6. Physiological and Environmental Causes of Freezing Injury in Red Spruce

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For many, concerns about the implications of "environmental change" conjure up scenarios of forest responses to global warming, enrichment of greenhouse gases, such as carbon dioxide and methane, and the northward migration of maladapted forests. From that perspective, the primary focus of this chapter, that is, causes of freezing injury to red spruce (*Picea rubens* Sarg.), may seem somewhat counterintuitive and inconsistent with the overall theme of the book. However, the dramatically increased incidence of freezing injury to northern montane red spruce forests over the past four decades is, in fact, largely a function of human-induced environmental change. "Environmental change" in the context of this chapter includes both changing climatic patterns and chemical changes in the atmospheric, forest canopy, and/or soil environment that may directly or indirectly result from atmospheric wet (precipitation or cloud water) or dry (direct deposition of gases or aerosols) deposition.

Winter injury to red spruce is now recognized as a significant factor in the decline of montane red spruce forests in northeastern North America. It is well documented that red spruce has been subject to repeated, severe, regionwide winter injury over at least the past 50 years, with this injury most commonly observed in montane forests (Friedland et al., 1984; Johnson et al., 1986; Peart et al., 1991), although it also occurs at lower

elevations (Morgenstern, 1969; Peart et al., 1991; DeHayes et al., 1990). In recent years, the frequency of major regionwide winter injury events has averaged about four years with more moderate, localized injury occurring in intervening years. It has been demonstrated that red spruce winter injury is caused by subfreezing temperatures rather than foliar desiccation (DeHayes, 1992) and that the species exhibits only modest levels of midwinter cold tolerance barely sufficient to protect foliage from minimum temperatures commonly encountered in northern montane habitats. As a result, any "environmental change" that might impair the cold tolerance of red spruce foliage by just a few degrees would be expected to result in an increased frequency of freezing injury.

Well-documented environmental changes in the northeastern United States include increased pollutant deposition and exposure, especially in high elevation spruce–fir forests exposed to high concentrations of ozone and cloud water heavily laden with pollutant ions (Mohnen, 1992). There is also evidence that climatic perturbations, such as the frequency of winter thaws, have also increased in at least some locations in the northeastern U.S. (Strimbeck et al., 1995; Schaberg et al., 1996). Because red spruce grows in regions where air temperatures usually remain below 0°C throughout the winter, extended thaws that allow melting and mobilization of water in the plant may stimulate precocious dehardening. Freezing injury would be expected if a prolonged thaw is followed by extreme cold or another form of cold stress resulting from temperature fluctuations, such as rapid freezing or repeated freeze–thaw cycles (Perkins and Adams, 1995; Lund and Livingston, 1998). Such environmental changes could conceivably lead to physiological impairment that may alter the phenological development and/or the full extent of cold tolerance achieved by northern montane red spruce trees, thereby increasing susceptibility to freezing injury. The primary focus of this chapter is to describe the implications of environmental change on red spruce freezing injury, with particular emphasis on environmentally predisposing factors and physiological mechanism(s) responsible for pollution- and/or climate-induced alterations in cold tolerance.

Measurement of Red Spruce Cold Tolerance

Because of the numerous experimental and logistical challenges of assessing cold tolerance of trees *in situ*, most of our understanding of the causes of freezing injury and the physiology of cold tolerance development in red spruce has been derived from laboratory examinations of foliar freezing tolerance. Such assessments are pertinent to both trees under ambient conditions in natural forests and seedlings or trees exposed to altered environmental conditions applied as experimental treatments. However, because different cold tolerance laboratory protocols are often

used by differing investigators, the potential exists for different methodologies to influence assessments of red spruce cold tolerance and interpretations of potential causal or predisposing factors that may influence freezing injury.

Laboratory Cold Tolerance Measurements

Laboratory cold tolerance experiments usually involve exposing plant tissue (e.g., current-year needles) to a series of decreasing subfreezing temperatures in a systematic manner that includes controlling both the rate of freezing and subsequent thawing of the tissue. The extent of cellular disruption or injury is then assayed for tissue exposed to each test temperature and some protocol is used to describe a temperature or range of temperatures associated with freezing injury to the tissue under the conditions of the experiment. Numerous assays for freezing injury exist and several have been utilized in red spruce research, including visible tissue discoloration, electrolyte leakage, chlorophyll fluorescence, chlorophyll degradation, and photosynthetic gas exchange (DeHayes and Williams, 1989; Adams and Perkins, 1993; Hadley et al., 1993; Manter and Livingston, 1996). Although all of these procedures have been shown to effectively detect freezing injury, each appears to be measuring some different aspect or element of cell injury. For instance, post-freezing electrolyte leakage is an assessment of the loss of plasma membrane integrity, which may include alterations of membrane permeability or membrane rupture. In contrast, chlorophyll fluorescence is a reflection of low temperature-induced reduction in photochemical efficiency and is expected to be indicative of a disruption within chloroplasts or, more specifically, the thylakoid membrane. Photosynthetic gas exchange and chlorophyll content measurements likely reflect yet another set of temperature–induced physiological perturbations.

Another important procedural element that may influence cold tolerance interpretations is the handling of post-freezing injury data and designation of a meaningful temperature that is reliably associated with actual cold tolerance and freezing injury. Data handling and reporting procedures vary widely. In many cases, tissue injury estimates from control temperatures are compared with those from subfreezing test temperatures in a series of statistical tests (effectively an array of *t*-tests) to estimate a temperature range associated with injury (Jacobson et al., 1992; Manter and Livingston, 1996). Alternatively, the degree of tissue injury across trees or treatments is compared at an arbitrarily chosen point of comparison, such as an LT_{20} or LT_{50} (Sheppard et al., 1989; Adams and Perkins, 1993). We have used an analysis of variance approach of normalized electrolyte leakage data to compute a critical temperature (T_c) of tissue injury, which is defined as the highest temperature at which tissue freezing injury can be detected (DeHayes and Williams, 1989). In addition,

we have also developed a curve-fitting procedure that fits a function to each tree or treatment and allows computation of the temperature (T_m) at the midpoint of a sigmoid curve fit to electrolyte leakage data (Strimbeck, 1997). Although T_c and T_m have slightly different applications, the two procedures produce similar results in both an absolute and relative sense. A comparative study conducted in our laboratory revealed an extremely high correlation ($r^2 = 0.84$, $P < 0.01$) between cold tolerance estimates derived using T_c and T_m calculations.

Relevance of Cold Tolerance Measurements to Freezing Injury

Numerous tissue viability and data handling protocols appear to provide useful cold tolerance information and no one method appears to be "best." The selection of procedure may vary with the objectives of the assessment and the expertise and equipment of the research team involved. An additional important consideration, however, should be the relevance of the laboratory cold tolerance estimates to the actual or relative cold tolerance of trees under field conditions.

We have examined the relationship between laboratory-generated cold tolerance estimates (T_c) for red spruce derived from electrolyte leakage assessments and actual visible freezing injury measured under both field (Fig. 6.1a) and laboratory conditions (Fig. 6.1b). We have routinely demonstrated strong correlations between T_c and field freezing injury even though correlations are skewed downward because of variation in T_c among trees sufficiently cold tolerant to escape injury under field conditions (note the range in T_c among trees with 0% injury in Fig. 6.1a). The latter also points out the value of laboratory cold tolerance assessments in providing critical cold tolerance information in the absence of injury-producing conditions in the field. It is important to emphasize that the strong correlations between T_c and field freezing injury indicate a strong relative relationship, and a good predictive value of cold tolerance estimates generated using this laboratory procedure. That is, laboratory cold tolerance assessments using this procedure effectively distinguish trees or treatments with greater or less cold tolerance than others and accurately discern increases or decreases in cold tolerance over time or in response to environmental disturbance or manipulations. However, the extent to which cold tolerance estimates generated in the laboratory using any protocol represent the actual temperature of freezing injury under field conditions is considerably less clear.

In a separate study, we have also demonstrated the efficacy of electrolyte leakage as an assay for freezing-induced tissue injury. Using image analysis as an objective measure of tissue injury detected through foliar discoloration, Strimbeck (1997) found a strong correspondence between increased electrolyte leakage and the initiation of foliar reddening associated with red spruce freezing injury (see Fig. 6.1b). As expected,

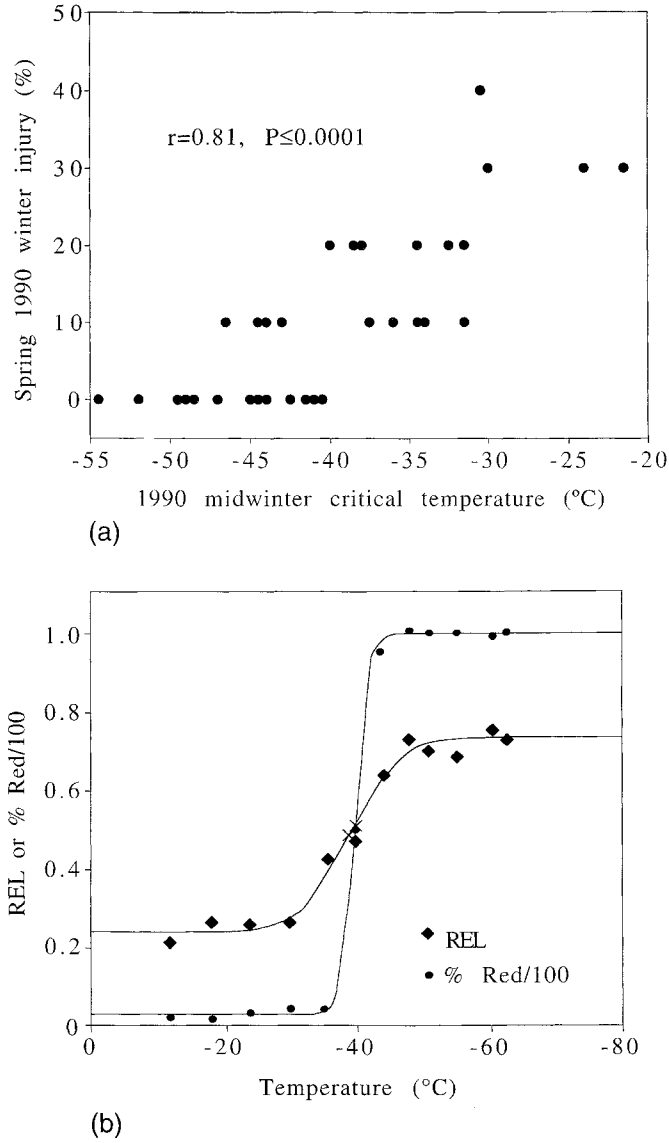

Figure 6.1. (a) Relationship between critical temperatures derived from laboratory freezing experiments with 36 red spruce trees and quantitative estimates of foliar winter injury of the same trees recorded in a provenance test of red spruce near Colebrook, NH. (b) Comparison of relative electrolyte leakage (REL) and image analysis of needle reddening (Red/100).

electrolyte leakage detected injury at temperatures a few degrees higher (i.e., warmer) than was evident through computer image analysis of foliar discoloration. We believe that this pre-visual injury represents real low

temperature–induced physiological perturbation at the cell membrane level even though it does not translate into visual symptoms of injury until temperatures are a few degrees lower. Some evidence suggests that this initial injury, evident by electrolyte leakage, but not foliar discoloration, may represent a level of injury that is sublethal and repairable (e.g., Palta et al., 1977; Hadley et al., 1993). Although certainly a possibility, the evidence for this in red spruce or other north temperate conifers is not abundant. Even if such membrane disturbance is repairable, it is likely that there would be an energy cost to the plant. It is more likely that this initial injury detectable by electrolyte leakage is too subtle to be detected visually, as is indicated by the unusually steep visual injury response curve (appearing as a threshold response in Fig. 6.1b) that suggests even image analysis is not sensitive to the very early phases of freezing injury. Microscopic anatomical analyses of injured red spruce needles are consistent with cell-to-cell variation and a gradation in freezing injury at the cellular level (Adams et al., 1991).

Relevance of Cold Tolerance Estimates to Actual Injury Temperatures

A remaining critical question is the extent to which laboratory estimates of cold tolerance reflect the actual temperature of injury under ambient field conditions. At first pass, one might suspect that laboratory estimates might slightly underestimate actual cold tolerance because tissue viability assays often focus on the initiation of freezing injury because it is difficult to determine when tissue is dead. In contrast, however, recent evidence has suggested the opposite is more likely. That is, laboratory estimates more likely overestimate cold tolerance because the systematic and controlled nature of laboratory freezing conditions (e.g., slow and consistent rate of freezing and thawing) potentially represents a less stressful set of conditions than the natural environment, which includes a diverse and interacting set of environmental conditions and inconsistent fluctuations in ambient temperatures.

Data from Lund and Livingston (1998), for instance, suggest that multiple freeze–thaw events, which are common under ambient conditions, may enhance the freezing injury susceptibility of red spruce. Thus, T_c would be expected to be higher under ambient conditions in which temperatures fluctuated above and below freezing. Furthermore, Manter and Livingston (1996) have reported that rapid post-freezing thaw rates can also exacerbate tissue injury that originally results from freezing, but would not be detected in controlled studies that typically utilize a slow post-freezing rate of thaw. Similarly, Hadley et al. (1996) noted that post-freezing environmental conditions dramatically influenced the extent of freezing injury detected by both electrolyte leakage and foliar discoloration. Prefrozen shoots subsequently exposed to the ambient atmosphere

with or without direct sunlight had much greater freezing injury symptoms than shoots placed in a constant 3°C refrigerator. In these experiments, red spruce shoots exposed to ambient atmospheric conditions after freezing to lethal temperatures exhibited LT_{10}s (based on visible injury) that were 5 to 15°C higher than those for shoots maintained in a controlled environment.

These data collectively indicate that the actual midwinter freezing tolerance of red spruce is likely less than that reported in controlled freezing tests. Although laboratory freezing studies provide conservative estimates of red spruce cold tolerance, they are strongly correlated with freezing injury under field conditions. Therefore, for consistency throughout this chapter, we will report laboratory-generated estimates of T_c or T_m as the measures of cold tolerance.

Midwinter Cold Tolerance of Red Spruce

Numerous cold tolerance assessments of red spruce have been conducted in several laboratories over the past decade. Despite varying objectives and the use of different procedures and populations in these assessments (all factors that would be expected to produce dramatically different results), a remarkably consistent pattern of midwinter maximum cold tolerance for red spruce has emerged. The average temperature in which freezing injury occurs to current-year needles of red spruce in northern New England and New York laboratory studies during winter is in the −40 to −45°C range (Table 6.1). The most comprehensive of these assessments, which examined the January cold tolerance of 60 native trees across an elevational range on Mt. Mansfield, Vermont, revealed an

Table 6.1. Estimates of the Cold Tolerance of Red Spruce Current-year Foliage Using Various Assessment Techniques

Citation	Assessment Technique	Date	Number of Trees	Estimated Cold Tolerance (°C)
Sheppard et al., 1989	Visual injury	Jan 1987	20	$LT_{10} \cong -37$[a] $LT_{50} \cong -42$[a]
DeHayes, 1992	Electrolyte leakage	Jan 1988	9	$T_c \cong -40$
Hadley and Amundson, 1992	Electrolyte leakage	Jan 1990	10	$T_c \cong -41$[a]
Perkins et al., 1993	Visual injury	Jan 1992	5	$LT_{20} \cong -46$[a]
Adams and Perkins, 1993	Chlorophyll fluorescence	Jan 1992	5	F_0 inflection $\cong -50$[a,b]
Strimbeck et al., 1995	Electrolyte leakage	Jan 1995	10	$T_c \cong -47$
Schaberg et al., 2000b	Electrolyte leakage	Jan 1996	60	$T_m \cong -42$

[a] Estimated from figure.
[b] Occurs after 20% visible injury.

average injury temperature of −41.7°C with a range of −30 to −54°C (Schaberg et al., 2000b). Importantly, 36% of the sampled trees exhibited maximum cold tolerance between −30 and −40°C. Minimum winter temperatures on the mountain fall within this temperature range during 91% of winters. Red spruce trees from southern New England are even more susceptible to freezing injury (DeHayes et al., 1990). Midwinter critical temperatures for 20 trees from a southern New Hampshire and a Massachusetts population averaged −38.7°C with a range from −21 to −49°C. In this case, 45% of the sampled trees exhibited maximum cold tolerance less than −40°C. Furthermore, trees from these provenances consistently suffered the highest incidence of winter injury in a rangewide provenance test located in northern New Hampshire (DeHayes et al., 1990).

Importantly, the maximum depth of midwinter red spruce cold tolerance is considerably less than balsam fir (*Abies balsamea* [L.] Miller), a sympatric associate that does not suffer freezing injury, and is only barely sufficient to avoid freezing injury under normal winter conditions. For example, cold tolerance assessments in our laboratory consistently demonstrate midwinter cold tolerance estimates for balsam fir below −60 to −65°C with some trees escaping injury at temperatures at or below −90°C. Historical temperature records from both Whiteface Mountain, NY, and Mt. Mansfield, VT, make it clear that red spruce, rather than balsam fir, appears to represent the outlier species with respect to cold tolerance. These records indicate that ambient montane temperatures approaching the maximum cold tolerance of at least some red spruce trees occur most winters, again illustrating the unique freezing injury susceptibility of red spruce. Also, freezing injury in red spruce is confined to current-year needles, which are about 10°C less cold tolerant than year-old needles. The latter indicates that red spruce trees have the physiological capacity to develop greater cold tolerance, but current-year needles lag behind older needles in cold tolerance development. In fact, if current-year needles were equal in cold tolerance to year-old needles of red spruce, it is expected that the species would escape freezing injury most years.

Given that laboratory-generated cold tolerance estimates are likely conservative and accurately reflect at least relative cold tolerance, freezing injury to the current-year foliage of some red spruce trees would be expected in many winters in northern montane environments. Field observations of red spruce winter injury support this contention (Morganstern, 1969; Friedland et al., 1984; Johnson et al., 1986, 1988; DeHayes et al., 1990; DeHayes, 1992). If specific pollution events (e.g., high acidic deposition inputs) or unusual climatic conditions (winter thaws) further reduce red spruce cold tolerance during some winters, as laboratory and field experiments have demonstrated (Fowler et al., 1989; DeHayes et al., 1991; DeHayes, 1992; Vann et al., 1992; Strimbeck et al.,

1995; Schaberg et al., 1996), the frequency and extent of red spruce freezing injury would be expected to dramatically increase in years such impacts are prevalent.

Potential Cold Tolerance Perturbations

Gaseous Pollutants

Considerable research has evaluated the impact of natural and anthropogenic factors on foliar cold tolerance. Among the factors assessed, changes in the concentrations of two gaseous agents (ozone and carbon dioxide) have been reported to alter the cold tolerance of spruce.

Although some work has suggested that ozone (O_3) exposure may increase freezing injury for red spruce during autumn (Fincher et al., 1989), the preponderance of evidence indicates that O_3 does not reduce red spruce foliar cold tolerance or increase freezing injury susceptibility. For example, we found no differences in the cold acclimation, depth of winter hardiness, or deacclimation of seedlings exposed to ambient (± 54 nl l^{-1}) versus reduced O_3 (± 24 nl l^{-1}) (DeHayes et al., 1991). In addition, in a study that included 3 independent experiments, we detected no reductions in autumn, winter, or spring cold tolerance for seedlings exposed to O_3 concentrations as high as 4 times ambient levels (Waite et al., 1994). In fact, in 2 of the experiments, seedlings exposed to elevated O_3 were actually more cold tolerant in January than seedlings that received low O_3 (Fig. 6.2).

Controlled exposures of other spruce species to elevated carbon dioxide (CO_2) levels provide an uncertain view of the potential impacts of CO_2 enrichment on red spruce cold tolerance. Margolis and Venzina (1990) evaluated the impact of either a continuous 10-week, or a series of 2-week, treatments of 1000 µl l^{-1} CO_2 on bud development and autumn cold tolerance of containerized black spruce seedlings (*Picea mariana* [Mill.] B.S.P.). They found that all CO_2 enrichments resulted in reduced cold tolerance (Margolis and Venzina, 1990). Because late growing season CO_2 enrichment delayed bud development and reduced autumn cold tolerance for black spruce (Margolis and Venzina, 1990), it seemed possible that cold tolerance perturbations resulted from a phenological disruption of cold acclimation. However, CO_2 enrichment has also been shown to decrease foliar nitrogen (N) concentrations in black spruce (Campagna and Margolis, 1989). And, considering the reported positive influence of N on foliar cold tolerance (DeHayes et al., 1989; Klein et al., 1989), it is also plausible that CO_2 enrichment reduced cold hardiness indirectly via a reduction of N (Margolis and Venzina, 1990). A preliminary evaluation of the combined influence of CO_2 enrichment and N nutrition on the cold tolerance of spruce seedlings was undertaken by Dalen et al. (1997). They evaluated the impact of CO_2 exposure and N fertilization on the autumn

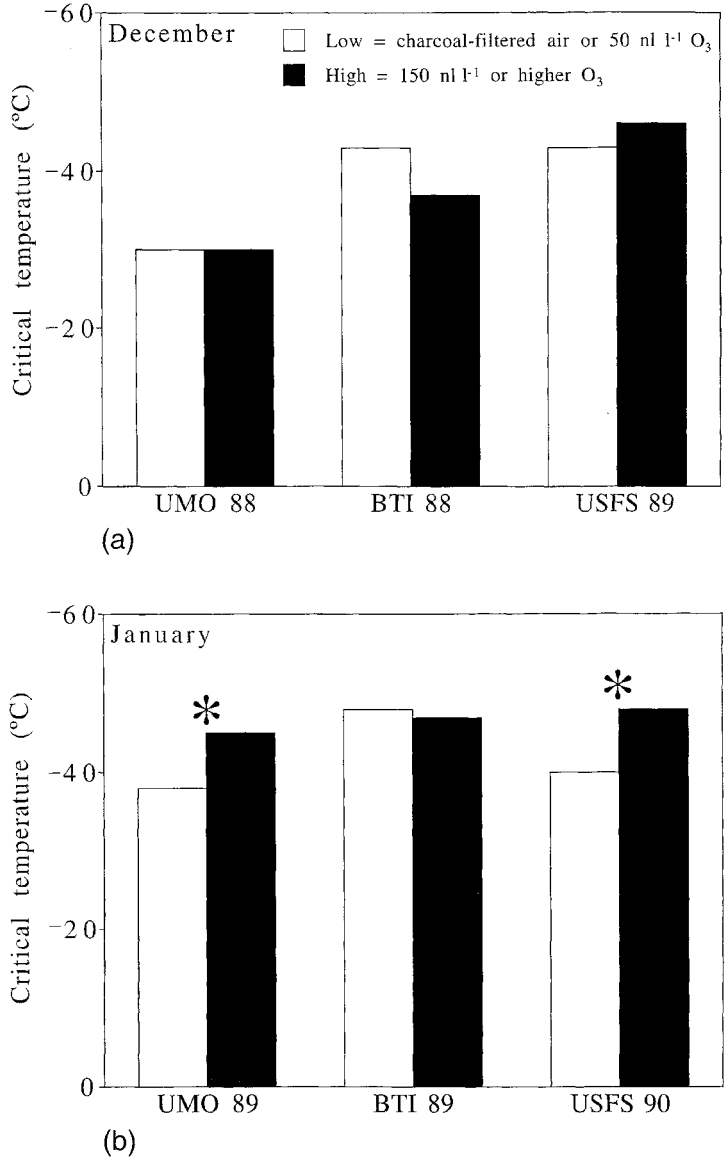

Figure 6.2. Critical temperatures during (a, b) winter (1988 and 1989) and (c) early spring (1989 and 1990) of current-year needles from red spruce exposed to charcoal-filtered air or ozone at the University of Maine (UMO), Boyce Thompson Institute (BTI), and the U.S. Forest Service Research Laboratory in Delaware, Ohio (USFS). An asterisk indicates significant treatment differences ($P \leq 0.05$).

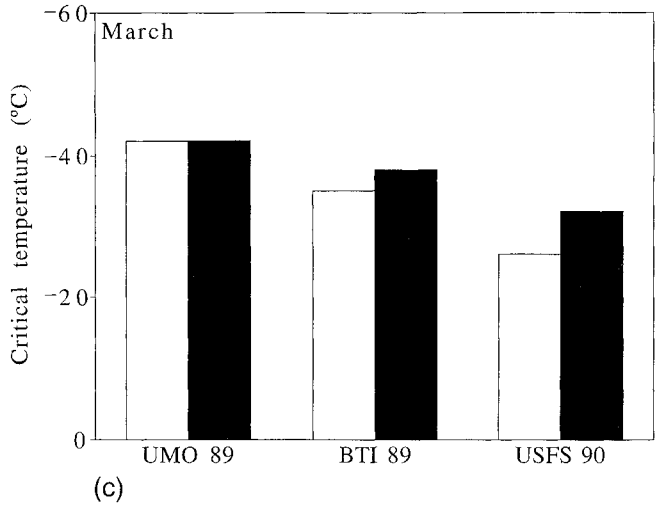

Figure 6.2. (*Continued*)

bud set and winter cold tolerance of Norway spruce (*Picea abies* [L.] Karst.) seedlings. Although CO_2 enrichment and/or N fertilization had no impact on the timing of bud set, elevated CO_2 increased the cold tolerance of high N-treated plants (Dalen et al., 1997). These results suggest that CO_2 enrichment and N nutrition may interact to influence winter cold tolerance through mechanisms separate from those controlling bud development.

Solar Warming and Rapid Freezing

Solar desiccation has been ruled out as the mechanism of winter injury in red spruce (Peart et al., 1991; Perkins et al., 1991; DeHayes, 1992). However, in part because winter injury can be greatest on the sun-exposed, south side of trees (Hadley et al., 1991; Perkins et al., 1991; Boyce, 1995), solar warming has been implicated as a contributor to foliar freezing injury (Hadley et al., 1991; Perkins et al., 1991; Hadley and Amundson, 1992; Strimbeck et al., 1993; Perkins and Adams, 1995). Field measurements have documented that the temperature of sun-exposed foliage can exceed that of the ambient air by over 20°C and that, similar to reports of the directionality of injury, solar warming is greatest for the southern aspect of crowns (Strimbeck et al., 1993).

It has been hypothesized that radiational warming could result in freezing injury if low temperatures follow sun-induced foliar dehardening (Hadley et al., 1991; Hadley and Amundson, 1992) or if rapid freezing occurs when solar illumination ends (Hadley et al., 1991; Perkins et al., 1991; Strimbeck et al., 1991; Perkins et al., 1993; Strimbeck et al., 1993).

Although radiational warming from protracted artificial illumination can result in foliar dehardening (Hadley and Amundson, 1992), it is less certain that radiational dehardening would occur in the field because solar warming there is often "transitory" and does not always raise needle temperatures above the freezing point (Strimbeck et al., 1993). In contrast, the rapid freezing of foliage following solar warming has been documented in the field (Perkins et al., 1991; Strimbeck et al., 1993), and results of experimental simulations indicate that rapid freezing can induce needle discoloration similar to natural winter injury (Perkins and Adams, 1995). However, the span of foliar temperature drop required to induce rapid freezing injury ($\geq$15°C) has not been recorded under field conditions (Strimbeck, 1997). The current absence of field verification for the temperature drops that induce rapid freezing may indicate that these conditions rarely occur in nature (Strimbeck, 1997), although it is also possible that current sampling has been insufficient to fully characterize field conditions. The apparent ability of rapid freezing to induce injury on year-old foliage is also inconsistent with reports from the field. Freezing injury occurs almost exclusively on the current-year foliage of red spruce (DeHayes et al., 1990; DeHayes, 1992). Yet, rapid freezing can result in injury to year-old foliage when experimental conditions cause severe injury in current-year foliage (Strimbeck, 1997).

To fully elucidate the significance of solar warming and rapid freezing to red spruce winter injury, more comprehensive monitoring of needle microclimate during winter will be required. Still, even with the current level of scientific uncertainty, Strimbeck (1997) concluded that rapid freezing stress may help explain localized injury concentrated on sun-exposed branches. However, it seems improbable that rapid freezing following solar warming could account for reports of injury on shaded foliage (DeHayes et al., 1990; Hadley et al., 1991; Peart et al., 1991) or explain regionwide injury events, which have occurred in the northeastern U.S. at a rate of two to three times per decade (Johnson et al., 1988; DeHayes, 1992).

Precocious Dehardening and Winter Thaws

Although brief daytime thaws during winter appear to have little impact on red spruce cold tolerance (Perkins et al., 1993), considerable evidence indicates that significant precocious dehardening can occur in response to longer thaws. Several studies have documented reductions in cold tolerance of up to 14°C for red spruce seedlings exposed to simulated thaws (5 to 10°C) lasting four to five days (DeHayes, 1992; Schaberg et al., 1996). And, Strimbeck et al. (1995) recently documented that the current-year foliage of mature montane red spruce dehardened an average of 9°C after only three days of exposure to above-freezing (0 to 10°C) temperatures (Fig. 6.3). Individual trees dehardened as much as 14°C (Strimbeck

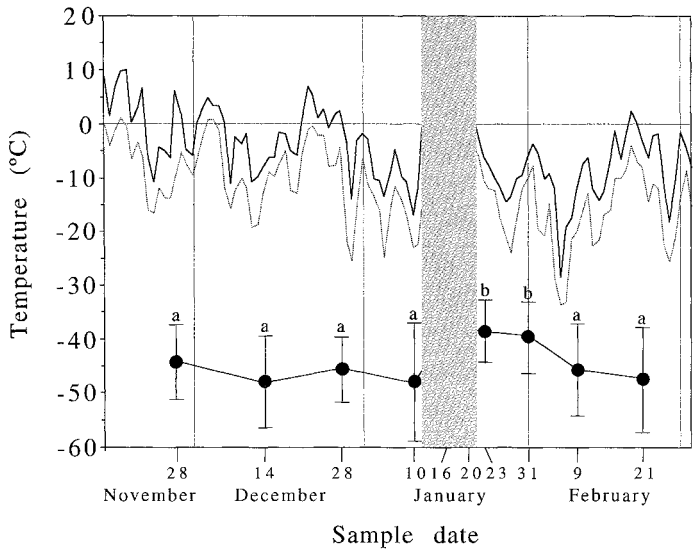

Figure 6.3. Cold tolerance of current-year foliage from mature red spruce trees and ambient air temperatures for Mt. Mansfield, VT. Solid and dotted lines are daily maximum and minimum temperatures, respectively, at the elevation of the sample stand (1000 m). Points and bars are mean, maximum, and minimum critical temperatures (T_c) of current-year foliage from 10 trees. Shaded area indicates extent of thaw weather. Mean T_c values with the same letter are not significantly different at $P \leq 0.01$, using the Duncan's multiple range test.

et al., 1995). More importantly, trees remained partially dehardened and more vulnerable to freezing injury for up to 19 days after consistent subfreezing ambient temperatures resumed (Strimbeck et al., 1995).

Data also indicate that the dehardening response to winter thaws may be unique to red spruce. For example, although red spruce experienced substantial and persistent reductions in cold tolerance during the January 1995 thaw, companion balsam fir trees showed no evidence of dehardening (Strimbeck et al., 1995). Results of a replicated experiment we conducted show the same response for seedlings exposed to simulated thaw treatments (DeHayes, 1992). Red spruce and balsam fir seedlings were exposed to either 5°C for five days, 10°C for five days, these treatments followed by five days of ambient (subfreezing) conditions, or continuous ambient conditions (the control). Red spruce seedlings experienced significant reductions in cold tolerance in response to thaw, whereas no reductions in cold tolerance were detected for balsam fir (Table 6.2). Similar to trees in the field, thaw-induced dehardening often persisted for the red spruce seedlings despite reexposure to subfreezing temperatures.

Table 6.2. Reduction in Cold Tolerance of Current-year Needles from Red Spruce and Balsam Fir Seedlings Exposed to 4 Temperature Treatments Compared with Seedlings Exposed to Ambient Subfreezing Conditions during Winter 1989

Temperature, Treatments	Reduction in Cold Tolerance (°C) on:					
	1/23/89		2/17/89		3/27/89	
	Spruce	Fir	Spruce	Fir	Spruce	Fir
10°C, 5 days	12.0[a]	5.7	13.5[a]	3.1	11.9[a]	1.3
5°C, 5 days	11.1[a]	0.3	8.3[a]	2.1	5.9[a]	–
10°C, 5 days followed by 5 days at ambient	4.3	1.9	7.9[a]	0.5	7.3[a]	–
5°C, 5 days followed by 5 days at ambient	5.8[a]	1.3	4.7	1.9	1.3	−1.1
Ambient	−45.2	−62.9	−41.1	−63.7	−40.5	−49.5

[a] Significant reduction in cold tolerance from seedlings exposed to ambient subfreezing conditions.

Because red spruce reach midwinter (pre-thaw) hardiness levels that are dangerously near ambient temperature lows, protracted reductions in cold tolerance following thaws greatly increase the risk of freezing injury if thaws are followed by extreme cold. In fact, the findings of Strimbeck et al. (1995) may exemplify a consequence of this elevated risk: the least cold tolerant tree following the January 1995 thaw exhibited the greatest amount of freezing injury after minimum temperatures at the site reached −34°C about 16 days after the thaw ended (Strimbeck et al., 1995). Even with thaw-induced dehardening, however, overall freezing injury among study trees was not severe (Strimbeck et al., 1995). From a broader perspective, examination of climatic records shows that midwinter thaws were associated with only two of the last four episodes of regionwide freezing injury (Tobi et al., 1995). Thus, although possibly an important contributor, midwinter thaws alone do not account for all recent episodes of red spruce freezing injury. More likely, combinations of predisposing factors including thaw-induced dehardening, air pollution stress, rapid freezing, and even repeating cycles of freezing and thawing (Lund and Livingston, 1998) may differentially contribute to injury development over time (Strimbeck et al., 1995).

Plant and Soil Nutrition

Nitrogen

Numerous studies have shown that short-term nitrogen (N) additions either have no impact on red spruce freezing tolerance or may even improve hardiness levels. For example, we fertilized red spruce seedlings with one of four concentrations of soil-applied ammonium nitrate

(NH_4NO_3) (0, 300, 1500 or 3000 kg N ha^{-1}) during early-, mid-, or late-summer and observed that seedlings generally acclimated more rapidly, achieved a greater depth of cold tolerance in winter, and deacclimated more slowly with increasing N treatment (DeHayes et al., 1989). The timing of N application also had an impact: N applications in early summer resulted in a slight increase in midwinter cold tolerance, whereas mid- and late-summer additions resulted in considerable increases in hardiness (DeHayes et al., 1989). Klein et al. (1989) reported that misting nutrient-deficient and fertilized red spruce seedlings with simulated cloud water containing either ammonium (NH_4), nitrate (NO_3), or both, had no effect on foliar cold tolerance. However, improving the nutrient status of seedlings that were initially N-deficient reduced their sensitivity to freezing injury (Klein et al., 1989). In addition, L'Hirondelle et al. (1992) reported that N fertilization increased the late autumn cold tolerance of red spruce seedlings, but that the low doses of N applied as part of a simulated acid mist had no impact.

Data also suggest that, at least during the first years of application, N additions have only a limited impact on the cold tolerance of mature trees in the field. White (1996) evaluated the cold tolerance of red spruce trees within two experimental watersheds in Maine: an untreated reference watershed and an adjacent watershed that received granular ammonium sulfate [$(NH_4)_2SO_4$] at a dose that supplied approximately 32 kg ammonium (NH_4) ha^{-1} $year^{-1}$ and 86 kg sulfate (SO_4) ha^{-1} $year^{-1}$. Following four years of treatment, the midwinter cold tolerance of 8 dominant or codominant red spruce trees from each watershed was assessed using electrolyte leakage. No difference between watersheds was detected regarding the temperature at which freezing damage occurred (White, 1996). However, there was some indication that $(NH_4)_2SO_4$ treatment may have increased cold tolerance slightly: relative damage at −45°C was lower in trees from the treated watershed (White, 1996).

Although it is clear that short-term additions of N do not reduce red spruce cold tolerance, this might not hold true for prolonged N additions. Recent studies have determined that chronic N fertilization can result in growth reductions and increased mortality for red spruce trees (McNulty et al., 1996). Alterations in foliar cation nutrition and carbon relations have been associated with and may contribute to N-induced decline (Schaberg et al., 1997). Still, some data suggest that increases in freezing injury may also occur in response to long-term N addition (Perkins et al., 2000), although the early appearance of injury during this study made it impossible to fully accredit injury to any one stressor (Perkins et al., 2000).

Sulfur

Sulfur (S) additions appear to have a variable impact on hardiness levels during autumn, but no impact on winter cold tolerance. For instance,

although they did not measure winter cold tolerance, Cape et al. (1991) found that application of sulfate-containing mists reduced the cold tolerance of red spruce seedlings on one of four autumn dates assessed. Using data from this study as a foundation, Sheppard (1994) then proposed a mechanism whereby foliar assimilation of sulfate (SO_4^{2-}) could result in reduced foliar cold tolerance. However, other findings indicate that foliar assimilation of SO_4^{2-} is very limited (Lindberg and Lovett, 1992; McLaughlin et al., 1996). In a separate study, Jacobson et al. (1992) reported that the anionic (SO_4^{2-} and NO_3^-) composition of mist treatments significantly impacted red spruce cold tolerance in the fall, but that the specific impact differed between the two dates assessed: in early October nitrate NO_3^--treated plants had lower cold tolerance than SO_4^{2-}-treated ones, while in mid-October the opposite was true. During the winter, differences in cold tolerance were associated with treatment pH and not the anionic composition of treatments (Jacobson et al., 1992). In a separate acid mist experiment, L'Hirondelle et al. (1992) found that SO_4^{2-}-treated seedlings had greater frost hardiness at the start of cold acclimation, but that the anionic composition of mists had no impact on hardiness levels thereafter. In addition, recent studies have documented significant reductions in cold tolerance for red spruce saplings exposed to acid mists that had either equalized SO_4^{2-} concentrations (Schaberg et al., 2000a) or contained no additional SO_4^{2-} (DeHayes et al., 1999). Data from mature trees fertilized with $(NH_4)_2SO_4$ for 4 years also showed no changes or slight increases in winter cold tolerance following treatment (White, 1996).

Aluminum

High concentrations of aluminum (Al) within soil solutions have been implicated as a contributing factor in the decline of red spruce in the northeastern U.S. (Shortle and Smith, 1988; Lawrence et al., 1995). Considering the preeminent importance of freezing injury to red spruce decline in this region (Johnson et al., 1988; Wilkinson, 1990; DeHayes, 1992; Tobi et al., 1995), it seems likely that if Al influences decline here, this influence could be through some perturbation in cold tolerance.

Schaberg et al. (2000a) recently tested the influence of soil solution Al at a level close to the maximum concentration reported for native red spruce forest soils (e.g., about 210 μM Al, Joslin and Wolfe, 1992) on the mineral nutrition, gas exchange, growth, and foliar cold tolerance of red spruce saplings. Saplings were treated with one of four Ca (0, 25, 75, or 225 μM) and two Al (0 and 200 μM) soil watering treatments in a factorial arrangement and were split between two aerial mist treatments (pH 3 or 5). Al treatment altered the growth, gas exchange, and foliar chemistry of saplings (Table 6.3), but no differences in autumn or winter cold tolerance were found (Table 6.4). Because Al may contribute to the decline of red

Table 6.3. Statistical Significance of Soil Solution Al (0 or 200 μM Al) and Ca (0, 25, 75 or 225 μM Ca) Treatments on Foliar Cation Concentrations, Diameter, Height and Shoot Length, and Gas Exchange of Red Spruce Seedlings

Foliar Elemental Concentration (mg kg^{-1})							
	Al	B	Ca	Mg	Mn	P	Zn
Soil Al	*	NS	*	*	*	**	*
Soil Ca	NS	NS	*	NS	NS	NS	*

Growth (mm)			
	Stem Diameter	Total Height	Shoot Length
Soil Al	*	*	*
Soil Ca	NS	NS	NS

Gas Exchange (μmol m^{-2} s^{-1})				
	Jul 1994		Sep 1994	
	Photo	Resp	Photo	Resp
Soil Al	**	*	*	NS
Soil Ca	NS	*	NS	NS

NS = not significant. * = $P \leq 0.05$. ** = $P \leq 0.01$.

spruce through a disruption in calcium (Ca) nutrition (Shortle and Smith, 1988; Lawrence et al., 1995), it is important to note that Al treatment dramatically reduced total foliar Ca concentrations (see Table 6.4). Interestingly, however, Al treatment did not decrease Ca concentrations specifically associated with the plasma membranes of mesophyll cells (Table 6.4). This membrane-associated Ca (mCa) is of particular importance to the physiology of conifer needles (DeHayes et al., 1997). Although of possible importance to other aspects of physiology, realistically high soil solution Al concentrations neither reduced critical pools of mCa nor increased the risk of foliar freezing injury.

Calcium

Many factors, including the leaching loss of Ca due to acid precipitation (Likens et al., 1996) and N deposition (Aber et al., 1995), Ca removal via intensive harvesting (Federer et al., 1989), and recent reductions in Ca deposition (Hedin et al., 1994) may be contributing to a depletion of Ca from forest ecosystems within the eastern U.S. Although the long-term impact of Ca depletion has yet to be determined, the decline of red spruce at certain locations has already been linked to a disruption of Ca nutrition (McLaughlin and Kohut, 1992; McNulty et al., 1996; Schaberg et al., 1997). Red spruce in the southern Appalachian Mountains provide

Table 6.4. Influence of Soil Al, Soil Ca, and Acidic Mist Treatments on Current-year Foliar Ca Leaching and Concentration, Membrane-associated Ca (mCa), and Cold Tolerance in Red Spruce

Treatments	Foliar Ca Leaching (μg/l)			Total Foliar Ca (mg kg^{-1})				mCa Fluorescence			Cold Tolerance (°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 Al													
0 μmol l^{-1}	247	162	113	2417[a]	2803[a]	2562[a]	2931[a]	0.21	0.18	0.14	−46.5	−54.0	−47.8
200 μmol l^{-1}	328	133	96	1523	2040	1926	2091	0.23	0.18	0.15	−45.9	−54.6	−46.6
Soil Ca													
0 μmol l^{-1}	249	172	84	1560[a]	2042	1733[a]	1815[a]	0.21	0.18	0.15	−45.4	−53.8	−45.6
225 μmol l^{-1}	373	150	106	2335	2514	2743	3142	0.23	0.17	0.15	−47.2	−55.8	−46.7
Acidic Mist													
pH 3	526[a]	233[a]	148[a]	1969	2289	2262	2488	0.21	0.16[b]	0.12[b]	−44.5[a]	−51.5[b]	−44.9[b]
pH 5	66	64	60	1971	2553	2246	2534	0.22	0.19	0.18	−48.0	−57.0	−49.4

[a] $P \leq 0.01$.
[b] $P \leq 0.05$.

a prime example of this: considerable research has indicated that acid deposition-induced alterations in Ca nutrition and carbon relations have contributed to spruce decline in this region (McLaughlin and Kohut, 1992; McLaughlin et al., 1991, 1993).

Freezing injury of northern red spruce could also be related to a disruption in Ca nutrition. Calcium is important to freezing injury avoidance in herbaceous plants (Pomeroy and Andrews, 1985; Monroy et al., 1993; Crotty and Poole, 1995), and, a possible link between Ca nutrition and the cold tolerance of red spruce is supported by some data. Perkins and Adams (1995) reported that the concentration of several cations, including Ca, was related to freezing injury susceptibility: trees sensitive to injury had significantly lower ($P < 0.10$) Ca, magnesium (Mg) and manganese (Mn) concentrations than resistant trees. Results from cloud water exclusion studies on Whitetop Mountain, VA, also suggest an association between cation nutrition and cold tolerance. Native red spruce seedlings were exposed to or excluded from ambient acid cloud water and then physiologically and chemically assessed (Thornton et al., 1990; DeHayes et al., 1991). Although no treatment differences were found in growth or photosynthetic characteristics, seedlings exposed to acid cloud water had significantly lower concentrations of Ca and Mg in current-year foliage (which is susceptible to freezing injury) but not in year-old needles (which are not typically injured) (Thornton et al., 1990). More importantly, current-year needles of acid-exposed seedlings were also significantly less cold tolerant than comparable foliage from protected plants (DeHayes et al., 1991).

A more specific link between the Ca content and hardiness of red spruce foliage was discovered as part of a preliminary experiment conducted in our laboratory. We conducted laboratory cold tolerance and foliar Ca measurements on 19 mature trees that were part of cold tolerance studies in previous winters. The Ca content of current-year foliage was significantly correlated with the temperature of freezing injury on that sample date ($r = -0.64$; $P = 0.003$; Fig. 6.4) as well as the degree of freezing injury experienced the previous winter ($r = 0.44$; $P = 0.10$). Thus, trees with relatively low foliar Ca were generally less cold tolerant and suffered greater freezing injury the past winter. Interestingly, the correlation between Ca content and cold tolerance appeared to be driven primarily by trees with low rather than high Ca (Fig. 6.4). Trees with low Ca had relatively mild critical temperatures, whereas above some apparent Ca threshold there was no linear relationship between cold tolerance and Ca.

Despite evidence supporting a Ca–cold tolerance relationship, recent data by Schaberg et al. (2000a) indicate that total foliar Ca and cold tolerance levels are not always related. In this study, red spruce saplings received controlled additions of Ca and/or Al via the soil solution and received either pH 3.0 or 5.0 aerial mist treatments (see previous section on

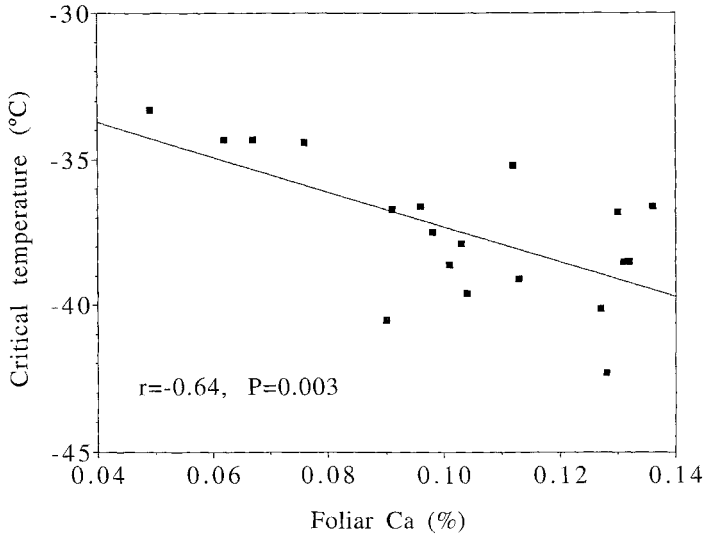

Figure 6.4. Relationship between foliar critical temperature (°C) and foliar calcium concentrations (%) of 31-year-old red spruce trees growing in northwestern NH. Observations represent means of three samples from each of 19 trees.

Aluminum for details). Calcium treatment significantly increased foliar Ca incorporation and resulted in higher respiration rates while shoots were elongating (see Table 6.3). Similar to the Al treatments in this study, and perhaps because Ca treatment did not alter physiologically important concentrations of mCa (Table 6.4), Ca additions had no discernible impact on cold tolerance.

Acidic Cloud Deposition

Strong evidence now indicates that exposure to acid cloud water increases the risk of foliar freezing injury for red spruce (Table 6.5). Numerous studies have shown that treatment of red spruce with simulated acid deposition reduces the cold tolerance of current-year needles anywhere from 5 to 12°C (Fowler et al., 1989; Jacobson et al., 1992; Sheppard et al., 1993; Waite et al., 1994; DeHayes et al., 1999; Schaberg et al., 2000a) (see Table 6.5). In addition, studies of seedlings (DeHayes et al., 1991) or branches (Vann et al., 1992) exposed to ambient cloud water show that reductions in cold tolerance comparable to those documented for controlled studies also occur in the field. For example, in a mist exclusion study conducted on Whitetop Mountain, VA, native red spruce seedlings exposed to ambient cloud water were 5°C less cold tolerant in winter than seedlings that had cloud water excluded (DeHayes et al., 1991). Similarly, in a study on Whiteface Mountain, NY, airborne chemicals were excluded

Table 6.5. Studies of Mist pH Effects on the Cold Tolerance of Red Spruce Current Year Foliage

Citation	Subject	Treatments	Treatment ions	Season	Mean Δ cold tolerance
Fowler et al. 1989	seedlings	pH 2.5 to 5.0	H^+, NH_4^+, NO_3^-, SO_4^{2-}	autumn	12°C
DeHayes et al. 1991	seedlings	ambient cloud vs. no cloud	ambient mix	late autumn to winter	3 to 5°C
Vann et al. 1992	mature tree branches	ambient cloud vs. no cloud	ambient mix	winter	10°C
Jacobson et al. 1992	seedlings	pH 2.5 to 4.5	H^+, NO_3^-, SO_4^{2-}	autumn	6 to 7.5°C[a]
Sheppard et al. 1993	seedlings	pH 2.5 vs. 5.0	H^+, NH_4^+, NO_3^-, SO_4^{2-}	autumn	6°C
Sheppard et al. 1993	seedlings	pH 2.7 vs. 5.1	H^+, NH_4^+, NO_3^-, SO_4^{2-}	autumn	8°C
Waite et al. 1994	seedlings	pH 3.0 vs. 4.2	H^+, NO_3^-, SO_4^{2-}	winter	6°C
DeHayes et al. 1999	seedlings	pH 3.0 vs. 5.0	H^+, Cl^-	winter	10°C
Schaberg et al. 2000a	saplings	pH 3.0 vs 5.0	H^+, equalized SO_4^{2-}	winter	8°C

[a] Estimated from figure.

from select branches of 75-year-old red spruce trees using exclusion chambers (Vann et al., 1992). Current-year foliage on branches exposed to ambient chemicals was approximately 10°C less cold tolerant during winter and experienced more freezing injury than comparable foliage protected from ambient inputs for three months during the previous growing season (Vann et al., 1992). Because maximum winter cold tolerance levels of red spruce current-year needles are barely sufficient to protect foliage from the minimum temperatures encountered (DeHayes, 1992), disturbances that decrease cold tolerance even a few degrees are important.

Although the chemical composition of cloud water in the eastern U.S. is dominated by 4 ionic constituents, NO_3^-, SO_4^{2-}, NH_4^+, and hydrogen ions (H^+) (Mohnen, 1992), evidence suggests H^+ ions are responsible for reducing the foliar cold tolerance of red spruce during winter. Studies have shown that misting with solutions containing NO_3^- (L'Hirondelle et al., 1992) or NH_4^+ and NO_3^- (Cape et al., 1991) do not decrease the foliar cold tolerance of treated seedlings. Cape et al. (1991) reported that misting with solutions containing SO_4^{2-} reduced foliar cold tolerance on one out of four autumn dates assessed (see previous section on Sulfur), whereas L'Hirondelle et al. (1992) found that misting with SO_4^{2-} actually increased the frost hardiness of seedlings at the start of cold acclimation. However, neither of these studies examined the impacts of SO_4^{2-} on winter hardiness levels (Cape et al., 1991; L'Hirondelle et al., 1992). Jacobson et al. (1992) showed that, although SO_4^{2-} and/or NO_3^- additions reduced cold tolerance somewhat in autumn, these treatments had no influence on winter hardiness levels. Reductions in winter cold tolerance reported for this study were specifically associated with mist acidity and not the anionic composition of treatments (Jacobson et al., 1992). Similar findings were reported by L'Hirondelle et al. (1992). Furthermore, recent work has shown that misting with an acid treatment that contained no NO_3^-, SO_4^{2-}, or NH_4^+ significantly reduced the autumn and winter cold tolerance of red spruce saplings (DeHayes et al., 1999). In this study, 12 saplings were evenly distributed between two mist treatments: base nutrient solutions acidified with hydrochloric acid (HCl) to pH 3.0 or 5.0 applied for a total of 118 hours from September through November, 1993. Average reductions in cold tolerance (7.2°C in November and 10.4°C in February) were comparable to those reported for studies in which mist treatments included NO_3^-, SO_4^{2-}, NH_4^+, and H^+ (see Table 6.5). Another study also highlighted the importance of H^+ rather than SO_4^{2-} to treatment-induced reductions in cold tolerance (Schaberg et al., 2000a). Here, pH 3.0 mist application resulted in an 8°C reduction in cold tolerance relative to pH 5.0 treatment even though mist solutions contained equal SO_4^{2-} concentrations. The similarity in response across different anionic solutions coupled with reduced H^+, but not SO_4^{2-} (Joslin et al., 1988; McLaughlin et al., 1996), concentrations in throughfall suggest that acid mist–induced

Ca leaching and cold tolerance reductions are likely the result of cation exchange driven by differential H^+ exposure.

Interactions with Acid Deposition

Although it is well established that exposure to acid deposition reduces the cold tolerance of red spruce (DeHayes, 1992), other environmental factors could interact with acid deposition and/or each other to ameliorate, intensify, or in some other way alter its impact on hardiness. Because of this, knowledge of the interactions among stressors is essential to fully assess red spruce's risk of freezing injury during this period of rapid environmental change. Unfortunately, little is currently known about the influence of integrated stress on tree physiology in general, let alone specific influences on the cold tolerance of red spruce. Still, there is some evidence that other environmental perturbations can interact with acid deposition to modify plant physiology, including, at times, freezing tolerance.

A case in point: the interactive impact of acid mist and soil Al on red spruce mineral nutrition and growth. It is well established that soil pH and Al can interact to influence the availability and uptake of Al and base cations (Johnson and Fernandez, 1992). Consequently, pH and Al levels influence potentials for Al toxicity and/or cation deficiencies within plants (Marschner, 1986). Schaberg et al. (2000a) evaluated the interactive impact of soil solution Al and acid mist treatments on red spruce saplings exposed to soil Ca, soil Al and aerial mist treatments (see previous section on Aluminum). In addition to treatment main effects (see Tables 6.3 and 6.4), in a few specific instances the relative influence of Al differed between the two pH mist treatments. For instance, Al-induced reductions in Ca, Mg, zinc (Zn) and phosphorus (P) concentrations were comparatively greater for pH 5–treated saplings than for pH 3–treated plants (Schaberg et al., 2000a). Despite documented influences of Al × pH interactions on foliar cation concentrations, and even though cation chemistry (especially Ca) may have links to cold tolerance physiology (Pomeroy and Andrews, 1985; Monroy et al., 1993; Perkins and Adams, 1995), no interactive impacts of acid mist and Al treatment on cold tolerance were detected.

In contrast to results for acid mist and soil Al treatments, some data indicate that acid mist exposure and N fertilization may interact and influence foliar cold tolerance (L'Hirondelle et al., 1992). As independent factors, both N fertilization and acid mist application influence cold tolerance: N fertilization can enhance cold hardiness (DeHayes et al., 1989) whereas acid mist reduces freezing tolerance (DeHayes, 1992). L'Hirondelle et al. (1992) examined the possible combined effects of N and acid mist treatments by fertilizing pH 3–misted seedlings with either 100 (high-N) or 20 (low-N) mg $N\,l^{-1}$ and evaluating the influence of treatment on autumn cold acclimation. They found that high-N treatment partially mitigated the impact of acid exposure during the later stages of

acclimation (L'Hirondelle et al., 1992). Although N treatment had no influence on cold tolerance during September and October, high-N seedlings were slightly but significantly more cold tolerant than low-N seedlings by November (L'Hirondelle et al., 1992). Because no assessment of cold hardiness was made after November 7 (L'Hirondelle et al., 1992), it is unknown if N treatment would have improved freezing tolerance during winter. Even if N additions do temper the influence of acid mist on cold tolerance, other data suggest that N additions do not fully compensate for acid-induced reductions in hardiness. Several studies have reported that applications of acid mists that include N reduce foliar cold tolerance levels (Fowler et al., 1989; Waite et al., 1994). However, differences in N concentrations among mist treatments may have been insufficient to influence foliar cold tolerance.

Of all the possible combinations of factors that influence red spruce cold tolerance, interactions between acid mist and thaw have the greatest potential to increase the risk of freezing injury during winter. Mean reductions in cold tolerance of 12°C (Fowler et al., 1989) can occur in response to acid mist treatment, and average drops in hardiness up to 14°C have been reported for red spruce exposed to simulated winter thaws (Schaberg et al., 1996). If the combination of acid mist and thaw produced a cold tolerance response that was additive of the individual impacts of these stressors, resulting reductions in hardiness would dramatically increase the risk of freezing injury for red spruce current-year foliage. Possible interactions between acid mist and thaw are of practical importance because atmospheric additions of acid-producing compounds are likely to continue, while midwinter thaws are predicted to occur with greater frequency and intensity in the decades ahead (MacCracken et al., 1991). Although of great potential consequence to the health and survival of red spruce in the northeastern U.S., it is unknown if acid mist and thaw interact to heighten the risk of freezing injury for red spruce.

Potential Explanations of Freezing Injury in Red Spruce

Physiological Responses to Winter Warming

Phenology of Bud Development and Foliar Cold Tolerance

Although numerous climate models predict that mean surface temperatures could increase 1 to 3°C over the next 50 years, it seems unlikely that these changes will be spatially and seasonally uniform. Temperature changes are likely to be greatest at higher latitudes (Ramanathan, 1988; Lorius et al., 1990) where autumn, winter, and spring temperatures could be disproportionately altered (Kettunen et al., 1987; MacCracken et al., 1991).

Potential increases in temperature during the dormant period have raised questions regarding the likelihood of precocious budbreak, and

several researchers have used computer models to simulate the impact of climatic warming on bud phenology and growth initiation for trees in northern Europe (Hanninen, 1991; Kellomaki et al., 1995). These models predict that climatic warming could speed ontogenetic development following the chilling period and induce budbreak as early as midwinter (Hanninen, 1991; Kellomaki et al., 1995). Because temperatures below 0°C are likely to occur in winter and early spring despite overall warming, precocious budbreak would likely increase the risk of frost injury (Hanninen, 1991; Kellomaki et al., 1995). Although the assumptions used in these models require further evaluation, the predictions of Hanninen (1991) and Kellomaki et al. (1995) raise concerns about the possible influence of climate change on bud development for all northern tree species, including red spruce. However, it seems unlikely that red spruce would be uniquely impacted. In fact, because red spruce typically begins growth later in the spring than most sympatric species (Burns and Honkala, 1990), it might actually be less likely to experience frost injury as a result of precocious budbreak.

The development and maintenance of foliar cold tolerance is also tied to environmental cues: short days and relatively mild subfreezing temperatures in the fall which initiate the cold acclimation process and low subfreezing temperatures which induce and perpetuate the attainment of maximum cold tolerance levels during winter (Sakai and Larcher, 1987). Warming during the dormant season (autumn through spring) could disrupt the timing and rate of foliar cold acclimation in the autumn, the depth of cold tolerance attained during winter, or the timing and rate of dehardening in the spring. Although disruptions of any of these processes seem possible, evidence suggests that, compared with other northern conifers, red spruce may be most susceptible to perturbations of winter cold tolerance (DeHayes, 1992). For example, levels of cold tolerance for red spruce are comparable with sympatric species such as balsam fir during the autumn and spring, but fall below the hardiness levels of other species during winter (DeHayes, 1992). The fact that red spruce deharden during thaws whereas sympatric balsam fir do not (Strimbeck et al., 1995) may highlight the unique potential for increased freezing injury to red spruce due to warming winter climates.

Midwinter Thaws

In northern latitudes, the greatest disruption of temperature is predicted to occur during winter (Kettunen et al., 1987; MacCracken et al., 1991). Importantly, it is predicted that temperature extremes for winter will increase as thaws become more common while contemporary temperature minima persist (MacCracken et al., 1991). Increases in the number (Schaberg et al., 1996) and severity (Strimbeck et al., 1995) of winter thaws have already been reported for portions of Vermont. The trend in

winter thawing degree days (TDD, the summation of daily temperature means for all days from 21 December through 21 March with average temperatures >0°C) for Mt. Mansfield, VT, provides an example of this (Fig. 6.5). The slope of the linear relationship between TDD and year ($m = 0.6185$) is significantly greater than zero ($P \leq 0.002$) for the period following measurement initiation in 1954 (see Fig. 6.5), indicating that TDD have generally increased over this time. Considering the documented sensitivity of red spruce to freezing injury during winter (DeHayes, 1992) and its propensity for precocious dehardening during thaws (Strimbeck et al., 1995), perturbations of winter temperatures could be of particular concern for this species. However, red spruce do not just deharden during thaws. Significant changes in carbon metabolism also occur.

Northern red spruce typically have relatively low rates of net photosynthesis in the fall and spring, and rates close to zero for much of the winter (Schaberg et al., 1995, 1998). Yet, photosynthetic activity can increase substantially during extended thaws (Schaberg et al., 1995, 1996, 1998). For example, in their evaluation of field photosynthesis for 46 plantation-grown trees in northwestern VT, Schaberg et al. (1995) found that photosynthetic rates more than tripled during two protracted thaws (Fig. 6.6). In addition, although average rates of photosynthesis reached only 14% of the mean for red spruce during the growing season, rates for

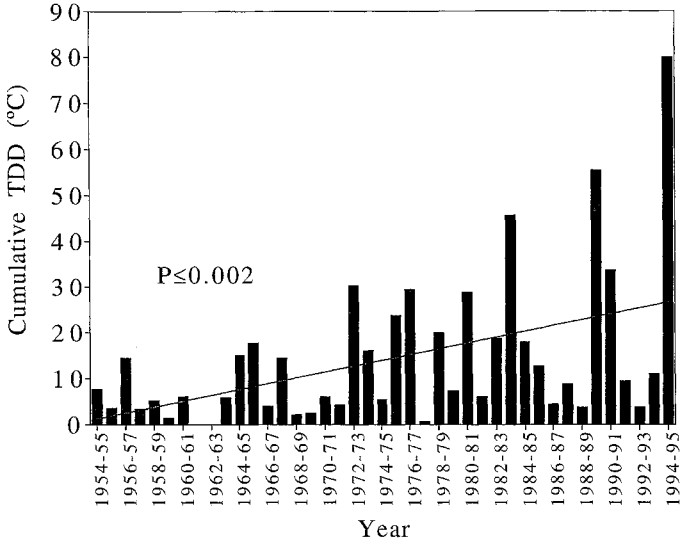

Figure 6.5. Cumulative thawing degree days (TDD) for 21 December through 21 March recorded on Mt. Mansfield, Stowe, VT, from 1954 through 1995 (NOAA records). Cumulative TDD are the summation of mean daily temperatures for days when average temperatures exceeded 0°C. Slope of the linear trend of TDD over time is positive and significantly different from zero ($P \leq 0.002$).

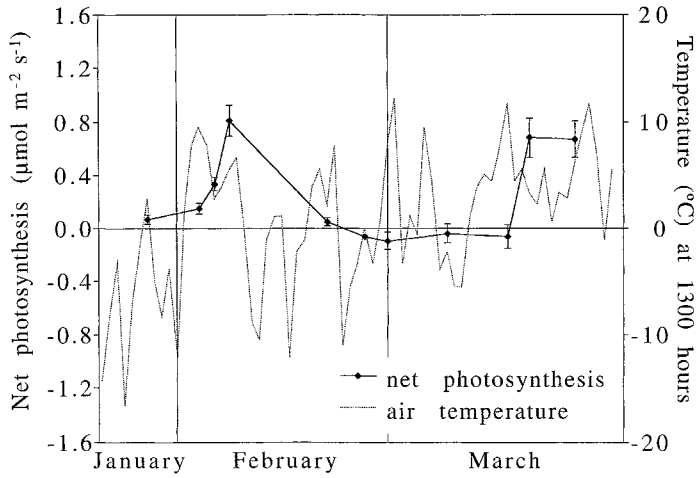

Figure 6.6. Average midday CO_2 exchange rates for red spruce from 3 Vermont seed sources plotted with air temperatures at 1300 hours for the sampling period (January through March 1991). Error bars are ±1 SE from means for all seed sources combined.

some trees approximated growing season levels (Schaberg et al., 1995). Increases in *in situ* photosynthesis can occur within four days of thaw inception (Schaberg et al., 1996), whereas increases in photosynthetic capacity (maximum photosynthetic rates measured on rehydrated shoots under near-optimal growing season temperature and light conditions) can occur within 48 hours (natural thaw) or even 3 hours (simulated thaw) (Schaberg et al., 1998). The delayed rise in field photosynthesis relative to photosynthetic capacity suggests that other environmental limitations (possibly low water availability) retard the photosynthetic response to thaw in the field (Schaberg et al., 1998). Thaw-induced increases in respiration have also been noted for red spruce seedlings exposed to simulated thaw (Schaberg et al., 1996). Initial increases in respiration were followed by rises in carbon capture so that plants exhibited net carbon uptake by the fourth day of thaw treatment (Schaberg et al., 1996).

Despite likely thaw-induced increases in respiration and potential delays in photosynthetic gain, increases in total carbohydrate and foliar sugar concentrations have been reported for red spruce seedlings in the field following a winter thaw (Snyder, 1990). The physiological importance of possible carbon gain during winter is still unknown. However, winter photosynthesis could benefit leaves by augmenting energy stores when more distal carbon reserves are less available due to cold-induced reductions in phloem transport (Grusak and Minchin, 1989). Increases in foliar carbon reserves during winter may also help offset the metabolic costs of maintaining evergreen foliage.

The temporal association of thaw-induced changes in photosynthesis and cold hardiness raises interesting questions regarding survival and selection. Because both potentially positive (increased carbon capture) and negative (decreased cold hardiness) changes in physiology can occur, red spruce's response to thaw could be characterized as a "tradeoff," with the adaptive consequences of thaw-induced changes in physiology dependent upon long-term probabilities of "costs" vs. "benefits."

This tradeoff could be particularly pertinent within the context of potential climate change. Pollution-induced climatic warming could have broad impacts on the health and distribution of forest trees (Krauchi, 1993), with montane and alpine trees and species with limited genetic diversity among the groups most likely to be affected (Krauchi, 1993). The distribution (Burns and Honkala, 1990) and unusually low genetic variability of red spruce (DeHayes and Hawley, 1992; Hawley and DeHayes, 1994) may place this species at particular risk. Data on the range of maximum cold tolerance levels achieved by red spruce, balsam, and Fraser fir in rangewide provenance tests support the possibility that this limited genetic variability extends to cold tolerance (DeHayes, 1992). In midwinter, the fir provenances differed considerably in cold tolerance whereas pure red spruce provenances had similar average cold tolerances (DeHayes, 1992). However, the specific impacts of climate change would likely depend on the mix of environmental changes experienced. For example, if the number and/or duration of winter thaws increased and winter low temperatures were moderated, then red spruce might benefit from enhanced carbon capture without an increased risk of freezing injury. A more likely possibility, however, is that pollution-induced climate change could cause winter thaws to become increasingly common while existing low temperature extremes persist (MacCracken et al., 1991). Under this scenario, the increased risk of freezing injury following thaw could offset any physiological benefit of winter carbon capture. In fact, if thaw-induced reductions in cold tolerance resulted in foliar injury and loss, these losses would decrease potentials for current and future carbon capture and remove access to carbon reserves within lost foliage. If this occurred, thaw-associated alterations in physiology could actually be detrimental to the carbon reserves of red spruce.

The adaptive consequences of climate change on the health and distribution of red spruce are particularly difficult to predict due to the potential impacts of other pollution-induced stresses. Even if northern climates become uniformly warmer during winter, acid mist–induced reductions in hardiness (Fowler et al., 1989; DeHayes et al., 1991; Vann et al., 1992) could cause red spruce to be vulnerable to freezing injury at temperatures currently thought to be safe. And, if red spruce respond as black spruce seedlings have in a controlled test (Margolis and Venzina, 1990), atmospheric CO_2 enrichment could also reduce cold tolerance and increase the risk of freezing injury, although pollutant additions of N

could partially offset acid- and/or CO_2-induced reductions in cold tolerance. In addition, acid deposition- and/or N-induced alterations in mineral nutrition could also disrupt growing season energy relations (McLaughlin et al., 1993; Schaberg et al., 1997), which, in turn, could alter the adaptive significance of winter carbon capture. Furthermore, red spruce's limited genetic variability could greatly limit its ability to respond to changing pollution-induced stress (DeHayes and Hawley, 1992).

Possible Influence of Glacial Refugia

If indeed red spruce face a tradeoff between carbon capture and cold hardiness, then it is reasonable to question how selection may have influenced the initial development and continued presence of this response to thaw. One possibility is that this response is a remnant of selection during the last ice age. Compared with other conifers of the region, red spruce is thought to have had a restricted glacial refugia that extended from the Mid-Atlantic states eastward through an area that is now submerged continental shelf (White and Cogbill, 1992). Maritime influences likely moderated temperatures within the refugium, and may have reduced the adaptive advantage conveyed by the development of deep cold hardiness (White and Cogbill, 1992). Prolonged exposure to moderate coastal temperatures may also have promoted an extended period for carbon capture, including winter photosynthesis if the risk of freezing injury was low. Conifers from the maritime regions of the Pacific Northwest may provide a contemporary example of this; they are known to be photosynthetically active and have only limited cold tolerance during the mild winters typical of that region (e.g., Larcher and Bauer, 1981; Hawkins et al., 1995).

In addition to the potential impacts of refugia location, the small size of red spruce's glacial refugium may have reduced gene flows and increased levels of inbreeding, resulting in reduced genetic variability. Combined with the enhanced potential for environmental homogeneity (and thus more uniform selection pressure) within a restricted refugium, breeding limitations associated with small refugium size may help account for red spruce's low genetic variability relative to other conifers (White and Cogbill, 1992; Hawley and DeHayes, 1994). This reduction in variability may also be one reason why red spruce's physiological response to thaw has persisted over time: selective forces are now interacting with a more limited gene pool.

Acid Mist–Induced Modifications of Membrane-Associated Calcium

Considerable research has now established that exposure of red spruce foliage to both simulated (Fowler et al., 1989; Waite et al., 1994) and ambient acidic cloud water (DeHayes et al., 1991; Vann et al., 1992)

during the growing season or autumn results in a significant reduction in late autumn and midwinter freezing tolerance of current-year needles (see Table 6.5). Such reductions are sufficient to explain the dramatic increase in freezing injury to red spruce observed in the northern montane forests over the past 40 years (Johnson et al., 1988, 1996; DeHayes, 1992) and growth and vigor losses typical of the decline (Wilkinson, 1990; Tobi et al., 1995). Although several studies have examined the role of specific pollutant ions in cold tolerance reductions (Cape et al., 1991; Sheppard, 1994), no empirically based physiological mechanism for this phenomenon has been established. Specific, highly repeatable findings that are critical to understanding the influence of acidic mist on red spruce freezing tolerance and that must be accounted for in any viable physiological mechanism are:

1. Freezing injury in red spruce is specific to current-year foliage, which is about 10°C less freezing tolerant in winter than year-old foliage.
2. Acid mist–induced reductions in cold tolerance persist throughout the winter even when the acid mist is removed.
3. Relatively short-term exposure to acidic mist in autumn only has an equal or greater influence on winter freezing tolerance than growing season exposure.
4. Simulated mists, which include a base solution with an ionic composition patterned after regional cloud chemistry analyses, reduce cold tolerance whether mist pH adjustments are made with HCl or H_2SO_4.

Potential Role of Calcium in Cold Tolerance

We have examined acid mist–induced alterations in Ca physiology as a potential mechanism for low temperature sensitivity and injury in red spruce foliage. Calcium is an abundant element in trees and a major cation in soil and surface waters and has been a focus of recent attention because of depletion resulting from acid deposition, diminished base cation deposition, foliar leaching, N saturation, and competitive interactions with Al (Joslin et al., 1988; Shortle and Smith, 1988; Lawrence et al., 1995; Hedin et al., 1994; Aber et al., 1995; Likens et al., 1996). Empirical information has demonstrated an inverse relationship between foliar Ca concentration, freezing tolerance, and freezing injury among native red spruce trees and experimental work has shown reduced foliar Ca in response to exposure to acidic mist (Joslin et al., 1988, DeHayes et al., 1991; McLaughlin et al., 1993). McLaughlin et al. (1991, 1993) have also implicated acid deposition-induced calcium deficiency as a potential causal factor in dark respiration increases and altered carbon metabolism associated with red spruce decline in the southern Appalachians, where ambient winter temperatures preclude freezing injury. McLaughlin et al. (1993) observed a partial reversal of acid mist–induced respiration increases through soil Ca fertilization, and suggest that foliar as well as

soil-driven reactions may be important in altered carbon metabolism resulting from acid deposition.

Calcium is highly compartmentalized within cells and tissues and this partitioning is critical to its physiological function in plants. The major fraction of Ca in conifer needles is insoluble extracellular Ca oxalate and pectate crystals (Fink, 1991), while Ca ions associated with the plasma membrane region (including some free and displaced apoplastic Ca from the cell wall) are labile and of major physiological importance. This pool of mCa, although a relatively small fraction of total foliar Ca ion pools, strongly influences the response of cells to changing environmental conditions and stress (Hepler and Wayne, 1985; Dhindsa et al., 1993; McLaughlin et al., 1993). Membrane-associated Ca influences plasma membrane structure and function, stabilizing membranes and influencing permeability by bridging phosphate and carboxylate groups of membrane phospholipids and proteins (Palta and Li, 1978; Davies and Monk-Talbot, 1990; Steponkus, 1990). The plasma membrane plays a critical role in mechanisms of cold acclimation and low temperature injury in plants (Pomeroy and Andrews, 1985; Davies and Monk-Talbot, 1990; Steponkus, 1990). By influencing membrane architecture, mCa influences solution movement across membranes, the ability of cells to resist dehydration, extracellular ice damage, and perhaps intracellular freezing during cold acclimation (Guy, 1990). This labile mCa pool (Atkinson et al., 1990) may also play an important role as a second messenger ("messenger Ca") in the perception and transduction of stress signals, including low temperature signals (Dhindsa et al., 1993; Monroy et al., 1993; Crotty and Poole, 1995), across membranes by binding to proteins such as calmodulin (Hepler and Wayne, 1985). Calcium bridges can be broken in acidic environments resulting in foliar Ca leaching, which could potentially lead to destabilization of membranes, depletion of a pool of messenger Ca, and enhanced susceptibility to environmental stress such as low temperature.

Evidence of Acid-Induced Calcium and Cold Tolerance Perturbations

We have conducted a series of *in vivo* acidic mist experiments that consistently demonstrate significantly greater Ca leaching (Fig. 6.7a) from current-year needles of red spruce in response to pH 3.0 vs. 5.0 (base mist solution adjusted with HCl) mist throughout summer and autumn (DeHayes et al., 1999). Ca concentrations in foliar leachate range about 2 to 10 times greater in foliage exposed to the more acidic mist. Furthermore, an *in vitro* experiment designed to partition throughfall Ca has conclusively verified that most (~85%) of the acid-leached Ca is derived from needles rather than stems and nearly 50% more Ca is leached from current-year than year-old foliage (Fig. 6.7b). Analysis of throughfall

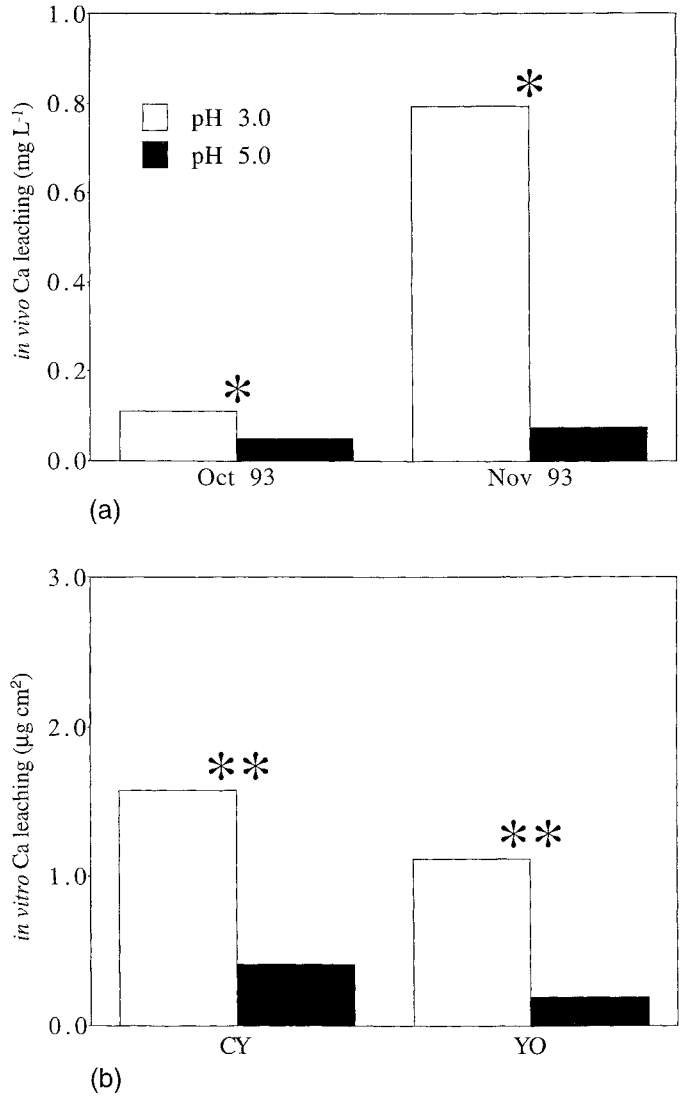

Figure 6.7. Impacts of acid mist treatment on (a) *in vivo* Ca leaching, (b) *in vitro* Ca leaching, (c) total foliar Ca, and (d) cold tolerance of foliage from red spruce. All data for current-year foliage unless indicated otherwise. CY, Current-year foliage; YO, year-old foliage. ($* = P \leq 0.05$; $** = P \leq 0.01$).

also indicate nearly 60 times greater uptake of H^+ in response to pH 3.0 vs. 5.0 mist. Acid mist–induced Ca losses are accompanied by reductions in membrane stability (DeHayes et al., 1999) and a significant 4 to 10°C decrease in freezing tolerance during late autumn and winter subsequent to acid mist exposure (Fig. 6.7d).

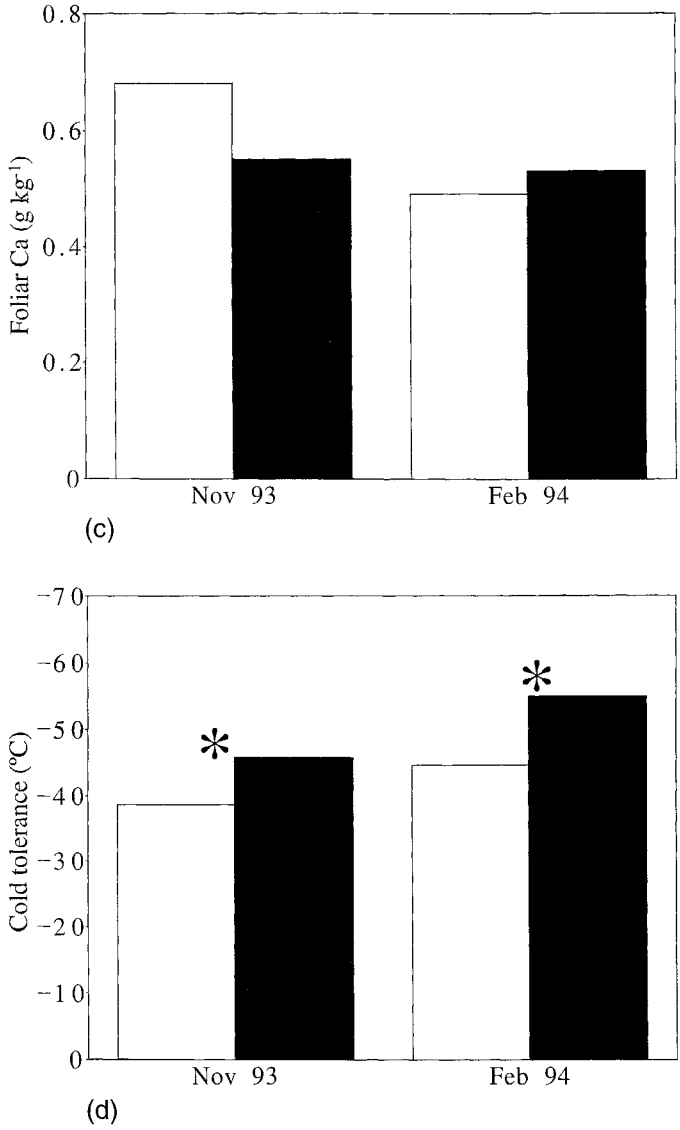

Figure 6.7. (*Continued*)

Despite up to 10-fold differences in foliar Ca leaching between pH treatments, a reduction in total foliar Ca pools of pH 3–treated foliage is typically not evident (Fig. 6.7c; see Table 6.4). It is likely that total foliar Ca estimates primarily reflect the dominant insoluble and immobile extracellular Ca pool, which may mask relatively subtle, but critical, shifts in the labile and environmentally sensitive mCa pool. If acid-induced

foliar Ca leaching preferentially removes mCa, then membrane dysfunction and a concomitant increase in freezing injury susceptibility would be expected.

Using an analytical procedure allowing localization and quantification of subcellular Ca (Borer et al., 1997), we have examined the specific influence of acid deposition on the mCa pool. In a comprehensive experiment designed to examine freezing tolerance, mCa, total foliar Ca, and foliar Ca leaching responses to a factorial combination of Ca perturbation treatments, we demonstrated that acid rain displaces Ca ions specifically associated with the plasma membrane–cell wall compartment in red spruce mesophyll cells (Schaberg et al., 2000a). This membrane alteration is accompanied by a 4–10°C reduction in foliar freezing tolerance. Treatments were applied as soil Ca amendments, enhanced soil aluminum, and acid mist (base mist solutions adjusted with H_2SO_4) applications from June through September (pH 3.0 vs. 5.0). Although reduced soil Ca and enhanced soil Al treatments resulted in significant and predictable reductions in total foliar Ca, they had no consistent influence on foliar leaching, mCa, or freezing tolerance (see Table 6.4), which are critical red spruce decline precursors in northern montane forests. In contrast, pH 3.0 acid mist treatments applied in summer and early autumn resulted in significant foliar Ca leaching, and a consistent, significant, and parallel reduction in late fall and winter freezing tolerance and mCa in current-year needles of red spruce (see Table 6.4). Although relatively minor in late summer, acid mist-induced reductions in mesophyll cell mCa reached a maximum of about 35% between mist treatments by midwinter (see Table 6.4), even though the acid mist treatments had been removed months earlier.

The persistence of the acid mist influence on mCa and cold tolerance throughout winter and the responsiveness to short-term acid mist applications in autumn is a reflection of the lack of mobility of Ca in the phloem (Marschner, 1986) and the dependence upon xylem transport to replenish depleted mCa. It appears that the physiological implications of acid deposition are exacerbated in autumn when post–growing season losses of mCa cannot be replaced via the transpiration stream. These results conclusively demonstrate that acid deposition 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 Ca pools.

Other Evidence Implicating mCa in Red Spruce Freezing Injury

Considerable additional empirical data implicates mCa involvement or an association between mCa and freezing tolerance/injury in red spruce. For instance, the pronounced needle age class difference in both cold tolerance and freezing injury susceptibility is consistent with the pattern of

developmental variation in mCa in current-year needles as well as age class differences in foliar Ca leaching. There is little or no detectable mCa in current-year needles of red spruce during early summer (Fig. 6.8; DeHayes et al., 1997) and mCa levels in current-year needles are inherently lower than in year-old needles throughout summer and early autumn when acid mist–induced Ca leaching is substantial. The substantial age class difference in foliar Ca leaching (see Fig. 6.7) further accentuates the mCa and cold tolerance differences between red spruce needle age classes and offers a viable explanation for the unique low temperature sensitivity of red spruce current-year foliage.

Furthermore, unlike total foliar Ca 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 in mCa reflect an increase in Ca ion exchange sites associated with short day–induced increases in membrane phospholipids, while apparent frost-initiated reductions in mCa are likely associated with changes in the fatty acid composition of membrane lipids (DeHayes et al., 1997) and perhaps a role for mCa in the perception and transduction of the low temperature cold acclimation signal as has been suggested for some agronomic crops (Dhindsa et al., 1993; Monroy et al., 1993; Crotty and Poole, 1995).

We have also documented abrupt, but temporary, mCa reductions in current-year needles of both red spruce seedlings and native trees in response to midwinter thaws (Table 6.6). Cold tolerance was examined during one of these thaws and verified that thaw-induced mCa reductions were accompanied by significant precocious dehardening over only 3 days (DeHayes et al., 1997; Strimbeck et al., 1995). After the thaw, both mCa and freezing tolerance returned to prethaw levels. Sequential changes in mCa and frost tolerance levels, coupled with the well documented changes in membrane structure in leaf tissue of north temperate conifers during cold acclimation (e.g., DeYoe and Brown, 1979; Senser and Beck, 1982, 1984), strongly support the contention that mCa is important to cold tolerance and that acid mist alterations of mCa cause membrane destabilization and a concomitant loss in freezing tolerance.

Alterations to mCa also seem pertinent to the documented physiological impairment and growth decline of red spruce in the southern Appalachians, which is attributed to acid mist–induced alterations in Ca nutrition and carbon relations (McLaughlin and Kohut, 1992; McLaughlin et al., 1991, 1993) rather than to freezing injury. Although they have not examined the mCa pool specifically, McLaughlin et al. (1993) suggest that tissue respiration increases in response to acid deposition-induced Ca losses may be associated with the critical regulatory role of Ca in membrane permeability. Acid deposition–induced alteration of mCa that we have documented and that leads to enhanced freezing injury

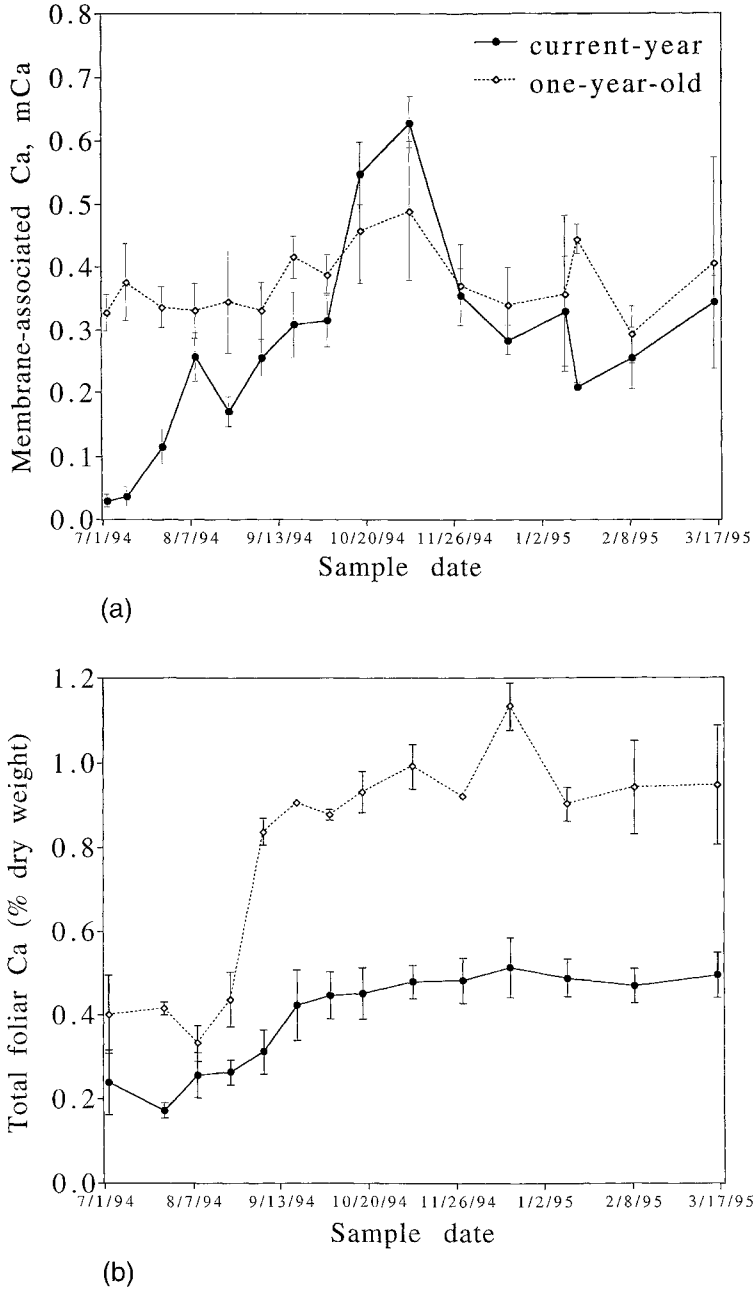

Figure 6.8. Seasonal patterns of (a) membrane-associated Ca (mCa) and (b) total foliar Ca of current-year and 1-year-old needles of red spruce seedlings. Error bars are ±1 SE from means. In some cases, error bars are obscured by symbols representing means.

Table 6.6. Mesophyll Cell Membrane-associated Ca (mCa) Levels in Current-year Foliage of Red Spruce Seedlings (1995) and Mature Trees (1997) before and after Winter Thaws

Year	Relative mCa Concentration		*P*
	Pre-thaw	Post-thaw	
1995	0.328	0.209	0.065
1997	0.232	0.116	0.001

susceptibility in the north would also be expected in response to acidic deposition in the southern Appalachians. As such, the acid deposition–mCa leaching–membrane alteration explanation for red spruce decline that we describe represents a comprehensive explanation for acid-induced red spruce decline throughout the montane forests of eastern North America.

Model for Enhanced Freezing Injury and Membrane-associated Calcium Alteration

Our results provide an empirically supported mechanistic explanation of acid mist– and winter thaw–induced reductions in freezing tolerance and overall health of red spruce forests (Fig. 6.9). Acid deposition–induced cation exchange results from H^+ replacement of Ca on exchange sites at the cell wall–membrane interface. This pool of mCa, unlike other insoluble extracellular Ca pools, is readily available for acid-induced cation exchange resulting in Ca leaching, membrane destabilization, depletion of a potential pool of messenger Ca, and significant reductions in the freezing tolerance of current-year needles of red spruce. Although SO_4^{2-} has been suggested as the critical pollutant ion in cold tolerance reductions (Shepherd, 1994; Cape et al., 1991), we have demonstrated similar cold tolerance reductions and significant mCa leaching in response to mists containing equalized SO_4^{2-} concentrations and with different anionic constituents (DeHayes et al., 1999; Schaberg et al., 2000a). This similarity in response across different anionic solutions coupled with evidence of reduced H^+, but not SO_4^{2-} (Joslin et al., 1988; McLaughlin et al., 1996), concentrations in throughfall suggest that acid mist-induced Ca leaching and cold tolerance reductions are likely the result of cation exchange driven by differential H^+ exposure. There is strong empirical support for most elements of the model and it effectively accounts for each of the aforementioned unique and specific highly repeatable factors that are integral components of the red spruce freezing injury and decline phenomenon.

Using this model, one would predict that sites exposed to high H^+ inputs and winter thaws followed by very low air temperatures would

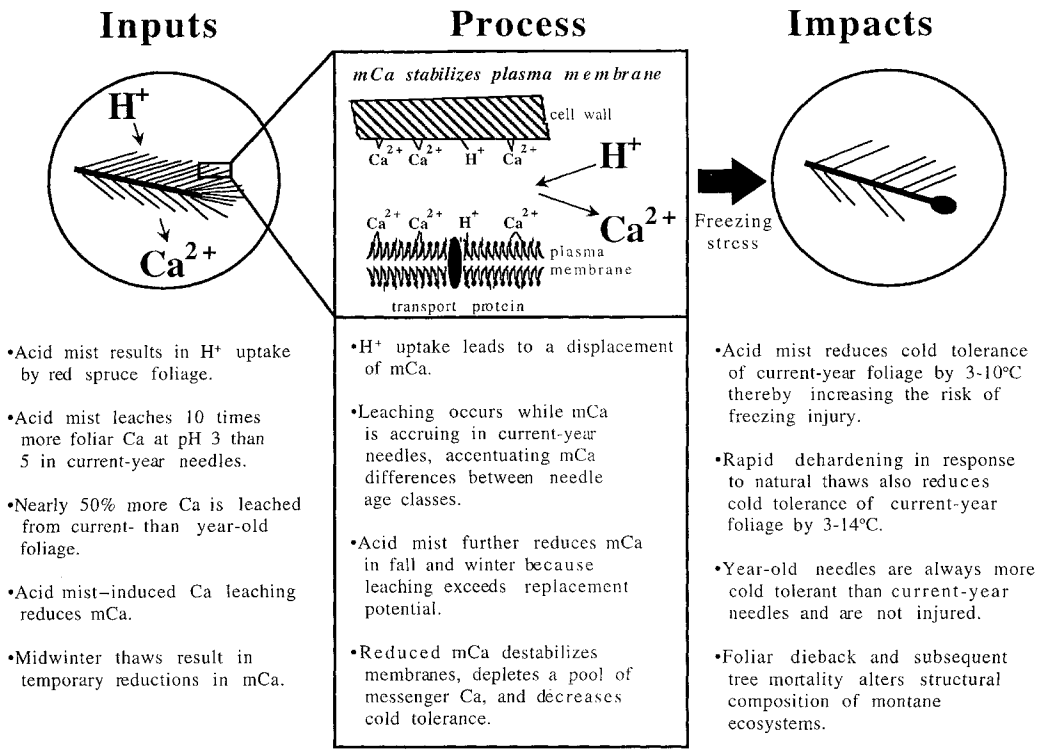

Figure 6.9. Empirically based mechanistic model depicting acid mist–induced Ca perturbations, destabilization of plasma membranes, and reduction in red spruce foliar cold tolerance.

experience freezing injury with the greatest severity and frequency. Indeed, montane populations in the northeastern U.S. meet these criteria and commonly experience freezing injury (Friedland et al., 1984; Johnson et al., 1986; Peart et al., 1991). However, lower elevation forests are not without risk and currently experience some freezing injury (Morgenstern, 1969; Peart et al., 1991; DeHayes et al., 1990). In addition, continued H^+ inputs, predicted increases in the frequency and extent of winter thaws, and possibly even soil Ca depletion (because soils ultimately supply membranes with mCa) could be expected to increase the risk of freezing injury above current levels.

Although of some potential value in predicting trends in injury, the real importance of the model we present is its utility in synthesizing scientific information and thought regarding freezing injury of red spruce. We believe this model provides a physiological explanation for the dramatic increase in red spruce freezing injury over the past 40 years that coincided with increased pollution emissions and for the well documented decline of red spruce in northern montane forest ecosystems.

Conclusions

The unique freezing injury susceptibility of red spruce is, in part, a function of the modest depth of midwinter cold tolerance attained by the species, which appears to not be well adapted to northern montane temperature conditions. Historical analyses, however, indicate that freezing injury to red spruce occurred only sporadically during the late 19th century and first 50 years or so of this century and then increased dramatically over recent decades (Johnson et al., 1986). Because there is no evidence that winters in New England or New York have become colder (in fact, some evidence suggests a slight warming trend) during this recent period when freezing injury has increased (Hamburg and Cogbill, 1988), one must conclude that environmental changes that have impaired freezing tolerance during the approximate period 1950 to the present are primarily responsible for the enhanced freezing injury documented over the past several decades. A substantial body of evidence has demonstrated that both acid deposition and increasingly frequent winter thaws result in a consistent and significant reduction in midwinter cold tolerance, which dramatically increases the probability of freezing injury. Cold tolerance reductions averaging up to 10°C have been documented in response to either acidic deposition or thaw-induced precocious dehardening in red spruce trees under both ambient and experimental conditions. The extent and implications of interactions between these environmental conditions are not yet known, but are currently under study. Regardless of the relationship between acid- and temperature-impacts on cold tolerance, forests are exposed to such a heterogeneous mix of acid inputs and

temperature perturbations (including thaws, rapid freezing, and cycles of thaw and freezing) that levels and patterns of injury predictably appear complex and temporally and/or spatially variable.

Equally as important to the understanding of red spruce freezing injury are environmental changes that have not resulted in cold tolerance impairments for this species. Based on the current state of knowledge, there appears to be no compelling evidence that exposure to high concentrations of tropospheric O_3 or Al in soil solution has any direct impact on the development or attainment of freezing tolerance in red spruce. Given the documented importance of freezing injury as an initiating and predisposing factor in red spruce decline in northern montane forests, it would appear that these potential stresses are not principally associated with the deterioration of red spruce forests. Although considerable empirical evidence has demonstrated either neutral or positive cold tolerance responses to N supplements, we are hesitant to discount a potential physiological impairment associated with atmospheric N deposition over the long-term, especially in high-elevation forests. Recent evidence has shown that long-term N supplements can negatively impact red spruce forest health and nutrient dynamics in ways that could conceivably alter plant cold tolerance development (Schaberg et al., 1997; Perkins et al., 2000).

Both acid mist-induced and thaw-induced reductions in red spruce cold tolerance appear to involve alterations to the structure and/or function of mCa in mesophyll cells. The physiologically critical and labile mCa pool, although a relatively small fraction of the total foliar Ca ion pool, strongly influences the response of cells to changing environmental conditions and stress. With respect to acidic mist, it appears that H^+ displaces Ca on exchange sites at the cell wall–membrane interface leading to membrane destabilization, depletion of a pool of messenger Ca, and enhanced susceptibility to low temperature stress. The extent to which acid mist alteration of mCa also influences the apparent hypersensitive red spruce dehardening response to winter thaws is not as yet known, but evidence indicates that mCa is involved in the dehardening response. It is important to emphasize that the commonly measured total foliar Ca ion pool is not correlated with or a meaningful indicator of the physiologically important mCa pool (DeHayes et al., 1997). In fact, it is likely that environmentally induced shifts in the critical mCa pool would not be detected in an analysis of total foliar Ca content. As such, it is critical that the mCa pool specifically be monitored in woody plant leaf tissue (Borer et al., 1997), especially in studies examining plant responses to pollution- or climate-induced environmental change.

Of greater concern relative to overall forest health is the strong likelihood that acid rain alteration of mCa and membrane integrity is not unique to red spruce, but simply exacerbated in this species because of its low temperature sensitivity. It is now well established that Ca plays a critical role in plant

responses to numerous stresses, such as low temperature, salt, drought, and low light. (Hepler and Wayne, 1985; Dhindsa et al., 1993; Monroy et al., 1993; Sheen, 1996). It is also likely that the relatively small and environmentally sensitive mCa pool serves a critical and active role in such plant stress responses. Pollution-induced alteration to the mCa pool in forest trees would be expected to lead to physiological impairment of the plant stress response system and a predisposition to an array of environmental and biological stresses. The potential forest stress implications are further compounded by the lasting influence of acid deposition-induced leaching, depletion, and cycling disruption of Ca in forest soils. Estimates show that the pool of Ca in the soil complex in northeastern forests may have shrunk by as much as 50% during the past 45 years as a result of acidic deposition (Likens et al., 1996). As a result, the available pool of soil Ca for mCa replenishment and reconstruction of the plant stress response system may not be sufficient to stabilize healthy forests. Ironically, at a time of unprecedented abiotic and biotic stress loading from numerous sources, including atmospheric pollutants, changing climatic conditions, exotic pests, pathogens, and nutrient depletion, acidic inputs may be depleting a critical pool of Ca within plants that helps them respond to stress.

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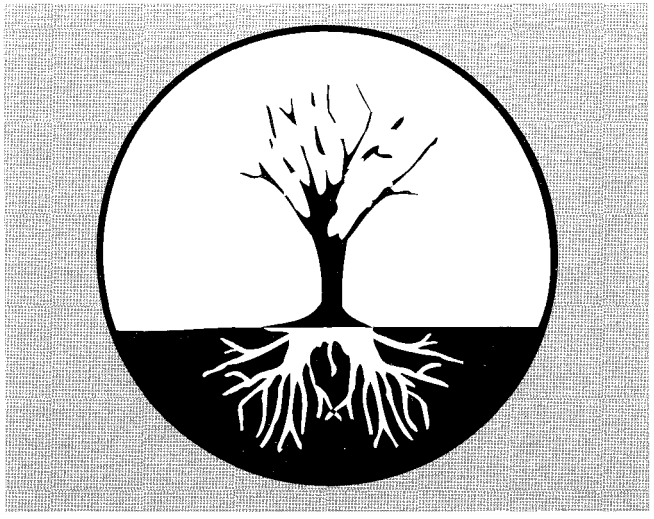

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