Gas exchange of 20-year-old black spruce families displaying a genotype × environment interaction in growth rate

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Abstract: Gas exchange and xylem water potential were measured on 20-year-old black spruce (*Picea mariana* (Mill.) B.S.P.) trees from four full-sib families from a 7×7 diallel. These families constitute a 2 parent $\times$ 2 parent factorial breeding structure. One female parent (59) produced families that have displayed high productivity on three plantation sites, whereas the other female parent (63) produced families that had high growth rates on two of the sites but not on the third, least productive, site. In 1991 (a hot, dry year) measurements were obtained from trees at two sites; in 1992 (a cool, wet year) measurements were obtained from only one site. Gas exchange was measured on detached branches cut from the upper third of the crown; measurements were initiated within 2 min from cutting. In 1991, daytime xylem water potential, predawn xylem water potential, net photosynthesis (P_n), and needle conductance (g_{wv}) were all lower in trees growing on the least productive site (2) than in trees growing on the most productive site (3), indicating that trees on site 2 were under greater water stress than those on site 3. Progeny of female 59 displayed higher P_n than progeny of female 63 over both years of contrasting rainfall and on both the wetter and drier sites during the first year. However, progeny of the two female parents did not differ with respect to g_{wv} in either year nor on either site during the first year. The genetic variation in P_n/g_{wv}, observed over sites and years, is positively related to growth rate only on the dry site. Thus, the genotype $\times$ environment interaction in growth appears to be due to relatively stable genetic differences in P_n. We hypothesize that water stress on the dry site reduces P_n below a threshold value at which point genetic variation in P_n impacts on productivity and results in genetic variation in growth rate. Furthermore, we hypothesize that on the wet site carbon gain per unit leaf area is not as limiting to growth rate; thus, the observed genetic differences in P_n do not influence genetic rankings in growth rate.

Résumé : Nous avons mesuré les échanges gazeux et le potentiel hydrique du xylème sur des épinettes noires (*Picea mariana* (Mill.) B.S.P.) âgées de 20 ans provenant de quatre fratries provenant d'un test 7×7 en diallèle. Ces fratries sont issues de croisements de 2 parents $\times$ 2 parents en structure factorielle. Un parent femelle (59) a produit des familles avec une productivité élevée sur trois sites de plantation. L'autre parent femelle (63) a produit des familles avec une productivité élevée sur deux des sites de plantation, mais pas sur le troisième site plus pauvre. Nous avons obtenu des mesures sur deux sites en 1991 (été chaud et sec), mais sur seulement un site en 1992 (été frais et humide). Les échanges gazeux ont été mesurés sur des branches détachées provenant du tiers supérieur de la cime, en dedans de 2 min de l'excision. En 1991, les valeurs de potentiels hydriques du xylème diurne et pré-aube, de photosynthèse nette (P_n) et de conductance stomatique (g_{wv}) étaient plus faibles chez les arbres poussant sur le site moins productif (2) que chez ceux poussant sur le site plus productif (3), indiquant que les arbres sur le site 2 subissaient un stress hydrique plus élevé que ceux sur le site 3. Les valeurs de P_n étaient plus élevées chez la descendance de la femelle 59 que chez la descendance de la femelle 63 au cours des deux années de régimes pluviométriques contrastants, et sur les deux sites durant la première année de l'étude. Les valeurs de g_{wv} n'ont cependant pas montré de différences significatives. La variabilité génétique de P_n/g_{wv}, telle qu'observée sur les deux sites et pendant les 2 années, n'a pu être reliée positivement au taux de croissance que sur le site sec. L'interaction génétique $\times$ environnement observée dans le taux de croissance semble donc être due à des

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différences génétiques relativement stables de P_n. Nous proposons comme hypothèse que le stress hydrique sur le site sec réduit P_n sous une valeur seuil au-dessous de laquelle la variation génétique de P_n a un impact sur la productivité et engendre une différence génétique du taux de croissance. Nous proposons aussi comme hypothèse que, sur le site humide, le gain en carbone par unité de surface foliaire ne limite pas autant la croissance; les différences génétiques observées pour P_n n'influencent donc pas le classement génétique des taux de croissance.

[Traduit par la Rédaction]

Introduction

A standard statistical analysis of the growth of a 7×7 black spruce (*Picea mariana* (Mill.) B.S.P.) diallel planted on three sites at the Petawawa National Forestry Institute indicated an important genotype $\times$ environment interaction (G $\times$ E) component of variance (Boyle 1987). Carbon isotope discrimination of a subset of interacting families was examined to test the hypothesis that the G $\times$ E interaction was, at least partly, related to differences in photosynthetic gas exchange characteristics among families growing on sites of varying water availability (Flanagan and Johnsen 1995). Results indicated that one of the three sites was drier than the other two, and families that grew relatively well on the dry site discriminated less against ^{13}C (on all sites) than families that grew poorly on the dry site.

Carbon isotope discrimination is theoretically and empirically related to the ratio of net photosynthesis (P_n) to stomatal conductance (g_{wv}) (Farquhar et al. 1982, 1989). Thus, the above-mentioned family differences in carbon isotope discrimination might be due to family differences in P_n and (or) g_{wv}. As pointed out by Meinzer et al. (1990), "appropriate background physiological data must be acquired before the results of leaf carbon isotope analyses can be fully interpreted."

This paper presents 2 years of gas exchange and xylem pressure potential measurements of 20-year-old black spruce trees from the same four families studied by Flanagan and Johnsen (1995). The purpose was twofold: (1) to further test the hypothesis that the observed G $\times$ E interaction is due to differences in gas exchange characteristics and (2) to attempt to discern if the observed family differences in carbon isotope discrimination are related to family differences in P_n and (or) g_{wv}. This work, as a whole, represents a prototype approach toward the characterization of G $\times$ E interactions at the physiological level.

Materials and methods

Seed sources and site locations
The four full-sib families under study are a subset of a complete 7×7 diallel experiment established, with 2-year-old stock, on three sites located at the Petawawa National Forestry Institute (46°N, 77°30'W) in 1973 (Morgenstern 1974; Boyle 1987). The parent trees came from the Lake Simcoe – Rideau region of Ontario (Hills 1960), but the exact origin within that region is unknown. After 20 years, the three sites displayed a range of productivity; mean heights were 676, 625, and 732 cm for sites 1, 2, and 3, respectively (Fig. 1). Of the two sites used in this current study, foliar carbon isotope discrimination (Flanagan and Johnsen 1995) and soil analyses (O. Hendrickson, S. Brown

Fig. 1. Family mean height versus site mean height for four full-sib families from a 7×7 black spruce diallel experiment grown on three sites at the Petawawa National Forestry Institute. Site means are based on all trees from the diallel experiment except the four full-sib families studied (7122, 7125, 7143, and 7146) and selfs. Measurements were taken in 1990.
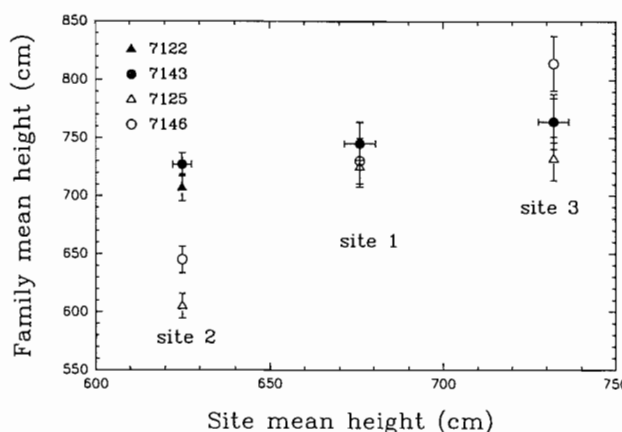
and R. Ponce-Hernandez, unpublished data) indicate that site 2 is drier than site 3.

The four families used are the same as those studied by Flanagan and Johnsen (1995) and constitute a 2 parent $\times$ 2 parent breeding structure as shown in Table 1. Full-sib families 7122 and 7143 maintained 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). Thus, families 7122 and 7143 represent productive "stable" families, whereas families 7125 and 7146 represent "unstable" families.

1991: Measurements on two sites
Trees from all four full-sib families were measured 2 days per week over a 5-week period commencing July 22, 1991. Each site was measured on a separate day each week, with the first site measured each week chosen at random. Weather forecasts were used to facilitate choosing 2 days during the week with similar climatic conditions.

Gas exchange was measured on detached branches within 2 min of excision. Meng and Arp (1993) demonstrated that although P_n and g_{wv} of branches cut from 30- to 40-year-old red spruce (*Picea rubens* Sarg.) trees declined continuously over a 2-h period following cutting, the impact on both measures within 6 min from cutting was minor and not statistically significant. Our tests with 20-year-old trees, conducted under a variety of field conditions, also support the validity of gas exchange measurements on black spruce branches taken within several minutes of

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

Male	Female	
	59	63
52	7122	7125
62	7143	7146

cutting (data not presented). The detached branch method has also been shown to be reliable in studies on mature *Picea abies* (L.) Karst. (Lange et al. 1986), 19-year-old *Pseudotsuga menziesii* (Mirb.) Franco (Zhang et al. 1993), 21-year-old *Abies balsamea* (L.) Mill. (Gage and Dehayes 1992), and 8-year-old *Pinus taeda* L. (Ginn et al. 1992).

On each date, sampling began at approximately 09:00 and progressed through the day with a break at 12:00. Both plantations have 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). Sampling proceeded from one side of a plantation (block 1) to the other (blocks 3 or 4), returning to block 1 at the end of a cycle. During each cycle, one tree per family was measured. Individual trees were randomly selected for measurement on each date and were measured only once on each date. Four cycles of measurements were completed on each date on site 2 (4 trees per family per block) and 5 cycles on site 3 (5 trees per family per block).

Approximately 30 cm terminal sections of branches were cut, using a pole clip, from the upper third of the crown (southern exposure). Shoots were immediately placed in a plastic bag containing moist paper towels and taken to a central location within the plantation. Measurements at site 2 were taken inside a fully vented tent, whereas measurements at site 3 were taken outside in the shade. Gas exchange was measured using a Li-6200 portable photosynthesis system (LI-COR Inc., Lincoln, Nebr.) and a 0.25-L sample cuvette. Measurements of P_n and g_{wv} were made under a portable cool incandescent light (12-V, 75-W General Electric EYF bulb) that provided approximately 2200 $\mu mol \cdot m^{-2} \cdot s^{-1}$ photosynthetically active radiation. Measurements were made on a 3-cm terminal section of the current year's growth. To provide a good seal, needles were removed from the stem at the point where the branch entered the cuvette. Measurements were initiated within 2 min of removing the shoot from a tree.

Immediately following measurement of gas exchange, xylem water potential (ψ_x) of the shoot section was measured using a pressure chamber (model 3005, Soil Moisture Corp., Calif.). Shoots were placed in a plastic bag and kept in a cooler until they were transported to a laboratory to determine the projected leaf surface area using a Li-3100 area meter (LI-COR Inc., Lincoln, Nebr.). The projected needle surface area was multiplied by 4 to estimate the total needle surface area. Needles collected during the first 2 weeks were also dried (65°C) to a constant weight and weighed.

On three occasions (during weeks 1, 3, and 4) predawn xylem water potential (ψ_{pd}) measurements were made on

Table 2. Weekly mean and overall mean gas exchange cuvette relative humidity (RH) and temperature and mean weather station high temperatures for 1991 measurement days, by week and site (SD in parentheses).

Week	Site	Mean cuvette RH (%)[a]	Mean cuvette temp. (°C)[a]	Mean high air temp. (°C)[b]
1	2	51.87 (5.23)	27.16 (1.95)	29.25
	3	57.98 (9.66)	25.74 (1.18)	26.50
2	2	60.56 (11.35)	26.20 (2.87)	24.00
	3	55.16 (11.53)	23.96 (2.58)	26.75
3	2	54.64 (4.62)	25.81 (2.72)	25.25
	3	55.80 (7.87)	23.71 (2.23)	24.00
4	2	45.98 (3.98)	27.87 (3.10)	29.25
	3	50.01 (7.70)	26.04 (3.22)	26.50
5	2	55.09 (5.80)	31.33 (2.49)	28.25
	3	49.87 (11.15)	30.34 (2.66)	31.50
Overall means[c]	2	53.62 (5.31)	27.67 (2.19)	27.20 (2.43)
	3	53.76 (3.64)	25.96 (2.66)	27.05 (2.73)

[a]During each week, data were collected at each site on a separate day of the week. Weekly values were calculated using individual tree measurements.

[b]Weekly values represent the mean of an a.m. and p.m. high temperature recorded for the particular day at the Petawawa National Forestry Institute weather station located approximately 5 km from the sites.

[c]Calculated using weekly values.

one tree per family per block per site between 04:00 and 05:30. The order of site measurement on each of the three dates was chosen at random. Branches were sampled from the middle third of the crown.

Net photosynthesis and g_{wv} were expressed and analyzed on a needle surface area and needle dry weight basis for the first 2 weeks and on a needle surface area basis only for the last 3 weeks. The internal CO_2 concentration (C_i)/atmospheric CO_2 concentration (C_a) ratio was estimated using Li-6200 algorithms. As some, but not all, individual trees were remeasured over multiple weeks, the data were analyzed by week. Analysis of variance (ANOVA) was used to assess the influence of the male parent, female parent, site, as well as interactions, using a randomized complete block design. The male parent, female parent, site, and block were all considered to be fixed effects.

1992: Measurements on one site

Gas exchange measurements were made on the same full-sib families as those measured in 1991 but only on site 2. Measurements were taken on nine separate dates between July 15 and August 11. On each date, one tree per family per block was measured. Trees within family/block plots were randomly assigned to each date; individual trees were only measured on one date. On each date individual trees were measured four times during the approximate time intervals of 09:00–10:00, 10:30–12:00, 12:30–14:00, and 14:00–15:00. As the spring and the early part of the summer of 1992 were extremely cold and wet, shoot phenology was retarded and the current year's foliage was not mature enough to be used for excised measures of gas exchange.

Table 3. Sources of variation, degrees of freedom (df), mean squares (MS), and p-values from ANOVA of 1991 net photosynthesis ($\mu mol \cdot m^{-2} \cdot s^{-1}$), by week.

Source[a]	df	Week 1 MS	p	Week 2 MS	p	Week 3 MS	p	Week 4 MS	p	Week 5 MS	p
M	1	0.3643	0.2196	0.1506	0.3756	0.1291	0.3544	0.1318	0.3861	0.0002	0.9782
F	1	1.6006	**0.0113**	0.9343	**0.0290**	0.1313	0.3505	0.9621	**0.0204**	3.0721	**0.0004**
S	1	7.0982	**0.0001**	0.5406	0.0950	9.7509	**0.0001**	0.1395	0.3727	6.5394	**0.0001**
M×F	1	0.2357	0.3226	0.0463	0.6228	0.0892	0.4414	0.9530	**0.0210**	0.1033	0.5012
M×S	1	0.6466	0.1032	0.1161	0.4363	0.3411	0.1335	0.0019	0.9169	0.0390	0.6793
M×F×S	1	0.0468	0.6586	0.0000	1.0000	0.0265	0.6743	0.0125	0.7890	0.1584	0.4050
Block(S)	5	0.7619	**0.0110**	0.1601	0.5228	0.1228	0.5364	0.2651	0.1880	0.5789	**0.0316**
Error	111	0.2379	—	0.1899	—	0.1495	—	0.1742	—	0.2268	—

Note: p-values ≤0.05 are in bold print.
[a]M, male; F, female; S, site.

Table 4. Sources of variation, degrees of freedom (df), mean squares (MS), and p-values from ANOVA of 1991 needle conductance ($mmol \cdot m^{-2} \cdot s^{-1}$), by week.

Source[a]	df	Week 1 MS	p	Week 2 MS	p	Week 3 MS	p	Week 4 MS	p	Week 5 MS	p
M	1	490.32	0.5144	614.75	0.7053	20.55	0.7261	416.25	0.4466	121.52	0.5605
F	1	178.33	0.6939	793.73	0.6674	202.08	0.2730	5.44	0.9306	119.33	0.5640
S	1	36 287.93	**0.0001**	5676.93	0.2519	117.90	0.4019	3454.36	**0.0298**	1121.61	0.0788
M×F	1	264.58	0.6318	0.74	0.9895	21.37	0.7209	2214.34	0.0808	19.44	0.8158
M×S	1	5.08	0.9470	291.73	0.7944	99.49	0.4412	71.86	0.7516	177.09	0.4824
F×S	1	171.82	0.6993	112.87	0.8712	82.26	0.4837	27.72	0.8441	36.60	0.7493
M×F×S	1	0.12	0.9919	970.16	0.6348	0.16	0.9751	1029.55	0.2322	387.12	0.2996
Block(S)	5	446.30	0.8540	1980.89	0.8023	149.34	0.4860	924.26	0.2708	297.30	0.5283
Error	111	1 143.18	—	4271.26	—	166.64	—	713.93	—	356.50	—

Note: p-values ≤0.05 are in bold print.
[a]M, male; F, female; S, site.

Therefore, second-year foliage (expanded in 1991) was measured. Shoots were again sampled from the upper third of the crown, but from that portion exposed to maximum illumination at the particular measurement period. Gas exchange, ψ_x, and needle surface area measurements were obtained as in 1991.

On five occasions (once per week) ψ_{pd} measurements were taken on trees measured for gas exchange. Measurements were taken on one tree per family per block between 04:00 and 05:00. Branches were sampled from the middle third of the crown.

Gas exchange and daily ψ_x means were calculated for individual trees using the four measurements per tree per date. Data from all nine dates were subjected to ANOVA to assess the influence of the male parent, female parent, date, as well as interactions, using a randomized complete block design. All effects were considered to be fixed.

Results

1991: Measurements on two sites

To make appropriate treatment comparisons of gas exchange traits, specific leaf areas (SLA) between sites and among families were compared. During week 1, across all families, trees from site 2 had a mean SLA of 156.76 $m^2 \cdot g^{-1}$, significantly lower ($p = 0.0162$) than the mean SLA for trees from site 3 (162.69 $m^2 \cdot g^{-1}$). Results in week 2 were similar, with the mean SLA being 155.95 and 160.34 $m^2 \cdot g^{-1}$ for trees from sites 2 and 3, respectively ($p = 0.0350$). Over these first 2 weeks of measurements, there were no significant genetic differences or genetic interactions in SLA. Since analysis of gas exchange traits as functions of needle weight and needle surface area resulted in nearly identical trends of means and statistical significance, gas exchange traits for both years are reported only as functions of needle surface area.

Although the two sites were measured on separate days of the week, temperature and relative humidity of the paired dates, by week, were similar (Table 2). Trees from site 2 displayed lower P_n and g_{wv} each week than trees from site 3 (Figs. 2A, 2B). These gas exchange differences were statistically significant during 3 of the 5 weeks for P_n (Table 3) and 2 of the 5 weeks for g_{wv} (Table 4). There were significant differences between sites with respect to the C_i/C_a ratio during 4 of the 5 weeks (Table 5). The greatest difference occurred during week 1, when trees

Table 5. Sources of variation, degrees of freedom (df), mean squares (MS), and p-values from ANOVA of 1991 internal CO_2/atmospheric CO_2 (C_i/C_a), by week.

Source[a]	df	Week 1 MS	Week 1 p	Week 2 MS	Week 2 p	Week 3 MS	Week 3 p	Week 4 MS	Week 4 p	Week 5 MS	Week 5 p
M	1	0.0006	0.7897	0.0002	0.8667	0.0015	0.5136	0.0010	0.6242	0.0029	0.3896
F	1	0.0041	0.5018	0.0215	0.1192	0.0099	0.0974	0.0098	0.1360	0.0366	**0.0027**
S	1	0.2181	**0.0001**	0.0206	0.1264	0.0758	**0.0001**	0.0205	**0.0321**	0.0637	**0.0001**
M×F	1	0.0001	0.9390	0.0005	0.8308	0.0008	0.6438	0.0201	**0.0337**	0.0080	0.1534
M×S	1	0.0006	0.7953	0.0002	0.8751	0.0002	0.8018	0.0014	0.5673	0.0009	0.6323
F×S	1	0.0094	0.3098	0.0005	0.8053	0.0013	0.5447	0.0004	0.7608	0.0003	0.7776
M×F×S	1	0.0020	0.6376	0.0000	0.9453	0.0006	0.6845	0.0046	0.3066	0.0065	0.1982
Block(S)	5	0.0052	0.7120	0.0025	0.9180	0.0046	0.2715	0.0042	0.4463	0.0044	0.3443
Error	111	0.0090	—	0.0087	—	0.0036	—	0.0043	—	0.0039	—

Note: p-values ≤0.05 are in bold print.

[a]M, male; F, female; S, site.

Table 6. Sources of variation, degrees of freedom (df), mean squares (MS), and p-values from ANOVA of 1991 net photosynthesis/needle conductance ($\mu mol \cdot mol^{-1}$), by week.

Source[a]	df	Week 1 MS	Week 1 p	Week 2 MS	Week 2 p	Week 3 MS	Week 3 p	Week 4 MS	Week 4 p	Week 5 MS	Week 5 p
M	1	25.59	0.8033	32.52	0.7612	55.14	0.5274	34.10	0.6497	133.86	0.3349
F	1	129.10	0.5762	924.49	0.1074	393.48	0.0930	360.49	0.1413	1389.01	**0.0023**
S	1	10 988.26	**0.0001**	726.24	0.1530	2515.23	**0.0001**	463.75	0.0957	1814.96	**0.0005**
M×F	1	0.80	0.9648	12.14	0.8526	27.66	0.6543	859.33	**0.0240**	331.40	0.1304
M×S	1	15.67	0.8455	3.33	0.9225	9.99	0.5982	45.92	0.5982	46.99	0.5672
F×S	1	513.93	0.2661	31.17	0.7660	86.81	0.8032	10.26	0.8032	4.68	0.8566
M×F×S	1	81.37	0.6571	4.57	0.9093	22.88	0.3348	154.21	0.3348	283.66	0.1614
Block(S)	5	231.70	0.7262	108.45	0.9058	209.66	0.4582	154.47	0.4582	171.17	0.3141
Error	111	409.74	—	349.88	—	137.29	—	164.44	—	142.72	—

Note: p-values ≤0.05 are in bold print.

[a]M, male; F, female; S, site.

from site 2 had a C_i/C_a ratio of 0.63 compared with 0.73 on site 3. Over the remaining 4 weeks the site rankings were inconsistent, with site 2 having lower values than site 3 during week 4 and site 3 having lower values than site 2 during weeks 2, 3, and 5. The magnitude of the differences was much less during the last 4 weeks, with the differences in the C_i/C_a ratio between sites being less than 0.04. Site rankings with respect to the P_n/g_{wv} ratio were opposite those of the C_i/C_a ratio. Otherwise, the pattern of statistical significance over time (Table 6), as well as the magnitude of site differences with respect to P_n/g_{wv}, were nearly identical with those of C_i/C_a. Site 2 had lower ψ_x over all weeks (Fig. 3A), and the means were significantly different during weeks 1, 2, and 5. Site 2 also had significantly lower ψ_{pd} (Fig. 3B) over all 3 weeks measured.

The predominant genetic differences in physiological measurements were between progeny of the two female parents. Progeny of female 59 had higher P_n over all 5 weeks (Fig. 4A), and the differences were significant during 4 of the 5 weeks (Table 3). The P_n performance of the progeny of the parents was consistent over the two sites (Table 3). There were no significant differences among

parents with respect to g_{wv} on any date (Table 4, Fig. 4B) nor any significant contribution of a G × E interaction (Table 4). Progeny of female 59 had lower C_i/C_a as well as higher P_n/g_{wv} over all 5 weeks, and the differences were significant during week 5 and approached statistical significance during weeks 2, 3, and 4 (Tables 5 and 6). Across both sites and all dates, progeny of female 59 had a mean C_i/C_a of 0.706 and a mean P_n/g_{wv} of 54.17 $\mu mol \cdot mol^{-1}$, while progeny of female 63 had a mean C_i/C_a of 0.728 and a mean P_n/g_{wv} of 49.89 $\mu mol \cdot mol^{-1}$.

1992: Measurements on one site

Again, the predominant genetic differences in physiological measures were associated with the two female parents. Across all dates, progeny of female 59 had significantly higher P_n than progeny of female 63 (Table 7, Fig. 5A). The female × male × date interaction term in the ANOVA of P_n was statistically significant, indicating differential performance of specific full-sib families over different dates (Table 7); no interpretable pattern was observed (data not shown). Across all dates, progeny of female 59 also had a lower mean g_{wv} than progeny of female 63, but this

Fig. 2. (A) Mean site net photosynthesis (P_n) and (B) mean site needle conductance (g_{wv}), by week, for 20-year-old black spruce trees from four full-sib families measured in 1991. Asterisks indicate weeks during which site means were different at $p \leq 0.05$.

Fig. 3. (A) Daytime xylem water potential (ψ_x) and (B) predawn xylem water potential (ψ_{pd}), by week, for 20-year-old black spruce trees from four full-sib families measured in 1991. Asterisks indicate weeks during which site means were different at $p \leq 0.05$. Predawn xylem water potential was not measured during weeks 2 or 5.
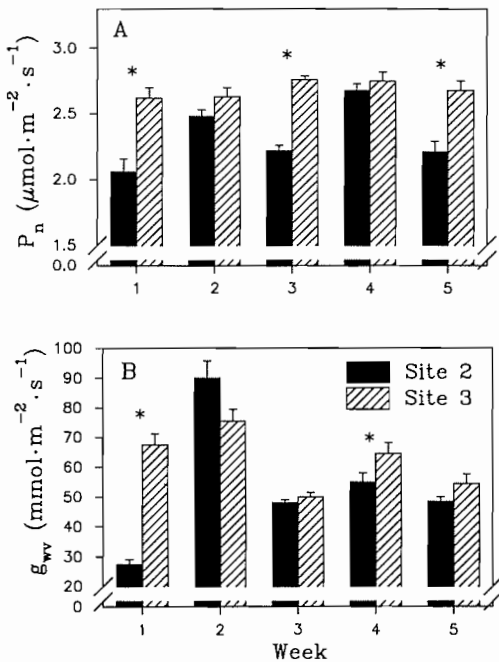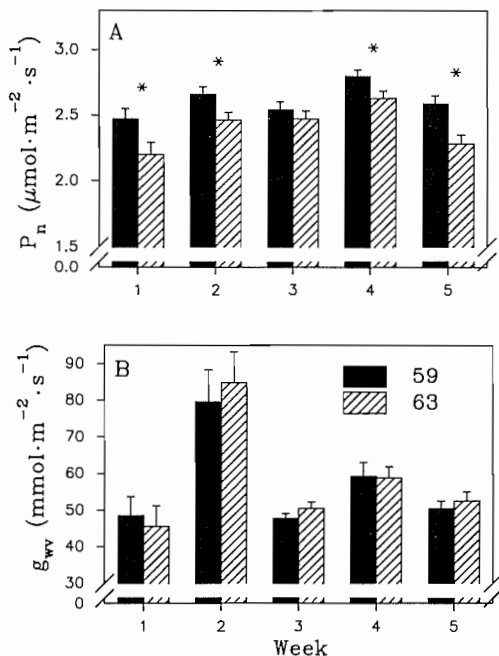

difference was not consistent over time, nor was it statistically significant (Table 7, Fig. 5B).

Progeny of female 59 had a significantly lower C_i/C_a than progeny of female 63 (Table 7); the mean C_i/C_a was 0.711 for progeny of female 59 and 0.736 for progeny of female 63. Progeny of female 59 had significantly higher P_n/g_{wv} than progeny of female 63 (Table 7). The mean P_n/g_{wv} was 52.5 $\mu mol \cdot mol^{-1}$ for progeny of female 59 and 47.8 $\mu mol \cdot mol^{-1}$ for progeny of female 63. ANOVA of ψ_x and ψ_{pd} indicates significant variation among sample dates ($p = 0.0000$). There were also statistically significant differences between progeny of the two male parents with respect to ψ_x ($p = 0.0007$). Progeny of male 52 had a mean ψ_x of -1.33 MPa, and progeny of male 62 had a mean ψ_x of -1.39 MPa.

Discussion

In an attempt to elucidate the physiological basis of a G × E interaction, we have used a prototype approach in which three main elements were addressed. First, we wanted to identify the environmental factor(s) contributing to the interaction. Second, we wanted to clearly establish that the selected subset of genetic entries was interacting with site. Third, we wanted to identify mechanisms, interpretable with regard to the environmental response, that are under genetic control.

Xylem water potential and gas exchange data collected in 1991, shown here, support the contention that site 2 is drier than site 3. Xylem water potential, both daytime and predawn, were lower in trees growing on site 2 than in
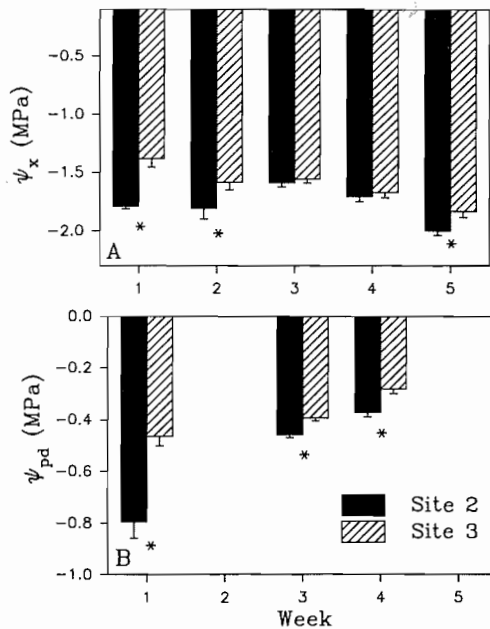
trees growing on site 3. Net photosynthetic rates of site 2 trees ranged from 3 to 22% lower than those of site 3 trees, with a mean difference of -14.0%. Needle conductance was also lower in trees growing on site 2 than in trees growing on site 3, by an average 14.4% over all dates. Lower gas exchange rates occurred on site 2 despite the fact that during the 2 weeks when needle weights were measured, trees from site 2 had slightly lower SLA than trees from site 3, a trend consistent with site 2 being a drier site (Kozlowski et al. 1991).

These data agree with original site observations, and preliminary soil analyses (O. Hendrickson, unpublished data), suggesting that the differences in productivity between sites 2 and 3 are due, at least in part, to differences in water availability. Analyses of soil structure and profiles clearly showed site 2 to be far better drained than site 3 (S. Brown and R. Ponce-Hernandez, unpublished data). Analyses of foliar stable isotope discrimination in 1991 and 1992 indicated that trees from site 2 discriminated less against the heavier isotope of carbon than trees on the two other sites, also consistent with the hypothesis that site 2 is a drier site (Flanagan and Johnsen 1995). In addition, the difference in stable isotope discrimination between the two sites was greater following a hot, dry summer (1991) than a cool, wet summer (1992).

Although gas exchange was generally lower in trees on site 2 than on site 3, site rankings with respect to the P_n/g_{wv} ratio were inconsistent over the study period. Estimations based on stable isotope discrimination predict that site 2 trees would have higher P_n/g_{wv} than site 3 trees (Flanagan and Johnsen 1995). This inconsistency may be

Fig. 4. (A) Mean net photosynthesis (P_n) and (B) mean needle conductance (g_{wv}) for progeny of female 59 and female 63, by week, of 20-year-old black spruce trees from four full-sib families measured in 1991. Data were averaged across both sites. Asterisks indicate weeks during which female progeny means were different at $p \leq 0.05$.

Fig. 5. (A) Mean net photosynthesis (P_n) and (B) mean needle conductance (g_{wv}) for progeny of female 59 and female 63, by day, of 20-year-old black spruce trees from four full-sib families measured in 1992. Inserts show means across all dates and use the same units as the main graph. Asterisks indicate where female progeny means were different at $p \leq 0.05$.

due, at least in part, to the timing of the gas exchange measurements. Measurement of foliar carbon isotope discrimination provides an indication of the assimilation-weighted average of P_n/g_{wv}. The foliage assessed for carbon isotope discrimination was fully expanded and hardened prior to the July–August gas exchange measurements. June, during both 1991 and 1992, was by far the driest month of the growing season and represents the period of greatest needle expansion. Thus, site differences with respect to water stress may have been greater during this period, when needle expansion was greatest, and less during the period when gas exchange measurements were conducted. Phenology, leaf development, and the timing of gas exchange measurements have been shown to influence the relationship between P_n/g_{wv} estimates made via instantaneous gas exchange and those estimated via stable isotopes (Meinzer et al. 1992).

Thus, there is multifaceted evidence (ψ_x, ψ_{pd}, soil analysis, gas exchange, and carbon isotope discrimination) to suggest that differences in productivity between the sites are largely due to differences in water availability and, thus, water stress. We recognize that in our examinations into the causes of the site differences in growth rate, all evidence is circumstantial. No argument is exhaustive enough to exclude the possibility that factors other than differential water stress contribute to the observed site differences in growth rate. Nevertheless, considering the consistent responses of such varied measures of trees on the sites as well as further conformation via analysis of physical characteristics of the sites, we feel it is probable that water
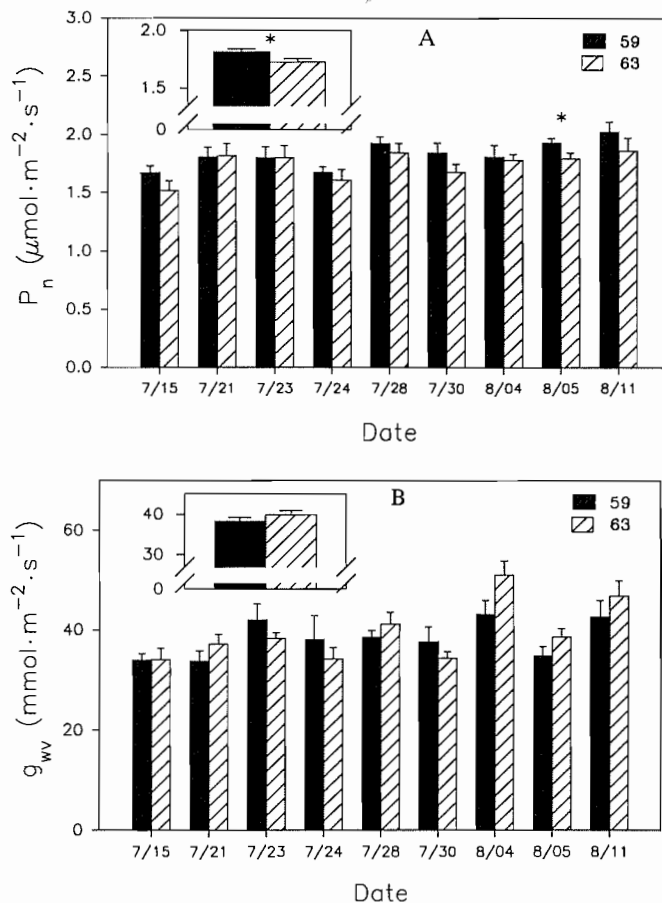
stress is to a large degree responsible for the environmental component of the G × E interaction.

The families selected for study here were chosen for their stability or lack of stability with respect to growth rate on three different sites. These families varied significantly in carbon isotope discrimination, with progeny of female 59 having lower discrimination than progeny of female 63 (Flanagan and Johnsen 1995). These differences were consistent over all three sites and were highly repeatable over 2 years representing both a hot, dry year and a cool, wet year. In addition, on the dry site, family mean carbon isotope discrimination was strongly negatively correlated with growth. Thus, families with higher P_n/g_{wv} (intrinsic water use efficiency (Meinzer et al. 1990)) grew better on the dry site than families with lower P_n/g_{wv}. This strongly supports the hypothesis that the selected families are truly interacting with site and that the physiological basis is, at least partly, related to differences in the balance of carbon gain to water loss.

The gas exchange data obtained indicate that the observed genetic differences in carbon isotope discrimination

Table 7. Sources of variation, degrees of freedom (df), mean squares (MS), and p-values from ANOVA of 1992 net photosynthesis (P_n, $\mu mol \cdot m^{-2} \cdot s^{-1}$), needle conductance ($g_{wv}$, $mmol \cdot m^{-2} \cdot s^{-1}$), internal CO_2/atmospheric CO_2 (C_i/C_a), and P_n/g_{wv} ($\mu mol \cdot mol^{-1}$).

Source[a]	df	P_n		g_{wv}		C_i/C_a		P_n/g_{wv}	
		MS	p	MS	p	MS	p	MS	p
M	1	0.0847	0.1869	109.88	0.1234	0.0057	0.1074	159.46	0.1654
F	1	0.2653	**0.0206**	81.54	0.1838	9.0207	**0.0025**	775.57	**0.0026**
D	8	0.1949	**0.0003**	310.08	**0.0000**	0.0040	0.0755	166.06	**0.0496**
F×M	1	0.0816	0.1953	33.26	0.3947	0.0022	0.3110	112.48	0.2434
F×D	8	0.0192	0.9182	58.23	0.2625	0.0013	0.7759	50.18	0.7642
M×D	8	0.0380	0.6107	52.47	0.3353	0.0029	0.2257	113.77	0.2084
F×M×D	8	0.1117	**0.0244**	96.20	**0.0409**	0.0031	0.1860	119.17	0.1815
Block	3	0.0231	0.6958	82.91	0.1479	0.0011	0.6766	50.58	0.6042
Error	104	0.0480	—	45.53	—	0.0022	—	81.71	—

Note: p-values ≤0.05 are in bold print.

[a]M, male; F, female; D, date.

are largely due to differences in P_n. In 1991, a hot, dry summer (Flanagan and Johnsen 1995), the current year's foliage from progeny of female 59 had, over all weeks, approximately 9% higher P_n than progeny of female 63. The differences were consistent over weeks and between sites and were statistically significant during 4 of the 5 weeks. In 1992, a cool, wet summer (Flanagan and Johnsen 1995), the ranking based on second year's foliage was identical, with progeny of female 59 having approximately 5% higher P_n, on average, over all dates. Neither consistent nor significant differences in g_{wv} were observed between progeny of female trees in 1991 or 1992.

Tan et al. (1992) performed a controlled-environment experiment on the same families used in this current study using polyethylene glycol induced water stress in a hydroponics system. They found families differences in gas exchange only under intermediate stress, and that the progeny of female 59 maintained both higher P_n and higher g_{wv} than the progeny of female 63; thus, no differences existed among the families in the P_n/g_{wv} ratio. In contrast, our results, as well as results of Flanagan and Johnsen (1995), indicate mature tree family differences in P_n/g_{wv} and P_n under both wetter and drier conditions. It is unknown whether this discrepancy represents a maturation effect (Greenwood et al. 1989; Hutchinson et al. 1990; Sandquist et al. 1993) or is an artifact of experimental conditions perhaps related to the use of polyethylene glycol induced drought in a hydroponics system (Tan et al. 1992).

In most cases examined so far involving other plant species, genetic differences in carbon isotope discrimination have been attributed to differences in g_{wv} and not P_n. These examples include examinations of clones of crested wheatgrass (*Agropyron desertorum* (Fischer ex Link) Schultes) (Read et al. 1992, 1993), wheat (*Triticum turgidum* L.) genotypes (Farquhar and Richards 1984), and coffee (*Coffea arabica* L.) genotypes (Meinzer et al. 1990). In *Pseudotsuga menziesii*, provenance differences in carbon isotope discrimination also appeared to be due to differences in g_{wv} and not P_n (Zhang et al. 1993). The results presented in the current study on black spruce are not without precedence,

however, as differences in carbon isotope discrimination in peanut (*Arachis* spp.) were shown to be due to differences in P_n and not g_{wv} (Hubick et al. 1986; Wright et al. 1988).

As discussed by Farquhar et al. (1989), carbon isotope discrimination is likely to be a polygenic trait, as it is influenced by genes related to both carbon gain and water loss. With this in mind, and considering the contrasting cases in the literature, it is also likely that the physiological basis for genetic differences in carbon isotope discrimination varies among species and may even be dependent on the specific population examined within a species. Selection pressures among populations of trees might result in different genetic correlations between carbon isotope discrimination and gas exchange traits than genetic correlations found within populations of trees. Such trends have been observed in studies on shoot growth, another composite trait (Kremer and Larson 1983; Bongarten 1986). The relative importance of the component traits, stem unit number and stem unit length, in contributing to differences in shoot growth is dependent on the population structure being studied; the number of stem units is more important among populations and the stem unit length is more important within populations (Kremer and Larson 1983; Bongarten 1986).

The results of 2 years of gas exchange analyses and 2 years of carbon isotope discrimination analyses provide compelling evidence that this particular case of G × E interaction is related to genetic differences in P_n. As growth differences among these families occur only on the dry site, it was originally hypothesized that any genetic differences in gas exchange traits would also be expressed only under conditions of drought. Instead, genetic differences in both P_n and carbon isotope discrimination have been quite stable over 2 sites of varying water availability and over 2 years of greatly contrasting temperature and rainfall. Similar stability in carbon isotope discrimination has been observed among wheat (Condon et al. 1987; Condon and Richards 1992) and cow-pea (*Vigna sinensis* (L.) Endl.) (Hall et al. 1994) genotypes.

It appears that genetic variation in P_n/g_{wv}, which is constant over the study sites, has been manifested into growth

differences only on the dry site. As these physiological differences were observed on both a wetter site and a drier site, as well as during a very dry year and a very wet year, they do not represent differences in soil drought tolerance, per se, although they result in differences in productivity on the dry site. Thus, the G × E interaction with respect to growth appears to be due, at least in part, to genetic differences in P_n.

The literature is replete with studies examining the relationship between P_n and growth rate. Some studies indicate no or even a negative correlation between P_n and growth (Sorenson and Ferrell 1973; Nelson 1988; Larson and Wellendorf 1990; Zhang et al. 1993, 1994), while others resulted in positive correlations (Logan 1971; Boltz et al. 1986; Blake and Yeatman 1989). Sulzer et al. (1993), using open-pollinated families of black spruce in a retrospective study, observed a positive correlation between seedling P_n and seedling growth but no correlation between P_n and 10-year height of the same families planted in the field.

It is apparent that the genetic correlation between P_n and growth rate in forest trees is complicated and is dependent on many factors, including the specific population being studied, the age of the trees, the time(s) of year P_n is measured, and the growth conditions. The correlation of P_n with growth will probably depend on the extent and (or) sign of genetic correlations with other traits influencing growth (such as dry matter partitioning, respiration, etc.), which is likely a function of the specific population being studied. These relationships may or may not be stable over all ages. In addition, the correlation between P_n, measured at any one given time, with yield can depend on the seasonal pattern of P_n and its influence on the integration of carbon gain. The relationship of P_n with growth may also be dependent on the particular environmental conditions. Some environmental conditions may result in no expression of genetic variation in P_n, precluding any relationship with growth. In other situations, varying environments may simply alter the extent that stable genetic differences in carbon gain per unit leaf area limits growth.

In this particular case, we hypothesize that water stress on the dry site reduces P_n below a threshold value at which point genetic variation in P_n impacts productivity and results in genetic variation in growth rate. Furthermore, we hypothesize that on the wet site, carbon gain per unit leaf area is not as limiting to growth rate; thus, the observed genetic differences in P_n do not influence genetic rankings in growth rate.

In addition, other traits not studied here may also be contributing to the observed G × E interaction in growth. This prototype approach to the characterization of a G × E interaction at the physiological level is ongoing and is being developed by intensifying work with the families used here and by expanding the number of families under study.

Acknowledgments

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