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Acidic mist reduces foliar membrane-associated calcium and impairs stomatal responsiveness in red spruce

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Summary Acidic deposition can leach essential pools of calcium (Ca) directly from plant foliage. Because of the central role of Ca in environmental signal transduction, disruptions of labile foliar Ca pools could impair physiological responses to a variety of environmental stimuli and stressors. We investigated the possibility that acidic mist-induced depletion of membrane-associated Ca (mCa), which is one form of labile Ca, may alter stomatal responsiveness to water stress, a process known to include Ca in signal transduction cascades. Red spruce (*Picea rubens* Sarg.) seedlings were exposed to either pH 3.0 or pH 5.0 mist treatments for one growing season. Foliar nutrition was assessed following treatments, and declines in stomatal conductance and net photosynthesis were measured on current-year shoots following stem excision. Seedlings exposed to pH 3.0 acidic mist treatments had reduced mCa relative to the pH 5.0 treated seedlings. Seedlings subjected to the pH 3.0 acidic mist treatment exhibited impaired stomatal functions, including a smaller maximum aperture, slower closure and an increased lag time between stomatal closure and photosynthetic decline following experimental water stress. Delayed stomatal closure could undermine desiccation avoidance mechanisms. Previous work has demonstrated that acidic mist treatments deplete mCa in red spruce and impair cold tolerance, with similar effects in other species. The results we present provide further evidence that acidic mist-induced mCa depletion may cause disruption of a broad range of plant stress responses.

Keywords: acid rain, foliar nutrients, mCa, Picea rubens, stomata, water stress.

Introduction

Widespread reductions in environmental calcium (Ca) availability have been documented in a variety of forest ecosystems throughout the United States (e.g., Johnson et al. 1994, Hallett and Hornbeck 1997, Lawrence et al. 1997, Likens et al. 1998, Driscoll et al. 2001) and Europe (e.g., Hütte 1989, Stoddard et al. 1999). Reduced environmental Ca availability may be caused by numerous factors, including biomass removal (Tritton et al. 1987, Federer et al. 1989), reduced particulate deposition (Likens et al. 1996), soil acidification (Butler et al. 2001, Driscoll et al. 2001), nitrogen saturation (Aber et al. 1998), and increased Al solubility, which can inhibit Ca uptake (Cronan 1991) and accelerate losses of soil Ca by leaching (Lawrence et al. 1995). Further, direct acidic leaching from plant foliage can selectively deplete the labile and physiologically available pool of membrane-associated Ca (mCa), without measurably altering total foliar Ca concentrations (Schaberg et al. 2000), which are likely dominated by crystalline deposits of sparingly soluble Ca oxalate (Fink 1991).

Acidic leaching of mCa from foliage is associated with a variety of physiological impairments in red spruce (*Picea rubens* Sarg.), including reductions in plasma membrane stability and cold hardiness, which have been implicated in the decline of montane red spruce (DeHayes et al. 1999, Schaberg et al. 2000). Calcium enhances the structural stability of cell membranes through electrostatic interactions with plasma membrane phospholipids and proteins, and is an important component of the cell wall (van Steveninck 1965, Hanson 1984, Marschner 1995). Acidic mist selectively leaches Ca from the labile apoplasmic pool (measured as mCa), leaving a reduced supply of Ca to stabilize the plasma membranes and cell walls, despite significant amounts of Ca bound as Ca oxalate, which is insoluble and therefore unavailable for structural maintenance. Depletion of the labile Ca pool leaves cells susceptible to damage from mechanical stress during low-temperature-induced winter injury events (DeHayes et al. 1999, Schaberg et al. 2000).

Not all functional impairment as a result of foliar Ca leaching is structural, however. Labile Ca is an important second messenger in the perception and transduction of environmental cues, including low temperatures that trigger normal physiological responses such as autumnal cold acclimation (e.g., Arora and Palta 1988, Monroy et al. 1993, Knight 2000). Reduced mCa could limit the physiological responsiveness of foliage to environmental changes, thereby exacerbating the structural deficits that accompany Ca loss. If depletion of mCa is indicative of a depleted pool of messenger Ca, acidic leaching of labile foliar Ca could similarly impair numerous plant responses to environmental cues (Schaberg et al. 2001). Cal-

cium-dependent signaling mediates a wide variety of physiological processes, including regulation of ion channels in guard cells (Mansfield et al. 1990, MacRobbie 1997). This allows precise control of stomatal aperture in response to changes in CO_2 concentration (Webb et al. 1996), water availability (Knight et al. 1997, Knight 2000) and temperature (Wilkinson et al. 2001). Thus, plants with reduced or insufficient mCa may have impaired stomatal control and a compromised ability to acclimate to environmental alterations in such factors as light, temperature, CO_2 concentration and water availability. This, in turn, could affect a plant's ability to respond to environmental fluctuation via adjustments in rates of photosynthesis and transpiration.

Depletion of Ca from foliar pools that are essential in specific signal transduction pathways is expected to impair plant responsiveness to environmental changes (DeHayes et al. 1999, Schaberg et al. 2001). Guard cell turgor, and thus stomatal aperture, is regulated by Ca-dependent signal transduction, and mCa is known to be depleted by acidic leaching. This study was designed to determine whether acidic mist-induced depletion of foliar mCa impairs stomatal responsiveness to water stress.

Materials and methods

Plant material and acidic mist treatments

Red spruce seed from a bulk Vermont seed collection was germinated in 1997 and planted in a greenhouse potting soil mixture in 20-cm-diameter pots. Seedlings were grown under ambient environmental conditions at the USDA Forest Service Northeastern Research Station, South Burlington, VT, and were 4 years old at the time of the experiment. Four seedlings were assigned by ascending height to each of six blocks. Within each block, two seedlings were randomly assigned to each of two mist treatments: pH 3.0 or pH 5.0 (control) artificial precipitation solutions. The pH 3.0 treatment was within the range of cloud acidity that has been measured in high elevation spruce–fir forests in the eastern United States (Saxena and Lin 1990) and was less acidic than the fog measured on the summit of Mount Mansfield, VT, which had a mean pH of 2.8 during the summer of 1998 (DeHayes et al. 1999).

The base mist solution was designed to simulate the ionic composition of rainwater in the northeastern United States, as described by Schaberg et al. (2000), and contained 150 μM NH_4NO_3; 12.5 μM $(NH_4)_2SO_4$; 10 μM NaCl; 5 μM KCl; and 2.5 μM $MgSO_4 \cdot 7H_2O$. So that all plants would receive the same sulfate concentration, both pH 3.0 and pH 5.0 mist solutions were adjusted to pH 3.0 with H_2SO_4, and then the pH 5.0 solution was titrated back to pH 5.0 with NaOH. Solutions were not adjusted with HNO_3 because N additions have been documented to alter foliar Ca concentrations in red spruce (Swan 1971, Schaberg et al. 1997). Plots received 6 mm of mist on four nights each week, for a total of 24 mm weekly, approximately the mean weekly rainfall in the northeastern United States (Wood and Bormann 1977, Raynal et al. 1982, Houle et al. 1999). Mist treatments started in July 2001 and continued for 12 weeks through the period of data collection for this study.

Foliar Ca analyses

Relative membrane-associated calcium was compared between acidic mist treatments, using standard fluorescence techniques (Borer et al. 1997). Four current-year needles per plant were sectioned and stained with chlorotetracycline (CTC) and evaluated via epifluorescence microscopy and digital image analysis. Chelation of CTC to divalent cations in close proximity to an apolar environment, such as a biological membrane, results in a conformational change in the CTC molecule, substantially increasing fluorescence intensity over that found in an entirely polar environment (Caswell and Hutchinson 1971). Techniques and instrumentation have been fully evaluated to verify that Ca is the cation assessed by these methods (Borer et al. 1997).

Total foliar Ca and potassium (K) concentrations were also measured. Samples were oven-dried (65 °C), ground (2-mm mesh) and digested by heating with nitric acid and hydrogen peroxide (adapted from Jones and Case 1990). Sample digestion was followed by analysis via Inductively Coupled Plasma Atomic Emission Spectrometry (ICP/AES) with a Perkin Elmer Optima 3000DV ICP/AES (Perkin Elmer Analytical Instruments, EG&G, Wellesley, MA). Pine needles (SRM 1575) from the National Institute of Standards and Technology, sample duplicates and blanks were analyzed for procedural verification.

Measurements of stomatal and photosynthetic responsiveness to water stress

Stomatal and photosynthetic responsiveness to water stress was assessed by observing time course changes in current-year foliage of excised branches (Meng and Arp 1993) of acidic mist-treated plants, held under constant conditions of light, CO_2 concentration and temperature. Measurements were made with a Li-Cor LI-6400 portable photosynthesis system (Li-Cor, Lincoln, NE). Stomatal conductance, net photosynthetic rate and internal CO_2 concentration (C_i) were calculated from measured parameters. Stomatal conductance was used as a proxy for stomatal aperture. Photosynthetic photon flux (PPF) was above saturation (1000 μmol m^{-2} s^{-1}), CO_2 concentration was 350 μmol mol^{-1} and temperature was held at a value (usually 20–25 °C) reflecting the conditions of the particular measurement day.

Before shoots were sampled for measurement, plants were removed from mist enclosures, dried gently with paper towels, and allowed to acclimate in full sun for at least 45 min. Measurements, which began after 1000 h and were usually completed before 1700 h, were made on clear sunny days toward the end of the growing season.

Assessment of each twig lasted approximately 1.5 h. Because only four series of measurements could be made each day, seedlings were assessed in pairs (one from each treatment from each block, ordered randomly) to control for day-to-day differences in environmental conditions. Measurements were made on needles attached to the distal 1.5 cm of one sun-ex-

posed current-year shoot from each plant. Immediately proximal to the retained needles, a 1–2 cm section of the shoot was defoliated with scissors. The needle-free section of stem allowed proper closure and sealing in the 2 × 3-cm cuvette. Photosynthetic rate and stomatal conductance were recorded every 20 s for about 10 min to establish baseline values of stomatal conductance during active photosynthesis. The stem of the twig was then excised at the edge of the cuvette, leading to increasing water stress as the foliage transpired. Stomatal conductance and photosynthetic rate were recorded every 20 s until stomata closed and photosynthesis ceased.

Following the gas exchange measurements, the foliage was carefully removed from the excised shoot and its projected area measured with a Li-Cor LI-3100 area meter. All data were expressed per unit leaf area.

Data analyses

Blocked one-way analyses of variance were performed to compare mCa and total foliar Ca between mist treatments and among blocks. All stomatal closure and photosynthetic decline data were corrected so that T_0 was the time of stem excision. The decline in both stomatal aperture and net photosynthetic rate over the course of the measurements was most appropriately described by a nonlinear curve (Figure 1):

$$Y = Y_{min} + \frac{Y_{max} - Y_{min}}{1 + e^{k(T_m - \text{time})}} \quad (1)$$

where Y_{max} is the pre-excision background value of the measured parameter, Y_{min} is the ending minimal value, k represents the slope of the linear portion of the decline, and T_m is the time after stem excision at which the Y is midway between Y_{max} and Y_{min}. Curves were fit to data for each excised shoot, and the resultant parameters were compared. The lag time between stomatal closure and photosynthetic decline (i.e., the T_m difference between these two variables) was calculated for each shoot. The relatively long duration of the measurements and likelihood that responses were influenced by differences in environmental conditions on multiple days of measurement (Meng and Arp 1993) necessitated paired assessments of seedlings from the two treatments. Data for each response curve parameter were thus analyzed by paired t-tests. When model assumptions, such as normality, were violated and a suitable transformation was not found, the non-parametric Wilcoxon signed-rank test was used. Rates of photosynthetic decline and stomatal closure were compared by means of covariance and regression analyses.

Results and discussion

Foliar Ca

Foliage from seedlings in the pH 3.0 mist treatment had significantly less mCa than seedlings in the pH 5.0 control treatment, but total foliar Ca did not differ between treatments (Table 1). The potting mix in which seedlings were grown provided ample Ca for uptake, and foliage from all plants had greater than 3000 mg kg^{-1} Ca, which is well above the documented "sufficiency" thresholds for red spruce of 1200 mg kg^{-1} (Swan 1971, DeHayes et al. 1999, Borer et al. 2004) or 1700 mg kg^{-1} (van Miegroet et al. 1993). As with previous

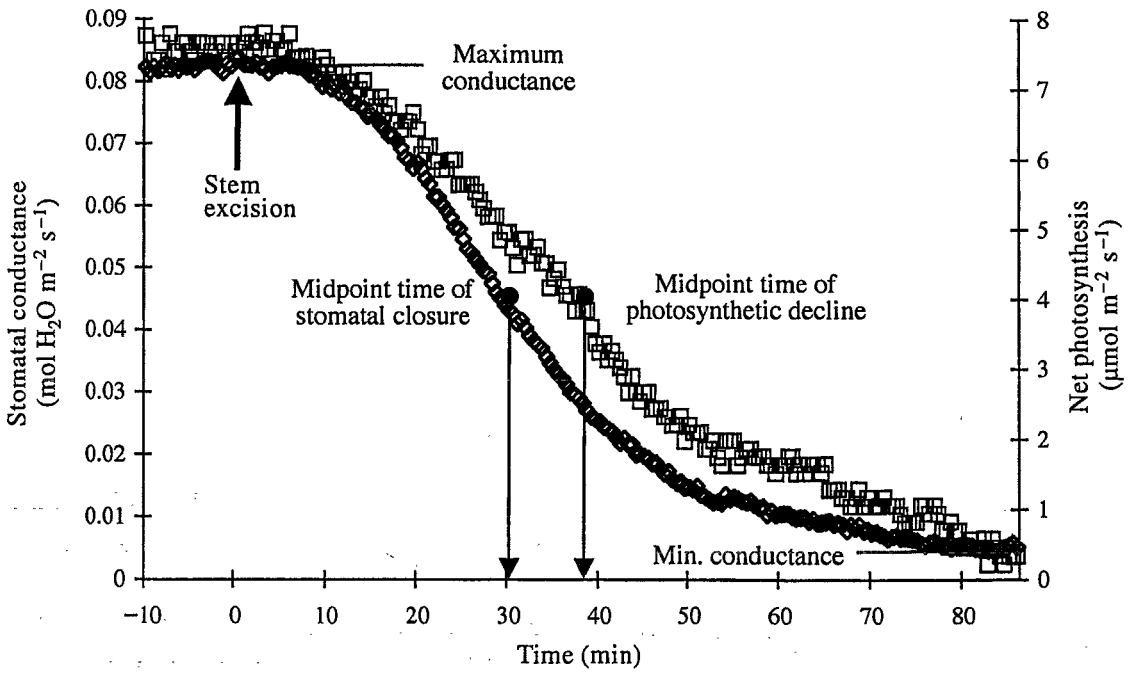

Figure 1. Stomatal conductance (◇) and net photosynthesis (□) of one seedling: stability before and decline after stem excision. Time values have been adjusted so that zero represents the time of stem excision. Pre-excision maximum stomatal conductance, post-closure minimum stomatal conductance and midpoint time values are indicated.

work (e.g., Schaberg et al. 2000), direct foliar leaching by acidic mist treatments significantly reduced the small, but labile and physiologically important, pool of mCa without significantly altering the total foliar Ca concentration. Leaching likely occurs by a passive combination of ion exchange with the apoplast and diffusion through the cuticle (Mecklenburg et al. 1966, Lovett and Hubbell 1991, Turner and van Broekhuizen 1992).

This reduction in mCa without concurrent alteration of total foliar Ca reflects the well-known physiological partitioning of Ca into distinct foliar pools. Chemically sequestered, physiologically unavailable Ca oxalate can represent a large proportion of total foliar Ca (Fink 1991, Borer et al. 2004); thus measures of total foliar Ca may have minimal physiological relevance. In contrast, Ca is maintained at low concentrations in the cytoplasm of all cells, which allows small, localized fluctuations of cytoplasmic Ca to be used as signals. These signals result in physiological changes in response to a wide range of environmental and hormonal cues (e.g., Hanson 1984, Bush 1995, Knight 2000, Roos 2000) and help regulate guard cell turgor and stomatal aperture (e.g., Mansfield et al. 1990, Irving et al. 1992, McAinsh et al. 1995, Li et al. 1998, Grabov and Blatt 1999). Thus, reductions in the labile and physiologically active pool of mCa as a result of leaching by acidic mist may set the stage for additional physiological impairment, even without measurable differences in total foliar Ca concentrations.

Impairment of stomatal responsiveness

Previous work with red spruce showed that acidic mist exposure leaches Ca from foliage, depletes mCa, destabilizes foliar plasma membranes, reduces foliar cold tolerance and increases susceptibility to freezing injury (DeHayes 1999, Schaberg et al. 2000). The current study extends this work by demonstrating that exposure to acidic mist also impairs stomatal responsiveness to water stress. Maximum stomatal aperture and maximum net photosynthetic rate were both in the range previously reported for red spruce (Amundson et al. 1992, Meng and Arp 1993, Eamus 1993), and the minimum values for fully closed stomata and fully arrested photosynthesis did not differ between the treatments. In addition to having less mCa, plants in the pH 3.0 mist treatment had impaired stomatal functioning compared with seedlings in the pH 5.0 mist treatment. For example, maximum stomatal aperture was significantly reduced in the low pH mist treatment (Table 1). This was likely a result of the reduced pool of labile Ca necessary for stomatal control, as demonstrated by the depletion of foliar mCa. Our measurements of mCa are specific to mesophyll cells, which are inherently more protected from leaching by acidic mist than are guard cells, which are part of the epidermis. Thus, the differences we measured in mCa may be conservative estimates of the effect of the mist treatments on the labile apoplasmic pool of Ca surrounding guard cells.

Because all seedlings were actively photosynthesizing, the reduced stomatal aperture in the low pH treatment resulted in significantly reduced C_i (Table 1). However, the lower initial C_i appeared not to be rate limiting for photosynthesis, as indicated by the lack of a treatment difference in the maximum net photosynthetic rate (Table 1). Calcium signaling helps regulate stomatal aperture in response to changes in C_i (Mansfield et al. 1990, Webb et al. 1996). Although lower C_i should stimulate stomatal opening, plants in the low pH treatment had a reduced capacity to respond to low C_i, as seen by their smaller

Table 1. Effects of acidic mist treatment on foliar calcium (Ca) and potassium (K), and on calculated parameter means from nonlinear curve-fitting procedures for gas exchange responses to water stress induced by stem excision. Means and differences were calculated from 12 pairs of trees. The P values < 0.1 are shown in bold. Abbreviation: mCa = membrane-associated calcium.

Parameter	pH 3.0	pH 5.0	Mean difference (seedling pairs)	P value
Relative mCa	0.109	0.148	–	**0.05**[1]
Total foliar Ca (mg kg^{-1})	4529	4255	–	0.50[1]
Total foliar K (mg kg^{-1})	8796	9316	–	0.37[1]
Maximum stomatal conductance (mol H_2O m^{-2} s^{-1})	0.122	0.135	0.017	**0.02**[2]
Minimum stomatal conductance (mol H_2O m^{-2} s^{-1})	0.002	0.004	0.001	0.19[2]
Rate (slope) of stomatal closure	−0.118	−0.150	−0.036	**0.04**[2,3]
Midpoint time of stomatal closure (T_m; min)	37.74	32.62	−6.82	0.19[2]
Maximum photosynthetic rate (μmol CO_2 m^{-2} s^{-1})	10.62	10.27	−0.142	0.37[2,3]
Minimum photosynthetic rate (μmol CO_2 m^{-2} s^{-1})	0.085	0.178	−0.042	0.37[2,3]
Rate (slope) of photosynthetic decline	−0.115	−0.143	−0.029	**0.06**[2]
Midpoint time of photosynthetic decline (T_m; min)	44.48	38.27	−8.34	0.15[2]
Internal CO_2 (C_i; μmol CO_2 mol^{-1} air)	193.4	204.2	10.8	**0.01**[2]
Stomatal closure and photosynthetic decline (lag time between T_m difference; min)	6.74	5.65	−1.27	**0.04**[2]

[1] Analysis of variance (treatment and block) for Ca parameters and K.

[2] One-tail paired t-tests for stomatal and photosynthetic parameters.

[3] The non-parametric Wilcoxon signed-rank was used when model assumptions were violated.

maximum stomatal apertures. This suggests that the acid-induced reduction in labile Ca resulted in impaired stomatal functioning, perhaps by disrupting Ca-dependent signaling pathways. In contrast to our results, Eamus and Fowler (1990) found greater conductances when red spruce was exposed to pH 2.5 mist than when exposed to pH 5.0 mist. Mansfield et al. (1990) indicated that increasing Ca in the apoplast can stimulate stomatal closure and inhibit opening in various species. Because both positive (Eamus and Fowler 1990) and negative (Table 1) impacts on stomatal conductance have been documented after acidic leaching of foliage, it is likely that differences reflect differing methodologies and growth conditions among studies. Further, instantaneous measurements may not fully capture treatment differences that are apparent in our measurements of stomatal closure over time.

Seedlings in the low pH mist treatment had a significantly lower rate of stomatal closure than controls (Table 1) in response to water stress following shoot excision. Stomata from seedlings in the low pH treatment took an average of 4.5 min longer than control seedlings to progress from 90% open to 10% open, a 15% lower rate of stomatal closure. Under conditions of extreme water stress, impaired stomatal responsiveness may leave plants vulnerable to desiccation during periods of limited water availability. Eamus (1993) found impaired stomatal responsiveness to changing photon flux after acidic mist treatments, which could also have been indicative of acid-induced depletion of foliar Ca pools involved in Ca-dependent signaling in guard cells. Stomatal closure is slower at low temperatures (Meng and Arp 1993), so physiological drought during the winter could be accelerated by an acid-induced reduction in stomatal responsiveness. However, during normal winter conditions, and even during an artificial midwinter thaw, Schaberg et al. (1998) found stomatal conductance remained low in red spruce foliage.

Acidic mist treatments could alter factors other than Ca-dependent processes involved in regulation of stomatal aperture. For example, acidic deposition can erode cuticular wax and delay wax synthesis (Turunen et al. 1995). This would be expected to result in faster water loss, and thus faster stomatal closure in the low pH treatment, but our results demonstrated the opposite pattern. In addition, we found no significant differences in total foliar K concentrations between plants in the pH 3.0 and pH 5.0 mist treatments (Table 1). Potassium movements into and out of the cytoplasm are essential in osmotic and turgor regulation in guard cells (Blatt 2000), but it appears that treatment-induced alterations of K had no role in influencing our results. It is also possible that apoplasmic acidification impaired the process of cytoplasmic pH regulation that is essential in guard cell movements. However, degradation of such a fundamental cell function would likely have killed the cells, and direct cell mortality has not been associated with foliar H^+ additions. Thus impairment of Ca-specific processes was likely the primary cause of the slowed stomatal response that we observed.

Additional effects of impairment

There is little consensus in the literature about the impact of acidic mist treatments on photosynthetic rate. McLaughlin et al. (1993) found that photosynthetic rate in red spruce was reduced in low pH treatments, but others (e.g., Eamus and Fowler 1990, Kohut et al. 1990) found enhanced photosynthesis in low pH treatments, or found no alteration of photosynthetic rates as a result of acidic mist treatments (e.g., Lee et al. 1990, Schaberg et al. 2000). These inconsistencies may reflect differences in growth, treatment and instantaneous measurement conditions rather than Ca physiology. Likewise, we found no acid-induced alterations in maximum net photosynthetic rate (Table 1). The current study is unique, however, in that we also assessed photosynthetic changes in response to an imposed water stress. Although the initial C_i was lower, the slower stomatal closure in the pH 3.0 treatment resulted in a slower depletion of CO_2 within leaves. As a result, there was a significant 22.5% greater lag between stomatal closure and photosynthetic decline (Table 1) in pH 3.0- than in pH 5.0-misted seedlings. However, an analysis of covariance between the rates of photosynthetic decline and stomatal closure demonstrated that there was no difference in the relationship between these parameters as a result of the mist treatments ($P = 0.374$). In addition, stomatal closure and photosynthetic decline occurred at the same rate (slope of regression = 1.02; $P < 0.001$). Stomatal closure thus appeared to be the primary factor determining the decline in net photosynthetic rate for these seedlings. The treatment-induced reductions in labile Ca and stomatal function help explain both the lower initial C_i and the greater lag in photosynthetic decline subsequent to stomatal closure for pH 3.0- versus pH 5.0-misted seedlings.

Information from other work supports the likelihood that reduced apoplasmic Ca availability decreases stomatal responsiveness. Calcium-based regulation of guard cell responses to drought, ABA and cold includes Ca flux from a number of cellular locations, including the apoplast (Knight et al. 1997, MacRobbie 2000, Wilkinson et al. 2001). Several studies have shown that, under certain conditions, Ca influx from the apoplast is of primary importance or essential for stomatal regulation (e.g., Webb et al. 1996, Knight et al. 1997, MacRobbie 2000, Wilkinson et al. 2001). External pools of labile Ca are likely more vulnerable to leaching by acidic mist losses than the labile Ca that is sequestered and protected inside organelles. In addition to stomatal regulation, Ca is known to play other roles in photosynthesis. Light causes Ca uptake by chloroplasts (Plieth et al. 1998), which helps balance charges during electron transport in the light reactions, and may help stabilize and aggregate thylakoid membranes (Webb et al. 1988). Because we saw no difference in the maximum net photosynthetic rate between plants in the two mist treatments, these functions of Ca appeared to be unaffected by acidic mist treatment. Movement of Ca into chloroplasts occurs within cells, and should be protected from apoplasmic acidic leaching by layers of cellular membranes, through which the passage of ions is strictly regulated.

Because of the highly conserved physiological functions of Ca and evidence of Ca leaching from the foliage of numerous species, it is unlikely that acid-induced stomatal impairment is limited to red spruce. Calcium leaching by acidic deposition

has been demonstrated for many tree species, such as white spruce (*Picea glauca* (Moench) Voss; Scherbatskoy and Klein 1983), eastern white pine (*Pinus strobus* L.; Lovett and Hubbell 1991), yellow birch (*Betula alleghaniensis* Britton; Scherbatskoy and Klein 1983), chestnut oak (*Quercus prinus* L.; Lovett et al. 1985), European beech (*Fagus sylvatica* L.; Leonardi and Flückiger 1989), sugar maple (*Acer saccharum* Marsh.; Lovett and Hubbell 1991) and red maple (*Acer rubrum* L.; Potter 1991). If species differ in their reliance on multiple physiological pathways for stomatal regulation (MacRobbie 1997, 2000, Blatt 2000), species with comparatively few alternative pathways could be at a distinct competitive disadvantage when exposed to continued acidic deposition and soil Ca depletion.

The study reported here provides insight into the physiology and health of trees exposed to the combined and ongoing environmental stresses of Ca depletion, acidic deposition and water stress. The results provide evidence to support the hypothesis (Schaberg et al. 2001) that anthropogenically caused depletion of mCa may disrupt physiological processes that require labile foliar Ca, thus impairing the ability of plants to respond adaptively to environmental cues. This vulnerability could be exacerbated in areas with limited exchangeable soil Ca (McLaughlin et al. 1993, Schaberg et al. 2001), and may interact with physiological impairments caused by other forms of air pollution such as ozone (McLaughlin and Percy 1999). Previous work highlighted mechanisms by which anthropogenic Ca depletion leads to diminished freezing tolerance in red spruce (DeHayes et al. 1999, Schaberg et al. 2002). This study helps build a more comprehensive understanding of a mechanism through which acid-induced biological Ca depletion may reduce physiological responsiveness to environmental changes, leading to amplified declines in tree health following environmental stress events. A growing number of field-based examples support this proposed mechanism (e.g., McLaughlin and Wimmer 1999, Schaberg et al. 2001).

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