

## Associations of calcium and aluminum with the growth and health of sugar maple trees in Vermont

Paul G. Schaberg<sup>a,\*</sup>, James W. Tilley<sup>b</sup>, Gary J. Hawley<sup>b</sup>,  
Donald H. DeHayes<sup>b</sup>, Scott W. Bailey<sup>c</sup>

<sup>a</sup> USDA Forest Service, Northeastern Research Station, 705 Spear Street,  
South Burlington, VT 05403, USA

<sup>b</sup> The University of Vermont, Rubenstein School of Environment and Natural Resources,  
81 Carrigan Drive, Burlington, VT 05405, USA

<sup>c</sup> USDA Forest Service, Northeastern Forest Experiment Station, Hubbard Brook Experimental Forest,  
RFD #1, Box 779, Campton, NH 03223, USA

Received 22 July 2005; received in revised form 26 October 2005; accepted 27 October 2005

### Abstract

We compared tree growth and crown condition with soil and foliar elemental composition in 14 sugar maple (*Acer saccharum* Marsh.) stands in VT, USA, to evaluate if deficiencies or imbalances in cation nutrition were associated with growth and health reductions in native stands. The Till Source Model (TSM) was used to select study sites potentially high or low in calcium (Ca) by predicting the relative Ca concentration of soil parent material derived from glacial till. The TSM successfully identified high or low levels of soil Ca ( $P = 0.031$ ) and foliar Ca ( $P = 0.011$ ) among stands. Although soil Ca, potassium (K), phosphorus (P), and iron (Fe) concentrations were all associated with stand growth and health, the influence of K, P, and Fe on health appeared indirect and potentially a result of autocorrelations between Ca and these nutrients. The only cases where improved health coincided with increased soil element content involved Ca and molar ratios of Ca and aluminum (Al). Among foliar nutrients, significant relationships with elevated branch dieback were only found for low foliar Ca ( $P = 0.021$ ) and high foliar Al ( $P = 0.035$ ). Average annual basal area growth during the decade prior to sampling was lower for trees in stands with low foliar Ca ( $P = 0.050$ ) and high foliar Al ( $P = 0.017$ ). These data emphasize the importance of Ca and Al to sugar maple health and highlight the vulnerability of sugar maple stands to declines in growth and vigor following continued anthropogenic Ca depletion.

Published by Elsevier B.V.

**Keywords:** Calcium; Aluminum; Sugar maple; *Acer saccharum*; Growth; Decline

### 1. Introduction

Sugar maple (*Acer saccharum* Marsh.) decline has been documented throughout parts of the northeastern USA and Quebec during the 1960s, 1980s, and 1990s (Mader and Thompson, 1969; Kelley, 1988; Allen et al., 1992a; Wilmot et al., 1995). These declines were characterized using various measures, including crown deterioration, increased leaf chlorosis, and reduced growth. Stress factors such as drought (Payette et al., 1996), freezing (Robitaille et al., 1995), and insect defoliation (Allen et al., 1992b) have been implicated with the decline and mortality of sugar maple. Regardless of

stressor, decline also appears to be associated with deficiencies or imbalances of various elements including nitrogen (N), phosphorus (P), potassium (K), magnesium (Mg), manganese (Mn), or calcium (Ca) (Mader and Thompson, 1969; Bernier and Brazeau, 1988a; Pare and Bernier, 1989; Ouimet and Fortin, 1992; Wilmot et al., 1995; Horsley et al., 2000). Although the specific elements associated with decline can vary among sites, deficiencies in Ca have been highlighted as a potential contributor to sugar maple decline in recent studies throughout the region (e.g., Ellsworth and Liu, 1994; Ouimet and Camire, 1995; Wilmot et al., 1996; Long et al., 1997; Moore et al., 2000). These Ca deficiencies have generally been attributed to regional soil cation shortages and the mobilization of aluminum (Al) caused when acidic deposition disrupts natural weathering and nutrient cycling processes (e.g., Shepard et al., 1990; Richter et al., 1992; Ouimet et al., 2001).

\* Corresponding author. Tel.: +1 802 951 6771; fax: +1 802 951 6368.

E-mail address: [pschaberg@fs.fed.us](mailto:pschaberg@fs.fed.us) (P.G. Schaberg).

Altered Ca nutrition has also been linked to the decline of red spruce (*Picea rubens* Sarg.) (Schaberg and DeHayes, 2000). Recent investigations of Ca depletion in red spruce have focused on the disruption of a relatively small, but physiologically important pool of Ca specifically associated with plasma membranes of mesophyll cells (DeHayes et al., 1999; Jiang and Jagels, 1999; Schaberg et al., 2000; Jagels et al., 2002). This highly compartmentalized and labile pool of membrane-associated Ca (mCa) supports at least two important functions: (1) structural support and (2) environmental signal perception and transduction (Marschner, 1995). Acidic deposition leaches mCa from red spruce mesophyll cells, causes membrane destabilization, a reduction of foliar cold tolerance, and could inhibit the role of Ca as second messenger in response to environmental stimuli (DeHayes et al., 1999; Schaberg et al., 2000). This mechanism of acid-induced Ca disruption has also been experimentally verified for eastern white pine (*Pinus strobus* L.), eastern hemlock (*Tsuga canadensis* L.), and balsam fir (*Abies balsamea* (L.) Mill.) (Schaberg et al., 2001).

Studies have demonstrated that inadequate levels of foliar Ca can reduce photosynthetic carbon fixation (McLaughlin et al., 1991), inhibit low temperature acclimation (Monroy et al., 1993), and diminish a plant's ability to cope with a variety of abiotic stresses such as high temperatures (Gong et al., 1998), oxidative stress (Christie and Jenkins, 1996), mechanical injury (Trewavas and Knight, 1994), salinity, and drought (Knight et al., 1997). Considering this, it has been proposed that depletion of the labile Ca pool may suppress stress response systems and predispose plants to exaggerated injury following secondary stress events. Sugar maple decline may provide an example of Ca deficiency leading to inadequate physiological response to the variety of stress factors (e.g., insect defoliation, drought, and freezing injury) also associated with decline (McLaughlin and Wimmer, 1999; Schaberg et al., 2001).

To better assess the role of site nutrition, especially Ca, as a potential contributor to maple decline, we assessed the soil and foliar nutrition, diameter growth, crown transparency, vigor

index, and percent crown dieback in 14 sugar maple stands throughout VT, USA. Foliar mCa was also measured on trees for all sites to more specifically evaluate the influence of subcellular pools of Ca on health and productivity. To help assure that stands included a broad range of soil Ca contents, the Till Source Model (TSM: a computer-based model for estimating soil parent material composition based on bedrock geology and patterns of glacial deposition; Bailey and Hornbeck, 1992) was used to identify sites of predicted high and low Ca status for investigation. If effective in locating sites inherently low in Ca, the TSM could be helpful in identifying stands prone to physiological decline following continued anthropogenic Ca depletion.

## 2. Materials and methods

### 2.1. Till Source Model predictions and stand selection

This study was conducted in 14 sugar maple stands that have been regularly monitored by the North American Maple Project (NAMP) (Cooke et al., 1996) or Hardwood Health Survey (HHS) (Kelley et al., 1992). Based on site accessibility, similar current management practices, permission of use by private landowners, and dominance (>50% basal area) of sugar maple, 8 HHS sites and 18 NAMP sites were selected for TSM predictions. The TSM uses latitudinal and longitudinal coordinates to generate a source envelope that incorporates the most recent digitized bedrock geology maps and the flow direction of the Wisconsin glacier that crossed Vermont approximately 14,000 years ago (Bailey and Hornbeck, 1992). Of the 26 sites selected for TSM predictions, 6 were predicted to contain low levels of Ca, 8 were predicted to have high levels of Ca, and the remaining 12 were predicted to have moderate Ca. The 14 stands predicted to contain high or low Ca were used for this study. They represented a range in elevation and average stand age, and were located throughout Vermont (Table 1). At each of the 14 sites, 6 sugar maple trees (>10 cm diameter at DBH (1.3 m)) were randomly selected for nutritional and vigor assessments.

Table 1  
Predicted soil Ca (TSM method), latitude, longitude, elevation, and the range of tree ages of sugar maple stands examined in this study

Stand	TSM Ca prediction	North latitude <sup>a</sup>	West longitude <sup>a</sup>	Elevation (m)	Range of tree ages (years)
1	High	44°04'36"	72°33'30"	482	84–113
2	Low	44°35'20"	72°41'16"	491	89–108
3	Low	44°06'01"	72°45'48"	549	89–133
4	High	44°30'29"	72°13'18"	533	83–109
5	High	44°46'20"	72°16'35"	352	71–86
6	Low	42°50'57"	72°49'34"	609	64–103
7	Low	42°59'16"	72°41'21"	466	59–106
8	Low	43°28'59"	72°54'36"	305	61–92
9	High	43°57'27"	72°37'45"	384	49–129
10	Low	43°33'50"	72°48'36"	883	45–80
11	High	44°38'32"	72°15'13"	607	44–90
12	High	44°56'51"	71°55'59"	634	73–91
13	Low	44°55'59"	72°15'19"	320	73–126
14	Low	42°47'51"	72°52'16"	427	125–174

<sup>a</sup> Latitude and longitude values from the World Geodetic System (WGS84) datum.

## 2.2. Foliar sampling and chemistry

Foliage was collected from the six selected trees per plot between July 22 and August 14, 2001. Small branches were removed by pruning pole from a southwest and southeast-facing limb at mid-crown. Approximately 12 sun leaves (as judged from crown exposure and foliar thickness) including petioles were pooled per branch, sealed into plastic bags, and kept cool for transport. Each leaf was examined for the presence of insect damage, and ones with extensive damage were discarded. In the laboratory, four leaves per tree were selected for mCa analysis and remaining leaves were oven-dried at 55 °C and ground using a Wiley mill.

Ground foliage was digested using nitric acid and hydrogen peroxide (Jones and Case, 1990). Digestions of 0.5 g of tissue were performed in 75-ml reflux tubes in an AD-4020 block digester (Westco Scientific Instruments, Danbury, CT). Foliar samples were predigested in 5 ml of concentrated nitric acid at room temperature overnight, and then digested for 1 h at 120 °C, followed by two incubations in 5 ml of 30% hydrogen peroxide at 120 °C for 30 min. Digests were diluted to 75 ml with distilled deionized water and analyzed for a broad spectrum of cations (Al, Ca, K, Fe, Cu, Mn, and Mg) using inductively coupled plasma atomic emission spectroscopy (ICP-AES, PlasmaSpec 2.5, Perkin-Elmer Optima, Lowell, MA). Peach leaf standards from the National Institute of Standards and Technology (SRM 1547) were digested and analyzed for comparison. Standards were generally within 5% of NIST certified values for all cations except Al, which was corrected for 78% recovery.

## 2.3. Membrane-associated calcium

For each site, four leaves per tree were randomly selected and sectioned to produce three sections per leaf for mCa evaluation. Sectioning, staining, microscopy, and image analysis procedures used were adapted from Borer et al. (1997) and DeHayes et al. (1997). These procedures incorporate epifluorescence microscopy using the fluorescent probe chlorotetracycline (CTC) with computer image processing to quantify the intensity of mCa-specific fluorescent emissions. Previous studies verified that Ca is the cation associated with CTC fluorescence in mesophyll cells (Borer et al., 1997; Lazarus et al., 2002).

## 2.4. Soil sampling and chemistry

Four soil samples from the mineral rooting zone (10–30 cm, excluding Oa/A and E horizons if present) were augered from positions approximately 2 m from the stem in the cardinal directions around each sample tree. Samples were dried at 35 °C for 1 week and then sieved with a 2 mm mesh to remove roots and coarse fragments. The four samples surrounding each tree were weighed to 5 g and composited to create one 20 g sample.

Ammonium acetate (1.25 M, pH 4.8; McIntosh, 1969) was used to extract nutrients from soils. Soil samples were weighed

( $4.000 \pm 0.005$  g) and 20 ml of extraction solution was added to each flask. Samples were shaken for 30 min, and then filtered using Whatman 2 filter paper. Extraction solutions were analyzed for a broad spectrum of cations (Al, Ca, K, Fe, Cu, Mn, and Mg) using ICP-AES. Samples from a bulk homogenized soil were also analyzed as an internal standard to evaluate extraction consistency. A standard deviation of less than 10% for all cations from the bulked soil indicated that extractions were consistent among sample runs.

## 2.5. Evaluation of canopy health and diameter growth

Protocols for tree health evaluation were those used by the NAMP (Cooke et al., 1996). Crown vigor index was assessed using the scale: (1) healthy (no major branch mortality); (2) light decline; (3) moderate decline; (4) severe decline; (5) dead (for complete definitions, see Cooke et al., 1996). Percent branch dieback, including dead branches less than 2.5 cm in diameter, where mortality began at the terminal portion of the limb and progressed inward, was estimated for each tree using a 12-class system (Cooke et al., 1996). Percent crown transparency was estimated with the same system as percent branch dieback (Cooke et al., 1996).

Two increment cores were taken at 90° angles from each sample tree per plot to provide a history of radial growth. In the laboratory, cores were mounted and prepared as described by Cook and Kairiukstis (1989). Measurements were decadal rather than annual to reduce the variability of growth trends between sites. Each core was measured at least twice and cross-dating between cores was undertaken to calibrate year assessments. The average decadal growth of the two cores from each tree was used to reduce differences caused by core location. Because current site nutrition would likely only influence recent growth, site nutrient status was compared only to data from the most recent (1992–2001) decade of growth. Tree-ring data are presented as basal area increments (BAI).

## 2.6. Statistical analyses

Analyses of variance were used to test for differences among sites predicted to contain high or low Ca in the soil and foliage. The analysis of the TSM followed a nested design that included TSM Ca prediction and site within TSM Ca prediction as sources of variation. Stand averages for health, growth, and soil and foliage nutrient concentrations were used to evaluate differences among sites. Foliar Al, Ca, K, Fe, Cu, Mn, and Mg data were categorized as deficient, sufficient, or toxic based on nutritional ranges for healthy forest sugar maples reported by previous studies (Table 2). When the assumption of equal-variances was violated, the non-parametric van der Waerden test was employed. Pearson correlations of stand averages were also utilized to analyze relationships between crown condition, growth, and nutrient concentrations. Stand-based mCa means and variances were also compared to health, growth, and nutrient concentration parameters. To adjust for unequal variances among stands, mCa data were transformed using the slope of the linear relationship of stand means and standard

Table 2

Distribution of study plots among nutrient sufficiency categories established using the published range in foliar nutrient concentrations for healthy sugar maples (Kolb and McCormick, 1993)

Element	Published range for healthy trees (mg kg <sup>-1</sup> )	Number of study plots ( <i>n</i> ) per nutrient category		
		Low	Sufficient	Toxic
Ca	5000–21900	5	9	0
Mg	1100–4000	4	10	0
Al	31–60	9	5	0
K	5500–10400	2	12	0
Fe	59–130	11	3	0
Cu	3–9	0	14	0
Mn	632–1630	2	6	6

Kolb and McCormick (1993) ranges were based on data from the following references: Mader and Thompson (1969), Ellis (1975), Lea et al. (1980), Morrison (1985), McLaughlin et al. (1987), Bernier and Brazeau (1988a,b,c), and Kocman et al. (1991). Nutrient summaries are for foliage collected in July or August from forest-grown sugar maple trees that were not fertilized and had no apparent dieback or nutrient deficiency symptoms.

deviations as a power function (Montgomery, 2001). Unless otherwise noted, results were considered statistically significant when  $P \leq 0.05$ .

### 3. Results

#### 3.1. Till class predictions of stand nutrition and relationships to health and growth

Significant differences in soil Ca ( $P = 0.031$ ) and foliar Ca concentrations ( $P = 0.011$ ) were found between TSM Ca classes (Fig. 1). Predicted high Ca sites had nearly twice the foliar Ca and six times the soil Ca compared to sites predicted to be low in Ca. Significant differences were also found between TSM predictions regarding stand health and growth. Sites predicted to have higher Ca showed greater average growth during the decade preceding this study (1992–2001) compared with sites predicted to contain lower Ca ( $P = 0.039$ ). High Ca sites exhibited marginally less branch dieback ( $P = 0.058$ )

Table 3

Mean ( $\pm 1$ S.E.) soil element concentrations from ammonium acetate extractions at a 10–30 cm depth for the 14 sugar maple stands examined

Site	Ca (mg kg <sup>-1</sup> )	Mg (mg kg <sup>-1</sup> )	K (mg kg <sup>-1</sup> )	Mn (mg kg <sup>-1</sup> )	Al (mg kg <sup>-1</sup> )	Fe (mg kg <sup>-1</sup> )
1	534.3 $\pm$ 110.7	16.8 $\pm$ 2.9	19.2 $\pm$ 2.7	37.9 $\pm$ 6.0	168.8 $\pm$ 15.3	12.8 $\pm$ 6.6
2	138.5 $\pm$ 32.7	16.3 $\pm$ 3.4	29.5 $\pm$ 5.4	30.4 $\pm$ 8.4	334.9 $\pm$ 26.9	84.0 $\pm$ 16.3
3	35.3 $\pm$ 2.8	20.2 $\pm$ 1.9	67.6 $\pm$ 6.5	68.2 $\pm$ 19.2	435.1 $\pm$ 40.2	147.0 $\pm$ 34.7
4	446.9 $\pm$ 378.3	11.9 $\pm$ 6.3	26.9 $\pm$ 2.1	29.2 $\pm$ 12.3	466.0 $\pm$ 88.8	20.1 $\pm$ 5.9
5	336.7 $\pm$ 89.9	39.1 $\pm$ 13.7	35.9 $\pm$ 7.6	9.4 $\pm$ 3.7	397.4 $\pm$ 99.8	94.8 $\pm$ 21.2
6	89.2 $\pm$ 9.8	15.7 $\pm$ 2.3	49.6 $\pm$ 5.6	15.1 $\pm$ 3.9	460.4 $\pm$ 44.1	132.8 $\pm$ 18.3
7	34.2 $\pm$ 4.8	14.9 $\pm$ 1.8	58.1 $\pm$ 9.4	54.0 $\pm$ 23.6	583.2 $\pm$ 40.6	107.7 $\pm$ 44.7
8	336.6 $\pm$ 90.0	30.0 $\pm$ 4.3	40.7 $\pm$ 6.4	16.4 $\pm$ 5.7	295.6 $\pm$ 60.4	39.2 $\pm$ 17.3
9	253.8 $\pm$ 81.5	18.0 $\pm$ 3.5	31.0 $\pm$ 8.0	51.9 $\pm$ 24.8	356.4 $\pm$ 55.8	58.0 $\pm$ 19.8
10	63.6 $\pm$ 8.3	16.0 $\pm$ 1.4	44.8 $\pm$ 3.6	5.8 $\pm$ 1.3	583.2 $\pm$ 55.9	74.8 $\pm$ 15.3
11	1684.9 $\pm$ 560.0	48.1 $\pm$ 13.9	34.4 $\pm$ 4.8	26.6 $\pm$ 5.7	222.7 $\pm$ 57.8	10.3 $\pm$ 3.4
12	21.1 $\pm$ 3.0	6.50 $\pm$ 1.0	27.8 $\pm$ 2.3	15.8 $\pm$ 1.8	834.7 $\pm$ 82.4	93.1 $\pm$ 23.1
13	38.0 $\pm$ 6.0	11.6 $\pm$ 1.9	33.3 $\pm$ 2.8	14.3 $\pm$ 1.5	750.6 $\pm$ 76.1	96.7 $\pm$ 15.1
14	40.9 $\pm$ 4.5	12.8 $\pm$ 1.1	45.6 $\pm$ 1.9	2.2 $\pm$ 0.2	565.8 $\pm$ 31.9	186.9 $\pm$ 35.0

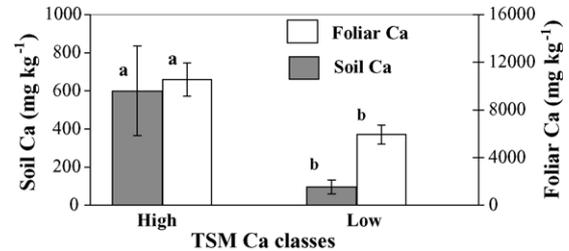


Fig. 1. Means ( $\pm 1$ S.E.) of foliar and soil Ca for Till Source Model (TSM) Ca classes from sugar maple study sites throughout Vermont. Analyses of soil and foliar Ca were independent. For comparisons of soil and foliar Ca means between TSM classes, means without common letters differed significantly at  $P \leq 0.05$  based on ANOVA tests.

than sites predicted to contain low Ca, but no differences in crown transparency or vigor index were found between TSM groups.

#### 3.2. Soil and foliar nutrient concentrations and relationships to crown condition

Concentrations of soil and foliar elements are presented in Tables 3 and 4, respectively. Average branch dieback, crown transparency, and vigor index for stands ranged between 0 and 50%, 10 and 60%, and 1 and 4 (with 1 as most vigorous), respectively (Table 5). Significant positive correlations were found between percent branch dieback and concentrations of soil K ( $r = 0.54$ ,  $P = 0.045$ ) and Fe ( $r = 0.56$ ,  $P = 0.039$ ). Soil K also showed a strong correlation with vigor class ( $r = 0.71$ ,  $P = 0.007$ ). However, autocorrelations between Ca and other nutrients (see Section 3.4) raise the possibility that apparent associations between K and Fe with health measures were spurious and reflected the overarching influence of Ca.

ANOVA results indicated that the mean percent branch dieback was higher for sites that exhibited deficient foliar Ca ( $P = 0.021$ ) and Mg ( $P = 0.052$ ) relative to sites with concentrations of those elements within the range reported for healthy sugar maple trees (Kolb and McCormick, 1993). In contrast, sites classified as having low foliar Al levels demonstrated less branch dieback ( $P = 0.035$ ) than sites with

Table 4

Mean ( $\pm 1$ S.E.) element and relative mCa concentrations, and mCa variance of foliage from sugar maple trees at the 14 study sites

Site	Ca (g kg <sup>-1</sup> )	Mg (g kg <sup>-1</sup> )	K (g kg <sup>-1</sup> )	Mn (g kg <sup>-1</sup> )	Al (mg kg <sup>-1</sup> )	Fe (mg kg <sup>-1</sup> )	Cu (mg kg <sup>-1</sup> )	mCa <sup>a</sup>	mCa variance
1	15.3 $\pm$ 1.1	1.8 $\pm$ 0.1	3.7 $\pm$ 0.3	0.9 $\pm$ 0.1	14.6 $\pm$ 1.3	46.8 $\pm$ 3.9	4.0 $\pm$ 0.3	159 $\pm$ 8	439
2	8.2 $\pm$ 0.5	1.5 $\pm$ 0.1	6.2 $\pm$ 0.4	2.4 $\pm$ 0.3	27.3 $\pm$ 1.9	67.0 $\pm$ 8.3	6.7 $\pm$ 0.5	80 $\pm$ 12	811
3	3.6 $\pm$ 0.4	0.9 $\pm$ 0.1	6.5 $\pm$ 0.6	3.0 $\pm$ 0.5	46.7 $\pm$ 3.7	72.5 $\pm$ 7.2	5.7 $\pm$ 0.5	128 $\pm$ 4	94
4	9.7 $\pm$ 0.9	1.3 $\pm$ 0.2	7.5 $\pm$ 0.2	2.0 $\pm$ 0.5	22.0 $\pm$ 2.7	58.4 $\pm$ 7.7	6.7 $\pm$ 0.2	118 $\pm$ 12	952
5	7.6 $\pm$ 0.9	1.6 $\pm$ 0.2	5.6 $\pm$ 0.6	0.6 $\pm$ 0.0	18.0 $\pm$ 1.0	46.0 $\pm$ 8.5	5.6 $\pm$ 1.0	185 $\pm$ 11	530
6	5.6 $\pm$ 1.0	1.2 $\pm$ 0.2	8.0 $\pm$ 0.7	1.4 $\pm$ 0.2	35.5 $\pm$ 2.4	42.1 $\pm$ 4.7	4.6 $\pm$ 0.4	156 $\pm$ 8	401
7	4.4 $\pm$ 0.2	1.1 $\pm$ 0.2	6.0 $\pm$ 0.3	3.2 $\pm$ 0.4	32.8 $\pm$ 3.8	40.5 $\pm$ 8.4	5.8 $\pm$ 0.7	157 $\pm$ 8	347
8	9.5 $\pm$ 0.8	1.5 $\pm$ 0.2	7.8 $\pm$ 0.5	0.8 $\pm$ 0.2	18.1 $\pm$ 2.0	43.9 $\pm$ 10.7	7.5 $\pm$ 1.2	93 $\pm$ 13	1057
9	11.6 $\pm$ 1.6	1.8 $\pm$ 0.2	3.8 $\pm$ 0.4	1.6 $\pm$ 0.5	28.0 $\pm$ 3.3	62.9 $\pm$ 6.7	4.3 $\pm$ 0.3	101 $\pm$ 14	1244
10	3.7 $\pm$ 0.5	0.9 $\pm$ 0.1	9.2 $\pm$ 0.5	1.0 $\pm$ 0.2	26.5 $\pm$ 2.0	34.5 $\pm$ 3.7	7.8 $\pm$ 0.6	110 $\pm$ 10	563
11	11.9 $\pm$ 0.8	1.2 $\pm$ 0.1	7.0 $\pm$ 0.3	0.4 $\pm$ 0.1	14.4 $\pm$ 1.5	37.8 $\pm$ 5.4	5.7 $\pm$ 0.3	124 $\pm$ 12	825
12	4.2 $\pm$ 0.6	0.8 $\pm$ 0.1	5.8 $\pm$ 0.2	2.9 $\pm$ 0.4	29.5 $\pm$ 2.9	56.7 $\pm$ 9.4	5.0 $\pm$ 0.5	99 $\pm$ 9	528
13	7.8 $\pm$ 0.6	1.4 $\pm$ 0.2	5.6 $\pm$ 0.5	3.0 $\pm$ 0.5	49.6 $\pm$ 4.6	54.7 $\pm$ 2.8	5.5 $\pm$ 1.2	108 $\pm$ 10	657
14	4.7 $\pm$ 0.7	0.7 $\pm$ 0.1	8.1 $\pm$ 0.9	0.9 $\pm$ 0.1	40.0 $\pm$ 3.8	44.1 $\pm$ 11.0	7.0 $\pm$ 1.0	147 $\pm$ 11	683

<sup>a</sup> A ratio of relative brightness associated with Ca-specific fluorescence.

higher, but regionally typical, Al concentrations (Fig. 2). The average vigor index was also marginally higher for sites that contained deficient levels of foliar Ca ( $P = 0.063$ ) and Mg ( $P = 0.058$ ). A positive correlation was found when comparing foliar Al to percent branch dieback ( $r = 0.68$ ,  $P = 0.008$ ). Regardless of the categorization of other elements as deficient or toxic, no significant relationships were found between canopy condition or growth with foliar concentrations of K, Fe, Cu, and Mn.

### 3.3. Soil and foliar nutrient concentrations and relationships to growth

Average annual growth for the decade ending in 2001 was significantly correlated with soil nutrient concentrations in the rooting zone for Ca ( $r = 0.60$ ,  $P = 0.024$ ) and marginally with K ( $r = -0.49$ ,  $P = 0.073$ ). Although significant associations between Ca, Al, and Ca/Al molar ratios in the soil and canopy condition were not evident, significant correlations were found when comparing soil Ca/Al molar ratios with growth for the 1992–2001 decade ( $r = 0.57$ ,  $P = 0.031$ ; Fig. 3). The average Ca/Al molar ratio for each of the 14 sites was divided into 3 categories that were based on those established for soil solutions by Cronan and Grigal (1995):  $<0.2$  (expected to show a high risk of adverse impacts on tree growth and nutrition;  $n = 7$ ),  $0.2$ – $1.0$  (expected to show a moderate risk of adverse impacts;  $n = 4$ ), and  $>1.0$  (expected to show no negative impact;  $n = 3$ ). Under this categorization, marginally significant differences between the highest and lowest Ca/Al categories were found for basal area growth ( $P = 0.064$ ).

Mean basal area growth was positively correlated with mean foliar Ca concentration ( $r = 0.53$ ,  $P = 0.050$ ). In contrast, mean basal area growth decreased as mean foliar Al concentration of a site increased ( $r = -0.63$ ,  $P = 0.017$ ) (Fig. 4). An improved correlation was found when the foliar Ca/Al molar ratio was compared with growth ( $r = 0.67$ ,  $P = 0.009$ ) (Fig. 3). No other significant relationships were found between foliar nutrient concentrations and growth.

### 3.4. Foliar and soil relationships

The only significant correlations found between soil elements and their foliar counterparts were Ca ( $r = 0.63$ ,  $P = 0.016$ ) and Al ( $r = 0.63$ ,  $P = 0.015$ ). Additional strong correlations were found between foliar Ca and soil Al ( $r = -0.72$ ,  $P = 0.004$ ), soil Fe ( $r = -0.78$ ,  $P = 0.0009$ ), and soil K ( $r = -0.70$ ,  $P = 0.005$ ). There were strong linear relationships between foliar Ca and foliar Al ( $r = -0.63$ ,  $P = 0.015$ ), foliar K ( $r = -0.55$ ,  $P = 0.043$ ), and foliar Mg ( $r = 0.80$ ,  $P = 0.0006$ ). Although linear relationships were not strong, logarithmic (natural log of soil Ca) correlations were found between soil Ca and soil Al ( $r = -0.83$ ,  $P = 0.0003$ ), soil K ( $r = -0.54$ ,  $P = 0.046$ ), and Fe ( $r = -0.78$ ,  $P = 0.001$ ), suggesting non-linear interactions or antagonistic relationships between these elements and Ca. Soil K and Fe were also significantly correlated ( $r = 0.66$ ,  $P = 0.011$ ).

### 3.5. Membrane-associated calcium

At the tree level, there was no correlation between foliar Ca and mCa ( $r = -0.011$ ,  $P = 0.717$ ). However, a weak correlation between these parameters was found when trees with foliar Ca concentrations below the Ca deficiency threshold ( $<5000$  mg kg<sup>-1</sup>) were evaluated ( $r = 0.126$ ,  $P = 0.049$ ). Correlations of foliar mCa and growth also showed no relationship ( $r = -0.001$ ,  $P = 0.361$ ), and only a weak relationship at the lower half of the mCa range ( $r = -0.051$ ,  $P = 0.093$ ). At the plot level, significant relationships were not found between foliar mCa concentrations and other variables except mCa variance. The variance of mCa for each site was calculated using the mean mCa value for each tree within the site. A strong correlation was found between mean foliar Ca and mCa variance among sites ( $r = 0.82$ ,  $P = 0.0006$ ) (Fig. 5). Differences among mCa variances were found when sites were evenly divided into either high ( $n = 7$ ) or low ( $n = 7$ ) quality stands based on vigor class ( $P = 0.042$ ), crown transparency ( $P = 0.048$ ), and, marginally, for basal area growth from 1992 to 2001 ( $P = 0.076$ ).

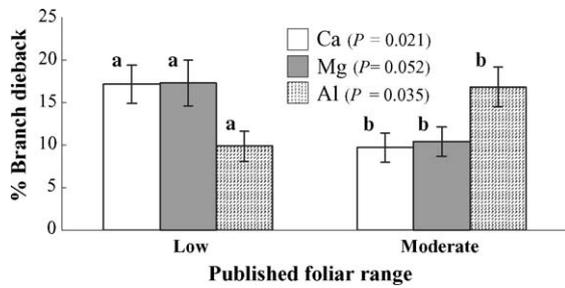


Fig. 2. Means ( $\pm$ S.E.) of percent branch dieback for sugar maple study sites that exhibited differences between low and moderate levels of foliar Ca, Mg, and Al. Previously published foliar nutrient data were used to classify stands as either low or moderate in concentrations of foliar Ca (low below 5000 mg kg<sup>-1</sup>,  $n = 5$  and 9), Mg (low below 1100 mg kg<sup>-1</sup>,  $n = 4$  and 10) and Al (low below 31 mg kg<sup>-1</sup>,  $n = 9$  and 5; Kolb and McCormick, 1993). Per foliar nutrient, means without common letters differ significantly based on ANOVA tests.

4. Discussion

The TSM generally predicted the relative soil and foliar Ca status of sites used in this study (Fig. 1). In addition, TSM classes significantly differed in branch dieback and growth. Combined, these results suggest that it may be possible to identify stands on glaciated soils that are susceptible to decline based on TSM predictions of Ca content prior to signs of health deterioration or specific nutrient evaluation.

The ability of the TSM to predict extractable Ca in the rooting zone was good but not perfect, and some sites expected to contain high Ca concentrations fell within the range of predicted low Ca sites. This limitation was not unexpected as TSM predicts bulk Ca in the soil parent material, not available Ca in the rooting zone. Numerous biotic and abiotic processes influence soil nutrient levels. Recent disturbance, such as anthropogenic Ca depletion, may also affect the relationship

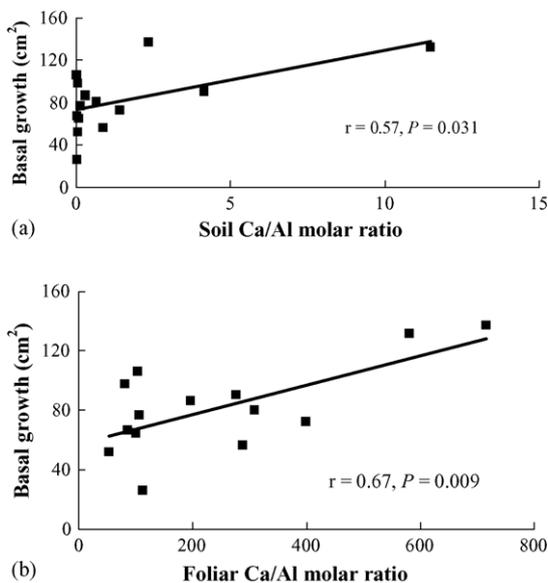


Fig. 3. Correlations of: (a) mean soil Ca/Al molar ratio and average basal area growth for 1992–2001 and (b) mean foliar Ca/Al molar ratio and average basal area growth for 1992–2001 for the 14 sugar maple stands assessed. Regression lines are presented to assist with the visual interpretation of data.

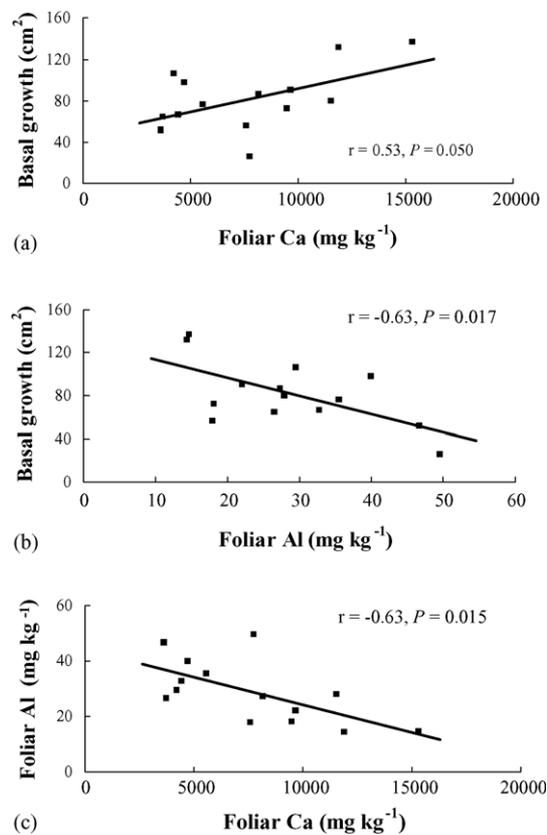


Fig. 4. Correlations of: (a) mean foliar Ca concentration and average basal area growth for 1992–2001, (b) mean foliar Al concentration and average basal area growth for 1992–2001, and (c) mean foliar Ca and mean foliar Al concentrations for the 14 sugar maple stands assessed. Regression lines are presented to assist with the visual interpretation of data.

between native soil quality and available nutrient supplies (Likens et al., 1998). For example, inputs of Ca by weathering (1–2 kg/ha/year) and deposition (1–3.2 kg/ha/year) can be negligible compared with outputs by leaching (4–49 kg/ha/year) and biomass removal (200–1100 kg/ha/year) (Federer et al., 1989; Adams, 1999; Huntington, 2000). Despite these complications, the TSM appeared effective at delineating high and low Ca categories for Vermont.

Concentrations of extractable Al, Ca, Mg, K, Fe, and Cu in soil (Table 3) were generally within the range reported for other

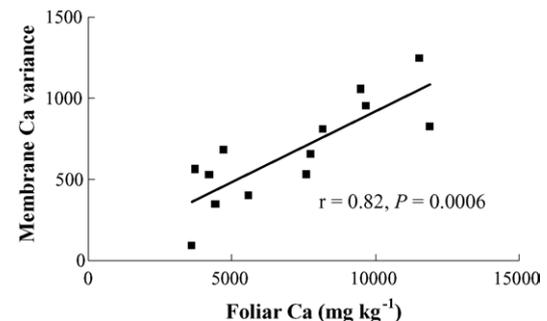


Fig. 5. The correlation of the variance of membrane associated calcium (mCa) and mean foliar Ca among sites. The mCa variance for each site was calculated using measured mCa values for each tree within a site ( $n = 6$ ). A regression line is presented to assist with the visual interpretation of data.

Table 5  
Sugar maple health and growth data (mean  $\pm$  S.E.) for the 14 study sites

Site	Transparency (%)	Branch dieback (%)	Tree vigor	Mean annual BAI (cm) 1992–2001
1	30.0 $\pm$ 5.8	9.2 $\pm$ 0.8	3.0 $\pm$ 0.36	13.7 $\pm$ 3.9
2	26.7 $\pm$ 3.3	8.3 $\pm$ 2.5	2.0 $\pm$ 0.25	8.7 $\pm$ 2.5
3	26.7 $\pm$ 3.3	23.3 $\pm$ 7.5	3.5 $\pm$ 0.22	5.2 $\pm$ 1.2
4	13.3 $\pm$ 2.1	5.0 $\pm$ 1.3	1.5 $\pm$ 0.22	9.0 $\pm$ 2.9
5	25.0 $\pm$ 2.9	6.3 $\pm$ 1.3	2.9 $\pm$ 0.16	5.6 $\pm$ 1.6
6	20.0 $\pm$ 0.0	4.2 $\pm$ 0.8	1.7 $\pm$ 0.17	7.6 $\pm$ 1.6
7	31.7 $\pm$ 6.0	16.7 $\pm$ 7.0	2.5 $\pm$ 0.42	6.7 $\pm$ 2.6
8	23.3 $\pm$ 2.1	15.0 $\pm$ 5.5	2.3 $\pm$ 0.33	7.2 $\pm$ 1.8
9	23.3 $\pm$ 2.1	14.2 $\pm$ 3.7	2.3 $\pm$ 0.33	8.0 $\pm$ 2.0
10	30.0 $\pm$ 4.5	12.5 $\pm$ 2.5	2.5 $\pm$ 0.22	6.5 $\pm$ 1.9
11	18.3 $\pm$ 3.1	6.7 $\pm$ 1.1	2.0 $\pm$ 0.26	13.2 $\pm$ 3.1
12	21.7 $\pm$ 3.1	11.7 $\pm$ 2.8	2.2 $\pm$ 0.31	10.6 $\pm$ 3.4
13	31.7 $\pm$ 4.0	18.3 $\pm$ 3.1	3.0 $\pm$ 0.26	2.6 $\pm$ 0.5
14	21.7 $\pm$ 1.7	21.7 $\pm$ 4.8	3.7 $\pm$ 0.21	9.8 $\pm$ 4.5

Health measurements included percent crown transparency, percent branch dieback, and crown vigor index, which ranged from 1 (healthy) to 4 (almost dead). Growth was measured as mean basal area increment (BAI).

sugar maple and hardwood stands in eastern North America (Morrison, 1990; Foster et al., 1992; Kolb and McCormick, 1993; Heisey, 1995; Wilmot et al., 1995), although concentrations of Ca and Mg were sometimes near the low end of the range. Study sites also represented a broad range in sugar maple health (Table 5). Many soil elements were significantly related to growth and health measurements. For example, stands with higher concentrations of soil Fe and K tended to experience greater branch dieback. However, soil Fe and K were also highly correlated with Ca and each other, suggesting that Ca concentrations or other inherent soil properties (e.g., pH, clay content, cation exchange capacity, and organic content), may have influenced both soil nutrient relations and tree health. The only cases where higher sugar maple growth coincided with higher soil element concentrations involved Ca and Ca/Al ratios.

In contrast to soil elements, only foliar Ca and Al were consistently related to both stand health and productivity. Even though it occurred at potentially toxic levels in six stands (Table 2), for unknown reasons, foliar Mn was not associated with crown deterioration or growth declines. ANOVA analyses indicated that stands categorized as having sufficient foliar Ca had lower branch dieback (Fig. 2) and greater basal area growth than stands with foliar Ca concentrations below the deficiency threshold. Foliar Ca concentration was also positively correlated with growth (Fig. 4). High foliar Al was correlated with increased branch dieback and decreased basal area growth within stands (Fig. 4). The specificity of foliar Ca and Al relations to health and growth measures emphasizes the likely biological importance of these elements to sugar maple vigor in Vermont. This specificity also supports the possibility that associations of soil elements other than Ca and Al with stand health and productivity are the result of autocorrelations among soil constituents brought about by inherent soil or climatic properties, and are not indicative of their direct influence on vigor at these sites.

Associations between soil and foliar elements highlight the incongruities between these two pools. Foliar Ca and Al were

the only elements that exhibited significant correlations with their soil counterparts. For foliar and soil Ca, negative correlations were found with soil Al, Fe, and K. Negative relationships have been previously documented for soil and foliar Ca with soil Al (Lawrence et al., 1995; Marschner, 1995), Fe (Agarwala and Mehrotra, 1984), and K (Tomlinson, 1990). It is unlikely these soil nutrients directly contributed to decline within our study sites because soil and foliar concentrations of Al, Fe, and K generally fell within the published range for maple forests judged to be healthy based on a lack of decline or nutrient deficiency symptoms (Tables 2–4, and see Burton et al., 1993; Kolb and McCormick, 1993). Instead, inverse relationships of soil and foliar Ca concentrations with soil Al, Fe, and K may better represent nutrient antagonism at soil exchange sites and the soil–root interface, as well as anthropogenic disruptions of nutrient cycling (Marschner, 1995; Likens et al., 1998).

Hydrogen (H) loading by acidic deposition can mobilize Al in the soil solution and these ions may compete with Ca for adsorption at soil cation-exchange sites (Lawrence et al., 1995; Brady and Weil, 2002). Once displaced to the soil solution, Ca may be absorbed by roots or lost by leaching. High Al levels can also inhibit Ca uptake at the soil–root interface (Andersson, 1988) or directly interfere with root growth (Joslin and Wolfe, 1988). Several studies have suggested that low Ca/Al ratios may be indicative of potential decline or productivity loss (see review by Cronan and Grigal, 1995). Evidence from the present study supports this contention.

Despite the associations found between sugar maple health and soil Ca/Al ratios, results from this study suggest that foliar Ca, Al, and Ca/Al concentrations may be better indicators of tree health. This is in contrast to results put forth by Cronan and Grigal (1995). The disparity is likely due to methodological differences between the studies, including age and location of study trees, measurements of soil concentrations, and the focus on sugar maple in the current study. Cronan and Grigal (1995) examined studies of several tree species that typically focused on soil solution nutrient concentrations of seedlings grown in

greenhouses rather than extractable nutrients of mature stands in natural soils. The low levels of soil Ca for many of the sites we studied may have provided foliar Ca concentrations low enough to diminish Ca-oxalate accumulation (Fink, 1991; Zindler-Frank et al., 2001) that otherwise could have confounded assessment of the relationship of total foliar Ca with tree health and growth. Further field studies are needed to determine whether soil or tree Ca/Al ratio measurements are better to identify nutrient thresholds that meaningfully influence tree health.

Whether due to the influence of soil parent material, the antagonistic effects of different soil nutrients, or other inherent soil and climatic factors, the Ca and Al status of stands (particularly of foliage) was consistently associated with growth and health measures (Figs. 2–4). This is accordant with recent literature examining sugar maple decline (e.g., Wilmot et al., 1995; Lyon and Sharpe, 1999), and the potential Ca deficiency thresholds noted by others were reflected in the low Ca–low vigor relationships we documented. While relationships of Ca and Mg concentrations with tree vigor were consistent with previous results, foliar Al concentrations (Table 4) that were associated with poor vigor in this study (Fig. 2) were considerably lower than typical toxicity levels reported for maple ( $>60 \text{ mg kg}^{-1}$ ) (Thornton et al., 1986; Kolb and McCormick, 1993; Cote and Camire, 1995). This suggests that Al was not directly toxic to study trees, but indirectly impacted growth and vigor, probably through well-documented influences on Ca nutrition (e.g., Shortle and Smith, 1988; Lawrence et al., 1995).

The strong influence of Ca status on stand productivity and vigor highlights the direct significance of Ca to stress response physiology and tree growth. Environmental stimulation such as wounding, drought, pathogen infection, or a sudden temperature change causes an influx of Ca into the cytoplasm, primarily from the plasma membrane (Pinos and Tester, 1997; White, 1998) and vacuole (Knight et al., 1997). Temporary elevations in cytoplasmic Ca mediate an assortment of cellular functions, including gene expression, cell extension, carbohydrate metabolism, ionic balance, and mitosis (Hepler and Wayne, 1985). Because the magnitude of a cellular response is proportional to Ca concentration, compromised Ca availability may inhibit cellular response because influx machinery is not properly activated (Palta, 1990). In addition, Ca provides cellular structural stability by bridging phosphate and carboxylate groups of phospholipids (Caldwell and Haung, 1981) and proteins at the membrane surface (Legge et al., 1982), and by cross-linking pectic chains in the middle lamella of cell walls. In the absence of an adequate Ca supply, cell extension may cease (Marschner and Richter, 1974) or solute leakage may occur from the cytoplasm (Arora and Palta, 1988).

To more specifically evaluate the influence of Ca on stand health and potential stress response, mCa was also measured for sites. Correlations of tree means supported past findings that mCa limitations are biologically relevant: there was a significant relationship between foliar Ca and mCa only below the established Ca deficiency threshold of  $5000 \text{ mg kg}^{-1}$

( $r = 0.126$ ,  $P = 0.049$ ), and there was a weak association with growth at the lower half of the mCa range ( $r = -0.051$ ,  $P = 0.093$ ). However, no differences in mCa means were found among sites. This lack of differentiation among sites could be an artifact of sampling. Studies evaluating mCa of trees sampled on a single date have consistently indicated that mCa can be a better indicator of biological Ca availability and associated physiology than total foliar pools (DeHayes et al., 1999; Schaberg et al., 2000; Borer et al., 2004). Yet, mCa is also seasonally variable and sensitive to fluctuations of environmental (e.g., temperature) cues (DeHayes et al., 1997; Schaberg and DeHayes, 2000). Due to unavoidable logistical constraints, foliar sampling extended over a 3-week period when seasonal accrual of mCa also occurred (Lazarus et al., 2002). Thus, differences in biological mCa pools were potentially confounded with seasonal mCa accrual patterns.

Despite shortcomings in sampling, some data suggest that the capacity of mesophyll cells to maintain Ca on membranes differed among sites coincident with foliar Ca levels. At sites containing trees with low foliar Ca concentrations, mCa levels were low, and variation around mCa means was limited (Fig. 5). Although mCa averages were not significantly higher at sites with elevated foliar Ca concentrations, variation around means was greater (Fig. 5). Evidence with red spruce suggests that Ca deficient trees have uniformly low mCa levels, whereas Ca sufficient trees have high mCa levels before stress events but low mCa levels indistinguishable from Ca deficient trees following stress signaling (Borer, 2004). Adequate foliar stores of Ca may allow for expedited recharge of mCa following signaling. A capacity for enhanced mCa recharge would help stabilize membranes, expedite continued Ca-dependent signaling, provide for appropriate physiological response to stress, and better support continued tree health and growth. Consistent with this possibility, the higher variance in mCa at high Ca sites was significantly associated with greater tree vigor ( $P = 0.042$ ), reduced crown transparency ( $P = 0.048$ ), and greater basal area growth ( $P = 0.076$ ).

In some respects, the strong and consistent relationships of foliar Ca and Al concentrations with stand growth and vitality measures are surprising. Assessed stands included a broad geographic and elevation range within the state, and encompassed a wide variety of site factors (e.g., differences in drainage, aspect, etc.) that were not accounted for by TSM site selection criteria, yet which likely affected tree growth and health. Furthermore, stands differed in average tree age and site history. Despite these and other dissimilarities among sites that added unaccounted variability, the underlying importance of Ca and Al nutrition to sugar maple growth and vigor remained evident.

## 5. Conclusions

Numerous past studies have examined the relationships between foliar and/or soil nutrient status with the decline of sugar maple forests (e.g., Bernier and Brazeau, 1988a; Ouimet and Fortin, 1992; Kolb and McCormick, 1993; Wilmot et al., 1995; Long et al., 1997), and the variation of results suggest that

nutrient imbalances may be localized and dependent on some combination of anthropogenic impacts, soil properties, and climate. Although several soil element concentrations showed some relation to tree growth or health in the current study, only Ca and Al (particularly foliar) concentrations were consistently and strongly related to maple growth and crown condition. The particular relevance of stand Ca status to vigor in the 14 stands we evaluated has important management implications: at least some Vermont forests appear to be close to crossing thresholds of meaningful Ca deficiency. It is anticipated that more forests will approach deficiency levels as acidic deposition (Likens et al., 1996), declines in atmospheric base cation deposition (Hedin et al., 1994), soil Al mobilization (Lawrence et al., 1995), nitrogen saturation (Aber et al., 1998), and other anthropogenic factors continue to deplete environmental Ca stores.

### Acknowledgements

We thank Rock Ouimet (MRNFP—Québec), Stephen Horsley (USDA Forest Service), Timothy Wilmot (University of Vermont), and three anonymous reviewers for valuable input on earlier drafts of this manuscript. Thanks are also extended to Sandy Wilmot (Vermont Agency of Natural Resources) for providing data from the Northern Hardwood Health Survey and North American Maple Project. We are indebted to T. Coe, B. Lazarus, P. Murakami, H. Heitz, M. Turner, and S. Nijensohn for their technical assistance in the field and laboratory. We are especially grateful to the Vermont landowners who generously granted permission to collect samples from their property. License to sample from public lands was granted by Vermont's Agency of Natural Resources, Department of Forests, Parks and Recreation, and Department of Fish and Wildlife. This research was supported by funds provided by the Northeastern Ecosystem Research Cooperative, the U.S. Environmental Protection Agency, the Northeastern States Research Cooperative, and the USDA CSREES McIntire-Stennis Forest Research Program.

### References

- Aber, J., McDowell, W., Nadelhoffer, K., Magill, A., Berntson, G., Kamakea, M., McNulty, S., Currie, W., Rustad, L., Fernandez, I., 1998. Nitrogen saturation in temperate forest ecosystems. *Bioscience* 48, 921–934.
- Adams, M.B., 1999. Acidic deposition and sustainable forest management in the central Appalachians, USA. *For. Ecol. Manage.* 122, 17–28.
- Agarwala, S.C., Mehrotra, S.C., 1984. Iron–magnesium antagonism in growth and metabolism of radish. *Plant Soil* 80, 355–361.
- Allen, D.C., Barnette, C.J., Millers, I., Lachance, D., 1992a. Temporal change (1988–1990) in sugar maple health, and factors associated with crown condition. *Can. J. For. Res.* 22, 1776–1784.
- Allen, D.C., Bauce, E., Barnett, J.C., 1992b. Sugar maple declines—causes, effects, and recommendations. In: Manion, P.D., Lachance, D. (Eds.), *Forest Decline Concepts*. APS Press, New York, pp. 123–136.
- Andersson, M., 1988. Toxicity and tolerance of aluminum in vascular plants: a literature review. *Water Air Soil Pollut.* 39, 439–462.
- Arora, R., Palta, J.P., 1988. In vivo perturbation of membrane-associated calcium by freeze–thaw stress on onion bulb cells. Simulation of this perturbation in extracellular KCL and alleviation by calcium. *Plant Physiol.* 85, 622–628.
- Bailey, S.W., Hornbeck, J.W., 1992. Lithologic Composition and Rock Weathering Potential of Forested, Glacial-Till Soils. USDA Forest Service Research Pa NE-662.
- Bernier, B., Brazeau, M., 1988a. Foliar nutrient status in relation to sugar maple dieback and decline in the Quebec Appalachians. *Can. J. For. Res.* 18, 754–761.
- Bernier, B., Brazeau, M., 1988b. Nutrient deficiency symptoms associated with sugar maple dieback and decline in Quebec Appalachians. *Can. J. For. Res.* 18, 1265–1269.
- Bernier, B., Brazeau, M., 1988c. Magnesium deficiency symptoms associated with sugar maple dieback and decline in Quebec Appalachians. *Can. J. For. Res.* 18, 762–767.
- Borer, C.H., 2004. Calcium physiology in the foliage of red spruce: implications for forest health. Ph.D. Dissertation. University of Vermont, Burlington.
- Borer, C.H., DeHayes, D.H., Schaberg, P.G., Cumming, J.R., 1997. Relative quantification of membrane-associated calcium in red spruce mesophyll cells. *Trees* 12, 21–26.
- Borer, C.H., Schaberg, P.G., DeHayes, D.H., Hawley, G.J., 2004. Accretion, partitioning and sequestration of calcium and aluminum in red spruce foliage: implications for tree health. *Tree Physiol.* 24, 929–939.
- Brady, N.C., Weil, R.R., 2002. *The Nature and Properties of Soil*. Pearson Education, Inc., NJ, USA.
- Burton, A.J., Pregitzer, K.S., MacDonald, N.W., 1993. Foliar nutrients in sugar maple forests along a regional pollution–climate gradient. *Soil Sci. Soc. Am. J.* 57, 1619–1628.
- Caldwell, C.R., Haug, A., 1981. Temperature dependence of the barley root plasma membrane-bound  $\text{Ca}^{2+}$  and  $\text{Mg}^{2+}$ -dependent ATPase. *Plant Physiol.* 53, 117–124.
- Christie, J.M., Jenkins, G.I., 1996. Distinct UV-B and UV-A/blue light signal transduction pathways induce chalcone synthase gene expression in *Arabidopsis* cells. *Plant Cell* 8, 1555–1567.
- Cook, E.R., Kairiukstis, L.A., 1989. *Methods of Dendrochronology: Applications in the Environmental Sciences*. Kluwer Academic Publishers, USA.
- Cooke, R., Pendrel, B., Barnett, C., Allen, D., 1996. *North American Maple Project Cooperative Field Manual*. USDA Forest Service.
- Cote, B., Camire, C., 1995. Application of leaf, soil, and tree ring chemistry to determine the nutritional status of sugar maple on sites of different levels of decline. *Water Air Soil Pollut.* 83, 363–373.
- Cronan, C.S., Grigal, D.F., 1995. Use of calcium/aluminum ratios as indicators of stress in forest ecosystems. *J. Environ. Qual.* 23, 209–226.
- DeHayes, D.H., Schaberg, P.G., Hawley, G.J., Borer, C.H., Cumming, J.R., Stimpbeck, G.R., 1997. Physiological implications of seasonal variation in membrane-associated calcium in red spruce mesophyll cells. *Tree Physiol.* 17, 687–695.
- DeHayes, D.H., Schaberg, P.G., Hawley, G.J., Stimpbeck, G.R., 1999. Alteration of membrane-associated calcium leads to membrane destabilization and foliar injury in red spruce. *Bioscience* 49, 789–800.
- Ellis, R.C., 1975. Sampling deciduous broadleaved trees for the determination of leaf weight and foliar elemental concentrations. *Can. J. For. Res.* 5, 310–317.
- Ellsworth, D.S., Liu, X., 1994. Photosynthesis and canopy nutrition of four sugar maple forests on acid soils in northern Vermont. *Can. J. For. Res.* 24, 2118–2127.
- Federer, C.A., Hornbeck, J.W., Tritton, L.M., Martin, C.W., Pierce, R.S., Smith, C.T., 1989. Long-term depletion of calcium and other nutrients in eastern US forests. *Environ. Manage.* 14, 593–601.
- Fink, S., 1991. The micromorphological distribution of bound calcium in needles of Norway spruce [*Picea abies* (L.) Karst.] *New Phytol.* 119, 33–40.
- Foster, N.W., Morrison, I.K., Xiwei, Y., Arp, P.A., 1992. Impact of soil water deficits in a mature sugar maple forest: stand biogeochemistry. *Can. J. For. Res.* 22, 1753–1760.
- Gong, M., van der Luit, A.H., Knight, M.R., Trewavas, A.J., 1998. Heat-shock-induced changes in intracellular  $\text{Ca}^{2+}$  level in tobacco seedlings in relation to thermotolerance. *Plant Physiol.* 116, 429–437.
- Hedin, L.O., Granat, L., Likens, G.E., Bulshand, T.A., Galloway, J.N., Butler, T.J., Rodhe, H., 1994. Steep declines in atmospheric base cations in regions of Europe and North America. *Nature* 367, 351–354.

- Heisey, R.M., 1995. Growth trends and nutritional status of sugar maple stands on the Appalachian plateau of Pennsylvania, USA. *Water Air Soil Pollut.* 82, 675–693.
- Hepler, P.K., Wayne, R.O., 1985. Calcium and plant development. *Ann. Rev. Plant Physiol.* 36, 397–439.
- Horsley, S.B., Long, R.P., Bailey, S.W., Hallet, R.A., Hall, T.J., 2000. Factors associated with the decline disease of sugar maple on the Allegheny Plateau. *Can. J. For. Res.* 30, 1365–1378.
- Huntington, T.G., 2000. The potential for calcium depletion in forest ecosystems of southeastern United States: review and analysis. *Global Biogeochem. Cycles* 14, 623–638.
- Jagels, R., Jiang, M., Marden, S., Carlisle, J., 2002. Red spruce canopy response to acid fog exposure. *Atmos. Res.* 64, 169–178.
- Jiang, M., Jagels, R., 1999. Detection and quantification of changes in membrane-associated calcium in red spruce saplings exposed to acid fog. *Tree Physiol.* 19, 909–916.
- Jones Jr., J.B., Case, V.W., 1990. Sampling, handling, and analyzing plant tissue samples. In: Westerman, R.L. (Ed.), *Soil Testing and Plant Analysis: Science of America Book Series*. Soil Science Society of America, Inc., Madison, WI.
- Joslin, J.D., Wolfe, M.H., 1988. Responses of red spruce seedlings to changes in soil aluminum in six amended forest soil horizons. *Can. J. For. Res.* 18, 1614–1623.
- Kelley, R.S., 1988. The relationship of defoliators to recent hardwood dieback and decline in Vermont. In: *Proceedings of the 21st Annual Northeast Forest Insect Work Conference*, State University of New York Albany, New York, 2–3 March, p. 47.
- Kelley, R.S., Smith, E.L., Cox, S.M., 1992. Vermont Hardwood Tree Health in 1991 Compared to 1986. Vermont Department of Forests, Parks and Recreation, Waterbury, VT, 25 pp.
- Knight, H., Trewavas, A.J., Knight, M.R., 1997. Calcium signaling in *Arabidopsis thaliana* responding to drought and salinity. *Plant J.* 12, 1067–1078.
- Kocman, V., Peel, T.E., Tomlinson, G.H., 1991. Rapid analysis of macro and micro nutrients in leaves and vegetation by automated X-ray fluorescence spectrometry (a case study of an acid-rain affected forest). *Commun. Soil Sci. Plant Anal.* 22, 2063–2075.
- Kolb, T.E., McCormick, L.H., 1993. Etiology of sugar maple decline in four Pennsylvania stands. *Can. J. For. Res.* 23, 2395–2402.
- Lawrence, G.B., David, M.B., Shortle, W.C., 1995. A new mechanism for calcium loss in forest-floor soils. *Nature* 378, 162–164.
- Lazarus, B.E., Schaberg, P.G., Murakami, P.F., Coe, T., Hawley, G.J., 2002. Understanding calcium deficiency in sugar maple (*Acer saccharum*) by assessing foliar membrane-associated calcium. In: *Proceedings of the 17th North American Forest Biology Workshop and the Western Forest Genetics Association*, Washington State University, Pullman, Washington, 15–18 July, p. 53.
- Lea, R., Tierson, W.C., Bickelhaupt, D.H., Leaf, A.L., 1980. Differential foliar response of northern hardwood fertilization. *Plant Soil.* 54, 419–439.
- Legge, R.L., Thompson, J.E., Baker, J.E., Lieberman, M., 1982. The effect of calcium on the fluidity and phase properties of microsomal membranes isolated from postclimacteric Golden Delicious apples *Malus*. *Plant Cell Physiol.* 23, 161–169.
- Likens, G.E., Driscoll, C.T., Buso, D.C., 1996. Long-term effects of acid rain: response and recovery of a forest ecosystem. *Science* 272, 244–246.
- Likens, G.E., Driscoll, C.T., Buso, D.C., Siccama, T.G., Johnson, C.E., Lovett, G.M., Fahey, T.J., Reiners, W.A., Ryan, D.F., Martin, C.W., Bailey, S.W., 1998. The biogeochemistry of calcium at Hubbard Brook. *Biogeochemistry* 41, 89–173.
- Long, R.P., Horsley, S.B., Lilja, P.R., 1997. Impacts of forest liming on growth and crown vigor of sugar maple and associated hardwoods. *Can. J. For. Res.* 27, 1560–1573.
- Lyon, J., Sharpe, W.E., 1999. An assessment of Ca:Al ratios of selected Pennsylvania forest soils. *Water Air Soil Pollut.* 109, 53–65.
- Mader, D.L., Thompson, B.W., 1969. Foliar and soil nutrients in relation to sugar maple decline. *Soil Sci. Soc. Am. Proc.* 33, 794–800.
- Marschner, H., 1995. *Mineral Nutrition of Higher Plants*. Academic Press, New York.
- Marschner, H., Richter, C., 1974. Calcium-transport in Wurzeln von Mais- und Bohnenkeimpflanzen. *Plant Soil* 40, 193–210.
- McIntosh, J.L., 1969. Bray and Morgan soil test extractants modified for testing acid soils from different parent materials. *Agron. J.* 61, 259–265.
- McLaughlin, D.L., Linzon, S.N., Dimma, D.E., McIlveen, W.D., 1987. Sugar maple decline in Ontario. In: Hutchinson, T.C., Meema, K.M. (Eds.), *Effects of Atmospheric Pollutants on Forests, Wetlands, and Agricultural Ecosystems*. Springer-Verlag, New York, pp. 101–116.
- McLaughlin, S.B., Anderson, C.P., Hanson, P.J., Tjoelker, M.G., Roy, W.K., 1991. Increased dark respiration and calcium deficiency of red spruce in relation to acidic deposition at high elevation southern Appalachian mountain sites. *Can. J. For. Res.* 21, 1234–1244.
- McLaughlin, S.B., Wimmer, R., 1999. Calcium physiology and terrestrial ecosystem processes. *New Phytol.* 142, 373–417.
- Monroy, A.F., Sarhan, F., Dhindsa, R.S., 1993. Cold-induced changes in freezing tolerance, protein phosphorylation, and gene expression: evidence for the role of calcium. *Plant Physiol.* 102, 1227–1235.
- Montgomery, D.C., 2001. *Design and Analysis of Experiments*. John Wiley and Sons, New York.
- Moore, J.D., Camire, C., Ouimet, R., 2000. Effects of liming on the nutrition, vigor, and growth of sugar maple at the Lake Clair Watershed, Quebec, Canada. *Can. J. For. Res.* 30, 725–732.
- Morrison, I.K., 1985. Effect of crown position on foliar concentrations of 11 elements in *Acer saccharum* and *Betula alleghaniensis* trees on a till soil. *Can. J. For. Res.* 15, 179–183.
- Morrison, I.K., 1990. Organic matter and mineral distribution in an old-growth *Acer-saccharum* forest near the northern limit of its range. *Can. J. For. Res.* 20, 1332–1342.
- Ouimet, R., Camire, C., 1995. Foliar deficiencies of sugar maple stands associated with soil cation imbalances in Quebec Appalachians. *Can. J. Soil Sci.* 75, 169–175.
- Ouimet, R., Duchesne, L., Houle, D., Arp, P.A., 2001. Critical loads of atmospheric S and N deposition and current exceedances for Northern temperate and boreal forests in Quebec. *Water Air Soil Pollut.: Focus* 1, 119–134.
- Ouimet, R., Fortin, J.M., 1992. Growth and foliar nutrient status of sugar maple: incidence of forest decline and reaction to fertilization. *Can. J. For. Res.* 22, 699–706.
- Palta, J.P., 1990. Stress interactions at the cellular and membrane levels. *HortScience* 25, 1377–1382.
- Pare, D., Bernier, B., 1989. Origin of the phosphorus deficiency observed in declining sugar maple stands in Quebec Appalachians. *Can. J. For. Res.* 19, 24–34.
- Payette, S., Fortin, M.J., Morneau, C., 1996. The recent sugar maple decline in southern Quebec: probable causes deduced from tree rings. *Can. J. For. Res.* 26, 1069–1078.
- Pineros, M., Tester, M., 1997. Calcium channels in higher plants: selectivity, regulation and pharmacology. *J. Exp. Bot.* 48, 551–577.
- Richter, D.D., Johnson, D.W., Dai, K.H., 1992. Cation exchange reactions in acid forest soils: effects of atmospheric pollutant deposition. In: Johnson, D.W., Lindberg, S.E. (Eds.), *Atmospheric Deposition and Forest Nutrient Cycling*. Springer-Verlag, Berlin, Heidelberg, pp. 340–358.
- Robitaille, G., Boutin, R., Lachance, D., 1995. Effects of soil freezing stress on sap flow and sugar maple content of sugar maples (*Acer saccharum*). *Can. J. For. Res.* 25, 577–587.
- Schaberg, P.G., DeHayes, D.H., 2000. Physiological and environmental causes of freezing injury in red spruce. In: Mickler, R.A., Birdsey, R.A., Hom, J.L. (Eds.), *Responses of Northern US Forests to Environmental Change*. Springer-Verlag, New York, (Chapter 6), pp. 181–227.
- Schaberg, P.G., DeHayes, D.H., Hawley, G.J., 2001. Anthropogenic calcium depletion: a unique threat to forest ecosystem health? *Ecosyst. Health* 7, 214–228.
- Schaberg, P.G., DeHayes, D.H., Hawley, G.J., Strimbeck, G.R., Cumming, J.R., Murakami, P.F., Borer, C.H., 2000. Acid mist, soil Ca and Al alter the mineral nutrition and physiology of red spruce. *Tree Physiol.* 20, 73–85.
- Shepard, J.P., Mitchell, M.J., Scott, T.J., Driscoll, C.T., 1990. Soil solution chemistry of an Adirondack Spodosol: lysimetry and N dynamics. *Can. J. For. Res.* 20, 818–824.

- Shortle, W.C., Smith, K.T., 1988. Aluminum-induced calcium deficiency syndrome in declining red spruce. *Science* 240, 238–240.
- Thorton, F.C., Schaedle, M., Raynal, D.J., 1986. Effect of aluminum on the growth of sugar maple in solution culture. *Can. J. For. Res.* 16, 892–896.
- Tomlinson, G.H., 1990. Nutrient disturbances in forest trees and the nature of the forest decline in Quebec and Germany. *Water Air Soil Pollut.* 54, 61–74.
- Trewavas, A., Knight, M.R., 1994. Mechanical signaling, calcium and plant form. *Plant Mol. Biol.* 26, 1329–1341.
- White, P.J., 1998. Calcium channels in the plasma membrane of root cells. *Ann. Bot.* 81, 173–183.
- Wilmot, T.R., Ellsworth, D.S., Tyree, M.T., 1995. Relationships among crown condition, growth, and stand nutrition in seven northern Vermont sugar-bushes. *Can. J. For. Res.* 25, 386–397.
- Wilmot, T.R., Ellsworth, D.S., Tyree, M.T., 1996. Base cation fertilization and liming effects on nutrition and growth of Vermont sugar maple stands. *For. Ecol. Manage.* 84, 123–134.
- Zindler-Frank, E., Hönow, R., Hesse, A., 2001. Calcium and oxalate content of the leaves of *Phaseolus vulgaris* at different calcium supply in relations to calcium oxalate crystal formation. *J. Plant Physiol.* 158, 139–144.