Chapter 18

Red Spruce (*Picea rubens* Sarg.) Cold Hardiness and Freezing Injury Susceptibility

Donald H. DeHayes[1], Paul G. Schaberg[2], and G. Richard Strimbeck[1]
[1]School of Natural Resources, University of Vermont, Burlington, VT 05405, USA; [2]USDA Forest Service, Northeastern Research Station, P.O. Box 968, Burlington, VT 05402, USA

Key words: red spruce, freezing injury, cold hardiness, sugars, water relations, dehardening, acid deposition, membrane-associated calcium

1. INTRODUCTION

To survive subfreezing winter temperatures, perennial plant species have evolved tissue-specific mechanisms to undergo changes in freezing tolerance that parallel seasonal variations in climate. As such, most northern temperate tree species, including conifers, are adapted to the habitat and climatic conditions within their natural ranges and suffer little or no freezing injury under normally occurring environmental conditions. In fact, the maximum depth of cold hardiness in most well-adapted tree species is substantially greater than minimum temperatures encountered naturally, and cold hardening and dehardening are typically coordinated with environmental conditions so that plants are not injured by early or late season frosts or subfreezing temperatures that follow temporary winter thaws. Indeed, most examples of freezing or 'winter injury' in northern temperate woody plants involve species or provenances that are planted off-site. Furthermore, most cases of freezing injury are associated with improper timing or an insufficient rate of cold acclimation during autumn, resulting in injury at temperatures that a species could probably withstand at a more advanced stage of cold hardiness (Smithberg and Weiser 1968; Cannell 1985).

F.J. Bigras and S.J. Colombo (eds.), Conifer Cold Hardiness, 495–529.

Based on our current understanding of developmental cold hardiness and freezing injury, it appears that red spruce (*Picea rubens* Sarg.) violates each of the aforementioned premises about cold hardiness. That is, red spruce frequently suffers winter injury in both native and planted forests throughout the northern half of its native range, especially in northern montane forests (DeHayes 1992). Furthermore, although the rate and timing of cold acclimation in autumn and deacclimation in spring for red spruce appear to protect it from freezing injury during these transitional seasons, the maximum depth of cold hardiness that red spruce attains in mid-winter is barely sufficient for it to withstand ambient mid-winter temperatures encountered in many high elevation northern forests (DeHayes 1992; DeHayes et al. 1999; Schaberg and DeHayes 2000). In addition, precocious dehardening in response to winter thaws occurs frequently in red spruce and further accentuates its susceptibility to freezing injury during mid-winter (December through March) when most well-adapted tree species are expected to be protected from ambient environmental conditions (Strimbeck et al. 1995; Schaberg and DeHayes 2000). This chapter provides a comprehensive summary of the physiology of cold hardiness in red spruce and the nature, history, and causes of freezing injury that has led to the widespread decline of red spruce forests throughout much of eastern North America.

1.1 Geographic distribution of red spruce

Red spruce is distributed throughout the maritime provinces of eastern Canada and coastal forests of Maine, the boreal and mixed hardwood forests of northern New England, and the high elevation spruce-fir forests throughout the Appalachian and Adirondack Mountains that extend from northern New York and New England to North Carolina and Tennessee (Little 1971). The montane red spruce forests from southern New England southward are characterized largely by isolated populations at elevations above about 1500 m in the southern Appalachians and about 700 to 800 m in southern New England (White and Cogbill 1992). As with most eastern montane tree species, the elevational distribution of red spruce is inversely related to latitude. Because the latitudinal temperature gradient is steepest in winter, this suggests that the northern limit of the red spruce distribution is set by its ability to tolerate minimum winter temperatures (White and Cogbill 1992). Despite an elevational gradient from north to south, the mean January temperature is lower in northern than southern red spruce forests (White and Cogbill 1992). The northern limit of the species is correlated with a mean annual temperature of 2°C, a mean annual January temperature of -13°C, and an absolute minimum temperature of -40°C (Arris and

Eagleson 1989). Unlike many of its sympatric associates and other species of *Picea* native to eastern North America, red spruce forests do not extend northward into the boreal forests of interior Canada or westward into the Great Lakes region of the north-central United States.

2. HISTORY AND INTERPRETATIONS OF WINTER INJURY IN RED SPRUCE

What is commonly referred to as 'winter injury' in red spruce appears as an obvious red-brown necrosis of current-year needles in mid-winter to early spring, followed by gradual abscission in late spring and summer (DeHayes 1992). Older needles are almost always unaffected, and needles that survive their first winter may persist on the tree for up to 10 years. It is well-documented that red spruce forests have been subjected to repeated, severe, regionwide winter injury over at least the past 50 years (DeHayes 1992; Schaberg and DeHayes 2000), with the injury most commonly observed in montane forests. Notably, this period is coincident with the onset of regional air pollution (DeHayes 1992; DeHayes et al. 1999). The severity of injury is highly variable, with readily apparent differences between years, locations, individual trees, branches within trees, shoots within branches, and even needles within shoots. Individual needles may also have necrotic flecks or bands of injured cells scattered through an otherwise healthy needle (Adams et al. 1991). Over the past 20 years or so, the frequency of major regionwide winter injury events to red spruce has averaged about 4 years, with more moderate, localized injury occurring in intervening years (DeHayes 1992; Schaberg and DeHayes 2000). More importantly, winter injury has a direct impact on the growth and survival of trees, and the severity of winter injury in red spruce is correlated with subsequent decreases in basal area increment and height growth (Wilkinson 1990; Tobi et al. 1995).

When injury is severe, some trees may lose nearly 100% of their current-year foliage, and nearly all trees in a stand are affected to some extent. In a survey of severe injury in 1989, Peart et al. (1991) reported increasing injury with elevation from a mean of 20% of current-year foliage at 880 m to 41% at 1140 m. Boyce (1995) reported 20% of current-year foliage injured for stands at 1050 m on Whiteface Mountain in 1993. Both studies found that injury increased with height in the canopy, and that damage was greatest on south to west aspects. However, Peart et al. (1991) noted that substantial injury also occurred on shaded foliage. As with severe injury, moderate injury is concentrated on the sun-exposed south and west sides of the canopy, suggesting that exposure to solar radiation may play a role in producing injury (Hadley et al. 1991). A detailed description of macroscopic

and cellular symptoms of foliar freezing injury in red spruce is provided by DeHayes (1992).

2.1 Winter desiccation *vs* freezing injury as causal agents

Initial reports of winter injury to red spruce attributed injury to 'winter drying' or 'winter desiccation' (*e.g.* Curry and Church 1952; Morgenstern 1969; Herrick and Friedland 1991) that may occur when transpirational water losses are not replaced because of restricted water movement in either frozen soil or xylem. These reports, however, did not provide specific evidence that injury was caused by foliar desiccation rather than subfreezing temperatures. The assumption of desiccation as the cause of winter injury in red spruce seems to have evolved largely from studies of various subalpine conifer species in Europe and North America (Tranquillini 1982; DeLucia and Berlyn 1984), which suggested that foliar mortality due to winter desiccation limits tree growth at treeline. However, many have argued that winter desiccation is rarely sufficient to produce injury in red spruce (Marchand and Chabot 1978; Kincaid and Lyons 1981), except in extreme circumstances when abrasion by wind-borne snow and ice particles damages cuticles (Hadley and Smith 1986). Field experiments further demonstrated that desiccation is not the primary mechanism of injury (Perkins et al. 1991), and that injury from subfreezing temperatures, rather than desiccation, produces the necrosis observed in the field (Strimbeck et al. 1991). Freeze-injured needles do lose water rapidly (Strimbeck et al. 1991), but this is, presumably, a result of injury to stomatal guard cells (Adams et al. 1991). Such freezing injury appears to account for the foliar drying observed by Herrick and Friedland (1991).

There is now substantial concrete evidence indicating that subfreezing temperatures rather than desiccation are the primary cause of the 'winter injury' so frequently observed in red spruce, and measures of mid-winter cold hardiness in red spruce are usually strongly correlated with freezing injury under both laboratory and field conditions (DeHayes 1992; Schaberg and DeHayes 2000). It is important to emphasize, however, that freezing injury in red spruce probably occurs as a result of freeze-dehydration when water is drawn from living cells to extracellular ice masses. However, this type of low temperature injury is not caused by winter drying or desiccation. Environmental conditions that provoke freezing injury in the field likely include low temperature extremes, rapid changes in temperature (Perkins and Adams 1995), and exposure to a series of freeze-thaw cycles (Lund and Livingston 1998).

It has been suggested that rapid changes in temperature may also produce foliar injury at temperatures too high to produce injury at slower freezing rates. This may explain a concentration of freezing injury on sun-exposed foliage of some conifers (White and Weiser 1964). Indeed, Strimbeck et al. (1993) recorded red spruce needle temperatures as much as 23°C above ambient air temperature and drops as great as 9°C in 1 minute, averaging 0.8 and 0.6°C min^{-1} over 10 and 15 minute intervals, respectively. Perkins and Adams (1995) reported that experimental rapid freezing of red spruce can produce symptoms of injury similar to those observed in the field at freezing rates greater than 10°C min^{-1} through a temperature range of -4 to -10°C. Recently, Strimbeck and DeHayes (2000) monitored rapid freezing responses of montane red spruce trees throughout autumn and winter. In autumn, experimental rapid freezing produced injury only at temperatures considerably lower than what would normally be encountered at that time of year. In winter, rapid freezing caused occasional, moderate injury to fully hardened trees that were also susceptible to slow freezing injury. Once again, experimental rapid freezing occurred at temperature spans more extreme than those recorded in the field. Unlike field freezing injury symptomology that includes only current-year needles, year-old foliage was usually damaged when rapid freezing stress regimes caused injury to current-year foliage. Although it does not appear that rapid freezing is responsible for the severe and widespread injury observed in red spruce over the past 50 years, it does appear that solar warming of red spruce foliage followed by rapid freezing can cause light to moderate foliar injury in specific locations in some years.

3. VARIATION IN COLD HARDINESS AND FREEZING INJURY IN RED SPRUCE

3.1 Seasonal patterns of cold hardiness development

Seasonal patterns of cold hardiness development for trees from three red spruce provenances are illustrated in *Figure 1A*. Cold acclimation of red spruce throughout autumn appears to proceed at a rate sufficient to protect the species from low temperatures encountered from late summer to perhaps early December. In fact, cold tolerance of red spruce from August through November is comparable to that of balsam fir (*Abies balsamea* (L.) Mill.), a species that does not suffer freezing injury (DeHayes 1992). Low temperature vulnerability for red spruce appears through the winter months

of December through March because most red spruce trees do not achieve the depth of mid-winter cold hardiness expected for northern montane forest tree species. Numerous cold hardiness studies conducted over the past decade using various test procedures and sampling different northern populations have consistently demonstrated a remarkable pattern of mid-winter freezing injury vulnerability in red spruce. These studies collectively indicate that the average temperature at which current-year needles of red spruce in northern New England and New York are injured is in the range of -37 to -47°C (*Table 1*). Because controlled freezing environments are thought to be less stressful than natural conditions (Schaberg and DeHayes 2000), these laboratory freezing studies may actually overestimate the mid-winter cold hardiness of red spruce.

Table 1. January estimates of the cold hardiness of current-year foliage of red spruce from several populations using various assessment techniques.

Citation	Assessment technique[a]	Year	Number of trees	Estimated cold hardiness (°C)
Sheppard et al. 1989	VI	1987	20	-37
DeHayes 1992	EL	1988	9	-40
Hadley and Amundson 1992	EL	1990	10	-41
Perkins et al. 1993	VI	1992	5	-46
Adams and Perkins 1993	CF	1992	5	-50[b]
Strimbeck et al. 1995	EL	1995	10	-47
Schaberg et al. 2000b	EL	1996	60	-42

[a] CF, chlorophyll fluorescence; EL, electrolyte leakage; VI, visual injury.
[b] estimated from figures and occurs after 20% visible injury.

The most comprehensive assessment of cold hardiness in red spruce, which examined 60 native red spruce trees across a range of elevations on Mt. Mansfield, Vermont, USA, revealed an average injury temperature of -41.7°C and a range from -30 to -54°C (DeHayes et al. 1999; Schaberg et al. 2000b; *Figure 1B*). Approximately 36% of these trees were injured at temperatures warmer than -39°C. Climatic records show that ambient temperatures from Mt. Mansfield drop to -35 and -30°C in about 30% and 91% of winters, respectively. These data demonstrate that ambient temperatures approach the maximum cold hardiness of at least some red spruce trees during most winters, accentuating the unique freezing injury susceptibility of red spruce during mid-winter.

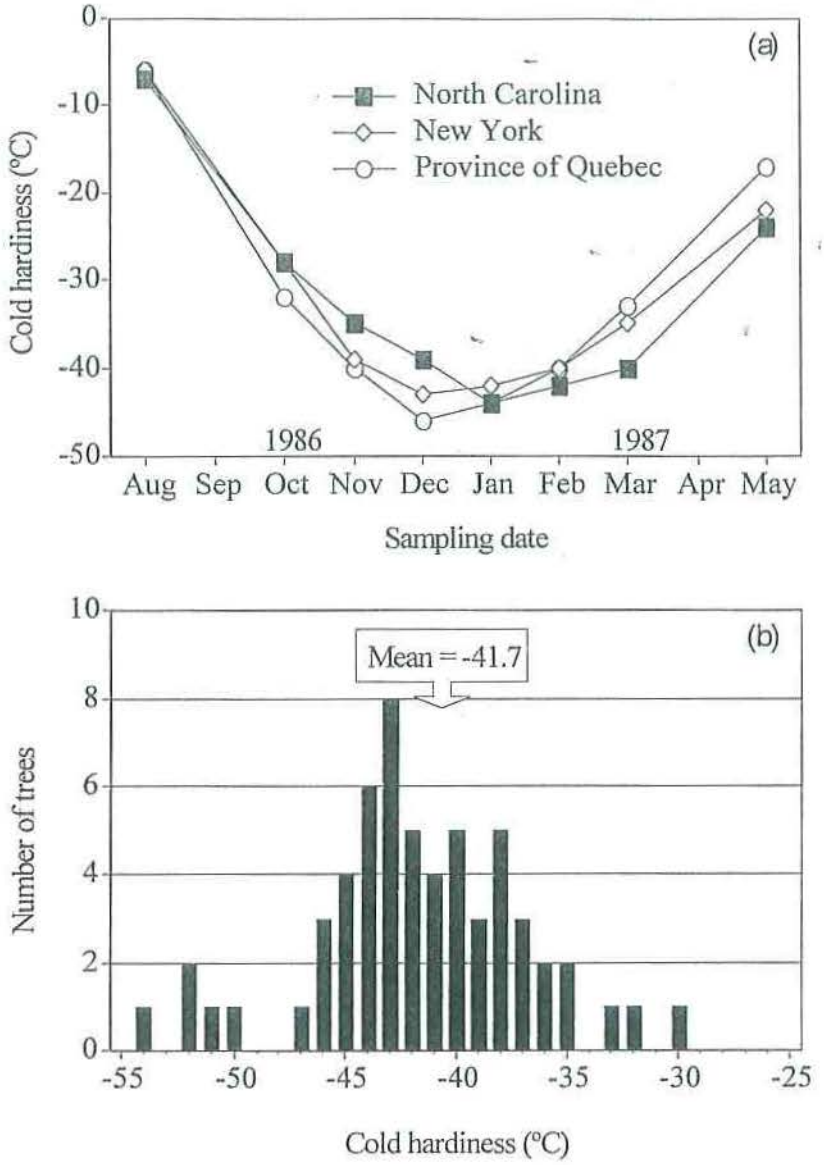


Figure 1. Cold hardiness of red spruce trees. (A) Seasonal patterns of cold hardiness development of trees from 3 provenances grown in common garden in northern New Hampshire, USA. (From Waite and DeHayes 1992). (B) Winter cold hardiness of 60 native red spruce trees from Mt. Mansfield, Vermont, USA. (From Schaberg et al. 2000b).

3.2 Interspecific comparisons

The mid-winter low temperature sensitivity of red spruce is unique relative to other northern temperate conifers with similar geographic and ecological distributions. Late winter laboratory cold hardiness comparisons between red spruce and four other native conifers in north-central Vermont revealed that red spruce was the least cold tolerant of the five species

examined (*Table 2*). For example, eastern white pine (*Pinus strobus* L.) and eastern hemlock (*Tsuga canadensis* (L.) Carrière) trees were on average almost 22°C more cold tolerant than native red spruce in the same mixed conifer forests. White spruce (*Picea glauca* (Moench) Voss) and red pine (*Pinus resinosa* Aiton) from the same forest escaped injury at temperatures as low as -90°C. Mid-winter cold tolerance of red spruce is similar to that of Fraser fir (*Abies fraseri* (Pursh) Poir.), which is endemic to the montane forests of the southern Appalachians (DeHayes 1992). However, Fraser fir forests experience consistently milder winter temperatures than northern red spruce forests.

Table 2. A comparison of the cold hardiness of 5 northern-temperate conifers sampled in Wolcott, Vermont, USA, on March 3, 1998.

Species	Number of trees sampled	Mean cold hardiness (critical temperature, °C)
Red spruce	5	-38
Eastern white pine	5	-60
Eastern hemlock	5	-61
White spruce	5	<-90
Red pine	5	<-90

Low temperature sensitivity and freezing injury susceptibility of red spruce is almost always limited to current-year foliage, which is approximately 10°C less cold tolerant in winter than year-old foliage (DeHayes 1992; Schaberg and DeHayes 2000; Strimbeck and DeHayes 2000). Once again, this phenomenon is unique to red spruce, at least as compared with balsam fir (*Abies balsamea*), which exhibits similar cold hardiness levels between tissues and a much greater mid-winter cold tolerance than red spruce (DeHayes et al. 1990). The cold hardiness difference between current-year and year-old needles in red spruce indicates that this species has the physiological capacity to develop greater levels of cold hardiness. However, it appears that this capacity is not achieved in current-year needles of most red spruce trees by mid-winter.

3.3 Provenance variation

Provenance variation in freezing injury of red spruce has been quantified in a rangewide provenance test plantation located in northwestern New Hampshire, USA (DeHayes et al. 1990; DeHayes 1992; Waite and DeHayes 1992). Freezing injury to at least some trees has occurred most years in this plantation and trees from all provenances suffered some freezing injury each year that injury was assessed. Trees from Quebec, New York, and New

Brunswick provenances consistently suffered the least freezing injury, while trees from Massachusetts and New Hampshire provenances consistently suffered the most freezing injury (DeHayes et al. 1990). The Quebec provenance was consistently the most cold tolerant of all red spruce provenances studied (DeHayes et al. 1990; Waite and DeHayes 1992) (*Figure 1A*). Southern Appalachian provenances were highly variable in freezing injury susceptibility from year to year, which appeared to be related to provenance variation in the timing of cold acclimation during autumn and early winter. Trees from southern provenances acclimated to the cold more slowly than those from northern provenances and suffered substantial freezing injury during winters with low temperatures in December and early January (DeHayes et al. 1990). By mid-winter, trees from the southern provenances reached levels of cold hardiness similar to those of most northern provenances (*Figure 1A*).

Unlike most other tree species, provenance variation in both freezing injury and mid-winter cold tolerance in red spruce is at best only weakly correlated with geographic and climatic characteristics of the original seed source. Furthermore, the magnitude of provenance variation in mid-winter cold tolerance of red spruce was relatively small for a species with a largely latitudinal geographic distribution. For example, the range in average mid-winter cold hardiness among 12 red spruce provenances was only 8 to 10°C compared to 14 to 18°C among balsam fir provenances. When the very cold hardy Quebec provenance, which is thought to be introgressed with black spruce (*Picea mariana* (Mill.) B.S.P.), is excluded from the analysis, there is only about a 4°C range in cold tolerance among red spruce provenances in mid-winter. In fact, red spruce appears to exhibit mid-winter cold hardiness levels more fitting to southern Appalachian winters than the northern montane habitats that this species typically occupies.

4. PHYSIOLOGY OF COLD TOLERANCE DEVELOPMENT IN RED SPRUCE

Numerous modifications of plant biochemistry and physiology occur coincidentally with seasonal changes in cold hardiness. The co-occurrence of these alterations has led to substantial conjecture about possible functional linkages between changes in plant biochemistry, nutrition, structure, function, and cold hardiness. However, very few of these associations have been subject to independent experimental manipulation in order to demonstrate that a given factor has a controlling role in the development and/or maintenance of cold hardiness. Factors that are consistently associated with developmental cold hardiness across a range of plant species

and levels of cold hardiness include the concentration and type of plant sugars, soluble proteins, lipids, membrane structure and stability, cellular water relations, and plant nutrition (Levitt 1956; Levitt 1980; Sakai and Larcher 1987; Davies and Monk-Talbot 1990). Data specifically relating many of these factors to red spruce cold hardiness and injury susceptibility have recently been generated (Strimbeck 1997; DeHayes et al. 1999; Schaberg et al. 2000a, b).

4.1 Influence of nitrogen supplements

Despite hypotheses suggesting a negative influence of nitrogen (N) supplements, especially late growing season supplements, on cold hardiness development (*e.g.* Friedland 1984), most evidence for red spruce has revealed a positive cold hardiness response to N additions in both seedlings (DeHayes et al. 1989) and mature trees (White 1996). Red spruce seedlings fertilized with ammonium nitrate (at 0 *vs* either 300, 1500, or 3000 kg N ha^{-1}) during late spring, mid-summer, or late summer acclimated more rapidly in autumn, achieved a greater depth of cold hardiness in winter, and deacclimated more slowly with increasing N treatment (DeHayes et al. 1989). The positive cold hardiness response to N supplements occurred even though foliar N concentrations for seedlings receiving low doses of N exceeded levels associated with N deficiency (Swan 1971). Timing of N application also had an impact on cold hardiness development: N applications in late spring resulted in a small increase (3.5 to 7.5°C) in cold hardiness the following fall and winter, whereas mid- and late-summer additions resulted in substantial increases (10 to 18°C) on cold hardiness that persisted throughout the winter. Seasonal variation in response to N supplements appears to be related to differential incorporation and utilization of N by red spruce over time. Late spring N supplements were incorporated into biomass and accompanied by a growth enhancement. In contrast, N applied later resulted in no such growth enhancements, but may have contributed to the production of cryoprotectant proteins (Close 1996) that enhance cold hardiness.

Although evidence shows that short-term N additions seem to enhance red spruce cold hardiness, this may not be true for prolonged N additions. Chronic N additions have been shown to result in growth reductions and increased mortality of red spruce trees (McNulty et al. 1996). It is plausible that N saturation could disrupt cold hardiness by altering foliar calcium and carbon relations, both of which influence cold hardiness development in red spruce.

4.2 Water relations and cold tolerance

Although low temperature injury in red spruce is thought to result from freeze-induced dehydration of cells, water withdrawn from cells remains in leaves so that no overall change in tissue water content is expected. As a result, foliar water content may not be a good measure of relative cold hardiness among red spruce trees, even though water content may co-vary with cold hardiness development on a seasonal basis and freeze-injured foliage may dry out as a result of injury. In fact, correlations between freezing injury and weekly measures of water content and water potential were consistently nonsignificant in red spruce throughout winter (DeHayes 1992).

Strimbeck (1997) investigated the inter-relationships between cold hardiness, osmotic pressure, and foliar water content across a range of red spruce trees, leaf tissues (current-year and year-old needles), and seasons (autumn, winter and spring) selected to maximize the magnitude of variation in red spruce cold hardiness. Consistent differences in the three parameters were found among sample dates, needle age classes, and individual trees, and the three measures co-varied over the course of the winter. As cold hardiness increased in winter and in year-old as opposed to current-year needles, water content tended to decrease and osmotic pressure increased. Correlation analyses among values for each tree/tissue age class/sample date combination were significant between cold hardiness and osmotic pressure, but not for cold hardiness and water content. The relationship between cold hardiness and osmotic pressure was further explored using analysis of covariance to determine if the well-documented variation in cold hardiness among trees, needle age classes, and seasons could be explained by a unique relationship between cold hardiness and osmotic pressure (Strimbeck 1997). These analyses demonstrated that the relationship between osmotic pressure and cold hardiness can explain a significant portion, but not all, of the cold hardiness variance. This evidence suggests that changes in osmotic pressure or concentrations of solutes in cell solutions may play a direct role in the development and maintenance of cold hardiness in red spruce needles. In theory, cells with a higher initial osmotic concentration will always retain a higher proportion of total cell water when in equilibrium with ice at a given temperature than will cells with lower osmotic concentrations (Levitt 1980; Franks 1985). Of the many solutes that contribute to variation in osmotic pressure gradients, sugars are thought to play a key role.

4.3 Role of carbohydrates

Increases in organic solutes (especially sugars) have long been associated with the development of cold tolerance in a wide range of plants (Levitt 1956; Levitt 1980). Of the many classes of solutes that have been investigated, the increases in sugar concentration that accompany hardening are among the most consistently observed (Levitt 1956; Levitt 1980; Sakai and Larcher 1987), particularly in conifers (*e.g.* Parker 1959; Aronsson et al. 1976; Hinesley et al. 1992), including red spruce (Amundson et al. 1992; Schaberg et al. 2000c). In conifer foliage, this appears to happen primarily by conversion of starch to sugar, mainly sucrose, but with smaller amounts of other sugars, including the oligosaccharides raffinose and stachyose, which often show the greatest proportional increase during hardening (*e.g.* Parker 1959; Hinesley et al. 1992). These and other oligosaccharides are widely associated with the development of cold or desiccation tolerance (Kandler and Hopf 1980).

Seasonal changes in sugar and starch concentrations within montane red spruce seedlings were recently documented (Schaberg et al. 2000c). These seedlings experienced steep increases in foliar sugar concentrations in the fall, maintained high but somewhat variable sugar concentrations during winter, and exhibited a sharp decline in foliar sugars during spring. Autumnal patterns provide a good example of the increases in sugars that accompany cold hardiness development. Overall sugar concentrations approximately tripled in current-year (<1-year-old) needles (going from about 50 mg g^{-1} dry weight in August to about 150 mg g^{-1} dry weight in December), and approximately doubled in older (≥1-year-old) needles (increasing from 100 mg g^{-1} dry weight to almost 200 mg g^{-1} dry weight during the same period) (Schaberg et al. 2000c). Foliar sugar concentrations followed a pattern of change synchronous to the well-defined pattern of red spruce cold acclimation, maintenance, and deacclimation (*Figure 1A*). In addition, differences in the sugar concentrations of current-year and older needles correspond to discrepancies in cold hardiness between these needle age classes. Older needles have markedly higher sugar concentrations (Schaberg et al. 2000c) and are consistently more cold hardy (DeHayes 1992) than current-year needles. Because older red spruce foliage also has a lower water content (Strimbeck 1997), age-class related differences in sugar concentrations within cell solutions might be even greater than those reported using a dry weight standardization.

Numerous studies have shown that foliar sugar and cold hardiness levels often fluctuate together (Levitt 1980). However, the confounding influence of season (*e.g.* Parker 1959; Hinesley et al. 1992) or experimental treatment (*e.g.* Ögren 1997; Ögren et al. 1997) have frequently complicated

determination of any causal association between these parameters. For example, although cold hardiness and foliar sugar concentrations change dramatically and somewhat synchronously with season, it is possible that these are independent responses to environmental cues such as light and temperature. To remove the possible confounding of potentially unrelated, but concurrent, seasonal trends in physiology, DeHayes (1992) evaluated the relationship between total foliar sugar concentrations of 40 red spruce trees in December 1989 with December cold hardiness estimates or freezing injury data. He found a low, but highly significant, negative correlation between sugar concentration and both critical temperature (a measurement of the temperature of freezing injury) ($r = -0.43$, $P < 0.01$, 38 d.f.) and visible foliar injury ($r = -0.41$, $P < 0.01$, 38 d.f.). In this study, trees with relatively high foliar sugar concentrations tended to be more cold hardy and suffer less freezing injury.

A more specific look at the association between foliar cold hardiness and carbohydrate concentrations was recently provided by Schaberg et al. (2000b). They also measured foliar cold tolerance and sugar concentrations on a single date (January 1996). However, they evaluated 60 trees and analyzed foliage for an array of specific sugars (sucrose, glucose, fructose and raffinose) that have been experimentally implicated as being potentially important to plant cold hardiness. As in the above study, Schaberg et al. (2000b) found a significant negative correlation between the estimated temperature of freezing injury and total foliar sugar concentration ($r = -0.379$, $P < 0.05$, 58 d.f.). However, they noted that this relationship was apparently driven by significant relationships with two sugar types: raffinose ($r = -0.59$, $P < 0.01$, 58 d.f.) and, to a lesser extent, sucrose ($r = -0.30$, $P < 0.02$, 58 d.f.). Correlations between cold hardiness and fructose or glucose concentrations were not significant. Because some sugars are thought to have specific protective effects, understanding associations between cold hardiness and particular sugar constituents may help elucidate mechanistic relationships.

Among the many suggested mechanisms whereby sugars may influence plant cold hardiness, three have received the most scientific attention. These involve (1) enhanced osmotic control; (2) vitrification; and (3) direct cryoprotection.

The colligative effect of freezing point depression is generally considered unimportant in deeply cold hardy plants because it lowers the freezing point by only a few degrees at the osmotic concentrations typically observed in plants (Levitt 1980). Still, the lowering of water potential by biological solutes such as sugars may play a role in freeze-dehydration avoidance in cold tolerant plant tissues. Levitt (1980) interprets the role of sugars in

cryoprotection as a colligative mechanism of desiccation avoidance in moderately tolerant plants.

A more important form of dehydration avoidance that occurs in deeply cold tolerant plants may involve the vitrification of cell solutions. Vitrification is the transition of a solution from a more or less free-flowing liquid state to a highly viscous 'glassy' state. In vitrified solutions, the kinetics of water movement are so slow that, even if the loss of water is thermodynamically favored by strong gradients in water potential, the tissue may be able to resist further water loss for months or even years. The kinetics of diffusion and reaction processes leading to the denaturation of cell components may be similarly reduced, thereby delaying or preventing many of the deleterious effects of freeze dehydration. As such, vitrification is considered the likely mechanism by which cells retain a proportion of their total water content as bound or unfrozen water (Franks 1985), and may be an important overall mechanism for the survival of plant tissues that contain high concentrations of biological solutes, especially sugars (Burke 1985; Koster 1991; Close 1996).

Another mechanism whereby soluble carbohydrates may influence cold hardiness involves the direct interaction of cryoprotective sugars with dehydration-sensitive macromolecules, such as proteins (Arakawa and Timasheff 1982; Close 1996) or membrane lipids (Anchorduguy et al. 1987; Crowe et al. 1994). Cryoprotective solutes may stabilize (Franks 1985; Carpenter and Crowe 1988; Close 1996) or replace (Clegg 1986; Caffrey et al. 1988) water during dehydration, allowing biomolecules and membranes to maintain a functional configuration, or preventing the irreversible denaturation of proteins or phase changes in membrane lipids.

The mechanisms whereby sugars specifically influence red spruce cold hardiness have received little evaluation. Strimbeck (1997) found a consistent relationship between cold hardiness and osmotic pressure in red spruce foliage over the course of a winter and spring, and he speculated that solutes contributing to overall osmotic pressure (notably sugars) may contribute to cold hardiness by promoting vitrification or *via* the direct cryoprotection of cellular components. Similarly, significant correlations between foliar cold hardiness and raffinose and sucrose concentrations reported for red spruce trees led Schaberg et al. (2000b) to speculate that these particular sugars may be supporting vitrification and/or cryoprotection. Indeed, experimental evidence for a variety of plant species and more simplified systems provides strong biological support for mechanisms hypothesized for red spruce, especially those regarding the possible importance of raffinose and sucrose (*e.g.* Anchorduguy et al. 1987; Carpenter and Crowe 1988). Still, direct verification of the suspected role(s) sugars play in influencing red spruce cold hardiness awaits further study.

Whatever physiological mechanism(s) may be involved, if high sugar concentrations are important to the maintenance of deep cold hardiness in red spruce, then the sustenance of these sugar levels throughout winter would be important to foliar and tree survival. Despite this presumed importance, red spruce experiences net reductions in foliar sugars during typical sub-freezing winter periods (Schaberg et al. 2000c), possibly as a result of respiratory losses that predominate during cold periods (Schaberg et al. 1995, 1998). Although sugar reductions are at times substantial (*e.g.* about 25% of the total sugar concentration in older needles; Schaberg et al. 2000c), foliar sugar stores can also be augmented if winter temperatures moderate. A significant increase in the sugar content of older needles has been documented for native red spruce during a winter thaw (Schaberg et al. 2000c). This increase occurred without any reduction in localized starch concentrations or reductions in sugar or starch concentrations in new needles, stems or roots (Schaberg et al. 2000c). It is possible that increased sugar concentrations in old needles could have resulted from conversions from a non-carbohydrate fraction. However, it seems more likely that this increase resulted from photosynthesis because sugar increases (during a protracted thaw) occur precisely when winter photosynthesis has been observed in this species (Schaberg et al. 1995, 1998). Indeed, the preferential accumulation of sugars in older foliage may help account for the sustained hardiness of this foliar age-class during thaws relative to current-year needles that do not increase in sugar content (Schaberg et al. 2000c) and deharden during thaws (Strimbeck et al. 1995).

4.4 Role of calcium in red spruce cold tolerance development

Another factor that may affect cold tolerance development in red spruce is calcium (Ca) nutrition. Calcium is a major cation in soil and surface waters and an abundant macronutrient in trees. Within plants, Ca is highly compartmentalized, and this partitioning is critical to its physiological function. For example, Ca is of vital importance for cell wall formation and plays an important role in membrane structure and function, stabilizing membranes by bridging phosphate and carboxylate groups of membrane phospholipids and proteins (Palta and Li 1978; Davies and Monk-Talbot 1990; Steponkus 1990). In contrast, cytosolic Ca is phytotoxic and cytoplasmic concentrations are extremely low (Hanson 1984), although intracellularly Ca acts as a metabolic regulator for many processes and as a second messenger in binding to proteins such as calmodulin (Hepler and Wayne 1985). Despite its critical roles in physiology, the major fraction of Ca in conifer needles exists in insoluble Ca oxalate crystals that occur

extracellularly on the outside walls of mesophyll cells and, to a lesser extent, within cell walls of epidermal cells (Fink 1991). In addition, Ca pectate occurs in high concentration in the cell walls of sieve cells and transfusion parenchyma (Fink 1991). Measures of total foliar pools of Ca ions in conifer foliage may primarily reflect these large stored pools rather than the small, but physiologically critical and labile, Ca ions associated with the plasma membrane-cell wall complex. In particular, membrane-associated Ca (mCa), although a relatively small fraction of total foliar Ca ion pools, strongly influences the response of cells to changing environmental conditions, including freezing stress (Hanson 1984; Hepler and Wayne 1985; Dhindsa et al. 1993).

The plasma membrane plays a central role in cold acclimation and low temperature injury in plants (Pomeroy and Andrews 1985; Davies and Monk-Talbot 1990; Steponkus 1990), and mCa is an integral component of cell membrane structure and function. By influencing membrane architecture, mCa influences solution movement across membranes, the ability of cells to resist dehydration, extracellular ice damage, and, at least in some cases, intracellular freezing during cold acclimation. Extracellular Ca, including mCa (Atkinson et al. 1990), also serves as an important second messenger and plays a crucial role in the perception and transduction of low temperature signals during cold acclimation (Dhindsa et al. 1993). Studies with crop plants have demonstrated that the loss or restriction of Ca enhances susceptibility to freezing injury (Pomeroy and Andrews 1985; Monroy et al. 1993; Crotty and Poole 1995), whereas the addition of Ca to the extracellular solution can prevent freezing injury (Arora and Palta 1988).

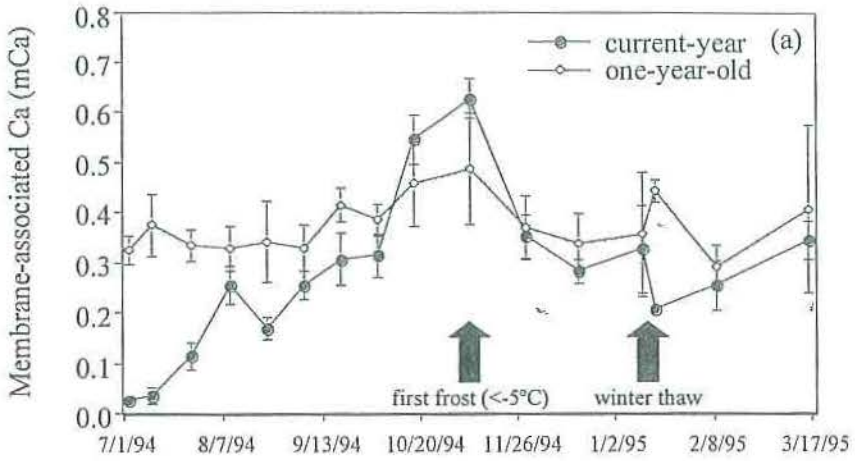
Recent procedural advances have allowed for the specific assessment of mCa in tissues of woody plants, including red spruce (Borer et al. 1997). Using these protocols, we evaluated seasonal variations of mCa and total foliar Ca pools in red spruce mesophyll cells (DeHayes et al. 1997). We found that total Ca concentrations in current-year foliage vary little with season and appear unresponsive to environmental cues (DeHayes et al. 1997). However, mCa levels in these same tissues were seasonally dynamic and varied in response to temporal changes in the environment (DeHayes et al. 1997, *Figure 2A*). Seasonal changes in mCa appear to reflect the dynamic and environmentally responsive nature of Ca ion exchange sites in the plasma membrane-cell wall compartment of red spruce mesophyll cells, especially in current-year needles. For example, the increase in mCa from June through October was likely a reflection of increased Ca binding sites in both the developing cell wall and plasma membrane. The latter is associated with seasonal increases in polar lipids of plasma membranes in late summer and early autumn (Senser and Beck 1982). In contrast, changes in mCa that occurred throughout the winter appeared to coincide with changing

environmental conditions (*e.g.* fall frost) and are probably a reflection of seasonal augmentation of membrane phospholipids and a concomitant incorporation of polyunsaturated fatty acids into membrane lipids (Senser and Beck 1982).

Seasonal fluxes in mCa may also reflect the important second messenger role of extracellular Ca. There is increasing evidence that mCa plays a critical role in the transduction of low temperature signals during cold acclimation (Dhindsa et al. 1993), and possibly in the dehardening response associated with climatic warming (Woods et al. 1984). Indeed, our data specifically indicates that mCa levels in red spruce mesophyll cells are responsive to winter warming. We have documented abrupt, but temporary, mCa reductions in current-year needles of both red spruce seedlings (DeHayes et al. 1997) and native trees (Schaberg and DeHayes 2000) in response to a mid-winter thaw (*Table 3*). Cold hardiness was examined during one of these thaws and verified that thaw-induced mCa reductions were accompanied by significant precocious dehardening over only 3 days (Strimbeck et al. 1995; DeHayes et al. 1997). After the thaw, both mCa and freezing tolerance returned to prethaw levels. Sequential changes in mCa and cold hardiness levels, coupled with the well-documented changes in membrane structure in leaf tissue of northern temperate conifers during cold acclimation (*e.g.* DeYoe and Brown 1979; Senser and Beck 1982), strongly support the contention that mCa is important to cold hardiness physiology. In *Figure 2B*, we propose a model that depicts seasonal variation in mCa of red spruce mesophyll cells within a context of seasonal alterations to membrane structure and functional inter-relationships with red spruce cold hardiness. It seems likely that factors which significantly alter mCa levels would also disrupt the development and maintenance of hardiness.

Table 3. Mesophyll cell membrane-associated Ca (mCa) levels in current-year foliage of red spruce foliage of red spruce seedlings (From DeHayes et al. 1997) and mature trees (D.H. DeHayes et al., unpublished data) before and after winter thaws.

Year	Tissue type	Relative mCa concentration		*P* value
		Pre-thaw	Post-thaw	
1995	Potted seedlings	0.328	0.209	0.065
1997	Native trees	0.232	0.116	0.001



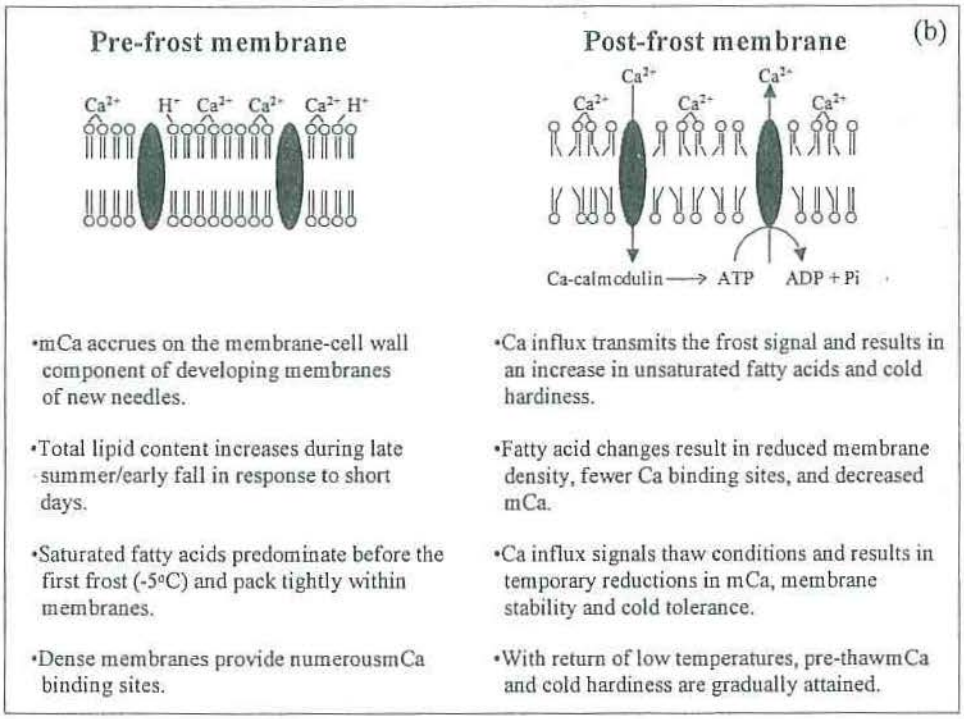


Figure 2. Membranes and cold hardiness (From DeHayes et al. 1997). (A) Seasonal patterns of membrane-associated Ca (mCa) of current-year and year-old needles from red spruce seedlings. Error bars are ±1 SE from means. (B) Hypothetical model depicting interrelated alterations in mCa and membrane structure.

5. UNIQUE SUSCEPTIBILITY TO ENVIRONMENTAL STRESS

5.1 Precocious dehardening in response to winter thaws

Considerable evidence has now verified that, in addition to alterations in mCa concentrations, meaningful reductions in red spruce cold hardiness occur in response to prolonged, multi-day thaws during winter. Several laboratory studies have documented mean reductions in cold hardiness of up to 14°C for red spruce seedlings exposed to simulated thaws (5 to 10°C) lasting 4 to 5 days (DeHayes 1992; Schaberg et al. 1996). More importantly, comparable levels of precocious dehardening have been documented for mature trees in the field. Strimbeck et al. (1995) found that the current-year foliage of montane red spruce dehardened an average of 9°C after only 3 days of exposure to above-freezing (0 to 10°C) temperatures (*Figure 3*). Trees in this study dehardened as much as 14°C and remained partially dehardened for up to 19 days after subfreezing ambient temperatures returned (Strimbeck et al. 1995). Thus, although the thaw itself was limited to 9 days, the resulting physiological impact was far more persistent, greatly extending the period of increased vulnerability to freezing injury.

Data from both field and laboratory studies also suggest that this dehardening response may be unique to red spruce. For example, although red spruce trees exhibited substantive and persistent reductions in cold hardiness during the January 1995 thaw, sympatric balsam fir showed no evidence of dehardening (Strimbeck et al. 1995). This same pattern of response was evident in results of a replicated experiment we conducted using seedlings exposed to simulated thaw treatments (DeHayes 1992). Red spruce and balsam fir were exposed to either 5°C for 5 days or 10°C for 5 days; these treatments were followed by 5 days of ambient (subfreezing) conditions, or continuous ambient conditions (the control). Similar to mature trees in the field, red spruce seedlings experienced sizable (6 to 12°C) thaw-induced reductions in cold hardiness, whereas no reductions in cold hardiness were detected for balsam fir (*Table 4*). As with the field results, thaw-induced dehardening frequently persisted for the red spruce seedlings following re-exposure to subfreezing temperatures.

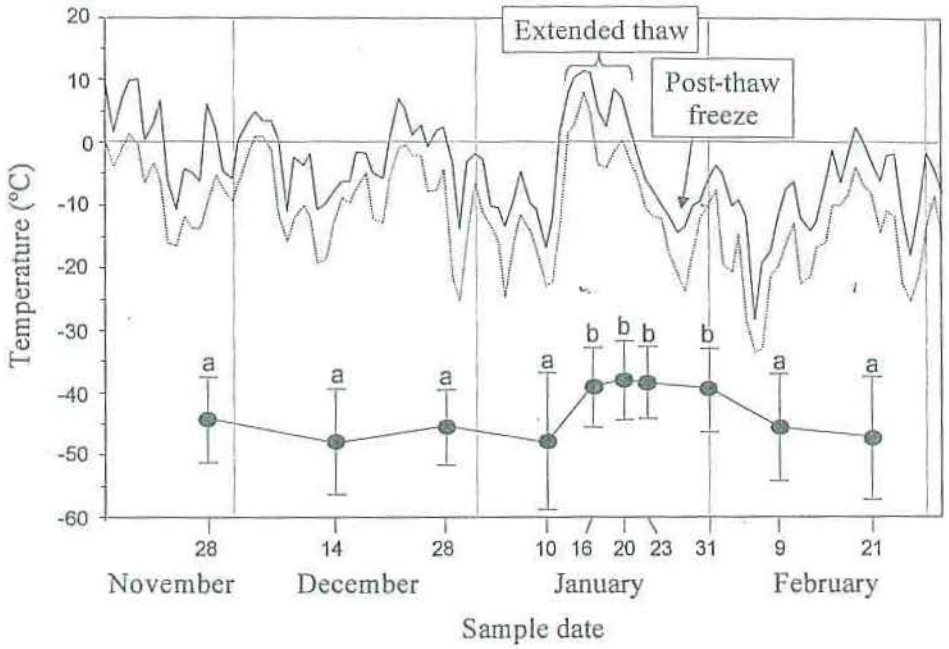


Figure 3. Cold hardiness of current-year foliage from mature red spruce trees and ambient air temperatures for Mt. Mansfield, Vermont, USA. Solid and dashed lines are daily maximum and minimum temperatures at the study site (1000 m). Points and bars are mean, maximum and minimum cold hardiness levels for 10 trees. Means with the same letter are not significantly different ($P < 0.01$), based on Duncan's multiple range test. (From Strimbeck et al. 1995).

Table 4. Reduction in cold hardiness 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. (From DeHayes 1992).

	Reductions in cold hardiness (°C), winter 1989					
Temperature	January		February		March	
treatments	Spruce	fir	Spruce	fir	spruce	fir
10°C 5 d	12.0[a]	5.7	13.5[a]	3.1	11.9[a]	1.3
5°C 5 d	11.1[a]	0.3	8.3[a]	2.1	5.9[a]	-
10°C 5 d then 5 d at ambient	4.3	1.9	7.9[a]	0.5	7.3[a]	-
5°C 5 d then 5 d 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 hardiness from seedlings exposed to ambient sub-freezing conditions.

The combination of rapid (within 3 days), substantial (3 to 14°C), and prolonged (up to 25 days after first detection) dehardening significantly

increases the risk of freezing injury for red spruce because this species attains pre-thaw hardiness levels that are barely sufficient to protect current-year foliage from freezing injury (Strimbeck et al. 1995). However, red spruce does not only deharden during winter thaws, it can also become photosynthetically active (Schaberg et al. 1995, 1998) and experience increases in foliar sugar concentrations (Schaberg et al. 2000a).

The co-occurrence of changes in photosynthesis and cold hardiness during thaws raises questions regarding the ecology and survival of red spruce. Because both potentially beneficial (increased carbon capture) and detrimental (reduced cold hardiness) changes in physiology can occur, the response of red spruce to thaw could be characterized as a tradeoff, with the adaptive consequences of thaw-induced changes in physiology dependent upon the long-term balance of costs and benefits. This tradeoff could be particularly consequential if current predictions of climate change hold true. It is predicted that pollution-induced climate change could cause winter thaws to become more common in northern latitudes, while existing low temperature extremes may persist (MacCracken et al. 1991). If this occurs, any physiological benefit of winter carbon capture could well be offset by an increased incidence of freezing injury following thaw-induced dehardening. Resulting foliar losses would reduce future potentials for carbon capture and remove access to carbon stored in lost foliage. As a result, thaw-induced changes in physiology could actually deplete red spruce carbon stores.

Thaw-induced reductions in cold hardiness are most likely to result in freezing injury if extreme cold, rapid freezing, or cycles of freezing and thawing occur while foliage is partially dehardened (Perkins and Adams 1995; Strimbeck et al. 1995; Lund and Livingston 1998). Indeed, the field observations of Strimbeck et al. (1995) may illustrate a consequence of this elevated risk; the least cold hardy tree following the January 1995 thaw also exhibited the most freezing injury after minimum temperatures reached -34°C about 16 days after the thaw ended (Strimbeck et al. 1995). Still, overall freezing injury at the site was not severe, even though thaw-induced dehardening was substantial and low ambient temperatures quickly followed the thaw (Strimbeck et al. 1995). In fact, climatic records show that mid-winter thaws were associated with only two of the last four episodes of regionwide freezing injury (Tobi et al. 1995). Clearly, thaw-induced dehardening does not readily account for all recent episodes of red spruce freezing injury. However, winter thaw is not the only environmental perturbation that has been documented to reduce the freezing tolerance of red spruce. Inputs of acid deposition also significantly increase the risk of foliar freezing injury for this species.

5.2 Acidic deposition-induced reductions in cold hardiness

Early studies on possible links between acid deposition and decline emphasized soil processes. However, there is at present no published direct evidence linking soil mediated stresses to cold hardiness or winter injury in red spruce, although it remains a possibility that soil and root processes may be important predisposing factors. In contrast, there is good experimental evidence that acid deposition in the canopy has direct effects on foliar cold hardiness.

In what has become a highly repeatable experiment, numerous studies have shown that treatment of red spruce with simulated acid deposition reduces the cold hardiness of current-year foliage anywhere from 5 to 12°C (Fowler et al. 1989; DeHayes et al. 1991; Jacobson et al. 1992; Vann et al. 1992; Sheppard et al. 1993; Waite el al. 1994; DeHayes et al. 1999; Schaberg et al. 2000a; *Table 5*). Significantly, evaluations of seedlings (DeHayes et al. 1991) or branches of mature trees (Vann et al. 1992) exposed to ambient acid deposition have documented that reductions in cold hardiness comparable to those found in simulation studies also occur in the field. For example, Vann et al. (1992) used small exclusion chambers to protect select branches of 75-year-old red spruce on Whiteface Mountain, New York, USA, from exposure to airborne chemicals, including high doses of acid cloud water. Current-year foliage of branches exposed to ambient inputs were about 10°C less cold hardy during winter than foliage protected from airborne chemicals for 3 months during the previous growing season (Vann et al. 1992). Results from this study supported the contention that pollutant-induced reductions in cold hardiness occur in nature, and suggested that these reductions are mediated through direct changes in foliar physiology. Similar to the branch study, DeHayes et al. (1991) found that native red spruce seedlings on Whitetop Mountain, Virginia, USA, exposed to ambient cloud water were 5°C less cold hardy in winter than seedlings for which cloud water was excluded. Notably, through the use of charcoal filtration in some exclusion chambers, this study confirmed the results of simulation studies indicating that cold hardiness reductions were the result of cloud water, and not ozone exposure (DeHayes et al. 1991). Subsequent studies observed that ozone exposure had little impact on the cold hardiness of red spruce foliage (Waite et al. 1994). Other than reductions in cold hardiness, the only other consistent impact cloud water had on the seedlings in the Whitetop Mountain experiment was a reduction in foliar Ca concentrations (Thornton et al. 1990). The possibility that cloud water-induced reductions in cold hardiness and Ca levels could be linked became a central focus of later research (DeHayes et al. 1999; Schaberg et al. 2000a).

Table 5. Studies of the influence of acid mist on the cold hardiness of red spruce current-year foliage.

Citation	Treatments	Treatments ions	Season	Mean Δ in cold hardiness (°C)
Fowler et al. 1989	pH 2.5 to 5.0	H^+, NH_4^+, NO_3^-, SO_4^{2-}	Fall	12
DeHayes et al. 1991	Ambient cloud *vs* No cloud	Ambient mix	Fall to winter	3 to 5
Jacobson et al. 1992	pH 2.5 to 4.5	H^+, NO_3^-, SO_4^{2-}	Fall	6 to 7.5[a]
Vann et al. 1992	Ambient cloud *vs* No cloud	Ambient mix	Winter	10
Sheppard et al. 1993	pH 2.7 *vs* 5.0	H^+, NH_4^+, NO_3^-, SO_4^{2-}	Fall	8
Waite et al. 1994	pH 3.0 *vs* 4.2	H^+, NO_3^-, SO_4^{2-}	Winter	6
DeHayes et al. 1999	pH 3.0 *vs* 5.0	H^+, Cl^-	Winter	10
Schaberg et al. 2000a	pH 3.0 *vs* 5.0	H^+, equalized SO_4^{2-}	Winter	8

[a] estimated from figure.

5.3 Proposed mechanism for acid-induced reductions in cold hardiness

Studies with crop plants have shown that Ca can play a prominent role in preventing freezing injury (Pomeroy and Andrews 1985; Arora and Palta 1988; Monroy et al. 1993; Crotty and Poole 1995). Evidence from Europe and North America indicates that acid precipitation can disrupt Ca cycling and nutrition within forest ecosystems (Shortle and Smith 1988; Schulze 1989; Likens et al. 1996). Cationic (H^+) and anionic (NO_3^- and SO_4^{2-}) components of acid precipitation can act to deplete soil pools of base cations and limit the uptake and incorporation of Ca^{2+} and Mg^{2+} by trees (Likens et al. 1996). Soil acidification and NO_3^- and SO_4^{2-} additions can also increase the availability of Al in the soil solution (Johnson and Fernandez 1992), which competes with and inhibits Ca^{2+} uptake by tree roots (Reuss and Johnson 1986; Shortle and Smith 1988). In addition, acid precipitation can leach cations from foliage (Joslin et al. 1988; Klemm et al. 1989), with Ca having the greatest rate of efflux (Klemm et al. 1989). When soil availability of Ca is low, acid-induced foliar leaching may contribute to the development of Ca deficiencies (Joslin et al. 1988). Some empirical information has linked Ca deficiency with freezing injury susceptibility for red spruce. For example, we found that red spruce trees with very low foliar Ca concentrations are less cold tolerant than trees within the normal range of foliar Ca (DeHayes et al. 1999). We have also shown that trees that are consistently susceptible to freezing injury had lower Ca concentrations in current-year, but not year-old, foliage than frost-resistant trees (DeHayes et

al. 1999). However, until recently, the possible connection between Ca nutrition and red spruce freezing injury had not been experimentally tested.

We have examined acid mist-induced alterations of Ca physiology as a potential mechanism for freezing injury in red spruce foliage through a series of experiments. Our initial studies documented that current-year foliage of red spruce saplings exposed to pH 3 mist experienced more foliar leaching of Ca, greater cellular electrolyte loss (a sign of membrane disruption), and reduced cold hardiness (4 to 10°C) compared to plants exposed to pH 5 mist (*Figure 4*; DeHayes et al. 1999; Schaberg et al. 2000a). A separate *in vitro* leaching experiment verified that approximately 85% of the Ca leached comes from foliage, and that more is leached from current-year needles (which are susceptible to freezing injury) than year-old needles (which are resistant) (DeHayes et al. 1999). Although total foliar Ca concentrations were not altered by acid additions (DeHayes et al. 1999), additional study verified that foliar Ca leaching reduced the physiologically active pool of Ca associated with plasma membranes of mesophyll cells (mCa) (Schaberg et al. 2000a). This specific pool of mCa may perform critical functions, including membrane stabilization (*e.g.* Guy 1990) and low temperature signal transduction (*e.g.* Atkinson et al. 1990), that help plant cells survive freezing stress. Furthermore, empirical data specifically implicates mCa involvement in the developmental cold hardiness of red spruce (see Section 4.3). Indeed, our recent data has shown that the direct leaching loss of mCa is accompanied by significant reductions in membrane stability and cold hardiness (Schaberg et al. 2000a). Verification of an acid-induced disruption of foliar mCa provides new insight on how acid deposition disrupts red spruce cold hardiness.

Using our recent data and the findings of others as a foundation, we have developed a detailed hypothesis of the mechanism by which acid deposition reduces the cold hardiness of red spruce current-year foliage. This mechanism focuses on the impact of ionic atmospheric inputs on foliar mCa levels. We propose that direct acidic deposition on red spruce foliage preferentially displaces calcium ions specifically associated with plasma membranes of mesophyll cells. As a result, mCa is reduced, plasma membranes are destabilized, and messenger calcium is depleted. This physiological impairment leads to the more commonly observed secondary symptoms of freezing injury in northern regions and potentially enhanced susceptibility to other stresses that compromise overall forest health.

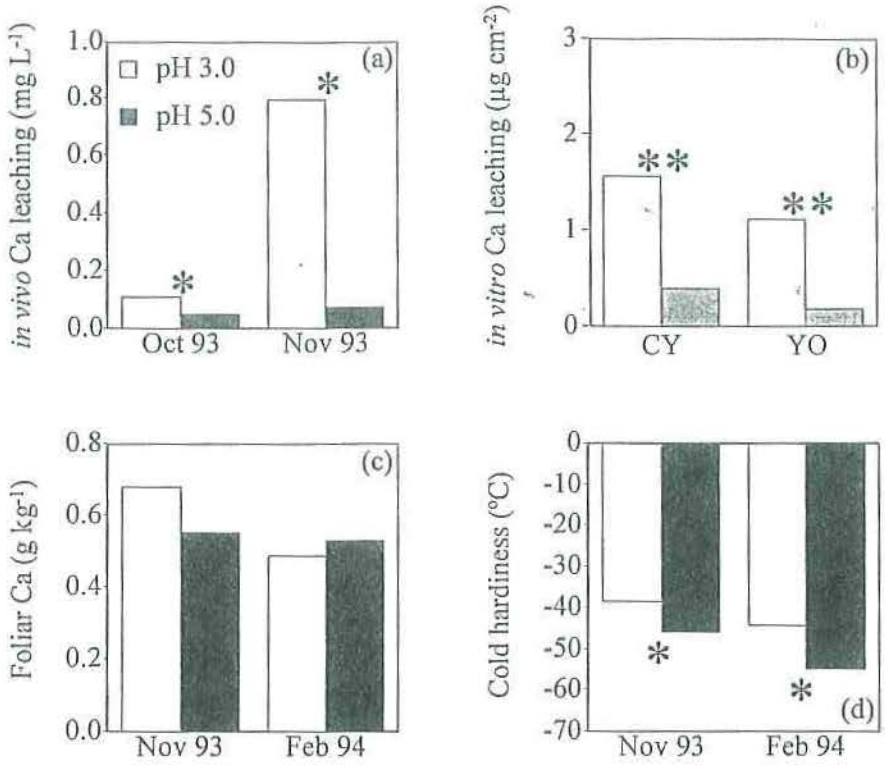


Figure 4. Effects of acid mist treatment on (a) *in vivo* Ca leaching, (b) *in vitro* Ca leaching, (c) total foliar Ca concentrations, and (d) cold hardiness of red spruce foliage. (From DeHayes et al. 1999). ** and * indicate P≤0.01 and 0.05, respectively.

Although acid precipitation in the eastern United States is dominated by four ionic constituents (NO_3^-, SO_4^{2-}, NH_4^+, and H^+) (Mohnen 1992), evidence indicates that H^+ ions are responsible for reducing the foliar cold hardiness of red spruce during winter (Jacobson et al. 1992; Schaberg et al. 2000b). In addition, acid deposition-induced foliar Ca leaching could result from inputs of H^+, SO_4^{2-}, or both (Edwards et al. 1995). However, reductions in H^+ concentrations in throughfall following foliar contact without similar changes in SO_4^{2-} levels (Joslin et al. 1988; McLaughlin et al. 1996) indicate that leaching losses are not driven by SO_4^{2-} inputs, but are more likely the result of H^+-induced cation exchange. Our data verifies that foliar H^+ uptake accompanies Ca leaching loss, and that this leaching is independent of SO_4^{2-} content of treatment solutions (DeHayes et al. 1999; Schaberg et al. 2000a). Furthermore, we have documented that Ca leaching specifically reduces mCa, and that this loss coincides with significant reductions in membrane stability and cold hardiness (DeHayes et al. 1999; Schaberg et al. 2000a).

Each step of this sequential, mechanistic explanation for acid-induced reductions in cold hardiness is supported by data. Moreover, this mechanism

specifically accounts for established unique features of red spruce freezing injury. For example, it accounts for freezing tolerance differences between current-year foliage (which increase mCa only gradually during the growing season and have a high rate of acid-induced Ca leaching) and year-old foliage (which have an established mCa pool and experience less Ca leaching loss) (DeHayes et al. 1999). The mechanism also accounts for the predisposing (rather than the direct stress and injury) influence of acid deposition that is uniquely compatible with empirical assessments of red spruce decline symptomology. Generalized growth impairments would be expected if direct sulfate toxicity or carbon metabolism disruption were primary factors in red spruce decline (*e.g.* Cape et al. 1991; Sheppard 1994) because pollution-induced changes in physiology would likely have a metabolic cost, even in the absence of freezing injury. However, most controlled environment studies have documented unimpaired or even improved growth for red spruce exposed to acid mist in the absence of another stress factor (*e.g.* low temperature) (Laurence et al. 1989; Kohut et al. 1990; Lee et al. 1990). Indeed, growth reductions associated with red spruce decline in northern montane forests appear to be directly associated with freezing injury events (Wilkinson 1990; Tobi et al. 1995) rather than exposure to the predisposing acidic conditions. Overall, this mechanism explains the direct link between acid deposition and the dramatic increase in red spruce freezing injury in northern montane forests and range-wide growth declines observed over the past 40 years, a time period that coincides with increased pollution emissions.

5.4 Soil Ca depletion

It is now well-established that soil Ca is being depleted in numerous temperate zone forest ecosystems throughout the northern hemisphere (*e.g.* Schulze 1989; Richter et al. 1994; Kirchner and Lydersen 1995). Although it is often difficult to quantify the extent of soil Ca depletion over time, recent reports indicate that the magnitude of Ca losses may be substantial. For example, based on long-term chronological records and detailed biogeochemical studies, Likens et al. (1996) have estimated that the pool of Ca in the soil complex at the Hubbard Brook Experimental Forest (New Hampshire, USA) may have shrunk by more than 50% over the past 45 years. Soil losses of Ca were attributed to leaching caused by acid rain inputs, decreasing Ca deposition, and to changing amounts of net storage of Ca in biomass. Such soil Ca losses have resulted in forest ecosystems that continue to be sensitive to continuing inputs of acids in atmospheric deposition, despite long-term reductions in sulfur deposition (Likens et al. 1998). Although high acid loading leading to Ca leaching is an important

and consistent explanation for soil Ca depletion in northern temperate forests (*e.g.* Kirchner and Lyderson 1995; Likens et al. 1996; Likens et al. 1998), other potential contributing factors include declines in atmospheric base cations (Hedin et al. 1994), soil aluminum mobilization (Shortle and Smith 1988; Lawrence et al. 1995), intensive forest harvesting (Mann et al. 1988; Federer et al. 1989), nitrogen saturation (Aber et al. 1995; Schaberg et al. 1997) and changing climatic conditions (Tomlinson 1993).

Direct foliar leaching of mCa and resulting reductions in cold hardiness have now been firmly established for red spruce (DeHayes et al. 1999; Schaberg et al. 2000a). In comparison, the impacts of soil-mediated Ca perturbations on cold hardiness are far less certain. Schaberg et al. (2000a) added Ca and/or Al to the soil solutions of red spruce seedlings grown in a low Ca soil (52 $\mu g\ g^{-1}$). As expected, soil Ca additions significantly increased Ca incorporation within foliage, and soil Al addition reduced foliar Ca (Schaberg et al. 2000a). However, reduced foliar Ca due to low soil Ca availability or soil Al additions did not reduce foliar cold hardiness, presumably because mCa levels remained sufficient for anatomical support and normal physiological function (Schaberg et al. 2000a). Yet, because soils are the source of foliar Ca, including mCa, we expect that soil Ca would have an effect on mCa and cold hardiness in more severely limited soil Ca environments. Indeed, we have documented a significant association between foliar Ca concentration and reduced cold hardiness for red spruce trees with very low foliar Ca concentrations growing in Ca depauperate soils (DeHayes et al. 1999). Here, deficiencies in total foliar Ca likely resulted in mCa levels insufficient to support full cold hardiness development (DeHayes et al. 1999). Despite logical connections among soil Ca availability, foliar mCa increase and cold hardiness physiology, data directly linking soil Ca deficiency with mCa deficiencies and red spruce freezing injury are currently lacking. If they occur, soil-mediated foliar Ca deficiencies would be expected to exacerbate the direct foliar leaching loss of mCa, increasing the risk and potential scope of red spruce freezing injury.

6. CONCLUSION

Red spruce achieves a minimal level of mid-winter cold hardiness for a northern temperate conifer, predisposing the species to freezing injury. Recent evidence indicates that the frequency of freezing injury in red spruce has increased over the past 50 years, even though there is no evidence that winters have become colder. In addition, several environmental stress factors are individually linked to freezing injury in red spruce, which appears to further enhance its susceptibility. Other than the inherent sensitivity of red

spruce to low temperature in mid-winter, no single factor has been identified as the sole causal agent to all recent incidences of regionwide red spruce freezing injury. This raises the possibility that freezing injury under ambient conditions is not the result of reductions in cold hardiness caused by a single dominant factor, but rather results from some combination or interaction of environmental perturbations.

Of all the possible combinations of environmental factors that influence red spruce cold hardiness, interactions between acid mist and mid-winter thaws have the greatest potential to increase the risk of freezing injury. Mean reductions in cold hardiness 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). Both of these environmental factors can reduce mCa and cold hardiness levels in red spruce, and perhaps more importantly, may predispose trees to other environmental stresses. Furthermore, it is possible that mCa disruption may be exacerbated by soil Ca depletion and the interactions of soil nitrogen saturation and Ca physiology in red spruce.

If the combination of acid mist and thaw produces a cold hardiness response that is additive of the individual impacts of these stress factors, resulting reductions in hardiness would dramatically increase the risk of freezing injury for red spruce current-year foliage and would collectively explain the high incidence of freezing injury documented in recent years. Possible interactions between acid mist and thaw are of practical importance because atmospheric additions of acid-producing compounds and soil Ca depletion are likely to continue, while mid-winter 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 eastern North America, it is currently unknown whether acid mist and thaw interact to heighten the risk of freezing injury for red spruce or not. It seems likely that combinations of, and possibly interactions among, predisposing factors such as thaw-induced dehardening, acid deposition, rapid freezing following solar warming, repeated cycles of freezing and thawing, and possibly N saturation, or other Ca-depleting factors could differentially contribute to freezing injury susceptibility at various locations. By enhancing freezing injury susceptibility, combinations of environmental stress factors may ultimately impact the productivity and long-term viability of the red spruce forests that define a unique ecological entity in eastern North America.

ACKNOWLEDGEMENTS

We thank Gary Hawley, Paula Murakami and Amy Burfeind for their considerable help in the preparation of this chapter.

REFERENCES

Aber, J.D., Magill, A., McNulty, S.G., Boone, R.D., Nadelhoffer, K.J., Downs, M., and Hallet, R. 1995. Forest biogeochemistry and primary production altered by nitrogen saturation. Water Air Soil Pollut. **85**: 1665-1670.

Adams, G.T., and Perkins, T.D. 1993. Assessing cold tolerance in *Picea* using chlorophyll fluorescence. Environ. Exp. Bot. **33**: 377-382.

Adams, G.T., Perkins, T.D., and Klein, R. 1991. Anatomical studies on first-year winter injured red spruce foliage. Am. J. Bot. **78**: 1199-1206.

Amundson, R.G., Hadley, J.L., Fincher, J.F., Fellows, S., and Alscher, R.G. 1992. Comparisons of seasonal changes in photosynthetic capacity, pigments, and carbohydrates of healthy sapling and mature red spruce and of declining and healthy red spruce. Can. J. For. Res. **22**: 1605-1616.

Anchorduguy, T.J., Rudolph, A.S., Carpenter, J.F., and Crowe, J.H. 1987. Modes of interaction of cryoprotectants with membrane phospholipids during freezing. Cryobiology, **24**: 324-331.

Arakawa, T., and Timasheff, S.N. 1982. Stabilization of protein structure by sugars. Biochemistry, **21**: 6536-6544.

Aronsson, A., Ingestad, T., and Lööf, L.-G. 1976. Carbohydrate metabolism and frost hardiness in pine and spruce seedlings grown at different photoperiods and thermoperiods. Physiol. Plant. **36**: 127-132.

Arora, R., and Palta, J.P. 1988. *In vivo* perturbation of membrane-associated calcium by freeze-thaw stress in onion bulb cells. Plant Physiol. **87**: 622-628.

Arris, L.L., and Eagleson, P.S. 1989. Evidence for a physiological basis for the boreal-deciduous forest ecotone in North America. Vegetatio, **82**: 55-58.

Atkinson, M.M., Keppler, L.D., Orlandi, E.W., Baker, C.J., and Mischke, C.F. 1990. Involvement of plasma membrane calcium influx in bacterial induction of the K^+/H^+ and hypersensitive responses in tobacco. Plant Physiol. **92**: 215-221.

Borer, C.H., DeHayes, D.H., Schaberg, P., and Cumming, J.R. 1997. Relative quantification of membrane-associated calcium in red spruce mesophyll cells. Trees, **12**: 21-26.

Boyce, R. 1995. Patterns of foliar injury to red spruce on Whiteface Mountain, New York, during a high-injury winter. Can. J. For. Res. **25**: 166-169.

Burke, M.J. 1985. The glassy state and survival of anhydrous biological systems. *In* Membranes, metabolism, and dry organisms. *Edited by* A.C. Leopold. Comstock Publishing Associates, Ithaca, NY. pp. 358-363.

Caffrey, M., Fonseca, V., and Leopold, A.C. 1988. Lipid-sugar interactions. Relevance to anhydrous biology. Plant Physiol. **86**: 754-758.

Cannell, M.G.R. 1985. Autumn frost damage on young *Picea sitchensis*. 1. Occurrence of autumn frosts in Scotland compared with western North America. Forestry, **58**: 131-143.

Cape, J.N., Leith, I.D., Fowler, D., Murray, M.B., Sheppard, L.J., Eamus, D., and Wilson, R.H.F. 1991. Sulphate and ammonium in mist impair the frost hardening of red spruce seedlings. New Phytol. **125**: 119-126.

Carpenter, J.F., and Crowe, J.H. 1988. The mechanism of cryoprotection of proteins by solutes. Cryobiology, **25**: 244-255.

Clegg, J.S. 1986. The physical properties and metabolic status of *Artemia* cysts at low water contents: the "water replacement hypothesis". *In* Membranes, metabolism, and dry Organisms. *Edited by* A.C. Leopold. Comstock Publishing Associates, Ithaca, NY. pp. 169-187.

Close, T.J. 1996. Dehydrins: emergence of a biochemical role of a family of plant dehydration proteins. Physiol. Plant. **97**: 795-803.

Crotty, C.M., and Poole, R.J. 1995. Activation of an outward rectifying current by low temperature in alfalfa protoplasts. Plant Physiol. **108** (Supplement): 38.

Crowe, J.H., Leslie, S.B., and Crowe, L.M. 1994. Is vitrification sufficient to preserve liposomes during freeze-drying? Cryobiology, **31**: 355-366.

Curry, J.R., and Church, J.W. 1952. Observations on winter drying of conifers in the Adirondacks. J. For. **50**: 114-116.

Davies, H.V., and Monk-Talbot, L.S. 1990. Permeability characteristics and membrane lipid composition of potato tuber cultivars in relation to Ca^{2+} deficiency. Phytochemistry, **29**: 2833-2835.

DeHayes, D.H. 1992. Winter injury and developmental cold tolerance in red spruce. *In* Ecology and decline of red spruce in the eastern United States. *Edited by* C. Eagar and M.B. Adams. Ecological Studies Vol. 96. Springer-Verlag, Berlin. pp. 296-337.

DeHayes, D.H., Ingle, M.A., and Waite, C.E. 1989. Nitrogen fertilization enhances cold tolerance of red spruce seedlings. Can. J. For. Res. **19**: 1037-1043.

DeHayes, D.H., Waite, C.E., and Ingle, M.A. 1990. Storage temperature and duration influence cold tolerance of red spruce foliage. For. Sci. **36**: 1153-1158.

DeHayes, D.H., Thornton, F.C., Waite, C.E., and Ingle, M.A. 1991. Ambient cloud deposition reduces cold tolerance of red spruce seedlings. Can. J. For. Res. **21**: 1292-1295.

DeHayes, D.H., Schaberg, P.G., Hawley, G.J., Borer, C.H., Cumming, J.R., and Strimbeck, G.R. 1997. Physiological implications of seasonal variation in membrane-associated calcium in red spruce mesophyll cells. Tree Physiol. **17**: 687-695.

DeHayes, D.H., Schaberg, P.G., Hawley, G.J., and Strimbeck, G.R. 1999. Acid rain impacts on calcium nutrition and forest health. Bioscience, **49**: 789-800.

DeLucia, E.H., and Berlyn, G.P. 1984. The effect of increasing elevation on leaf cuticle thickness and cuticular transpiration in balsam fir. Can. J. Bot. **62**: 2423-2431.

DeYoe, D.R., and Brown, G.N. 1979. Glycerolipid and fatty acid changes in eastern white pine chloroplast lamellae during the onset of winter. Plant Physiol. **64**: 924-929.

Dhindsa, R.S., Monroy, A., Wolfraim, L., and Dong, G. 1993. Signal transduction and gene expression during cold acclimation of alfalfa. *In* Advances in plant cold hardiness. *Edited by* P.H. Li and L. Christersson. CRC Press, Boca Raton, FL. pp. 57-72.

Edwards, G.S., Sherman, R.E., and Kelly, J.M. 1995. Red spruce and loblolly pine nutritional responses to acidic precipitation and ozone. Environ. Pollut. **89**: 9-15.

Federer, C.A., Hornbeck, J.W., Tritton, L.M., Martin, C.W., Pierce, R.S., and Smith, C.T. 1989. Long-term depletion of calcium and other nutrients in eastern US forests. Environ. Manage. **13**: 593-601.

Fink, S. 1991. The micromorphological distribution of bound calcium in needles of Norway spruce (*Picea abies* (L.) Karst.). New Phytol. **119**: 33-40.

Fowler, D., Cape, J.N., Deans, J.D., Leith, I.D., Murray, M.B., Smith, R.I., Sheppard, L.J., and Unsworth, M.H. 1989. Effects of acid mist on the frost hardiness of red spruce seedlings. New Phytol. **113**: 321-335.

Franks, F. 1985. Biophysics and biochemistry at low temperatures. Cambridge University Press, Cambridge, UK.

Laurence, J.A., Kohut, R.J., and Amundson, R.G. 1989. Response of red spruce seedlings exposed to ozone and simulated acid precipitation in the field. Arch. Environ. Contam. Toxicol. **18**: 285-290.

Lawrence, G.B., David, M.B., and Shortle, W.C. 1995. A new mechanism for calcium loss in forest-floor soils. Nature, **378**: 162-165.

Lee, W.S., Chevone, B.I., and Seiler, J.R. 1990. Growth response and drought susceptibility of red spruce seedlings exposed to simulated acidic rain and ozone. For. Sci. **36**: 265-275.

Levitt, J. 1956. The hardiness of plants. Academic Press, Inc., New York.

Levitt, J. 1980. Responses of plants to environmental stresses. Vol. 1. 2nd ed. Academic Press, Inc., New York.

Likens, G.E., Driscoll, C.T., and Buso, D.C. 1996. Long-term effects of acid rain: response and recovery of a forest ecosystem. Science, **272**: 244-246.

Likens, G.E., Driscoll, C.T., Buso, D.C., Siccama, T.G., Johnson, C.E., Lovett, G.M., Fahey, T.J., Reiners, W.A., Ryan, D.F, Martin, C.W., and Bailey, S.W. 1998. The biogeochemistry of calcium at Hubbard Brook. Biogeochemistry, **41**: 89-173.

Little, E.L. Jr. 1971. Atlas of United States trees. Vol 1. Conifers and important hardwoods. U.S. Dep. Agric. Misc. Publ. No. 1146.

Lund, A.E., and Livingston, W.H. 1998. Freezing cycles enhance winter injury in *Picea rubens*. Tree Physiol. **19**: 65-69.

MacCracken, M., Cubasch, U., Gates, W.L., Harvey, L.D., Hunt, B., Katz, R., Lorenz, E., Manabe, S., McAvaney, B., McFarlane, N., Meehl, G., Meleshko, V., Robock, A., Stenchikov, G., Stouffer, R., Wang, W.-C., Washington, W., Watts, R., and Zebiak, S. 1991. A critical appraisal of model simulations. *In* Greenhouse-gas-induced climatic change: a critical appraisal of simulations and observations. Development in atmospheric science. *Edited by* M.F. Schlesinger. Elsevier, Amsterdam. pp. 583-591.

Mann, L.K., Johnson, D.W., West, D.C., Cole, D.W., Hornbeck, J.W., Martin, C.W., Riekerk, H., Smith, C.T., Swank, W.T., Tritton, L.M., and Van Lear, D.H. 1988. Effects of whole-tree and stem-only clearcutting on postharvest hydrologic losses, nutrient capital, and regrowth. For. Sci. **34**: 412-428.

Marchand, P.J., and Chabot, B.F. 1978. Winter water relations of tree-line plant species on Mt. Washington, New Hampshire. Arct. Alp. Res. **10**: 105-116.

McLaughlin, J.W., Fernandez, I.J., and Richards, K.J. 1996. Atmospheric deposition to a low-elevation spruce-fir forest, Maine, USA. J. Environ. Qual. **25**: 248-259.

McNulty, S.G., Aber, J.D., and Newman, S.D. 1996. Nitrogen saturation in a high elevation New England spruce-fir stand. For. Ecol. Manage. **84**: 109-121.

Mohnen, V.A. 1992. Atmospheric deposition and pollutant exposure of eastern U.S. Forests. *In* Ecology and decline of red spruce in the eastern United States. *Edited by* C. Eagar and M.B. Adams. Ecological Studies Vol. 96. Springer-Verlag, Berlin. pp. 64-124.

Monroy, A.F., Sarhan, F., and Dhindsa, R.S. 1993. Cold-induced changes in freezing tolerance, protein phosphorylation, and gene expression. Plant Physiol. **102**: 1227-1235.

Morgenstern, E.K. 1969. Winter drying of red spruce provenances related to introgressive hybridization with black spruce. Can. For. Serv. Bi-Mon. Res. Notes 25.

Ögren E. 1997. Relationship between temperature, respiratory loss of sugar and premature dehardening in dormant Scots pine seedlings. Tree Physiol. **17**: 47-51.

Ögren, E., Nilsson, T., and Sundblad, L.-G. 1997. Relationship between respiratory depletion of sugars and loss of cold hardiness in coniferous seedlings over-wintering at raised temperatures: indications of different sensitivities of spruce and pine. Plant Cell Environ. **20**: 247-253.

Palta, J.P., and Li, P.H. 1978. Cell membrane properties in relation to freezing injury. *In* Plant cold hardiness and freezing stress. *Edited by* P.H. Li and A. Sakai. Academic Press, Inc., New York. pp. 93-115.

Parker, J. 1959. Seasonal variations in sugars of conifers with some observations on cold resistance. For. Sci. **5**: 56-63.

Peart, D.R., Jones, M.B., and Palmiotto, P.A. 1991. Winter injury to red spruce at Mount Moosilauke, New Hamphire. Can. J. For. Res. **21**: 1380-1389.

Perkins, T.D., and Adams, G.T. 1995. Rapid freezing induces winter injury symptomatology in red spruce foliage. Tree Physiol. **15**: 259-266.

Perkins, T.D., Adams, G.T., and Klein, R.M. 1991. Dessication or freezing? Mechanisms of winter injury to red spruce foliage. Am. J. Bot. **78**: 1207-1217.

Perkins, T.D., Adams, G.T., Lawson, S., and Hemmerlein, M.T. 1993. Cold tolerance and water content of current-year red spruce foliage over two winter seasons. Tree Physiol. **13**: 119-129.

Pomeroy, M.K., and Andrews, C.J. 1985. Effect of low temperature and calcium on survival and membrane properties of isolated winter wheat cells. Plant Physiol. **78**: 484-488.

Reuss, J.O., and Johnson, D.W. 1986. Acid deposition and the acidification of soils and waters. Ecological Studies Vol. 59. Springer-Verlag, Berlin.

Richter, D.D., Markewitz, D., Wells, C.G., Allen, H.L., April, R., Heine, P.R., and Urrego, B. 1994. Soil chemical change during three decades in an old-field loblolly pine (*Pinus taeda* L.) ecosystem. Ecology, **75**: 1463-1473.

Sakai, A., and Larcher, W. 1987. Frost survival of plants. Responses and adaptation to freezing stress. Ecological Studies Vol. 62. Springer-Verlag, Berlin.

Schaberg, P.G., and DeHayes, D.H. 2000. Physiological and environmental causes of freezing injury in red spruce. *In* Responses of northern U.S. forests to environmental change. *Edited by* R.A. Mickler, R.A. Birdsey, and L.L. Hom. Springer-Verlag, Berlin. pp. 181-227.

Schaberg, P.G., Wilkinson, R.C., Shane, J.B., Donnelly, J.R., and Cali, P.F. 1995. Winter photosynthesis of red spruce from three Vermont seed sources. Tree Physiol. **15**: 345-350.

Schaberg, P.G., Shane, J.B., Hawley, G.J., Strimbeck, G.R., DeHayes, D.H., Cali, P.F., and Donnelly, J.R. 1996. Physiological changes in red spruce seedlings during a simulated winter thaw. Tree Physiol. **16**: 567-574.

Schaberg, P.G., Perkins, T.D., and McNulty, S.G. 1997. Effects of chronic low-level N additions on foliar elemental concentrations, morphology, and gas exchange of mature montane red spruce. Can. J. For. Res. **27**: 1622-1629.

Schaberg, P.G., Shane, J.B., Cali, P.F., Donnelly, J.R., and Strimbeck, G.R. 1998. Photosynthetic capacity of red spruce during winter. Tree Physiol. **18**: 271-276.

Schaberg, P.G., DeHayes, D.H., Hawley, G.J., Strimbeck, G.R., Cumming, J.R., Murakami, P.F., and Borer, C.H. 2000a. Acid mist and soil Ca and Al alter the mineral nutrition and physiology of red spruce. Tree Physiol. **20**: 73-85.

Schaberg, P.G., Strimbeck, G.R., Hawley, G.J., DeHayes, D.H., Shane, J.B., Murakami, P.F., Perkins T.D., Donnelly, J.R., and Wong, B.L. 2000b. Cold tolerance and photosystem function in a montane red spruce population: physiological relationships with foliar carbohydrates. J. Sustain. For. (In press).

Schaberg, P.G., Snyder, M.C., Shane, J.B., and Donnelly, J.R. 2000c. Seasonal patterns of carbohydrate reserves in red spruce seedlings. Tree Physiol. (In press).

Schulze, E.-D. 1989. Air pollution and forest decline in a spruce (*Picea abies*) forest. Science, **244**: 776-783.

Senser, M., and Beck, E. 1982. Frost resistance in spruce (*Picea abies* (L.) Karst.). V. Influence of photoperiod and temperature on the membrane lipids of the needles. Z. Pflanzenphysiol. Bd. **108**: 71-85.

Sheppard, L.J. 1994. Causal mechanisms by which sulphate, nitrate and acidity influence frost hardiness in red spruce: review and hypothesis. New Phytol. **127**: 69-82.

Sheppard, L.J., Smith, R.I., and Cannell, M.G.R. 1989. Frost hardiness of *Picea rubens* growing in spruce decline regions of the Appalachians. Tree Physiol. **5**: 25-37.

Sheppard, L.J., Cape, J.N., and Leith, I.D. 1993. Influence of acidic mist on frost hardiness and nutrient concentrations in red spruce seedlings. 1. Exposure of the foliage and the rooting environment. New Phytol. **124**: 595-605.

Shortle, W.C., and Smith, K.T. 1988. Aluminum-induced calcium deficiency syndrome in declining red spruce. Science, **240**: 1017-1018.

Smithberg, M.H., and Weiser, C.J. 1968. Patterns of variation among climatic races of red-osier dogwood. Ecology, **49**: 495-505.

Steponkus, P.L. 1990. Cold acclimation and freezing injury from a perspective of the plasma membrane. *In* Environmental injury to plants. *Edited by* F. Katterman. Academic Press, Inc., New York. pp 1-16.

Strimbeck, G.R. 1997. Cold tolerance and winter injury of montane red spruce. Ph.D. thesis. University of Vermont, Burlington, VT, USA.

Strimbeck, G.R., and DeHayes, D.H. 2000. Rapid freezing injury in red spruce: seasonal changes in sensitivity and effects of temperature range. Tree Physiol. **20**: 187-194.

Strimbeck, G.R., Vann, D.R., and Johnson, A.H. 1991. *In situ* experimental freezing produces symptoms of winter injury in red spruce foliage. Tree Physiol. **9**: 359-367.

Strimbeck, G.R., Johnson, A.H., and Vann, D.R. 1993. Midwinter needle temperature and winter injury of montane red spruce. Tree Physiol. **13**: 131-144.

Strimbeck, G.R., Schaberg, P.G., DeHayes, D.H., Shane, J.B., and Hawley, G.J. 1995. Midwinter dehardening of montane red spruce during a natural thaw. Can. J. For. Res. **25**: 2040-2044.

Swan, H.S.D. 1971. Relationship between nutrient supply, growth, and nutrient concentrations in the foliage of white and red spruce. Woodlands Report WR/34. Pulp and Paper Res. Inst. of Canada, Pointe Claire, Canada.

Thornton, F.C., Pier, P.A., and McDuffie, C. Jr. 1990. Response of growth, photosynthesis, and mineral nutrition of red spruce seedlings to O_3 and acidic cloud deposition. Environ. Exper. Bot. **30**: 313-323.

Tobi, D.R., Wargo, P.M., and Bergdahl, D.R. 1995. Growth response of red spruce after known periods of winter injury. Can. J. For. Res. **25**: 669-681.

Tomlinson, G.H. 1993. A possible mechanism relating increased soil temperature to forest decline. Water Air Soil Pollut. **66**: 365-380.

Tranquillini, W. 1982. Frost drought and its ecological significance. *In* Encyclopedia of plant physiology. New Series, Vol. 12 B. *Edited by* O.L. Lange, P.S. Nobel, C.B. Osmond, and H. Ziegler. Springer-Verlag, Berlin. pp. 379-400.

Vann, D.R., Strimbeck, G.R., and Johnson, A.H. 1992 Effects of ambient levels of airborne chemicals on freezing resistance of red spruce foliage. For. Ecol. Manage. **51**: 69-79.

Waite, C.E., and DeHayes, D.H. 1992. Developmental cold tolerance of red spruce and balsam fir provenances. *In* Genetics in forest biology. Proceedings of the 1st Northern Forest Genetics Conference, 23-25 July 1991, Burlington, Vermont. *Edited by* D.H. DeHayes and G.J. Hawley. pp. 99-108.

Waite, C.E., DeHayes, D.H., Rebbeck, J., Schier, G.A., and Johnson, A.H. 1994. The influence of elevated ozone on freezing tolerance of red spruce seedlings. New Phytol. **126**: 327-335.

White, G.J. 1996. Effects of chronic ammonium sulfate treatments on forest trees at the Bear Brook Watershed in Maine. Ph.D. thesis. University of Maine, Orono, ME, USA.

White, P.S., and Cogbill, C.V. 1992. Spruce-fir forests of eastern North America. *In* Ecology and decline of red spruce in the eastern United States. *Edited by* C. Eagar and M.B. Adams. Ecological Studies Vol. 96. Springer-Verlag, Berlin. pp. 3-39.

White, W.C., and Weiser, C.J. 1964. The relation of tissue dessication, extreme cold, and rapid temperature fluctuations to winter injury of American arborvitae. Proc. Am. Soc. Hortic. Sci. **85**: 554-563.

Wilkinson, R.C. 1990. Effects of winter injury on basal area and height growth of 30-year-old red spruce from 12 provenances growing in northern New Hampshire. Can. J. For. Res. **20**: 1616-1622.

Woods, C.M., Polito, V.S., and Reid, M.S. 1984. Response to chilling stress in plant cells. II. Redistribution of intracellular calcium. Protoplasma, **121**: 17-24.