

Physiological changes in red spruce seedlings during a simulated winter thaw

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Summary We evaluated net photosynthesis, respiration, leaf conductance, xylem pressure potential (XPP) and cold hardiness in red spruce (*Picea rubens* Sarg.) seedlings exposed to either a continuous thaw (CT) or a daytime thaw with freezing nights (FN) for 8 days during mid-winter. Physiological differences between CT and FN seedlings were evident for all measured parameters. However, the temporal expression of treatment differences varied among parameters. When compared to FN seedlings, CT seedlings had higher rates of respiration following 24 h of treatment, and a higher net photosynthetic rate, leaf conductance and XPP after 48 h of treatment. The CT seedlings were significantly less cold tolerant than the FN seedlings following 4 days of thaw, whereas FN seedlings did not deharden over the 8 days of treatment. Examination of temporal trends among thaw-associated changes in physiology suggested that, although greater carbon exchange occurred as stomatal conductance increased, the transition from negative to positive net photosynthesis was not the result of increases in conductance, but may have been associated with thaw-induced increases in XPP. Because thaw-associated changes in gas exchange and cold hardiness were offset in time, we conclude that, if changes in these processes are physiologically linked, the linkage is indirect.

Keywords: cold hardiness, photosynthesis, *Picea rubens*, respiration, stomatal aperture, winter thaw, xylem pressure potential.

Introduction

Winter temperatures in the boreal forest region of the northern USA and Canada are not continuously low. Mid-winter thaws have become increasingly common and, at times, prolonged. For example, NOAA records for Burlington, VT indicate that during the months of December through March, winter thaw events (defined as four or more consecutive days with temperature maxima above freezing) have occurred with an average frequency of 1.4 per month during the past decade. This is an

increased frequency of approximately 30% compared to averages for the previous three decades.

Winter thaws induce at least two types of change in the physiology of red spruce (*Picea rubens* Sarg.): (1) plants may partially deharden; and (2) plants may increase their rates of net photosynthesis. Strimbeck et al. (1995) found that current-year foliage of montane red spruce dehardened an average of 9 °C during a 9-day natural thaw and the dehardened state persisted for up to 19 days during which plants experienced repeated subfreezing temperatures. DeHayes (1992) reported that red spruce seedlings can deharden as much as 12–14 °C in response to prolonged (5-day) exposure to temperatures of 5–10 °C, whereas an associated species, balsam fir (*Abies balsamea* (L.) Mill.), did not deharden under the same climatic conditions. Reductions in cold tolerance are important because the maximum winter cold hardiness attained by red spruce is barely sufficient to prevent freezing injury at the lowest temperatures encountered during most winters (DeHayes 1992). Although the vulnerability of fully hardened red spruce to injury from low temperatures has been questioned (Perkins et al. 1993), precocious dehardening could greatly increase the risk of freezing injury if a thaw is followed by low temperatures, or if foliage is subjected to other types of cold stress such as rapid freezing (Perkins and Adams 1995) or repeated cycles of freezing and thawing (J.L. Hadley, Harvard Forest, Harvard University, personal communication).

Red spruce can photosynthesize in winter during prolonged thaws (Schaberg et al. 1995). Assimilation during thaws could be responsible for increases in total carbohydrates and foliar sugars reported in red spruce foliage during winter (Snyder 1990). Although the physiological importance of winter assimilation in red spruce is unknown, winter carbon capture may benefit leaves by augmenting energy stores at a time when remote carbon reserves are unavailable because of stem freezing.

Thaw-associated changes in the winter physiology of northern conifers, such as red spruce, have received little attention. Considered in the context of potential global warming and

increases in the number or duration of winter thaws, the consequences of thaw-related changes in physiology could affect the overall health and survival of boreal montane red spruce. Examining the potential association between changes in cold hardiness and carbon exchange is an important first step in determining the overall impact of thaw-related alterations on conifer physiology. We evaluated carbon assimilation, respiration, foliar conductance of water vapor, shoot xylem pressure potentials (XPP) and foliar cold hardiness of potted red spruce seedlings during a simulated mid-winter thaw to determine the temporal relationship between thaw-induced changes in photosynthesis and cold hardiness and also to examine possible physiological connections among these processes.

Methods

Plant material

In September 1993, 93 five-year-old red spruce seedlings, were transplanted to 3-liter plastic pots containing coarse sand. Plant material was kept outdoors at the Northeastern Forest Experiment Station in South Burlington, VT so that natural winter hardening could take place. Pots were insulated with perlite to simulate a natural soil environment and protect roots from low air temperatures. Seedlings remained outdoors until the initiation of thaw treatments in late January 1994.

Simulated thaw treatments

In late January 1994, the seedlings were removed from the perlite insulation. Gas exchange rates, cold hardiness and XPP of nine seedlings were measured immediately. The remaining 84 seedlings were randomly assigned to one of two simulated thaw treatments: half were assigned to a continuous-thaw (CT) treatment and half were assigned to a freezing-night (FN) treatment. The CT seedlings were placed in one of two plant growth chambers (Sherer Scientific Products, Asheville, NC) maintained at approximately 10 °C, with an 8-h light period and a photosynthetic photon flux density (PPFD) of approximately 400 $\mu\text{mol m}^{-2} \text{s}^{-1}$. The FN seedlings were placed in the same chambers during the 8-h light period, but were moved to a freezer maintained at approximately -3 °C for a 16-h dark period following each thaw exposure. Soil temperature at the center of pots was recorded at the end of each light and dark period using six T-type soil thermocouples in each treatment group. Soils of CT seedlings thawed within 24 h of treatment initiation and reached an equilibrium with air temperature (10.6 ± 0.4 °C) within 48 h. In contrast, soils of FN seedlings oscillated between being thawed (6.8 ± 0.7 °C by the end of light periods in the growth chamber) or frozen (-0.2 ± 0.4 °C by the end of dark periods in the freezer). At the end of each light period, seedlings were watered to excess.

Treatment temperatures were more uniform, but no less extreme than field temperatures. Records kept by the NOAA for Burlington, VT indicate that air temperatures can remain continually above 0 °C and reach maxima over 10 °C during protracted winter thaws. Moreover, cumulative heat loading (the amount and duration of exposure to temperatures above 0 °C) in the field can greatly exceed that to which the CT

seedlings were exposed. For example, during a thaw in January 1995, except for a 12-h period during which temperatures dipped to -1.1 °C, air temperatures remained above freezing for 10 days and reached a high of 18.3 °C. Because of their limited thermal capacity, the soil-filled pots used in our study may have thawed more rapidly than frozen forest soils. However, forest soils often remain unfrozen during winter even in the absence of a thaw, especially when snow cover is present (Kimball et al. 1973).

Gas exchange and water relations

Gas exchange was measured for nine seedlings immediately before the imposition of thaw treatments and for nine seedlings from each treatment on Days 2, 4, 6 and 8 following the onset of treatment. In addition, gas exchange was measured for six seedlings in each treatment group after 1 day of treatment. Gas exchange measurements were taken indoors (at approximately 21 °C) with an LI-6200 portable photosynthesis instrument with a 0.25-liter cuvette (Li-Cor Inc., Lincoln, NE). The LI-6200 was calibrated each day using a span gas CO₂ concentration of 310 ppm. Zero settings of the IRGA and flow meter were adjusted regularly between sample measurements. Because regular occurrences of low or negative rates of net photosynthesis were anticipated, a 30-s time-interval measurement of gas exchange was used rather than a fixed-concentration drawdown. Gas exchange measurements were taken on one intact current-year shoot (5-cm long) from the upper 1/3 of the crown of each seedling. Branches were maintained in their natural orientations during measurement. A 110-130V/300W metal halogen lamp (General Electric Company, Cleveland, OH) was used to provide near-saturating photon flux densities (PPFD $\geq 500 \mu\text{mol m}^{-2} \text{s}^{-1}$, Alexander et al. 1995). Respiration measurements were made on the same branch approximately 1 min after darkening the cuvette with a doubled layer of cloth. Preliminary measurements indicated that dark respiration rates of red spruce reach a steady state within 1 min and remain steady for up to 10 min (cf. McLaughlin et al. 1990). Leaf conductance (mm s^{-1}) was calculated for each CO₂ exchange measurement from LI-6200-generated transpiration and temperature measurements as described by Schaberg et al. (1995).

Following gas exchange measurement, sample shoots were harvested and XPP was measured with a PMS model 1001 portable pressure chamber (PMS Instrument Co., Corvallis, OR) as described by Ritchie and Hinckley (1975). To adjust photosynthetic and respiration rates to a projected area basis, projected needle areas from gas exchange samples were measured with a Li-Cor Model LI-3100 area meter following XPP determination.

Cold tolerance

Following gas exchange and XPP measurements, the cold tolerance of the remaining current-year foliage was determined. Current-year foliage from each seedling was rinsed in iced distilled water, then chopped in 0.5-cm sections to prepare a single bulk sample. Subsamples were measured volumetri-

cally (approximately 0.3 ml or 0.1 to 0.2 g) and transferred to 64-cell styrene trays for freezing treatment in a computer-controlled liquid nitrogen freezer (Model KRYO 10, TS Scientific, Perkasi, PA). Freezing rate was $6\text{ }^{\circ}\text{C h}^{-1}$. Trays were held at eight to 12 preselected test temperatures, then removed from the freezer and placed in precooled styrene foam containers to thaw slowly to $4\text{ }^{\circ}\text{C}$.

Freezing injury was assessed by electrolyte leakage as determined by conductivity measurements. Conductivity data were used to calculate critical temperatures (the highest temperature at which freezing injury can be detected) for each bulk sample per seedling. Critical temperatures have been shown to be strongly correlated with field freezing injury and effective in discerning cold tolerance differences among imposed treatments, plant tissues, and needle age classes (DeHayes 1992). For detailed descriptions of freezing procedures and critical temperature calculations refer to DeHayes et al. (1990) and DeHayes and Williams (1989).

Statistical analyses

Analyses of variance were used to examine the significance of differences between treatments for the entire sampling period and for individual days. Correlation analyses were used to evaluate relationships among gas exchange, stomatal conduc-

tance, critical temperature and XPP measurements. Data were transformed when necessary to satisfy statistical assumptions. Unless otherwise noted, differences were considered statistically significant if $P \leq 0.05$.

Results and discussion

Gas exchange

Average rates of net carbon assimilation by seedlings in both treatments were negative and did not differ from one another during the first 24 h of treatment (Figure 1A). However, after 48 h, treatment means differed; the net photosynthetic rate of CT seedlings was approximately $0\text{ }\mu\text{mol m}^{-2}\text{ s}^{-1}$, whereas the net photosynthetic rate of FN seedlings remained negative. As the thaw progressed, the magnitude of the difference between treatments increased as the net photosynthetic rate increased in CT seedlings, but plateaued just below zero in FN seedlings. Following eight days of thaw, the average rate of photosynthesis for CT seedlings was approximately 45% of the average rate reported for red spruce in the field during the growing season (Gage and DeHayes 1992). During the later part of the thaw, photosynthetic rates for some individual CT seedlings approximated growing season rates. Although average rates of net photosynthesis for FN seedlings never exceeded $0\text{ }\mu\text{mol}$

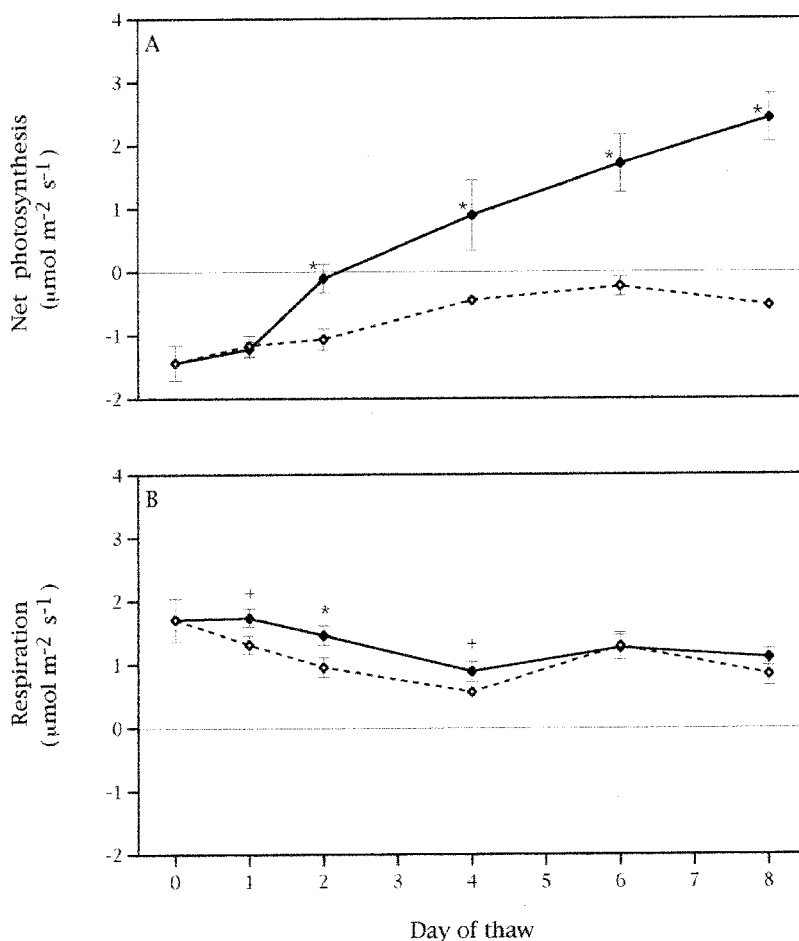


Figure 1. Average rates of net photosynthesis (A) and respiration (B) in the continuous-thaw (CT) and freezing-night (FN) treatments during an 8-day simulated thaw. Reported differences of $P > F = 0.05$ denoted by * and $P > F = 0.10$ by + between CT and FN means are the result of analyses of variance for data from individual days. Error bars are ± 1 SE of the treatment mean.

$\text{m}^{-2} \text{s}^{-1}$, a partitioning of the sums of squares for the day \times treatment interaction showed that rates of net photosynthesis for FN seedlings were significantly higher on Day 8 ($-0.54 \mu\text{mol m}^{-2} \text{s}^{-1}$) than on Day 0 ($-1.44 \mu\text{mol m}^{-2} \text{s}^{-1}$). Hadley et al. (1993) reported that cut and rehydrated red spruce shoots exposed to 20/12 °C day/night temperatures and a 12-h photoperiod exhibited more rapid increases in net photosynthetic rate than those we observed.

Although differences between treatments in dark respiration rate were small (Figure 1B), CT seedlings had significantly higher rates of respiration than FN seedlings on Day 1 ($P = 0.06$, $n = 5$), Day 2 ($P = 0.03$, $n = 9$) and Day 4 ($P = 0.08$, $n = 9$) of the thaw treatment. Because respiration rates are temperature dependent, and because rates for both CT and FN seedlings were measured at the same temperature (average cuvette temperature was 21 °C), our measurements do not fully account for likely differences in respiration attributable to differential temperature exposure; differences in respiration were almost certainly greater during periods when FN seedlings were exposed to -3 °C while CT seedlings were held at 10 °C. Observed respiration rates were high relative to winter rates measured in the field (typical mid-winter values for red spruce average about $0.3 \mu\text{mol m}^{-2} \text{s}^{-1}$, unpublished data). It is likely

that the high rates of respiration in this study were the result of the mild thaw and the cuvette temperatures. Partitioning of the sums of squares for the day \times treatment interaction revealed that, for both CT and FN seedlings, respiration rates were significantly lower on Day 8 than on Day 0.

Leaf conductance and XPP

Average leaf conductance for FN seedlings remained below 0.5 mm s^{-1} throughout the study (Figure 2A), suggesting that there was partial stomatal closure in FN seedlings throughout the study (cf. Nobel 1991). Although leaf conductances were higher for CT seedlings than for FN seedlings on Days 2, 6 and 8, leaf conductances for CT seedlings on Day 2 remained close to 0.5 mm s^{-1} .

Xylem pressure potentials were low at the initiation of the study, but CT seedlings experienced a large increase in XPP within 48 h (to values that were maintained throughout the study) (Figure 2B). Because the 48-h measurement of XPP was the first performed following thaw inception, it is not known when the initial increases in XPP in CT seedlings occurred. If low shoot XPP resulted from limitations in water uptake caused by freezing of the soil in the pots, then XPP may

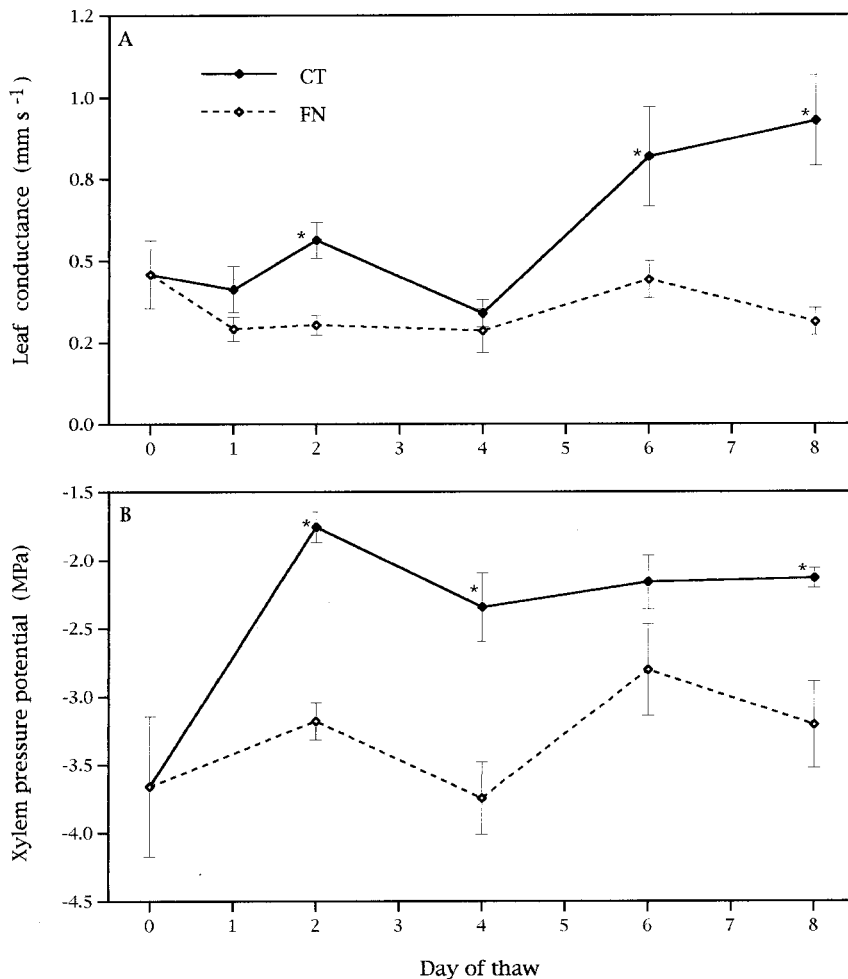


Figure 2. Average leaf conductance and xylem pressure potential (XPP) in the continuous-thaw (CT) and freezing-night (FN) treatments during an 8-day simulated thaw. Reported differences of $P > F = 0.05$ denoted by * between CT and FN means are the result of analyses of variance for data from individual days. Error bars are ± 1 SE of the treatment mean.

have increased before the 48-h measurement because soils of CT seedlings thawed within 24 h. The FN seedlings showed a smaller and more variable increase in XPP over time, and average XPP values for these seedlings were never higher than -2.8 MPa (Figure 2B). Anderson and McLaughlin (1991) reported that XPP for red spruce in the field can drop below -2.9 MPa during winter. However, Sperry and Tyree (1990) found that red spruce can experience a large increase in xylem embolism at similar xylem tensions (approximately -2.8 to -4.2 MPa). Based on the low XPP of many of our seedlings, it seems likely that at least some seedlings experienced a reduction in hydraulic conductivity as a result of xylem embolism. Even if some embolism occurred, however, any associated loss in hydraulic conductivity did not prevent the partial rehydration of CT shoots.

Cold hardiness

Seedlings were initially hardy to approximately -47 °C, and hardiness did not change significantly during the first 48 h of treatment (Figure 3). Differences between treatments were evident following 4 days of thaw; CT seedlings dehardened to approximately -35 °C, whereas hardiness of FN seedlings remained essentially unchanged. These treatment differences persisted and average hardiness ratings were -33 °C for CT seedlings and -44 °C for FN seedlings on Day 8. Although CT seedlings dehardened an average of 14 °C for the thaw overall, partitioning of the sums of squares for the day \times treatment interaction using contrasts revealed that hardiness in FN seedlings was not significantly different on Days 0 (-47 °C) and 8 (-44 °C).

Temporal differences in gas exchange, leaf conductance and XPP for CT seedlings

The changes in leaf conductance were not associated with the physiological transition from negative to positive net photosynthesis. Although net photosynthesis and leaf conductance

were significantly correlated for the thaw as a whole ($r = 0.44$, $P = 0.01$), when evaluated on an individual-day basis, associations between these parameters changed over time. Net photosynthesis and leaf conductance were not correlated during the initial days of the thaw (Day 1: $r = -0.29$, $P = 0.58$ and Day 2: $r = 0.08$, $P = 0.83$), but were significantly correlated thereafter (Day 4: $r = 0.77$, $P = 0.01$, Day 6: $r = 0.91$, $P = 0.01$, and Day 8: $r = 0.74$, $P = 0.04$). The transition from negative to positive net photosynthesis occurred during the initial phases of the thaw, the same period during which net photosynthesis and leaf conductance were not correlated. Although average net photosynthesis went from negative values on Day 2 to positive values on Day 4, average leaf conductance decreased between these dates, confirming that changes in stomatal aperture were not responsible for the changes in net photosynthetic rate.

It is assumed that the primary mechanism by which stomatal aperture influences the rate of photosynthesis is by regulating the availability and flow of CO_2 in and out of leaves (Nobel 1991). High stomatal resistance restricts CO_2 diffusion, creating a lag in CO_2 supply that can become rate limiting to photosynthetic carbon capture. Because our seedlings had negative net assimilation rates during the early stages of the thaw (through Day 2), CO_2 concentrations within needles must have been greater than the ambient CO_2 concentration. Thus, CO_2 availability (and stomatal aperture) did not limit photosynthesis during this period. On the contrary, because stomata can close when internal CO_2 concentrations are high (Nobel 1991), the negative net photosynthetic rates of seedlings during the early stages of the thaw may have been responsible for the low stomatal conductance (Wong et al. 1979).

Although the transition from negative to positive net photosynthesis was not related to changes in leaf conductance, the transition may have been associated with changes in XPP. The large increase in XPP on Day 2 for CT plants (Figure 2A) was coincident with the initial increase in net photosynthesis in this treatment (Figure 1A). Xylem pressure potential and net photosynthesis in CT seedlings were also positively correlated for the thaw as a whole ($r = 0.49$, $P = 0.01$) and for the first 2 days

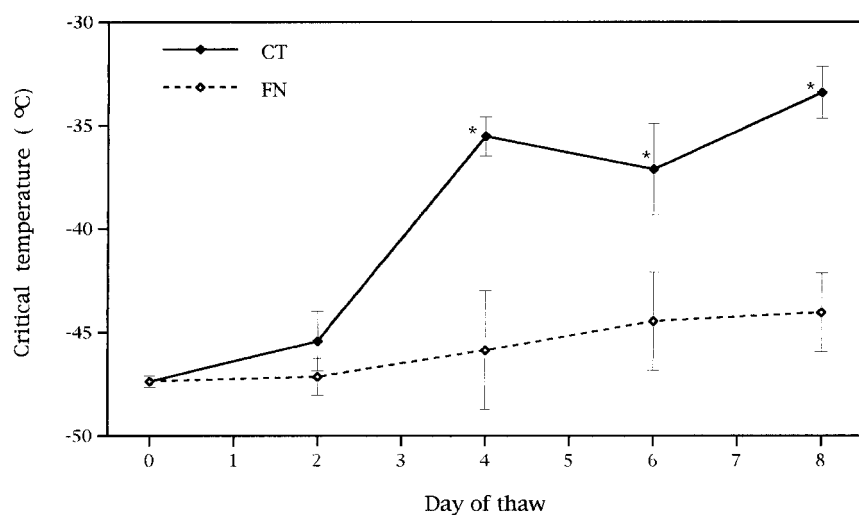


Figure 3. Average critical temperatures in the continuous-thaw (CT) and the freezing-night (FN) treatments during an 8-day simulated thaw. Reported differences of $P > F = 0.05$ denoted by * between CT and FN means are the result of analyses of variance for data from individual days. Error bars are ± 1 SE of the treatment mean.

($r = 0.58$, $P = 0.04$) and 4 days ($r = 0.51$, $P = 0.02$) of thaw (the transition from a period of negative net photosynthesis to one of positive net photosynthesis), but were not correlated thereafter. Numerous reports document nonstomatal limitations to photosynthesis associated with low water availability (Boyer 1971, Mohanty and Boyer 1976, Sharkey and Badger 1982, Teskey et al. 1986, Kaiser 1987). These limitations may result from reductions in electron transport (Mohanty and Boyer 1976, Epron and Dreyer 1992), photophosphorylation (Keck and Boyer 1974, Sharkey and Badger 1982), Calvin cycle or more generalized enzyme activity (Kaiser 1987, Martin and Ruiz-Torres 1992) or mesophyll conductance (Beadle et al. 1981, Grieu et al. 1988). Reductions in mesophyll conductance may become particularly limiting for red spruce as XPP drops below -2.0 MPa (Beadle et al. 1981).

Temporal differences in cold hardiness and gas exchange for CT seedlings

Critical temperatures were significantly correlated with net photosynthesis ($r = 0.65$, $P = 0.01$) and respiration ($r = -0.33$, $P = 0.04$) for CT seedlings. However, treatment-associated changes in gas exchange were evident earlier than treatment differences in cold hardiness (Figures 1 and 3). It is possible that thaw-associated changes in gas exchange and critical temperature were independent and that these parameters were correlated only because both responded to the same environmental factors. Nevertheless, physiological links between photosynthesis and cold tolerance involving changes in the lipid compositions of membranes (Öquist 1982) and alterations in foliar sugar concentrations (Parker 1963) have been proposed.

Öquist (1982) suggested that cold-induced changes in thylakoid lipid content could alter the miscibility, and thus either the mobility or the content of plastoquinone in thylakoid membranes (Öquist 1982), which, in turn, could account for reductions in the photosynthetic electron transport of conifers during winter (Öquist 1982, Öquist 1983, Öquist and Ögren 1985). However, if changes in membrane structure dominated the responses of seedlings in our study, then increases in photosynthesis would have occurred simultaneously with (if only membrane changes were involved), or following (if changes in other components such as plastoquinone were involved), reductions in cold hardiness. We found that increases in net photosynthesis preceded measured reductions in cold hardiness.

High foliar sugar concentrations during winter may enhance cold hardiness and limit photosynthetic capacity (Parker 1963). Thaw-induced increases in starch synthesis and respiration might, therefore, be expected to result in a reduction in foliar sugar concentrations, followed first by enhanced photosynthetic rates, then by dehardening. Experimental evidence suggests that osmotic adjustments associated with high sugar concentrations may provide limited protection from freezing (Yelenosky and Guy 1989). However, this direct osmotic impact may be less important in enhancing cold hardiness than sugar's cryoprotective impact on cellular components or its general contributions to energy metabolism (Sakai and Larcher 1987). Mechanisms by which high foliar sugar concentrations could limit photosynthesis by end-product inhibi-

tion have also been outlined (Foyer 1987, Stitt and Quick 1989, Krapp et al. 1993). Experimental evidence supporting these mechanisms has been obtained in studies with herbaceous plants (Foyer 1988, Stitt et al. 1988) and cell cultures (Krapp et al. 1993).

It has been found that starch grains can accumulate within the chloroplasts of red spruce needles during simulated winter thaws (J.L. Hadley, personal communication) and during early spring (Senser et al. 1975). In addition, Snyder (1990) reported that sugar concentrations decrease, while starch concentrations increase within red spruce needles during spring. Although we did not measure foliar sugar and starch concentrations, prolonged exposure of CT seedlings to above freezing temperatures could have promoted the conversion of foliar sugars to starch. This conversion, combined with decreases in foliar sugar concentrations resulting from treatment-induced increases in respiration (seedlings had negative net assimilation rates through Day 2), could have reduced feedback inhibition of photosynthesis and reduced concentrations of cryoprotective sugars. Metabolic activity associated with elevated rates of respiration (including potential alterations in membrane lipids and the possible repair or reorganization of cellular components) may also be required before cold hardiness is significantly affected.

Potential consequences of thaw-associated changes in physiology

Carbon capture during winter thaws could benefit red spruce by replenishing foliar carbon stores, thus offsetting the metabolic cost of maintaining evergreen foliage. However, reductions in cold tolerance during periods of winter thaw could reduce long-term carbon gains if they result in winter injury to foliage. Although CT seedlings had positive net assimilation rates after 4 days of thaw, they lost approximately 12°C in cold tolerance during the same period. For conifers such as balsam fir, which attain mid-winter hardiness that far exceeds that required to avoid freezing injury (DeHayes 1992), a 12°C loss of hardiness would not be detrimental. However, red spruce attains a degree of mid-winter hardiness that is, at best, barely sufficient to prevent freezing injury. A 12°C reduction in hardiness may, therefore, greatly increase the risk of winter injury. The risk of such injury could be increased by pollution-induced reductions in cold tolerance (DeHayes et al. 1991, DeHayes 1992). Any resulting injury will both decrease the potential for carbon gain and deny uninjured tissues access to carbon reserves in lost foliage. The overall impact of thaw-associated alterations in physiology may, therefore, be detrimental to the carbon economy of red spruce. The detrimental impact could be increasingly severe if prolonged winter thaws become more common, but existing winter temperature minima remain unchanged, as some climate models predict (MacCracken et al. 1991).

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References

- Alexander, J.A., J.R. Donnelly and J.B. Shane. 1995. Photosynthetic and transpirational responses of red spruce understory trees to light and temperature. *Tree Physiol.* 15:393–398.
- Anderson, C.P. and S.B. McLaughlin. 1991. Seasonal changes in shoot water relations of *Picea rubens* at two high elevation sites in the Smokey Mountains. *Tree Physiol.* 8:11–21.
- Beadle, C.L., R.E. Neilson, P.G. Jarvis and H. Talbot. 1981. Photosynthesis as related to xylem water potential and carbon dioxide concentration in Sitka spruce. *Physiol. Plant.* 52:391–400.
- Boyer, J.S. 1971. Nonstomatal inhibition of photosynthesis in sunflower at low leaf water potentials and high light intensities. *Plant Physiol.* 48:532–536.
- DeHayes, D.H. 1992. Winter injury and developmental cold tolerance in red spruce. *In* The Ecology and Decline of Red Spruce in the Eastern United States. Eds. C. Eager and M.B. Adams. Springer-Verlag, New York, pp 296–337.
- DeHayes, D.H. and M.W. Williams. 1989. Critical temperature: A quantitative method of assessing cold tolerance. Gen. Tech. Rep. NE-134. Broomall, PA. USDA Forest Service, Northeastern Forest Experiment Station, 6 p.
- DeHayes, D.H., C.E. Waite, M.A. Ingle and M.W. Williams. 1990. Winter injury susceptibility and cold tolerance of current and year-old needles of red spruce trees from several provenances. *For. Sci.* 36:982–994.
- DeHayes, D.H., F.C. Thorton, C.E. Waite and M.A. Ingle. 1991. Ambient cloud deposition reduces cold tolerance of red spruce seedlings. *Can. J. For. Res.* 21:1292–1295.
- Epron, D. and E. Dreyer. 1992. Effects of severe dehydration on leaf photosynthesis in *Quercus petraea* (Matt.) Liebl.: photosystem II efficiency, photochemical and nonphotochemical fluorescence quenching and electrolyte leakage. *Tree Physiol.* 10:273–284.
- Foyer, C.H. 1987. The basis for source–sink interaction in leaves. *Plant Physiol. Biochem.* 25:649–657.
- Foyer, C.H. 1988. Feedback inhibition of photosynthesis through source–sink regulation in leaves. *Plant Physiol. Biochem.* 26:483–492.
- Gage, S.F. and D.H. DeHayes. 1992. Variation in seasonal patterns of photosynthesis among red spruce and balsam fir provenances. *In* Proc. First Northern Forest Genetics Conference. Eds. D.H. DeHayes and G.J. Hawley. Univ. Vermont, Burlington, VT, pp 109–120.
- Griew, P., J.M. Guehl and G. Aussenac. 1988. The effects of soil and atmospheric drought on photosynthesis and stomatal control of gas exchange in three coniferous species. *Physiol. Plant.* 73:97–104.
- Hadley, J.L., R.G. Amundson, J.A. Laurence and R.J. Kohut. 1993. Physiological response to controlled freezing of attached red spruce branches. *Environ. Exp. Bot.* 33:591–609.
- Kaiser, W.M. 1987. Effects of water deficit on photosynthetic capacity. *Physiol. Plant.* 71:142–149.
- Keck, R.W. and J.S. Boyer. 1974. Chloroplast response to low water potentials. III. Differing inhibition of electron transport and photophosphorylation. *Plant Physiol.* 53:474–479.
- Kimball, S.L., B.D. Bennett and F.S. Salisbury. 1973. The growth and development of montane species at near-freezing temperatures. *Ecology* 54:168–173.
- Krapp, A., H. Bettina, C. Schafer and M. Stitt. 1993. Regulation of the expression of *rbS* and other photosynthetic genes by carbohydrates: a mechanism for the ‘sink regulation’ of photosynthesis? *Plant J.* 3:817–828.
- MacCracken, M., U. Cubasch, W.L. Gates, L.D. Harvey, B. Hunt, R. Katz, E. Lorenz, S. Manabe, B. McAvaney, N. McFarlane, G. Meehl, V. Meleshko, A. Robock, G. Stenchikov, R. Stouffer, W.-C. Wang, W. Washington, R. Watts and S. Zebiak. 1991. A Critical appraisal of model simulations. *In* Greenhouse-Gas-Induced Climatic Change: A Critical Appraisal of Simulations and Observations. Ed. M. F. Schlesinger. Developments in Atmospheric Science 19, Elsevier, Amsterdam, pp 583–591.
- Martin, B. and N.A. Ruiz-Torres. 1992. Effects of water-deficit stress on photosynthesis, its components and component limitations, and on water use efficiency in wheat (*Triticum aestivum* L.). *Plant Physiol.* 100:733–739.
- McLaughlin, S.B., C.P. Anderson, N.T. Edwards, W.K. Roy and P.A. Layton. 1990. Seasonal patterns of photosynthesis and respiration of red spruce saplings from two elevations in declining southern Appalachian stands. *Can. J. For. Res.* 20:485–495.
- Mohanty, P. and J.S. Boyer. 1976. Chloroplast response to low leaf water potentials. IV. Quantum yield reduced. *Plant Physiol.* 57:704–709.
- Nobel, P.S. 1991. Physicochemical and environmental plant physiology. Academic Press, New York, 635 p.
- Öquist, G. 1982. Seasonally induced changes in acyl lipids and fatty acids of chloroplast thylakoids of *Pinus sylvestris*. *Plant Physiol.* 69:869–875.
- Öquist, G. 1983. Low temperature effects on photosynthesis in conifers. *In* Effects of Stress on Photosynthesis. Eds. R. Marcelle, H. Clijsters and M. Van Poucke. Martinus Nijhoff/Dr. W. Junk Publishers, Boston, pp 211–218.
- Öquist, G. and E. Ögren. 1985. Effects of winter stress on photosynthetic electron transport and energy distribution between the two photosystems of pine as assayed by chlorophyll fluorescence kinetics. *Photosynthesis Res.* 7:19–30.
- Parker, J. 1963. Causes of the winter decline in transpiration and photosynthesis in some evergreens. *For. Sci.* 9:158–166.
- Perkins, T.D. and G.T. Adams. 1995. Rapid freezing induces winter injury symptomatology in red spruce foliage. *Tree Physiol.* 15:259–266.
- Perkins, T.D., G.T. Adams, S. Lawson and M.T. Hemmerlein. 1993. Cold tolerance and water content of current-year red spruce foliage over two winter seasons. *Tree Physiol.* 13:119–129.
- Ritchie, G.A. and T.M. Hinckley. 1975. The pressure chamber as an instrument of ecological research. *Adv. Ecol. Res.* 9:165–254.
- Sakai, A. and W. Larcher. 1987. Frost survival of plants: responses and adaptation to freezing stress. Springer-Verlag, New York, 321 p.
- Senser, M., F. Schötz and E. Beck. 1975. Seasonal changes in structure and function of spruce chloroplasts. *Planta* 126:1–10.
- Schaberg, P.G., R.C. Wilkinson, J.B. Shane, J.R. Donnelly and P.F. Cali. 1995. Winter photosynthesis of red spruce from three Vermont seed sources. *Tree Physiol.* 15:354–350.
- Sharkey, T.D. and M.R. Badger. 1982. Effects of water stress on photosynthetic electron transport, photophosphorylation, and metabolite levels of *Xanthium strumarium* mesophyll cells. *Planta* 156:199–206.
- Snyder, M.C. 1990. Seasonal patterns of carbohydrate reserves within red spruce seedlings in the Green Mountains of Vermont. MS Thesis, Univ. Vermont, Burlington, VT, 57 p.
- Sperry, J.S. and M.T. Tyree. 1990. Water-stress-induced xylem embolism in three species of conifer. *Plant Cell Environ.* 13:427–436.

- Stitt, M., I. Wilke, R. Feil and H.W. Heldt. 1988. Coarse control of sucrose-phosphate synthase in leaves: alterations of the kinetic properties in response to the rate of photosynthesis and the accumulation of sucrose. *Planta* 174:217–230.
- Stitt, M. and W.P. Quick. 1989. Photosynthetic carbon partitioning: its regulation and possibilities for manipulation. *Physiol. Plant.* 77:633–641.
- Strimbeck, G.R., P.G. Schaberg, D.H. DeHayes, J.B. Shane and G.J. Hawley. 1995. Midwinter dehardening of montane red spruce during a natural thaw. *Can. J. For. Res.* 25:2040–2044.
- Teskey, R.O., J.A. Fites, L.J. Samuelson and B.C. Bongarten. 1986. Stomatal and nonstomatal limitations to net photosynthesis in *Picea taeda* L. under different environmental conditions. *Tree Physiol.* 2:131–142.
- Wong, S.C., I.R. Cowan and G.D. Farquhar. 1979. Stomatal conductance correlates with photosynthetic capacity. *Nature* 282:424–426.
- Yelenosky, G. and C.L. Guy. 1989. Freezing tolerance of citrus, spinach, and petunia leaf tissue. Osmotic adjustment and sensitivity to freeze induced cellular dehydration. *Plant Physiol.* 89:444–451.