mass of root tissue and were used to calculate C/N ratios. We also calculated ratios of N with total soluble sugars, total carbohydrate (total soluble sugars plus starch), and total phenol.

Aluminum, Ca, Fe, K, Mg, Mn, and P in roots were extracted with a sulfuric acid – peroxide digestion as outlined in Parkinson and Allen (1975). Concentrations of these elements in the digests were measured with a Perkin Elmer Optima 3000 inductively coupled plasma spectrophotometer. Concentrations of Al, Ca, Mg, and Mn were used to calculate molar ratios for Al/Ca and Mn/Mg. Ground pine needle tissue (NIST) was used as a standard to maintain consistency of extraction and analysis.

### Vitality of woody roots

#### Response to wounding

The response of small-diameter woody roots to wounding was measured to assess vitality of woody roots. During the 1993 sampling, three additional buttress roots on each tree were chosen and a third- or fourth-order healthy woody branch was excavated on each buttress root. The distal end of each root was clipped off with pruning shears at 5–20 cm from the juncture with its “parent” root. The length of the clipped root depended on where the root branched into smaller (higher order) branches. Clipped roots ranged from 0.5 to 1.5 cm in diameter at their proximal ends. Clipped roots were tagged with plastic twist-ties, recovered with soil, and marked with surveyor flags for relocation and examination the following year.

Clipped roots were removed at the juncture with their parent root in September 1994, about 15 months after wounding. If discoloration in the wood continued into the parent root, this root also was sampled proximally in 15-cm sections (hinged cuts were used to maintain proximal-distal relationships) until discoloration ceased or five sections were cut. All root sections were placed in plastic bags on ice in coolers for transport to the laboratory where they were maintained at ~3°C until processed (maximum of 30 days).

In the laboratory, clipped roots were examined and living and dead branches (determined by texture and color after scraping into the bark and wood with a scalpel) within 2.5 cm of the cut end were counted. Branches that had formed from or near the callus tissue at the cut end were counted separately. The root section was then washed free of soil and the extent of discoloration in the bark and wood from the cut end was measured; if the whole section was discolored, the next section in a distal to proximal relationship to or of the parent root was evaluated until discoloration ceased or all sections were examined.

#### Response to root and butt rot fungi

Vitality of woody roots was also assessed by evaluating the response of small-diameter woody roots to wounding and challenge by root and butt rot fungi known to colonize red spruce (Rizzo and Harrington 1988). Four root and butt rot fungi were used: *Armillaria ostoyae* (Romagnesi) Herink, *Perenniporia subacida* (Pk) Donk., *Resinicum bicolor* (Alb., and Schw. Fr.) Parm., and *Scytonostroma galactinum* (Fr.) Donk. Inoculum was prepared by growing all four fungi on stem sections of red spruce. Stem sections (~5 cm long and ~1–2 cm in diameter) were placed in wide-mouth plastic jars, packed with moistened, presterilized peat moss (1 h at 120 pounds per square inch (psi) (1 psi = 6.895 kPa)), and autoclaved for 30 min at 120 psi. After cooling, stem sections were inoculated by placing a 9-cm-diameter disc of malt agar with multiple 2-week-old colonies of a fungus on top of the stem sections. Inoculated stem sections were incubated for at least 90 days to ensure establishment of the fungus in the bark and wood of the stem sections; sterile water was added as necessary to maintain adequate moisture.

In October 1993, nine trees (three three-tree clusters) that had not been used for root sampling were chosen for inoculation at each of the nine locations. Five buttress roots on each tree were selected at random for inoculation: four buttress roots were chosen for inoculation and randomly assigned one of the four fungi and the fifth root was the uninoculated control. Two small-diameter (0.4–0.8 cm) healthy appearing woody roots (third or fourth order) were carefully exposed on each buttress root system by excavation with handtools. One root was wounded with a scalpel by scraping the side of the root down to the cream-colored inner bark/cambial layer; the other root was not wounded. A colonized stem section (inoculum block) of one of the fungi was attached with two plastic twist-ties to the side of each root at the point of wounding on the wounded root and randomly on the unwounded root. Roots were re-covered with soil and marked with surveyor flags. The small-diameter roots on the control (uninoculated) buttress root were treated

similarly but sterile, uninoculated stem sections of red spruce were attached.

Inoculated and control roots were harvested in September 1994, ~1 year after inoculation. Roots were located and cut at points 15 cm proximally and distally from the inoculum; the inoculum block was left attached to the root. Root sections were placed in plastic bags on ice in coolers and returned to the laboratory where they were stored at ~3°C until processed (maximum of 60 days).

In the laboratory, roots were removed from their bags and the center position of the inoculum block was marked on the root. The inoculum block was removed and its status recorded as hard, soft, or mushy. The status of mycelium of each fungus on and in the inoculum block was assessed as present or not obvious. A random sample of three inoculum blocks of each fungus per site was processed for re-isolation of the fungus. Inoculum blocks were washed, brushed clean of soil debris in a mild detergent, and rinsed. They were sterilized by immersion in a 1% NaOCl (Al) in 10% ethanol solution for 5 min, blotted, rinsed in sterile water, blotted again, dipped in 95% ethanol, and flamed. Blocks were then split and tissues from the inner bark, outer wood, and inner wood were placed on an agar medium selective for basidiomycetes (15 g malt extract·L<sup>-1</sup>, 2 mg benomyl·L<sup>-1</sup>, 2 mg dichloran·L<sup>-1</sup>, and 100 mg streptomycin·L<sup>-1</sup>) (Worrall 1991). Plates were examined for evidence of fungal growth after 2 weeks of incubation in the dark at room temperature (~22°C). Isolates from the inoculum blocks were compared with a culture of the original fungus in the block.

The inoculated roots were washed, brushed clean of soil debris, and examined for evidence of infection and colonization. Pitching at the wound or inoculation site and evidence of fungus mycelium on the root surface at the inoculation site were recorded as present or absent. We measured the extent of external and internal (cambial zone) mycelial development, proximal and distal to the inoculation site, and the extent of proximal and distal discoloration in the bark, outer wood, and inner wood. Where there was evidence of colonization, root tissues were processed as described for the inoculum blocks to re-isolate and identify the inoculated fungus.

### Statistical analyses

Data on root vitality were analyzed by standard univariate ANOVA methods (SAS Institute Inc. 1990) with site as the independent variable and numbers of living root tips, percent dead root tips, percent mycorrhizal root tips, numbers of branches and length of discoloration on clipped roots, and length of colonization or discoloration on inoculated roots as the dependent variables. Data were analyzed separately by year for those variables measured for 2 years because measurements were taken for different trees each year. Data on root chemistry were analyzed with multivariate ANOVA procedures; the three diameter classes of roots, separately by year, were the multivariate observations for each tree. Where data were not normally distributed, a variety of transformations were performed; however, if transformations did not improve the distribution of the data, the untransformed data were used. On two sites, missing data for some of the organic chemistry measurements made it impossible to make comparisons across root diameter classes. This was cor-

rected by using estimated values for the missing data (Yates 1933). Differences among means were tested for significance ( $p \leq 0.05$ ) by the Bonferroni procedure. Spearman and Pearson correlation tests were performed on all dependent variable data and the independent variables of soil and soil solution chemistry (both forest floor and mineral soil) (David and Lawrence 1996; Lawrence et al. 1995) on a site basis. Spearman and Pearson correlation tests were performed on all dependent variables to determine relationships among them, particularly among root vitality variables and organic chemistry and element concentrations. Spearman correlations were used because some data were neither continuous variables nor distributed normally.

All data were plotted against the gradient of the ratio of the exchangeable Al to exchangeable Ca concentrations (cmol<sub>c</sub>·kg<sup>-1</sup>) of the Oa horizon to determine if there were relationships among root vitality variables and this Al/Ca ratio gradient. Although the Al/Ca ratio of soil solution has been proposed as an indicator of potential stress to forests (Cronan and Grigal 1995), exchangeable rather than dissolved concentrations were used in this study to avoid the complications of collecting and speciating dissolved Al species in the forest floor. Concentrations of dissolved Al in the forest floor can vary with the collection method (Lawrence and David 1996) and are influenced by high concentrations of dissolved organic C that readily complex with Al. Most of the experiments that led to the development of the Al/Ca ratio as a stress indicator, however, were conducted with seedlings under controlled laboratory conditions with minimal dissolved organic C concentrations (Cronan and Grigal 1995). Stress thresholds developed from these experiments, therefore, are not directly transferable to natural forest conditions. The procedure for measuring exchangeable concentrations of Al and Ca, although operational, is relatively straightforward and was found by David and Lawrence (1996) to be directly related to the ratio of dissolved concentrations. For these reasons, exchangeable concentrations of Al and Ca were chosen over dissolved concentrations to evaluate possible relationships with root mortality.

The Al/Ca ratio in the Oa horizon was measured at the same time that roots were collected for vitality and chemical analyses (David and Lawrence 1996). Chemical concentrations of the Oe horizon were not measured in this study.

Because the majority of the roots were collected from the Oe horizon, we estimated the Al/Ca concentrations in the Oe from data collected 1 year after the study from three of the 11 sites, Big Moose, Groton, and Hubbard Brook. Regression analysis yielded the following equations that relate exchangeable Al and Ca concentrations in the Oa horizon to those in the Oe horizon:

$$\text{Al: Oe} = 0.345(\text{Oa}) - 0.57; P = 0.008, n = 14,$$

$$R^2 = 0.43$$

$$\text{Ca: Oe} = 0.702(\text{Oa}) + 8.57; P = 0.003, n = 14,$$

$$R^2 = 0.65$$

where Oe is the Oe horizon concentration of acid-extractable Al or Ca (cmol<sub>c</sub>·kg<sup>-1</sup>) and Oa is the Oa horizon concentration of acid-extractable Al or Ca (cmol<sub>c</sub>·kg<sup>-1</sup>).

The Al/Ca ratios for the Oe horizon were calculated for each site from the estimated Al and Ca concentrations, and the root vitality variables were plotted against the gradient for the Oe Al/Ca ratio of the sites to determine whether there were relationships among the root vitality variables and this gradient. Where relationships were indicated, regression analyses were employed to determine whether the relationships were significant.

## Results

### Root vitality measurements

The number of root tips per nonwoody branch differed significantly among sites in both 1993 and 1994, but there was no relationship or trend among the site differences and the Al/Ca gradient in the Oe or Oa horizon (Fig. 1A). The 1994 root samples had more tips per branch than the 1993 samples (Fig. 1A). In 1993, Big Moose, Whiteface Mountain, Groton, and Cone Pond had the fewest root tips per branch; in 1994, Groton, Hubbard Brook, and Whiteface Mountain had the fewest root tips per branch, although these values were two or more times greater than in 1993. The Kossuth site had the most root tips per branch for both sample years (Fig. 1A).

The percentage of root tips that were mycorrhizal also differed significantly among sites in both 1993 and 1994 but not between years within a site (Fig. 1B). Percentages of mycorrhizae ranged from 25 to 55 in 1993 and from 29 to 46 in 1994. Among all sites, Whiteface Mountain had the lowest percent mycorrhizae in both years. This site was at the highest elevation (950 m), but there was no consistent trend of percent mycorrhizae with elevation (data not presented). There was no significant linear relationship between percent mycorrhizae and the soil Al/Ca gradient, but four of the five sites with the higher Al/Ca ratios had lower percentages of mycorrhizae (Fig. 1B).

There were significant differences in percent dead root tips among sites in 1993 and 1994 (Fig. 1C). Percentages were higher (two times or more) in 1993 than in 1994 for eight of the nine sites measured in both years; root tip mortality at Howland was higher in 1994. There was no relationship between percent dead root tips and either of the Al/Ca ratio gradients.

Nine mycorrhizal morphotypes were observed on nonwoody roots (Table 2). Type 9 was observed only in 1994 but was recorded at all sites, some at relatively high frequencies (Table 2). Types 2, 3, 6, 7, and 8 were infrequent or absent at some sites, while Types 1, 4, 5, and 9 were more frequent at all sites. Major shifts or changes in frequency of individual morphotypes were not significantly related to the Al/Ca ratio gradients (Table 2). However, there was a numerical trend for Types 1, 4, and 5 to change with the Al/Ca gradient. Except at Cone Pond, Type 1 was more frequent, while Types 4 and 5 were less frequent at the higher elevation sites (Hubbard Brook and Whiteface Mountain) or sites with higher soil Al/Ca ratios (above 0.8 in the Oa horizon and above 0.12 in the Oe horizon) (Table 2). The total number of occurrences of all morphotypes generally was similar among sites and greater in 1994 than in 1993. Two of the Maine sites, Kossuth and Bear Brook (unfertilized), had high counts in both years. There was no relationship between total

frequency of morphotype occurrence and either Al/Ca gradient (Table 2).

### Tree condition

There was no general relationship between crown condition and the Al/Ca ratio gradients in 1993 or 1994 (data not presented). Trees at Crawford Notch and Big Moose had the poorest crown conditions in both years (1.2 and 2.0 in 1993 and 1.5 and 1.7 in 1994, respectively, versus 1.0 for the other nine sites). There were no significant relationships of crown condition with root vitality or chemistry variables (data not presented).

### Root chemistry: carbohydrates, N, and phenols

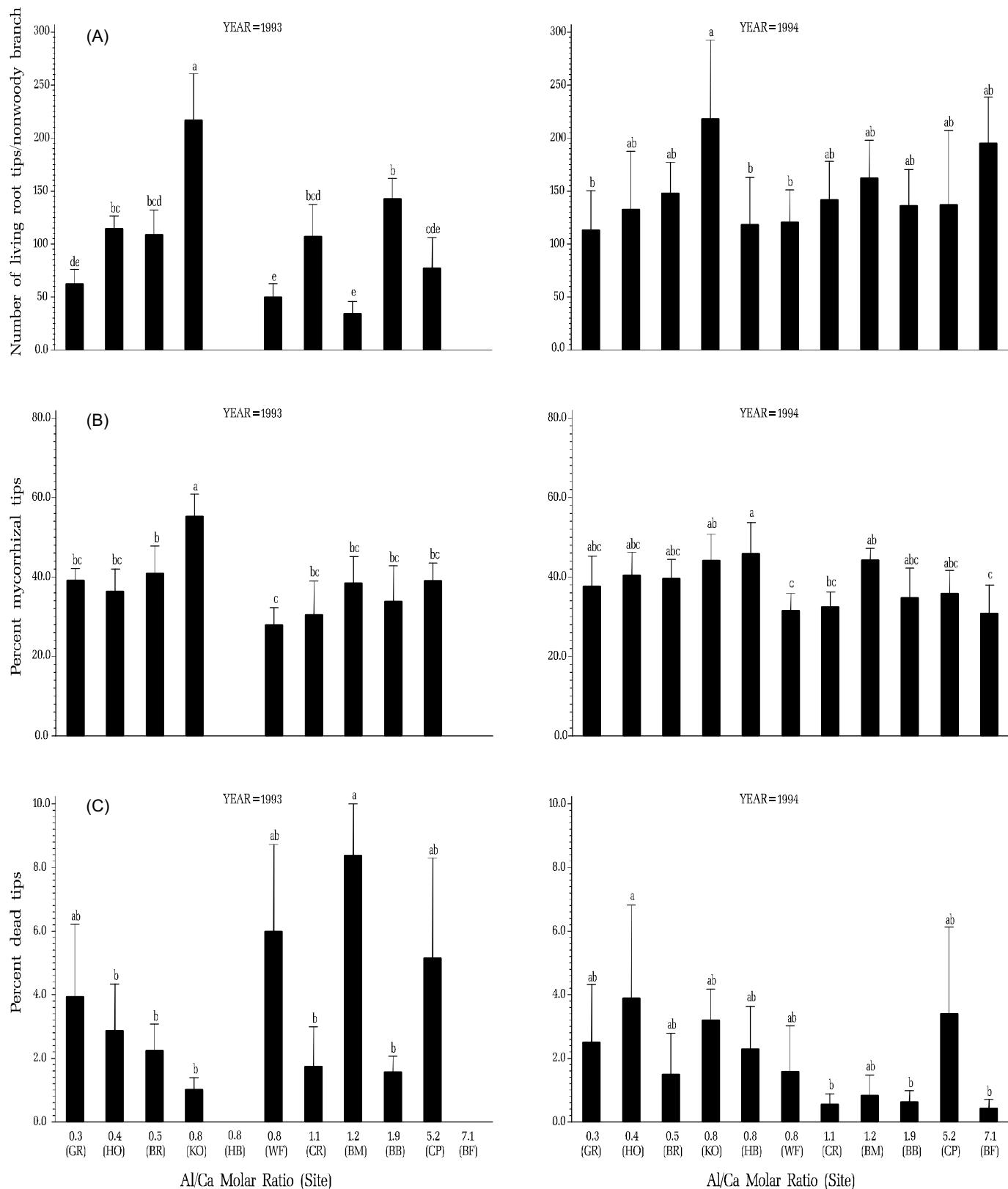
There were significant differences in starch concentrations among the sites, but the differences were related to the sampling time; there was a trend for trees in the earlier sampled stands to have higher starch levels than trees in stands sampled later (Fig. 2). In 1993, we sampled Maine first, New Hampshire second, and Vermont and New York last. In 1994, we reversed the order to compensate for differences in phenology. Differences among stands related to time of sampling were less in 1994, although the earlier harvested samples still had higher concentrations of starch. Starch was significantly lower in nonwoody roots than in woody roots at all sites in both years (Fig. 2). Differences between years within a site were mostly a function of time of harvest. There was no relationship between starch concentration and the Al/Ca ratio gradients.

Concentrations of total soluble carbohydrates did not differ among the sites but within sites were significantly lower in nonwoody than in woody roots in 1993 and generally lower in 1994 (data not presented). Concentrations in nonwoody roots ranged from 58 to 83 mg·g<sup>-1</sup> in 1993 and from 55 to 90 mg·g<sup>-1</sup> in 1994. In woody roots, concentrations ranged from 80 to 105 mg·g<sup>-1</sup> in 1993 and from 50 to 102 mg·g<sup>-1</sup> in 1994. There was no relationship between soluble carbohydrates and either of the Al/Ca ratio gradients (data not presented).

Percent C and N and C/N ratios differed significantly among sites in both years. Differences in N were large (Table 3) compared with C, which varied little (data not presented); percent C ranged from 47.2 to 51.4. Except for Cone Pond, there was a trend for N to increase with an increase in elevation or Al/Ca ratio of the Oa horizon (Table 3). Nonwoody roots had higher concentrations of N than either fine or coarse woody roots (Table 3). The C/N ratio decreased in proportion to the increase in N; among stands, the ratio ranged from 39.2 to 76.0 for nonwoody roots, from 59.8 to 103.0 for fine woody roots, and from 74.5 to 127.9 for coarse woody roots. There also was a trend for the total soluble sugars and total carbohydrates (total soluble sugars plus starch) to N ratios (data not presented) to decrease as the Al/Ca ratio increased; this trend was more evident in nonwoody roots.

There were significant differences in total phenol concentrations among the sites for all three root types, although the differences were not consistent, nor were they related to the Al/Ca ratio gradients (data not presented). Among sites, total phenol concentrations in 1993 generally were significantly lower in nonwoody roots (range 71.6–125.3 mg·g<sup>-1</sup>) than in

**Fig. 1.** Root vitality measurements of nonwoody roots of mature red spruce from nine sites in 1993 and 11 sites in 1994 across an Al/Ca ratio gradient in the northeastern United States. (A) Average number of root tips per nonwoody branch; (B) percent mycorrhizae; (C) percent dead root tips. Sites arranged from a forest floor (Oa horizon) Al/Ca ratio of 0.3–7.1 (applies to all figures). Values with different letters are significantly different at  $p = 0.05$  determined by a Bonferroni test.



**Table 2.** Occurrence and frequency of mycorrhizal morphotypes on nonwoody root systems of mature red spruce from nine sites in northeastern United States.

| Site <sup>a</sup> | Oa Al/Ca ratio | Mycorrhizal morphotype <sup>b</sup> |        |      |        |      |       |         |        |
|-------------------|----------------|-------------------------------------|--------|------|--------|------|-------|---------|--------|
|                   |                | 1*                                  |        | 2    |        | 3    |       | 4*      |        |
|                   |                | 1993                                | 1994   | 1993 | 1994   | 1993 | 1994  | 1993    | 1994   |
| GR                | 0.3            | 40de                                | 49d    | 0b   | 1d     | 3b   | 4bc   | 235abc  | 248a   |
| HO                | 0.4            | 19e                                 | 56d    | 1b   | 11dc   | 12b  | 8abc  | 254ab   | 224a   |
| BR                | 0.8            | 63cde                               | 89bcd  | 5b   | 40abc  | 13b  | 6abc  | 250abc  | 249a   |
| KO                | 0.8            | 44de                                | 69cd   | 21ab | 17bcd  | 31b  | 7abc  | 258a    | 241a   |
| HB <sup>c</sup>   | 0.8            | —                                   | 140ab  | —    | 5cd    | —    | 3bc   | —       | 216ab  |
| WF                | 0.8            | 186a                                | 82bcd  | 32a  | 31abcd | 12b  | 7abc  | 111e    | 144cd  |
| CR                | 1.1            | 141ab                               | 109bcd | 9ab  | 27bcd  | 0c   | 2bc   | 185cd   | 185abc |
| BM                | 1.2            | 99bcd                               | 113bcd | 2b   | 48ab   | 8b   | 25a   | 187bcd  | 200abc |
| BB                | 1.9            | 110bc                               | 189a   | 13ab | 37abc  | 115a | 10abc | 165de   | 147bcd |
| CP                | 5.2            | 58cde                               | 46d    | 2b   | 16bcd  | 2b   | 0c    | 232abcd | 211abc |
| BF <sup>c</sup>   | 7.1            | —                                   | 137abc | —    | 66a    | —    | 22ab  | —       | 101d   |

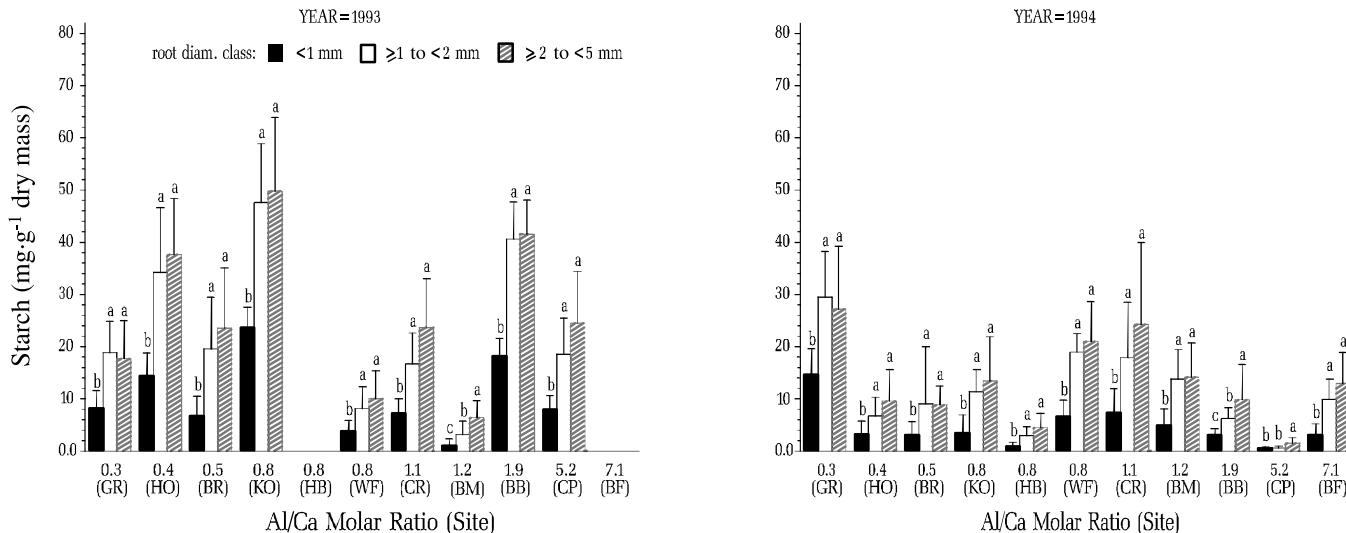
Note: Number of occurrences on nonwoody root systems out of a possible 270 per site per year (10 fine nonwoody root systems × 3 buttress roots/

<sup>a</sup>Sites are arranged in order of increasing forest floor (Oa horizon) Al/Ca ratio (Lawrence et al. 1995). Oa Al/Ca ratios for GR through BF are 0.04,

<sup>b</sup>Morphological types (1–7) as described in Wargo et al. (1993). Types 8 and 9 are described in the text. An asterisk indicates morphotypes that

<sup>c</sup>Stands not sampled in 1993.

**Fig. 2.** Average root starch concentrations for three diameter classes of roots of mature red spruce trees from nine sites in 1993 and 11 sites in 1994 across an increasing forest floor Al/Ca ratio gradient in the northeastern United States. Root diameter classes were <1 mm (nonwoody), 1 to <2 mm (fine woody), and 2 to <5 mm (coarse woody). Values with different letters are significantly different at  $p = 0.05$  determined by a Bonferroni test.



fine ( $110.9\text{--}183.3 \text{ mg}\cdot\text{g}^{-1}$ ) and coarse woody roots ( $99.9\text{--}167.6 \text{ mg}\cdot\text{g}^{-1}$ ). In 1994, total phenol concentrations in nonwoody ( $81.2\text{--}155.4 \text{ mg}\cdot\text{g}^{-1}$ ) and fine woody roots ( $105.1\text{--}173.1 \text{ mg}\cdot\text{g}^{-1}$ ) were similar and generally higher than in coarse woody roots ( $74.2\text{--}137.5 \text{ mg}\cdot\text{g}^{-1}$ ). Concentrations of oxidized phenol showed similar patterns and were proportional to total phenol concentrations (data not presented). There was no relationship between oxidized phenols or carbohydrate/phenol ratios (data not presented) and the Al/Ca ratio gradients.

#### Nonwoody root pathology

Few fungi were isolated consistently from putative dead root tips or sections of nonwoody roots. Most isolates were

dark-pigmented sterile fungi. Several of these isolates were characterized as *P. fortinii*-like by RFLP analysis of PCR-amplified rDNA (see Harney 1994; Harney et al. 1995). Neither numbers of isolates nor morphological types were significantly different among sites, nor were they related to either of the Al/Ca ratio gradients (data not presented).

#### Response of roots to wounding

There were no significant site differences in response of clipped roots to wounding. Discoloration in the bark and wood (outer and inner) differed among sites, but there was no consistent relationship with the Al/Ca ratio gradients. Discoloration in the inner wood was greatest in length. Proximal discoloration in the bark, outer wood, and inner wood

1993 and 11 sites in 1994 across an increasing forest floor (Oa horizon) Al/Ca ratio gradient in red spruce dominated stands in the

| 5*    | 6     | 7    | 8    | 9    | Total |      |      |      |       |
|-------|-------|------|------|------|-------|------|------|------|-------|
| 1993  | 1994  | 1993 | 1994 | 1993 | 1994  | 1993 | 1994 | 1993 | 1994  |
| 36abc | 60ab  | 12b  | 13ab | 0b   | 0a    | 12a  | 1ab  | 0a   | 19d   |
| 43ab  | 80a   | 27ab | 24ab | 20ab | 0a    | 8a   | 10a  | 0a   | 24cd  |
| 12bcd | 22bcd | 25ab | 20ab | 1ab  | 0a    | 2a   | 0b   | 0a   | 49bcd |
| 48a   | 46abc | 47a  | 13ab | 22a  | 1a    | 4a   | 0b   | 0a   | 95ab  |
| —     | 17cd  | —    | 7b   | —    | 1a    | —    | 0b   | —    | 26cd  |
| 4cd   | 8cd   | 16ab | 4b   | 4ab  | 2a    | 0a   | 0b   | 0a   | 109a  |
| 1d    | 8cd   | 18ab | 12ab | 2ab  | 0a    | 0a   | 0b   | 0a   | 82abc |
| 1d    | 6cd   | 12b  | 36a  | 5ab  | 2a    | 3a   | 0b   | 0a   | 84ab  |
| 0d    | 24bcd | 2b   | 9ab  | 10ab | 0a    | 0a   | 0b   | 0a   | 11d   |
| 38ab  | 27bcd | 9b   | 4b   | 4ab  | 0a    | 0a   | 0b   | 0a   | 20d   |
| —     | 1d    | —    | 4b   | —    | 0a    | —    | 1ab  | —    | 40bcd |
|       |       |      |      |      |       |      |      |      | 372a  |

tree × 9 trees per plot). Numbers within columns followed by different letters are significantly different at  $p = 0.05$  determined by a Bonferroni test. 0.06, 0.11, 0.09, 0.12, 0.09, 0.15, 0.15, 0.23, 0.52, and 0.52. See Table 1 for site definitions. changed with the Al/Ca ratio gradient.

**Table 3.** Mean percent N in three diameter classes of roots of red spruce from nine sites in 1993 and 11 sites in 1994 across a forest floor (Oa horizon) Al/Ca ratio gradient in red spruce dominated stands in the northeastern United States.

| Site <sup>a</sup> | Oa Al/Ca ratio | Root type and diameter class |             | Fine woody, ≥1 to <2 mm |             | Coarse woody, ≥2 to <5 mm |             |
|-------------------|----------------|------------------------------|-------------|-------------------------|-------------|---------------------------|-------------|
|                   |                | Nonwoody, <1 mm              |             | 1993                    | 1994        | 1993                      | 1994        |
|                   |                | 1993                         | 1994        | 1993                    | 1994        | 1993                      | 1994        |
| GR                | 0.3            | 0.91±0.04cd                  | 0.76±0.04de | 0.53±0.02c              | 0.48±0.02f  | 0.42±0.02d                | 0.38±0.02e  |
| HO                | 0.4            | 0.80±0.03d                   | 0.67±0.02e  | 0.52±0.01c              | 0.50±0.01ef | 0.42±0.02d                | 0.43±0.02d  |
| BR                | 0.5            | 0.98±0.06c                   | 0.81±0.03d  | 0.63±0.03b              | 0.61±0.02cd | 0.50±0.02bc               | 0.48±0.01c  |
| KO                | 0.8            | 1.10±0.12bc                  | 0.72±0.03e  | 0.58±0.02b              | 0.53±0.01de | 0.47±0.02cd               | 0.43±0.01d  |
| HB <sup>b</sup>   | 0.8            | —                            | 0.91±0.01c  | —                       | 0.63±0.01c  | —                         | 0.51±0.02c  |
| WF                | 0.8            | 1.13±0.06b                   | 1.13±0.03b  | 0.73±0.04a              | 0.71±0.01b  | 0.55±0.02ab               | 0.53±0.01bc |
| CR                | 1.1            | 1.10±0.06b                   | 1.15±0.07b  | 0.76±0.02a              | 0.66±0.02bc | 0.55±0.02ab               | 0.52±0.04bc |
| BM                | 1.2            | 0.95±0.02c                   | 1.19±0.05a  | 0.70±0.02ab             | 0.67±0.02bc | 0.50±0.02bc               | 0.54±0.01bc |
| BB                | 1.9            | 1.29±0.04a                   | 1.07±0.03b  | 0.76±0.03a              | 0.72±0.03b  | 0.59±0.09a                | 0.55±0.01bc |
| CP                | 5.2            | 1.06±0.06bc                  | 0.76±0.02de | 0.64±0.02b              | 0.57±0.01d  | 0.48±0.02c                | 0.47±0.01cd |
| BF <sup>b</sup>   | 7.1            | —                            | 1.27±0.04a  | —                       | 0.84±0.04a  | —                         | 0.67±0.03a  |

**Note:** Numbers followed by different lower case letters are significantly different when comparing within a column by year and root diameter class.

<sup>a</sup>Sites are arranged in order of increasing forest floor (Oa horizon) Al/Ca ratio (Lawrence et al. 1995). Oe Al/Ca ratios for GR through BF are 0.04, 0.06, 0.11, 0.09, 0.12, 0.09, 0.15, 0.15, 0.23, 0.52, and 0.52. See Table 1 for site definitions.

<sup>b</sup>Stands not sampled in 1993.

ranged from 1.3 to 4.9, from 1.0 to 5.1, and from 4.7 to 7.4 cm, respectively. Results were similar for numbers of living and dead branches within 2.5 cm of the cut end and adventitious branches at the cut end. Adventitious branching on wounded roots was poorest at Big Moose, where none were formed; trees there also had the poorest crown conditions.

#### Inoculation with root and butt rot fungi

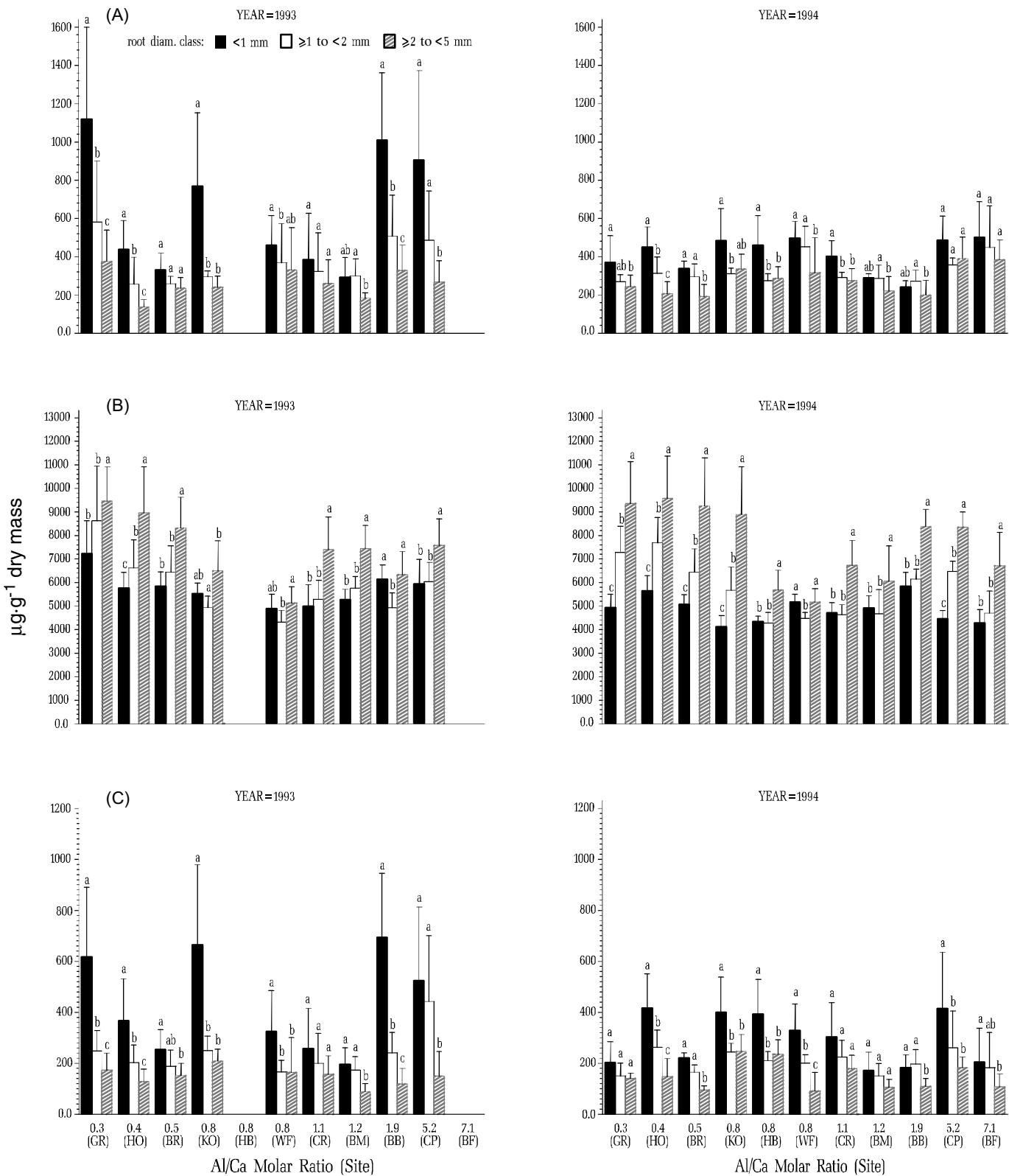
Infection of inoculated small-diameter woody roots by root and butt rot fungi ranged from 8% at Cone Pond to 30% at Bear Brook, but there was no relationship with the Al/Ca ratio gradients (data not presented). Of a possible 608 inoculated roots, 126 were infected. Most infections (78%) occurred on the wounded roots and colonization was primarily

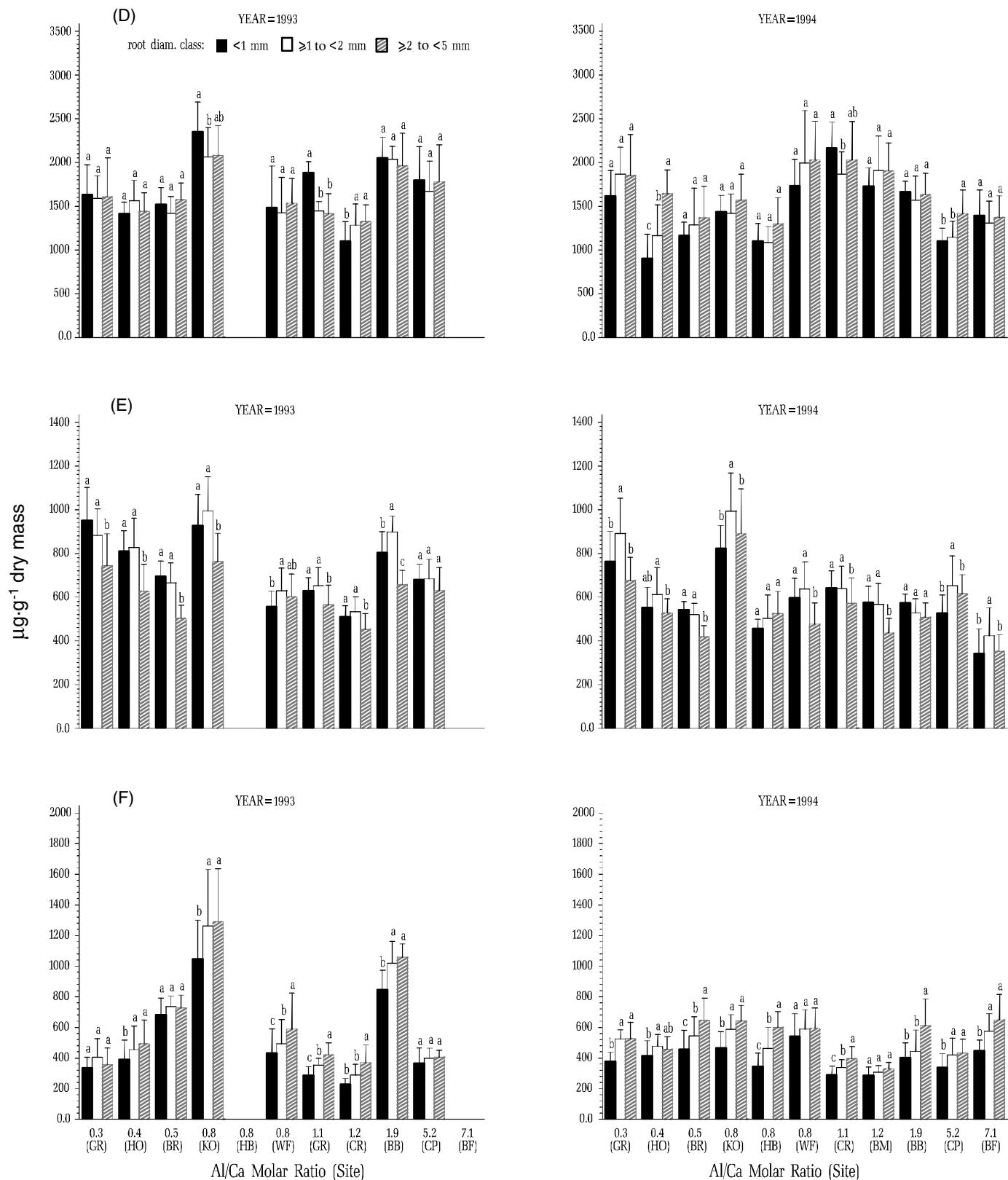
in bark tissues. Of these infected roots, 24% were colonized by *S. galactinum*, 16% by *A. ostoyae*, 31% by *P. subacida*, and 39% by *R. bicolor*. All wounded roots at all sites, whether inoculated or not, had discoloration in both bark and outerwood. All fungi were re-isolated from their inoculum blocks but not with equal frequency. Frequencies of isolation for *A. ostoyae*, *S. galactinum*, *P. subacida*, and *R. bicolor* were 18, 39, 48, and 48%, respectively.

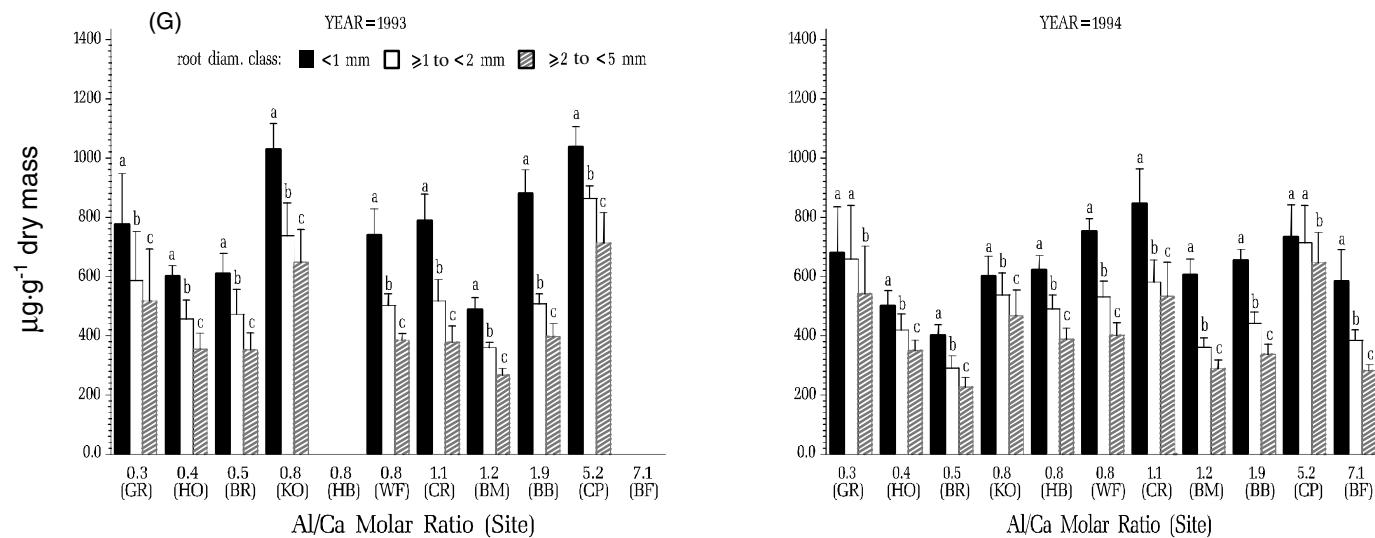
#### Elemental chemistry

There were significant differences in root element concentrations and molar ratios within each root diameter class among sites, but there was no significant relationship between root element concentrations (Fig. 3) and Al/Ca and Mn/Mg molar ratios (data not presented) and the forest floor

**Fig. 3.** Mean concentrations of elements for three diameter classes of roots from mature red spruce trees from nine sites in 1993 and 11 sites in 1994 across an increasing forest floor A/Ca ratio gradient in the northeastern United States. (A) Al; (B) Ca; (C) Fe; (D) K; (E) Mg; (F) Mn; (G) P. Root diameter classes were <1 mm (nonwoody), 1 to <2 mm (fine woody), and 2 to <5 mm (coarse woody). Values with different letters are significantly different at  $p = 0.05$  determined by a Bonferroni test.



**Fig. 3 (continued).**

**Fig. 3 (concluded).**

Al/Ca ratio gradients. For some sites, concentrations of elements in 1993 and 1994 were similar (within 10% of each other); for others, concentrations were substantially higher in 1993 depending on root diameter class. Variability among sites also was greater for 1993 element data (Fig. 3). Concentrations of Al, Fe, K, Mg, and P generally were higher in 1993 than in 1994 at Groton, Kossuth, Bear Brook, and Cone Pond (Figs. 3A and 3C–3G).

Differences in element concentrations among the three root classes were significant for some sites (Fig. 3). Concentrations of Al, Fe, Mg, and P generally were higher in nonwoody roots than in fine and coarse woody roots (Figs. 3A, 3C, 3E, and 3G). Phosphorus showed the most consistent relationship with root diameter class; concentrations decreased progressively from a high in nonwoody roots to a low in coarse woody roots (Fig. 3G). Concentrations of Ca and Mn tended to be higher in coarse woody roots, while concentrations of K tended to be similar for all root diameter classes (Figs. 3B, 3D, and 3F). There was a trend for Ca and Mn to increase progressively from a low in nonwoody roots to a high in coarse woody roots (Figs. 3B and 3F).

### Correlations of root vitality variables with root chemistry

There were few consistent significant relationships among root vitality variables and root chemistry variables on a site basis. In 1993, the number of living root tips per branch was correlated positively with Mn ( $r = 0.85$ ,  $0.83$ , and  $0.73$ ) and the Mn/Mg ratio ( $r = 0.70$ ,  $0.65$ , and  $0.62$ ) in nonwoody, fine, and coarse woody roots, respectively. In 1994, the number of living root tips was not significantly correlated with any element in the roots. In 1993, percent dead root tips in 1993 was negatively correlated with K, Mg, and Mn in nonwoody ( $r = -0.87$ ,  $-0.63$ , and  $-0.70$ , respectively) and fine woody roots ( $r = -0.67$ ,  $-0.67$ , and  $-0.64$ , respectively) but only with Mn in coarse woody roots ( $r = -0.68$ ); in 1994, there were no consistent correlations with any elements among root size classes. In 1993, percent mycorrhizae was negatively correlated with Al in fine ( $r = -0.60$ ) and coarse woody roots ( $r = -0.72$ ) and with the Al/Ca molar ra-

tio in all three root classes ( $r = -0.73$ ,  $-0.75$ , and  $-0.80$ , respectively); in 1994, there were no significant correlations of percent mycorrhizae with any root element by root size.

There were significant and consistent correlations of root vitality measures with organic chemistry variables but not between years. In 1993, the number of living root tips per branch was correlated positively with starch concentration for all root classes ( $r = 0.77$ ,  $0.92$ , and  $0.73$  for nonwoody, fine woody, and coarse woody, respectively); in 1994, the number of living root tips was not significantly correlated with any chemistry variable. In 1993, percent dead root tips was negatively correlated with starch for all root classes ( $r = -0.73$ ,  $-0.75$ , and  $-0.67$ , respectively). In 1994, percent dead root tips was highly correlated with N ( $r = -0.96$ ,  $-0.87$ , and  $-0.90$ , respectively), the C/N ratio ( $r = 0.93$ ,  $0.86$ , and  $0.90$ , respectively), and oxidized phenols ( $r = 0.74$ ,  $0.73$ , and  $0.67$ , respectively) for all root classes. In 1993, percent mycorrhizae was positively correlated with total phenol for all root classes ( $r = 0.70$ ,  $0.73$ , and  $0.67$ , respectively). In 1994, percent mycorrhizae was not correlated with any organic chemistry variable.

### Correlations of root variables with soil solution elements

No root vitality variables in 1993 or 1994 were consistently correlated with soil solution elements in the forest floor (Oa) or mineral soil. Some organic chemistry variables in the roots were highly correlated with some elements in both the forest floor and mineral soil solution, but there was no consistency. For example, starch concentration in 1993 was positively correlated with total Al, K, Mg, and total monomeric Al in the forest floor and negatively with the same elements in the mineral soil solution. Oxidized phenol showed a similar pattern: negatively correlated with total Al ( $r = -0.71$ ) and total monomeric Al ( $r = -0.63$ ) in the forest floor solution but positively correlated with Al ( $r = 0.80$ ) and total monomeric Al ( $r = 0.87$ ) in the mineral soil solution. In 1994, oxidized phenol correlations were essentially the reverse of those in 1993.

### Correlations of root elements with soil solution elements

Element concentrations in all root classes were not significantly correlated (Pearson coefficients) with the concentration of their counterpart element in the soil solution of the Oa horizon or mineral soil in 1993 or 1994 (data not presented). Elements of Ca, Mg, and K were consistently, although not highly or always significantly, correlated negatively with three Al measurements in the mineral soil solution, total exchangeable Al, inorganic Al, and total monomeric Al. For nonwoody roots in 1993, correlation coefficients for these three Al concentrations and Ca were  $-0.40$ ,  $-0.58$  and  $-0.53$ , respectively. Coefficients were  $-0.78$ ,  $-0.60$ , and  $-0.71$  for K and  $-0.64$ ,  $-0.86$ , and  $-0.82$  for Mg ( $n = 9$  sites,  $p = 0.10$ ). In 1994, root elements were mostly negatively correlated with these three Al concentrations; correlations were significant only for Mg ( $-0.63$ ,  $-0.77$ , and  $-0.74$ ;  $n = 11$  sites,  $p = 0.10$ ).

### Discussion

Changes in the concentrations of available Al and Ca in the soil solution seems to be one of the prime mechanisms by which acidic deposition affects forest growth, and the Al/Ca ratio (or its inverse, Ca/Al) has been proposed as an important indicator of potential stress in forest ecosystems (Cronan and Grigal 1995). Previous studies of red spruce have documented perturbations in biological processes that were related to the historic mobilization and leaching of Ca and Mg in the soil solution and that resulted in a conspicuous enrichment of Ca and Mg in wood formed during the 1960s compared with wood formed before and after this decade (Shortle and Smith 1988; Lawrence et al. 1995; David and Lawrence 1996; Shortle et al. 1997). Soil studies at the same sites showed that the concentrations of Ca and Mg in the soil and soil solutions were highly correlated with the Al concentrations (Lawrence et al. 1995; David and Lawrence 1996) and that there was a direct link of Al competing for exchange sites on the root tip (Shortle and Smith 1988; Smith et al. 1995), resulting in limited uptake of Ca and Mg, which are essential for root development and growth. Despite all of the research demonstrating these chemical changes (Al and Ca pools and fluxes) in vegetation and soil, and the fact that trees growing on sites with a high soil Al/Ca ratio were under more stress than those on sites with a low Al/Ca ratio (Minocha et al. 1996, 1997), few of the indicators monitored during this study consistently detected decreasing vitality of root systems of mature red spruce growing across a gradient of Al/Ca ratios, in both the Oa and the Oe horizons of the forest floor.

In this study, sites were selected to yield a broad gradient of forest floor Al/Ca ratios (David and Lawrence 1996) and tree stress (Minocha et al. 1997). Based on the soil solution Ca/Al molar ratio gradient proposed by Cronan and Grigal (1995) as an indicator of stress in forest ecosystems, negative growth effects should not be detected when sites have low Al/Ca ratios but should be detected when sites have much higher ratios. While the ratios used in our study (exchangeable soil Al and Ca) are not equivalent to the soil solution molar ratio of Cronan and Grigal, they are correlated with each other (David and Lawrence 1996) and we ex-

pected to measure some negative effects related to our ratio gradient. However, none of the putative higher risk sites showed significant signs of reduced vitality as indicated by crown condition or the root variables that we measured. The lack of consistent correlations of these tree vitality indicators with the soil Al/Ca ratio is interesting because these are variables that have been documented in other studies to change in roots of trees in response to stress (Mejstrik 1989; Meyer 1988; Schlegel 1992; Smith et al. 1995; Wargo et al. 1993). Polyamine levels in foliage of these trees in our sites with higher Al/Ca ratios indicated that they were stressed (Minocha et al. 1996, 1997).

In addition to the threshold values of Ca/Al ratios in soil solution, Cronan and Grigal (1995) also listed a soil base saturation value of 15% and a Ca/Al ratio in fine tissue of 0.2 as thresholds, below which effects on trees would become evident. Values at all of the sites exceeded these thresholds for both base saturation and fine root chemistry in the forest floor, although base saturation in the top 10 cm of the mineral soil was below 15% at all sites.

Root vitality measurements that showed a strong relationship with the Al/Ca soil ratio were percentage of root tips that were mycorrhizal and frequency of mycorrhizal morphological types (morphotypes). Differences in percentage of mycorrhizae and morphotypes related to stress were observed by Wargo et al. (1993) in red spruce stands in New York and New England. In that study, declining trees had significantly fewer mycorrhizae and also fewer morphotypes than putatively healthy trees. However, one morphotype (Type 3: dark brown mantle) was found in higher abundance on declining trees at Whiteface Mountain. This morphotype occurred with moderate frequency in our gradient study except at the Bear Brook unfertilized site, where it was a dominant type in 1993. Another morphotype (Type 1: creamy tan mantle) was observed as the dominant type on healthy trees on Whiteface Mountain in both Wargo et al. (1993) and our gradient study. This morphotype (Type 1) was also dominant at both Bear Brook sites, where exchangeable Al concentrations were high, suggesting an effect of Al on its abundance.

In this study, Type 4 (black and hairy mantle) observed on all sites in both years probably represents a complex of dematiaceous fungi that colonize red spruce roots and represent mycorrhizal, pseudomycorrhizal, and pathogenic relationships (Livingston and Blaschke 1984; Wang and Wilcox 1985; Wilcox and Wang 1987). Morphotypes were distinguished only by exterior features, and no attempt was made to separate them into true or pseudomycorrhizal partners. Several randomly chosen isolates of dematiaceous fungi isolated from putatively dead root tips from three sites in this study, Kossuth, Hubbard Brook, and Whiteface Mountain, were all identified as *P. fortinii*-like by rDNA analyses (Harney 1994; Harney et al. 1995), and all sites yielded isolates that were morphologically similar to these identified isolates. *Phialacephala fortinii* is considered an opportunistic pathogen on conifers and forms pseudomycorrhizal relationships (Wang and Wilcox 1985; Wilcox and Wang 1987). Harney (1994) demonstrated that *P. fortinii* was pathogenic on red pine (*Pinus resinosa* Ait.) seedlings, but in our study, there was no relationship between the frequency of this morphotype and percent root tip mortality.

Except for Cone Pond, the abundance of the Type 4 morphotype decreased as the elevation and soil Al/Ca ratio increased. The decrease in this morphotype with elevation and (or) increase in the soil Al/Ca ratio probably represents a shift in the population of mycorrhizal partners and perhaps a decrease in numbers of mycorrhizae because these same sites had lower percentages of mycorrhizal tips. In Sitka spruce (*Picea sitchensis* (Bong.) Carrière) stands in Scotland that were fertilized with ammonium sulfate, the production of fine roots and mycorrhizae was reduced as was percent mycorrhizal infection (Alexander and Fairley 1983). Fertilization also increased the number of mycorrhizae morphotypes and reduced the incidence of mycorrhizae formed by the dematiaceous fungus *Cenococcum geophilum*, an easily identifiable mycorrhiza and a dominant morphotype in Sitka spruce stands. Sites in our study that showed decreases in the dematiaceous morphotypes (Type 4) had higher concentrations of N in their root tissue, suggesting an effect of this element on the occurrence of this morphotype. The Bear Brook fertilized site (also with ammonium sulfate) had the highest N concentration among the sites and had the lowest occurrence of the dematiaceous morphotype and one of the lowest percentages of mycorrhizal tips.

The predominant location of red spruce roots in the forest floor should have increased our ability to detect or measure changes in the indicators being monitored during this study. The forest floor is the primary source of nutrients for coniferous trees in cold temperate and boreal forests (Cole and Rapp 1981; Vogt et al. 1981, 1986), and the importance of this horizon as the dominant site for nutrient capture increases as the stand ages and develops. In the Pacific Northwest, 70-year-old Douglas-fir (*Pseudotsuga menziesii* (Mirb.) Franco) forests obtained nearly all of their nutrients from the forest floor (Johnson et al. 1982). The few studies conducted in forests of the northeastern United States revealed that fine roots of conifers are predominantly in the forest floor, which in these forests can be 5–15 cm deep (Hopkins 1939; Fernandez 1992; Joslin and Wolfe 1992). More recently, studies in the same area, but over a greater range of sites than in the Hopkins (1939) study, showed that 70–80% of the conifer fine-root biomass (<2 mm in diameter) occurred in the forest floor, while more than half of the fine roots of hardwood species were in the mineral horizons (unpublished data). These studies suggest that perhaps changes in rooting capacity or hospitable horizons for root growth and nutrient capture precede changes in root vitality.

Most studies have shown that differences in root vitality measurements occur between healthy and declining trees (Bruck 1984; Meyer 1984, 1988; Mejstrik 1989; Wargo et al. 1993). Reduced nutrient capture due to reduced rooting environment or from competition for cations, e.g., Al versus Ca and Mg (Cronan 1991; Dahlgren et al. 1991; Cronan and Grigal 1995), would affect productivity of the tree, resulting in the shedding of plant parts that can no longer be maintained. However, our data indicated no strong relationships between root vitality and root chemistry and the soil Al/Ca ratio in the Oe or Oa horizon for putatively healthy or stressed trees at these sites. For example, trees at Big Moose were rated visually to be in the poorest health and had the highest root tip mortality in 1993. However, in 1994, trees at this site had one of the lowest levels of root tip mortality. In

1993, trees from this site also had the lowest number of root tips per branch, but in 1994, Big Moose trees ranked third highest for that variable. Concentrations of Al, Fe, K, Mg, Mn, and P in the roots of trees at Big Moose were lowest, or near lowest, among all sites in 1993 and 1994. All trees sampled at this site showed signs of decline disease, some in the initial stages and some in the moderate stages. Many other trees in the stand were in the advanced stages of decline or were dying. Crawford Notch was another site where mortality of red spruce was significant but most of the remaining trees were in good to fair health; vitality measurements on roots from trees from Crawford Notch were not different from those of other sites. It is possible that crown decline in these red spruce was related to winter injury (DeHayes et al. 1999) and preceded any major changes in root vitality.

A recent study on mycorrhizae and Ca in a mixed conifer-hardwood forest in New England found that apatite might be an important source of Ca in some base-poor forest sites (Blum et al. 2002). Apatite-derived Ca was utilized largely by ectomycorrhizal tree species such as red spruce and suggests that these mycorrhizal fungi may weather apatite and absorb the released Ca directly. Apatite weathering by ectomycorrhizal fungi could compensate for lost Ca in base-poor sites or in sites with high soil Al/Ca ratios. Thus, ectomycorrhizal fungi could sustain internal Ca concentrations sufficient to maintain vitality. Changes in root vitality and hence mycorrhizae could occur when changes in carbohydrate or growth regulator production would result in decreases in mycorrhizal populations generally or specifically. In our study, there was a decrease in percent mycorrhizal infection related to an increase in the Al/Ca ratio and elevation. High-elevation stands in the northeastern United States have been the site of major red spruce decline in the northeastern United States and these declines may reflect changes in the ectomycorrhizal-Ca relationship.

The Al/Ca ratio in the soil in either forest floor horizon had no apparent effect on the energy levels in roots as indicated by concentrations of starch, total soluble sugar, total phenolics, and oxidized phenol. Other studies have shown that changes in these C-based compounds generally were correlated with increased root deterioration and susceptibility to root pathogens (Wargo et al. 1993). No such relationship was observed in the current study. Although there were differences in these organic constituents among root classes, differences among sites were negligible. Sites with high Al/Ca ratios had carbohydrate and starch levels in the roots that were equal to or greater than the concentrations in roots from sites with much lower Al/Ca ratios. Carbohydrate concentrations measured in these trees were similar to those measured in red spruce in other studies (Wargo et al. 1993; Schaberg et al. 1999).

Nitrogen in the roots was the only element related to the Al/Ca gradient and it increased as the Al/Ca ratio increased. Increased N caused a corresponding decrease in the carbohydrate/N ratio in root tissue, potentially making the root tissue more susceptible or vulnerable to pathogen colonization (Goodman et al. 1967). A lower carbohydrate/N ratio generally increases the ability of microorganisms, particularly wood decay organisms, to colonize and degrade woody tissues (Levi and Cowling 1969). Changes in the phenol/N

ratios have similar effects and a higher ratio of N to phenol can lower the toxicity of polyphenols to certain fungi (Kirkham 1957a, 1957b). Phenol/carbohydrate ratios have similar effects. Entry et al. (1992) related infection rates by *A. ostoyae* to the ratio of phenol to sugar in roots of western conifers. Susceptible trees had higher concentrations of sugars and lower concentrations of phenols than more resistant trees. However, in our study, changes in the ratios of carbohydrates, N, and total phenol apparently had little effect on the susceptibility of root tissue to colonization by natural or introduced inoculum of root decay organisms. There were no significant differences among sites in the amount of discoloration and (or) decay 1 year after root wounding, nor were there significant differences among sites in susceptibility and colonization by the four root and butt rot organisms inoculated on wounded and unwounded woody roots. These fungi were recovered from the inoculum blocks, so there was adequate inoculum for colonization. Only trees at Cone Pond showed some resistance to colonization; of those sites where the inoculations were performed, this site had the highest Al/Ca ratio in both the Oe and Oa horizons. At Cone Pond, Al was two to five times higher than at the other sites where inoculations occurred (see David and Lawrence 1996); it is possible that this concentration was sufficient to inhibit fungal infection and colonization by butt rot fungi. These same fungi, particularly *Armillaria*, were inhibited (*in vitro*) by Al and other metals (Wargo and Carey 2001) at concentrations that occurred in soils in red spruce stands (Wargo et al. 1987). Decreases in the incidence of *Armillaria* root disease on Douglas-fir in the coastal and Cascade Mountains in Washington State were also related to increases in soil-extractable Al in the soils (Browning and Edmonds 1985).

The lack of a relationship of vitality measurements and element concentrations in the roots with the soil Al/Ca ratio may be a function of the sampling method used. We sampled roots primarily from the Oe and upper portion of the Oa horizons in the forest floor. Working in stands near some of our gradient stands (e.g., Kossuth and Howland and Whiteface Mountain), Smith et al. (1995) found that concentrations of Ca were higher and concentrations of Al were lower in root tips of red spruce from the F (equivalent to our Oe) horizon compared with root tips from the H (equivalent to our Oa) horizon. Working on Whitetop Mountain, Virginia, in red spruce stands at two elevations, Joslin and Wolfe (1992) found that the Oa horizon had less Ca and more Al than the Oe horizon as well as significantly less root density ( $\text{g}\cdot\text{m}^{-3}$ ) in the Oa horizon, suggesting a less hospitable environment for fine roots in the Oa horizon. Rustad and Cronan (1995) found that Al in the forest floor can increase with increased depth due to the affinity for Al in the organic material and to the progressively greater incorporation of mineral soil and (or) mineral soil Al into the organic horizon. The ratios of exchangeable Al/Ca concentrations in the top 10 cm of the mineral soil at our sites ranged from 5 to 52 (David and Lawrence 1996), values that were an order of magnitude greater than those in the Oa horizon.

Another possible reason for the lack of relationships among root vitality variables and the soil Al/Ca ratios in this study is that our elements were measured in composite samples for root size class from both the Oe and the Oa hori-

zons. Our nonwoody root class (<1 mm in diameter) included root tips and associated nonwoody branches that probably differed significantly from the tips in element concentration as indicated by Schelegel et al. (1992). In their studies on fine-root chemistry at Whiteface Mountain, New York, X-ray microanalysis of cations in root tissue revealed high concentrations of Al in the root cortex. However, Al was not detected in the stelar tissue. By contrast, Ca concentration was low in the cortex but higher in the stele. These data suggest that concentrations of cations in composite samples, especially of Al and Ca, were affected by the proportion of cortex to stelar tissue in the samples.

These differences in sampling procedures could have confounded relationships of root vitality with soil element chemistry and the Al/Ca ratio. Our calculation of the estimated Al/Ca ratio in the Oe horizon suggests that the Oe horizon is a more favorable environment chemically than the Oa horizon for many of the sites measured. As a result, composite root samples, which were from both horizons but predominantly from the Oe horizon, might have confounded both root cation and root vitality relationships with soil cations, particularly the Al/Ca ratio. Therefore, analyzing root vitality and chemistry for the Oe and Oa horizons and upper 10 cm of the B horizon separately may have revealed more information on relationships between root vitality and soil chemistry.

This research revealed significant variation in many of the measured variables between the two sampling years, suggesting a possible influence of the climatic conditions on the strength of the indicator being measured. In fact, Johnson et al. (1988) linked the period of greater red spruce mortality to a period when there was a co-occurrence of multiple disturbances (acid deposition and drought). We examined the Palmer drought severity index (PDSI) during the 1992, 1993, and 1994 study period for our study sites, but the differences in root vitality among sites were not related to the PDSI (for areas where our sites were located) in either the dormant season of the year before or in the growing season of the year of root vitality measurements.

Our studies suggest that the ability to detect early changes in tree vitality and susceptibility to root pathogens by monitoring changes in root vitality and chemistry in specific trees is inconsistent for red spruce growing on sites with varying forest floor Al/Ca ratios. Previous studies have documented the effectiveness of the indicators selected (e.g., starch) to measure the loss of root vitality (Wargo et al. 1993), but these measurements were taken on trees with a broader range of declining health than we observed. Most of the trees sampled in our studies were in good to fair health (visually), suggesting that the response of the measured variables to the adverse effects of high Al concentrations is not apparent until significant changes in tree health occur. The indicators monitored during our study varied but were not correlated directly with the forest floor Al/Ca ratios, the primary criteria used to select the study sites. Only the concentrations of N in roots and percent mycorrhizae and mycorrhizal morphotypes varied directly with the Al/Ca ratios in this study. The changes recorded for the last two variables suggest a link to root vitality, since both are important in contributing to the defensive mechanisms used by plants to control their susceptibility to pathogens and perhaps to

nutrient capture in base-poor soils. The effectiveness of these variables also varied with the sampling year, suggesting the importance of measuring under conditions (e.g., drought periods) that may aggravate the expression of existing disturbances.

## Acknowledgements

The authors acknowledge the technical assistance of Gregg Bradford and Susan Howell for root element analyses and Chris Fagan for data analyses and graphics preparation. David Randall, USDA Forest Service, Northeastern Research Station, retired, provided statistical advice and review. Kevin Smith and Chris Eagar, USDA, Forest Service, Northeastern Research Station, Durham, N.H., and Christopher Cronan, University of Maine, Orono, Me., reviewed the manuscript and provided helpful suggestions for improving it.

## References

- Alexander, I.J., and Fairley, R.I. 1983. Effects of N fertilization on populations of fine roots and mycorrhizae in spruce humus. *Plant Soil*, **71**: 49–53.
- Bauch, J., and Schroder, W. 1982. Zellularer Nachweis von Elementen in den Feinwurzeln gesunder und erkrankter Tanne (*Abies alba* Mill.). *Forstwiss. Centralbl.* **101**: 285–294.
- Bauch, J., Rademacher, P., Berneike, W., Kroth, J., and Michaelis, W. 1985a. Breite und Elementgehalt der Jahrringe in Fichten aus Waldschadensgebieten. In *Waldschadens-Einflussfaktoren und ihre Bewertung* Dusseldorf, FRG. VCI Berichte 560. pp. 943–959.
- Bauch, J., Stienen, H., Ulrich, B., and Matzner, E. 1985b. Einfluss einer Kalkung bzw. Dungung auf den Elementgehalt in Feinwurzeln und das Dickenwachstum von Fichten aus Waldschadensgebieten. *Allg. Forst Zeitschrift*, **43**: 1148–1150.
- Bloomfield, J., Vogt, K., and Wargo, P.M. 1996. Tree root turnover and senescence. In *Plant roots. The hidden half*. Edited by Y. Waisel, A. Eshel, and U. Kafkafi. Marcel Dekker, New York. pp. 363–381.
- Blum, J.D., Klaue, A., Nezat, C.A., Driscoll, C.T., Johnson, C.E., Siccama, T.G., Eagar, C., Fahey, T.J., and Likens, G.E. 2002. Mycorrhizal weathering of apatite as an important calcium source in base-poor forest ecosystems. *Nature (London)*, **417**: 729–731.
- Bremner, J.M. 1996. Nitrogen — total. In *Methods of soil analysis. Part 3. Chemical methods*. Edited by D.L. Sparks. Soil Science Society of America, Madison, Wis. SSSA Book Ser. 5. pp. 1085–1121.
- Browning, J.E., and Edmonds, R. 1985. Influence of soil aluminum on incidence of *Armillaria* in Douglas fir. In *Proc. of the 33rd Annual West. Int. For. Dis. Work Conf.*, 24–27 Sept. 1985, Olympia, Wash. Edited by W.G. Thies. Oregon State University, Corvallis, Oreg. pp. 95–97.
- Bruck, R.I. 1984. Decline of montane boreal ecosystems in central Europe and the southern Appalachian Mountains. *TAPPI Proc.* 1984. pp. 159–163.
- Cole, D.W., and Rapp, M. 1981. Elemental cycling in forest ecosystems. In *Dynamic properties of forest ecosystems*. Edited by D.E. Reichle. Int. Biol. Prog. 23. Cambridge University Press, Cambridge, U.K. pp. 341–409.
- Cook, E.R., and Johnson, A.H. 1989a. Climate change and forest decline: a review of the red spruce case. *Water Air Soil Pollut.* **48**: 127–140.
- Cook, E.R., and Johnson, A.H. 1989b. Threshold responses and climatic change: possible contributors to the decline of red spruce. In *Proceedings of the United States of America and the Federal Republic of Germany Research Symposium/Workshop: the Effects of Atmospheric Pollution on Spruce–Fir Forests in the Eastern United States and the Federal Republic of Germany*. U.S. For. Serv. Gen. Tech. Rep. NE-120. pp. 311–322.
- Cook, E.R., Johnson, A.H., and Blasing, T.J. 1987. Forest decline: modeling the effect of climate in tree rings. *Tree Physiol.* **3**: 27–39.
- Cronan, C.S. 1991. Differential adsorption of Al, Ca, and Mg by roots of red spruce (*Picea rubens* Sarg.). *Tree Physiol.* **8**: 227–237.
- 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.
- Cronan, C.S., and Schofield, C.L. 1979. Aluminum leaching response to acid precipitation: effect on high-elevation watersheds in the northeast. *Science (Washington, D.C.)*, **204**: 305–306.
- Dahlgren, R., Vogt, K.A., and Ugolini, F.C. 1991. The influence of soil chemistry on fine root aluminum concentrations and root dynamics in a subalpine spodosol. *Plant Soil*, **133**: 117–129.
- David, M.B., and Lawrence, G.B. 1996. Soil and soil solution chemistry under red spruce stands across the northeastern U.S. *Soil Sci.* **161**: 314–328.
- DeHayes, D.H., Schaberg, P.G., Hawley, G.J., and Strimbeck, G.R. 1999. Acid rain impacts on calcium nutrition and forest health. *Bioscience*, **49**: 789–800.
- Dubois, K., Gilles, K.A., Hamilton, J.K., Rebers, P.A., and Smith, F. 1956. Colorimetric method for determination of sugars and related substances. *Anal. Chem.* **28**: 350–356.
- Ellsworth, D.S., and Liu, S. 1994. Photosynthesis and canopy nutrition in four sugar maple forests on acid soils in northern Vermont. *Can. J. For. Res.* **24**: 2118–2127.
- Entry, J.A., Martin, N.E., Kelsey, R.G., and Cromack, K., Jr. 1992. Chemical constituents in root bark of five species of western conifer saplings and infection by *Armillaria ostoyae*. *Phytopathology*, **82**: 393–397.
- Fernandez, I.J. 1992. Characterization of eastern U.S. spruce–fir soils. In *Ecology and decline of red spruce in the eastern United States*. Edited by C. Eagar and M.B. Adams. Springer-Verlag, New York. pp. 40–63.
- Glenn, M.G., Wagner, W.S., and Webb, S.L. 1991. Mycorrhizal status of mature red spruce (*Picea rubens*) in mesic and wetland sites of northwestern New Jersey. *Can. J. For. Res.* **21**: 741–749.
- Goodman, R.N., Kiraly, Z., and Zaitlin, M. 1967. The biochemistry and physiology of infectious plant disease. D. van Nostrand, Princeton, N.J.
- Harney, S.K. 1994. Dematiaceous endophytes from plant roots: molecular characterization and interactions. Ph.D. dissertation, State University of New York, College of Environmental Science and Forestry, Syracuse, N.Y.
- Harney, S.K., Wentworth, T.S., and Wargo, P.M. 1995. *Phialocephala fortinii*, a potential fine root pathogen, isolated from red spruce. *Phytopathology*, **85**: 1141.
- Hertel, G. (Technical coordinator). 1988. Effects of atmospheric pollutants on the spruce–fir forests of the eastern United States and the Federal Republic of Germany. U.S. For. Serv. Gen. Tech. Rep. NE-120.
- Hopkins, H.T., Jr. 1939. The root distribution of forest trees in the central Adirondack region. Ph.D. dissertation, Cornell University, Ithaca, N.Y.
- Hornbeck, J.W., and Smith, R.B. 1985. Documentation of red spruce decline. *Can. J. For. Res.* **15**: 1199–1201.

- Johnson, A.H., Cook, E.R., and Siccama, T.G. 1988. Climate and red spruce growth and decline in northern Appalachians. *Proc. Natl. Acad. Sci. U.S.A.* **85**: 5369–5373.
- Johnson, D.W., Cole, D.W., Bledsoe, C.S., Cromack, K., Edmonds, R.L., Gessel, S.P., Grier, C.C., Richards, B.N., and Vogt, K.A. 1982. In Nutrient cycling in forests of the Pacific Northwest. Analysis of coniferous forest ecosystems in the western United States. Edited by R.L. Edmonds. Hutchinson Ross, Stroudsburg, Pa. pp. 186–232.
- 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.
- Kirkham, D.S. 1957a. Studies on the significance of polyphenolic host metabolites in the nutrition of *Venturia inaequalis* and *Venturia pirina*. *J. Gen. Microbiol.* **17**: 120–134.
- Kirkham, D.S. 1957b. The significance of polyphenolic metabolites of apple and pear in the host relations of *Venturia inaequalis* and *Venturia pirina*. *J. Gen. Microbiol.* **17**: 491–504.
- Lawrence, G.B., and David, M.B. 1995. 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 (London)*, **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*, **38**: 19–39.
- Levi, M., and Cowling, E.B. 1969. Role of nitrogen in wood deterioration. VII. Physiological adaptation of wood-destroying and other fungi to substrates deficient in nitrogen. *Phytopathology*, **59**: 460–468.
- Likens, G.E., and Bormann, F.H. 1974. Acid rain: a serious regional environmental problem. *Science (Washington, D.C.)*, **184**: 1176–1179.
- Livingston, W.H., and Blaschke, H. 1984. Deterioration of mycorrhizal short roots and occurrence of *Mycelium radicum atrovirens* on declining Norway spruce in Bavaria. *Eur. J. For. Pathol.* **14**: 340–348.
- McLaughlin, S.B., Downing, D.J., Blasing, T.J., Cook, E.R., Johnson, A.H., and Adams, H.S. 1987. An analysis of climate and composition as contributors to decline of red spruce in high elevation Appalachian forests of the eastern United States. *Oecologia*, **72**: 487–501.
- Mejstrik, V. 1989. Ectomycorrhizas and forest decline. *Agric. Ecosyst. Environ.* **28**: 325–337.
- Meyer, F.H. 1984. Mykologische Beobachtungen zum Baumsterben. *Allg. Forstz.* **89**: 212–228.
- Meyer, F.H. 1988. Ectomycorrhiza and decline of trees. In *Ectomycorrhiza and acid rain*. Edited by A.E. Jansen, J. Dighton, and A.H.M. Bresser. Res. Rep. 12. Eur. Comm. Air Pollut., Bilthover, Germany. pp. 9–31.
- 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, R., Shortle, W.C., Lawrence, G.B., David, M.B., and Minocha, S.C. 1997. Relationships among foliar chemistry, foliar polyamines, and soil chemistry in red spruce trees growing across the northeastern United States. *Plant Soil*, **191**: 109–122.
- Parkinson, J.A., and Allen, S.E. 1975. A wet oxidation procedure suitable for the determination of nitrogen and mineral nutrients in biological material. *Commun. Soil Sci. Plant Anal.* **6**: 1–11.
- Rizzo, D.M., and Harrington, T.C. 1988. Root and butt rot fungi on red spruce and balsam fir in the White Mountains, New Hampshire. *Plant Dis.* **72**: 329–331.
- Rosenblatt, M., and Peluso, J.V. 1941. Determination of tannins by photometer. *J. Assoc. Off. Agric. Chem.* **24**: 170–181.
- Rustad, L.E., and Cronan, C.S. 1995. Biogeochemical controls on aluminum chemistry in the O horizon of a red spruce (*Picea rubens* Sarg.) stand in central Maine, U.S.A. *Biogeochemistry*, **29**: 107–129.
- Rustad, L.E., Fernandez, I.J., David, M.B., Mitchell, M.J., Nadelhoffer, K.J., and Fuller, R.B. 1996. Experimental soil acidification and recovery at the Bear Brook Watershed in Maine. *Soil Sci. Soc. Am. J.* **60**: 1933–1943.
- SAS Institute Inc. 1990. SAS/STAT user's guide, version 6.01 ed. SAS Institute Inc., Cary, N.C.
- Schaberg, P.G., Snyder, M.C., Shane, J.B., and Donnelly, J.R. 1999. Seasonal patterns of carbohydrate reserves in red spruce seedlings. *Tree Physiol.* **20**: 549–555.
- Schaberg, P.G., DeHayes, D.H., Hawley, G.J., Strimbeck, G.R., Cumming, J.R., Murakami, P.F., and Borer, C.H. 2000. Acid mist and soil Ca and Al alter the mineral nutrition and physiology of red spruce. *Tree Physiol.* **20**: 73–85.
- Schlegel, H., Amundson, R.G., and Huttermann, A. 1992. Element distribution in red spruce (*Picea rubens*) fine roots; evidence for aluminum toxicity at Whiteface Mountain. *Can. J. For. Res.* **22**: 1132–1138.
- Schroeder, 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.
- Scott, J.T., Siccama, T.G., Johnson, A.H., and Breisch, A.R. 1984. Decline of red spruce in the Adirondacks, New York. *Bull. Torrey Bot. Club*, **111**: 438–444.
- Shortle, W.C., and Smith, K.T. 1988. Aluminum-induced calicum deficiency syndrome in declining red spruce. *Science (Washington, D.C.)*, **240**: 1017–1018.
- Shortle, W.C., Smith, K.T., Minocha, R., Lawrence, G.B., and David, M.B. 1997. Acidic deposition, cation mobilization, and biochemical indicators of stress in healthy red spruce. *J. Environ. Qual.* **26**: 871–876.
- Siccama, T.G., Bliss, M., and Vogelmann, H.W. 1982. Decline of red spruce in the Green Mountains of Vermont. *Bull. Torrey Bot. Club*, **109**: 163–168.
- Smith, K.T., Shortle, W.C., and Ostrofsky, W.D. 1995. Aluminum and calcium in fine root tips of red spruce collected from the forest floor. *Can. J. For. Res.* **25**: 1237–1242.
- Smith, K.T., Cufar, K., and Levani, T. 1999. Temporal stability and dendroclimatology in silver fir and red spruce. *Phyton (Horn, Austria)*, **39**: 117–122.
- Stienen, H., Barckhausen, R., Schaub, H., and Bauch, J. 1984. Mikroskopische und rontgenenergiedispersiv Untersuchungen an Feinwurzeln gesunder und erkrankter Fichten (*Picea abies* (L.) Karst.) verschiedener standorte. *Forstwiss. Centralbl.* **103**: 262–274.
- Ulrich, B. 1983. Soil acidity and its relation to acid deposition. In *Effects of accumulation of air pollutants in ecosystems*. Edited by B. Ulrich and J. Pankratz. D. Reidel, Boston, Mass. pp. 127–146.
- Vogt, K.A., Edmonds, R.L., and Grier, C.C. 1981. Seasonal changes in biomass and vertical distribution of mycorrhizal and fibrous-textured conifer fine roots in 23- and 180-year-old subalpine *Abies amabilis* stands. *Can. J. For. Res.* **11**: 223–229.
- Vogt, K.A., Grier, C.C., and Vogt, D.J. 1986. Production, turnover, and nutrient dynamics of above- and belowground detritus of world forests. *Adv. Ecol. Res.* **15**: pp. 303–377.
- Walter, W.M., Jr., and Purcell, A.E. 1980. Effect of substrate levels and polyphenol oxidase activity on darkening in sweet potato cultivars. *J. Agric. Food Chem.* **28**: 941–944.

- Wang, C.J.K., and Wilcox, H.E. 1985. New species of ectendomycorrhizal and pseudomycorrhizal fungi: *Phialophora finlandia*, *Chloridium paucisporum*, and *Phialocephala fortinii*. *Mycologia*, **77**: 951–958.
- Wargo, P.M., and Carey, A.C. 2001. Effects of metals and pH on *in vitro* growth of *Armillaria ostoyae* and other root and butt rot fungi of red spruce. *For. Pathol.* **31**: 5–24.
- Wargo, P.M., Carey, A.C., Geballe, G.T., and Smith, W.H. 1987. Occurrence of rhizomorphs of *Armillaria* in soils from declining red spruce stands in three forest types. *Plant Dis.* **71**: 163–167.
- Wargo, P.M., Bergdahl, D.R., Tobi, D.R., and Olson, C.W. 1993. Root vitality and decline of red spruce. *Contributions biologicae arborum*. Vol. 4. ECOMED Verlogsgesellschaft. Landsberg Lech, Germany.
- Weiss, M.J., McCreery, L., Miller, I., O'Brien, J.T., and Miller-Weeks, M. 1985. Red spruce and balsam fir decline and mortality. NA-TP-11. USDA For. Serv., Northeastern Area, State and Priv. For., Broomall, Pa.
- Wilcox, H., and Wang, C.J.K. 1987. Mycorrhizal and pathological associations of dematiaceous fungi in roots of 7-month-old tree seedlings. *Can. J. For. Res.* **17**: 884–899.
- Worrall, J.J. 1991. Media for selective isolation of hymenomycetes. *Mycologia*, **83**: 296–302.
- Yates, F. 1933. The analysis of replicated experiments when the field results are incomplete. *Emp. J. Exp. Agric.* **1**: 129–142.