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Selfing results in inbreeding depression of growth but not of gas exchange of surviving adult black spruce trees

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Summary In most tree species, inbreeding greatly reduces seed production, seed viability, survival and growth. In a previous large-scale quantitative analysis of a black spruce (*Picea mariana* (Mill.) B.S.P.) diallel experiment, selfing had large deleterious effects on growth but no impact on stable carbon isotope discrimination (an indirect measure of the ratio of net photosynthesis (A) to stomatal conductance (g_{wv})). It was hypothesized that selfing has no effect on carbon (C) fixation at the leaf level but impairs subsequent utilization of C. Alternatively, A and g_{wv} may be impacted by selfing to the same extent. However, no gas exchange data were collected to test these hypotheses. We have now obtained photosynthetic gas exchange data from three selfed families and three outcrossed families (all the result of controlled pollination) from the same diallel experiment. Photosynthetic responses to intercellular CO_2 concentration (A–C_i curves) were generated on four replicates per family, one block per day, over a 4-day period in July. There were no differences between selfed and outcrossed families in maximum carboxylation rate, maximum electron transport, A or g_{wv} (both estimated at 370 ppm CO_2), or the ratio A/g_{wv}. Because selfed trees had higher mortality than outcrossed trees during the experiment, we cannot exclude the possibility that previously living selfed progeny had low A. Nevertheless, the data indicate that inbreeding can result in trees that have low productivity despite high A, supporting our hypothesis that gas exchange is similar between selfed and outcrossed progeny trees. We conclude that utilization of fixed C is modified in the surviving selfed progeny.

Keywords: carbon isotope discrimination, photosynthesis, Picea mariana, stomatal conductance.

Introduction

Most outcrossing forest trees suffer deleterious effects of inbreeding, including reduced seed set, survival, growth and productivity (Samuel et al. 1972, Snyder 1972, Eriksson et al. 1973, Orr-Ewing 1974, Sorenson et al. 1976, Park and Fowler 1984, Griffin and Lindgren 1985, Geburek 1986, Denti and Schoen 1988, Morgante et al. 1993, Wu et al. 1998, Koelewijn et al. 1999, Sorensen 1999, Wang 1999). Progeny of self-pollinated trees that survive in the field are typically substantially smaller than progeny of outcrossed trees. This difference occurs during initial stand establishment and can be further accentuated in the course of stand development (Williams and Savolainen 1996) as the smaller inbred trees are increasingly outcompeted for light and other resources by faster-growing outcrossed trees.

Decreased seed set, survival and vigor of selfed progeny result from increases in the frequency of deleterious recessive genes. Although the mode of gene action contributing to inbreeding depression has been studied (Park and Fowler 1984, Griffin and Lindgren 1985, Wu et al. 1998, Kuang 1999, Wang 1999), specific impacts on component traits that contribute to decreased vigor (such as changes in net photosynthesis (A), stomatal conductance (g_{wv}) and biomass partitioning) have rarely been studied. One exception is a study by Blake and Yeatmen (1989), who examined water relations, gas exchange and early growth rates of outcrossed and selfed jack pine (*Pinus banksiana* Lamb.) from a controlled cross diallel experiment. The smaller selfed families had lower A, higher g_{wv}, and thus lower water-use efficiency (WUE) than the larger outcrosssed families, particularly under stressful conditions (dry and hot).

In contrast, Johnsen et al. (1999) conducted a quantitative analysis of growth and foliar stable carbon isotope discrimination of one diagonal (i.e., no reciprocals were tested) in a 7 × 7 diallel of field-grown 22-year-old black spruce (*Picea mariana* (Mill.) B.S.P.) trees. Although inbreeding depressed height growth by 32% and diameter growth (at breast height (DBH)) by 36%, it had no impact on stable carbon isotope discrimination. That is, the breeding value for stable C isotope discrimination of selfed trees was equal to twice the genetic value derived for the parent tree, suggesting totally additive behavior. The authors hypothesized that, because the selfed trees had greatly depressed growth although stable isotope discrimination was not impacted, the use of assimilated C must have been modified by inbreeding. Alternatively, because stable C isotope discrimination is an integrated proxy measure of the ratio A/g_{wv}, both A and g_{wv} may have been impacted by selfing. No gas exchange data were available to distinguish between these hypotheses.

Here we report photosynthetic gas exchange measurements conducted on a subset of selfed and outcrossed families from the same diallel experiment when the trees were 29 years old. In combination with the carbon isotope study (Johnsen et al. 1999), these data reveal that different traits are differentially impacted by inbreeding.

Materials and methods

The 7 × 7 black spruce diallel experiment was established in 1973 at the Petawawa Research Forest (PRF) located in Chalk River, Ontario (46° N, 77°30′ W). The details on breeding and the field experiment have been described (Morgenstern 1974, Boyle 1987, Johnsen et al. 1999). The parent trees were from the Lake Simcoe–Rideau region of Ontario, but the exact origins are unknown. The diallel experiment was established over three separate sites at the PRF. Gas exchange measurements were conducted on three selfed and three outcrossed families on Site 2. Previous gas exchange and water relations research on these sites characterized Site 2 as the driest of the three sites because of rapid drainage (Flanagan and Johnsen 1995, Johnsen and Major 1995, 1999, Major and Johnsen 1996, 1999, 2001). Site 2 was established as a randomized complete block design with four blocks and 16 trees per plot planted at a spacing of 1.8 × 1.8 m; no artificial thinning has been conducted in the stand.

Three selfed families (7137, 7145 and 7153) and three outcrossed families (7143, 7144 and 7146) were utilized (parentage is shown in Table 1). Gas exchange of trees was measured on four consecutive days beginning July 9, 2002. On each day, between about 1000 and 1400 h, gas exchange of one tree per family per block was measured. Whole detached branches (cut with a pole pruner) from above the point of canopy closure were removed from the upper whorl for measurement. Branches were immediately recut under water and the cut end was maintained in a large water reservoir. Gas exchange was measured on a lateral shoot near the end of each branch.

Previous-year shoot sections from secondary lateral branches at the distal end of the main branch were placed in the chamber of an LI-6400 system (Li-Cor, Lincoln, NE). Two LI-6400s were used simultaneously so that all trees in each block could be measured during a short interval each day. Gas exchange was measured across a range (150 to 1000 ppm) of chamber atmospheric CO_2 concentrations for the purpose of constructing individual tree response curves to intercellular CO_2 concentration (i.e., A–C_i curves). The LI-6400 internal controls were used to maintain photosynthetically active radiation (PAR) at 1600 µmol m^{-2} s^{-1}, leaf temperature at 22.0 °C and vapor pressure deficit (VPD) at 1.00 kPa. Mean and standard deviations for chamber temperature and VPD, by cross class, are shown in Table 2.

The external CO_2 concentrations used for gas exchange measurements, and their order of measurement, were the same as described by Maier et al. (2002). Following completion of the A–C_i curves, shoot sections located in the chamber were removed and stored on ice until needle surface area (Regent Instruments, Quebec, Canada) and needle dry mass were measured. Maximum carboxylation rate (V_{cmax}) and maximum electron transport (J_{max}) were calculated based on the Farquhar biochemical model of photosynthesis (Farquhar and von Caemmerer 1982) as in Maier et al. (2002). In addition, mean A and g_{wv} were calculated based on values measured at an external CO_2 concentration of 370 ppm. The height and DBH of all sampled trees, and total surviving trees per block, were determined the week after the photosynthetic measurements. Stem volume per tree was estimated as $1/3\pi r^2 h$, where r = stem radius and h = stem height.

All variables were subjected to analysis of variance (ANOVA) with cross class (selfed or outcrossed), family nested within cross class, and block. Percent survival data were log-transformed. Cross class (one degree of freedom) was statistically tested by using cross class × block (three degrees of freedom) as the error term.

Results and discussion

Differential survival rates between selfed and outcrossed progeny have been large in the diallel experiment at the PRF. Although full complements of seedlings were planted on the site in 1973, only 37% have survived in the selfed plots compared with 95% in the outcrossed plots. Most mortality in the selfed plots occurred well before crown closure (data not shown), which is just occurring at the current stand age. Thus, until now, mortality of the selfed progeny was not substantially impacted by shading from neighbors.

At age 29, the three selfed families that we studied had 58% fewer surviving trees in the plantation than the three outcrossed families (Table 3). The measured selfed trees were, on average, 38, 39 and 76% smaller in height, DBH and volume, respectively, than the outcrossed trees (Table 3).

For all gas exchange traits, there were no statistically significant differences between selfed and outcrossed trees, and P-values were generally high (Table 4). There was no difference in leaf area ratio between cross classes (P = 0.160), and rela-

Table 1. Parentage of the six full-sib families of black spruce used to study inbreeding impacts on growth, survival and gas exchange.

Male	Female			
	59	60	62	63
60		7137		
62	7143	7144	7145	7146
63				7153

Table 2. Mean chamber temperature and vapor pressure deficit (VPD), with standard deviation in parentheses, by cross class.

Cross class	Temperature (°C)	VPD (kPa)
Self	21.75 (1.33)	1.07 (0.11)
Outcrossed	21.91 (1.29)	1.04 (0.08)

Table 3. Mean (with standard error in parenthesis) tree survival per plot (all trees in plantations), and mean tree height, diameter at breast height (DBH) and volume (only measured trees), by cross class. The *P*-values are from ANOVA of differences between cross classes.

Trait	Selfed progeny	Outcrossed progeny	*P*-value
Survival (%)	37.00 (6.2)	94.80 (2.8)	0.008[1]
Height (m)	6.82 (0.35)	10.96 (0.42)	0.004
DBH (cm)	8.6 (0.40)	14.1 (0.60)	< 0.001
Volume ($\times 10^4$ cm^3)	1.413 (0.18)	5.958 (0.67)	0.005[1]

[1] ANOVA performed on log-transformed data.

tive differences in *A* and g_{wv}, and associated *P*-values, were similar when expressed either on a per unit leaf area basis or on a leaf mass basis (only area based values are presented).

Thus, the results for a subset of families on Site 2 support the hypothesis that, although inbreeding greatly decreases survival and aboveground growth, photosynthetic gas exchange of selfed and outcrossed progeny does not differ. Furthermore, the results of an analysis based on carbon isotope discrimination, a more integrated estimate of A/g_{wv}, support our findings, and individual tree carbon isotope discrimination ranking was similar among years (Johnsen et al. 1999).

In contrast, Blake and Yeatman (1989) observed lower *A* and higher g_{wv} in selfed progeny relative to outcrossed progeny of jack pine populations, particularly when conditions were hot and dry. Compared with the study of Blake and Yeatmen (1989), our measurements were made on older (29 years old) trees on a well-drained site where trees are subject to chronic drought stress, although the VPD used for our measurements was low. Studies on the genetic basis of inbreeding depression indicate that there are a small number of genes that confer large deleterious effects, with most other deleterious effects being minor (Crow and Simmons 1983). It is possible that the selfed trees that died before we conducted our experiment had impaired *A*. Low *A* may have even been an important contributor to tree death if homozygosity of genes directly impacting *A* was highly deleterious. We speculate that the remaining selfed progeny in the plantation are survivors that, although much smaller than the outcrossed progeny, can still fix C normally on a per unit leaf basis.

Table 4. Mean (with standard error in parenthesis) leaf area ratio (LAR), net photosynthesis (*A*), stomatal conductance (g_{wv}) and A/g_{wv} (each measured at an atmospheric CO$_2$ concentration of 370 ppm), mean maximum carboxylation rate (V_{cmax}), mean maximum electron transport (J_{max}) and dark respiration (R_d), by cross class. The *P*-values are from ANOVA of differences between cross classes.

Trait	Selfed progeny	Outcrossed progeny	*P*-value
LAR	68.222 (2.159)	72.935 (1.343)	0.1600
A	2.148 (0.113)	2.200 (0.160)	0.8493
g_{wv}	0.030 (0.002)	0.032 (0.003)	0.6238
A/g_{wv}	76.184 (3.487)	69.900 (3.881)	0.1948
V_{cmax}	10.863 (0.544)	11.458 (0.603)	0.6269
J_{max}	23.453 (1.264)	24.653 (1.062)	0.6105
R_d	–0.6483 (0.098)	–0.5300 (0.103)	0.1982

Selfed progeny are much smaller than outcrossed progeny, a difference that will impact physiology if there is an allometric relationship between physiology and size (Johnsen and Bongarten 1991). A difference in size could either magnify or compensate for any intrinsic differences in gas exchange. This seems unlikely, however, because a stable isotope discrimination study indicated that the breeding values of the selfed progeny were almost exactly twice the sum of the genetic values of the parent tree (Johnsen et al. 1999).

It is noteworthy that, in a subset of outcrossed families in the same field experiment, family photosynthetic performance was strongly related to growth (Johnsen and Major 1995, Major and Johnsen 1996). Although minor differences in gas exchange can be difficult to measure, extensive analyses of carbon isotope discrimination in the same experiment (Johnsen et al. 1999) revealed subtle, repeatable and highly heritable variation that was strongly correlated to growth.

Preliminary analysis by ground-penetrating radar of root biomass of one selfed and one outcrossed family (Butnor et al. 2001, 2003) indicated that selfed progeny have much greater allocation to root biomass than outcrossed progeny (data not shown). If confirmed with a larger sample, this could account for the smaller aboveground size of selfed progeny compared with outcrossed progeny. A similar combination of traits was observed in a seedlot from the Yukon that had extremely low growth rates, lower survival and higher gas exchange rates than seedlots from Ontario when grown in a greenhouse or common garden studies at the PRF in Ontario (Johnsen and Seiler 1996, Johnsen et al. 1996). Yukon seedlings also partitioned more biomass to roots than the Ontario provenances. Reduced height growth and increased allocation of biomass to roots of the Yukon seedlot was associated with a shorter period of shoot growth compared with the Ontario seedlots. Similarly, selfing may also impact phenology and contribute to greatly decreased aboveground growth (including leaf area, observed but not quantified in this study) and increased allocation below ground.

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