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Calcium addition at the Hubbard Brook Experimental Forest reduced winter injury to red spruce in a high-injury year

Gary J. Hawley, Paul G. Schaberg, Christopher Eagar, and Catherine H. Borer

Abstract: Laboratory experiments have verified that acid-deposition-induced calcium (Ca) leaching reduces the foliar cold tolerance of red spruce (*Picea rubens* Sarg.) current-year foliage, increasing the risk of winter injury and crown deterioration. However, to date no studies have shown that ambient losses in soil Ca have resulted in increased winter injury in the field. In 2003, a year of severe region-wide winter injury to red spruce, we measured the nutrition and winter injury of current-year foliage and bud mortality for red spruce on two watersheds at the Hubbard Brook Experimental Forest in Thornton, New Hampshire: (1) a reference watershed that has undergone considerable Ca loss attributed to acid-deposition-induced leaching and (2) a watershed that was fertilized with $CaSiO_3$ in 1999 to replace lost Ca. For all crown classes combined, winter injury was significantly greater (P = 0.05) for red spruce on the reference watershed than for spruce on the Ca-addition watershed. Differences in foliar injury were particularly evident for dominant and codominant trees. For these crown classes, red spruce on the reference watershed lost about 75% of their current-year foliage to winter injury, about three times more than foliar losses for the Ca-addition watershed (P = 0.01). Patterns of bud mortality followed that of foliar injury. The only difference in foliar cation nutrition detected was a significantly greater concentration of Ca in red spruce foliage from the Ca-addition watershed relative to spruce from the reference watershed (P = 0.001). Differences in Ca concentration, foliar winter injury, and bud mortality that occurred coincident with watershed Ca treatment provide the first evidence that ambient Ca depletion is associated with elevated winter injury of red spruce trees.

Résumé : Des expériences en laboratoire ont démontré que le lessivage du calcium (Ca) causé par les dépôts acides diminue la tolérance au froid du feuillage de l'année de l'épinette rouge (*Picea rubens* Sarg.), ce qui augmente les risques de dommages causés par le froid et de dépérissement de la cime. Cependant, à ce jour aucune étude n'a démontré que les pertes ambiantes de Ca dans le sol ont eu pour effet d'accroître les dommages dus au froid sur le terrain. En 2003, une année où l'épinette rouge a subi des dommages sévères dus au froid dans l'ensemble de la région, nous avons mesuré les nutriments et les dommages causés par le froid au feuillage de l'année ainsi que la mortalité des bourgeons de l'épinette rouge dans deux bassins versants à la Forêt expérimentale de Hubbard Brook à Thornton, au New Hampshire : un bassin versant témoin qui a subi des pertes considérables de Ca à cause du lessivage dû aux dépôts acides et un autre bassin qui a été fertilisé avec du $CaSiO_3$ en 1999 pour remplacer le Ca perdu. Pour toutes les classes de cime combinées, les dommages dus au froid étaient significativement plus sévères (P = 0,05) chez les épinettes rouges du bassin témoin que chez celles du bassin fertilisé en Ca. La différence dans la sévérité des dommages foliaires était particulièrement évidente chez les arbres dominants et codominants. Dans ces classes de cime, les épinettes rouges du bassin témoin avaient perdu environ 75 % de leur feuillage de l'année à cause des dommages dus au froid, soit environ trois fois plus de pertes de feuillage que dans le bassin fertilisé en Ca (P = 0,01). Les patrons de mortalité des bourgeons suivaient ceux des dommages foliaires. La seule différence qui a été détectée dans la nutrition foliaire en cation était une concentration significativement plus grande de Ca dans le feuillage des épinettes rouges du bassin fertilisé en Ca comparativement aux épinettes du bassin témoins (P = 0,001). Les différences dans la concentration en Ca, les dommages foliaires causés par le froid et la mortalité des bourgeons qui coïncident avec l'apport de Ca

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G.J. Hawley[1] and C.H. Borer.[2] The University of Vermont, The Rubenstein School of Environment and Natural Resources, Burlington, VT 05405, USA.
P.G. Schaberg. USDA Forest Service, Northern Research Station, South Burlington, VT 05403, USA.
C. Eagar. USDA Forest Service, Northern Research Station, Durham, NH 03824, USA.

[1]Corresponding author (e-mail: gary.hawley@uvm.edu).
[2]Present address: Berry College, Department of Biology, Mount Berry, GA 30149, USA.

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dans un bassin versant constituent la première preuve que la perte de Ca ambiant est associée à l'augmentation des dommages causés par le froid chez l'épinette rouge.

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Introduction

Red spruce (*Picea rubens* Sarg.) winter injury results from freezing damage that leads to the reddening, mortality, and eventual abscission of the most recent foliar age-class (DeHayes 1992). Exposure to low temperatures (DeHayes et al. 1990), freeze–thaw cycles (Hadley and Amundson 1992; Lund and Livingston 1998), and rapid freezing (Perkins and Adams 1995) can all induce winter injury damage in laboratory studies. Red spruce current-year foliage is particularly vulnerable to freezing injury because it achieves reduced levels of cold tolerance compared with older red spruce foliage or foliage of other sympatric species (DeHayes et al. 2001). However, experimental evidence suggests that elevated injury occurs after further reductions in cold tolerance following exposure to anthropogenic factors, such as acidic deposition and protracted nitrogen (N) addition, which deplete critical pools of calcium (Ca) in the foliage (Schaberg and DeHayes 2000).

The mechanism through which Ca depletion reduces red spruce cold tolerance was first described through controlled exposures of seedlings to simulated acidic deposition (DeHayes et al. 1999; Schaberg et al. 2000). These studies showed that acid mist leached Ca from foliage and depleted Ca specifically associated with mesophyll cell membranes. This loss of membrane-associated Ca (mCa) destabilizes cells, reduces foliar cold tolerance, and increases the risk of freezing injury (DeHayes et al. 1999; Schaberg et al. 2000). The same mechanistic sequence of depleted mCa, reduced membrane stability, and cold tolerance resulted in elevated winter injury for native, mature red spruce following protracted soil-based N fertilization (Schaberg et al. 2002).

Numerous anthropogenic factors appear to be depleting Ca from forest ecosystems (Likens et al. 1996, 1998), including red spruce stands (Joslin et al. 1992). High acid loading that promotes Ca leaching is an important and consistent explanation for soil Ca depletion in north temperate forests (Likens et al. 1996, 1998). Other potential contributing factors include declines in atmospheric base cation deposition (Hedin et al. 1994), soil aluminum (Al) mobilization (Lawrence et al. 1995), intensive forest harvesting (Federer et al. 1989), nitrogen saturation (Aber et al. 1998), and soil warming (Tomlinson 1993). Despite evidence of soil Ca depletion and detailed experimental verification that reductions in labile Ca reduce red spruce foliar cold tolerance, no studies have shown that ambient losses in soil Ca have resulted in increased winter injury in the field.

The severe, region-wide winter injury event of 2003 (Lazarus et al. 2004) provided a unique opportunity to test the influence of existing soil Ca loss on red spruce winter injury. In 2003, we measured foliar nutrition, foliar winter injury, and bud mortality for red spruce on two nearby watersheds at the Hubbard Brook Experimental Forest (HBEF, Thornton, New Hampshire): a reference (watershed 6, for which substantial Ca loss has been documented; Likens et al. 1996, 1998) and a Ca-addition watershed (watershed 1, which was fertilized with pelletized wollastonite ($CaSiO_3$) in 1999 to replace lost Ca; Peters et al. 2004). By comparing Ca nutrition and winter injury levels for red spruce from these watersheds we conducted the first specific evaluation of whether ambient Ca leaching is associated with greater winter injury of red spruce.

Methods

Watershed treatments

The Ca-addition Watershed Experiment at HBEF was designed to evaluate the role of Ca supply in regulating the structure and function of base-poor forest ecosystems. In October 1999, $CaSiO_3$ was added to watershed 1 (Ca-addition watershed) at a rate of 850 kg Ca/ha to increase the base saturation of the soil from about 10% to approximately 19% — a value thought to be close to the base saturation of those soils prior to acid deposition. Fifty-six metric tons of VANSIL-10, a dry commercial form of wollastonite, was crushed, pelletized with a lignin sulfonate binder (approximately 2% wet weight), and applied by helicopter to the 11.8 ha Ca-addition watershed. Silicon (Si), which was added along with the Ca in wollastonite, is the second-most abundant element in the Earth's crust and is typically not limiting in soils (Marschner 2002). The increase in exchangeable Ca is expected to develop over a decade or more as the wollastonite gradually weathers. These south-facing watersheds are located in the Hubbard Brook Valley and have a 20%–30% average slope. For details of the wollastonite application study see Peters et al. (2004). Likens et al. (1996, 1998) have documented a substantial soil-available-Ca loss in the 13.2 ha reference watershed.

Foliar winter injury assessments

Winter injury was assessed on 27 May 2003 on both the Ca-addition and reference watersheds at HBEF. Circular plots (0.1 ha) containing dominant or codominant red spruce were randomly chosen in each watershed. All red spruce trees in these plots above breast height (1.2 m) were assessed and categorized into the following crown classes: dominant, codominant, intermediate, suppressed, and understory. A total of 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 (an average of 6.3 trees per plot). All spruce trees in each plot were visually evaluated for the reddening of current-year foliage and rated on a scale of 0–10 by two observers (Lazarus et al. 2004). A score of 1 represented 1%–10% injury, a score of 2 represented 11%–20% injury, etc.

Bud mortality

Bud mortality associated with foliar winter injury was visually assessed on both watersheds using binoculars on 28

Table 1. Example of nested statistical design using foliar winter injury data.

Source of variation	*n*	df	Statistical *F* test
Treatment (watershed)	2	1	Treatment MS / plot within treatment MS
Plot within treatment	13	11	Plot within treatment MS / tree within plot MS
Tree within plot	82	69	

Note: MS refers to mean squares from analyses of variance.

October 2003. Because earlier assessments of foliar winter injury were conducted on plots that were located by GPS and were not permanently marked, we were able to return to the same plot locations, but not necessarily to the same exact trees. A total of 30 trees in six plots from the Ca-addition watershed and 34 trees in seven plots from the reference watershed were examined (an average of 4.9 trees per plot). Bud mortality measurements were conducted using the same 0–10 injury scale used for foliar assessments. For each red spruce tree assessed, two measurements were made: the proportion of current-year foliage (elongated in 2002) shoots that were winter injured in 2003 and the proportion of those shoots that failed to break bud in 2003. These two measurements were multiplied to estimate the proportion of all buds produced in 2002 that were lost in association with winter injury (see Lazarus et al. 2004).

Foliar nutrition

Associated with bud mortality assessments, branch samples from the upper third of the crown of dominant and codominant trees were collected using shotguns for foliar cation analysis. Foliar samples were collected on 28 October 2003. Samples from winter injury plots were supplemented with dominant and codominant trees in immediately adjacent plots to produce a total sample of 31 trees in six plots from the Ca-addition watershed and 37 trees in seven plots from the reference watershed (an average of 5.2 trees per plot). Current-year shoots were excised from branches, bulked per tree, sealed in plastic bags, and transported to the laboratory for processing. Current-year foliar samples were oven-dried (65 °C), ground, and digested by heating with nitric acid and hydrogen peroxide (adapted from Jones and Case 1990) using a block digester (Westco Scientific Instruments, Danbury, Connecticut). Digests were analyzed for total foliar cation concentrations by inductively coupled plasma atomic emission spectroscopy (ICP-AES, PlasmaSpec 2.5, Leeman Labs, Lowell, Massachusetts). We report data for Al, Ca, Fe, K, Mg, and Mn. Eastern white pine needles from the National Bureau of Standards and Technology (SRM 1575), sample duplicates, and blanks were analyzed for procedural verification. Standards were within 5% of certified values.

Statistical analysis

Analyses of variance (ANOVA), using a nested design (see Montgomery 2001), were used to test for treatment differences among means. All data were analyzed using JMP Statistical Software (2002, SAS Institute Inc., Cary, North Carolina). With this nested design, we used plot within treatment means (six to seven randomly selected plots per treatment (watershed)) and variance estimates to test for the significance of treatment differences, and tree within plot (average of 5.2 trees per plot) mean and variance estimates to test for the significance of plot within treatment differences. Table 1 shows an example of the statistical design used in this study. All data were analyzed using individual tree as the unit of observation. Foliar reddening and bud mortality means were calculated from damage class percentage midpoints. These data were treated as continuous because damage classes represented increasing levels of injury and damage class ranges were small and of equal size.

Results and discussion

Watershed differences in winter injury and foliar nutrition

For all crown classes combined, foliar winter injury was significantly greater ($P = 0.05$) for trees on the reference watershed than for trees on the Ca-addition watershed (Fig. 1A). Winter injury was greatest for dominant and codominant trees at the Hubbard Brook watersheds, which was consistent with region-wide findings that year (Lazarus et al. 2004). Differences in winter injury between watersheds were most evident for these crown classes. Dominant and codominant red spruce on the reference watershed lost about 75% of their current-year foliage to winter injury, which is about three times greater than the same crown classes on the Ca-addition watershed (Fig. 1A). Although the same trend for elevated injury existed for intermediate, suppressed, and understory red spruce on the reference relative to the Ca-addition watershed, levels of injury for these smaller trees with less crown exposure were much lower and more variable, and thus means were not significantly different (Fig. 1A).

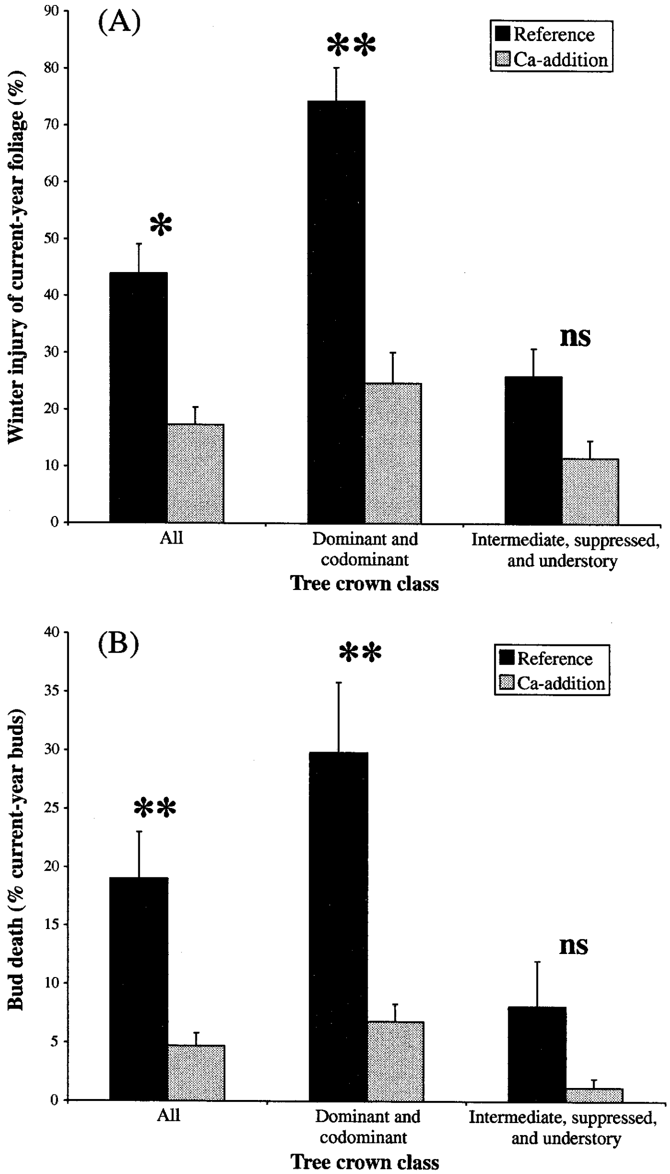
As found in other locations in 2003 (Lazarus et al. 2004), patterns of bud mortality generally paralleled foliar injury levels. For all crown classes combined, bud mortality was significantly greater ($P = 0.01$) for trees on the reference watershed than for trees on the Ca-addition watershed (Fig. 1B). Differences in bud mortality between the watersheds were significant for dominant and codominant trees, but not for intermediate, suppressed, and understory crown classes (Fig. 1B).

We measured foliar cation concentrations for dominant and codominant trees in the Ca-addition and reference watersheds (Table 2) to explore whether differences in foliar and bud mortality were associated with differences in tree nutrition. Results of this analysis showed significantly greater ($P = 0.001$) concentrations of Ca in foliage from the Ca-addition watershed relative to the reference watershed. However, no other differences in foliar cation concentrations were detected (Table 2).

Mechanistic explanation of response

Data from replicated experiments have shown that acidic mist directly leaches Ca from red spruce current-year fo-

Fig. 1. Means (±1 SE) of current-year red spruce winter injury in reference and Ca-addition watersheds and among crown classes; foliar injury (A) and bud mortality (B). Watershed means were either not significantly different (ns) or statistically different at $P \le 0.05$ (*) or $P \le 0.01$ (**) based on nested analyses of variance.



liage, preferentially displaces mCa from the plasma membranes of mesophyll cells, destabilizes these membranes, depletes Ca thought to be important to plant stress response systems, and significantly reduces foliar cold tolerance (DeHayes et al. 1999; Schaberg et al. 2000). Additional work has verified that Ca depletion instigated through protracted soil nitrogen additions can induce this same mechanistic sequence and result in increased winter injury and experimentally induced stand decline (Schaberg et al. 2002). Building upon this understanding, we propose that Ca addition to watershed one not only increased foliar Ca levels in red spruce, but specifically added to critical mCa pools, which stabilized membranes, increased foliar cold tolerance, and resulted in reduced freezing injury. Specific evaluation of this mechanistic sequence is ongoing.

Despite evidence that experimentally induced Ca depletion can increase red spruce winter injury, to date no data have shown that ambient Ca depletion contributes to winter injury in the field. Spatial analysis of patterns of winter injury in New York, Vermont, New Hampshire, and Massachusetts for 2003 indicated that winter injury was greatest at high elevations, in western portions of the region, and on west-facing slopes — all areas that receive higher atmospheric inputs of H^+ and would be prone to Ca depletion (Lazarus et al. 2006). However, the current study is unique in that greater winter injury was not associated with treatment-induced Ca depletion or likely depletion levels across the landscape, but was associated with documented ambient Ca loss contrasted with experimental Ca addition. This addition increased soil pH in the spruce–fir zone of the Ca-addition watershed from pretreatment levels of 3.6 to 4.3 in 2002 (Fisk et al. 2006).

Implications for forest health

Our data provide the first evidence that ambient Ca depletion is associated with elevated winter injury of red spruce foliage. Regionally, winter injury was unusually high in 2003 (Lazarus et al. 2004). Levels of winter injury for dominant and codominant trees on the reference watershed resembled the regional means for 2003 (Lazarus et al. 2004). However, injury levels for dominant and codominant trees on the Ca-addition watershed were less than half the injury levels on the reference watershed and were close to means reported for the northeast in 1989 and 1993 — the next most recent years of high injury (Peart et al. 1991; Boyce 1995; Lazarus et al. 2004). Injury levels above historic means on the reference watershed raise the possibility that recent ambient Ca losses may have driven some trees below biological Ca thresholds and predisposed them to increased winter injury as documented in the reference watershed and regionally in 2003.

Because the movement of labile Ca is a critical component of plant stress response systems (Sanders et al. 1999; Pandey et al. 2000), we have hypothesized that anthropogenic disruptions of biologically available Ca (e.g., mCa) may impair the ability of plants to recognize and respond to environmental stresses such as freezing, and thus predispose forests to decline (Schaberg et al. 2001). Reduced stress response would predispose plants to amplified injury following natural or anthropogenic stress events and increase the probability of injury and decline of individual plants and populations. The greater foliar-freezing injury and bud mortality and lower foliar Ca concentrations of red spruce on the reference watershed relative to trees on the Ca-addition watershed are consistent with our hypothesis. However, this hypothesis is not limited to any one species or decline scenario. Indeed, some recent data suggest that the abundance and vigor of sugar maple seedlings is also greater on the Ca-addition watershed than the reference watershed (Juice et al. 2006), perhaps because Ca-sufficient seedlings are better able to sense and respond to the many environmental (e.g., light, water, and temperature) cues and stress events that influence regeneration success.

Table 2. Cation concentrations and standard errors (SE) of the means for current-year foliage of dominant and codominant red spruce trees from the Ca-addition and reference watersheds at the Hubbard Brook Experimental Forest.

	Mean foliar element concentrations (mg·kg^{-1})					
Treatment	Ca	Al	Mg	K	Mn	Fe
Ca-addition						
Mean	2225.8	25.2	901.8	6664.9	1715.0	40.9
SE	123.4	8.9	44.4	632.1	182.1	3.2
Reference						
Mean	1704.6	40.7	804.7	7896.1	1440.3	37.8
SE	108.4	6.8	39.0	555.1	159.9	2.8
Significance	***	ns	ns	ns	ns	ns

Note: *** indicates that differences between watershed means are significantly different at $P \leq 0.001$ and ns indicates that means are not significant based on ANOVA results.

Whatever the implications for other species, the differences in injury coincident with Ca treatment we report are relevant to the health and productivity of red spruce forests in the northeastern United States and adjacent Canada. Continued acid-induced soil and foliar Ca depletion would be expected to drive more red spruce forests beyond Ca deficiency thresholds and increase the likelihood of high foliar and bud mortality levels like those documented for 2003. The thinning of crowns following foliar and bud mortality has been associated with the reduced growth (Wilkinson 1990; Tobi et al. 1995) and increased mortality (Lazarus et al. 2004) of red spruce — changes that could alter ecosystem processes (e.g., optimal net primary productivity, controlled nutrient cycling, seed production, and habitat) beyond those pertinent to a single tree species.

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