DISRUPTION OF CALCIUM NUTRITION AT HUBBARD BROOK EXPERIMENTAL FOREST (NEW HAMPSHIRE) ALTERS THE HEALTH AND PRODUCTIVITY OF RED SPRUCE AND SUGAR MAPLE TREES AND PROVIDES LESSONS PERTINENT TO OTHER SITES AND REGIONS

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Abstract.—Pollution-induced acidification and other anthropogenic factors are leaching calcium (Ca) and mobilizing aluminum (Al) in many forest soils. Because Ca is an essential nutrient and Al is a potential toxin, resulting depletions of Ca and increases in available Al may significantly alter the health and productivity of forest trees. Controlled experiments on red spruce (*Picea rubens* Sarg.) and sugar maple (*Acer saccharum* Marsh.) seedlings – species experiencing decline in the field – have documented physiological disruptions specific to Ca deficiency. More importantly, field studies at Hubbard Brook Experimental Forest (HBEF) in New Hampshire have shown that Ca additions that increase soil Ca levels to those estimated to exist prior to pollutant-induced Ca leaching, reverse many of the symptoms of red spruce and sugar maple decline. For example, Ca addition to Watershed 1 at the HBEF increased the Ca nutrition, sugar storage, antioxidant enzyme activity, and cold tolerance of the current-year foliage of mature red spruce trees and dramatically reduced foliar winter injury during a high-injury year. Similarly, soil Ca additions in a replicated plot-based study at HBEF increased foliar Ca concentrations in sugar maple trees and simultaneously alleviated the two characteristic symptoms of maple decline – branch dieback and reductions in woody growth. Improvements in Ca nutrition here also significantly increased levels of stem wound closure. Previous research with red spruce saplings in the Southern Appalachian Mountains and sugar maple trees across the Allegheny Plateau of north central Pennsylvania are consistent with our findings for mature trees at HBEF and suggest that the influence of Ca depletion on tree function and decline are pertinent to many sites and regions. Although species like red spruce and sugar maple may be particularly susceptible to Ca deficiency and related decline, results of studies on other tree species in the laboratory and field suggest a broader influence of Ca deficiency. Because Ca deficiency and Al toxicity particularly disrupt plant carbon (C) relations (i.e., the biochemistry of photosynthesis, respiration, and growth) alterations in the availability of these cations may also disproportionately reduce levels of C sequestration within forest systems.

INTRODUCTION

Growing evidence from around the globe indicates that anthropogenic factors including pollution-induced acidification, associated aluminum (Al) mobility, and nitrogen (N) saturation are disrupting natural nutrient cycles and depleting base cations from forest ecosystems (Lawrence et al. 1995; Likens et al. 1996, 1998; Aber et al. 1998, 2003). Although cation depletion can have varied and interacting influences on ecosystem function (e.g., altering nutrient cycling, species composition, and food webs in forested systems Schaberg et al. 2001, in press), it is the loss of calcium (Ca) that may be particularly limiting to tree health and productivity. Ca plays unique roles in plant metabolic pathways that allow plants to recognize and acclimate to biotic and abiotic stresses (Sanders et al. 1999, Pandey et al. 2000, Roos 2000) and that regulate cellular energy relations (Pan and Dilley 2000, Dilley 2004, Miqyass et al. 2007, Tozawa et al. 2008) – two processes critical to the long-term survival and growth of trees.

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Historically, much of the research regarding the influence of Ca on plant health has involved basic research with herbaceous crops, especially Arabidopsis (e.g., Cheong et al. 2003, Pandey et al. 2004, Liu et al. 2005). However, laboratory-based experiments with red spruce (*Picea rubens* Sarg.) and sugar maple (*Acer saccharum* Marsh.) seedlings – two tree species that are experiencing decline in the field – have also been conducted. For example, experiments with red spruce saplings have shown that the loss of biologically available Ca 1) reduces the stability of foliar membranes and decreases foliar cold tolerance (Schaberg et al. 2000a); and 2) slows the closure of stomata during water stress – predisposing plants to drought damage (Borer et al. 2005). Similar work with pot-grown sugar maple seedlings has shown that photosynthesis and growth are depressed when Ca is limited (St. Claire and Lynch 2005a) and that low cation (especially Ca) nutrition may also reduce levels of mycorrhizal colonization – exacerbating nutritional deficits (St. Claire and Lynch 2005b). Results of these controlled studies lay the foundation for understanding the fundamental changes in physiology that result from pollution-induced plant Ca deficiencies. Nonetheless, complementary experimentation must also occur in the field to evaluate whether laboratory results have bearing under the more complicated conditions that exist in heterogeneous forest systems.

The Hubbard Brook Experimental Forest (HBEF) in New Hampshire is an ideal location to evaluate the broader, real-world risks of Ca depletion to forest health and productivity in the field. HBEF is where acid deposition was first measured and defined in the United States (Likens et al. 1972), and it has provided some of the most convincing evidence connecting acid deposition to Ca depletion (Likens et al. 1996, 1998). Furthermore, once the nature and extent of Ca depletion were known, HBEF established two long-term experiments to assess the influence of ambient Ca depletion on nutrient cycling, forest health, and productivity. One of these experiments involved the replacement of all the Ca estimated to have been lost through acid-induced pollutant leaching back to an entire forested watershed (Groffman et al. 2004). The second was a replicated plot-based experiment that provided a broad spectrum of soil Ca levels (Berger et al. 2001).

Here we describe the results of several studies that evaluated the influence of Ca manipulation of native forest soils on mature red spruce and sugar maple health and productivity in the field. Although the studies described vary in the treatment scale (from entire watershed to replicated 45 m plots), the important commonality is that treatment impacts in both were compared to an ambient control – soils that have been depleted of Ca largely as a result of acid deposition-induced Ca leaching (Likens et al. 1996, 1998). Thus, the subjects of comparison in these experiments are the nutrition, health, and productivity of trees under ambient conditions (Ca partially depleted due to acidic inputs) versus the nutrition and function of trees on sites that received added Ca (intended to bring soil Ca levels to pre-pollution levels). This design helps to identify current deficits in tree status attributable to pollution-induced Ca depletion. The replicated plot study also includes a soil Al treatment. Because Al competes with and reduces Ca uptake, the addition of the Al treatment provides a look at the consequences of further reductions in Ca availability beyond those currently experienced under ambient conditions. Tree assessments focus on foliar nutrition (to verify treatment-induced changes in Ca availability) and physiological/health measures that have previously been shown to be Ca-dependent (i.e., foliar sugar concentrations; the activity of the antioxidant enzyme ascorbate peroxidase [APX]; cold tolerance; winter injury and bud mortality for red spruce; and foliar nutrition, crown vigor and dieback, xylem growth and wound closure for sugar maple).

STUDY AREA

Watershed-level Study of Red Spruce

HBEF is divided into multiple small headwater watersheds which serve as either reference or treatment sites. Watershed 6 is the biogeochemical-reference watershed of the forest for which extensive Ca depletion has been documented (Likens et al. 1996, Likens et al. 1998). Watershed 1 was fertilized in 1999 with 38 g m^{-2} $CaSiO_3$ (wollastonite, a form of Ca that is inherently slow-releasing) to increase the availability of Ca to pre-industrial levels (Groffman et al. 2004). Because red spruce was a common component of the forests at the upper reaches of both the reference and treated watersheds, these locations were used to evaluate the influence of soil Ca depletion on red spruce physiology and health.

Replicated-plot Study of Sugar Maple

We also used the Nutrient Perturbation (NuPert) study comprising 12 forested plots (45 m x 45 m) where sugar maple is the dominant canopy tree species (Berger et al. 2001). In 1995, the 12 plots were equally and randomly divided among three treatments: 1) control (no treatment), 2) soil Ca addition (to boost Ca to pre-pollution levels of availability), and 3) soil Al addition (to compete with and reduce Ca availability), resulting in four replicates of the treatments. Treatment applications of $CaCl_2$ or $AlCl_3$ occurred in fall and spring to coincide with leafless periods. Annual additions of $CaCl_2$ were discontinued in 1999 and replaced with a one-time application of wollastonite pellets (38 g m^{-2}) to provide a slow release of Ca to the soil as done elsewhere at the HBEF (Peters et al. 2004). At the time that nutritional and health measures reported here were made, the NuPert plots had received a total of 48 g m^{-2} of Ca and 8.1 g m^{-2} Al via treatment additions (Huggett et al. 2007).

METHODS

Foliar Winter Injury and Bud Mortality of Red Spruce

Winter injury was assessed in May 2003 on both the Ca-addition and reference watersheds at HBEF. One-tenth-ha circular plots containing dominant or co-dominant red spruce were randomly chosen in each watershed. All red spruce trees in these plots above breast height (1.3 m) were assessed and categorized into the following crown classes: dominant, co-dominant, intermediate, suppressed, and understory. Six plots containing 38 trees were examined for winter injury in the Ca-addition watershed and seven plots containing 44 trees were assessed in the reference watershed. All spruce trees in each plot were visually evaluated for the reddening of current-year foliage and rated on a scale of 0 to 10 by two observers (Lazarus et al. 2004). A score of 1 represented 1-10 percent injury, a score of 2 represented 11-20 percent injury, etc. Bud mortality associated with foliar winter injury was visually assessed on both watersheds using binoculars on 28 October 2003; we used the same 0-10 injury scale as for foliar assessments.

Cation Concentrations in Red Spruce Foliage

To measure cation nutrition, we sampled foliage in late October 2003 from a sub-sample of trees assessed for winter injury in May 2003. We used shotguns to collect current-year, sunlit foliage from dominant and co-dominant trees from six plots from the Ca-addition watershed (31 trees total) and seven plots from the reference watershed (37 trees total). In November 2005 six south-facing plots per watershed were established containing five mature dominant or co-dominant red spruce each (n = 30 per watershed, total n = 60 for all measures). Samples of sunlit, current-year foliage from the upper crowns of trees were collected for the measurement of cation concentration in November 2005. These same trees were used for foliar collections in February 2006 to measure soluble sugar concentrations and APX activity. For both the 2003 and 2005 collections, samples used for cation analysis were sealed in plastic bags for transport and dried for 2 weeks at 65 ˚C upon return to the laboratory. Samples were then ground to pass a 2 mm sieve and digested by heating with nitric acid and hydrogen peroxide using a block digester (adapted from Jones and Case 1990), and analyzed for total foliar Ca, Al, potassium (K), magnesium (Mg), and manganese (Mn) by inductively coupled plasma atomic emission spectroscopy (ICP-AES, PlasmaSpec 2.5, Leeman Labs, Lowell, MA). Eastern white pine needles from the National Bureau of Standards and Technology (SRM 1575), sample duplicates, and blanks were analyzed for procedural verification. Tissue standards were within 5 percent of certified values.

Soluble Sugar Analysis of Red Spruce Foliage

Samples analyzed for soluble sugar concentration were packed in ice in the field, freeze-dried, ground, and ultimately stored at -80 ˚C until ready for assay. Cuticular waxes were removed with hexane, and sugars were extracted with 80 percent ethanol (Hinesley et al. 1992). Methods described by

Schaberg et al. (2002) were followed to analyze concentrations of stachyose, glucose, sucrose, xylose, fructose, and raffinose using a Waters HPLC with a 510 pump, a 410 differential refractometer, and a Waters Sugar-Pak column. Data were analyzed for both individual and total sugar concentrations using Waters Millenium™ 2000 software (Advanced Chemistry Development, Inc., Toronto, ONT), and expressed as milligrams per gram dry mass.

Ascorbate Peroxidase Activity in Red Spruce Foliage

Current-year foliage was packed in ice in the field. In the laboratory, samples were homogenized in extraction buffer modified from Schwanz and Polle (1998) and stored at -80 °C until ready for assay. Enzyme activity was monitored spectrophotometrically with a Beckman DU 800 (Beckman Coulter, Inc., Fullerton, CA). Total soluble protein was analyzed with a brilliant-blue total protein kit (TP0100, Sigma-Aldrich Co., St. Louis, MO). The methods of Nakano and Asada (1981) were followed as samples were spectrophotometrically analyzed at 290 nm, and the linear decrease in absorbance for 2 minutes was recorded to determine the activity of APX as ascorbate scavenged hydrogen peroxide (H_2O_2). Ascorbate oxidase activity was measured via the same methods with the omission of H_2O_2, and subtracted from APX activity to yield APX specific activity.

Cold Tolerance of Red Spruce Foliage

Current-year foliage was collected in February 2006 to measure cold tolerance at the point in winter when red spruce is most vulnerable to freezing injury. Samples were bagged and packed in ice in the field, and stored overnight at 4 °C. The following day, samples were chopped and bulked. Subsamples per tree were exposed to decreasing temperatures at a rate of -6 °C h^{-1} and held at 14 preselected test temperatures ranging from -15 °C to -90 °C for 30 minutes (Halman et al. 2008). Following freezing tests, a 3.0 mL solution of 0.01-percent *v/v* Triton X-100 in deionized water (4 °C) was added and samples were shaken for 8 h at room temperature. Initial conductivity of the effusate was measured with a multielectrode instrument (Wavefront Technology, Ann Arbor, MI). Then samples were dried for 72 h at 45 °C to kill the tissue and soaked in fresh detergent solution for 24 h. Final conductivity was measured. Relative electrolyte leakage (REL), a measure of membrane permeability calculated as the proportion of initial to final conductivity at a given test temperature, was used to calculate T_m, the temperature at the midpoint of a sigmoid curve fit to REL data for all test temperatures (Schaberg et al. 2000b, Strimbeck and DeHayes 2000).

Nutrition of Sugar Maple Foliage

Foliar cations were measured using the methods described above for red spruce foliage, except that peach leaves from the National Bureau of Standards and Technology (SRM 15547) were used as a procedural check rather than white pine needles.

Evaluation of Canopy Health for Sugar Maple

Tree health evaluations were 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 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 Sugar Maple Basal Area Growth

Two increment cores were taken from the main stems of sample trees at breast height in October 2004. Two cores were collected at 180 degrees for each tree. Cores were mounted and prepared according to 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 were based on a ratio of the basal area increment (BAI) of each post-treatment year (1996-2004) divided by the basal area increment of the pre-treatment year of 1995. There was no significant difference in the growth of trees among plots prior to treatment imposition in 1995 (P = 0.349).

Evaluation of Sugar Maple Wound Closure

Each of the five sample trees per plot was initially wounded in July and August 2004 with a 15-mm cork borer. Calipers were used to measure the width of each wound on 1 November 2005. This date was chosen to allow at least one full growing season of response for sample trees.

Statistical Analyses

For almost all the data from the watershed and replicated plots studies, 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). For the replicated plot study, to further assess the differences among Ca perturbation treatments, differences among means were analyzed using two mutually exclusive orthogonal contrasts: 1) Ca vs. Al and Control and 2) Al vs. Control. Sugar maple crown vigor, represented as categorical data, was analyzed using chi-square analysis. Percent branch dieback in sugar maple trees was treated as continuous data but was analyzed using a Wilcoxon/Kruskal-Wallis test because the data were not normally distributed.

RESULTS

Watershed-level Study of Red Spruce

Calcium addition significantly increased the Ca concentration of current-year foliage when measured in 2003 and 2005 (Table 1). Synchronous with these increases in foliar Ca, we detected significant changes in a number of Ca-dependent processes (Table 2). For example, the winter injury of current-year foliage from dominant and co-dominant trees was dramatically reduced for trees from the Ca-addition watershed relative to those on the control (Hawley et al. 2006). Significant reductions in freezing-induced bud mortality were also found for trees on the Ca-addition site compared to the control (Hawley et al. 2006). Ca treatment was also associated with significantly higher foliar sucrose and total sugar concentrations and higher APX activity relative to trees from the control (Halman et al. 2008). These increases in sugar concentration and antioxidant (APX) activity provide physiological explanations for the greater cold tolerance (Halman et al. 2008) and reduced winter injury (Hawley et al. 2006) found for trees on the Ca-treated watershed.

Replicated-plot Study of Sugar Maple

Ca addition increased Ca and reduced Al concentrations in sugar maple foliage at the NuPert site, but we detected no differences in foliar nutrition associated with Al treatment (Table 3). Ca treatment was also associated with an amelioration of the common symptoms of sugar maple decline: crown thinning and xylem growth reductions (Table 4). Relative to trees on Al-addition and control plots, trees on Ca-addition plots expressed greater crown vigor, a lower percent crown dieback, and greater xylem growth (Huggett et al. 2007). Furthermore, wounds imposed on trees on Ca-addition plots were approximately one-half closed 1 year after wounding, whereas wounds on trees from the control and Al-treated plots showed relatively few signs of healing (Huggett et al. 2007).

DISCUSSION

The results of field-based Ca manipulations we report complement the results of previously reported laboratory-based experiments, but extend these findings by verifying that adequate Ca nutrition is needed to support vital stress response and energy relations of mature red spruce and sugar maple trees in native forests.

Results from laboratory studies and correlative data from the field have linked Ca deficiency to reduced foliar cold tolerance, increased foliar winter freezing injury, crown deterioration, and decline of red spruce in the northern portions of its range (DeHayes 1992, DeHayes et al. 1999, Schaberg et al. 2000a, Lazarus et al. 2004), and to impaired energy relations and decline of red spruce saplings in the south (McLaughlin et al. 1991, McLaughlin et al.1993). Our data from long-term Ca additions at the watershed level also emphasize the importance of adequate Ca nutrition in bolstering foliar cold tolerance and reducing freezing injury. However, our data further highlight the broader influence of adequate Ca nutrition to functional

Table 1.—Mean and SE of cation concentration of current-year red spruce (*Picea rubens*) foliage from Ca-addition and reference watersheds at the Hubbard Brook Experimental Forest on two measurement dates. Significant differences between watershed means based on ANOVA are indicated by asterisks: *, P<0.10; ** P<0.05; and ***P<0.001. October 2003 data from Hawley et al. 2006, and November 2005 data from Halman et al. 2008.

	Foliar cation concentration (mg kg^{-1} dry mass)				
Treatment	Ca	Al	K	Mg	Mn
October 2003					
Ca-addition	2,225.8 ***	25.2	6,664.9	901.8	1,715.0
SE	123.4	8.9	632.1	44.4	182.1
Reference	1,704.6 ***	40.7	7,896.1	804.7	1,440.3
SE	108.4	6.8	555.1	39.0	159.9
November 2005					
Ca-addition	2,035.4 **	35.4	5,385	715.4	881.8 **
SE	78.2	1.8	193	17.1	64.0
Reference	1,796.3 **	36.0	5,242	681.0	1,330.8 **
SE	77.0	1.6	178	19.8	106.0

Table 2.—Mean and SE of winter injury, bud mortality, ascorbate peroxidase (APX) activity (expressed per mg of protein), sucrose, total sugars, and cold tolerance (T_m) of current-year red spruce (*Picea rubens*) foliage from Ca-addition and reference watersheds at the Hubbard Brook Experimental Forest on two measurement dates. Significant differences between watershed means based on ANOVA are indicated by asterisks: *, P<0.10; ** P<0.05; and ***P<0.01. 2003 data from Hawley et al. 2006, and 2006 data from Halman et al. 2008.

Treatment	**Winter Injury**	**Bud mortality**	**APX**	**Sucrose**	**Total sugars**	**T_m**
	2003	**2003**	**2006**	**2006**	**2006**	**2006**
	(%)	**(%)**	**(µmol mg^{-1} min^{-1})**	**--------- (mg g^{-1} dry mass) -----**		**(°C)**
Ca-addition	74.3***	6.8***	25.8*	4.00**	84.85**	-43.5**
SE	5.9	1.5	3.9	0.30	2.34	1.4
Reference	24.7***	29.8***	15.1*	2.68**	74.85**	-32.2**
SE	5.4	6.0	2.8	0.29	3.05	1.1

Table 3.—Treatment differences in foliar element concentrations (mean±SE) for sugar maple (*Acer saccharum*) trees in 2004 from Ca-addition, reference, and Al-addition study plots in the NuPert Study at the Hubbard Brook Experimental Forest. Means within element followed by the same letter are not significantly different (P<0.05), based on data from Huggett et al. 2007.

	Foliar cation concentration (mg kg^{-1} dry mass)				
Treatment	**Ca**	**Al**	**K**	**Mg**	**Mn**
August 2004					
Ca-addition	7,959.6 a	30.5 a	6,940.5 a	1,127.9 a	1,107.4 a
SE	477.5	1.4	226.8	70.8	78.7
Reference	5,505.2 b	37.1 b	7,595.7 a	1,006.5 a	1,113.1 a
SE	439.8	1.7	190.5	73.5	107.0
Al-addition	4,506.6 b	40.2 b	7,492.7 a	882.2 a	1,096.8 a
SE	392.3	2.4	278.5	82.2	107.5

Table 4.—Treatment differences in crown vigor, branch dieback, BAI ratio 2002, BAI ratio 2004, and wound closure (mean±SE) for sugar maple (*Acer saccharum*) trees in Ca-addition, reference and Al-addition study plots in the NuPert Study at Hubbard Brook Experimental Forest. Means within measure followed by the same letter are not significantly different (P<0.05), based on data from Huggett et al. 2007.

Treatment	Crown Vigor	Branch dieback (%)	BAI ratio 2002	BAI ratio 2004	Wound closure (mm)
Ca-addition	1.05 a	2.50 a	4.3 a	2.6 a	0.615 a
SE	0.05	0.57	0.5	0.3	0.313
Reference	1.60 b	5.50 b	2.1 b	1.9 b	-0.303 b
SE	0.20	1.08	0.3	0.3	0.260
Al-addition	1.30 b	5.25 b	2.2 b	1.6 b	0.060 b
SE	0.13	0.99	0.2	0.2	0.025

foliar antioxidant systems that protect against a range of oxidative stresses – not just those associated with the cold. Furthermore, Ca addition increased the storage of sucrose and total sugars in red spruce foliage – providing direct evidence of how adequate Ca availability improves the energy relations (here sugar storage) in mature trees in the field. Separate measures of foliar chlorophyll fluorescence for mature red spruce on the Ca-addition and reference watersheds at HBEF also highlight the influence of Ca on carbon (C) relations – here a reduction in photosystem function during summer when Ca is limiting (Boyce 2007).

Sugar maple decline is characterized by a slow loss of crown vigor, the dieback of fine twigs, and reduced radial increment over a period of years, frequently ending in tree death (Horsley et al. 2002). Both reductions and/or imbalances in base cation nutrition and stress imposed by some secondary biotic or abiotic factors have been linked to the onset and severity of decline (Horsley et al. 2002). Here we document that long-term Ca addition reduced the primary symptoms of sugar maple decline and increased crown vigor, reduced the percent of branch dieback, and increased basal area growth relative to trees on ambient (Ca-depleted) and Al-addition plots. In addition, Ca addition significantly improved the rate of stem wound closure – a quality that would benefit sugar maple trees that are regularly wounded for maple syrup production. Deficiencies or imbalances in other cations (e.g., Mg and K) undoubtedly also influence sugar maple health in the field (Horsley et al. 2002, St. Clair et al. 2008). However, our data showing significant reversals in decline symptoms with Ca-specific treatments are consistent with other reports that highlight the particular importance of Ca nutrition to sugar maple growth and survival (Juice et al. 2006, Moore and Ouimet 2006, Schaberg et al. 2006).

Overall, Ca addition to the soils studied was associated with a measurable increase in tree stress response capabilities and increased C storage (expressed as either increased woody growth or elevated concentrations of soluble sugars in foliage). Importantly, because comparisons for Ca treatment for both watershed and plot-based experiments were sites that experienced ambient Ca depletion, the reduced function and vigor of trees on control plots represent the decreased vigor and capacity of native trees due to soil Ca depletion.

Although our data highlight the influence of Ca nutrition on the health and productivity of red spruce and sugar maple trees in native forests in New Hampshire, other data indicate that these same processes have relevance to the vigor of these species in more southern locales. For example, numerous studies have shown that acidic deposition is associated with reduced foliar Ca nutrition and altered C relations (especially increased foliar respiration) among red spruce saplings in the Southern Appalachians (McLaughlin et al. 1990, McLaughlin et al. 1991, McLaughlin and Tjoelker 1992, McLaughlin et al. 1993). In addition, deficiencies of Ca and Mg were associated with the decline in sugar maple trees along the Allegheny Plateau in Pennsylvania (Kolb and McCormick 1992), and additions of Ca and Mg improved growth and crown vigor in these sites (Long et al. 1997). Our data from HBEF show that reversals in sugar maple decline occur with Ca addition alone and that the association of Ca nutrition and tree health is not limited to a specific geographic region. Indeed, new associations between the Ca status and health of trees periodically emerge – particularly in regions that have experienced prolonged pollution-induced Ca leaching (e.g., Europe) or where acid inputs are on the rise (e.g., China) (Schaberg et al. in press).

Because one common consequence of Ca deficiency is a disruption in C storage, our results also highlight a potentially important connection between two pollution-induced threats to forest health and sustainability: acid deposition and climate change. If further testing confirms a general connection between acidic deposition-induced Ca depletion and reduced C sequestration in trees, then the effects of one pollution-based stressor (acidic deposition) would be implicated in exacerbating the driver of another stress agent (climate change) by limiting tree C sequestration and storage. As such, our findings suggest one mechanism through which two anthropogenic stress factors can interact to jeopardize forest health and ecosystem function.

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