Family variation in photosynthesis of 22-year-old black spruce: a test of two models of physiological response to water stress

John E. Major and Kurt H. Johnsen

Abstract: Gas exchange and water potential were measured in 22-year-old black spruce (*Picea mariana* (Mill.) BSP) trees from four full-sib families on two sites (one drier and one wetter) at the Petawawa National Forestry Institute, Ontario. Based on an observed genotype × environment interaction and earlier work with seedlings, a hypothesis was formed that at high soil moisture availability, no genetic differences in net photosynthesis (P_n) would exist and as soil moisture decreases, genetic differences in P_n would increase. From results of initial research with mature trees we formed an alternative hypothesis that genetic differences in P_n are constantly maintained under an array of soil moisture conditions. The two models were rigorously tested over a range of soil moisture conditions using two physiological measurement crews who switched sites throughout the day. Second-year foliage P_n of mature black spruce was more affected by nonstomatal limitations than by stomatal limitations. Progeny of one female had 12.5% and 7.4% higher P_n than progeny of another female on the dry and wet site, respectively. Genetic variation in P_n was consistent over a range of soil water potential. Thus, the first hypothesis was rejected in favor of the alternative hypothesis. Genetic variation in P_n appeared to be due to differential response to vapor pressure deficit. Suggestions as to how to reconcile the observed genotype × environment interaction in growth with the genetic differences in P_n are discussed.

Résumé : Les auteurs ont mesuré les échanges gazeux et le potentiel hydrique chez des épinettes noires (*Picea mariana* (Mill.) BSP) de 22 ans représentatives de quatre descendances biparentales et plantées sur un site sec et un site humide à la forêt expérimentale de l'Institut forestier national de Petawawa, en Ontario. À partir de l'observation d'une interaction entre le génotype et l'environnement et des travaux antécédents portant sur des semis, une hypothèse a été émise, voulant qu'à humidité élevée du sol, il n'y ait pas de différences génétiques au niveau de la photosynthèse nette (P_n) et que lorsque l'humidité du sol diminue, les différences génétiques en P_n augmentent. À partir de résultats préliminaires de recherche portant sur des arbres matures, les auteurs ont formulé une seconde hypothèse voulant que les différences génétiques en P_n soient maintenues sous une variété de conditions d'humidité du sol. Ces deux hypothèses furent rigoureusement testées sous un éventail de conditions d'humidité du sol avec deux équipes qui prenaient des mesures physiologiques en alternance sur chacun des sites durant chaque journée de mesurage. La photosynthèse nette du feuillage âgé de 2 ans d'épinettes noires matures était plus affectée par des facteurs limitatifs qui n'étaient pas reliés à la transpiration par les stomates. La descendance d'une femelle démontrait une photosynthèse nette supérieure à celle de la descendance d'une autre femelle, avec des valeurs accrues de 12,5% sur site sec et de 7,4% sur site humide. Les variations génétiques en photosynthèse nette se maintenaient sous un éventail de valeurs de potentiel hydrique du sol. La première hypothèse de travail fut donc rejetée aux dépens de la seconde hypothèse. Les auteurs estiment que cette variabilité génétique en photosynthèse nette pourrait être la résultante d'une réponse différentielle à des déficits de pression de vapeur. Ils suggèrent également des moyens de réconcilier les observations d'interaction génotype × environnement en croissance avec la variabilité génétique observée en photosynthèse nette.

[Traduit par la Rédaction]

Introduction

Water is a predominant factor in determining the geographic distribution of vegetation, and thus drought tolerance would appear to be an important yield factor (Schulze 1986). Water stress has long been known to decrease net photosynthesis (P_n), but the precise mechanisms controlling this effect are not completely understood (Briggs et al. 1986; Schulze 1986; Losch and Schulze 1994; Pallardy et al. 1995). Stomatal limitation to P_n during water stress is varied and depends on a number of genetic and environmental factors (Running 1976; Pezeshki and Chambers 1986; Sala and Tenhunen 1994). Water stress, both soil and atmospheric, can also reduce P_n via influences on nonstomatal processes (Teskey et al. 1986; Seiler and Cazell

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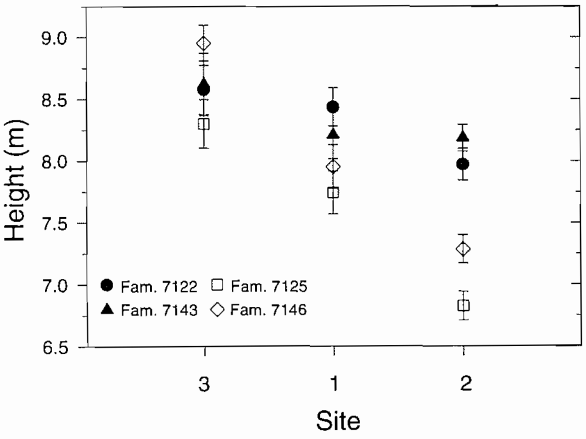
Table 1. Parentage of the four full-sib families (7122, 7125, 7143, and 7146) of black spruce used to study gas exchange.

	Female	
Male	59	63
52	7122	7125
62	7143	7146

Fig. 1. Family mean height for four full-sib families from three sites located at the Petawawa National Forestry Institute. Families 7122 and 7143 are progeny from female 59 (closed symbols) and families 7125 and 7146 are progeny from female 63 (open symbols). Measurements were taken in 1992.



1990; Stewart et al. 1995). Literature describing conifer genetic variation in the relationship of gas exchange in relation to water stress is limited, and all of it involves the use of seedlings (Bilan et al. 1977; Sands et al. 1984; Seiler and Johnson 1988; Tan et al. 1992*a*; Wang and Macdonald 1993; Zine El Abidine et al. 1994; Zhang and Marshall 1994).

A standard quantitative genetic analysis of a 7 × 7 black spruce (*Picea mariana* (Mill.) BSP) diallel on three sites at the Petawawa National Forestry Institute, Ontario, indicated an important genotype × environment (G × E) interaction in growth characteristics (Boyle 1987). Four families that exhibited this growth variation between sites were selected for further examination (Fig. 1). These families compose a 2 parent × 2 parent factorial breeding structure (Table 1). One female parent (59) produced families that displayed relatively high productivity on the three sites, whereas the other female parent (63) produced families that had high growth rates on two of the sites (1 and 3) but not on the third, least productive, site (2) (Fig. 1). Initial evidence suggested that site variation was largely due to differences in soil moisture availability. Collected on different days, predawn xylem water potential (ψ_{pd}), daytime xylem water potential (ψ_x), P_n, and needle conductance (g_{wv}) were lower on site 2 than on site 3 (Johnsen and Major 1995). Foliar stable carbon isotope discrimination analysis indicated that trees from site 2 discriminated less against the heavier carbon isotope than trees on the other two sites, which was indicative of greater water stress on site 2 relative to site 3 (Flanagan and Johnsen 1995).

Tan et al. (1992*a*, 1992*b*), working with seedlings of the same families, suggested that the higher growth rate of the faster growing families on the dry site is due to higher P_n, which was caused by greater g_{wv}, attributable to greater osmotic adjustment. They found that there was little progeny variation in any of the measured parameters in unstressed seedlings. Hence, a hypothesis of a genetic differential response in drought tolerance was formed (Fig. 2, model A). At high soil moisture availability, no P_n difference between progeny of females would be observed, and as soil moisture decreases, P_n difference between the two sets of progeny would increase.

It was observed in both 1991 and 1992 that progeny of female 59 displayed lower foliar stable carbon isotope discrimination than progeny of female 63 on all three sites, indicating that progeny of female 59 had a higher P_n/g_{wv} ratio (Flanagan and Johnsen 1995). Midsummer analysis of gas exchange data collected from sites 2 and 3 on different days in 1991, a dry year, found that progeny of female 59 had significantly higher P_n than progeny of female 63 on both sites (Johnsen and Major 1995). No significant differences in g_{wv} were detected between progeny of the two female parents (Johnsen and Major 1995). Hence, we formed a alternative hypothesis: P_n differences among these families are constantly maintained under an array of soil moisture conditions (Fig. 2, model B).

This paper presents gas exchange and water potential measurements in 22-year-old black spruce trees measured in 1993 from the same four full-sib families studied above. To rigorously test these hypothetical physiological models, data were collected simultaneously on sites 2 and 3, which represent sites with the lowest and highest productivity, respectively, over a range of soil moisture conditions. Our objectives were to (1) more rigorously test the hypothesis that the environmental (site) variation is attributable to differences in water availability; (2) further test for genetic variation in gas exchange traits between progeny of the two female parents; (3) test the two hypothetical physiological models to see which best described the genetic response to water stress; and (4) examine possible physiological mechanisms that further explained the genetic variation in gas exchange.

Materials and methods

The four full-sib families of black spruce are a subset of a complete 7 × 7 diallel experiment established, with 2-year-old stock, on three sites found within a 4.8-km (3-mi) radius located at the Petawawa National Forestry Institute (46°00′N, 77°30′W) in 1973 (Morgenstern 1974; Boyle 1987). The four families used compose a 2 female parent × 2 male parent breeding structure (Table 1). The exact location of parent trees is unknown; however, it is known that they originated from the Lake Simcoes – Rideau Region of Ontario (Hills 1960). Measured in 1992, the three sites displayed a range of productivity; mean heights for the four families were 8.09, 7.57, and 8.63 m for sites 1, 2, and 3, respectively. Full-sib families 7122 and 7143 maintained relatively

Fig. 2. Two hypothetical models describing the net photosynthesis (P_n) relationship to increasing soil moisture deficit.

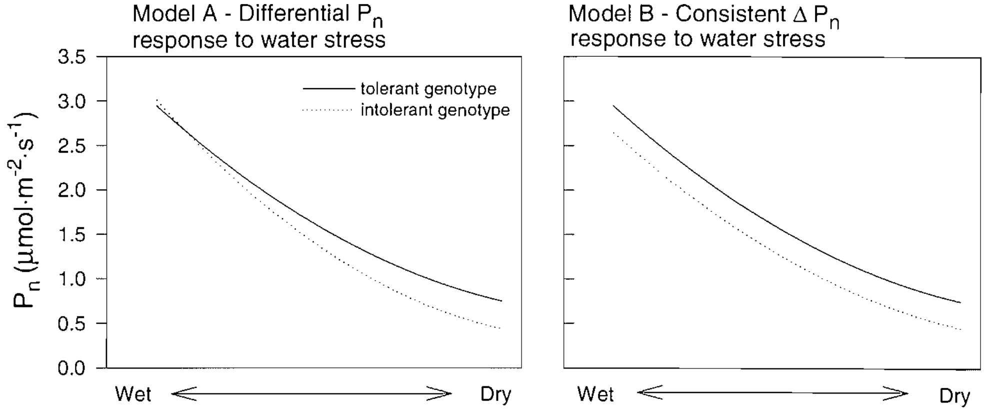


Fig. 3. Daily environmental conditions at Petawawa National Forestry Institute for (A) precipitation and (B) mean, maximum, and minimum air temperature at 1.3 m. Dates on x-axes (month/day) are the dates on which physiological measurements took place.

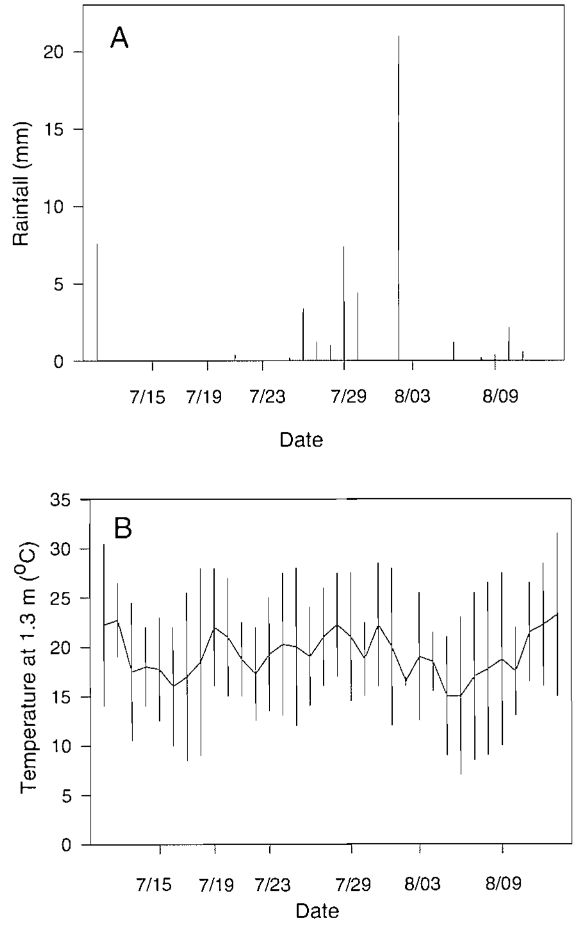
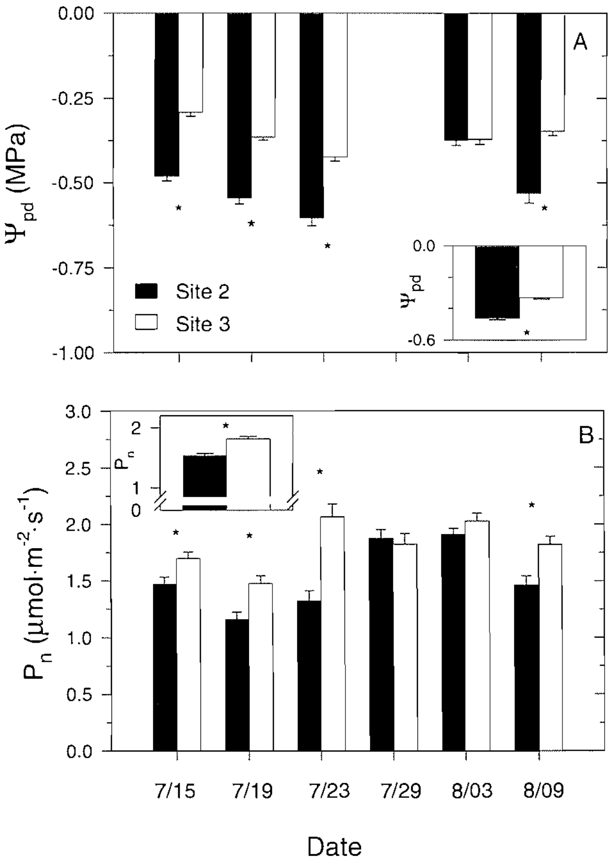


Fig. 4. (A) Mean site predawn xylem water potential (ψ_{pd}) and (B) mean site net photosynthesis (P_n), by measurement date (month/day) for 22-year-old black spruce from four full-sib families. Insert shows mean across all dates. Asterisks indicate dates during which site means were different at $p = 0.05$.



high productivity across all three sites, whereas families 7125 and 7146 had high growth rates on sites 1 and 3 but significantly lower growth rates on site 2 (Fig. 1).

Sites 2 and 3, representing growth extremes, were selected to test the hypotheses. Both sites have plantations with randomized complete block designs: site 2 has four blocks of 16-tree plots (4 × 4) and site 3 has three blocks of 9-tree plots (3 × 3). Trees were planted at a 1.8 × 1.8 m spacing. Physiological measurements were taken on six separate dates between July 15 and August 9, 1993. Trees within family/block plots were randomly assigned to each date; individual trees were

Table 2. Sources of variation, degrees of freedom (df), and *p*-values from ANOVA of 1993 physiological traits.

Source*	df	Trait†									
		P_g	R_d	P_n	C_i/C_a‡	g_m	l_g	g_{wv}	P_n/g_{wv}	ψ_x	ψ_{pd}
F	1	**0.0002**	0.5875	**0.0004**	0.0642	**0.0230**	0.0643	**0.0456**	0.0794	0.1095	0.0541
M	1	0.2072	0.2648	0.0932	0.2689	**0.0071**	0.3487	**0.0134**	0.3693	**0.0035**	0.9791
S	1	**0.0000**	0.5162	**0.0000**	**0.0000**	0.3695	**0.0000**	0.1936	**0.0000**	**0.0000**	**0.0000**
D	5	**0.0000**	**0.0000**	**0.0000**	**0.0000**	**0.0000**	**0.0000**	**0.0000**	**0.0000**	**0.0000**	**0.0000**
F×M	1	0.1015	0.8928	0.0843	0.4308	0.8385	0.3458	0.5941	0.5396	0.5715	0.7812
F×S	1	0.8561	0.1365	0.5695	0.4918	0.7790	0.5011	0.9640	0.4855	0.1331	**0.0271**
F×D	5	0.2907	0.7775	0.3209	0.6424	0.5192	0.5890	0.6164	0.6550	0.1000	0.1146
M×S	1	0.9677	0.0907	0.5959	0.6290	0.2369	0.7739	0.0977	0.5271	0.4064	**0.0110**
M×D	5	0.3334	0.2987	0.3383	0.0948	0.9571	0.1141	0.9767	0.0695	0.8895	0.1526
S×D	5	**0.0000**	**0.0018**	**0.0000**	**0.0244**	**0.0001**	0.1310	**0.0000**	**0.0144**	**0.0020**	**0.0000**
F×M×S	1	0.8358	0.2825	0.5957	0.4792	0.5756	0.5380	0.7420	0.5483	0.2553	0.2317
F×M×D	5	0.8472	0.8079	0.8920	0.8613	0.7286	0.8170	0.5788	0.8813	0.5002	0.6924
F×S×D	5	0.7492	0.2551	0.8696	0.4906	0.8860	0.4665	0.7917	0.3481	0.2674	0.7979
M×S×D	5	0.9583	0.2701	0.9365	0.3802	0.4625	0.3606	0.2730	0.2974	0.8063	0.4470
Block(site)	5	**0.0227**	0.3671	**0.0068**	**0.0130**	**0.0001**	**0.0133**	**0.0001**	**0.0063**	**0.0000**	**0.0452**
Error	120										
Total	167										

Note: *p*-values < 0.05 are in bold print.

*F, female, M, male; S, site; D, date.

†P_g, gross photosynthesis; R_d, dark respiration; P_n net photosynthesis; C_i/C_a, internal CO_2/atmospheric CO_2; g_m, mesophyll conductance to CO_2; l_g, gas phase limitation to CO_2; g_{wv}, stomatal conductance to H_2O; ψ_x, xylem water potential; ψ_{pd}, predawn xylem water potential.

‡Arcsine square root transformation performed for analysis.

only measured on one date. On each date, one tree per family per block was measured. On each date, trees were measured four times during the approximate time intervals of 08:30–09:30, 10:30–11:30, 12:30–13:30, and 14:30–15:30. Air temperature (1.3 m) and rainfall data were taken from a weather station located within 8 km (5 mi) of the study sites.

Two Li-6200 portable photosynthesis systems (LI-COR Inc., Lincoln, Nebr.) were used to measure P_n, g_{wv}, dark respiration (R_d), vapor pressure deficit (VPD), and needle temperature. Two pressure chambers (PMS Instruments Co., Corvallis, Oreg.) were used to measure ψ_x on both sites (2 and 3) on the same day. Instruments and crews were switched so that each site was measured by both crews twice during a day. Gas exchange was measured on detached branches within 2 min of excision (Meng and Arp 1993; Johnsen and Major 1995). Current-year foliage was not mature enough to be used for excised measures of gas exchange; therefore, 1-year-old foliage was measured. Approximately 30 cm sample branches with 1-year-old foliage were detached, using a pole clip, from the upper third of the crown, which was exposed to maximum illumination at that particular time of measurement. Shoots were immediately placed in a plastic bag containing moist paper towels and taken to a central location within the plantation. Gas exchange was measured on 3-cm samples of 1-year-old foliage under incandescent light (12 V, 75 W General Electric EYF bulb) that provided approximately 2200 $\mu mol \cdot m^{-2} \cdot s^{-1}$ (Johnsen and Major 1995). Following measurement under light, the chamber was completely covered with a black opaque plastic sheet for approximately 90 s before measurement of R_d (Johnsen et al. 1995). Gross photosynthesis (P_g) was estimated as $P_n + R_d$. Internal CO_2 concentration (C_i) and mesophyll conductance to CO_2 (g_m) were estimated using Li-6200 algorithms. Simultaneously, ψ_x was measured on an adjacent branch sample. Predawn xylem water potential (ψ_{pd}), sampled from the middle third of the crown, was also measured for each tree between 04:00 and 05:30 and was used as a measure of soil water potential (Dougherty and Hinckley 1981; Lucier and Hinckley 1982). The order of site measurement on each date was chosen at random.

Shoots were placed in a plastic bag and kept in a cooler until they were transported to a laboratory to determine projected leaf surface area using a Li-3100 area meter set at high resolution (0.1 mm^2) (LI-COR Inc.). Projected needle surface area (the mean of three measurements per sample) was multiplied by 4 to estimate total needle surface area (Grossnickle and Major 1994; Johnsen and Major 1995). Gas exchange and ψ_x daily means were estimated for individual trees using the four measurements per tree per date. Analysis of variance (ANOVA) was used to assess the influence of female parent, male parent, site, date, as well as their interactions using a randomized complete block design (Table 2). Female parent, male parent, site, date, and block were all considered to be fixed effects. Covariate regression analysis, using female parent, was used to examine P_n and g_{wv} response to ψ_{pd} and VPD. Effects of female parent were tested analogous to the analysis of covariance of family effects used by Johnsen and Bongarten (1991), using the model $Y_{ij} = B_0 + B_{0i} + B_1X_{ij} + B_{1i}X_{ij} + E_{ij}$, where Y_{ij} is the dependent variable (P_n or g_{wv}) of the *j*th plant of the *i*th female parent, B_0 and B_1 are average regression coefficients, B_{0i} and B_{1i} are female parent treatment coefficients, X_{ij} is the independent variable (VPD, ψ_x or ψ_{pd}), and E_{ji} is the error term. In this analysis three sources of variation are identified: (1) VPD, ψ_x or ψ_{pd} (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

Table 3. Daily and overall mean and standard error of physiological traits, by site, for each measurement day.

Trait*	Site	July 15	July 19	July 23	July 29	August 3	August 9	Overall
P_g (μmol·m^{-2}·s^{-1})	2	2.01±0.04	1.63±0.08	1.63±0.09	2.16±0.08	2.17±0.05	1.77±0.09	1.89±0.04
	3	2.11±0.06	1.94±0.09	2.43±0.10	2.18±0.09	2.32±0.07	2.09±0.08	2.18±0.04
R_d (μmol·m^{-2}·s^{-1})	2	0.54±0.03	0.48±0.03	0.31±0.02	0.31±0.02	0.26±0.02	0.31±0.02	0.37±0.01
	3	0.41±0.03	0.47±0.02	0.36±0.02	0.36±0.01	0.29±0.01	0.27±0.02	0.36±0.01
C_i/C_a	2	0.808±0.006	0.791±0.007	0.775±0.009	0.823±0.006	0.793±0.005	0.745±0.012	0.789±0.004
	3	0.801±0.007	0.750±0.005	0.751±0.011	0.777±0.014	0.776±0.008	0.745±0.006	0.767±0.004
g_m (mmol·m^{-2}·s^{-1})	2	32.6±2.1	19.4±0.9	20.8±1.1	37.8±2.1	33.1±1.3	17.6±1.2	26.9±1.0
	3	32.0±1.8	21.4±0.9	27.1±0.7	30.5±2.7	31.6±1.6	23.3±1.2	27.7±0.8
l_g (%)	2	19.2±0.6	20.9±0.7	22.5±0.9	17.7±0.6	20.7±0.5	23.1±0.9	20.7±0.3
	3	19.9±0.7	25.0±0.5	24.9±1.2	22.3±1.4	22.4±0.8	25.1±0.6	23.3±0.4
g_wv (mmol·m^{-2}·s^{-1})	2	50.1±1.5	31.3±1.6	33.6±1.7	61.9±3.5	53.5±1.9	28.4±1.9	43.1±1.5
	3	51.9±2.8	34.8±1.5	43.8±1.1	49.4±4.6	51.4±2.7	37.6±2.0	44.8±1.3
P_n/g_wv (μmol·mol^{-1})	2	38.0±1.3	40.1±1.6	45.4±1.9	34.2±1.2	40.6±1.2	49.4±2.6	41.3±0.9
	3	39.6±1.5	49.4±1.2	51.2±2.6	46.2±3.2	45.5±1.7	49.9±1.4	47.0±0.9
ψ_x (MPa)	2	−1.63±0.03	−1.73±0.02	−1.62±0.03	−1.46±0.03	−1.38±0.03	−1.77±0.04	−1.60±0.02
	3	−1.56±0.03	−1.66±0.03	−1.40±0.02	−1.20±0.04	−1.28±0.04	−1.59±0.03	−1.45±0.02
VPD (kPa)	2	1.52±0.03	2.27±0.03	1.48±0.02	1.17±0.04	1.15±0.02	1.84±0.04	1.57±0.03
	3	1.30±0.02	1.90±0.01	1.36±0.01	1.01±0.02	0.93±0.02	1.52±0.02	1.33±0.03

*P_g, gross photosynthesis; R_d, dark respiration; C_i/C_a, internal CO_2/atmospheric CO_2; g_m, mesophyll conductance to CO_2; l_g, gas phase limitation to CO_2; g_wv, stomatal conductance to H_2O; P_n, net photosynthesis; ψ_x, Xylem water potential; VPD, vapor pressure deficit.

the regression slopes (B_{1i} coefficients) between female parent progeny. The response surface relationship of P_n with ψ_pd and VPD was modeled using multivariate linear least squares regression analysis and based on partial *F*-tests on each model component (Kleinbaum et al. 1988). Models in which all components significantly contributed ($p = 0.05$) and possessed the highest r^2 values were considered the best fit.

Results

Only one minor rain event occurred over the first three sampling dates (Fig. 3A). Rainfall preceded measurement days July 29 (precluding measurement of ψ_pd on this date) and August 3, but little rain occurred the week prior to the final sampling date. On days that measurements were taken, temperatures experienced were typical of the 1993 seasonal pattern (Fig. 3B).

The significant site × date interactions (Table 2) indicate that the physiological differences between trees on the two sites were largely dependent on date. On all days except July 29 and August 3, site 2, compared with site 3, had lower P_n and ψ_pd (Fig. 4), and lower P_g and g_wv (Table 3). Mesophyll conductance to CO_2 displayed the same pattern as P_n except for on the first measurement date when means were similar (Tables 2 and 3). The significant site × date interaction for the ratios internal CO_2 concentration to atmospheric CO_2 concentration (C_i/C_a) and P_n/g_wv were due to scale effects rather than rank changes. Trees on site 2 had higher C_i/C_a and lower P_n/g_wv than trees on site 3 on all dates. On all days except July 29 and August 3 the higher C_i/C_a and lower P_n/g_wv of site 2 trees, compared with site 3 trees, were due to P_n differences being greater than g_wv differences. On July 29 and August 3, the higher C_i/C_a and lower P_n/g_wv of site 2 trees were due to equal P_n on both sites and higher g_wv on site 2 than on site 3. On average, site 2 had a slightly higher air temperature (25.3°C versus 24.4°C), lower relative humidity (53.6% versus 58.2%), and higher VPD (1.57 versus 1.34 kPa) (Table 3) than site 3.

The predominant genetic differences in gas exchange were between progeny of the two female parents (Tables 2 and 4). Progeny of female 59 had equal or greater P_n than progeny of female 63 on both sites and on all dates (Fig. 5). Across both sites and over all dates progeny of female 59 had 10.2% higher P_n than progeny of female 63 ($p = 0.0004$). Although there was no statistically significant site × female effect, progeny of female 59 had 12.5% and 7.4% higher P_n than progeny of female 63 on sites 2 and 3, respectively (Fig. 5). Progeny of the two female parents had similar rates of shoot R_d (Tables 2 and 4).

Mesophyll conductance to CO_2 was significantly different, with values of 28.2 ± 1.0 (SE) and 26.2 ± 0.9 mmol·m^{-2}·s^{-1} for progeny of females 59 and 63, respectively (Table 4). The progeny of female 59 had 6.1% higher

Fig. 5. Mean net photosynthesis (P_n) on site 2 (A) and site 3 (B), by measurement date (month/day), for progeny of females 59 and 63 from 22-year-old black spruce trees from four full-sib families. Insert shows means across all dates. Over all sites and dates, P_n of progeny of female 59 was significantly (p = 0.0004) higher than P_n of progeny of female 63.

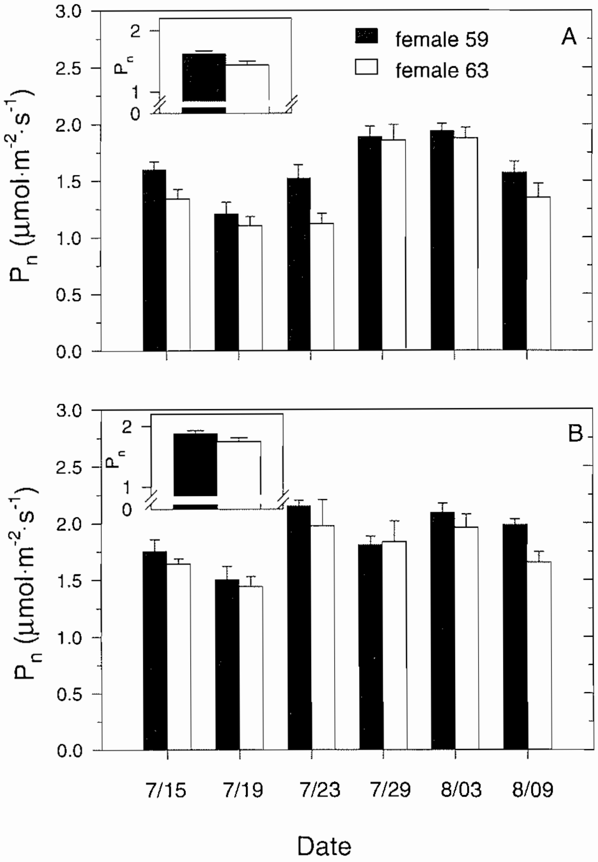
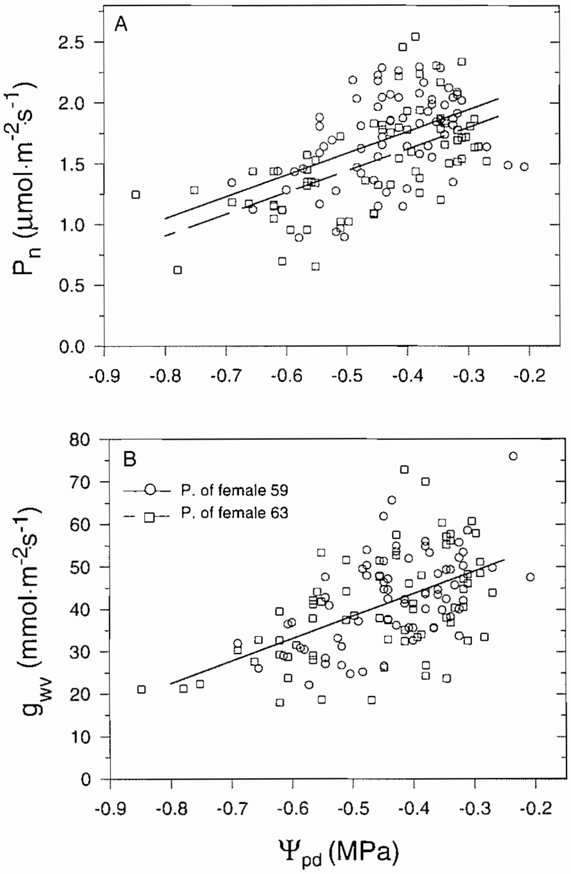


Fig. 6. (A) Net photosynthesis (P_n) and (B) needle conductance (g_{wv}) response for progeny of females 59 and 63 to predawn xylem water potential (ψ_{pd}) from 22-year-old black spruce trees from four full-sib families. Equations from analysis of covariance are as follows: (A) progeny of female 59, $y = 2.479 + 1.785x$; progeny of female 63, $y = 2.335 + 1.785x$; $r^2 = 0.329$, and (B) progeny of females 59 and 63, $y = 64.93 + 55.04x$; $r^2 = 0.292$.



g_{wv} than progeny of female 63 (p = 0.0456) (Table 2). The relative differences in P_n were greater than those of g_{wv}, so that progeny of female 59 still maintained higher P_n/g_{wv} (44.7 versus 42.7 μmol·mol^{-1}, p = 0.0794) and a lower C_i/C_a ratio (0.775 versus 0.784, p = 0.0642) than progeny of female 63 (Tables 2 and 4). Using the same equation as Teskey et al. (1986) to calculate stomatal limitation to P_n ($l_g = ((C_a - C_i)/C_a)100$) based on the assumption of a linear P_n to C_i relationship over the range measured for black spruce (Johnsen 1993), l_g had a mean of 22.2 ± 0.4% and 21.3 ± 0 .4% for progeny of females 59 and 63, respectively (Table 4).

Bulking data across sites and dates, P_n for progeny of both females displayed a positive linear relationship with ψ_{pd} (Fig. 6A). Analysis of covariance indicated no differences in slopes between progeny of the two females (p = 0.7247 for female × ψ_{pd}), and it indicated that female 59 had a higher P_n across all ψ_{pd} (p = 0.0116). There was a decrease in g_{wv} with ψ_{pd} (Fig. 6B), but analysis of covariance indicated no differences in slopes between progeny of the two females (p = 0.5316). Although there were significant g_{wv} in the ANOVA (Table 2), g_{wv} differences between progeny of the two females were not significant in the analysis of covariance (p = 0.4149).

Mean diurnal patterns (using data from all dates) of VPD, ψ_x, and P_n for progeny of both females from sites 2 and 3 are shown in Figs. 7A, 7B, and 7C, respectively. VPD increased steadily from morning to afternoon with no differences between progeny of females on each site. Site 2 versus site 3 showed a 0.17-kPa higher VPD in the morning and a 0.30-kPa difference in the afternoon measurement. Xylem water potential showed a sharp decline until 11:00, a moderate decrease until 13:00, and remained approximately constant until 15:00. Throughout the day site ψ_x differences were maintained, while little or no differences were found between progeny of female 59 and 63. Net photosynthesis for progeny of the two females from both sites were identical at 09:00. By 11:00 P_n from site 3 increased, while P_n from site 2 decreased. As time progressed site P_n differences were maintained, while mean values decreased slightly or remained the same. On site 2, differences between females began by 11:00 and continued, whereas on site 3 differences between females

Table 4. Daily and overall mean and standard error of physiological traits, by progeny of females 59 and 63, for each measurement day.

Trait*	Progeny of female:	July 15	July 19	July 23	July 29	August 3	August 9	Overall
P_g (μmol·m^{-2}·s^{-1})	59	2.16±0.05	1.81±0.10	2.12±0.11	2.18±0.07	2.27±0.05	2.06±0.08	2.10±0.04
	63	1.95±0.04	1.72±0.09	1.83±0.16	2.15±0.09	2.19±0.07	1.75±0.09	1.93±0.04
R_d (μmol·m^{-2}·s^{-1})	59	0.49±0.03	0.47±0.02	0.33±0.02	0.33±0.02	0.26±0.01	0.31±0.02	0.37±0.01
	63	0.48±0.04	0.47±0.04	0.34±0.02	0.34±0.02	0.28±0.02	0.27±0.02	0.36±0.01
P_n (μmol·m^{-2}·s^{-1})	59	1.66±0.06	1.34±0.08	1.79±0.11	1.85±0.06	2.00±0.05	1.75±0.08	1.73±0.04
	63	1.47±0.07	1.25±0.07	1.49±0.15	1.85±0.11	1.91±0.07	1.48±0.09	1.58±0.05
C_i/C_a	59	0.803±0.007	0.774±0.007	0.752±0.007	0.796±0.012	0.784±0.006	0.742±0.009	0.775±0.004
	63	0.807±0.006	0.774±0.010	0.777±0.012	0.811±0.010	0.788±0.007	0.750±0.011	0.784±0.004
g_m (μmol·m^{-2}·s^{-1})	59	34.4±2.5	21.2±1.0	24.4±1.1	35.3±3.0	32.1±1.0	22.1±1.4	28.2±1.0
	63	30.2±1.1	19.4±0.9	22.7±1.5	34.1±2.1	32.8±1.6	18.1±1.3	26.2±0.9
l_g (%)	59	19.7±0.7	22.6±0.7	24.8±0.7	20.4±1.3	21.6±0.6	24.3±0.8	22.2±0.4
	63	19.3±0.6	22.6±1.0	22.3±1.2	18.9±1.0	21.2±0.7	23.7±1.0	21.3±0.4
g_{wv} (μmol·m^{-2}·s^{-1})	59	52.5±2.3	34.1±1.7	39.4±1.7	57.4±5.0	52.0±1.6	35.6±2.2	45.2±1.50
	63	49.3±1.9	31.5±1.5	36.6±2.4	55.7±3.5	53.2±2.7	29.1±2.0	42.6±1.50
P_n/g_w (μmol·mol^{-1})	59	39.2±1.3	44.0±1.5	50.4±1.8	41.0±2.9	43.1±1.4	50.5±2.0	44.7±0.9
	63	38.1±1.5	44.2±2.4	45.4±2.6	37.7±2.4	42.3±1.6	48.7±2.5	42.7±1.0
ψ_x (MPa)	59	−1.58±0.03	−1.69±0.03	−1.53±0.04	−1.29±0.04	−1.33±0.04	−1.72±0.04	−1.52±0.02
	63	−1.62±0.02	−1.71±0.02	−1.51±0.04	−1.41±0.05	−1.36±0.03	−1.67±0.05	−1.55±0.02
ψ_{pd} (MPa)	59	−0.38±0.03	−0.45±0.03	−0.50±0.03	—	−0.39±0.01	−0.43±0.03	−0.43±0.01
	63	−0.42±0.03	−0.48±0.03	−0.55±0.04	—	−0.36±0.02	−0.47±0.04	−0.46±0.02

*See Table 2 for definition of traits.

progressed more slowly and were not as pronounced by 15:00.

Bulking data across all sites and dates, no relationship was observed between P_n and ψ_x ($r^2 = 0.048$) and a weak one was observed between P_n and g_{wv} ($r^2 = 0.150$). A negative linear relationship was found between P_n and VPD (Fig. 8). Analysis of covariance indicated that P_n of progeny of female 59 decreased less steeply with increasing VPD than progeny of female 63 (Fig. 8, $p = 0.0025$ for female × VPD). Although g_{wv} also decreased with increasing VPD ($p = 0.0000$, $r^2 = 0.338$), progeny of the two females displayed similar responses ($p = 0.3099$ for female × VPD, $p = 0.1373$ for female). No relationship was found between l_g and ψ_{pd} nor between C_i and ψ_{pd} ($r^2 < 0.03$).

Three-dimensional response surfaces were produced to visually examine the interaction of soil and atmospheric drought on P_n. A multiple linear model best described the relationship of P_n to ψ_{pd} and VPD. The model of P_n to VPD and ψ_{pd} for progeny of female 59 is shown overlaying the model for progeny of female 63 in Fig. 9. This analysis again indicates similar P_n responses to ψ_{pd} and different responses to VPD between progeny of the two females. Differences in P_n between progeny of two females increased with increasing VPD at any given ψ_{pd}. Although the relationship of P_n with ψ_x by itself showed no interpretable trends, the relationship of P_n with ψ_x and VPD indicates that P_n has a linear decline with ψ_x at all VPD levels and that as VPD increased so did the differences between female progenies similar to that seen in Fig. 9.

Other statistically significant results appear to have no or minor biological importance. There was significant variation in mean chamber CO_2 concentrations between progeny of the two females (not shown). However, the differences were minor, with mean concentration values for progeny of female 59 and 63 being 360.3 and 362.6 ppm, respectively. Female × site was a significant source of variation in ψ_{pd} (Table 2). On site 2, progeny of female 59 had higher ψ_{pd} (−0.42 MPa) than progeny of female 63 (−0.46 MPa). On site 3, ψ_{pd} was −0.32 MPa for both progenies. The female and male interaction with site showed no interpretable trends. Xylem water potential between progeny of the two males was a statistically significant source of variation (Table 2); mean values were −1.51 and −1.56 MPa for progeny of males 52 and 62, respectively.

Discussion

Stomatal versus nonstomatal limitations

Calculations of l_g indicated that over the observed range of environmental conditions, P_n of fully developed black spruce foliage from mature trees was predominately limited

Fig. 7. Mean seasonal diurnal pattern of (A) vapor pressure deficit (VPD), (B) xylem water potential (ψ_x), and (C) net photosynthesis (P_n) for progeny of females 59 and 63 from site 2 (open symbols) and site 3 (closed symbols).

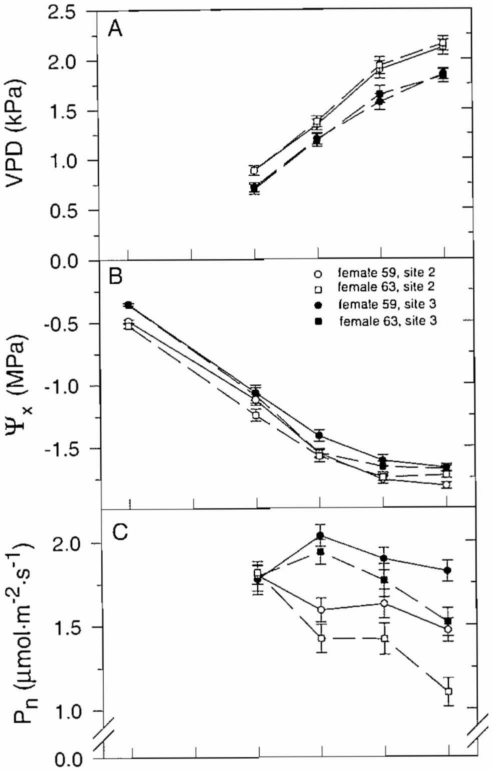


Fig. 8. Net photosynthesis (P_n) response for progeny of females 59 and 63 to vapor pressure deficit (VPD) from 22-year-old black spruce trees from four full-sib families. Equations from analysis of covariance are as follows: progeny of female 59, $y = 2.203 - 0.322x$; progeny of female 63, $y = 2.309 - 0.497x$; $r^2 = 0.258$.

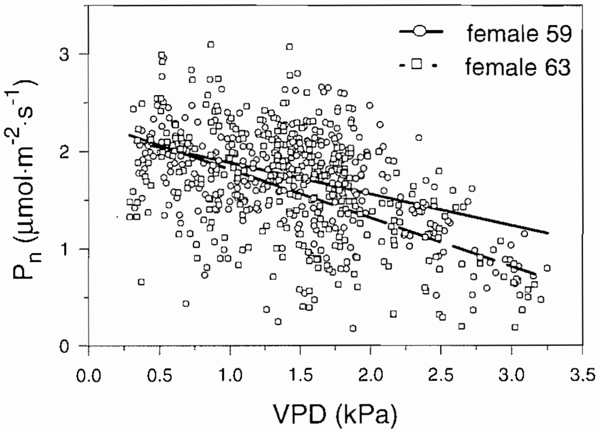
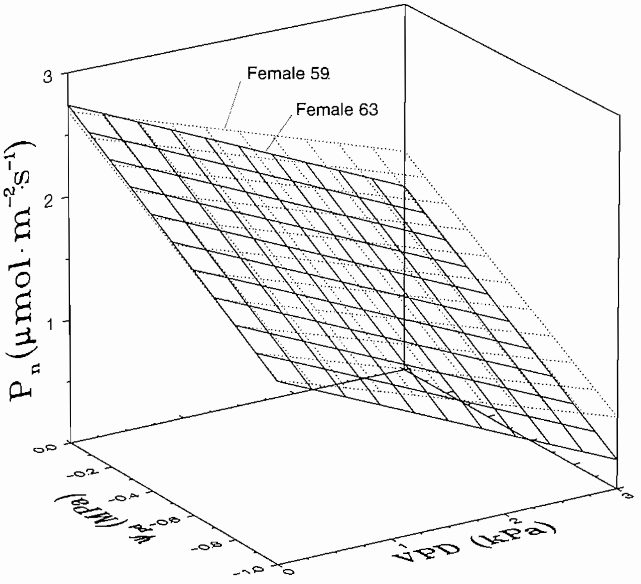


Fig. 9. Net photosynthesis (P_n) response for progeny of females 59 and 63 to predawn xylem water potential (ψ_{pd}) and vapor pressure deficit (VPD) from 22-year-old black spruce trees from four full-sib families. Equations are as follows: progeny of female 59, $P_n = 2.689 + 1.223(\psi_{pd}) - 0.296(\text{VPD})$; $r^2 = 0.240$; progeny of female 63, $P_n = 2.739 + 1.279(\psi_{pd}) - 0.408(\text{VPD})$; $r^2 = 0.350$.



by nonstomatal, rather than stomatal, factors. Studies have found that stomatal limitations can significantly influence P_n rates (Wang et al. 1995*a*; Tognetti et al. 1995). However, our results were very similar to those reported by Stewart et al. (1995) working with black spruce seedlings and Teskey et al. (1986) studying loblolly pine (*Pinus taeda* L.) seedlings. As Teskey et al. (1986) and others (Farquhar and Sharkey 1982; Briggs et al. 1986; Guehl and Aussenac 1987) have discussed, although linear relationships are often observed between P_n and g_{wv} during water stress, the limitation imposed on P_n by stomata is often small compared with nonstomatal factors. As such, the role of nonstomatal limitations is central in discussions of both site and genetic variation.

Site variation

All evidence indicated that site 2 experienced greater soil moisture stress than site 3, as in 1991 (Johnsen and Major 1995). On measurement days July 29 and August 3, when it had rained just previously, site 3 had, on average, only a 1.8% greater P_n than site 2. On the four other measurement days site 3 had on average 23% greater P_n than site 2. This evidence is stronger than that amassed in 1991, because trees on the two sites were measured simultaneously over a range of soil moisture conditions and the analysis took into account any variation inherent between individual Li-6200s and operators. The rapid onset of water stress symptoms on site 2 relative to site 3 following a rain event appears to be due to differences in drainage between sites (S. Brown and R. Ponce-Hernandez, unpublished). These findings indicate that differential water stress is largely responsible for the varying growth rates of trees on the two sites and is potentially a major environmental factor driving the G × E interaction.

Across all measurement dates, trees on the dry site (2) had rates of P_n that were 15.3% lower than those of trees on the wet site (3), while rates of g_{wv} were only 3.9% lower.

As a result, trees on the dry site (2) had a lower mean P_n/g_{wv} and a higher C_i/C_a than trees on the wet site (3); site rankings were consistent across all measurement dates. This is in contrast with estimates derived from carbon isotope analyses in 1991 and 1992, where dry site (2) trees were estimated to have higher P_n/g_{wv} and lower C_i/C_a than wet site (3) trees (Flanagan and Johnsen 1995). Estimates from 1991 gas exchange indicated similar discrepancies with stable isotope estimates (Johnsen and Major 1995). Water stress will result in lower C_i/C_a "if the 'supply function' of photosynthesis (leaf conductance) decreases at a faster rate under stress than the 'demand function' (photosynthetic dependence on C_i)" (Farquhar et al. 1989). Johnsen and Major (1995) suggested that the 1991 discrepancies between carbon isotope estimates and gas exchange measurements in site rankings of P_n/g_{wv} and C_i/C_a were due to variable water stress at the respective times of carbon assimilation. Foliage sampled for isotope analyses was largely assimilated in June, a period when soil water deficits were greater than in July and August, when gas exchange was measured. We now amend this explanation and suggest that stomatal limitations to P_n may be more important in succulent undeveloped foliage than in fully developed black spruce foliage. Stomata in developing black spruce foliage may be more sensitive to water stress than stomata in fully developed foliage. Thus, in developing foliage, the "supply function" may be more impacted by soil water stress than the "demand function," resulting in a decreased C_i/C_a ratio and the observed decreased carbon isotope discrimination. This hypothesis will be tested.

Genetic variation

As in 1991 and 1992 (Johnsen and Major 1995), the predominant genetic differences in gas exchange were between progeny of females 59 and 63. Across all dates and both sites, progeny of female 59 had P_n rates 10.2% greater than progeny of female 63. In 1991, differences were 9% and in 1992 differences were 5% (Johnsen and Major 1995). As a result of compounding, P_n differences of this magnitude can easily explain the family growth differences observed on the dry site. Because no differences were observed between progeny of the two females in R_d, P_n differences appear to be a result of differences in P_g. As photorespiration was not measured or accounted for, we have no estimate of whether differences in photorespiration contributed to differences in P_n.

Unlike 1991 and 1992, progeny of female 59 had 6.1% higher mean g_{wv} than progeny of female 63. Although g_{wv} has consistently responded to environmental stresses as expected, genetic variation in g_{wv} in these studies has been inconsistent among dates and seasons of measurements (Johnsen and Major 1995). Furthermore, here we observed no genetic differential response to ψ_{pd} or VPD; thus indicating a nil or a minor contribution to our observed genetic P_n differences. As the relative genetic difference in P_n was greater than that in g_{wv}, progeny of female 59 had higher P_n/g_{wv} and lower C_i/C_a than progeny of female 63. Genetic rankings in P_n/g_{wv} and C_i/C_a appear quite stable, as rankings were consistent with results from 1991 and 1992 gas exchange (Johnsen and Major 1995) and carbon isotope studies (Flanagan and Johnsen 1995).

Model comparison

There was a decrease in P_n with decreasing ψ_{pd}, as found in the literature (Grossnickle et al. 1991; Stoneman et al. 1994). Progeny of the two females displayed similar decreases in P_n in response to ψ_{pd} over the range examined. This analysis supports physiological model B (Fig. 2), where families maintained consistent P_n differences with increasing soil moisture stress. Hence, the hypothesis of differential tolerance to increasing soil moisture stress by families over the range tested was rejected (model A, Fig. 2).

Tan et al. (1992*a*), using seedlings subjected to polyethylene glycol (PEG), found gas exchange differences between the same families used here at mild and moderate water stress levels but not at low or extreme water stress levels. This was not found in our physiological response data, where genetic differences in P_n were consistent across a range of ψ_{pd}. It can be argued that Tan et al. (1992*a*), using controlled conditions, were better able to exert a larger range of water stress on their seedling material. Their high stress treatment using 25% PEG approximated −2.0 MPa. However, over three field seasons, the minimum mean ψ_{pd} we have measured on any day was −0.9 MPa. This is despite the fact that the period of July 1 to August 14, 1993, was extremely dry (second driest recorded since planting) with only 70.1 mm of rain and with 21.0 mm falling on August 2. Thus at the extreme water stress levels used by Tan et al. (1992*a*), gas exchange of all families was very low as a result of water stress and may have simply precluded the detection of genetic differences. In addition, this explanation does not reconcile a basic difference between our conclusions and those of Tan et al. (1992*a*). Tan et al. (1992*a*) attributed family P_n differences directly to differences in g_{wv}. In contrast, our data using mature trees over a wide range of environmental conditions indicate that genetic differences in P_n are due to differences in nonstomatal limitations. Stewart et al. (1995), working with black spruce seedlings, also found that P_n decline due to water stress was caused largely by nonstomatal factors.

Potential mechanisms contributing to genetic variation in P_n

As seen in Fig. 7, progeny of the two females had almost identical rates of P_n at 09:00 but the values diverged as the day progressed. Simultaneously, with the progress of time, ψ_x decreased and VPD increased. No general relationship was observed between P_n and ψ_x, which has also been observed for other tree species (Hall 1982; Schulze and Hall 1982; Teskey et al. 1994). Net photosynthesis did decline with increasing VPD, as in other conifers (Meinzer 1982; Grieu et al. 1988; Major et al. 1994). As discussed above, g_{wv} responded to environmental stresses as expected but provided little contribution to our observed genetic P_n differences. Thus it would appear that progeny of female 59 displayed a less severe inhibition of P_n as a result of higher VPD, at all ψ_{pd}, than did progeny of female 63. Therefore, the observed differences in P_n between progeny of females 59 and 63 appear to be a result of differential tolerance to atmospheric drought (VPD).

There are two, often interacting, control loops proposed for describing P_n response to VPD: feedback and feedforward

(Meinzer et al. 1984; Schulze 1986). Feedback response describes how VPD directly or indirectly results in a loss of turgor in guard cells, resulting in stomatal closure, thus decreasing C_i and therefore P_n. Feedforward response of P_n to VPD generally describes nonstomatal factors. A possible nonstomatal explanation for P_n decline to increasing VPD was illustrated by Sharkey (1984). By measuring C_i directly it was observed that P_n decreases were not due to changes in stomatal aperture. The author instead suggested that increased transpiration with higher VPD induced water deficits at sites of evaporation and thus inhibited P_n. Other studies have found relationships between CO_2 diffusion through the mesophyll and leaf anatomy, which can often interact with transpiration and (or) VPD (Evans et al. 1994; Syvertsen et al. 1995). In our study, there was no genetic variation of g_{wv} in response to VPD. The observed genetic differences in P_n appear to be due to differences in mechanisms associated with feedforward rather than feedback responses.

We now hypothesize that daily mean differences in P_n between progeny of females 59 and 63 are due to differential P_n responses to VPD. The dry site had on average 0.25 kPa (18%) higher VPD than the wet site. A difference of this magnitude and based only on the VPD model was enough to produce the same overall P_n differences found between progeny of females measured from each site. We recognize that it is still possible that a minimum threshold ψ_{pd} and (or) ψ_x may be required to produce enough stress (i.e., at leaf mesophyll cells) for a VPD effect on P_n to be manifested. It is difficult to separate ψ_{pd} and (or) ψ_x from VPD because they are highly correlated under field conditions. We will be further testing these relationships using grafts of the original mother trees in controlled environments.

Relationship of net photosynthesis with growth

Many studies have attempted to find positive relationships between P_n and growth, and this has met with limited success (Ceulemans and Saugier 1991; Pereira 1994). Where photosynthesis has been related to growth, more often it has been where P_n has been scaled up with estimates of total leaf area (Michael et al. 1990; Wang et al. 1995*b*). As discussed by Johnsen and Major (1995), it is clear that the genetic correlations between P_n and growth rate in forest trees is complicated and dependent on many factors.

In our current study, P_n differences among the families have been extremely consistent over 3 study years (Johnsen and Major 1995; Flanagan and Johnsen 1995). Why does genetic variation in P_n, which is expressed to varying degrees both over the wet and dry sites and over wet and dry years, only correlate with growth on the dry site? We speculate at least four possible reasons: (1) It is possible that the correlation is spurious and is not indicative of a causative relationship. (2) Perhaps our midsummer sampling does not properly reflect the average seasonal VPD conditions found on both sites; that is, perhaps the overall mean seasonal VPD of site 3 is lower, and hence integrated seasonal P_n differences between progeny of females on the wet site would be lower. (3) It may be that high P_n response to VPD is one of a suite of traits responsible for stable high growth across sites suffering varying degrees of water stress. High P_n might be required for such growth stability, but other traits may also be required. These other traits may also display a G × E interaction and contribute together to the observed growth differences. (4) Perhaps genetic differences in P_n only confer a growth advantage when P_n rates are generally depressed, below a "threshold," as seen on our dry site, as a result of water stress. Above this P_n threshold, growth may be more limited by variation in other traits such as nutrient uptake, meristematic properties, and shoot structure; variation in these traits might only confer growth advantages if photosynthesis per unit leaf is not limiting. Below this P_n threshold, variation in P_n itself may result in genetic variation in growth. The last three hypotheses imply that genetic variation in growth is dependent on genetic variation in component traits and that there is a hierarchical importance of traits dependent on limitations imposed by the environment. These various hypotheses will be tested by continuing intensive fieldwork on a subset of families, by conducting more extensive genetic analyses, and by subjecting families to manipulative experiments in controlled environments.

Conclusions

Under the range of conditions studied, P_n of second-year foliage from mature black spruce trees was more affected by nonstomatal limitations than by stomatal limitations. Gas exchange measurements simultaneously conducted on both sites indicate that P_n on site 2 is depressed as a result of water stress, compared with site 3. Genetic differences in P_n were observed between progeny of the two females, and the differences were similar in sign and magnitude to those detected the previous 2 years. The genetic variation in P_n was consistent across both sites and over a range of ψ_{pd}. Thus, model A, differential response to water stress, was rejected in favor of model B, consistent genotype P_n differences over a range of water stress. Analysis of response curves indicate genetic variation in P_n may be due to differential response to VPD and that this response is found across a range of ψ_{pd}. The VPD necessary to elicit genetic differences are common on both the wet and dry sites over many days of the growing season. However, the dry site had on average 0.25 kPa (18%) higher VPD than the wet site, and this difference, with an associated genetic response of P_n, may be contributing to the observed G × E interaction in growth rate.

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References

Bilan, M.V., Hogan, C.T., and Carter, H.B. 1977. Stomatal opening, transpiration, and needle moisture in loblolly pine seedlings from two Texas sources. For. Sci. **23**: 457–462.

Boyle, T.J.B. 1987. A diallel cross in black spruce. Genome, **29**: 180–186.

Briggs, G.M., Jurik, T.W., and Gates, D.M. 1986. Non-stomatal limitation of CO_2 assimilation in three tree species during natural drought conditions. Physiol. Plant. **66**: 521–526.

Ceulemans, R.J., and Saugier, B. 1991. Photosynthesis. *In* Physiology of trees. *Edited by* A.S. Raghavendra. John Wiley & Sons, New York. pp. 21–50.

Dougherty, P.M., and Hinckley, T.M. 1981. The influence of a severe drought on net photosynthesis of white oak (*Quercus alba*). Can. J. Bot. **59**: 335–341.

Evans, J.R., von Caemmerer, S., Setchell, B.A., and Hudson, G.S. 1994. The relationship between CO_2 transfer conductance and leaf anatomy in transgenic tobacco with a reduced content of rubisco. Aust. J. Plant Physiol. **21**: 475–495.

Farquhar, G.D., and Sharkey, T.D. 1982. Stomatal conductance and photosynthesis. Annu. Rev. Plant Physiol. **33**: 317–345.

Farquhar, G.D., Ehleringer, J.R., and Hubick, K.T. 1989. Carbon isotope discrimination and photosynthesis. Annu. Rev. Plant Physiol. Plant Mol. Biol. **40**: 503–537.

Flanagan, L.B., and Johnsen, K.H. 1995. Genetic variation in carbon isotope discrimination and its relationship to growth under field conditions in full-sib families of *Picea mariana*. Can. J. For. Res. **25**: 39–47.

Grieu, P., Guehl, J.M., and Aussenac, G. 1988. The effects of soil and atmospheric drought on photosynthesis and stomatal control of gas exchange in three coniferous species. Physiol. Plant. **73**: 97–104.

Grossnickle, S.C., and Major, J.E. 1994. Interior spruce seedlings compared with emblings produced from somatic embryogenesis. II. Stock quality assessment prior to field planting. Can. J. For. Res. **24**: 1385–1396.

Grossnickle, S.C., Arnott, J.T., and Major, J.E. 1991. Influence of dormancy induction treatment on western hemlock seedlings. 2. Physiological and morphological response during the first growing season on a reforestation site. Can. J. For. Res. **21**: 175–185.

Guehl, J.M., and Aussenac, G. 1987. Photosynthesis decrease and stomatal control of gas exchange in *Abies alba* Mill. in response to vapor pressure difference. Plant Physiol. **83**: 316–322.

Hall, A.E. 1982. Mathematical models of plant water loss and plant water relations. *In* Physiological plant ecology. II. Water relations and carbon assimilation. *Edited by* O.L. Lange, P.S. Nobel, C.B. Osmond, and H. Ziegler. Springer, New York. pp. 181–230.

Hills, G.A. 1960. Regional site research. For. Chron. **36**: 401–412.

Johnsen, K.H. 1993. Growth and ecophysiological responses of black spruce seedlings to elevated CO_2 under varied water and nutrient additions. Can. J. For. Res. **23**: 1033–1042.

Johnsen, K.H., and Bongarten, B.C. 1991. Allometry of acetylene reduction and nodule growth of *Robinia pseudoacacia* families subjected to varied root zone nitrate concentrations. Tree Physiol. **9**: 507–522.

Johnsen, K.H., and Major, J.E. 1995. Gas exchange of 20-year-old black spruce families displaying a genotype × environment interaction in growth rate. Can. J. For. Res. **25**: 430–439.

Johnsen, K.H., Seiler, J.R., and Major, J.E. 1996. Growth, shoot phenology and physiology of diverse seed sources of black spruce: II. 23-year-old field trees. Tree Physiol. **16**: 375–380.

Kleinbaum, D.G., Kupper, L.L., and Muller, K.E. 1988. Applied regression analysis and other multivariable methods. 2nd ed. PWS-Kent, Boston, Mass.

Losch, R., and Schulze, E.-D. 1994. Internal coordination of plant responses to drought and evaporational demand. *In* Ecophysiology of photosynthesis. *Edited by* E.-D. Schulze and M.M. Caldwell. Springer-Verlag, Berlin. pp. 185–204.

Lucier, A.A., and Hinckley, T.M. 1982. Phenology, growth and water relations of irrigated and non-irrigated black walnut. For. Ecol. Manage. **4**: 127–142.

Major, J.E., Grossnickle, S.C., and Arnott, J.T. 1994. Influence of dormancy induction treatments on the photosynthetic response of field planted western hemlock seedlings. For. Ecol. Manage. **63**: 235–246.

Meinzer, F.C. 1982. The effect of vapor pressure on stomatal control of gas exchange in Douglas-fir (*Pseudotsuga menziesii*) saplings. Oecologia, **54**: 236–242.

Meinzer, F., Goldstein, G., and Jaimes, M. 1984. The effect of atmospheric humidity on stomatal control of gas exchange in two tropical coniferous species. Can. J. Bot. **62**: 591–595.

Meng, F., and Arp, P.A. 1993. Net photosynthesis and stomatal conductance of red spruce twigs before and after twig detachment. Can. J. For. Res. **23**: 716–721.

Michael, D.A., Dickmann, D.I., Isebrands, J.G., and Nelson, N.D. 1990. Photosynthesis patterns during the establishment year within two *Populus* clones with contrasting morphology and phenology. Tree Physiol. **6**: 11–27.

Morgenstern, E.K. 1974. A diallel cross in black spruce, *Picea mariana* (Mill.) B.S.P. Silvae Genet. **23**: 67–70.

Pallardy, S.G., Cermak, J., Ewers, F.W., Kaufmann, M.R., Parker, W.C., and Sperry, J.S. 1995. Water transport dynamics in trees and stands. *In* Resource physiology of conifers—acquisition, allocation and utilization. *Edited by* W.K. Smith and T.M. Hinckley. Academic Press, New York. pp. 301–389.

Pereira, J.S. 1994. Gas exchange and growth. *In* Ecophysiology of photosynthesis. *Edited by* E.-D. Schulze and M.M. Caldwell. Springer-Verlag, Berlin. pp. 147–181.

Pezeshki, S.R., and Chambers, L. 1986. Stomatal and photosynthetic response of drought-stressed cherrybark oak (*Quercus falcata* var. *pagodaefolia*) and sweetgum (*Liquidambar styraciflua*). Can J. For. Res. **16**: 841–846.

Running, S.W. 1976. Environmental control of leaf water conductance in conifers. Can. J. For. Res. **6**: 104–112.

Sala, A., and Tenhunen, J.D. 1994. Site-specific water relations and stomatal response of *Quercus ilex* in a Mediterranean watershed. Tree Physiol. **14**: 601–617.

Sands, R., Kriedemann, P.E., and Cotterill, P.P. 1984. Water relations and photosynthesis in three families of radiata pine seedlings known to differ in their response to weed control. For. Ecol. Manage. **9**: 173–184.

Schulze, E.-D. 1986. Carbon dioxide and water vapor exchange in response to drought in the atmosphere and in the soil. Annu. Rev. Plant Physiol. **37**: 247–274.

Schulze, E.-D., and Hall, A.E. 1982. Stomatal responses, water loss and CO_2 assimilation rates of plants in contrasting environments. *In* Physiological plant ecology. II. Water relations and carbon assimilation. *Edited by* O.L. Lange, P.S. Nobel, C.B. Osmond, and H. Ziegler. Springer, New York. pp. 231–262.

Seiler, J.R., and Cazell, B.H. 1990. Influence of water stress on the physiology and growth of red spruce seedlings. Tree Physiol. **6**: 69–77.

Seiler, J.R., and Johnson, J.D. 1988. Physiological and morphological responses of three half-sib families of loblolly pine to water-stress conditioning. For. Sci. **34**: 487–495.

Sharkey, T.D. 1984. Transpiration-induced changes in the photosynthetic capacity of leaves. Planta, **160**: 143–150.

Stewart, J.D., Zine El Abidine, A., and Bernier, P.Y. 1995. Stomatal and mesophyll limitations of photosynthesis in black spruce seedlings during multiple cycles of drought. Tree Physiol. **15**: 57–64.

Stoneman, G.L., Turner, N.C., and Dell, B. 1994. Leaf growth, photosynthesis and tissue water relations of greenhouse-grown *Eucalyptus marginata* seedlings in response to water deficits. Tree Physiol. **14**: 633–646.

Syvertsen, J.P., Lloyd, J., McConchie, C., Kriedemann, P.E., and Farquhar, G.D. 1995. On the relationship between leaf anatomy and CO_2 diffusion through the mesophyll of hypostomatous leaves. Plant Cell Environ. **18**: 149–157.

Tan, W., Blake, T.J., and Boyle, T.J.B. 1992*a*. Drought tolerance in faster- and slower-growing black spruce (*Picea mariana*) progenies: I. Stomatal and gas exchange responses to osmotic stress. Physiol. Plant. **85**: 639–644.

Tan, W., Blake, T.J., and Boyle, T.J.B. 1992*b*. Drought tolerance in faster- and slower-growing black spruce (*Picea mariana*) progenies: II. Osmotic adjustment and changes of soluble carbohydrates and amino acids under osmotic stress. Physiol. Plant. **85**: 645–651.

Teskey, R.O., Gholz, H.L., and Cropper, W.P., Jr. 1994. Influence of climate and fertilization on net photosynthesis of mature slash pine. Tree Physiol. **14**: 1215–1227.

Teskey, R.O., Fites, J.A., Samuelson, L.J., and Bongarten, B.C. 1986. Stomatal and nonstomatal limitations to net photosynthesis in *Pinus taeda* L. under different environmental conditions. Tree Physiol. **2**: 131–142.

Tognetti, R., Johnson, J.D., and Michelozzi, M. 1995. The response of European beech (*Fagus sylvatica* L.) seedlings from two Italian populations to drought and recovery. Trees, **9**: 348–354.

Wang, T., Hagqvist, R., and Tigerstedt, P.M.A. 1995*a*. The relationships between yield and carbon fixation in selected hybrid families after crossing selfed lines of *Betula pendula* Roth. For. Genet. **2**: 77–86.

Wang, T., Tigerstedt, P.M.A., and Vihera-Aarnio, A. 1995*b*. Photosynthesis and canopy characteristics in genetically defined families of silver birch (*Betula pendula*). Tree Physiol. **15**: 665–671.

Wang, Z.M., and Macdonald, S.E. 1993. Peatland and upland black spruce populations in Alberta: morphology and ecophysiology. Can. J. For. Res. **23**: 33–40.

Zhang, J.W., and Marshall, J.D. 1994. Population differences in water-use efficiency of well-watered and water-stressed western larch seedlings. Can. J. For. Res. **24**: 92–99.

Zine El Abidine, A., Bernier, P.Y., Stewart, J.D., and Plamondon, A.P. 1994. Water stress preconditioning of black spruce seedlings from lowland and upland sites. Can. J. Bot. **72**: 1511–1518.