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Shoot water relations of mature black spruce families displaying a genotype × environment interaction in growth rate. III. Diurnal patterns as influenced by vapor pressure deficit and internal water status

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Summary Pressure–volume curves were constructed and shoot water potentials measured for +20-year-old black spruce *(Picea mariana* (Mill.) BSP) trees from four full-sib families growing on a moist site and a dry site at the Petawawa Research Forest, Ontario, to determine whether differences in diurnal water relations traits were related to productivity. To assess the basis for the observed diurnal patterns, we analyzed effects of environmental and internal water stress variables on diurnal water relations traits. Among the water relations traits examined, turgor pressure was the most sensitive, responding to site, family and environmental variables and displaying the strongest diurnal responses to varying soil water availability and atmospheric vapor pressure deficit (VPD). Overall, there was an 84% drop in turgor pressure with increasing VPD: turgor pressure fell 46% in response to the first 0.75 kPa increase in VPD, and 9.7% in response to a second 0.75 kPa increase in VPD. The families differed in water relations responses to moderate water stress, but not in responses to minor or more extreme water stresses. Thus, at a VPD of 0.5 kPa, there was an estimated 83% greater family difference in turgor pressure on the dry site compared with the moist site. Soil and atmospheric water stress appeared to exert effects in tandem to elicit these responses ($r^2 = 0.728$). A comparison of the mechanisms of response to water deficit indicated that osmotic adjustment was more important than change in cell wall elasticity. We used a conceptual water relations model to illustrate the differences between tolerant and intolerant families in their mechanisms of water stress response. We conclude that, because genetic responses to site factors are dynamic, the integrated response over time contributes to the observed genetic × environmental interaction in growth.

Keywords: diurnal variation, genetic variation, modeling, Picea mariana, turgor pressure, water stress.

Introduction

From 1991 through 1993, studies were carried out to elucidate the physiological processes underlying genotypic and environmental (site) variations in growth observed within a subset of black spruce *(Picea mariana* (Mill.) BSP) comprising a 7 × 7 genetic diallel experiment planted across sites of varying water availability. These studies focused on gas exchange (Johnsen and Major 1995, Major and Johnsen 1996), carbon isotope discrimination (Flanagan and Johnsen 1995), and yearly and seasonal water relations (Johnsen and Major 1999, Major and Johnsen 1999). In all of the studies, consistent genetic variation between two female progenies was observed. Johnsen and Major (1999) studied mean yearly site and genetic effects on water relations traits and reported that turgor-related traits were closely correlated with productivity. Major and Johnsen (1999) studied seasonal variation and overall response of water relations traits to soil drought and found that, despite active osmotic adjustment, each family appeared to adjust in a characteristic way, thereby maintaining stable family differences. Because both studies were made on mature trees in the field, genetic and site factors, as well as temporal and environmental factors, affected each water relations trait to varying degrees.

Growth requires an increase in cell volume, which depends on maintenance of high cell turgor pressure. Relative to other processes, cell elongation is very sensitive to increasing water stress (Salisbury and Ross 1985, Grossnickle 2000). The water status of a tree can range from fully turgid to permanent wilting depending on the balance of water loss to water gain, and this balance changes at various temporal scales including seasonally and diurnally (Hinckley et al. 1978, Teskey and Hinckley 1986, Grossnickle 2000). For short-term water balance, vapor pressure deficit (VPD) can be an important environmental driver because it displays considerable diurnal variation (Grossnickle 2000) and increased VPD can sharply decrease water potential and relative water content (Grossnickle and Blake 1986).

In this paper, we focus on diurnal patterns in water relations traits and examine the responses to varied VPD and soil water deficits. Our objective was to examine how diurnal water relations traits differed among families and how they were related to productivity. We hypothesized that productivity differences are related to family differences in diurnal water relations traits. To test this hypothesis, we examined the effects of (1) high soil water deficits, (2) recovery from high soil water deficits, (3) high vapor pressure deficit, and (4) low soil water deficits on the diurnal water balance of putative tolerant and intolerant families growing at moist and dry sites. We also analyzed the responses of the water relations traits to varying VPD, water potential, and relative water content to elucidate the nature of the observed diurnal patterns. Finally, we used a conceptual water relations model to illustrate the differences between tolerant and intolerant families in their mechanisms of response to water stress.

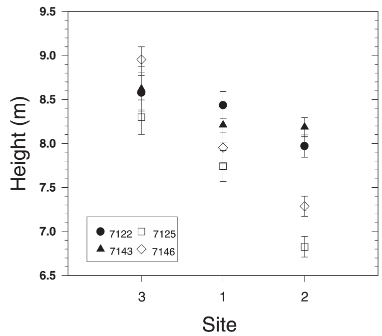

Figure 1. Family height (mean ± SD) of four full-sib families from three sites located at the Petawawa Research Forest, Ontario. Families 7122 and 7143 are progeny from Female 59 (filled symbols) and Families 7125 and 7146 are progeny from Female 63 (open symbols). Measurements were taken in 1992. Sites 3 and 2 are referred to as moist and dry sites, respectively.

Materials and methods

Site location and plant material

A complete 7 × 7 diallel genetic experiment of black spruce was established in 1973, with 2-year-old stock, on three sites located in the Petawawa Research Forest (46° N, 77°30′ W) (Morgenstern 1974, Boyle 1987). In the present study, a subset of four full-sib families, comprising a two-female parent × two-male parent breeding structure (Table 1) growing at two of the sites (Sites 2 and 3), was used. The families displayed a genotype × environment interaction (Figure 1), with Families 7122 and 7143 maintaining relatively high productivity across both sites, whereas Families 7125 and 7146 had high and moderate growth rates, respectively, on Site 1 and 3, but significantly lower growth rates on Site 2.

Because Sites 2 and 3 differ in water availability (Johnsen and Major 1999, Major and Johnsen 1999), they are hereafter referred to as the dry and the moist site, respectively. Both sites have plantations with randomized complete block designs: the dry site has four blocks of 16-tree plots (4 × 4) and the moist site has three blocks of 9-tree plots (3 × 3). Trees were planted at a spacing of 1.8 × 1.8 m. Three of the four blocks from the dry site and the three blocks from the moist site were used for sampling. Trees within family/block plots were randomly assigned to each sampling date; individual trees were only measured on one date.

Water relations

Water relations of 1-year-old foliage were measured during six periods between July 15 and August 9, 1993. On each occasion, predawn and daytime xylem water potentials (Ψ_{pd} and Ψ_x, respectively) and pressure–volume curves were determined on the same trees with pressure chambers (Soil Moisture Corp. model 3005, Santa Barbara, CA). Predawn xylem water potentials were sampled from the middle third of the crown, between 0400 and 0500 h and were also used as a measure of soil water potential (Dougherty and Hinckley 1981, Lucier and Hinckley 1982). Daytime xylem water potentials were measured at 0830–0930, 1030–1130, 1230–1330 and 1430–1530 h on detached branches within minutes after excision. For each sampling period, three replicates were used per family and site. Vapor pressure deficit was measured with an LI-6200 portable photosynthesis system (Li-Cor, Inc., Lincoln, NE) during each measurement time.

For pressure–volume measurements, which were completed within 1 day of the diurnal, *in situ* Ψ_x measurements, lateral shoots (15–20 cm) from the upper third of the crown were collected at dawn and allowed to rehydrate briefly by placing them in a beaker containing 4 cm of water. The beaker was placed on a tray and sealed in a large opaque plastic bag. After about 2 h, shoots were removed and their saturated weights were measured. A pressure–volume curve was determined by collecting measurements of shoot mass and Ψ_x at intervals. Between measurements, the shoot transpired outside the pressure chamber on the laboratory bench (22 ± 2 °C air temperature, 50 ± 10% relative humidity and 60 ± 20 μmol m^{-2} s^{-1} irradiance) (Hinckley et al. 1980, Grossnickle 1989). Shoot dry weights were measured after oven drying at 65 °C for 48 h and dry weight fraction (DWF) was determined by dividing dry weight by saturated weight. The pressure–volume curves were used to determine osmotic potential (Ψ_π) and modulus of elasticity (ε) (Schulte and Hinckley 1985, Grossnickle 1989). *In situ* shoot turgor pressure and relative water content (RWC) were determined for each Ψ_x measurement. Shoot turgor pressure was estimated as the difference between Ψ_x and the corresponding osmotic potential. Relative water content loss (RWCL) was calculated as the difference between 100% and actual RWC.

Table 1. Parentage of the four full-sib families (7122, 7125, 7143 and 7146) of black spruce used in the study.

Male	Female	
	59	63
52	7122	7125
62	7143	7146

Analyses

Because progeny of Female 59 (Families 7122 and 7143) had higher productivity on the dry site than progeny of Female 63 (Families 7125 and 7146), they are referred to as the tolerant and intolerant families, respectively. We note that the designations we have used for our sites and families do not necessarily reflect where these sites and families fit along the larger-scale environmental and genetic spectra of the species.

Four of the six diurnal sets of measurements were chosen to represent distinctly different environmental conditions. For the response analysis, we used the data from all six sampling dates. Correlation analysis was used to test the relationships between water relation traits, with VPD, RWCL and Ψ_x as independent variables. Covariate analysis, with female parent as a class variable, was used to examine responses of water relation traits to independent variables. Effects of female parent were tested in an analogous way to the analysis of covariance of family effects described by Johnsen and Bongarten (1991), based on the model $Y_{ij} = B_0 + B_{0i} + B_1X_{ij} + B_{1i}X_{ij} + E_{ij}$, where Y_{ij} is the dependent variable water relation trait of the jth plant of the ith female parent, B_0 and B_1 are mean regression coefficients, B_{0i} and B_{1i} are female parent treatment coefficients, X_{ij} is the independent variable (i.e., VPD), and E_{ji} is the error term. In this analysis, three sources of variation were identified: (1) VPD/RWCL (covariate), (2) female parent and (3) female parent × covariate. Significant female parent effects indicate differences in female parent progeny means (i.e., differences in B_{0i} coefficients, if B_{1i} coefficients are similar) and significant female parent × covariate effects indicate differences in the slopes (B_{1i} coefficients) between female parent progeny. When curvilinear responses occurred between variables, linearized models were employed in the covariate analysis.

Results

Environmental conditions

Among measurement days, the highest soil water deficit occurred on July 23 at both sites (Figures 2a and b) when mean Ψ_{pd} was –0.62 MPa on the dry site and –0.41 MPa on the wet site. Fifty mm of rain fell during the night of July 28–29, resulting in sharply increased water availability. With the exception of July 29, the lowest soil water deficit occurred on August 3, when both the dry and moist sites had a mean Ψ_{pd} of –0.38 MPa. Mean VPD was highest on July 19 (Figures 2c and d) with values of 2.3 kPa (range = 1.0–3.3 kPa) on the dry site and 1.9 kPa (range = 0.9–2.5 kPa) on the moist site. Because July 23, July 28–29, July 19 and August 3 illustrate periods of (1) high soil water stress, (2) recovery from high soil water stress (3) high VPD stress and (4) low soil water stress, respectively, we analyzed the diurnal water relations traits of the families during these four periods.

High soil water deficits

On July 23, there were minor differences in VPD between sites (Figure 3a). Throughout the day, RWC was lower on the dry site than on the moist site (Figure 3b) and trees at the dry site had significantly lower Ψ_{pd} than trees at the moist site (Figure 3c). Daytime Ψ_x showed minor or no differences between families, but site differences were consistent with those of Ψ_{pd}. The intolerant family on the dry site had higher Ψ_π and lower ε than the other site × family combinations (Figures 3d and 3e). Throughout the day, trees at the moist site had higher turgor pressures than trees at the dry site (Figure 3f). There were no differences in turgor pressure between families at the moist site; however, on the dry site, tolerant families had higher turgor pressures than intolerant families, particularly during the predawn to late-morning hours. During the afternoon, turgor pressure stabilized at about 0.8 and 0.4 MPa on the moist and dry sites, respectively.

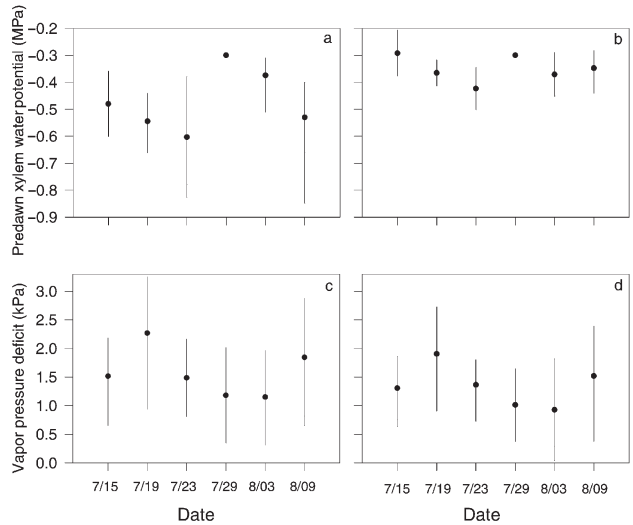

Figure 2. Environmental conditions on days of physiological measurements. Mean and range of predawn xylem water potentials in trees at the dry (a) and moist (b) sites, and mean and range of vapor pressure deficits at the dry (c) and moist (d) sites.

Recovery from high soil water deficits

Vapor pressure deficit increased steadily throughout July 29 and was slightly higher at the dry site than at the moist site (Figure 4a). Relative water content displayed a near linear drop from 100% to 89 and 83% on the moist and dry sites, respectively (Figure 4b). On the day after heavy rain, daytime Ψ_x remained slightly higher at the moist site than at the dry site (Figure 4c). On the dry site, Ψ_π of tolerant families was 0.35 MPa lower than that of intolerant families on the dry site, whereas there was only a 0.1 MPa difference on the moist site (Figure 4d). Throughout most of July 29, intolerant families on the dry site displayed significantly lower ε compared with the other family × site combinations (Figure 4e). Trees at the moist site had higher turgor pressure than trees at the dry site (Figure 4f). Turgor pressure of the tolerant families on the dry site recovered to the values of both families on the moist site on July 29. However, intolerant families on the dry site had consistently lower turgor pressure than tolerant families throughout the day.

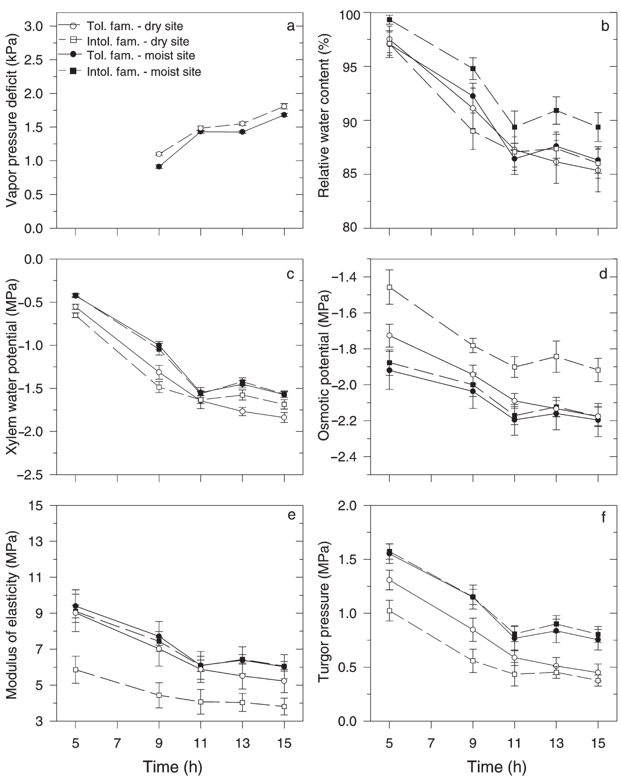

Figure 3. Mean and standard error of (a) vapor pressure deficit, (b) relative water content, (c) xylem water potential, (d) osmotic potential, (e) modulus of elasticity, and (f) turgor pressure of tolerant and intolerant families at the dry and moist sites on July 23, a day with high soil water deficits.

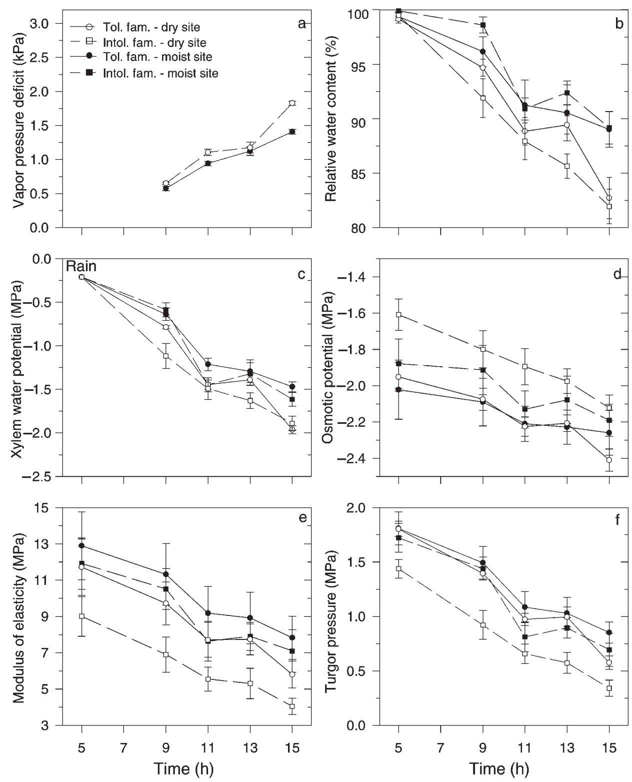

Figure 4. Mean and standard error of (a) vapor pressure deficit, (b) relative water content, (c) xylem water potential, (d) osmotic potential, (e) modulus of elasticity and (f) turgor pressure of tolerant and intolerant families at the dry and moist sites on July 29 during recovery from high soil water deficits.

High vapor pressure deficit and moderate soil water deficits

Vapor pressure deficit was significantly higher at the dry site compared with the moist site throughout July 19, particularly after midday, when values reached 3.1 and 2.4 kPa, respectively (Figure 5a). On the dry site, RWC fell quickly from 97 to 84% and remained nearly constant thereafter (Figure 5b). On the moist site, RWC fell at a more modest rate reaching about 85% by 1300 h. Predawn xylem water potential displayed significant site effects but no family effects (Figure 5c). Trees at the moist site had higher Ψ_x from predawn to 1100 h than at the dry site. After midday, there were no site or family differences in Ψ_x. Tolerant families from the moist site had higher ε and lower Ψ_π than the other family × site combinations (Figures 5d and 5e). Trees at the moist site had higher turgor pressure than trees at the dry site, and at both sites turgor pressure reached a plateau after 0900 h (Figure 5f). There were no significant differences in turgor pressure between families on the dry site; however, at the moist site, tolerant families displayed higher turgor pressures than intolerant families most of the day.

Low soil water deficits

On August 3, VPD was slightly higher at the dry site than at the moist site, reaching about 1.75 kPa by 1500 h (data not shown). Osmotic potential at both sites decreased 15%, from about –1.90 to –2.20 MPa. Throughout most of the day, mean ε decreased from a predawn value of 12 MPa to 7 MPa by 1500 h, and was higher in trees at the moist than the dry site. Turgor pressure displayed a modest decrease from predawn to 0900 h, then rapidly decreased until 1300 h and remained constant thereafter at about 0.7 MPa. Turgor pressure, Ψ_x and Ψ_π showed no consistent or significant differences between sites or families over the day.

Water relations versus environmental factors

Daytime Ψ_x decreased curvilinearly in response to increasing VPD ($P < 0.001$, $r^2 = 0.812$) (Figure 6a). Daytime xylem water potential decreased relatively quickly from 0.3 to 1.0 kPa VPD. At 1.5 kPa VPD, Ψ_x leveled to a mean value of about –1.90 MPa. Covariate analysis revealed no family differences in the response of Ψ_x to increasing VPD ($P = 0.193$).

Relative water content declined linearly with increasing VPD from 0.3 to 3.2 kPa ($P < 0.001$, $r^2 = 0.623$) (Figure 6b), but there was no family effect ($P = 0.381$) or family × VPD interaction ($P = 0.483$) even though RWC decreased to 80%. Xylem water potential decreased linearly with decreasing RWCL ($P < 0.001$, $r^2 = 0.687$) (Figure 6c), but there was no family effect ($P = 0.687$) or family × RWCL interaction ($P = 0.190$).

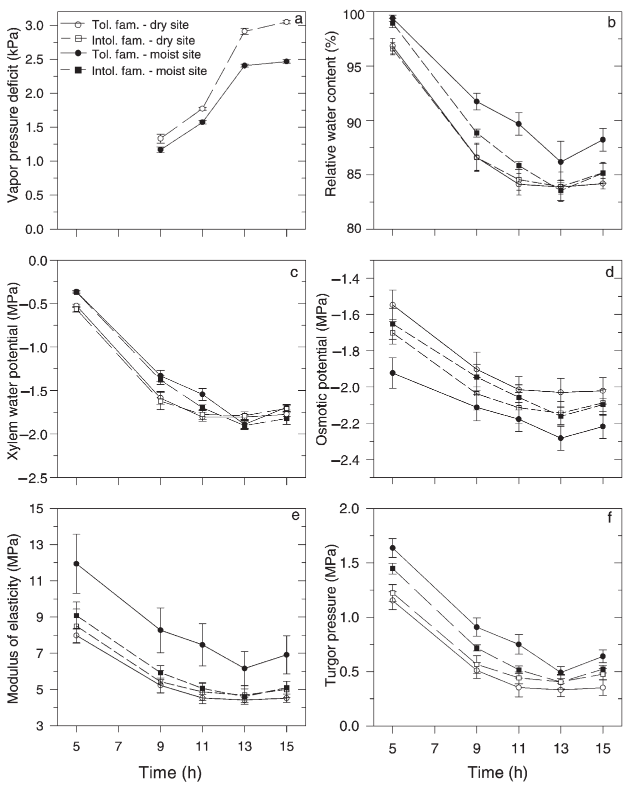

Figure 5. Mean and standard error of (a) vapor pressure deficit at the dry and moist sites on July 19, a day of high vapor pressure deficits and moderate soil water deficits. Mean and standard error of (b) relative water content, (c) xylem water potential, (d) osmotic potential, (e) modulus of elasticity and (f) turgor pressure of tolerant and intolerant families at the dry and moist sites on July 19, a day of high vapor pressure deficits and moderate soil water deficits.

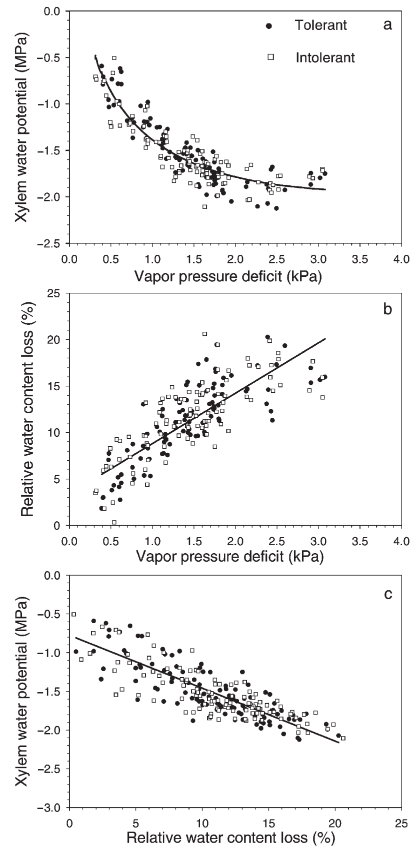

Figure 6. Relationships between (a) xylem water potential and vapor pressure deficit, $y = 1.63 + 0.247x + 0.93\ln(x)$: $r^2 = 0.812$, (b) relative water content loss and vapor pressure deficit, $y = 0.0386 + 0.0521x$: $r^2 = 0.623$ and (c) xylem water potential and relative water content loss, $y = -0.822 - 6.44x$: $r^2 = 0.687$.

Daytime Ψ_π decreased linearly with increasing VPD (Figure 7a). Although there was no difference in slope between families ($P = 0.357$), there was a significant family effect ($P = 0.000$, $r^2 = 0.275$). Osmotic potentials ranged from –2.05 to –2.30 MPa for tolerant families and from –1.95 to –2.20 MPa for intolerant families at 0.25 and 3.0 kPa VPD, respectively. Osmotic potentials displayed a similar response to increasing RWCL (data not shown). Although there was no difference in slope between families ($P = 0.623$), there was a significant family effect ($P = 0.000$, $r^2 = 0.334$). For tolerant families, Ψ_π values were lower, ranging from –1.95 to –2.30 MPa and from –1.85 to –2.20 MPa for intolerant families at 0 and 20% RWCL, respectively. Against either independent axis, tolerant families had a 0.1 MPa lower Ψ_π than intolerant families.

Modulus of elasticity displayed a significant negative linear relationship to VPD ($P < 0.001$, $r^2 = 0.494$) (Figure 7b). There were no differences in slopes between families ($P = 0.178$), but the tolerant family had a 1.0 MPa higher ε across all VPDs than the intolerant family ($P < 0.001$). Similarly, ε for both families displayed a significant negative linear relationship to RWCL ($P < 0.001$, $r^2 = 0.367$) (data not shown). There were no differences in slopes between families ($P = 0.500$), but the tolerant family had a 1.0 MPa higher ε across all RWCLs than the intolerant family ($P < 0.001$).

Turgor pressure displayed a significant positive linear relationship to ε ($P = 0.000$, $r^2 = 0.676$) (Figure 7c). Daytime ε ranged from 3.0 to 13.2 MPa and corresponding turgor pressures ranged from 0.25 to 1.50 MPa. Covariate analysis indicated no differences in slopes between families ($P = 0.287$) and no significant family effect ($P = 0.120$). Osmotic potential showed no significant relationship to elasticity ($P = 0.483$, $r^2 = 0.086$) and a statistically significant but trivial relationship to turgor pressure ($P = 0.002$, $r^2 = 0.069$).

Turgor pressure displayed a negative linear response to RWCL ($P < 0.001$, $r^2 = 0.581$) (Figure 8a). Covariate analysis indicated no differences in slopes between families ($P = 0.233$), but significant differences between families ($P = 0.003$). In contrast, turgor pressure displayed a strong negative curvilinear response to VPD ($P = 0.000$, $r^2 = 0.727$) (Figure 8b). There was a significant family effect ($P < 0.001$) and a family × VPD interaction ($P = 0.001$). The largest family differences were found at low VPD where tolerant families generated higher turgor pressures than intolerant families. At a VPD of 2.0 kPa, there were no turgor pressure differences between families.

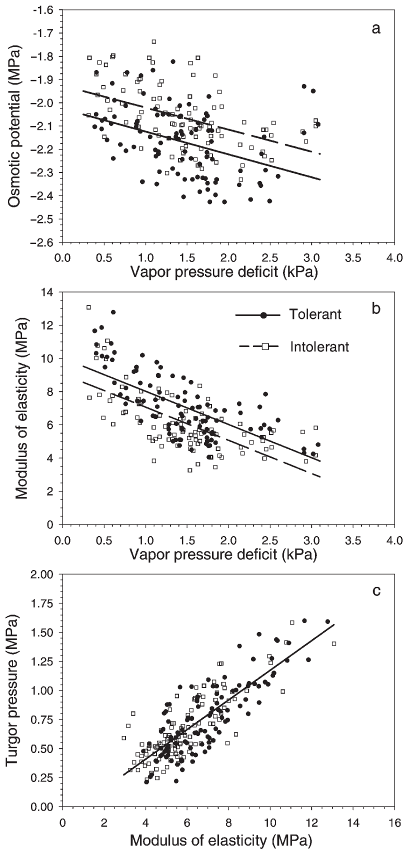

Figure 7. Relationships between (a) osmotic potential and vapor pressure deficit. Equations from analysis of covariance: tolerant families, $y = -2.025 - 0.097x$ and intolerant families, $y = -1.921 - 0.097x$: $r^2 = 0.275$. Relationships between (b) modulus of elasticity and relative water content loss. Equations from analysis of covariance: tolerant families, $y = -10.02 - 2.00x$ and intolerant families, $y = -9.06 - 2.00x$: $r^2 = 0.494$. Relationships between (c) turgor pressure and modulus of elasticity, $y = -0.102 + 0.128x$: $r^2 = 0.676$.

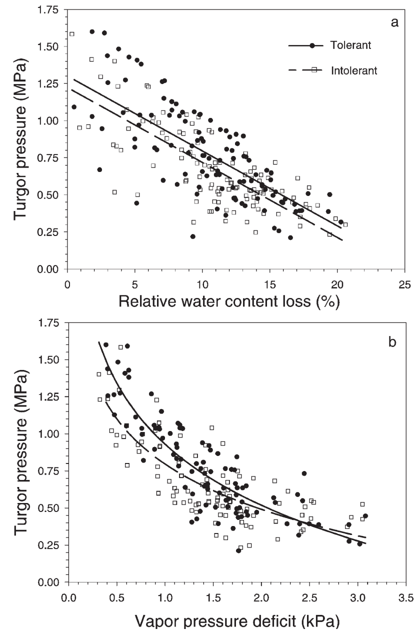

Figure 8. Relationships between (a) turgor pressure and relative water content loss. Equations from analysis of covariance: tolerant families, $y = -1.301 - 0.051x$ and intolerant families, $y = -1.223 - 0.051x$: $r^2 = 0.581$. Relationships between (b) turgor pressure and vapor pressure deficit. Equations from analysis of covariance: tolerant families, $y = 0.930 - 0.594\ \ln(x)$ and intolerant families, $y = 0.796 - 0.441\ \ln(x)$: $r^2 = 0.727$.

Three-dimensional response surfaces were produced for the interaction and tandem effects of soil and atmospheric pressure deficits on turgor pressure. The relationships of turgor pressure to VPD and Ψ_{pd} displayed a curvilinear surface with the tolerant family shown overlaying the intolerant family ($r^2 = 0.728$) (Figure 9a). Covariate analysis indicated significant VPD (ln(VPD), $P < 0.001$) and family effects ($P = 0.001$) and a family × VPD interaction ($P = 0.005$). Family × Ψ_{pd} ($P = 0.903$) and VPD × Ψ_{pd} ($P = 0.676$) interactions were not significant. The largest difference in turgor pressure between families was at low VPD, and no differences were observed at VPDs above 1.5 kPa.

Turgor pressure also displayed a significant interactive curvilinear surface with RWCL and VPD ($r^2 = 0.819$) (Figure 9b). The tolerant families are shown overlaying the intolerant families. Covariate analysis indicated significant VPD (ln(VPD), $P < 0.001$) and family effects ($P < 0.001$), and family × VPD ($P = 0.002$) and RWCL × VPD ($P < 0.001$) effects. The family × RWCL interaction was not significant ($P = 0.240$). Highest turgor pressure values were found at low VPD and low RWCL.

Discussion

Black spruce families display highly dynamic diurnal water relations and responses to environmental conditions that, when integrated over time, appear to contribute to genetic variation in growth (Johnsen and Major 1999). Previous studies showed that the relative performance of Sitka (*Picea sitchensis* (Bong.) Carrière) and interior spruces (Fan et al. 1997) and of western hemlock (*Tsuga heterophylla* (Raf.) Sarg.) and western redcedar (*Thuja plicata* Donn *ex* D. Don) (Grossnickle 1993) depend on the intensity of soil water stress. Similarly, the water relations responses of the black spruce families varied depending on prevailing environmental conditions at each site. Under moist conditions at both sites, there were no turgor pressure, Ψ_x, ε or Ψ_π differences between sites or families (August 3). However, at high soil water deficits, family, site and family × site interaction effects on turgor pressure were evident (July 23). These results support the conclusion that water availability is the dominant environmental source of variation between the sites (Major and Johnsen 1996). They also confirm that the progenies of these two female parents differ in drought tolerance (Johnsen and Major 1995, 1999, Major and Johnsen 1996, 1999, Flanagan and Johnsen 1995). Recovery from water stress also appears to contribute to the family × environment interaction (July 29).

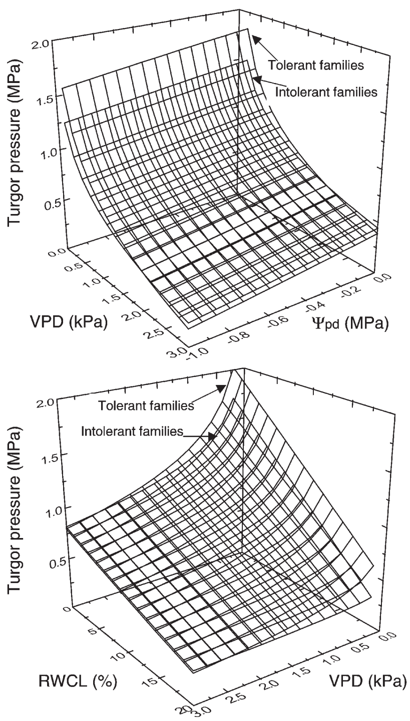

Figure 9. Relationships of (*a*) turgor pressure (*z*) to vapor pressure deficit (VPD, *x*) and predawn water potential (Ψ_{pd}, *y*). Equations from analysis of covariance: tolerant families, $z = 0.977 - 0.533 \ln(x) + 0.225y$ and intolerant families, $z = 0.881 - 0.400 \ln(x) + 0.225y$: $r^2 = 0.728$. Relationships between (b) turgor pressure (*z*) to relative water content loss (RWCL, *x*) and vapor pressure deficit (VPD, *y*). Equations from analysis of covariance: tolerant families, $z = 1.294 - 0.0422x - 0.451 \ln(y) + 0.0153x \ln(y)$ and intolerant families, $z = 1.184 - 0.0422x - 0.332 \ln(y) + 0.0153x \ln(y)$: $r^2 = 0.819$.

When water stress was removed, turgor pressure of tolerant families from the dry site immediately recovered to that of trees growing on the moist site, whereas intolerant families continued to have low turgor pressures. However, during a day of both high VPD and soil water deficits, family differences were observed at the dry site but not at the moist site (July 19). We conclude that these families express their genetic differences in water relations traits in response to moderate water stress, but not in response to low or severe water stress.

Vapor pressure deficit greatly impacted diurnal water relations (cf. Goldstein et al. 1985, Grossnickle and Reid 1985, Grossnickle and Blake 1986, Maier-Maercker 1998). Among the water relations traits measured, turgor pressure was the most sensitive, responding to site, family and environmental variables and displaying the most dramatic response to VPD. Although tolerant families had approximately 5% lower Ψ_π (0.1 MPa) than intolerant families over the range of environmental conditions, family had no effect on Ψ_{pd}. The convex relationship between Ψ_x and VPD indicates that Ψ_x is initially very sensitive to VPD but that it becomes less so at high VPD. When the VPD effect was ignored, Ψ_x had a linear relationship to RWC, indicating that, although the amount of water lost is linearly related to VPD, the amount of stress measured is nonlinear.

There was no difference between families in the rate of change of Ψ_π to increasing VPD or RWCL. The decrease in osmotic potential in response to an increase in VPD or RWCL is caused by solute concentration as a result of water loss or passive adjustment (Turner and Jones 1980, Hsiao and Jing 1987). If we assume that diurnal changes in Ψ_π were largely passive, the mean rate of passive osmotic adjustment was about –0.1 MPa kPa^{-1} VPD. This is equal to 0.25 MPa or 12.1% over the VPD range of 0.5–3.0 kPa, observed for the season. It is also equivalent to an approximate RWC range of 20%. If active adjustment is defined as the daily change in Ψ_π in response to increasing water stress, the daily rate of active adjustment from mild to moderate stress was approximately 18% (Major and Johnsen 1999).

Cell wall elasticity can influence turgor pressure (Kramer 1983, Colombo 1987, Grossnickle 2000). In a seedling study with the same families used here, Tan and Blake (1997) attributed family growth differences partly to variation in ε. Although we observed mean family differences in ε, our analyses suggest this was simply a correlated response to family differences in turgor pressure because the families shared the same turgor pressure/ε regression line. In addition, ε values were not correlated with growth (Johnsen and Major 1999), further suggesting that ε had played a passive role, at least during our midsummer measurement period.

In our study, VPD (log transformed) accounted for 68.9% of the daytime variation in turgor pressure. Because growth requires turgor pressure (Lockhart 1965*a*, Ray 1987), a primary effect of high VPD is to inhibit cell enlargement and growth (Hsiao and Jing 1987). If, for example, the turgor pressure threshold for cell enlargement is a modest 0.75 MPa, this value is reached at VPDs of about 1.0 kPa. Our results indicate that this VPD is often exceeded by 0900 h (in four of our six diurnal measurements).

Soil water deficits impact water relations traits (Kwon and Pallardy 1989, Grossnickle and Major 1994). In response to high soil water deficits, turgor pressure decreased approximately 38.6%, or approximately half of the decline observed in response to high VPD (Major and Johnsen 1999). The decrease occurred during the last half of the soil drought because active osmotic adjustment maintained turgor pressure during the first half of the soil drought. Vapor pressure deficits and soil water deficits acted in tandem and affected the turgor pressure of the families differently. Genetic differences in turgor pressure occurred at intermediate soil water deficits and were caused by differences in osmotic potential (Major and Johnsen 1999). Turgor pressure decreased with increasing VPD but family differences were greatest at low VPDs, which only occurred early in the morning. Irrespective of whether the water stress originated from below or above ground, it appears the effect was similar; however, atmospheric stress had a greater impact on turgor than soil water stress. Because photosynthetic differences between the families were not stomatally regulated (Johnsen and Major 1995, Major and Johnsen 1996), we postulate that the positive genetic relationship between turgor and growth is a function of the direct effects of turgor pressure on cell expansion, division and other biochemical processes.

Relative water content loss impacts turgor by removing available water (Levitt 1972, p 379). In relation to RWCL, the rate of turgor pressure decrease was –0.05 MPa per unit RWCL (%) with a family difference of 0.08 MPa or approximately 6%. The relationships of turgor pressure with VPD and RWCL and the interactive effect accounted for 82% of the variation in turgor pressure, with both main effects (VPD and RWCL) having similar impact. The significant results for both variables may reflect both a real time environmental effect (VPD) and a real time tree condition effect (RWC). These traits do not immediately follow one another. Vapor pressure deficit is almost always in flux, whereas relative water content is a cumulative result of water loss.

According to Lockhart's growth equation (Lockhart 1965*a*, 1965*b*, Cleland 1987, Okamoto 1996), turgor pressure must exceed a threshold value for growth to occur. We found that, at turgor pressures above this threshold, during a combination of intermediate soil water deficits and low VPD, genetic differences in turgor are most strongly expressed. For example, at a VPD of 0.5 kPa on the dry site, estimated turgor pressures were 1.36 and 1.03 MPa for tolerant and intolerant families, respectively, and the corresponding values on the moist site were 1.31 and 1.13 MPa (estimated by ANCOVA across all six dates). Thus, there was an 83% larger family difference on the dry site than on the moist site. In addition, turgor, especially before dawn, is highly correlated with growth (up to r = 0.904) (Johnsen and Major 1999).

Mean diurnal turgor pressure never fell below the turgor loss point on any measurement date and the turgor loss point was only surpassed on three of the over 670 individual measurements taken. On the day of high VPD and high soil water deficits, turgor pressure had already fallen to 0.5 MPa by 0900 h at the dry site when VPD was 1.6 kPa. Although VPD doubled by the afternoon, turgor pressure declined to only 0.3 MPa, indicating an increased resistance to turgor loss for black spruce. Stomatal aperture was reduced (Grossnickle and Blake 1986, Grossnickle and Major 1994), as indicated by the stomatal conductance measurements, but stomata were always open (Major and Johnsen 1996). Although reduced stomatal conductance was associated with lower P_n, in this study and others (Stewart et al. 1995, Major and Johnsen 1996) photosynthetic reduction to drought in black spruce is often largely non-stomatal (Stewart et al. 1995, Major and Johnsen 1996).

Figure 10 illustrates a conceptual water relations model of the differences in responses to water stress between the tolerant and intolerant families. The largest differences between families in turgor pressure occur at high RWC (Figure 10a). Family differences in osmotic potential contribute to the family differences in turgor pressure. As RWCL increases, differences between families narrow. At the turgor loss point, there are no family differences as measured by RWC or Ψ_π. In response to increasing soil water deficits, both families actively adjust Ψ_π (Figure 10b) (cf. Koppenaal et al. 1991, Major and

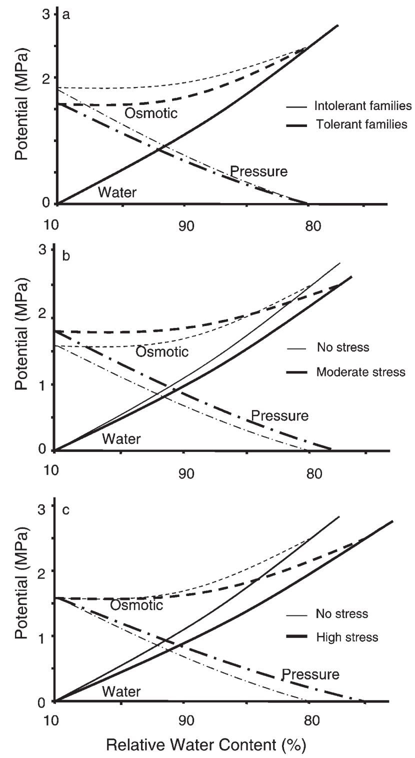

Figure 10. Conceptual Hofler water relations model illustrating comparisons between: (a) intolerant and tolerant genotypes: (b) no water stress and moderate water stress: and (c) no water stress and high water stress.

Johnsen 1999) to maintain turgor pressure. Although relative water content at the turgor loss point declines, no changes in Ψ_π occur at the turgor loss point. Elasticity of the cell walls increases with increasing water stress (Major and Johnsen 1999). In response to high water stress, Ψ_π reverses and becomes more positive (Figure 10c). Turgor pressure decreases and relative water content at the turgor loss point continues to decline, but there are no significant changes in osmotic potential at the turgor loss point.

Because the model integrates the physiological processes of black spruce responses to water stress, it might provide useful information about where tree improvement selection programs should focus efforts. The four full-sib families used here represent approximately 40% of the genetic variation in growth of the outcrossed families in the entire 7 × 7 diallel experiment (Figure 4, Johnsen et al. 1999). Although genetic variation in photosynthetic characteristics appears to be of paramount importance (Johnsen et al. 1999), within the diallel, these four families express a consistent genetic × environmental interaction in growth and water relations. As breeding programs advance, this significant and subtler variation may become increasingly important. Combining these traits by clonal selection may also be possible, depending on inheritance and the ability to screen large numbers of clones successfully.

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