Long-term calcium addition increases growth release, wound closure, and health of sugar maple (*Acer saccharum*) trees at the Hubbard Brook Experimental Forest

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Abstract: We surveyed and wounded forest-grown sugar maple (*Acer saccharum* Marsh.) trees in a long-term, replicated Ca manipulation study at the Hubbard Brook Experimental Forest in New Hampshire, USA. Plots received applications of Ca (to boost Ca availability above depleted ambient levels) or Al (to compete with Ca uptake and further reduce Ca availability). We found significantly greater total foliar and membrane-associated Ca in foliage of trees in plots fertilized with Ca when compared with trees from Al-addition and control plots ($P = 0.005$). Coinciding with foliar Ca differences, trees exhibited a significant difference in crown vigor and in percent branch dieback among treatments ($P < 0.05$), with a trend towards improved canopy health as Ca levels increased. Annual basal area increment growth for the years following treatment initiation (1998–2004) was significantly greater in trees subjected to Ca addition compared with trees in control and Al treatments. Treatment-related improvements in growth were particularly evident after overstory release following a 1998 ice storm. The amount of wound closure was also greatest for trees in Ca-addition plots relative to Al-addition and control plots ($P = 0.041$). These findings support evidence that ambient Ca depletion is an important limiting factor regarding sugar maple health and highlight the influence of Ca on wound closure and growth following release from competition.

Résumé : Nous avons recensé et blessé des tiges d'érable à sucre (*Acer saccharum* Marsh.) qui croissent en forêt dans le cadre d'une expérience à long terme dans laquelle des traitements visant à modifier la teneur en Ca dans le sol ont été répétés à la forêt expérimentale de Hubbard Brook dans l'État du New Hampshire, aux États-Unis. Les parcelles ont reçu des applications de Ca (pour augmenter la disponibilité de Ca au-dessus des niveaux ambiants déficients) ou de Al (pour compétionner avec l'absorption de Ca et réduire davantage la disponibilité de Ca). Nous avons observé significativement plus de Ca total associé aux feuilles et aux membranes dans le feuillage des arbres situés dans les parcelles fertilisées avec Ca comparativement aux arbres situés dans les parcelles témoins ou traitées avec Al ($P = 0,005$). Parallèlement aux différences dans le statut foliaire de Ca, la vigueur de la cime et le pourcentage de mort en cime étaient significativement différents selon le traitement ($P < 0,05$); l'état de santé de la canopée avait tendance à s'améliorer avec l'augmentation du niveau de Ca. L'accroissement annuel en surface terrière pendant les années qui sont suivi le traitement (1998–2004) était significativement plus élevé chez les arbres qui avaient reçu un supplément de Ca comparativement à ceux qui avaient été traités avec Al ou aux arbres témoins. L'amélioration de la croissance reliée aux traitements était particulièrement évidente après le dégagement survenu dans l'étage dominant à la suite du verglas de 1998. La fermeture des blessures était plus avancée chez les arbres situés dans les parcelles amendées avec Ca que chez les arbres situés dans les parcelles témoins ou amendées avec Al ($P = 0,041$). Ces résultats supportent le fait que la perte de Ca ambiant est un important facteur limitant pour la santé de l'érable à sucre et mettent en évidence l'influence de Ca sur la fermeture des blessures et la croissance à la suite d'une réduction de la compétition.

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Introduction

Calcium is a biologically essential element in soil and surface waters that serves as an important nutrient influencing forest structure and function. Long-term research has suggested that rates of Ca input through natural processes such as atmospheric deposition, cation exchange, mineral weathering, and mineralization of soil organic matter do not keep pace with leaching rates from forested ecosystems (Likens et al. 1996, 1998; Bailey et al. 2004). There is grow-

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ing evidence that various anthropogenic factors may be responsible for the depletion of Ca from forest ecosystems including N saturation (Aber et al. 1998), forest harvesting (Federer et al. 1989), changing climatic conditions (Tomlinson 1993), soil Al mobilization (Lawrence et al. 1995), and declines in atmospheric base cation deposition (Hedin et al. 1994). Most prominently, Ca leaching in northern temperate forests has been attributed to reductions in soil cation levels due to high acid loading (Likens et al. 1998; Ouimet et al. 2001). Because Ca is a biologically essential element, anthropogenic alterations of this cation may have serious implications for forest health.

A prominent example of the potential implications of Ca depletion to forest health involves the documented decline of sugar maple (*Acer saccharum* Marsh.). In the 1960s, 1980s, and 1990s, isolated occurrences of sugar maple decline were documented throughout the United States and Quebec (Canada), with incidences and severity increasing in recent decades (Horsley et al. 2002). The symptoms of sugar maple decline include a slow loss of crown vigor, dieback of fine twigs, and reduced radial increment growth over a period of years, frequently ending in tree death. It appears that a combination of abiotic and biotic stressors act in concert or consecutively to weaken a tree and induce decline. Current research and investigations of previous incidents of sugar maple decline support the hypothesis that alterations in mineral nutrition, particularly of base cations (Ca, Mg, Mn, and K), act as predisposing agents, weakening the ability of sugar maple to sense and respond to secondary abiotic or biotic stresses (Kolb and McCormick 1993; Horsley et al. 2002; Hallett et al. 2006). For example, reduced foliar Ca was linked to a reduction in photosynthesis of declining sugar maple (Ellsworth and Liu 1994). In the 1980s and 1990s, drought-stressed and moderately to severely defoliated trees in the Pennsylvania Allegheny National Forest were found to have foliar concentrations of Ca and Mg well below levels of healthy trees (Kolb and McCormick 1993). Furthermore, annual basal area growth trends (1958–1967 and 1978–1987) of overstory maple in southern New York and north-central Pennsylvania were found to be negatively correlated with soil Al (possibly due to the antagonistic relationship between Al saturation and plant Ca uptake) and positively correlated with soil Ca concentrations (Heisey 1995; McLaughlin and Wimmer 1999).

Although sugar maple decline has been associated with the availability of several base cations, recent insights concerning the partitioning and physiology of Ca support the hypothesis that recent disruptions in the growth and stress response of sugar maple trees may largely be attributable to deficiencies in this essential element. Calcium plays a critical role in regulating many of the physiological processes involved in plant growth and responsiveness to environmental stress (e.g., McLaughlin and Wimmer 1999; Knight 2000). Additionally, Ca has been linked to the production of callose and lignin, key compounds in wound response and repair of damaged tissue (Doster and Bostock 1988; Eklund and Eliasson 1990; Cui et al. 2001).

Considering the multiple roles of Ca nutrition in stress response physiology, plant Ca deficiencies could manifest as a variety of limitations in the ability of plants to respond to environmental stress factors (Schaberg et al. 2001). There is a particular need to evaluate this relationship for sugar maples,

Table 1. Treatment applications for study plots at the HBEF.

| | Treatment | |
| | Ca addition (four plots) | Al addition (four plots) |
Date	(g $CaCl_2 \cdot m^{-2}$)	(g $AlCl_3 \cdot m^{-2}$)
Oct. 1995	2	0.9
May 1996	3	0.9
Nov. 1996	2	0.9
May 1997	3	1.8
Oct. 1998	38 g wollastonite·m^{-2}	0.9
May 2001		0.9
Apr. 2002		0.9
May 2004		0.9

Note: There were also four untreated control plots.

which appear to be especially sensitive to Ca depletion, experience multiple natural stress exposures, and are periodically wounded (e.g., through tapping during maple syrup production).

To explore the relationship between Ca nutrition and stress response physiology, we surveyed and wounded forest-grown sugar maple trees in a long-term, replicated Ca manipulation study at the Hubbard Brook Experimental Forest (HBEF) in New Hampshire, USA. In addition to the commonly measured parameters of foliar nutrition, crown vigor index, percent branch dieback, and stem diameter growth, we evaluated wound closure amount and the wound-induced production of lignin and callose in trees subjected to applications of Ca (to boost Ca availability above depleted ambient levels) or Al (to compete with Ca uptake and further reduce Ca availability). Particular attention was given to the growth release of trees following a severe region-wide ice storm in 1998 (Jones and Mulherin 1998; Gyakum and Roebber 2001).

Materials and methods

Study site and treatment

Research was conducted at a preexisting long-term replicated Ca manipulation study at the HBEF. The study area was comprised of 12 forested plots (45 m × 45 m) with sugar maple as the dominant canopy tree species (Berger et al. 2001). In 1995, the 12 plots were equally and randomly divided among three treatments, (*i*) control (no treatment), (*ii*) soil Ca addition, and (*iii*) soil Al addition, resulting in four replicates of the treatments. Monitoring at reference watersheds at the HBEF has documented a long-term reduction in soil available Ca (Likens et al. 1998). Treatment applications of $CaCl_2$ or $AlCl_3$ occurred in fall and spring to coincide with leafless periods (Table 1). Annual additions of $CaCl_2$ were discontinued in 1999 and replaced with a one-time application of wollastonite pellets ($CaSiO_3$, 38·g^{-2}) to provide a slow release of Ca to the soil as done elsewhere at the HBEF (Peters et al. 2004).

In January 1998, trees at our study site were damaged during a severe ice storm that impacted many forests in regions of northern New York, New England, and southeastern Canada, resulting in broken branches or the collapse of trees due to heavy ice loads (Jones and Mulherin 1998; Gyakum and Roebber 2001). A damage survey of 340 trees

(10.6–60.0 cm diameter at breast height (DBH)) conducted at our study site shortly after the ice storm showed that 66% of trees suffered damage that ranged up to entire crown loss (data on file at the HBEF).

Foliar nutrition, health, stem increment growth, and wound response measurements for the study were conducted on five randomly chosen intermediate and codominant canopy class sugar maple trees (9.5–21 cm DBH) per plot. This DBH range provided the greatest number of trees for the randomized selection of five trees per plot.

Foliar sampling and chemistry

In mid-August 2004, sun-exposed leaves located in the midsection of the crown were removed from the southwest and southeast sides of trees. Approximately 12 leaves free of fungal damage or decay were collected per tree, stored in ziplock bags, and kept cool for transport. In the laboratory, leaves were oven-dried at 55 °C and ground using a Wiley mill.

Ground foliage was run through a series of nitric acid and hydrogen peroxide digestions (Jones and Vernon 1990). Cation levels (Al, Ca, K, Fe, Mn, and Mg) were measured using inductively coupled plasma atomic emission spectrometry (ICPAES) (PlasmaSpec 2.5; Perkin-Elmer Optima, Lowell, Massachusetts). Peach leaf standards from the National Institute of Standards and Technology (SRM 15547) were processed for comparison.

In a separate preliminary study, foliar membrane-associated Ca (mCa) was measured to better assess the biological relevance of previously published Ca deficiency thresholds for total foliar cation levels (Kolb and McCormick 1993). In August 2001, foliage was collected from four dominant sugar maple trees per plot. Small sunlit branches were removed from the southwest and southeast sides of tree crowns using shotguns. Approximately 12 sun-exposed leaves were collected from each branch, sealed into plastic bags, and kept cool for transport to the laboratory. Four leaves per tree were selected for measurement of foliar mCa levels using well-established fluorescence microscopy and computer image analysis methods (Borer et al. 1997; DeHayes et al. 1999; Schaberg et al. 2000) as modified for sugar maple leaves (Schaberg et al. 2006).

Evaluation of canopy health

Tree health evaluation was conducted on 27 August 2004 according to methods used by the North American Maple Project (Cooke et al. 1996). Crown vigor was estimated for each sample tree using the following scale: (1) healthy (no major branch mortality), (2) light decline, (3) moderate decline, (4) severe decline, and (5) dead. Branch dieback was also estimated for each sample tree using a 12-class system (for complete methods, see Cooke et al. 1996).

Evaluation of tree growth

To provide a history of radial stem growth, two increment cores were taken from the main stems of sample trees at breast height (1.3 m) in October 2004. Two cores were collected at 180° for each tree. Cores were mounted and prepared according to the methods of Cook and Kairiukstis (1989). Radial growth measurements included only the past 10 years of growth (1995–2004) to specifically evaluate treatment impacts. Analyses of growth for each sample tree was based on a ratio of the basal area increment (BAI) of each posttreatment year (1996–2004) divided by the basal area increment of the pretreatment year of 1995. There was no significant difference in the growth of trees among plots prior to treatment imposition in 1995 ($P = 0.349$).

Wounding

Each of the five sample trees per plot were initially wounded on 12 July 2004. Rectangular tubing (0.318 cm × 0.635 cm) was used to injure the bark down to the active bark cambium on the main trunk starting approximately 1 m above soil surface and spaced 14 cm apart in an ascending helical pattern starting on the northeast side of the tree (see Biggs (1984) for details). Randomly selected samples of tissue surrounding initial wounds were taken at 0, 3, 7, 9, 14, 21, and 30 days postwounding using a 15 mm diameter cork borer. Each bark disc was cut in half with a razorblade; one section was placed in methanol for laboratory extraction and analysis of lignin and callose and the other half was placed in formalin – acetic acid – alcohol for histochemical analysis of lignin and callose.

Evaluation of lignin and callose production

Adopting methods from Hammerschmidt (1984) and Doster and Bostock (1988), thioglycolic acid was used to isolate and characterize lignin from the bark tissue. After an extraction series using thioglycolic acid and hydrochloric acid, the absorbance of extracted solutions was measured for relative absorbance to dry mass of sample using a spectrophotometer at a wavelength of 280 nm (Beckman DU800; Beckman Coulter, Inc., Fullerton, California). Standardization was based on calculations by Doster and Bostock (1988) for absorbance relative to dried mass of lignin extract.

Callose was extracted using sodium hydroxide and resulting samples were quantified using relative fluorescence per dry mass of sample using a fluorescence spectrofluorometer at 400 nm excitation and 510 nm emission (Hitachi F-4500; Hitachi Ltd., Tokyo, Japan) (methods adopted from Kohle et al. (1985) and Kohler et al. (2000)). A standard curve was established using 1,3-β-glucan pachyman in 1 mol·L^{-1} NaOH. Amounts of callose were represented as pachyman equivalents.

For histochemical analysis, samples were dehydrated using the tertiary butyl alcohol series, embedded in paraffin, and sectioned at 10 μm on a rotary microtome (Spencer 820 microtome; American Optics Co., Buffalo, New York) (Jenson 1962). Adopting methods from Biggs (1985), bark tissue was analyzed on a bright-field microscope (Olympus BX60 optical microscope; Olympus Optical Co., Hamburg, Germany) fitted with a 546 nm filter and an X250 objective. Images of sectioned samples were taken pre- and poststaining for 5 min with the lignin-specific stain phloroglucinol plus HCl. All measurements were taken approximately 800 μm internal to the wound margin in the phelloderm–cortex region of the bark tissue (Biggs 1985). Lignin concentration was quantified using Scion image analysis software (Scion Corporation, Frederick, Maryland) to calculate the percent absorption of light, pre- versus poststaining, relative to the number of pixels evaluated.

Table 2. Treatment differences in foliar element concentrations (mean ± SE) for sugar maple (*Acer saccharum*) trees in 2004 from Ca-addition, control, and Al-addition study plots with a reference of the published range of foliar cations for healthy sugar maples (ranges from Kolb and McCormick 1993).

| Element | Treatment mean (mg·kg^{-1}) | | | Significance of contrasts (*P* value) | | Range for healthy sugar maple (mg·kg^{-1}) |
	Ca addition	Control	Al addition	Ca vs. control and Al	Control vs. Al	
Ca	7959.61±477.50	5505.24±439.81	4506.62±392.34	0.004	0.283	5000–21900
Al	30.55±1.41	37.14±1.70	40.17±2.41	0.029	0.410	32–60
Mg	1127.85±70.75	1006.45±73.53	882.23±82.24	0.304	0.538	1100–4000
Mn	1107.39±78.66	1113.10±106.96	1096.78±107.52	0.984	0.999	632–1630
Fe	44.81±1.98	45.39±1.20	48.08±2.08	0.443	0.356	59–130
K	6940.52±226.80	7595.73±190.52	7492.69±278.54	0.058	0.769	5500–10400

Table 3. Treatment differences in total foliar Ca and mCa levels (mean ± SE) for sugar maple trees in 2001 from Ca-addition, control, and Al-addition study plots.

| Element | Treatment mean (mg·kg^{-1}) | | | Significance of contrasts (*P* value) | |
	Ca addition	Control	Al addition	Ca vs. control and Al	Control vs. Al
Total foliar Ca	5767.20±275.22	3547.78±293.82	3466.12±235.47	<0.001	0.971
mCa	154.90±9.28	103.41±7.05	104.16±4.37	0.005	0.964

Evaluation of wound closure

Measurements of wound closure were taken on wounds made with the 15 mm cork borer. After collecting bark discs, the cork borer left a clean circular wound into the xylem appropriate for analysis of wound closure. Using calipers, the width of each wound was measured on 1 November 2005. This date was chosen to allow at least one full growing season of response for sample trees.

Statistical analyses

For continuous data, analysis of variance (ANOVA) was used to test for treatment differences. Significance tests utilized a nested design to test treatment differences with plot within treatment, and plot differences with tree within plot (Montgomery 2001). To further assess the differences among Ca perturbation treatments, differences among means were analyzed using two mutually exclusive orthogonal contrasts: (*i*) Ca versus Al and control and (*ii*) Al versus control. These contrasts maximized the statistical power for evaluating two important aspects of the study: (*i*) the influence of increased Ca availability above ambient and decreased levels and (*ii*) the influence of reduced Ca levels below ambient conditions. A multiple analysis of variance (MANOVA) was used to test for differences between treatments and years for increment growth and differences between treatments and days for lignin and callose production. In addition, growth data were analyzed using regression analysis for specific time periods.

Crown vigor, represented as categorical data, was analyzed using χ^2 analysis. Percent branch dieback was treated as continuous data but was analyzed using a Wilcoxon/Kruskal–Wallis test because the data were not normally distributed. Relationships between foliar Ca concentrations and crown vigor and percent branch dieback levels were assessed using an ANOVA with the Tukey–Kramer HSD test to evaluate specific differences among rating classes. For all tests, differences were considered statistically significant if $P \le 0.05$.

Results

Foliar nutrient concentrations

Significant differences in foliar Ca concentrations (*P* = 0.004) and foliar Al concentrations (*P* = 0.029) were found among treatment plots in 2004 (Table 2). Trees in plots treated with Ca exhibited significantly greater foliar Ca than trees from control and Al-treated plots. There was no difference in foliar Ca levels for trees in control versus Al-treated plots. Foliar Al levels were significantly lower in trees from plots treated with Ca compared with trees from control and Al-treated plots, whereas no differences in foliar Al levels were detected between trees in control and Al-treated plots.

Similar trends in total foliar Ca concentrations among treatments were found in our 2001 preliminary experiment (Table 3), although specific concentrations differed for these dominant trees sampled 4 years before the primary experiment. Importantly, mCa levels paralleled total foliar concentrations and established Ca deficiency thresholds. Levels of mCa were significantly higher in trees subjected to Ca treatment compared with trees subjected to Al or control treatments (*P* = 0.005). There was no difference in mCa levels between trees growing in control and Al-treated plots (*P* = 0.964).

### Canopy condition and health

Across the treatments, crown vigor rankings ranged between 1 and 3 (with 1 being most vigorous) and branch dieback spanned from 0% to 25% (Table 4). Crown vigor showed a significant difference among treatments (χ^2 = 10.19, *P* = 0.037) as did percent branch dieback (χ^2 = 6.92, *P* = 0.031). Analysis of mean foliar Ca concentration for each crown vigor rating class showed significantly lower foliar Ca for trees with reduced crown health (*P* = 0.007) (Fig. 1). Lower foliar Ca levels were also associated with increased percent branch dieback (*P* = 0.005) (Fig. 1).

Table 4. Sugar maple crown health measurements (mean ± SE) for all treatments.

Health analysis	Treatment mean			Differences among treatments
	Ca addition	Control	Al addition	
Crown vigor index	1.05±0.05	1.60±0.20	1.30±0.13	$\chi^2 = 10.19$, $P = 0.037$
% branch dieback	2.50±0.57	5.50±1.08	5.25±0.99	$\chi^2 = 6.92$, $P = 0.031$

Note: Results for crown vigor analysis using the crown vigor index are from a χ^2 test; results for percent branch dieback are from a Wilcoxon/Kruskal–Wallis test.

Fig. 1. Differences in mean ± SE foliar Ca concentration for (*a*) crown vigor ratings and (*b*) percent branch dieback for sugar maple trees (*Acer saccharum*). Crown vigor rating ranged from 1 (healthy) to 3 (moderate decline). Group means with different letters are significantly different based on a Tukey–Kramer HSD test.
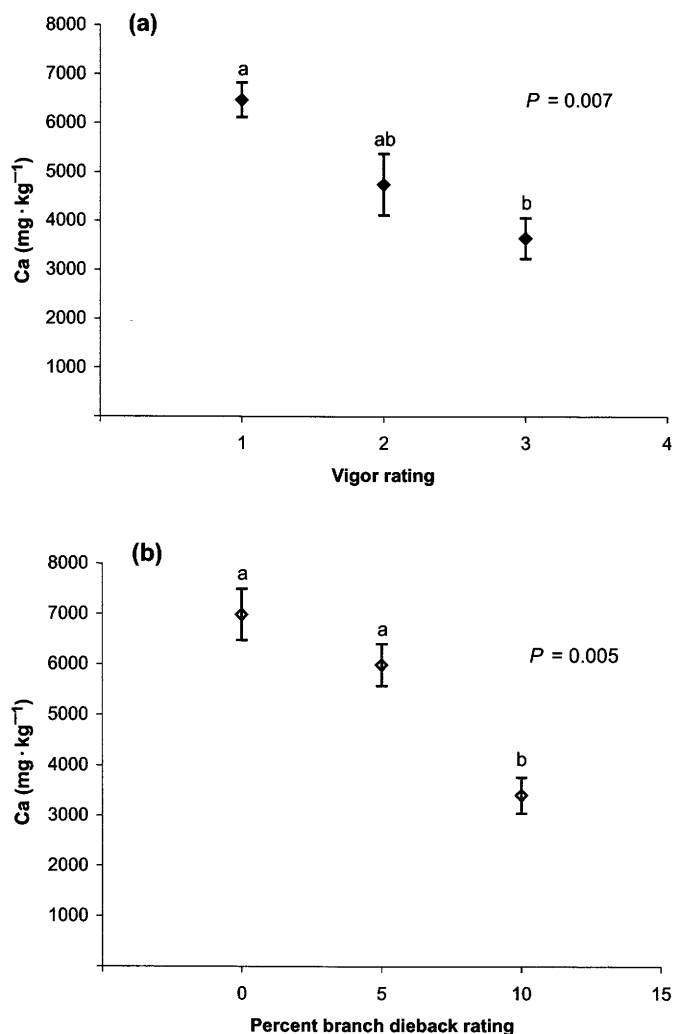
Tree growth

For analysis of tree growth, a MANOVA using repeated measures and a set alpha for all tests showed significant differences in treatment ($P < 0.001$), year ($P < 0.001$), and in the interaction between treatment and year ($P < 0.005$). Further analysis showed significantly greater growth in trees subjected to Ca treatment compared with trees subjected to control and Al treatments for each year from 1998 to 2004 (Fig. 2). For all years (1996–2004), there was no significant difference in the growth of trees subjected to either control or Al treatments.

As indicated in Fig. 2, the significant increase in growth among sample trees occurred following the 1998 ice storm. An analysis of growth trends for sugar maple trees immediately following the 1998 ice storm (1998–2002) and after possible poststorm crown closure (2002–2004) was calculated as the slope of the regression for individual trees (Table 5). In a comparison of growth trends immediately following the 1998 ice storm (1998–2002) and for growth trends after possible poststorm crown closure (2002–2004), there was a significant difference between trees growing in Ca treatments compared with those in the control and Al treatments ($P < 0.0001$ and $P = 0.001$, respectively) (Table 6). There was no significant difference in growth trends between control and Al treatments for 1998–2002 ($P = 0.386$) and 2002–2004 ($P = 0.140$).

Wound closure

Trees that received the Ca treatment exhibited a greater amount of average stem wound closure compared with Al and control treatment trees ($P = 0.041$) (Table 7). There was no difference in wound closure for trees growing on Al-treated plots compared with control plots ($P = 0.360$). Although Ca treatment was associated with greater wound closure overall, the influence of Ca appeared to be greatest for wounds positioned lower on stems (100–130 cm) ($P = 0.002$).

Lignin and callose production

Separate extraction assays for lignin and callose showed no significant difference among treatments ($P_{lignin} = 0.214$, $P_{callose} = 0.402$) or for the interaction between treatment and days postwounding ($P_{lignin} = 0.492$, $P_{callose} = 0.357$). Similar results were found for the microscopic analysis of lignin production. No significant difference among treatments ($P = 0.448$) or for the interaction between treatment and days postwounding ($P = 0.526$) was detected.

Discussion

Results from this study reinforce past findings on the importance of Ca nutrition on sugar maple health and growth (Long et al. 1997; Moore et al. 2000; Wargo et al. 2002; Moore and Ouimet 2006). Furthermore, this study revealed new connections between Ca nutrition and (*i*) basal area growth release following a natural disturbance and (*ii*) the stem wound response of trees.

Foliar cation concentrations in 2004 sample trees (Table 2) were generally within the range of previously reported levels for healthy sugar maples as compiled by Kolb and McCor-

Fig. 2. Treatment differences in BAI ratio (mean ± SE of growth for specific year/1995) of sugar maple trees on study plots from 1995 (pretreatments) to 2004. The timing of treatment initiation and the 1998 regional ice storm are indicated. Treatment means with different letters are significantly different based on the following orthogonal contrasts: Ca versus Al and control and Al versus control ($P < 0.05$).
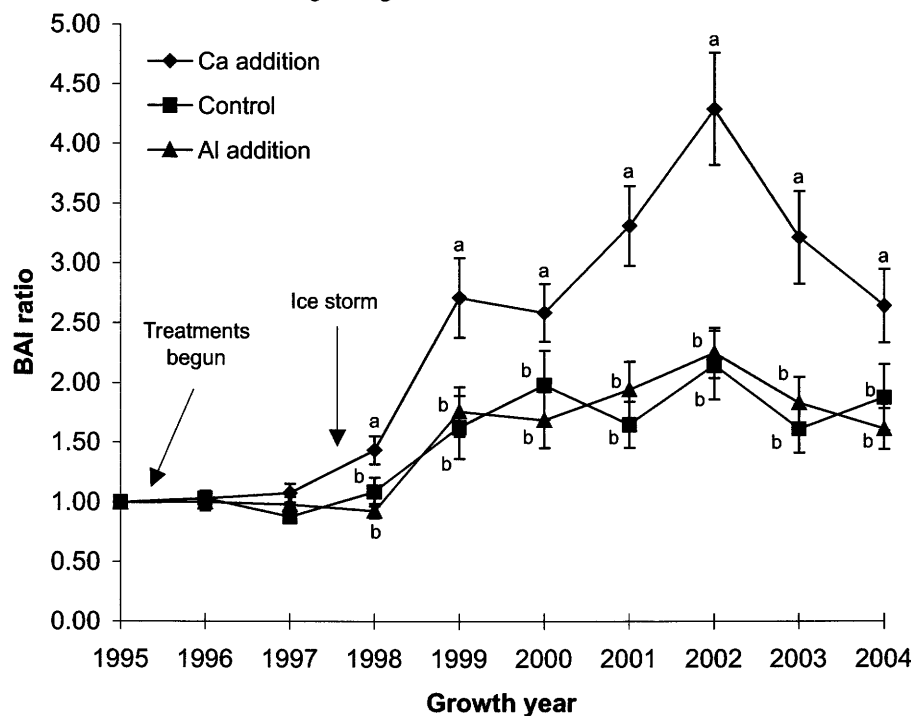
mick (1993). Exceptions include Ca, Al, Mg, and Fe, which dropped slightly below the reported range of each nutrient for some trees. Treatments did not significantly influence foliar cation concentrations for any elements other than Ca and Al. In the Ca-addition plots, the application of Ca elevated foliar Ca levels compared with trees on control and Al plots that had foliar Ca levels equal to, or just below, the deficiency threshold for healthy sugar maples (5000 mg·kg^{-1}). Although some studies have shown that variations in Mg and Mn nutrition often parallel differences in sugar maple growth and health (Horsley et al. 2002; Hallett et al. 2006), there were no significant differences in foliar concentrations of these cations among treatments in this study.

A comparison of total foliar Ca and mCa levels in 2001 (Table 3) revealed that trees with foliar Ca concentrations below the established threshold for healthy sugar maple trees also exhibited significantly lower levels of mCa. Membrane-associated Ca plays a vital role in membrane structure and function by stabilizing membranes and influencing permeability (Palta and Li 1978; Davies and Monk-Talbot 1990) and may be an important source of Ca used in stress signaling (Atkinson et al. 1990). Significantly lower levels of mCa for trees on control and Al-addition plots provide independent support that published deficiency thresholds established through field observations have biological relevance at the cellular level. These reductions in mCa may also indicate the physiological impacts of Ca deficiencies: that membrane stability and stress signaling capabilities are impaired when foliar Ca concentrations fall below critical thresholds.

Treatment-associated increases in foliar Ca levels were accompanied by significant changes in canopy health, tree growth, and stem wound closure amount. Analysis of tree health measures showed greater crown vigor and lower percent branch dieback as foliar Ca concentrations increased (Fig. 1). The χ^2 analysis of crown vigor showed a significant difference among treatments (Table 4), with the greatest vigor exhibited by trees in the Ca-addition plots. This trend is further supported by ANOVA of the differences in foliar Ca concentration among vigor ratings ($P = 0.007$) (Fig. 1). In addition, treatment applications resulted in differences in percent branch dieback (Table 4) with further analysis indicating that as mean foliar Ca concentrations increased, percent branch dieback was less evident ($P = 0.005$) (Fig. 1). Results suggest that poor crown condition and increased percent dieback are more evident when foliar Ca concentrations drop below established deficiency thresholds, which is consistent with previous reports on the association between Ca nutrition and sugar maple health (Wilmot et al. 1995; Schaberg et al. 2006; Juice et al. 2006).

Trees characterized as having higher foliar Ca levels also exhibited increased basal area growth (Fig. 2). In our study, Ca addition resulted in significantly greater growth for intermediate and codominant sugar maple trees over a 7-year period compared with trees on control and Al-treatment plots. Significant Ca-associated differences in growth were not detected until the third growing season after treatment began, which is consistent with previous reports on the timing of treatment impacts on sugar maple health and growth (Long et al. 1997; Moore et al. 2000). Although a treatment-specific increase in growth would be consistent with the gradual uptake and impact of Ca additions, an analysis of the slope of the BAI ratio for the years 1998–2002 (Table 5) revealed an increase in growth (measured as a significant positive slope coefficient for the regressions of growth

Table 5. Analyses of growth trends for sugar maple trees immediately following the 1998 ice storm (1998–2002) and after possible poststorm crown closure (2002–2004).

Time period	Ca addition	Control	Al addition
1998–2002	$r = 0.559, P < 0.001$	$r = 0.331, P = 0.030$	$r = 0.449, P < 0.001$
2002–2004	$r = -0.375, P = 0.015$	$r = -0.195, P = 0.082$	$r = -0.297, P = 0.359$

Note: Significant positive r values indicate increases in growth (slope coefficients greater than zero), whereas significant negative r values indicate reductions in growth (slope coefficients less than zero).

Table 6. Treatment differences in growth trends for sugar maple trees following the 1998 ice storm (1998–2002) and after possible poststorm crown closure (2002–2004) (treatment slope means ± SE).

Period	Treatment mean for linear slopes of BAI ratio			Significance of contrasts (P value)	
	Ca addition	Control	Al addition	Ca vs. control and Al	Control vs. Al
1998–2002	0.638±0.095	0.218±0.046	0.295±0.047	<0.0001	0.386
2002–2004	−0.757±0.14	−0.132±0.04	−0.316±0.052	<0.001	0.140

Table 7. Treatment differences in wound closure growth (mean ± SE) for sugar maple trees.

	Treatment mean (mm)			Significance of contrasts (P value)	
	Ca addition	Control	Al addition	Ca vs. control and Al	Control vs. Al
Wound closure	0.615±0.313	−0.303±0.26	0.060±0.25	0.041	0.360

Note: Negative numbers in wound closure are a result of the cracking and shrinking and (or) swelling of bark tissue following wounding.

over time) for trees in each treatment, implying a common response to some treatment-independent factor. We suspect that this observed pattern may reflect a generalized response to the opening of crown canopies caused by the 1998 ice storm (Jones and Mulherin 1998; Gyakum and Roebber 2001). Analysis of a poststorm damage survey of 340 trees at this study site showed that there was substantial injury to crowns, but that this damage was significantly greater ($P < 0.001$) in larger trees (>22 cm DBH) compared with smaller trees (10–21.9 cm DBH) (data on file at the HBEF). Importantly, this assessment showed that there were no differences in crown damage associated with soil treatment ($P = 0.17$). Thus, regardless of treatment, damage to the crowns of dominant trees likely created gaps in the canopy, allowing for increased light penetration and providing improved growing conditions for the less-damaged, codominant and intermediate trees that we studied. A release of codominant and intermediate trees following ice damage could have initiated the increased rate of growth from 1998 to 2002 documented for trees growing in each treatment (Table 5).

Although storm-related crown injury was indistinguishable among the treatments, growth responses following the ice storm differed significantly with treatment. Analysis of the slope in BAI ratio for 1998–2002 showed greater growth for trees growing in the Ca versus Al/control treatments (Table 6). Greater growth following the removal of overstory competition for trees on Ca-addition plots could reflect the importance of Ca in supporting physiological responses to changing resource conditions. In contrast, the limited growth response of trees on control and Al treatments following the ice storm suggests that ambient Ca depletion may have reduced the competitive vigor of trees relative to circumstances of greater Ca availability. Because adequate physiological response to disturbance factors is important to the growth and survival of long-lived tree species, the lim-

ited growth release of trees on control and Al-addition plots has ecological implications beyond those associated with the 1998 ice storm.

Because improvements in Ca nutrition associated with treatment remain evident (Table 2), the decline in growth from 2002 to 2004, as seen in the negative slope of the BAI ratio for trees subjected to Ca treatment (Table 6), suggests that this reduction in growth was in part a response to treatment-independent factors that persisted over several years. In particular, this could reflect the response of study trees to canopy closure as dominant trees refoliated following ice storm damage. The codominant and intermediate trees on Ca-addition plots could also have filled in canopy gaps in conjunction with the greater growth increase that they experienced after the ice storm.

Trees growing on plots subjected to Ca addition also exhibited greater amounts of wound closure than trees growing on control or Al-treated plots (Table 7). Although many studies have shown that Ca addition can increase vascular cambial activity and woody growth for sugar maple trees, this is the first evidence that Ca addition also improves bark cambium production and associated wound closure. The amount of stem wound closure is vital considering that the probability and severity of fungal infection have been found to diminish with increased wound healing (Biggs 1986). Thus, greater wound closure should lessen the likelihood of fungal infection. It should be noted that differences in wound closure amounts associated with Ca fertilization were most pronounced in wounds lower on the tree stem (100–130 cm, $P = 0.002$), a trend that warrants further investigation considering the implications for wound healing and tree health following the tapping of sugar maple trees for maple syrup production.

The production of callose and lignin is an important aspect of stem wound response in trees because they act to strengthen cell walls and form an impermeable barrier to

fungal ingress (Ride 1978; Cui et al. 2001). Although the production of callose and lignin has been shown to be Ca dependent at the cellular level (Kohle et al. 1985; Doster and Bostock 1988; Eklund and Eliasson 1990; Bach and Seitz 1997), they were not associated with perturbations in Ca nutrition in our study under field conditions. It is possible that differences in callose and lignin production attributable to treatment were not found because Ca levels in all treatments remained too high to influence these important physiological processes. However, it is also possible that artifacts of the measurement process reduced our ability to detect treatment-induced differences in production. Because the dry mass of each sample was used to calculate the results of lignin and callose extraction, wide differences in bark thickness within and among sample trees undoubtedly contributed a substantial and uncontrolled source of variability.

Although we hypothesized that Ca nutrition would be an important contributor to the vigor of the trees that we sampled, the strength of relationships between foliar Ca levels and tree health, growth, and wound closure measures was surprising. Sample trees included a range of DBH and canopy classes. Furthermore, differential exposures to environmental stress factors (e.g., drought, freezing injury, fungal infection, etc.) within and among plots could strongly increase the variability of growth and health measurements. Despite these likely sources of uncontrolled variability, associations between Ca and tree health and growth parameters remained consistent and significant.

The effects of Ca treatment on sugar maple growth, health, and wound closure documented in this study further support the hypothesis that Ca is a limiting factor in the overall health of sugar maple trees. Soil Ca amendments improved canopy health and appeared to play a role in facilitating the recovery and growth of trees following the 1998 ice storm. Although the relationship between Ca nutrition and the production of callose and lignin is unclear, Ca treatment did increase the amount of stem wound closure. The influence of Ca nutrition on wound closure has particular relevance for sugar maple trees because they are regularly wounded during maple syrup production.

The treatment comparisons of our study allowed us to focus on the influence of ambient Ca loss or potential further Ca depletion (control and Al-addition plots) relative to historically more typical levels of Ca availability (Ca-addition plots). These comparisons indicated that the cumulative depletion of Ca that has occurred (Likens et al. 1998) has resulted in significant reductions in vigor, growth, and wound closure for sugar maple trees at the HBEF. It is anticipated that depletion of Ca from forest soils will continue due to the effects of acidic deposition (Likens et al. 1996, 1998), soil Al mobilization (Shortle and Smith 1988; Lawrence et al. 1995), declines in atmospheric base cation deposition (Hedin et al. 1994), and other anthropogenic factors. As Ca becomes further depleted from hardwood forests of the eastern United States and adjacent Canada, the degradations in sugar maple health and productivity that we documented at the HBEF may become more evident throughout the region.

Acknowledgements

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