

# Acid Rain Impacts on Calcium Nutrition and Forest Health

*Alteration of membrane-associated calcium leads to membrane destabilization and foliar injury in red spruce*

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Forest ecosystems throughout the world are exposed to acid rain, a complex solution consisting largely of  $H^+$ ,  $SO_4^{2-}$ ,  $NH_4^+$ , and  $NO_3^-$  pollutant ions derived from sulfur and nitrogen oxides. Although the public in the United States may view acid deposition as a localized pollution issue specific to certain regions of North America and northern Europe, recent evidence of acidic deposition throughout much of Asia, including tropical forest regions, has demonstrated that acid deposition is actually a global phenomenon (Abate 1995).

Furthermore, despite downward trends in sulfate concentrations associated with reductions in sulfur dioxide emissions mandated by the 1990 Clean Air Act Amendments, the acidity of precipitation in eastern North America does not seem to be decreasing (Scherbatskoy et al. in press). For example, over the course of 10 fog events on the summit of Mt. Mansfield, Vermont (elevation 1237 m), during summer 1998, the cloud water had an average pH of 2.8, with several events of pH 2.1–

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## The stress response system and overall health of montane coniferous forest ecosystems may be compromised by a subtle but persistent acid-induced alteration of membrane-associated calcium

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2.5 (Sean Lawson and Timothy Scherbatskoy, personal communication, data on file at the School of Natural Resources, The University of Vermont). The median and minimum cloud water pH values from Mt. Mansfield are more acidic than comparable summer measurements made on nearby Whiteface Mountain, New York (elevation 1483 m), from 1982 to 1987 (pH 3.4–3.8; Mohnen 1992). These findings collectively highlight the severity of cloud water acidity engulfing montane forests in the eastern United States and the ongoing nature of acid deposition. The prevalence and persistence of acid deposition has fueled public concern about the widespread impact of acid rain on forest health.

It is now well documented that anthropogenic changes in precipitation chemistry and acidity have occurred over the past several decades

in North America. In addition, acid deposition-induced leaching, depletion, and cycling disruption of calcium in forest soils has recently been demonstrated (Lawrence et al. 1995, Likens et al. 1996, 1998). Cumulative estimates show that, as a result of acid deposition, the pool of calcium in the soil complex may have shrunk by as much as 50% during the past 45 years (Likens et al. 1996), a reduction that may have long-term implications even if the acidity of precipitation begins to ameliorate over time. Despite these well-documented and substantial physical changes, the extent and nature of the impact of these belowground changes on forest ecosystem health has been uncertain or only speculative. The National Acid Precipitation Assessment Program (NAPAP), in its 1992 *Report to Congress* (NAPAP 1992), declared that “there remains no evidence of a general, widespread decline of forest tree species caused by acidic deposition.” However, the report did conclude that “acidic deposition has been firmly implicated as a causal factor in northeastern high-elevation red spruce decline” (NAPAP 1992).

Indeed, numerous field and laboratory studies have demonstrated that red spruce (*Picea rubens*) has suffered widespread freezing injury (Peart et al. 1991, DeHayes 1992), growth reductions (Cook and Zedaker 1992), and mortality (Johnson et al. 1986, 1992, 1996) over the past 40 years. The cause(s) of enhanced freezing injury and decline of red spruce, including the possible influence of air pollution,

**Table 1.** Estimates of midwinter cold tolerance for current-year foliage of red spruce from northern US populations.

Location	Date	Number of trees	Injury temperature (°C) <sup>a</sup>	Reference
Whiteface Mountain, NY	January 1987	20	LT <sub>10</sub> = -37 <sup>b</sup>	Sheppard et al. 1989
Upper Jay, NY	January 1988	9	T <sub>c</sub> = -42	DeHayes 1992
Nesowdnhunk Lake, ME	January 1990	10	T <sub>c</sub> = -41 <sup>b</sup>	Hadley and Amundson 1992
Camel's Hump Mountain, VT	January 1992	5	LT <sub>20</sub> = -46 <sup>b</sup>	Perkins et al. 1993
Mt. Mansfield, VT	January 1995	10	T <sub>c</sub> = -47	Strimbeck et al. 1995
Mt. Mansfield, VT	January 1996	60	T <sub>m</sub> = -42	Schaberg et al. in press a

<sup>a</sup>LT<sub>10,20</sub> is the temperature associated with 10% or 20% foliar freezing injury, T<sub>c</sub> is the highest temperature at which statistically significant increase in freezing injury is detected, and T<sub>m</sub> is the temperature associated with the increase in slope of the injury response curve.

<sup>b</sup>Estimated from Sheppard et al. 1989.

were a major focus of NAPAP's Forest Response Program and have been studied intensively in both North America and Europe. Red spruce decline has, in fact, become synonymous with acid rain throughout much of the Northern Hemisphere.

Despite this general association, an empirically based physiological understanding of and explanation for red spruce decline have not been established. Several potential broad-based explanations have been proposed. For instance, perturbation of calcium nutrition has been identified as a potential stress factor contributing to red spruce decline (Shortle and Smith 1988, McLaughlin et al. 1993, Lawrence et al. 1995). Specifically, it has been proposed that aluminum that is mobilized in mineral soil by acid deposition reduces soil storage of calcium and its availability for root uptake (Shortle and Smith 1988, Bondietti et al. 1990, Lawrence et al. 1995). The resulting calcium deficiencies might be expected to lead to the reductions in Ca:Al ratios, sapwood area, and live crown volumes that are evident in some red spruce trees (Shortle and Smith 1988), leaving trees more vulnerable to secondary stresses, such as diseases, insect pests, and freezing stress. Acid-induced reductions in foliar calcium concentrations of red spruce in the southern Appalachians have also been linked to increases in dark respiration and reductions in net photosynthesis, which could also contribute to decline symptoms (McLaughlin et al. 1991, 1993). An alternative explanation for the effects of acid rain on red spruce (Sheppard 1994) holds that exposure of trees to elevated SO<sub>4</sub><sup>2-</sup> in acid rain may alter carbon metabolism of trees by consuming energy associated with sequestering SO<sub>4</sub><sup>2-</sup> ions and repairing injury that a

buildup of these ions might cause to membrane proteins. The "cost" of such carbon allocation is expected to be physiological impairment and increased vulnerability to secondary stresses, including freezing stress.

Whatever the mechanism for red spruce decline, a strong direct link between freezing injury and red spruce decline has been established in northern montane regions of the red spruce geographic range (DeHayes et al. 1990, DeHayes 1992, Johnson et al. 1996). The link is so strong that red spruce decline is actually attributed to freezing injury in northern regions (Wilkinson 1990, Tobi et al. 1995). Freezing injury results in a loss of foliage critical for photosynthate production and storage, and it leads to the reduced basal area, height growth, and live crown volume typical of the red spruce decline syndrome (Wilkinson 1990, DeHayes 1992, Tobi et al. 1995). Historical evidence and analysis indicate that the frequency of freezing injury episodes in red spruce forests has increased over the past 40 years (Friedland et al. 1984, DeHayes 1992, Johnson et al. 1992, 1996), a period that coincides with increased emissions of sulfur and nitrogen oxides and acid deposition.

Although much has been learned about the ecology and decline of red spruce (e.g., Barnard et al. 1990, Eagar and Adams 1992, NAPAP 1992, Johnson et al. 1996), including freezing injury symptomology and pathology, a clear understanding of the physiological and environmental causes of freezing injury in red spruce has not emerged. In particular, an empirically supported mechanism that explains how acid rain might enhance freezing injury susceptibility and lead to the deterioration of forest health has been

lacking. In this article, we synthesize existing and new information that demonstrates acid rain alteration of the foliar calcium pool specifically associated with the plasma membrane-cell wall compartment in red spruce mesophyll cells. In addition, we provide direct evidence that links this physiological disruption to red spruce freezing injury and decline throughout eastern North America. Finally, we consider the acid-induced alteration of this particular physiologically important pool of calcium in the context of other hypotheses for red spruce decline and discuss the broad implications of alteration of the foliar calcium pool to forest health and well-documented soil calcium depletion.

### Freezing injury to red spruce

Despite early reports of "winter drying" or "desiccation" as causes of the foliar dieback referred to as "winter injury" in red spruce (Curry and Church 1952, Pomerleau 1962, Morgenstern 1969), it is now well established that red spruce winter injury is caused by subfreezing temperatures (Strimbeck et al. 1991, Perkins et al. 1991, DeHayes 1992). Red spruce has been subjected to repeated, severe, regionwide freezing injury for many decades. In recent years, major regionwide freezing injury events have occurred at approximately 4-year intervals on average, with moderate, more localized injury occurring in intervening years.

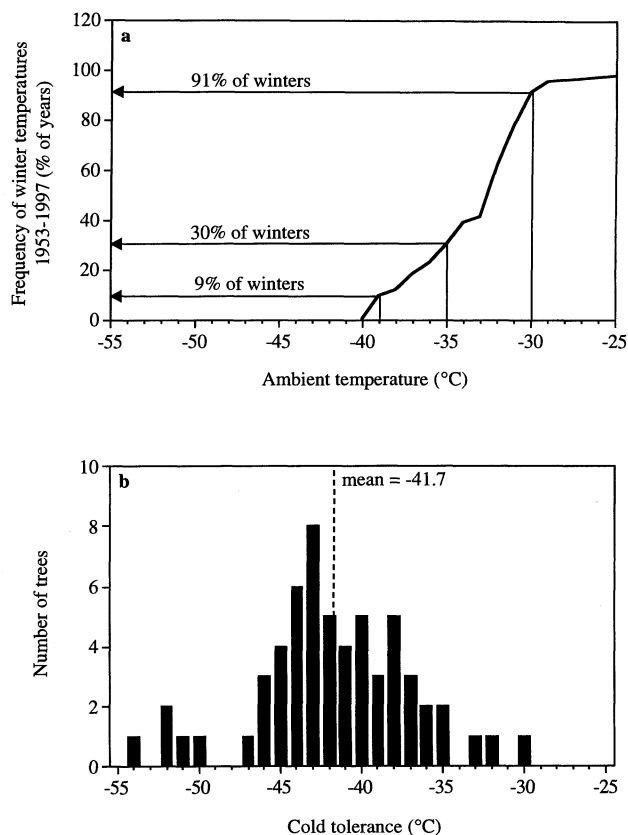
Numerous cold tolerance assessments of red spruce have been conducted over the past decade. Despite varying procedures and sampling of different northern montane populations, a remarkably consistent pattern of midwinter maximum cold tolerance for red spruce has emerged.

Laboratory studies indicate that the average temperature at which current-year needles of red spruce in northern New England and New York undergo freezing injury is in the range of  $-37^{\circ}\text{C}$  to  $-47^{\circ}\text{C}$  (Table 1). Because controlled freezing environments are thought to be less stressful than natural conditions (Hadley et al. 1996, Schaberg and DeHayes in press), these laboratory freezing studies probably overestimate the cold tolerance of red spruce.

The most comprehensive assessment of cold tolerance in red spruce, which examined the January cold tolerance of 60 native red spruce trees across an elevational range on Mt. Mansfield, revealed an average injury temperature of  $-41.7^{\circ}\text{C}$  and a range of  $-30^{\circ}\text{C}$  to  $-54^{\circ}\text{C}$  (Figure 1; Schaberg et al. in press a). A significant fraction of the trees (36%) were injured at temperatures warmer than  $-39^{\circ}\text{C}$  (Schaberg et al. in press a). Analysis of climatic records from Mt. Mansfield over the past 44 years indicates that temperatures rarely get as low as  $-40^{\circ}\text{C}$  but that they drop to as low as  $-35^{\circ}\text{C}$  and  $-30^{\circ}\text{C}$  in approximately 30% and 91% of winters, respectively (Figure 1). Thus, during most winters in northern montane habitats, ambient temperatures approach the maximum cold tolerance of at least some red spruce trees, accentuating the unique susceptibility of red spruce to freezing injury. Most northern coniferous species, by contrast, have winter cold tolerance levels well beyond minimum ambient temperatures (DeHayes 1992). Freezing injury susceptibility is greater for red spruce than sympatric species, such as balsam fir (*Abies balsamea*).

Freezing injury susceptibility of red spruce is almost always limited to current-year foliage, which is approximately  $10^{\circ}\text{C}$  less tolerant of freezing in winter than year-old foliage (DeHayes et al. 1990, DeHayes 1992). The difference in freezing tolerance between young and older needles indicates that red spruce has the physiological capacity to develop greater cold tolerance. In fact, if current-year needles of red spruce were equal in cold tolerance to year-old needles, then the species would escape freezing injury in all but the most severe winters. Considering the

Figure 1. Winter temperatures and cold tolerance on Mt. Mansfield. (a) Cumulative frequencies of minimum winter temperatures at 1200 m elevation over a 44-year period. (b) Midwinter cold tolerance of 60 native red spruce trees (Schaberg et al. in press a).



marginal cold tolerance of current-year foliage, any environmental disturbance, such as acid rain, that might reduce the cold tolerance of foliage by just a few degrees would be expected to result in a dramatic increase in the frequency of freezing injury.

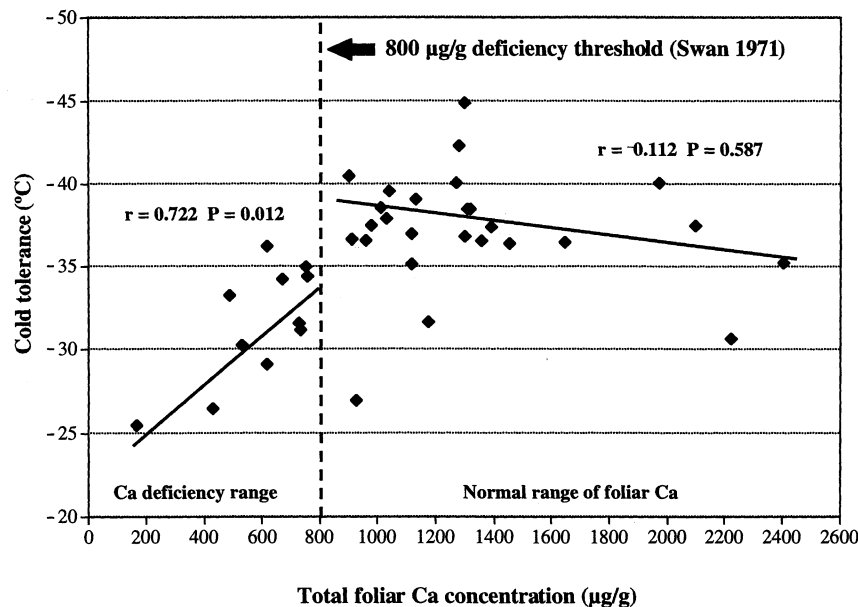
### Acid rain and cold tolerance

Considerable research has, in fact, established that exposure of red spruce foliage to both simulated (Fowler et al. 1989, L'Hirondelle et al. 1992, Waite et al. 1994) and ambient (DeHayes et al. 1991, Vann et al. 1992) acidic cloud water reduces the cold tolerance of current-year foliage. Experiments that we have conducted over several years under both laboratory and field conditions and involving mature trees as well as seedlings have consistently documented that exposure to acidic mist reduces the midwinter freezing tolerance of red spruce trees by  $3\text{--}10^{\circ}\text{C}$  (Schaberg and DeHayes in press). Cold tolerance reductions of this magnitude are sufficient to explain the widespread freezing injury to red spruce observed in northern montane forests over the past 40 years (DeHayes 1992, Johnson et al. 1996). That is, if seasons of high acid deposition loading reduced red spruce cold tolerance by an average of  $6\text{--}7^{\circ}\text{C}$ , then the reduction in average cold tolerance—from  $-41.7^{\circ}\text{C}$  to approximately  $-35^{\circ}\text{C}$ —would be expected to lead to freezing injury under ambient conditions approximately every 4 years on Mt. Mansfield (Figure

1). Acid deposition in the pH range currently experienced would be especially problematic for northern montane red spruce trees on the low end of the cold tolerance range (i.e., 36% of the red spruce trees on Mt. Mansfield).

### Potential involvement of foliar calcium

To examine how atmospheric pollution has led to enhanced freezing injury and the consequent decline of red spruce forests, we have studied the physiology of red spruce's response to acid deposition. In particular, we have examined acid mist-induced alterations in calcium physiology as a potential mechanism for low-temperature injury in red spruce foliage. Calcium is an abundant element in trees and a major cation in soil and surface waters. Acid deposition has a substantial and potentially long-lasting influence on leaching, depletion, and cycling of calcium in forest soils (Shortle and Smith 1988, Lawrence et al. 1995, Likens et al. 1996). Such findings highlight the belowground and atmospheric implications of base cat-

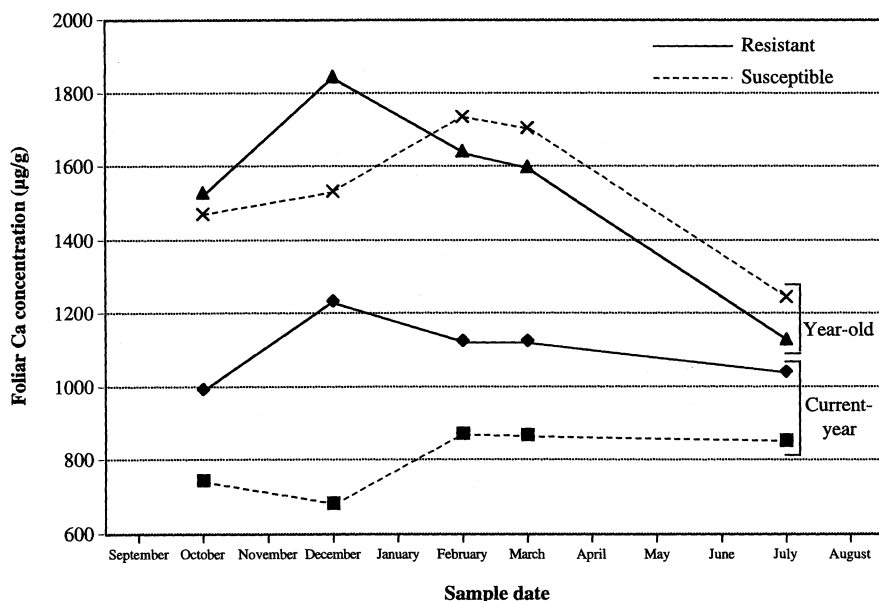


**Figure 2.** Relationships between foliar calcium concentration and midwinter cold tolerance for 30-year-old red spruce trees in northwestern New Hampshire that exhibit foliar calcium deficiency (less than 800 µg/g; Swan 1971) or are within the normal range of foliar calcium (Schaberg and DeHayes in press).

ion depletion and altered nutrient cycling. They also raise the possibility that acid deposition might have profound effects on calcium physiology of trees and overall forest health. The potential effects of a reduction in calcium reserves are especially intriguing for red spruce, the one North American tree species whose decline has been firmly linked to inputs of

acid deposition (NAPAP 1992).

Empirical information has demonstrated that red spruce trees with very low foliar calcium concentrations are less cold tolerant than trees whose foliar calcium levels fall in the normal range (Figure 2; Schaberg and DeHayes in press). In fact, there is a strong and significant positive association between cold tolerance



**Figure 3.** Relationships between foliar calcium concentration and freezing injury for current-year and year-old foliage of red spruce trees in northwestern New Hampshire with histories of susceptibility or resistance (Wilkinson 1990) to freezing injury (Donald H. DeHayes, Paul G. Schaberg, Gary J. Hawley, George R. Strimbeck, unpublished data).

and foliar calcium for trees exhibiting foliar calcium deficiency, whereas these parameters appear to be unrelated at higher calcium concentrations (Figure 2). Among 30-year-old red spruce trees growing in a low-calcium soil in northern New Hampshire, those trees that were consistently susceptible to freezing injury had significantly lower foliar calcium concentrations in current-year, but not year-old, foliage than frost-resistant trees (Figure 3; Donald H. DeHayes, Paul G. Schaberg, Gary J. Hawley, George R. Strimbeck, unpublished data). Furthermore, experimental work has documented that acid mist induces foliar calcium leaching (Joslin et al. 1988, DeHayes et al. 1991, McLaughlin et al. 1996, Schaberg et al. in press b). Acid-induced calcium deficiency has also been proposed to cause dark respiration increases and altered carbon metabolism associated with red spruce decline in the southern Appalachian Mountains, where freezing injury is not a problem (McLaughlin et al. 1990, 1991, 1993).

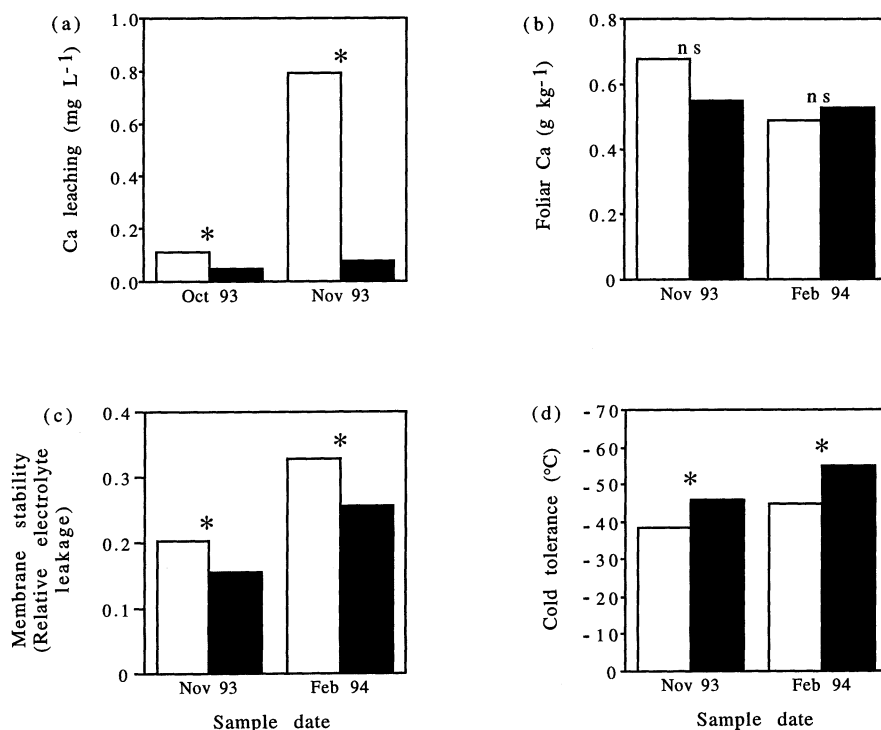
**Acid-induced foliar leaching and cold tolerance reductions.** Exposing red spruce trees to simulated acid mists that consisted of a base solution with an ionic composition patterned after regional cloud chemistry and realistic acidic pH levels (Schaberg and DeHayes in press) consistently demonstrated that calcium leaching from current-year needles during autumn (Figure 4a) was significantly greater in response to pH 3.0 than to pH 5.0 mist additions. Calcium concentrations were 2–10 times greater in leachates of foliage exposed to the more acidic mist. An *in vitro* experiment designed to partition the source of leached calcium showed that most (approximately 85%) of the acid-leached calcium was derived from needles rather than stems and that nearly 50% more calcium was leached from current-year foliage than from year-old foliage (Figure 5; Schaberg and DeHayes in press). Analysis of leachate also indicated that pH 3.0-treated foliage assimilated nearly 60 times more H<sup>+</sup> than pH 5.0-treated needles. Furthermore, acid mist-induced calcium losses were accompanied by significantly greater membrane disruption

(Figure 4c) and a 4–10 °C reduction in freezing tolerance at pH 3.0 than at pH 5.0 during late autumn and winter subsequent to acid mist exposure (Figure 4d). This result indicates that acid mist has a direct negative impact on membrane stability independent of low temperature, and it points to the plasma membrane as the potential site of acid-induced calcium disruption.

**Importance of membrane-associated calcium.** Although there were up to 10-fold differences in foliar calcium leaching between pH treatments, we have not detected a reduction in total calcium pools in foliage treated with simulated acid mists that mimic ambient pH levels and amounts (Figure 4b). However, calcium within cells and tissues is highly compartmentalized, and this partitioning is critical to its physiological function in plants. The majority of the calcium in conifer needles is insoluble extracellular calcium oxalate and pectate crystals, which are of little physiological importance (Fink 1991).

In contrast, the labile calcium ions in equilibrium within the plasma membrane region (including some freely displaced apoplastic calcium from the cell wall) are of major physiological importance. This pool of membrane-associated Ca (mCa), although a relatively small fraction of total foliar ion pools, strongly influences the response of cells to changing environmental conditions (Hepler and Wayne 1985, Dhindsa et al. 1993, Monroy et al. 1993, Sheen 1996). Because total foliar calcium estimates primarily reflect the dominant insoluble and immobile extracellular calcium pool, these estimates may mask relatively subtle, but critical, shifts in the labile and environmentally sensitive mCa pool. Indeed, recent experiments have demonstrated the relative seasonal stability of total foliar calcium pools compared with mCa and the lack of association between mCa and total foliar calcium pools (Figure 6; DeHayes et al. 1997). In fact, foliar calcium concentrations are not an accurate indicator of relative levels of mCa, especially for trees whose calcium concentrations fall within the normal range (Swan 1971).

The plasma membrane of plants



**Figure 4.** Impacts of acid mist treatment on physiological properties of red spruce. Open bars, pH 3.0; solid bars, pH 5.0. (a) In vivo calcium leaching. (b) Total foliar calcium. (c) Membrane stability as measured by cellular electrolyte loss. (d) Cold tolerance from red spruce foliage (Schaberg and DeHayes in press). Treatment means are either not significantly different (ns) or are significantly different at  $P \leq 0.05$  (\*) based on analyses of variance.

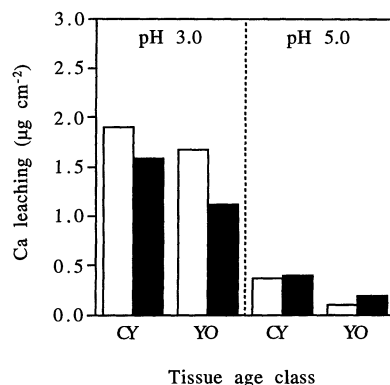
plays a critical role in mediating cold acclimation and low-temperature injury (Pomeroy and Andrews 1985, Steponkus 1990). Membrane architecture influences solution movement across membranes and the ability of cells to resist dehydration, extracellular ice damage, and, perhaps, intracellular freezing during cold acclimation (Arora and Palta 1986, 1988, Guy 1990). Membrane-associated calcium influences plasma membrane structure and function, stabilizing membranes and influencing permeability by bridging phosphate and carboxylate groups of membrane phospholipids and proteins (Figure 7; Palta and Li 1978, Legge et al. 1982, Davies and Monk-Talbot 1990). Thus, mCa influences cold tolerance, at least in part, through its effects on membrane structure.

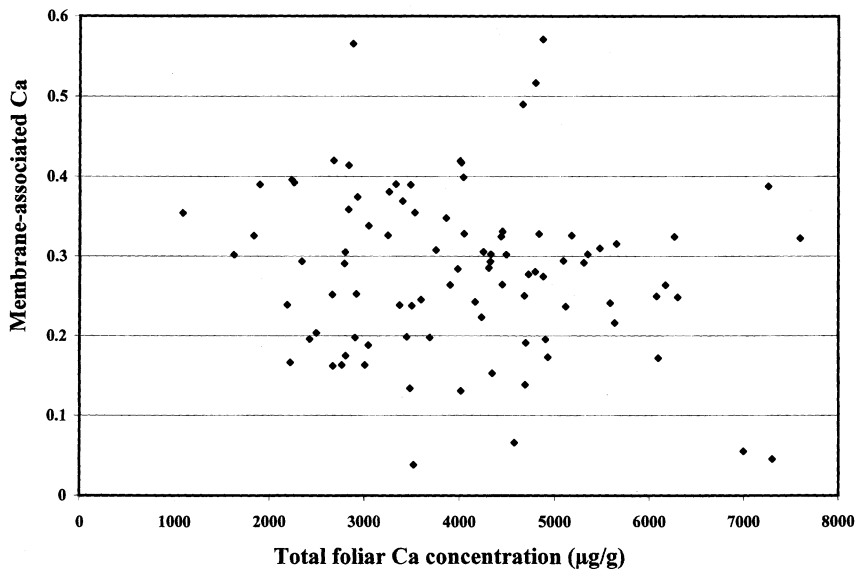
The labile mCa pool (Atkinson et al. 1990) also serves an important role as a second messenger in the perception and transduction of environmental signals, including low temperature (Figure 7; Hepler and Wayne 1985, Dhindsa et al. 1993, Monroy et al. 1993, Sheen 1996). Thus, low

temperatures may trigger mCa transport across the plasma membrane into the cytoplasm, where calcium interacts with biomolecules that help regulate physiological responses to freezing stress.

**Seasonal changes in mCa.** In addition to the considerable documenta-

**Figure 5.** Influence of pH 3.0 and pH 5.0 mists on in vitro calcium leaching of needles of red spruce seedlings. CY, current-year needles; YO, year-old needles; open bars, needles plus twigs; solid bars, needles alone (Schaberg and DeHayes in press).





**Figure 6.** Relationship between total calcium and membrane-associated calcium (mCa) concentrations for current-year foliage of red spruce seedlings (DeHayes et al. 1997). Parameters are not significantly correlated ( $r = -0.14$ ,  $P = 0.34$ ).

tion of calcium's general role in plant cold hardiness physiology, additional empirical data specifically implicate mCa involvement in the developmental cold tolerance of red spruce. Unlike total foliar calcium pools, mCa pools in current-year, but not year-old, needles are seasonally dynamic and responsive to temporal environmental changes that parallel seasonal changes in membrane structure related to cold acclimation. For example, late summer–early autumn increases of mCa in current-year needles (Figure 8; DeHayes et al. 1997) may reflect an increase in calcium ion exchange sites associated with short day-induced increases in membrane phospholipids, whereas apparent frost-initiated reductions in mCa are most likely associated with changes in the fatty acid composition of membrane lipids (DeHayes et al. 1997) and perhaps with a role for mCa in the perception and transduction of the low-temperature cold acclimation signal (Arora and Palta 1986, 1988, Guy 1990).

We have also documented abrupt, albeit temporary, reductions in mCa (Figure 8; DeHayes et al. 1997) and freezing tolerance (Strimbeck et al. 1995) in red spruce current-year, but not year-old, needles in response to a midwinter thaw. After the thaw, both mCa and freezing tolerance returned to pre-thaw levels. Sequential changes in mCa and cold tolerance levels, to-

gether with the well-documented changes in membrane structure in leaf tissue of north temperate conifers during cold acclimation (DeYoe and Brown 1979, Senser and Beck 1982), strongly support the contention that mCa is important to cold tolerance. Consequently, if acid mist reduces mCa through calcium leaching, then an alteration in membrane function, low-temperature signal transduction, and freezing injury susceptibility (and perhaps sensitivity to other stressors) would likely follow.

**Perturbations to mCa versus total foliar calcium pools.** Efforts to understand the physiological role of calcium in the reduced cold tolerance of acid mist–exposed red spruce were previously impeded by the difficulty of generating meaningful measures of the critical mCa compartment of the total foliar calcium pool. We have now developed and tested a protocol for measuring mCa in woody plant tissue (Borer et al. 1997) and have used it to evaluate the impact of acid mist and soil solution chemistry on red spruce physiology and nutrition (Schaberg et al. in press b). We examined freezing tolerance, mCa, total foliar calcium, and foliar calcium leaching of red spruce trees grown in a sand/perlite soil selected for its low pretreatment calcium content (52 µg/g; Schaberg et al. in press b). Plants were exposed in a factorial

arrangement to three treatments expected to result in biological calcium perturbation: soil solution calcium additions (0, 25, 75, or 225 µmol/L); soil solution aluminum (0 or 200 µmol/L), which can compete with and inhibit calcium uptake by roots (Shortle and Smith 1988); and pH 3.0 or pH 5.0 mist, which causes differential calcium leaching (Schaberg et al. in press b).

Soil calcium additions enhanced total foliar calcium concentrations and increased rates of respiration during the early growing season (Schaberg et al. in press b). As expected, soil aluminum treatment inhibited the incorporation of calcium and other cations, altered foliar gas exchange throughout the growing season, and reduced growth. Calcium additions increased foliar calcium incorporation but did not otherwise alter aluminum treatment effects; thus, the aluminum-induced changes in physiology appear to be direct and independent of foliar calcium nutrition. Neither calcium nor aluminum soil treatments had any discernible impact on foliar calcium leaching, mCa levels, or freezing tolerance, which implies that below-ground manipulations of soil and foliar calcium pools may not have important physiological consequences for red spruce trees.

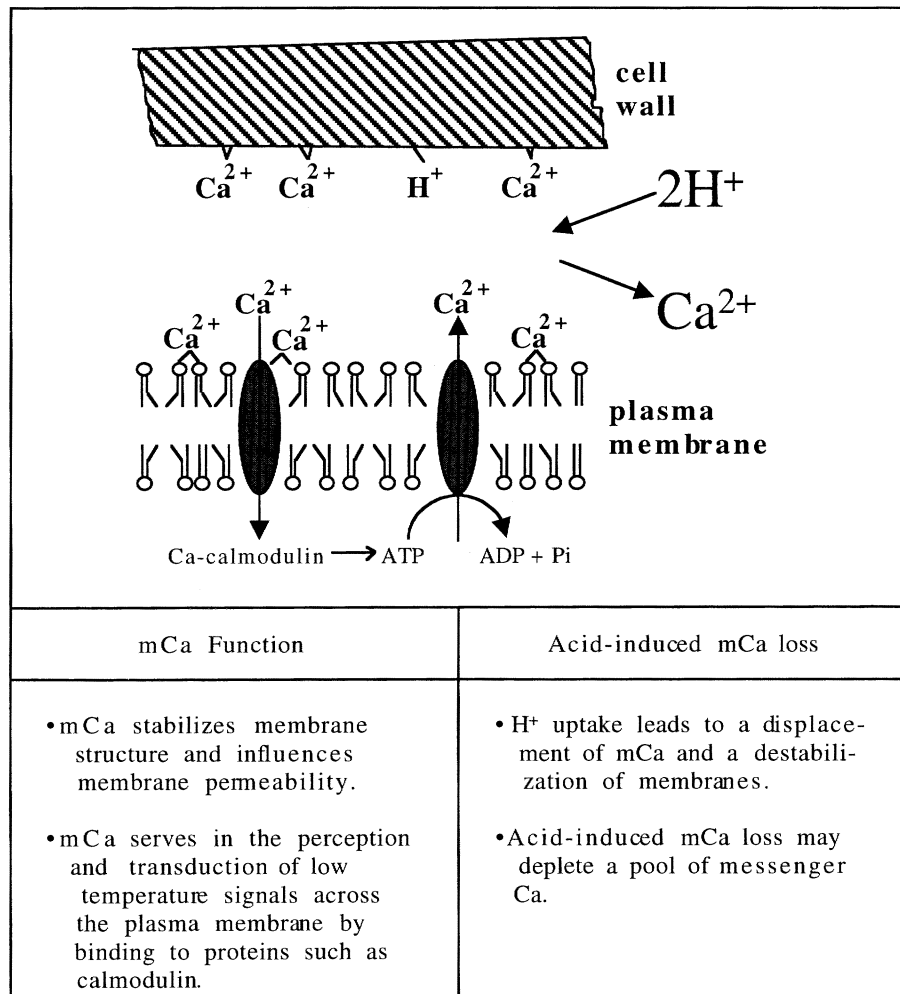
In contrast to the soil treatments, acid mist treatments had profound physiological effects. Compared to the pH 5.0 mist treatment, pH 3.0 mist applied to the entire plant, including soil, in summer and early autumn resulted in foliar calcium leaching and in consistent, significant, and parallel reductions in late fall and winter freezing tolerance and mCa levels of current-year needles (Table 2). Acid mist-induced reductions in mesophyll cell mCa were relatively minor in late summer but reached a maximum of 35% between mist treatments by midwinter (Table 2), even though acid mist treatment had been removed months earlier. A similar pattern was evident for the acid mist influence on cold tolerance (Table 2).

Although soil calcium and aluminum manipulations influenced calcium nutrition and physiology, these soil-driven reactions alone did not enhance foliar calcium leaching, al-

ter mCa, or reduce freezing tolerance, which appear to be critical precursors to red spruce decline in northern montane forests. Changes in foliar calcium from soil treatments probably reflected alterations in extracellular calcium pools (e.g., the concentration of calcium oxalate crystals in cell walls) that have low physiological availability. Changes in mCa from direct acid mist application to foliage, by contrast, reflected enhanced calcium leaching, leading to membrane instability and, perhaps, to disruption of calcium's role in environmental signal transduction.

The pronounced differences in cold tolerance and susceptibility to freezing injury in red spruce needles of different ages (DeHayes 1992) is consistent with the pattern of developmental variation in mCa in current-year needles (Figure 8) as well as age class differences in foliar calcium leaching (Figure 5). Even without acidic inputs, little or no mCa is detected in current-year needles of red spruce during early summer (Figure 8). Moreover, mCa levels in current-year needles are inherently lower than in year-old needles throughout summer and early autumn (Figure 8), when acid-induced calcium leaching is expected to be substantial. The substantial age class difference in foliar calcium leaching (Figure 5) further accentuates the mCa and cold tolerance differences between year-old and current-year red spruce needles, helping to explain the unique low-temperature sensitivity of red spruce current-year foliage.

The magnitude and persistence of the acid mist influence on mCa and cold tolerance throughout winter and the responsiveness to short-term acid mist applications in autumn are most likely a function of the amount of calcium leached, the lack of mobility of calcium in the phloem, and the dependence on transpiration to replenish depleted mCa. In effect, mesophyll cell mCa status reflects the seasonal dynamics of mCa accretion (Figure 8) and the net balance of foliar calcium additions (via xylem transport) versus calcium loss (from leaching). Consequently, the physiological implications of acid deposition may be exacerbated in late summer and autumn, when post-growing season transpiration is minimal and



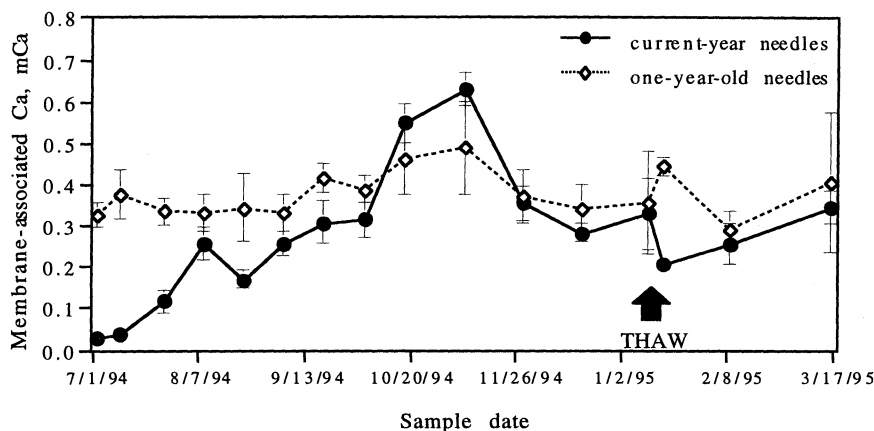
**Figure 7.** Schematic of a mesophyll cell plasma membrane illustrating physiological functions of membrane-associated calcium (mCa) and possible physiological consequences of acid-induced mCa loss.

ongoing mCa loss is not fully replaced via xylem transport.

Of the few significant interactions among the different treatments, almost all involved the influence of mist pH  $\times$  aluminum treatment on foliar nutrition (Schaberg et al. in press b). The trend was always the same for each element affected: Reductions in cation concentration associated with aluminum addition were greater for pH 5.0- than for pH 3.0-treated plants. Significant acid mist  $\times$  aluminum treatment interactions on mCa levels were also detected for two dates, when the degree of acid-induced reduction in mCa was greater for non-aluminum-treated trees (in September 1994) and 200  $\mu\text{mol/L}$  aluminum-treated trees (in January 1995). Still, interactions of pH and aluminum treatments did not mask overall acid-induced reductions of

mCa concentrations. In fact, one of the most physiologically meaningful findings was the conspicuous lack of interaction among other treatment combinations. The absence of any interaction between acid mist and soil calcium additions is particularly interesting because it indicates that acid-induced foliar calcium leaching, mCa depletion, and cold tolerance reductions were not ameliorated by supplemental soil calcium.

**Nature of calcium leaching and cold tolerance perturbations.** The pool of mCa, unlike other insoluble extracellular calcium pools, is readily available for leaching, resulting in membrane destabilization, depletion of a potential pool of messenger calcium, and significant reductions in the freezing tolerance of current-year red spruce needles. Acid deposition-



**Figure 8.** Seasonal patterns of membrane-associated calcium (mCa) in current-year and year-old needles of red spruce seedlings. The decrease in mCa during a midwinter thaw is significant for current-year, but not year-old, needles (DeHayes et al. 1997).

induced cation leaching may result from inputs of  $H^+$ ,  $SO_4^{2-}$ , or both (Edwards et al. 1995). Specifically, calcium leaching could follow either  $H^+$  displacement of  $Ca^{2+}$  on exchange sites or the binding and removal of free  $Ca^{2+}$  by  $SO_4^{2-}$  at the cell wall-membrane interface (Figure 7). Sulfate can be directly toxic to red spruce foliage independent of freezing stress (Cape et al. 1991), and it has been implicated as a critical pollutant ion in cold tolerance reductions in this species (Cape et al. 1991, Sheppard 1994). However, we have demonstrated similar cold tolerance reductions in response to acid mists that contained no  $SO_4^{2-}$  (because they were acidified with hydrochloric acid; Schaberg and DeHayes in press) or had equalized  $SO_4^{2-}$  concentra-

tions (Schaberg et al. in press b). These findings, coupled with reduced  $H^+$  but not  $SO_4^{2-}$  concentrations in foliar leachates (Joslin et al. 1988, McLaughlin et al. 1996), indicate that acid mist-induced calcium leaching and cold tolerance reductions are not  $SO_4^{2-}$  driven but are most likely the result of cation exchange driven by differential  $H^+$  exposure.

Our results demonstrate that direct acid deposition on needles represents a unique environmental stress in that it preferentially removes mCa, which is not readily replaced in autumn, resulting in a mCa deficiency that may not be detectable by examination of total foliar calcium pools. Destabilization of plasma membranes (Figure 4c) and a loss of freezing tolerance in current-year needles

accompany the mCa depletion. These findings verify a direct impairment of leaf physiology from acid deposition independent of well-documented belowground influences and provide a mechanistic explanation for cold tolerance reductions in native trees specifically associated with atmospheric deposition of acid on foliage (Vann et al. 1992).

### Proposed mechanism

In contrast to previous hypotheses, which involved aluminum- or  $SO_4^-$ -induced disruptions in carbon metabolism, the mechanism described above implicates the direct action of acid deposition on specific foliar calcium pools and cold tolerance. Our findings also emphasize the dynamic role of the physiologically active and labile mCa pool in plant tissues and demonstrate that pollution-induced shifts in this pool may not be detectable by typical analysis of total foliar calcium. In fact, quantification of mCa was essential in elucidating this mechanism for acid deposition perturbation of plant cold tolerance.

We propose that direct acidic deposition on red spruce foliage preferentially displaces calcium ions specifically associated with plasma membranes of mesophyll cells. As a result, mCa is reduced, plasma membranes are destabilized, and messenger calcium is depleted. This physiological impairment leads to the more commonly observed secondary symptoms of freezing injury in northern regions, elevated tissue respiration

**Table 2.** Influence of soil calcium, soil aluminum, and acid mist treatments on current-year foliar calcium leaching and concentration, membrane-associated calcium (mCa), and cold tolerance in red spruce.<sup>a,b</sup>

Treatments	Foliar calcium leaching ( $\mu\text{g/L}$ )			Total foliar calcium ( $\text{mg/kg}$ )				mCa fluorescence			Cold tolerance ( $^{\circ}\text{C}$ )		
	Jul 94	Aug 94	Sep 94	Jul 94	Sep 94	Nov 94	Feb 95	Sep 94	Nov 94	Jan 95	Nov 94	Jan 94	Feb 95
Soil calcium													
0 $\mu\text{mol/L}$	249	172	84	1560**	2042	1733**	1815**	0.21	0.18	0.15	-45.4	-53.8	-45.6
225 $\mu\text{mol/L}$	373	150	106	2335	2514	2743	3142	0.23	0.17	0.15	-47.2	-55.8	-46.7
Soil aluminum													
0 $\mu\text{mol/L}$	247	162	113	2417**	2803**	2562**	2931**	0.21	0.18	0.14	-46.5	-54.0	-47.8
200 $\mu\text{mol/L}$	328	133	96	1523	2040	1926	2091	0.23	0.18	0.15	-45.9	-54.6	-46.6
Acidic mist													
pH 3.0	526**	233**	148**	1969	2289	2262	2488	0.21	0.16*	0.12*	-44.5**	-51.5*	-44.9*
pH 5.0	66	64	60	1971	2553	2246	2534	0.22	0.19	0.18	-48.0	-57.0	-49.5

<sup>a</sup>Schaberg et al. in press b.

<sup>b</sup>Asterisks indicate cases in which soil calcium, soil aluminum, or acidic mist treatment means are significantly different at  $P \leq 0.05$  (\*) or  $P \leq 0.01$  (\*\*). Asterisks indicate cases in which soil calcium, soil aluminum, or acidic mist treatment means are significantly different at  $P \leq 0.05$  (\*) or  $P \leq 0.01$  (\*\*). Asterisks indicate cases in which soil calcium, soil aluminum, or acidic mist treatment means are significantly different at  $P \leq 0.05$  (\*) or  $P \leq 0.01$  (\*\*). Asterisks indicate cases in which soil calcium, soil aluminum, or acidic mist treatment means are significantly different at  $P \leq 0.05$  (\*) or  $P \leq 0.01$  (\*\*).

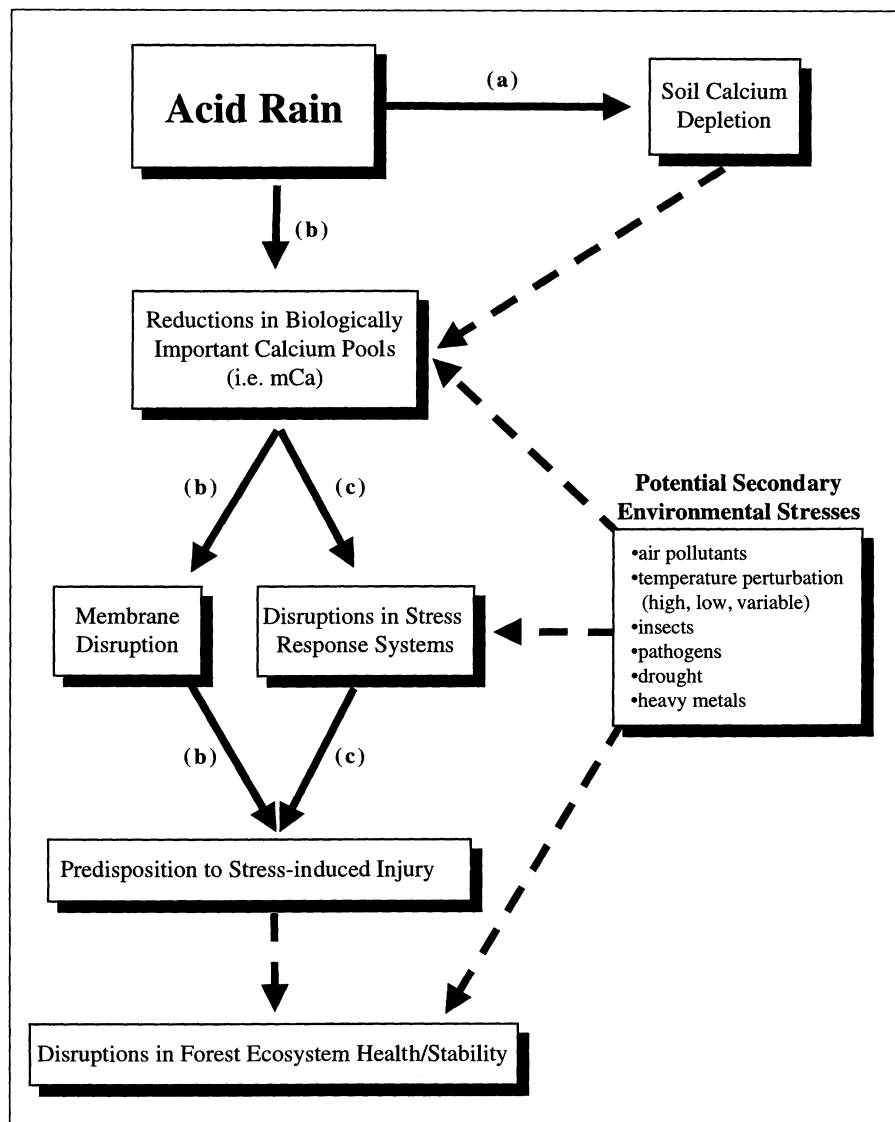


(McLaughlin et al. 1993), and enhanced susceptibility to other stresses that compromise overall forest health. This mechanism provides a direct link between acid deposition and dramatic increases in red spruce freezing injury in northern montane forests observed over the past 40 years, a period that coincides with increased pollution emissions.

Acid deposition-induced alteration of mCa and membrane stability documented in northern forests would also be expected in response to acidic deposition in the southern Appalachians. Although they did not examine the mCa pool specifically, McLaughlin et al. (1993) suggested that acid deposition-induced calcium losses and concomitant changes in membrane integrity may cause tissue respiration to increase. The acid deposition-mCa leaching-membrane alteration explanation for red spruce decline that we have proposed may therefore represent a single viable explanation for red spruce decline throughout the montane red spruce forests of eastern North America, where acid deposition is prevalent.

This mechanism specifically accounts for the unique features of the red spruce freezing injury syndrome, such as the freezing tolerance differences between current- and year-old foliage, the sensitivity of the cold tolerance response to short-term autumn mist applications, and the persistence of the acid-induced reduction in cold tolerance throughout winter (even when the source of acidity is removed). Furthermore, the sensitivity of the cold tolerance response to simulated acid mists regardless of the anion included in the pH adjustments or its concentration is consistent with the idea that  $H^+$  displaces  $Ca^{2+}$  on exchange sites but not with the hypothesis that  $SO_4^{2-}$  or some other pollutant ion enhances freezing injury susceptibility.

Finally, the predisposing (rather than the direct injury) influence of acid deposition implicit in the proposed mechanism is uniquely compatible with empirical assessments of red spruce decline symptomology. Generalized growth impairment would be expected if direct sulfate toxicity or carbon metabolism disruption were primary factors in red spruce decline (e.g., Cape et al. 1991,



**Figure 9.** The impacts of acid deposition on ecosystem calcium nutrition, health, and sustainability. Known impacts are indicated by solid lines, and potential impacts are indicated by dashed lines. Letters indicate references for the known components: a, Lawrence et al. (1995), Likens et al. (1996); b, Schaberg and DeHayes (in press), Schaberg et al. (in press b); c, Dhindsa et al. (1993), Monroy et al. (1993), Sheen (1996).

Sheppard 1994), because pollution-induced changes in physiology would most likely have a metabolic cost even in the absence of freezing injury. However, most controlled-environment studies have documented unimpaired or even improved growth in red spruce exposed to acid mist in the absence of another stressor, such as subfreezing temperatures (Taylor et al. 1986, Laurence et al. 1989, Kohut et al. 1990, Lee et al. 1990). Indeed, growth reductions associated with red spruce decline in northern montane forests appear to be the direct result of the freezing

injury (Wilkinson 1990, Tobi et al. 1995) rather than exposure to the predisposing acidic conditions.

### Broad forest health implications

Although it is well established that acid deposition has contributed to forest soil calcium depletion (Lawrence et al. 1995, Likens et al. 1996), our findings have shown that acid deposition can influence foliar base cation dynamics directly, independent of belowground processes. In fact, direct acid-induced disruption

**Table 3.** Relative stability of cell membranes in the current-year foliage of red spruce and balsam fir seedlings following autumn (September–November 1997) application of pH 5.0 or pH 3.0 simulated cloud water treatments.<sup>a,b,c</sup>

Species	Membrane stability (relative electrolyte leakage)		Change in membrane leakage (%)
	pH 5.0	pH 3.0	
Balsam fir	0.220	0.322**	46
Red spruce	0.254	0.338**	33

<sup>a</sup>Schaberg et al. in press b.

<sup>b</sup>Mist solutions equalized SO<sub>4</sub><sup>2-</sup> concentrations between pH treatments.

<sup>c</sup>Double asterisks (\*\*) indicate pH treatment means within species are significantly different at  $P \leq 0.01$  based on analyses of variance.

of mCa appears to override soil calcium influences, as evidenced by the fact that acid depletes mCa even in plants grown in soils with abundant calcium. Nevertheless, we expect that soil calcium would have an effect on mCa pools in more severely calcium limited soil environments. For example, red spruce trees with extremely low foliar concentrations of calcium growing in calcium-depleted soils show a reduced cold tolerance (Figure 2). The physiological impairment of trees exhibiting foliar calcium deficiency presumably reflects reduced mCa resulting from extremely low soil calcium availability. The substantial acid-induced soil calcium depletion that has recently been documented (Lawrence et al. 1995, Likens et al. 1996) would therefore be expected to exacerbate mCa deficiencies and compromise forest health and ecosystem stability (Figure 9).

The results discussed in this article also raise the strong possibility that acid rain alteration of mCa and, consequently, membrane integrity is not unique to red spruce but is simply exacerbated in this species because of its sensitivity to freezing injury. In fact, acid-induced foliar calcium leaching has been demonstrated in many other north temperate forest tree species, including yellow birch (*Betula alleghaniensis*; Scherbatskoy and Klein 1983), white spruce (*Picea glauca*; Scherbatskoy and Klein 1983), red maple (*Acer rubrum*; Potter 1991), eastern white pine (*Pinus strobus*), and sugar maple (*Acer saccharum*; Lovett and Hubbell 1991). Assessments of mCa, membrane integrity, cold tolerance, and other secondary stress impacts have not yet been made for these species.

However, we have recently demonstrated an acid mist-induced reduction in membrane integrity in balsam fir that is comparable to that of red spruce exposed to the same acidic solutions (Table 3; Schaberg et al. in press b). However, balsam fir hardiness levels exceeded detection limits so that cold tolerance effects, if present, could not be tested. But these data show that acid-induced membrane destabilization is not specific to red spruce and suggest that acid-induced foliar disruption may be a more general phenomenon than the highly visible freezing injury syndrome evident in red spruce.

Although it is well known that calcium plays a critical role in plant responses to numerous stresses (e.g., low temperature, salt, drought, and low light; Hepler and Wayne 1985, Dhindsa et al. 1993, Monroy et al. 1993, Sheen 1996), the biological implications of soil calcium depletion—especially its impact on the physiologically important mCa pool—remain uncertain at this time. Because most calcium in woody plant tissues is insoluble, immobile, and extracellular (Fink 1991), it is the relatively small and environmentally sensitive mCa pool that appears to serve a critical and active role in plant stress responses. If acid-induced soil calcium depletion also alters mCa and membrane stability, then the potential for calcium-mediated predisposition to stress-induced injury could have an extraordinary influence on forest health (Figure 9). In this context, the stress response system and overall health of montane coniferous forest ecosystems may be compromised by a subtle but persistent acid-induced alteration of mCa and cell membranes, leading to physi-

ological impairment and a predisposition to be affected by an array of environmental stresses. Because the mCa pool in woody plant leaf tissue can now be monitored, the potential for soil- or atmospheric-induced mCa alteration as a predisposing factor in other recent regionwide forest declines in eastern North America warrants investigation.

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