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Whole-plant nitrogen- and water-relations traits, and their associated trade-offs, in adjacent muskeg and upland boreal spruce species

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Abstract Black and white spruce (*Picea mariana* and *P. glauca*) exhibit a striking micro-geographic distribution pattern at the southern edge of the boreal forest. Black spruce grows in flooded nutrient-poor muskegs, while white spruce is found primarily on drier upland sites, and the two rarely form mixed stands. In an attempt to characterize the physiological and, hence, mechanistic basis of this pattern, we sampled five adjacent populations of black and white spruce from northern British Columbia and measured a suite of physiological and allocative characteristics, and associated trade-offs, that may be important to survival in habitats limited in nutrient or water availability. Two laboratory experiments were conducted: a greenhouse dry-down experiment to assess relative degree of drought tolerance; and a 2×2 nested factorial experiment in which seedlings were subjected to varying water and nitrogen regimes for approximately 16 weeks. White spruce was more drought-tolerant (i.e., maintained positive net photosynthesis at lower shoot water potential) and more efficient in water-use (as indicated by carbon isotopic composition) than black spruce. Black spruce was found to be significantly less sensitive to nitrogen stress, exhibited greater plasticity in nitrogen-use efficiency (measured as the carbon-to-nitrogen ratio in total plant tissue), and had a greater specific N absorption rate under high-N conditions than white spruce. Trade-offs hypothesized to be associated with these nitrogen and water relations traits were examined, but few were confirmed. Water-use efficiency and nitrogen-use efficiency did not trade-off between species, but did trade-off plastically (i.e., across treatments) within species. When exposed to simultaneous limitations of N and water both species were forced to utilize each re-

source with suboptimal efficiency. The change in isotopic composition per unit change in C/N ratio was not the same in the two species. This difference may reflect optimization of the trade-off, whereby each species maximizes the use efficiency of the most limiting resource (relative to its habitat), while minimizing the concomitant reduction in the use efficiency of the other resource.

Key words Water-use efficiency · Nitrogen-use efficiency · Carbon isotope discrimination · *Picea mariana* · *Picea glauca*

Introduction

Mechanisms that improve plant growth and chances of survival under resource limitation may incur costs which reduce growth or survivability in unstressful conditions (Grime 1979; Chapin 1980; Tilman 1988). As a result of such trade-offs, adaptation to low-resource environments may preclude successful occupation of high-resource environments and *vice versa* (Grime 1979). Similarly, adaptive demands set by one type of resource-limited environment may trade off with the adaptive demands necessary for occupation of other types of low-resource environments (Tilman 1988). For example, theory suggests that there should be a trade-off between water-use efficiency (WUE) and nitrogen-use efficiency (NUE). Because leaf intercellular space CO₂ concentration (C_i) is typically not saturating for carbon assimilation, any increase in C_i will result in increased carbon gain per unit leaf nitrogen (i.e., the photosynthetic NUE). This could be achieved through an increased stomatal conductance, but transpiration (E) would simultaneously increase over and above what is necessary for optimal water use. Adaptive specializations and associated trade-offs have been hypothesized to be principal mechanisms accounting for species distributions across resource gradients (e.g., Grime 1979; Tilman 1988). Through phenotypic alterations (plasticity), however, plants may be able to ameliorate or eliminate the costs of particular traits

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across environments. This study investigates whether variations in genetically based physiological and allometric characteristics, their associated trade-offs, and phenotypic plasticity may have contributed to a conspicuous ecological pattern found near the southern border of the boreal forest.

In the boreal zone, as a result of extreme climatic conditions, localized variations in slope, aspect, and drainage translate into large changes in nutrient and water availability over very short distances. Characteristic forest types tend to be strikingly correlated with these patterns. Towards the southern reaches of the boreal forest, stands of black spruce, *Picea mariana* (Mill.) B.S.P., are found mainly in lowland "muskegs" which are poorly drained and extremely low in available nutrients, particularly nitrogen (Vincent 1965; Larsen 1980; Elliott-Fisk 1988). In contrast, stands dominated by white spruce, *P. glauca* (Moench) Voss, are generally associated with drier upland sites which are not as limited in nutrient availability (Larsen 1980; Elliott-Fisk 1988). Although black spruce and white spruce exhibit similar levels of shade-tolerance (Elliott-Fisk 1988) and stands in this region are frequently found growing contiguously, they rarely form mixed stands (Vincent 1965; DeLong et al. 1991). This local distribution pattern suggests that selection pressures acting on either species in the other's habitat are intense.

In this study we attempted to characterize a potential mechanistic basis for the unique ecological pattern observed between black spruce and white spruce near the southern border of the boreal forest. Being congeneric species of similar growth form, gross phenological and morphological differences between black spruce and white spruce are not likely (Dudley 1996). Thus, adaptive responses in physiology and allocation may be the primary mechanisms by which these species cope with the environmental characteristics of their respective habitats. Black and white spruce seedlings from adjacent muskeg and upland populations located in northeastern British Columbia were grown under varying moisture and N regimes (under controlled conditions) for a length of time approximating the first (establishment) growing season in their native habitats. Because physiological traits may augment or counter each other and may similarly interact with morphological traits, an array of long-term, whole-plant N- and water-relations traits, and their potential trade-offs, were measured. Long-term whole-plant measures of physiological characteristics, being averaged over the life-time of the seedling, may have greater ecological relevance than instantaneous leaf-level traits (Donovan and Ehleringer 1994; Vaitkus and McLeod 1995). Of particular interest was the expected compromise between NUE and WUE. Although this trade-off is expected to occur in all land plants, the plastic balance between an incremental change in NUE and an incremental change in WUE may vary, particularly at the whole-plant level.

Materials and methods

Plant material

Open pollinated seeds from five pairs of adjacent stands of black spruce and white spruce were obtained from the boreal forest near Fort Nelson, British Columbia (59°N, 123°W). Each pair was at least 10 km from the next. The white spruce seeds were from previous collections maintained by the British Columbia Ministry of Forests (seedlots 8504, 8505, 8506, 35075, 37044), whereas the black spruce seeds were freshly collected (35 trees per stand, all more than 10 m apart to avoid repeated sampling of the same genet).

The landscape in the Fort Nelson area is characterized by a mosaic of level lowlands (muskeg) dominated by black spruce, and sloping uplands dominated by white spruce. Total mean annual precipitation (rain and snow) for the region is 446 mm of which only 200–290 mm falls as rain (DeLong et al. 1991), suggesting that well-drained upland sites are fairly dry in summer.

The white spruce seeds were stratified at 2°C for 3 weeks after soaking for 12 h. Black spruce seeds were not stratified. Randomly selected seeds from each population of both black spruce and white spruce were planted in 66-cm³ Cone-tainers (Stuewe and Sons Inc., Corvallis, Ore., USA) filled with a mixture of two parts peat, one part Perlite by volume, and 5.3 g l⁻¹ dolomite topped with grit (Target Forestry Sand, Burnaby, Canada). All ten populations were evenly represented in the drought tolerance and the long-term whole-plant nitrogen- and water-relations experiments outlined below.

Drought-tolerance experiment

All the seeds in this experiment were germinated and grown in a greenhouse under high water (watered four times a week) and nutrient conditions (fertilized twice a week; 150 mg l⁻¹ N, 60 mg l⁻¹ P, 150 mg l⁻¹ K, and 10 mg l⁻¹ S, with 1 mg l⁻¹ micronutrient mix containing 1.3% Bo, 0.1% Cu, 2.0% Mn, 0.6% Mo, and 0.4% Zn). The day/night temperature was approximately 20/14°C, and daytime relative humidity was about 35%. Natural sunlight was supplemented with high-intensity halide lamps to maintain a 20-h photoperiod. Irradiance varied from 350 to 1000 μmol m⁻² s⁻¹ photosynthetically active radiation (PAR) depending on weather. After 4 months, water was withheld and the seedlings were tested for relative drought tolerance by monitoring net carbon assimilation (A) and shoot xylem water potential (ψ_{xylem}) daily as the soil dried over about 7 days. Net carbon assimilation was measured with a LI-6200 closed photosynthesis system (LI-COR Inc., Lincoln, Neb., USA) under 650 μmol m⁻² s⁻¹ PAR. Relative humidity was maintained at 35–45%, CO₂ concentration at 350±10 μl l⁻¹, and leaf and air temperature at 23°C. Immediately after the photosynthesis measurement, the ψ_{xylem} of each seedling was measured with a pressure bomb (Soil Moisture Equipment, Santa Barbara, Calif., USA). The experiment was terminated when the seedlings had ceased positive carbon uptake. Projected area of leaves used to measure photosynthesis was determined using a LI-COR LI-3100 area meter.

Long-term, whole-plant nitrogen- and water-relations experiment

Seedlings for this experiment were grown in a PGV36 growth chamber (Conviron, Winnipeg, Canada). There were two N levels and two water levels, yielding four treatment combinations: (1) +H₂O+N, (2) +H₂O -N, (3) -H₂O+N, and (4) -H₂O -N (where "+" indicates high and "-" indicates low level of water or N). Five seedlings from each population were randomly allocated to each treatment. The photoperiod was 20 h with an irradiance of 350–400 μmol m⁻² s⁻¹ PAR (from both fluorescent and incandescent lamps), relative humidity was approximately 35%, and day/night temperature was maintained at 24/14°C. Fans were directed at the seedlings to minimize the boundary layer and ensure

isotopic uniformity of the source air. Cone-tainer trays were randomized daily to avoid positional effects within the growth chamber.

Treatments were initiated when the seedlings had reached about 2 cm in height after 2 weeks of well-watered and fertilized conditions. The high-moisture treatments (+H₂O+N, +H₂O -N) received 5–10 ml of water daily (to replace losses over the 24-h period since last watering, without leaching). The low-water treatments (-H₂O+N, -H₂O -N) were watered when the water potential of the soil in ten randomly selected cones dropped to about -1.0 MPa, at which time all treatments were watered to the drip point and fertilized. All four treatments received identical concentrations of phosphorus (625 mg l⁻¹ P), potassium (94 mg l⁻¹ K), sulfur (10 mg l⁻¹ S), and micro-nutrients (1 mg l⁻¹). The high-N treatments (+H₂O+N, -H₂O+N) were fertilized with 225 mg l⁻¹ N, and the low N treatments (+H₂O -N, -H₂O -N) received 25 mg l⁻¹ N.

The experiment lasted for 16 weeks during which the droughted seedlings went through 28 watering cycles. All seedlings were harvested at the end of the last drought cycle. Roots and shoots were separated at the root collar and ψ_{xylem} of each seedling was measured as above. After washing roots to remove the potting medium, roots and shoots of each seedling were freeze-dried and weighed, then re-combined and pulverized in a planetary ball mill (Pulverisette, Fritsch GMBH, Germany) to sub-micron particle size. The pulverized tissue was combusted, and nitrogen and carbon content determined with a Model 1106 Elemental Analyzer (Carlo Erba, Valencia, Calif., USA). Liberated CO₂ then passed to a Prism triple-collecting ratio mass spectrometer (VG Isogas, Middlewich, UK) for determination of $\delta^{13}\text{C}$ values (‰):

$$\delta^{13}\text{C} = \left[\frac{^{13}\text{C}\text{CO}_2 / ^{12}\text{C}\text{CO}_2(\text{sample})}{^{13}\text{C}\text{CO}_2 / ^{12}\text{C}\text{CO}_2(\text{standard})} - 1 \right] \times 1000$$

where the standard is VPDB (Vienna Peedee belemnite).

Farquhar et al. (1982) identified the relationship between $\delta^{13}\text{C}$ and the ratio of intercellular to atmospheric CO₂ concentration in C3 plants, a major determinant of WUE. The $\delta^{13}\text{C}$ of total plant tissue gives a relative index of the integrated WUE of a plant over the course of its life (Farquhar and Richards 1984). More positive $\delta^{13}\text{C}$ values indicate more water-use efficient plants. This relationship has been confirmed for white spruce (Sun et al. 1996). Plant $\delta^{13}\text{C}$ values can be converted to per mil discrimination values (Δ) if the $\delta^{13}\text{C}$ of the source air is known (Farquhar and Richards 1984). However, the $\delta^{13}\text{C}$ of air can vary, particularly under greenhouse and growth chamber conditions (Guy et al. 1986). We therefore do not present Δ values here.

Total plant carbon to nitrogen (C/N) was calculated as an estimate of whole-plant, long-term NUE. This ratio indicates the amount of carbon fixed per unit nitrogen, and is an index of the efficiency by which N is distributed and utilized to acquire carbon (Chapin and Van Cleve 1990). Specific N absorption rate (SAR) was determined by dividing total plant N content by root biomass (Schlesinger et al. 1989).

Statistical analysis

All statistical analyses were performed using SuperANOVA-Accessible General Linear Modeling Package and Stat View SE+ Graphics (Abacus Concepts 1990). Growth, SAR, carbon-to-nitrogen ratio (C/N), $\delta^{13}\text{C}$, and root-to-shoot ratio (R/S) were analyzed using a three-way nested factorial analysis of variance (ANOVA). Species, nitrogen, and water were main effects and populations were nested within species. Within each treatment the two species were compared using four one-way ANOVAs (one for each treatment), with the species effect tested against the nested population term. Treatment means were compared using Tukey's compromise means separation procedure. Analyses of covariance (ANCOVA) were performed for line comparisons. Probability plots and histograms were examined for normality, and residuals were also plotted against cell mean estimates to check for homoscedasticity. For C/N data a log transformation was used to meet the assumptions of ANOVA, but non-transformed results are presented in the figures.

Results

Drought tolerance

Figure 1 shows that A decreased as water stress increased (lower ψ_{xylem}) in both species. The 95% confidence intervals of the regression lines do not overlap at all below -0.8 MPa, indicating that white spruce maintained a marginally greater A than black spruce across most ψ_{xylem} values. Zero A was reached at a ψ_{xylem} of about -2.15 MPa for black spruce, and about -2.60 MPa for white spruce.

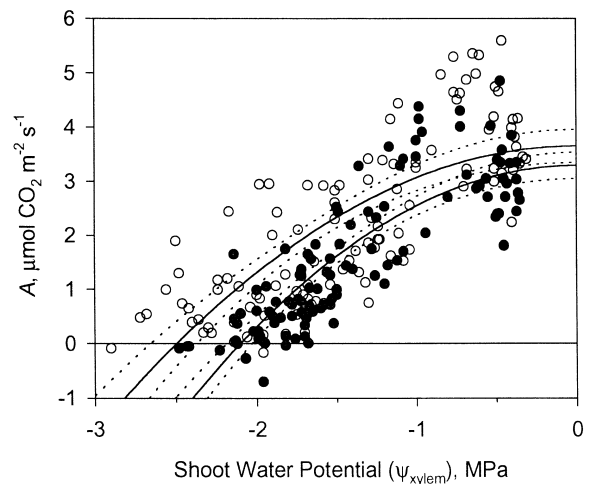


Fig. 1 Relationship between net photosynthetic rate, A ($\mu\text{mol CO}_2 \text{ m}^{-2} \text{ s}^{-1}$) and shoot water potential, ψ_{xylem} (MPa) in seedlings of black spruce (lower solid line) and white spruce (upper solid line). Dotted lines are 95% confidence intervals about the regressions. The x -intercepts of the species lines were significantly different at $P=0.0075$. Equations and coefficients of determination are: $y=0.72x^2+3.29$, $r^2=0.70$ for black spruce, and $y=0.58x^2+3.65$, $r^2=0.57$ for white spruce

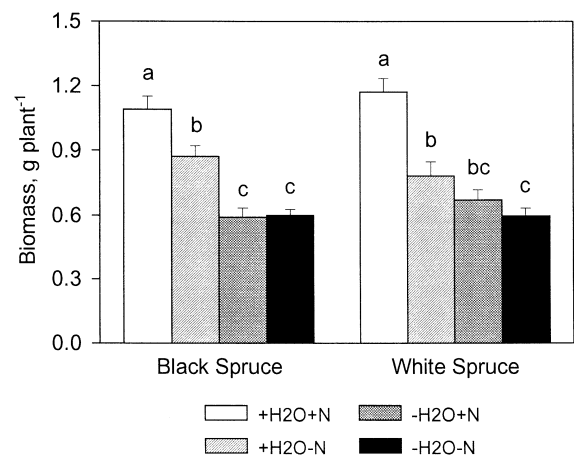


Fig. 2 Growth (g/plant) of black spruce and white spruce after approximately 16 weeks under various nitrogen and water regimes. Each bar represents the average of about 25 seedlings (\pm SEM). Species were statistically the same within each treatment. Significantly different ($P<0.05$) treatments within a species are denoted by different letters above the bars

Table 1 Main and interaction effects of species (*Sp*), nitrogen (*N*) and water regime (H_2O) on biomass, specific N absorption rate (*SAR*), carbon-to-nitrogen ratio (*C/N*), total tissue $\delta^{13}C$, and root-to-shoot ratio (*R/S*) of *Picea mariana* and *P. glauca* seedlings. Numbers indicate probabilities of type I error as determined by ANOVA

Dependent variable	Sp	N	H_2O	$H_2O \times N$	$Sp \times N$	$Sp \times H_2O$	$Sp \times H_2O \times N$
Biomass	0.7708	0.0001	0.0001	0.0033	0.0108	0.3611	0.6331
SAR	0.0006	0.0001	0.2258	0.5054	0.0001	0.7345	0.7174
C/N	0.0047	0.0001	0.0001	0.5818	0.0006	0.0495	0.0100
$\delta^{13}C$	0.0005	0.0001	0.0001	0.2592	0.0774	0.0331	0.0439
R/S	0.0073	0.0001	0.0014	0.2493	0.4378	0.0090	0.6774

Long-term whole-plant nitrogen- and water-relations

Levels of internal water stress, as indicated by ψ_{xylem} measurements, were very similar between black spruce and white spruce at the end of this experiment. White spruce had a mean ψ_{xylem} of -0.64 MPa in the high-water treatments ($+H_2O+N$ and $+H_2O-N$), -0.97 MPa in the water-stress treatment ($-H_2O+N$), and -0.99 MPa in the simultaneously water- and nitrogen-limited treatment ($-H_2O-N$); while for black spruce these values were -0.63 , -1.09 and -1.10 MPa, respectively.

The two species did not differ significantly in total growth within any one treatment (Fig. 2). Nevertheless, species responses to N-stress, but not water-stress, differed (as indicated in Table 1 by the significant interaction effect between species and N treatment, but not between species and H_2O). The biomass of white spruce was significantly more responsive to N than that of black spruce (Fig. 2). White spruce maintained a significantly greater R/S than black spruce in the well-watered treatments, but the two species had equal R/S in the droughted treatments (Fig. 3). Black spruce increased R/S with drought and N-stress, whereas white spruce responded only to N-stress (Fig. 3; $P < 0.01$ for species-by-water interaction in Table 1).

In general, N- and water-stress had opposing effects on C/N, and the combined stresses ($-H_2O-N$) partially canceled each other. However, C/N in the $-H_2O-N$ treatment was still significantly greater than in the control, i.e., $+H_2O+N$ (Fig. 4). The two species responded differently to both N- and water-stress, as indicated by the significant species-by-N and species-by-water interaction terms (Table 1). Compared to white spruce, black spruce had a greater increase and decrease in C/N with N-stress and water-stress, respectively (Fig. 4).

Variation in water supply did not have a significant effect on specific N absorption rate (SAR), but N stress significantly reduced SAR (Table 1; Fig. 5). Black spruce exhibited a greater SAR under the high-N regimes than white spruce, but the two species had similar SAR under low N supplies (Fig. 5).

The effects of N- and water-stress on $\delta^{13}C$ were highly significant (Table 1). Water- and N-stress acting independently ($-H_2O+N$ and $+H_2O-N$, respectively) had opposing effects on $\delta^{13}C$; i.e., water-stress increased but N-stress decreased $\delta^{13}C$ in both species (Fig. 6). In combination, however, water- and N-stress canceled out, as reflected by more similar $\delta^{13}C$ values in the double-stress ($-H_2O-N$) and control ($+H_2O+N$) treatments compared

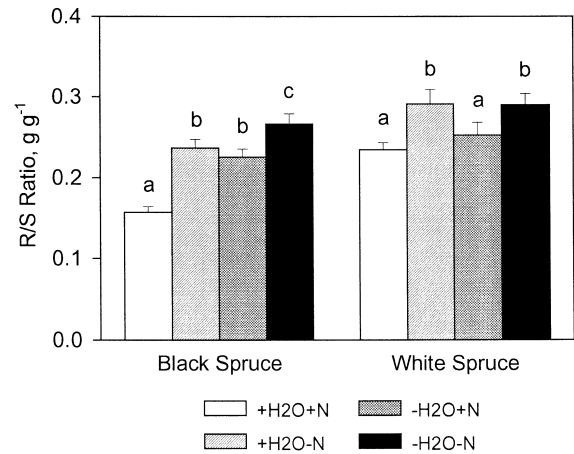


Fig. 3 Root-to-shoot ratio, R/S (g/g), of black spruce and white spruce seedlings grown for approximately 16 weeks under various nitrogen and water regimes. Each bar represents the average of about 25 seedlings (\pm SEM). Species were significantly different in $+H_2O+N$ ($P=0.0005$) and $+H_2O-N$ ($P=0.0400$) treatments, but not in $-H_2O+N$ and $-H_2O-N$ treatments. Significantly different ($P < 0.05$) treatments within a species are denoted by different letters above the bars

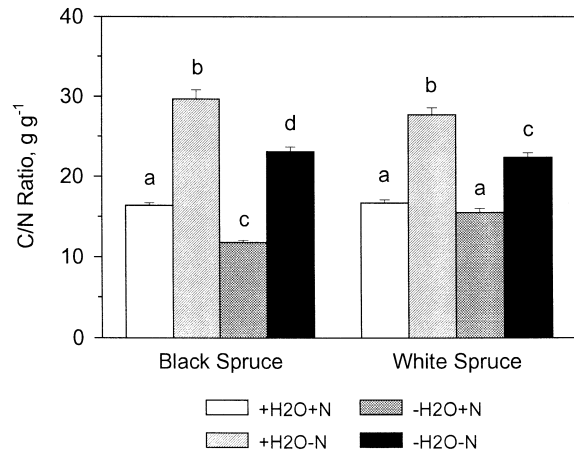


Fig. 4 Carbon-to-nitrogen ratio, C/N (g/g), of black spruce and white spruce seedlings grown for approximately 16 weeks under various nitrogen and water regimes. Each bar represents the average of about 25 seedlings (\pm SEM). Species were significantly different only in the $-H_2O+N$ treatment ($P=0.0001$). Significantly different ($P < 0.05$) treatments within a species are denoted by different letters above the bars

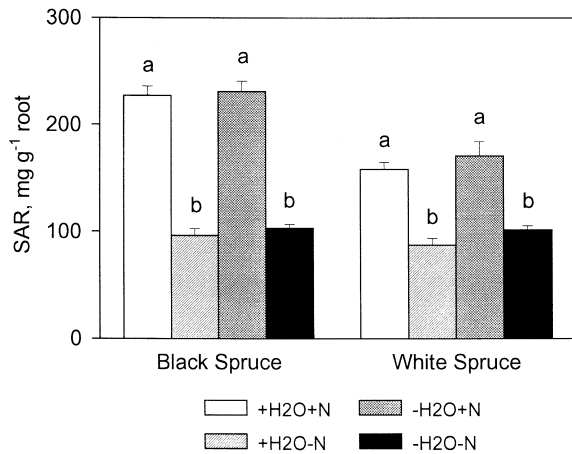


Fig. 5 Specific N absorption rate, SAR (mg N/g root), of black spruce and white spruce seedlings grown for approximately 16 weeks under various nitrogen and water regimes. Each bar represents the average of about 25 seedlings (\pm SEM). Species were significantly different in +H₂O+N ($P=0.0005$) and -H₂O+N ($P=0.0066$) treatments, but not in +H₂O -N and -H₂O -N treatments. Significantly different ($P<0.05$) treatments within a species are denoted by different letters above the bars

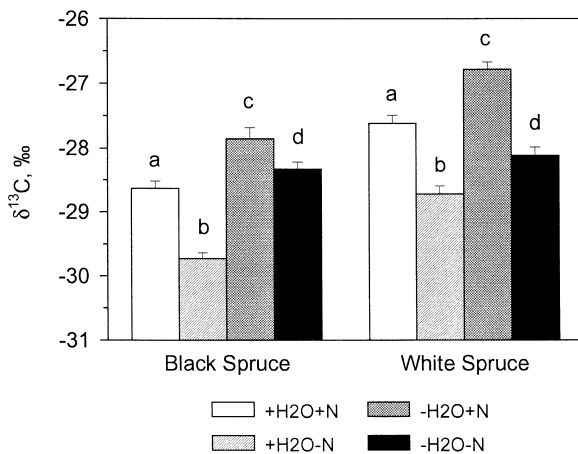


Fig. 6 Carbon isotope composition, $\delta^{13}\text{C}$ (‰), of black spruce and white spruce seedlings grown for approximately 16 weeks under various nitrogen and water regimes. Each bar represents the average of about 25 seedlings (\pm SEM). Species differences were significant in +H₂O+N ($P=0.0004$), +H₂O -N ($P=0.0001$), and -H₂O+N ($P=0.0165$) treatments, but marginally insignificant in the -H₂O -N ($P=0.0563$) treatment. Significantly different ($P<0.05$) treatments within a species are denoted by different letters above the bars

to the single-stress treatments (Fig. 6). White spruce had more positive $\delta^{13}\text{C}$ values than black spruce in every treatment (Fig. 6; Table 1).

Carbon isotopic composition and C/N were not significantly correlated within any given treatment, but were negatively correlated ($P=0.0001$) when plotted across all treatments for each species (Fig. 7). Black spruce had a significantly steeper slope for this relationship than did white spruce ($P=0.0259$; Fig. 7).

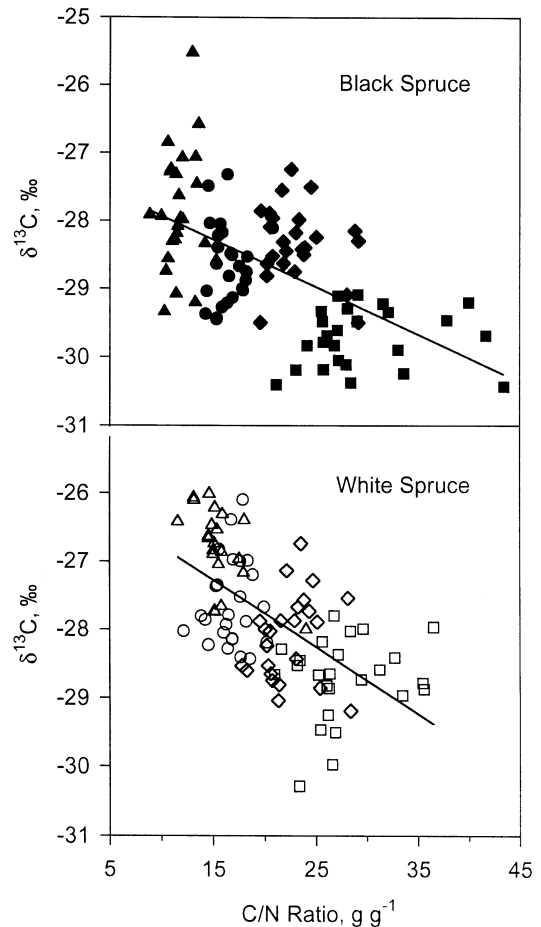


Fig. 7 Relationship between carbon isotope composition, $\delta^{13}\text{C}$ (‰), and carbon-to-nitrogen ratio, C/N (g/g), in black spruce and white spruce seedlings grown for approximately 16 weeks under various nitrogen and water regimes (+H₂O+N, circles; +H₂O -N, squares; -H₂O+N, triangles; -H₂O-N, diamonds). The linear regression equations and coefficients of determination are: $y = -0.069x - 27.233$, $r^2=0.322$ for black spruce, and $y = -0.098x - 25.807$, $r^2=0.354$ for white spruce. The slopes of these regressions were significantly different from zero ($P=0.0001$), and from each other ($P=0.0259$)

Discussion

Environmental stresses, and the unavoidable trade-offs plants face in adapting to them, are believed to be principal factors responsible for generating and maintaining plant species distributions (Grime 1979; Tilman 1988). Because muskeg and upland habitats contrast strongly in availability of nutrients and water, it is likely that differences in N- and water-relations traits, and their associated trade-offs, may influence the distinct topographic separation of black spruce and white spruce near the southern limits of the boreal forest. It should be noted, however, that muskeg and upland habitats differ in other important ways. In particular, muskeg soils are cold and poorly aerated and black spruce may be more tolerant of these harsh conditions (Conlin and Lieffers 1993). This caveat aside, traits that could be important to survival in the in-

fertile muskeg include: (1) low maximal relative growth rate, (2) high physiological capacity to extract nutrients from the soil, (3) high metabolic efficiency in the use of nutrients to produce new biomass, and (4) greater biomass allocation to roots. On the other hand, characteristics that could be important to survival in water-limited, upland habitats include: (1) high degree of drought tolerance, (2) highly efficient utilization of water, and (3) greater biomass allocation to roots.

Nitrogen relations

Maximal relative growth rate

Maximal relative growth rate (RGR_{max}) is defined as relative growth rate under optimal growth conditions. A low RGR_{max} may be an advantage in infertile habitats because of reduced need, or demand, for nutrients (Chapin 1980; Shipley and Keddy 1988; Fichtner and Schulze 1992). Low RGR_{max} , however, may prevent a species from occupying highly fertile environments that favor rapid growth (Grime 1979). The seeds of black spruce and white spruce were germinated at approximately the same time and were of comparable size, suggesting that their similar growth under high resource conditions ($+H_2O+N$ in Fig. 2) resulted from having similar RGR_{max} . Therefore, variation in RGR_{max} is probably not a major cause of the mutually exclusive distributions of black spruce and white spruce.

Nitrogen-stress sensitivity

Stress-sensitivity is the relative decrease in growth in response to a given decline in resource supply (Shipley and Keddy 1988). Fichtner and Schulze (1992) have found N-stress sensitivity to be more important than absolute growth in infertile habitats because non-nitrophilic species do not grow faster than nitrophilic species under limited N supply. In this study, black spruce exhibited less decrease in growth in response to N limitation (i.e., significant species-by-N interaction, Table 1; Fig. 2) and is, thus, less sensitive to N stress than white spruce. Black spruce, therefore, may tolerate nutrient stress better than white spruce, an attribute which may be critical for survival in the nutrient limited muskegs. A complementary interpretation of these results is that white spruce was more responsive to high N than black spruce, consistent with the idea that species from rich environments are better able to increase their growth with increasing nutrient supply than species from poor environments (Chapin 1980).

Specific N absorption rate

A high SAR may be an important means by which plants cope with N stress by enabling rapid exploitation of

fleeting soil N reserves, promoting the maintenance of relatively high internal N status, and/or enhancing N diffusion from the soil to the root by creating a steeper concentration gradient (Welbank 1962; Chapin 1980). The particular SAR expressed by a plant is a function of N availability and root absorption capacity (RAC) (Welbank 1962). Under high-N conditions, black spruce had a greater SAR than white spruce (Fig. 5) and probably also had a greater RAC because N availability was equivalent. Under low-N conditions, SAR was similar for the two species indicating that N availability, rather than RAC, became the primary limitation to N uptake. An enhanced ability to extract soil N can lead to "luxury consumption" above immediate demand when supplies are high (Chapin 1980; Birk and Vitousek 1986). Luxury consumption may act as a stress avoidance mechanism by enabling the use of stored N when external supplies are low (Chapin 1980). The higher RAC exhibited by black spruce may indicate a greater ability to capitalize on spring nutrient flushes. Although upland sites also exhibit flushes followed by N deprivation, these cycles are believed to be proportionally more extreme in muskegs (Saebo 1969; Larsen 1980).

Tilman (1988) suggested that plants are faced with a trade-off for allocating protein towards photosynthesis or to nutrient uptake. This trade-off could reduce the competitive ability of a species on sites with a high, stable N supply (Grime 1977; Tilman 1988). In a separate study (Q. L. Dang et al., unpublished work) we found that black spruce had lower rates of photosynthesis than white spruce under most treatment conditions. Since black spruce may also have a greater RAC than white spruce, these results are consistent with Tilman's suggestion.

Nitrogen-use efficiency

When plants are grown under high-N conditions a low apparent NUE can result from luxury consumption, and thereby represent an adaptation to infertile habitats that have periodic nutrient flushes (Chapin 1980). Alternatively, a high NUE may reflect adaptation to habitats which have relatively high and/or constant rates of N mineralization (Vitousek 1982). But when grown under low N conditions individuals with greater NUE will, by definition, achieve greater growth and should be favored (Schlesinger et al. 1989). Under conditions of low N supply, neither species in this study was able to utilize N more efficiently than the other (Fig. 4). Nevertheless, black spruce did exhibit greater plasticity in C/N than white spruce (Table 1). This plasticity may promote luxury consumption during nutrient flushes, while still allowing efficient nitrogen use during periods of N deprivation. The results also reflect the greater growth response of white spruce over black spruce to increasing nutrient availability.

Water-relations

Drought tolerance

High tolerance to drought may be beneficial in xeric environments not only because carbon gain and growth are sustained, but also because soil water reserves may be more rapidly depleted forcing less tolerant species to cease photosynthesis earlier (Bunce et al. 1977; DeLucia and Heckathorn 1989; DeLucia and Schlesinger 1991). However, Orians and Solbrig (1977) hypothesized that a cost of high drought tolerance may be lower rates of photosynthesis when water is plentiful, reducing the ability of tolerant species to occupy moist sites. In this study, white spruce maintained higher rates of photosynthesis at a lower Ψ_{xylem} than black spruce (Fig. 1), perhaps offering a partial explanation for why white spruce is favored over black spruce on upland sites (cf. DeLucia and Heckathorn 1989; DeLucia and Schlesinger 1991; Richards et al. 1995). White spruce also maintained greater A while not water stressed (Fig. 1), ruling out the likelihood that a trade-off between drought tolerance and A constrains it from occupying muskegs. These results are similar to those of DeLucia and Heckathorn (1989) who found that more tolerant species were excluded from moist sites for entirely other reasons (such as nutrient availability) than the costs of high drought tolerance. Nevertheless, it should be pointed that white spruce has a greater R/S than black spruce under high-moisture conditions (Fig. 3). As a result, white spruce could have lower net photosynthesis at the whole-plant level due to greater root respiration.

Water-use efficiency

A high WUE is an adaptation to water-limited conditions because more carbon is assimilated per unit water utilized over time, allowing greater growth (Vaitkus and McLeod 1995; Dudley 1996). A genotype may have a greater WUE than another in one of four ways: (1) higher A and similar E , (2) similar A and lower E , (3) higher A and E , but proportionally higher A than E , and (4) lower A and E , but proportionally lower E than A . In this study, white spruce achieved a higher WUE (as indicated by significantly less negative $\delta^{13}\text{C}$ values) in at least three of the four treatment combinations (Fig. 6).

A potential trade-off associated with increased WUE is reduced growth rate under high water conditions (Cohen 1970; Meizner et al. 1990; Richards et al. 1995). For example, Meizner et al. (1990) concluded that coffee genotypes with higher WUE, resulting from lower A and E , would yield less when grown in well-irrigated soils than genotypes with lower WUE. However, since the two species in this study exhibited similar growth but different WUE, it is clear that this trade-off is not a concern here. Similarly, high WUE and less negative $\delta^{13}\text{C}$ values have been associated with faster-growing genotypes of wheat (Condon et al. 1987) and white spruce (Sun et al. 1996).

In such cases high WUE probably derives from higher photosynthetic capacity, rather than lower stomatal conductance.

Biomass allocation (R/S)

Because of increased water uptake and reduced water loss, a plant with greater R/S may be better able to maintain growth under water-limited conditions. In this study, black spruce and white spruce had similar R/S in the $-\text{H}_2\text{O}+\text{N}$ treatment (Fig. 3) suggesting that, in terms of biomass allocation, the two species are equally adapted to drought. Two possible explanations may account for these results. First, black spruce and white spruce tend each to be limited by a key edaphic resource (N and water, respectively) either of which would favor allocation to roots (Gleeson and Tilman 1994). Second, because white spruce already has relatively high WUE and drought tolerance, it may most efficiently increase growth by allocating resources to photosynthetic organs (Gleeson and Tilman 1994), assuming N is not limiting. The latter explanation agrees with many other studies (e.g., White et al. 1990; Richards et al. 1995) in suggesting that physiological and allocative drought resistance mechanisms are alternative ways of coping with drought.

The difference in R/S ratio between the species was more apparent when water was not limiting (Fig. 3). White spruce seemed less able to reduce its R/S under these conditions, which could be disadvantageous in a muskeg environment.

Nitrogen- and water-use efficiency trade-off

An intrinsic (i.e., genetic) trade-off between gas exchange measures of WUE and NUE has long been recognized (e.g., Field et al. 1983). Such a trade-off may help enforce species distributions across moisture and nutrient gradients. In this study, long-term whole-plant measures of WUE and NUE (i.e., $\delta^{13}\text{C}$ and C/N, respectively) did not significantly correlate within any treatment, indicating a lack of an intrinsic trade-off between the two traits either between or within species. This suggests that evolving a higher level of either WUE or NUE does not necessarily preclude improved efficiency in the use of the other resource. These results contrast with those of Field et al. (1983) who found a negative correlation between species means of instantaneous WUE (i.e., A/E) and photosynthetic NUE in five co-occurring California evergreens. DeLucia and Schlesinger (1991), on the other hand, found that Great Basin species with relatively high WUE also had greater NUE.

Despite the absence of an intrinsic trade-off between WUE and NUE, $\delta^{13}\text{C}$ and C/N were negatively correlated for each species when plotted across all treatments (Fig. 7). This demonstrates a plastic trade-off whereby in order to increase WUE, NUE is sacrificed, and *vice ver-*

sa. Other authors have observed a plastic trade-off between WUE and NUE at the leaf level (e.g., Reich et al. 1989), but there are apparently no such previous reports utilizing long-term, whole-plant measures of these traits. $\delta^{13}\text{C}$ values and C/N ratios in the double-stress treatment were intermediate to those of the single stresses alone. Thus, when black spruce and white spruce are exposed to simultaneous limitations in both water and N, they are forced to compromise the efficiency by which they utilize both resources.

Species differences in the slope of the $\delta^{13}\text{C}$ against C/N correlation (Fig. 7) and the occurrence of significant species-by-N-by-water interaction terms for these measures of WUE and NUE (Table 1) are related. That is, the species responded differently to the effects of water- and nitrogen-stress, and hence exhibited differences in the plastic trade-off. The mechanistic basis for this difference is unknown but it could, for example, arise from a species difference in the plasticity of N allocation to photosynthetic machinery or, say, to osmotic adjustment during drought. The observed pattern needs to be verified and extended, but it may have important ecological implications for these two species. For any given increase in WUE, white spruce will experience less of a decrease in NUE than black spruce, but any given increase in NUE will come at a greater cost in terms of WUE. For black spruce, on the other hand, any given increase in NUE will result in less of a decrease in WUE than for white spruce.

Conclusions

In this study we attempted to determine whether the mutually exclusive micro-geographic distributions of black spruce and white spruce near the southern border of the boreal forest might, in part, be explained by physiological and allocative differences between the two species. For this purpose the effects of water and nitrogen limitations were examined. The greater drought tolerance and WUE exhibited by white spruce likely contribute to its ability to occupy drier upland sites. On the other hand, the suite of N-relations characteristics exhibited by black spruce (less N-stress sensitive, greater plasticity in NUE, and greater SAR under high-N conditions) suggests a slight advantage in nutrient-limited conditions. In general, however, the differences between the two species seem small relative to the remarkable degree of habitat separation that occurs between them.

Although specific trade-offs that could affect the relative propensities of black spruce and white spruce to occupy upland and muskeg habitats were observed (e.g., between SAR and A), our data generally reveal an absence of many reputed trade-offs associated with the several N- and water-relations traits we measured. For example, trade-offs hypothetically associated with improved water-relations characteristics (e.g., drought tolerance vs. A; WUE vs. growth or A) were completely lacking, and NUE and WUE did not trade off intrinsical-

ly. There was, however, a species difference in the plastic trade-off between NUE and WUE. This difference may reflect an optimization process whereby each species maximizes the use efficiency of the resource which is most limiting in its normal habitat, while minimizing the subsequent reduction in the use efficiency of the other resource.

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References

- Abacus Concepts (1990) SuperANOVA-accessible general linear modeling package. StatView SE and Graphics. Abacus Concepts, Berkeley
- Birk EM, Vitousek PM (1986) Nitrogen availability and nitrogen use efficiency in loblolly pine stands. *Ecology* 67:69–79
- Bunce JA, Miller LN, Dumbroff EB, Webb DP (1977) Competitive exploitation of soil water by five eastern North American tree species. *Bot Gaz* 138:168–173
- Chapin FS III (1980) The mineral nutrition of wild plants. *Annu Rev Ecol Syst* 11:233–260
- Chapin FS III, Van Cleve K (1990) Approaches to studying nutrient uptake, uses, and loss in plants. In: Pearcy PW, Ehleringer JR, Mooney HA, Rundel PW (eds) *Plant physiological ecology: field methods and instrumentation*. Chapman and Hall, London, pp 185–207
- Cohen D (1970) The expected efficiency of water utilization in plants under different competition and selection regimes. *Isr J Bot* 19:50–54
- Condon AG, Richards RA, Farquhar GD (1987) Carbon isotope discrimination is positively correlated with grain yield and dry matter production in field-grown wheat. *Crop Sci* 27:996–1001
- Conlin TSS, Lieffers VJ (1993) Anaerobic and aerobic CO_2 efflux rates from boreal forest conifer roots at low temperatures. *Can J For Res* 23:767–771
- DeLong C, Annas RM, Stewart AC (1991) Boreal white and black spruce zone. In: Meidinger D, Pojar J (eds) *Ecosystems of British Columbia*. Ministry of Forests, Victoria, British Columbia, pp 237–251
- DeLucia EH, Heckathorn SA (1989) The effects of soil drought on water-use efficiency in contrasting Great Basin desert and Sierran montane species. *Plant Cell Environ* 12:935–940
- DeLucia EH, Schlesinger WH (1991) Resource-use efficiency and drought tolerance in adjacent Great Basin and Sierran plants. *Ecology* 72:51–58
- Donovan KA, Ehleringer JR (1994) Carbon isotope discrimination, water-use efficiency, growth, and mortality in a natural shrub population. *Oecologia* 100:347–354
- Dudley SA (1996) Differing selection on plant physiological traits in response to environmental water availability: a test of adaptive hypotheses. *Evolution* 50:92–102
- Elliot-Fisk DL (1988) The boreal forest. In: Barbour MG, Billings WD (eds) *North American terrestrial vegetation*. Cambridge University Press, Cambridge, pp 33–62
- Farquhar GD, Richards RA (1984) Isotopic composition of plant carbon correlates with water-use efficiency of wheat genotypes. *Aust J Plant Physiol* 11:539–552
- Farquhar GD, O'Leary MH, Berry JA (1982) On the relationship between carbon isotope discrimination and the intercellular carbon dioxide concentration in leaves. *Aust J Plant Physiol* 9:121–137

- Fichtner K, Schulze E-D (1992) The effect of nitrogen nutrition on growth and biomass partitioning of annual plants originating from habitats of different nitrogen availability. *Oecologia* 92:236–241
- Field C, Merino J, Mooney HA (1983) Compromises between water-use efficiency and nitrogen-use efficiency in five species of California evergreens. *Oecologia* 60:384–389
- Gleeson SK, Tilman D (1994) Plant allocation, growth rate and successional status. *Funct Ecol* 8:543–550
- Grime JP (1979) *Plant strategies and vegetation processes*. Wiley, Chichester
- Guy RD, Reid DM, Krouse HR (1986) Factors affecting $\delta^{13}\text{C}/^{12}\text{C}$ ratios of inland halophytes. I. Controlled studies on growth and isotopic composition of *Puccinellia nuttalliana* (Schultes) Hitch. *Can J Bot* 64:2693–2699
- Larsen JA (1980) *The boreal ecosystem*. Academic Press, New York
- Meinzer FC, Goldstein G, Grantz DA (1990) Carbon isotope discrimination in coffee genotypes grown under limited water supply. *Plant Physiol* 92:130–135
- Orians GH, Solbrig OT (1977) A cost-income model of leaves and roots with special reference to arid and semiarid areas. *Am Nat* 111:677–690
- Reich PB, Walters MB, Tabone TJ (1989) Response of *Ulmus americana* seedlings to varying nitrogen and water status. 2. Water- and nitrogen-use efficiency in photosynthesis. *Tree Physiol* 5:173–184
- Richards MB, Stock WD, Cowling RM (1995) Water relations of seedlings and adults of two fynbos *Protea* species in relation to their distribution patterns. *Funct Ecol* 9:575–583
- Saebø S (1969) On the mechanism behind the effect of freezing and thawing on dissolved phosphorus in *Sphagnum fuscum* peat bogs. *Sci Rep Agric Col Norway* 48:1–10
- Schlesinger WH, DeLucia EH, Billings WD (1989) Nutrient-use efficiency of woody plants on contrasting soils in the western Great Basin, Nevada. *Ecology* 70:105–113
- Shipley B, Keddy PA (1988) The relationship between relative growth rate and sensitivity to nutrient stress in twenty-eight species of emergent macrophytes. *J Ecol* 76:1101–1110
- Sun ZJ, Livingston NJ, Guy RD, Ethier GJ (1996) Stable carbon isotopes as indicators of increased water use efficiency and productivity in white spruce (*Picea glauca* (Moench) Voss) seedlings. *Plant Cell Environ* 19:887–894
- Tilman GD (1988) *Plant strategies and the dynamics and structure of plant communities*. Princeton Monographs, Princeton
- Vaitkus MR, McLeod KW (1995) Photosynthesis and water-use efficiency of two sandhill oaks following additions of water and nutrients. *Bull Torrey Bot Club* 122:30–39
- Vincent AB (1965) *Black spruce: a review of its silvics, ecology and silviculture* (Publication 1100). Canadian Department of Forestry, Ottawa, Ontario
- Vitousek P (1982) Nutrient cycling and nutrient use efficiency. *Am Nat* 119:553–572
- Welbank PJ (1962) The effects of competition with *Agropyron repens* and of nitrogen- and water-supply on the nitrogen content of *Impatiens parviflora*. *Ann Bot* 26:361–373
- White JW, Castillo JA, Ehleringer J (1990) Associations between productivity, root growth and carbon isotope discrimination in *Phaseolus vulgaris* under water deficit. *Aust J Plant Physiol* 17:189–198