RAPID COMMUNICATION / COMMUNICATION RAPIDE

# Midwinter dehardening of montane red spruce during a natural thaw

G.R. Strimbeck, P.G. Schaberg, D.H. DeHayes, J.B. Shane, and G.J. Hawley

**Abstract**: We documented 3 to 14°C of dehardening in current-year foliage of 10 mature, montane red spruce (*Picea rubens* Sarg.) trees during a natural thaw from 12 to 21 January 1995. Mean cold tolerance was about −47°C before the onset of thaw conditions, and individuals ranged from −38 to −52°C. After 3 days of thaw, mean cold tolerance dropped to −39°C, with a range of −32 to −44°C. Trees did not regain prethaw levels of cold tolerance until sometime between 31 January and 9 February, or 10 to 20 days after subfreezing temperatures resumed. The least cold tolerant tree was at risk of injury when temperature at the field site fell to an estimated −33.8°C on 6 February, and this same tree developed noticeably more injury than other trees when injury symptoms developed in late March. No evidence of dehardening was found in balsam fir (*Abies balsamea* (L.) Mill.) trees from the same stand. All red spruce trees also showed the potential for net assimilation of carbon during the thaw, as determined by measurement of photosynthetic capacity under laboratory conditions. From the abrupt and substantial dehardening and persistence of the dehardened state, we conclude that dehardening during periods of warm weather may be a significant factor in freezing injury and decline of montane red spruce populations.

**Résumé** : Un désendurcissement de 3 à 14°C a été observé sur du feuillage de l'année courante de 10 épinettes rouges matures (*Picea rubens* Sarg.) lors d'un redoux survenu entre le 12 et le 21 janvier 1995. La tolérance au gel des arbres variait entre −38 et −52°C avant le début du dégel avec une moyenne se situant autour de −47°C. Après 3 jours de dégel, la tolérance au gel a diminué entre −32 et −44°C avec une moyenne de −39°C. Les arbres ont retrouvé leur tolérance initiale au gel entre le 31 janvier et le 9 février, soit 10 à 20 jours après le retour des températures gélives. L'arbre le moins tolérant a développé des symptômes de gel à la fin du mois de mars à la suite d'une température de −33,8°C survenue le 6 février. Aucun signe de désendurcissement n'a été observé sur les sapins baumiers (*Abies balsamea* (L.) Mill.) du même peuplement. À l'aide de mesures de capacité photosynthétique faites en laboratoire, les épinettes rouges ont montré un potentiel d'assimilation du carbone pendant le redoux. En nous basant sur l'observation d'un désendurcissement abrupt et important ainsi que sur sa persistance pendant des périodes de redoux, nous concluons que le désendurcissement peut être un facteur significatif occasionnant des dégâts de gel et qui peut entraîner le dépérissement dans des populations d'épinettes rouges.

[Traduit par la Rédaction]

## Introduction

Winter injury to current-year foliage is recognized as a significant factor in the decline of montane populations of red spruce (*Picea rubens* Sarg.) in northeastern North America (Wilkinson 1990; Johnson et al. 1992). Over at least the last 15 years, red spruce has been subject to repeated, severe, region-wide winter injury at intervals averaging 4 years, with more moderate, localized injury occurring in intervening years (DeHayes 1992). It has been shown that red spruce winter injury is caused by subfreezing temperatures rather than desiccation (DeHayes 1992; Strimbeck et al. 1991), and that the maximum midwinter cold tolerance levels for current-year foliage of most red spruce are barely sufficient to protect foliage from minimum temperatures commonly encountered in northern montane habitats (DeHayes 1992). As a result, environmental disturbances that decrease cold tolerance of red spruce by just a few degrees would be expected to result in an

Received September 20, 1995. Accepted October 4, 1995.

**G.R. Strimbeck,[1] D.H. DeHayes, J.B. Shane, and G.J. Hawley.** School of Natural Resources, University of Vermont, Burlington, VT 05405, U.S.A.
**P.G. Schaberg.** USDA Forest Service, Northeastern Forest Experiment Station, Burlington, VT 05401, U.S.A.

[1] Author to whom all correspondence should be addressed.

increased frequency of freezing injury. A number of studies have demonstrated that exposure to acid mist can impair foliar cold tolerance, increasing the likelihood of freezing injury (DeHayes et al. 1991; Fowler et al. 1989; Vann et al. 1992).

Another disturbance that may affect cold tolerance is climatic perturbation, such as unusually warm temperatures in winter, as may be associated with global warming. Red spruce grows in regions where air temperatures typically remain below 0°C for most of the winter, but may be punctuated by thaws (intervals of a few hours to several days when temperatures rise above 0°C), which allow melting and mobilization of water in some or all plant parts, including foliage and fine branches of evergreen species such as red spruce. Brief daytime thaws appear to have little effect on red spruce cold tolerance (Perkins et al. 1993), but continuous thaw conditions for 2 or more days may stimulate precocious dehardening. Injury could occur if a prolonged thaw is followed by extreme cold or if foliage is subjected to other kinds of cold stress such as rapid freezing (Perkins and Adams 1995) or repeated cycles of freezing and thawing (J.L. Hadely, personal communication; W.H. Livingston, personal communication). Analysis of local climatic data, discussed in more detail below, reveals thaws of unprecedented magnitude during the last 15 years, including extreme events in the winters of 1981 and 1984, coincident with two of the last five severe red spruce winter injury events. Given the increased severity of winter thaws and frequency of red spruce freezing injury events over the past few decades, examination of the propensity for and extent of winter dehardening in red spruce seems particularly pertinent.

In laboratory experiments, DeHayes (1992) demonstrated that current-year foliage of red spruce, but not balsam fir (*Abies balsamea* (L.) Mill.), seedlings dehardens by 5 to as much as 14°C when exposed to simulated winter thaws of 5 or 10°C for 5 days. Seedlings that dehardened did not redevelop full cold tolerance after 5 days exposure to ambient subfreezing winter temperatures. DeHayes et al. (1990) observed rapid dehardening of excised branches from mature red spruce trees after 18 or 45 h of storage at 24 and 15°C, respectively. While these studies offer data on response of red spruce to simulated thaws, it is presently unknown whether natural thaws in montane environments result in a sufficient loss of cold tolerance to enhance the susceptibility of current-year needles of red spruce to freezing injury.

We had an opportunity to quantify the extent of winter dehardening in foliage of native red spruce trees during the 1994–1995 winter. We had generated detailed cold tolerance base-line curves throughout late fall and winter as part of an intensive winter water relations – cold tolerance study in native montane red spruce and were, therefore, able to accurately quantify changes in cold tolerance in response to a dramatic natural thaw that occurred in January 1995. In this paper, we report the winter cold tolerance of 10 native red spruce trees and quantify the short-term and long-term cold tolerance response of those trees to an extreme and extended winter thaw. We also demonstrate that during thaws montane trees have the capacity for net photosynthesis under favorable conditions.

## Methods

Foliar samples for cold tolerance assessments were collected from mature red spruce trees growing at approximately 1000 m elevation on a southeast-facing slope of Mount Mansfield, Vermont. We collected three sun-exposed foliar samples from the canopy of each of 10–12 trees, on each of 17 dates at approximately 2-week intervals between 1 October 1994 and 8 May 1995. In January 1995, during an extreme thaw event, we collected samples 3–4 days apart. Samples were packed in snow, transported to the laboratory, and prepared for controlled freezing within 12 h of collection, and freezing treatments were completed within 36 h of collection. Current-year needles were sliced into 5-mm sections to prepare a single bulk sample for each branch. Subsamples were measured volumetrically (approximately 0.1 to 0.3 mL) into 64 cell styrene trays for freezing. Freezing was performed at $\leq 6°C \cdot h^{-1}$ in steps of usually 5°C over a range from −10 or −15°C to as low as −90°C, for a total of up to 16 test temperatures per date. Freezer temperature was held at least 15 min at each test temperature, after which one replicate tray was removed from the freezer, placed in a precooled styrene foam container, and transferred to a separate freezer at −5°C. After all trays equilibrated at −5°C, they were transferred in foam containers to a refrigerator at 4°C and held until thawed. A mild detergent solution (3.5 mL of 0.25% v/v Triton X-100 – deionized water) at 4°C was added to each cell, and sample trays were held in a high-humidity cabinet at room temperature for 8 ± 1 h. Initial conductivity of the effusate was measured using a multielectrode instrument (Wavefront Technology, Ann Arbor, Michigan), then samples were dried at least 16 h at 50°C, soaked in fresh detergent solution for 24 ± 1 h, and the final conductivity was measured. Relative electrolyte leakage, a relative measure of cell injury, was calculated as the proportion of initial to final conductivity.

Critical temperature ($T_c$), an estimate of the warmest subfreezing temperature producing significant injury (DeHayes and Williams 1989), was calculated for each branch. Although no laboratory procedure can be an exact predictor of winter injury occurring in the field, midwinter $T_c$ has been strongly correlated with severity of winter injury in the spring and has been effective in discriminating cold tolerance differences between provenances or experimental treatments in a variety of studies (DeHayes 1992). Critical temperature data for 10 trees over 10 sample dates between 28 November 1994 and 21 February 1995 were analyzed for significant changes in $T_c$ associated with the January thaw. The raw data were transformed as $-T_c^{-0.25}$ to reduce heteroscedascity between dates. Repeated measures analysis of variance and Duncan's multiple range test were used to assess differences in $T_c$ between dates. Details of the analysis of variance are as follows: 10 dates × 10 trees × 3 branches per tree, with 2 missing values, for a total of 298 observations; treatment and error degrees of freedom and sums of squares, respectively: 9 and 0.019 54 for date; 9 and 0.019 48 for tree; 280 and 0.019 69 for error; model $R^2 = 0.67$.

On 3 dates during and 3 dates after the thaw, photosynthesis and transpiration were also monitored for two shoots per tree using a laboratory-based LI-6262 $CO_2$ /$H_2O$ IRGA (LI-COR Inc., Lincoln, Nebraska, U.S.A.) in an open system configuration. Gas exchange measurements were made within 12 h of sample collection. Sample shoots were recut under water and attached to a continuous supply of distilled water through latex tubing. Air temperatures within the cuvettes were maintained at approximately 20°C, and three 110–130 V (300 W) metal halogen lamps were used to provide near-saturating photon flux densities (PFD ≥ 500 $\mu mol \cdot m^{-2} \cdot s^{-1}$). Three cuvettes were sequentially monitored for 1-min intervals for a total of 40 min. A nonlinear curve fitting procedure was used to fit the Michaelis–Menten equation, giving an estimate of the stabilized maximum assimilation rate ($A_{max}$) for each shoot.

Daily maximum and minimum temperatures at the field site were estimated by linear interpolation, using National Oceanic and Atmospheric Administration, National Weather Service, data for the south summit of Mount Mansfield (1250 m) and data from a long-term monitoring site at 400 m on the west slope of Mount Mansfield (Vermont Monitoring Cooperative). To examine long-term trends in frequency and severity of winter thaws, climatic data for Mount Mansfield (1961–1995) and Burlington, Vermont (1920–1995), were obtained from the Northeast Regional Climate Center, Cornell University, Ithaca, New York. In the Mount Mansfield record, missing values were estimated by DeGaetano et al. (1995), and temperatures were adjusted to 1000 m elevation using a lapse rate of 6.5°C·$km^{-1}$. We calculated thawing degree-days (TDD) for 16 December through the end of February of each year from the mean of daily maximum and minimum temperature, only for days when mean temperature was >0°C. These were summed for periods when temperatures remained continuously above freezing to give an estimate of TDD accumulating during any one thaw event.

## Results and discussion

### Climatic conditions

As is typical for northern New England winters, maximum and minimum temperatures in the sample stand were consistently below 0°C most days from early December 1994 through early January 1995 (Fig. 1). In mid-January, a 9-day warm spell set a number of new climatic records throughout northern New England and adjacent Canada. From 12 through 21 January, estimated maximum temperatures in the sample stand were greater than 0°C and reached a maximum of 11°C on 15 January (Fig. 1). Estimated maximum and minimum stand temperatures remained above 0°C during the initial 4 days of the thaw and subsequently hovered between daylight maxima of about 2 to 7°C and night minima of 0 to −5°C. On the third day of the thaw, the estimated stand temperature remained above 5°C for at least 24 h. After 21 January, maximum and minimum temperatures were again consistently below 0°C for most of the next 2 months.

The 1995 thaw is one of a cluster of extreme thaw events occurring in the last 15 years. We estimate 31 cumulative TDD for the first peak of the thaw, and an additional 7 TDD for the second peak (Fig. 1). In the Mount Mansfield record for 1961–1980, only one thaw event exceeded 10 cumulative TDD (13 TDD over 4 days in December 1964). There were five events of >10 TDD between 1981 and 1995, including events of >30 TDD in 1981 (35 TDD over 9 days), 1984 (32 TDD over 8 days), and 1995 (31 TDD over 5 days). Data for Burlington, Vermont, for 1921–1995 also show thaws of unprecedented magnitude in the last 15 years.

### Red spruce cold tolerance response

The 10 sample trees varied significantly and consistently in cold tolerance of current-year foliage throughout fall and early winter. By 1 October, shortly after the first frost of the season, the average $T_c$ of the 10 trees was −23.7°C (range −27.1 to −17.3°C). All trees reached their maximum depth of cold tolerance by mid-December (Fig. 1). Prior to the thaw, midwinter cold tolerance (defined as the average of $T_c$ on 14 and 28 December and 10 January) for the 10 trees averaged −47°C and ranged from −37 to −52°C.

Three days after the onset of continuous thaw conditions (21 TDD), current-year foliage from all trees experienced a significant reduction in $T_c$ that averaged 8.6°C (Fig. 1). Dehardening varied considerably among trees, and there was a slight tendency for trees with relatively high prethaw $T_c$ to deharden the least. After 3 days of thaw, dehardening (calculated as the difference between mean $T_c$ on the three dates prior to thaw and two dates during the thaw) ranged from 2.6 to 14.1°C among the 10 trees as follows:

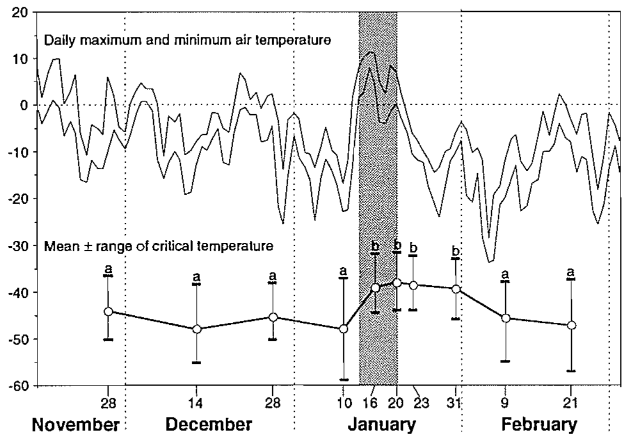
| Prethaw $T_c$ (°C) | Dehardening (°C) |
|---|---|
| −37.9 | 6.1 |
| −41.2 | 5.6 |
| −42.0 | 2.6 |
| −48.7 | 10.3 |
| −49.0 | 14.1 |
| −49.4 | 9.6 |
| −49.5 | 8.6 |
| −50.3 | 10.6 |
| −50.7 | 6.4 |
| −52.0 | 12.2 |

Despite a continuation of mild temperatures for 6 additional days, no appreciable or significant additional dehardening was evident in any of the trees (Fig. 1). Seven days after the onset of the thaw, the average critical temperature of the 10 trees was −37°C, and the range was −32 to −40°C.

During the thaw and associated period of dehardening, all trees were capable of net photosynthesis when favorable temperature and light conditions were supplied. Three days into the thaw, the mean $A_{max}$ was 1.0 $\mu mol \cdot m^{-2} \cdot s^{-1}$, which is about 17% of growing season rates measured in the field. By 9 February, mean $A_{max}$ was −0.8 $\mu mol \cdot m^{-2} \cdot s^{-1}$ (samples were net respiring), similar to $A_{max}$ of lowland trees after prolonged exposure to subfreezing temperatures (P.G. Schaberg, unpublished data). The photosynthetic data confirm that mild temperature conditions initiate physiological changes beyond the cold tolerance response, as found in lowland saplings (Schaberg et al. 1995).

The partially dehardened condition of current-year foliage persisted for at least 10 and perhaps as many as 19 days

**Fig. 1.** Midwinter mean, maximum, and minimum critical temperature ($T_c$) of current-year foliage from 10 trees, and estimated daily maximum and minimum temperatures at the elevation of the sample stand. 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 Duncan's multiple range test.




after consistently subfreezing temperatures resumed. Cold tolerance assessments on 23 and 31 January revealed average $T_c$ of −38 and −39°C, respectively. Critical temperatures did not drop to prethaw levels until 9 February, when the average $T_c$ for the 10 trees was −46°C and the range was −38 to −54°C. It is clear that there is a substantial lag in rehardening following a midwinter thaw and, therefore, an extended period of susceptibility to freezing injury by temperatures that would not otherwise cause freezing injury to fully hardened red spruce foliage. Both the abrupt dehardening and delayed rehardening are consistent with the findings of DeHayes (1992), who studied the response of red spruce seedlings to simulated midwinter thaws.

The estimated winter minimum site temperature of −34°C occurred on 6 February, or about 16 days after the thaw ended. On 31 January, the mean $T_c$ of the least cold tolerant tree was −33°C and three additional trees had $T_c$ in the −36 to −38°C range. Therefore, our data suggest that at least one tree was at risk of injury (Fig. 1). Visible symptoms of injury, a distinctive red–brown necrosis of current-year foliage, began appearing on trees at the Mount Mansfield site with the onset of longer and warmer days in March. Overall injury levels were slight in comparison to previous years, affecting <20% of current-year shoots in the most injured trees, with no visible injury in others. The least cold tolerant tree showed the greatest amount of injury, while injury was scant or absent in the other trees in the study.

These results demonstrate that dehardening by as much as 14°C can occur in montane red spruce populations in response to as little as 3 days of thaw conditions during midwinter, and that complete rehardening may be delayed for nearly 20 days after the thaw. Our results also suggest that this natural precocious dehardening phenomenon may be unique to red spruce. Although we did not monitor prethaw cold tolerance levels of balsam fir continuously during the 1994–1995 winter, we did assess the cold tolerance of four balsam fir trees from the same site on three dates during the thaw. $T_c$ for these trees was no higher than −70°C, and most balsam fir showed no detectable damage at −90°C.

Examination of climatic records shows that midwinter thaws cannot have played a role in all of the recent episodes of severe injury (Tobi et al. 1995), and the thaw and associated dehardening did not result in severe freezing injury this year. However, it is possible that the effects observed here, when coupled with predisposing factors such as air pollution stress, with extreme cold following a thaw, or with other forms of cold stress such as rapid freezing or repeated cycles of freezing and thawing, could play a role in the repeated episodes of severe injury observed in recent decades and could become increasingly important if global warming increases the frequency or severity of midwinter thaw events.

## Acknowledgments

This research was supported by funds from the Plant Responses to the Environment program of the National Research Initiative Competitive Grants Program, the USDA Forest Service Northern Global Change Program, and McIntyre–Stennis Research Program project MS-0075. C. Brown assisted extensively in the field and laboratory, and P. Cali, M. Kelley, D. Montminy, and A. Burfeind assisted in the laboratory.

## References

DeGaetano, A.T., Eggleston, K.L., and Knapp, W.W. 1995. A method to estimate daily maximum and minimum temperature observations. J. Appl. Meteorol. **34**: 371–380.

DeHayes, D.H. 1992. Winter injury and developmental cold tolerance of red spruce. *In* Ecology and decline of red spruce in the eastern United States. *Edited by* C. Eagar and M.B. Adams. Springer-Verlag, New York. pp. 295–337.

DeHayes, D.H., and Williams, M.W. 1989. Critical temperature: a quantitative method of assessing cold tolerance. USDA For. Serv. Gen. Tech. Rep. NE-134.

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.

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.

Johnson, A.H., McLaughlin, S.B., Adams, M.B., Cook, E.R. DeHayes, D.H., Eagar, C., Fernandez, I.J., Johnson, D.W., Kohut, R.J., Mohnen, V.A., Nicholas, N.S., Peart, D.R., Schier, G.A., and White, P.S. 1992. Synthesis and conclusions from epidemiological and mechanistic studies of red spruce decline. *In* Ecology and decline of red spruce in the eastern United States. *Edited by* C. Eagar and M.B. Adams. Springer-Verlag, New York. pp. 365–411.

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

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.

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.

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.

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.

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.