Cold Tolerance and Photosystem Function in a Montane Red Spruce Population: Physiological Relationships with Foliar Carbohydrates

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INTRODUCTION

Red spruce (*Picea rubens* Sarg.) growing in northern montane forests of eastern North America appears to be distinctive with respect to at least two aspects of winter physiology. First, red spruce attains only a modest level of midwinter cold tolerance compared to other north temperate conifers and appears barely capable of avoiding freezing injury at commonly occurring ambient winter temperatures (DeHayes, 1992). Second, red spruce is capable of net photosynthesis during winter in response to relatively brief exposure to mild temperatures (Schaberg et al., 1995). Despite considerable study, most of our understanding of red spruce cold tolerance and winter photosynthesis is derived from seedling studies in modified environments or examination of only a few mature trees in forests. As such, actual levels of attainment and the range among native trees are not well understood.

Foliar carbohydrate concentrations, especially sugars, may influence cold tolerance development (Sakai and Larcher, 1987), could limit photosynthesis via feedback inhibition (Krapp and Stitt, 1995) and enhance foliar respiration levels (Farrar, 1985). Although numerous studies have shown that foliar sugar and cold tolerance levels can be related, the extent of a causal association between these parameters has been complicated by the confounding influence of season (Parker, 1959; Hinesley et al., 1992) or experimental treatment (Ögren, 1997; Ögren et al., 1997). Cold tolerance and foliar sugar concentrations change dramatically and somewhat synchronously with season in north temperate tree species. It is not possible to effectively examine the extent of a potential functional relationship between these parameters by sampling across season. Furthermore, our current understanding of gas exchange-sugar relationships is based largely on growing season assessments of non-woody plants. In this paper, we document the range in midwinter cold tolerance and photosystem capacity (dark and light reactions) among 60 native red spruce trees in north central Vermont and examine the extent and nature of their physiological relationship with foliar sugar concentrations. We chose to focus measurement efforts across many individuals on a single date in midwinter when trees have reached their maximum depth of cold tolerance so that functional associations could be examined on an individual basis without the confounding influence of seasonal changes. The information reported provides a comprehensive assessment of red spruce freezing injury vulnerability and the role of winter carbon relations in the survival of the species.

MATERIALS AND METHODS

On January 16, 1996, over 20 current-year shoots per tree were collected from each of 60 mature red spruce on a southeast-facing slope of Mt. Mansfield, Vermont. Ambient air temperatures on the mountain were continually below 0°C for 48 days prior to sampling (NOAA records). To better determine the physiological range of parameters measured, 20 dominant or codominant trees were sampled from each of three elevations (650, 850 and 1050 m). Shoots were sealed in plastic bags, packed in snow, transported to the laboratory, and immediately prepared for assessment of cold tolerance, gas exchange and chlorophyll fluorescence. Foliage used in carbohydrate assessments was stored at −60°C prior to analysis. Cold tolerance was determined by controlled freezing tests using electrolyte leakage as a tissue injury assay and was estimated as T_m, the temperature at the midpoint of a sigmoid curve fitted to electrolyte leakage data (Strimbeck, 1997). Net photosynthetic capacity (P_{max}) was measured with a LI-6262 CO_2/H_2O IRGA (Li-Cor Inc., Lincoln NE, USA) using the methods of Schaberg et al. (1998). Shoots were taken directly from snow-packed storage, recut under water, attached to a supply of distilled water, and were placed in cuvettes maintained at near-optimal growing season temperature (16.7 ± 0.2°C) and light (582 ± 16 μmol m^{-2} s^{-1}) conditions (Alexander et al., 1995). Shoots processed in this manner reached stable photosynthetic maxima comparable to rates observed in freshly excised shoots within 20-40 minutes of initial illumination (Schaberg et al., 1998). Temperature-induced increases in P_{max} can occur within 3 h (Schaberg et al., 1998), but were avoided by timely photosynthetic assessment (within 45 min of removal from storage). The ratio of variable to maximum chlorophyll fluorescence (F_v/F_m) was used as an indicator of photosystem II function. Chlorophyll fluorescence was measured with a PK Morgan CF1000 fluorometer using the methods of Adams (1996). For carbohydrate samples, cuticular waxes were extracted (Hinesley et al., 1992), then sugars were extracted and sucrose, glucose, fructose and starch concentrations were enzymatically determined (Hendrix, 1993). Raffinose concentrations were evaluated using a Waters HPLC and Sugar-Pak column (Millipore Corp., Milford MA, USA) with a 50 mM Ca EDTA solvent.

Correlation analyses were used to evaluate relationships among measurement parameters. Elevational data were used in partial correlations with glucose and total sugar concentrations because these parameters increased slightly with increasing elevation ($P = 0.01$ for each as determined using analyses of variance). No other differences attributable to elevation were found. Differences were considered statistically significant when $P = 0.05$.

RESULTS AND DISCUSSION

Cold Tolerance

Mean cold tolerance was −41.7°C and ranged from −30.1 to −53.7°C (Table 1). Approximately 36% of trees evaluated had T_m values higher than −40°C. Because wintertime minimum temperatures on Mt. Mansfield are typically between −30 and −40°C (91% of the last 44 winters), our data support the conclusion of DeHayes

TABLE 1. Means, standard errors and ranges of cold tolerance (T_m), photosynthetic capacity (P_{max}), variable to maximum fluorescence (F_v/F_m), and sugar and starch concentrations for current-year foliage of red spruce on Mt. Mansfield, Vermont during midwinter.

Parameter	Mean ± se	Range among trees
Cold Tolerance		
T_m (°C)	−41.7 ± 0.6	−30.1-−53.7
Photosystem Function		
P_{max} (μmol $m^{-2}s^{-1}$)	−1.10 ± 0.10	0.62-−3.28
F_v/F_m	0.28 ± 0.01	0.22-0.40
Carbohydrate Concentrations (mg/g)		
Sucros	28.71 ± 0.93	15.83-45.99
Glucose	4.37 ± 0.28	1.24- 9.65
Fructose	12.89 ± 0.38	2.33-17.66
Raffinose	19.35 ± 0.43	14.42-31.21
Total Sugar	65.37 ± 1.50	38.91-93.94
Starch	33.07 ± 0.06	2.12- 4.12

(1992) that red spruce current-year foliage attains cold tolerance levels barely sufficient to protect it from minimum temperatures encountered in northern montane habitats. Given that acid deposition and winter thaws reduce red spruce cold tolerance by 4 to 10°C (DeHayes, 1992), the unique susceptibility and documented increase in freezing injury of red spruce over the past 50 years appears explainable.

Photosystem Function

Average P_{max} was -1.24 µmol m^{-2} s^{-1} (Table 1), and photosynthetic capacities for individual trees ranged from -3.28 (net respiration) to 0.63 µmol m^{-2} s^{-1} (net photosynthesis). Fewer than ten percent of trees showed any capacity for photosynthesis.

Average F_v/F_m was 0.28 and the range was 0.22 to 0.40. All values were well below the 0.832 level typical of foliage with a well functioning photosynthetic apparatus and indicate that a significant inhibition of photosystem II existed (Mohammed et al., 1995).

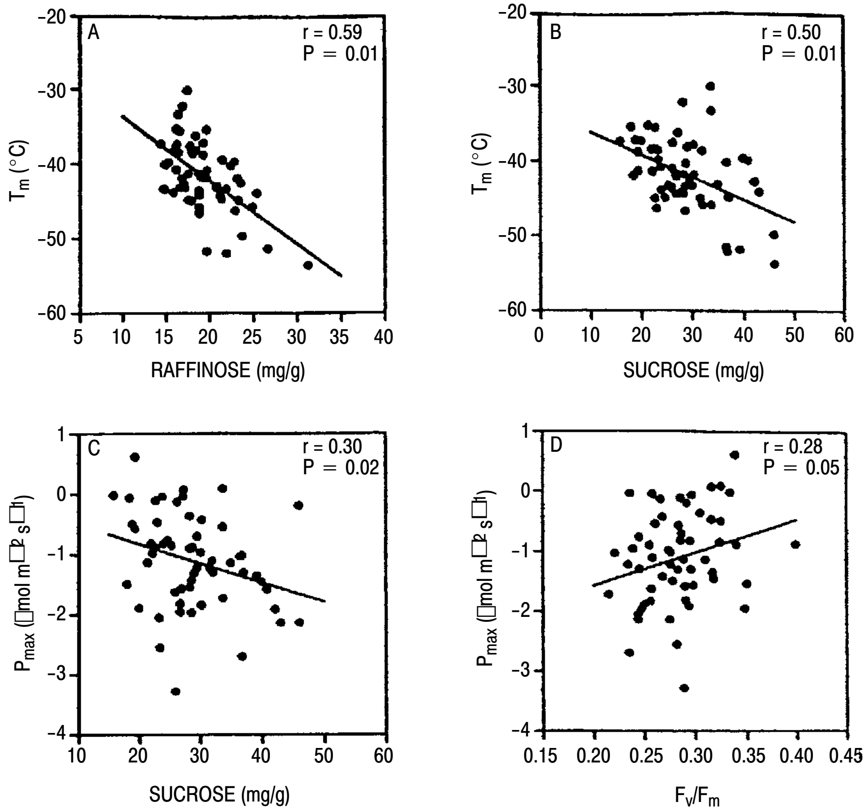
Carbohydrate Concentrations

Sucrose and raffinose are the dominant foliar carbohydrates (Table 1) as has been found in other spruces during winter (Neish, 1958; Parker, 1959), and are the primary sugars produced following experimentally induced winter photosynthesis (Neish, 1958).

Associations Among Parameters

Few correlations involving T_m and P_{max} were significant. Cold tolerance (T_m) was negatively correlated with raffinose (Figure 1A), sucrose (Figure 1B) and total sugar ($r = -0.38$, $P = 0.01$, not depicted) concentrations, indicating that foliage with higher sugar levels tended to be more cold tolerant. Sucrose, raffinose and other sugars protect both protein (Carpenter and Crowe, 1988) and membrane (Anchorduguy et al., 1987) systems from freezing injury *in vitro*. Sugars may also limit cellular freeze dehydration by their bulk colligative effects (Levitt, 1980), or by promoting vitrification of the partially dehydrated cell solution (Hirsh, 1987). The preferential accumulation of sugars in the cytoplasm (Koster and Lynch, 1992) may impart special protection to this region.

FIGURE 1. Data plots, correlation coefficients and P-values for associations of physiological parameters for red spruce during winter that were significantly correlated (P = 0.05) with cold tolerance (T_m) and photosynthetic capacity (P_{max}): T_m and raffinose concentration (A), T_m and sucrose concentration (B). P_{max} and sucrose concentration (C), and P_{max} and F_v/F_m (D).



Interestingly, F_v/F_m, often used as a measure of plant stress, was not correlated with individual tree cold tolerance (r = □0.031, P = 0.994). Given the energy intensive nature of cold tolerance development, one might expect that plants exhibiting greater levels of stress would achieve a lesser depth of cold tolerance.

P_{max} was negatively correlated with sucrose concentration (Figure 1C) and positively correlated with F_v/F_m (Figure 1D). Possible physiological links among these parameters have been proposed. For example, Parker (1963) suggested that the feedback inhibition of photosynthesis by foliar sugars (including sucrose) may be one factor limiting winter carbon gain in evergreen conifers. Reductions in photochemical efficiency (F_v/F_m) could follow the inhibition of enzymatic dark reactions (Mohammed et al., 1995). In addition, because sucrose is the major substrate for the respiratory network, the negative relationship between P_{max} and sucrose concentration could also reflect an enhancement of respiration due to higher substrate concentrations (Farrar, 1985). The weak correlations reported here for a period with temperatures continuously below 0°C provide little support for a functional relationship among parameters. However, such relationships could become more apparent during thaws when respiration rates increase, foliar sucrose concentrations decrease, and net photosynthetic rates rise relative to the midwinter baseline levels reported here.

CONCLUSION

Data presented here highlight the marginal cold tolerance of red spruce relative to ambient minimum temperatures typically encountered. Because our data do not include the confounding influences of season or treatment, strong correlations between cold tolerance and sugar levels provide unique support for the possibility that high raffinose and sucrose concentrations play an important role in enhancing the foliar cold tolerance of red spruce. The weak correlation of sucrose concentration and P_{max} may result from the low tree to tree variability of these parameters in midwinter, when foliar sucrose levels are generally high and capacities for photosynthesis are uniformly low.

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