Negative heterosis not apparent in 22-year-old hybrids of *Picea mariana* and *Picea rubens*

Kurt H. Johnsen, John E. Major, Judy Loo, and Donald McPhee

Abstract: Work from the 1970s indicated that, relative to either parent species, crosses between red spruce (*Picea rubens* Sarg.) and black spruce (*Picea mariana* (Mill.) B.S.P.) were inferior with respect to both growth and photosynthesis. We re-examined the hypothesis that there is negative heterosis in hybrids of red and black spruce using 22-year-old trees in a common garden study planted on two sites. The trees were the product of controlled crossings and represent a continuum from hybrid class 0 (pure black spruce) to hybrid class 1 (pure red spruce). Progeny of all controlled crosses were measured for height and diameter. A subset of families were measured for gas exchange and were assessed using a hybrid index based on needle color, needle configuration, twig ridges, twig bark color, vegetative bud color, and cone scale morphology. Tree growth rate linearly declined with the increasing proportion of red spruce germplasm (increasing hybrid index). In 1994, intermediate hybrid index classes did not differ in gas exchange from either pure black spruce (hybrid index class 0) or pure red spruce (hybrid index class 1), and in 1996, hybrids displayed slightly higher rates of gas exchange. Thus, negative heterosis was not apparent in 22-year-old trees. Individual tree hybrid index generally agreed with expectation based on midparent means, although the relationship was stronger on the higher productivity site ($r^2 = 0.91$) than the poorer productivity site ($r^2 = 0.54$).

Key words: black spruce, heterosis, hybrid, photosynthesis, red spruce.

Résumé : Au cours des années 70, on pensait que, comparativement aux deux parents, les plants issus de croisements entre épinettes rouges (*Picea rubens* Sarg.) et épinettes noires (*Picea mariana* (Mill.) B.S.P.) étaient inférieurs en termes de croissance aussi bien que de photosynthèse. Les auteurs ont ré-examiné cette hypothèse à l'effet qu'il y aurait une hétérosis négative chez les hybrides d'épinettes rouges et noires en utilisant des arbres âgés de 22 ans, situés dans un jardin d'étude commun et plantés sur deux sites. Les arbres proviennent de croisements contrôlés et représentent un continuum des hybrides de classe 0 (épinette noire pure) aux hybrides de classe 1 (épinette rouge pure). Les auteurs ont mesuré la hauteur et le diamètre de toutes les progénitures. Ils ont mesuré les échanges gazeux dans un sous-ensemble de familles et les ont évaluées en utilisant un index basé sur la couleur des aiguilles, la configuration des aiguilles, les cannelures sur les rameaux, la couleur de l'écorce des rameaux, la couleur des bourgeons végétatifs et la morphologie des écailles des cônes. La croissance des arbres édécroît de façon linéaire lorsque la proportion du germplasme d'épinette rouge augmente (augmentation de l'index d'hybridation). En 1994, les classes intermédiaires d'index d'hybridation ne montraient pas de différences dans les échanges gazeux, que ce soit des épinettes noires pures (classe 0 d'index d'hybridation) ou des épinettes rouges pures (classe 1 d'index d'hybridation), et en 1996 les hybrides ont montré des taux d'échanges gazeux légèrement plus élevés. Ainsi, l'hétérosis négative n'apparaît pas chez les arbres âgés de 22 ans. Les index d'hybridation des arbres individuels concordent généralement avec les attentes basées sur les moyennes entre parents bien que la relation soit plus forte sur le site plus productif (r^2 = 0,91) que sur le site moins productif (r^2 = 0,54).

Mots clés : épinette noire, hétérosis, hybride, photosynthèse, épinette rouge.

[Traduit par la rédaction]

Introduction

The geographic range of red spruce (*Picea rubens* Sarg.) is centered in the northeastern United States and Canadian maritimes, extends west into Ontario, and south along the upper elevations of the southern Appalachian mountains (Burns and Honkala 1990). Except in the southern part of its range, red spruce is largely sympatric with the transcontinental black spruce (*Picea mariana* (Mill.) B.S.P.). The two species appear closely related, as they can be artificially hybridized (Manley and Ledig 1979) and are similar in morphology (Manley 1971) as well as isozyme (Eckert 1989) and molecular marker (Bobola et al. 1992; Perron et al. 1995) profiles.

Two questions still exist regarding the co-existence of red and black spruce. First, to what extent do the two species naturally hybridize and introgress? Estimates of the extent of hybridization and (or) introgression range from large and medium (Perron and Bousquet 1997; Bobola et al. 1996*a*;

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Morgenstern and Farrar 1964; Manley 1972; Khalil 1987; Fowler et al. 1988) to small (Gordon 1976; Manley and Ledig 1979). A second unresolved question is what, if any, are the isolating barriers between the species? Manley (1975) demonstrated that interspecific crossability as well as crossability between hybrids was much lower than that of intraspecific matings. Manley and Ledig (1979) presented data that supported the idea that the two species are also ecologically isolated and that each exhibits physiological characteristics that favor different ecological niches. In a study of seedling photosynthetic responses, red spruce was shown to be superior under warmer temperatures with lower light conditions, while black spruce had higher photosynthetic rates under cooler temperatures and higher light conditions. These results were in general agreement with respective environments the two species typically inhabit when existing in sympatry; black spruce inhabits more open bog environments, which are sinks for cold air drainage during the night, while red spruce inhabits more upland sites and often develops under the canopy of other trees.

In addition, Manley and Ledig's (1979) results indicated that artificially produced red × black spruce hybrid seedlings displayed negative heterosis. Under almost all conditions reported, hybrids performed worse than either parent and F_1 hybrids generally had the poorest performance. They conjectured that hybrid inferiority was important for maintaining the two separate species identities. They also hypothesized that hybrids were more likely to survive under conditions where competition was reduced. Thus, hybrids would be more prevalent following disturbance such as clearcut logging.

We re-examined, by measuring growth, mortality, and gas exchange, the hypothesis that there is negative heterosis in hybrids of red × black spruce using 22-year-old trees in a common garden study planted on two sites. The experiment was originally established with the same full-sib families produced by Manley (1975) and studied by Manley and Ledig (1979). In addition, we morphologically assessed the mature progeny trees using a hybrid index to see if individual phenotypes conformed to expectation based on the original parent evaluations.

Materials and methods

The trees used are the product of a series of controlled pollinations described by Manley and Ledig (1979). Using a hybrid index system (Manley 1969, 1971) based on 19 leaf, twig, cone, and crown characteristics, individual trees were selected that approximated hybrid indices of 0, 0.25, 0.50, 0.75, and 1.00, where 0 represents pure black spruce and 1.00 represents pure red spruce. These trees were located on or near the Acadia Forest Experiment Station, New Brunswick, Canada (46°N, 66°15′W). Two sets of such trees were crossed in factorial combinations to produce 25 full-sib families that had expected hybrid indices of 0, 0.125, 0.25, 0.375, 0.50, 0.625, 0.75, 0.875, and 1.00 representing a continuum from pure black to pure red spruce. Three different series (three sets of full-sib families) of crosses were produced. In 1976, the 75 full-sib families were established in a progeny test, using 2-2 nursery stock, replicated on two sites at the Acadia Forest Experiment Station. Both sites were "upland" types. That is, they do not experience inundation at any point of the year. On both sites, a randomized complete block design, with three blocks and four tree plots (2 × 2) planted at 1.8 × 1.8 m spacing, was used. In the spring of 1994, 18 years after planting, all trees were measured for height and diameter at breast height.

Gas exchange was measured on a subset of families on both sites in July 1994. Three families from expected progeny classes 0, 0.25, 0.50, 0.75, and 1.00 were measured for a total of 15 full-sib families. Families were used that were the product of parents from identical index classes. For example, families of index class 0.50 were used from crosses of parents that were each index class 0.50 and thus represented putative F_2 hybrids.

Two LI-6200 portable photosynthesis systems (LI-COR Inc., Lincoln, Neb.) were used to measure net photosynthesis (P_n), needle conductance (g_{wv}), and dark respiration (R_d) on trees from both sites (1 and 2) on the same day. One block was measured each day over three consecutive days. Instruments and crews were switched so that each crew measured on each site twice during a day. On each day, one tree per family per block was measured during each of the approximate time intervals of 08:30–09:30, 10:30–11:30, 12:30–13:30, and 14:30–15:30. Gas exchange was measured on detached branches within 2 min of excision (Meng and Arp 1993; Johnsen and Major 1995). One-year-old foliage was measured from the upper third of the crown from a branch exposed to maximum illumination at the particular measurement time. Sampling protocol, measurement conditions, and leaf area and dry weight estimation were as described in Johnsen and Major (1995).

Gas exchange was measured again during the summer of 1996 only on site 1 on trees from four index class types: 0 (black spruce × black spruce), 0.5 (0.5 × 0.5; putative F_2), 0.5 (1.0 × 0.0 and 0.0 × 1.0; putative F_1), and 1.0 (red spruce × red spruce). Again, three families were measured per cross type. Measurements were made over a total of nine dates in July 1996. On each individual date, trees were only measured from one block and three dates were measured per block. A different individual tree was measured for each index class type – family – block – date combination. Each individual tree was measured four times on each date during the approximate time intervals listed above. Gas exchange was again measured on 1-year-old foliage from detached branches but using a LI-6400 portable photosynthesis system using a 6400-02 LED Light Source (LI-COR Inc., Lincoln, Neb.) set at 2000 $\mu mol \cdot m^{-2} \cdot s^{-1}$ photosynthetically active radiation.

The same subset of families used for 1994 gas exchange measurements were reassessed using a modification of the original hybrid index (Manley 1971) to examine how the current phenotypes conformed with expectation. One branch, from the upper third of the crown, was assessed for needle color, needle configuration, twig ridges, twig bark color, and vegetative bud color. When available, cones were assessed for morphology of cone scale edges. One-year-old foliage and branches were used for evaluation. Ratings were made as described by Manley (1971). Individual tree hybrid indices ranged from 0 (pure black spruce) to 1.00 (pure red spruce).

Expected hybrid index and site effects on height were examined using the analysis of covariance model:

$$[1] \qquad Y_{ij} = B_0 + B_{0i} + B_1X_{ij} + B_{1i}X_{ij},$$

where Y_{ij} is the height (or diameter) of the *j*th tree of site *i*, B_0 and B_1 are average regression coefficients, B_{0i} and B_{1i} are site coefficients, and X_{ij} is the expected hybrid index based on the original crosses. In this analysis, three sources of variation are identified: (*i*) expected hybrid index (the covariate), (*ii*) site, and (*iii*) the site × expected hybrid index interaction. The last indicates whether effects of expected hybrid index were consistent or variable between sites. In addition, data from each site were fitted with second order polynomial regressions.

The frequencies of plots occupied with living trees (as affected by initial missing plots at planting plus subsequent mortality), by hybrid index class, were examined using two-way cross-tabulation tables. Chi-square analysis, by site, was used to test for the dependence of percent occupied plots on hybrid index class.

Graphical analysis indicated no linear relationship between expected hybrid index and gas exchange traits. Gas exchange traits were analyzed using analysis of variance (ANOVA) using a randomized complete block design. For 1994 data, block and day were con-

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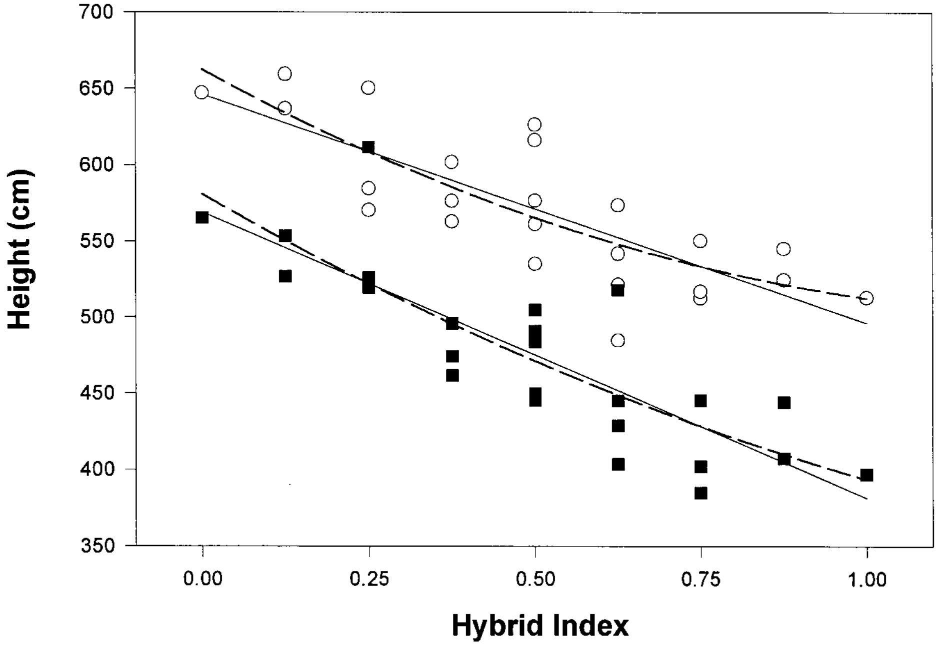
Fig. 1. Relationship between tree height and expected hybrid index (based on midparent means) for 22-year-old trees grown on site 1 (open circles) and site 2 (solid squares). Hybrid index 0 represents pure black spruce and hybrid index 1.00 represents pure red spruce with a continuum between. Linear regression r^2 values are 0.63 for site 1 and 0.68 for site 2. Second-order polynomials, by site, are also displayed. Note that, for clarity, family means are used for graphic representation, while individual tree data were used for analysis of covariance (see text).



founded, while for 1996 data, day was nested within block (three dates per block). Expected hybrid index and site were main effects and family was nested within expected hybrid index. All effects were considered fixed. The relationship between individual tree expected hybrid index and the phenotypically realized hybrid index was examined graphically and using linear regression.

Results

Analysis of covariance and graphical analysis (Fig. 1) indicate that tree height declined with increasing hybrid index values ($p = 0.0001$). Trees on site 1 generally were taller than site 2 trees ($p = 0.0001$). There was no interaction detected ($p = 0.1222$); the effect of expected hybrid index was the same on both slopes, as witnessed by parallel slopes for each site (Fig. 1). Results of the analysis of covariance of tree diameter resulted in the same regression trends and statistical significance as that for tree height. Second-order polynomials were also fitted to data from each site (Fig. 1) indicating a similar, but minor, non-linearity in the relationship between tree height and hybrid index on both sites.

The percentage of plots occupied by living trees was 80% for site 1 and 83% for site 2. There were no differences among hybrid index classes in mortality; chi-squared analysis indicated no dependence on the frequency of occupied sites on hybrid index class on site 1 ($p = 0.414$) or site 2 ($p = 0.949$).

In 1994, across all families, site 1 trees had higher rates of P_n expressed on a leaf area basis (Table 1). However, site 1 trees also had thicker leaves, as indicated by lower specific leaf area (SLA), and so, mean P_n expressed on a leaf weight basis was almost identical between sites (Table 1). Site differences in g_{wv} showed the same pattern as P_n (Table 1). There were no site differences detected in R_d (Table 1).

Table 1. Site means, with SE given in parentheses, of net photosynthesis expressed on a leaf area basis (P_n (LA)) and a leaf weight basis (P_n (DW)), dark respiration (R_d), needle conductance (g_{wv}), and specific leaf area (SLA) from 22-year-old trees measured in 1994.

		Site	
Trait	*p*	1	2
P_n (LA) (μmol·m^{-2}·s^{-1})	0.0015	1.82 (0.050)	1.66 (0.040)
P_n (DW) (μmol·g^{-1}·s^{-1})	0.7671	0.028 (0.001)	0.028 (0.001)
R_d (μmol·m^{-2}·s^{-1})	0.5727	0.25 (0.012)	0.26 (0.014)
g_{wv} (μmol·m^{-2}·s^{-1})	0.0006	65.55 (2.83)	56.91 (1.90)
SLA (cm^2·g^{-1})	0.0001	154.34 (1.68)	172.8 (3.35)

Note: Means were calculated across all five hybrid index types.

In 1994, differences among hybrid index classes in P_n, expressed on a leaf area basis, approached statistical significance (Table 2). The largest differences among P_n (leaf area basis) means was between pure black (hybrid index class 0) and pure red spruce (hybrid index class 1). Again, this difference in P_n expressed on a leaf area basis appears to be due to differences in SLA, which decreased with increasing hybrid index, and no genetic differences were apparent in P_n expressed on a leaf weight basis (Table 2). Hybrid index class was not a significant source of variation in any other gas exchange trait. The hybrid index class × site interaction was not a significant source of variation for any gas exchange trait.

In 1996, there were statistically significant differences in

Table 2. Means, with SE given in parentheses, of net photosynthesis expressed on a leaf area basis (P_n (LA)) and a leaf weight basis (P_n (DW)), dark respiration (R_d), needle conductance (g_{wv}), and specific leaf area (SLA) from 22-year-old trees measured in 1994 from five hybrid index classes ranging from pure black spruce (hybrid index class 0) to pure red spruce (hybrid index class 1).

		Hybrid index class				
Trait	*p*	0	0.25	0.50	0.75	1.00
P_n (LA) ($\mu mol \cdot m^{-2} \cdot s^{-1}$)	0.0628	1.66 (0.075)	1.77 (0.074)	1.67 (0.071)	1.70 (0.075)	1.87 (0.068)
P_n (DW) ($\mu mol \cdot g^{-1} \cdot s^{-1}$)	0.3804	0.028 (0.001)	0.029 (0.001)	0.028 (0.001)	0.027 (0.001)	0.029 (0.001)
*R*d ($\mu mol \cdot m^{-2} \cdot s^{-1}$)	0.5692	0.25 (0.019)	0.25 (0.025)	0.25 (0.017)	0.27 (0.024)	0.28 (0.019)
g_{wv} ($\mu mol \cdot m^{-2} \cdot s^{-1}$)	0.2928	57.65 (3.38)	59.75 (3.67)	60.63 (5.08)	62.00 (3.92)	66.17 (3.55)
SLA ($cm^2 \cdot g^{-1}$)	0.0124	170.26 (4.12)	165.68 (3.99)	165.82 (5.30)	159.38 (5.21)	156.72 (4.92)

Table 3. Means, with SE given in parentheses, of net photosynthesis expressed on a leaf area basis (P_n (LA)) and a leaf weight basis (P_n (DW)), dark respiration (R_d), needle conductance (g_{wv}), and specific leaf area (SLA) from 24-year-old trees from black spruce (hybrid index class 0), red spruce (hybrid index class 1), putative F_1 hybrids, and putative F_2 hybrids measured in 1996.

		Hybrid index class			
			0.50		
Trait	*p*	0	Putative F_1	Putative F_2	1.00
P_n (LA) ($\mu mol \cdot m^{-2} \cdot s^{-1}$)	0.0003	1.18 (0.050)	1.322 (0.044)	1.358 (0.050)	1.334 (0.043)
P_n (DW) ($\mu mol \cdot g^{-1} \cdot s^{-1}$)	0.0155	0.031 (0.001)	0.033 (0.001)	0.035 (0.001)	0.032 (0.001)
R_d ($\mu mol \cdot m^{-2} \cdot s^{-1}$)	0.8093	0.133 (0.012)	0.148 (0.011)	0.152 (0.012)	0.152 (0.013)
g_{wv} ($\mu mol \cdot m^{-2} \cdot s^{-1}$)	0.0002	18.33 (1.04)	22.48 (1.32)	23.94 (1.37)	24.05 (0.92)
SLA ($cm^2 \cdot g^{-1}$)	0.0420	266.06 (9.53)	251.78 (5.74)	259.04 (9.96)	244.32 (7.14)

P_n among hybrid index class types; black spruce P_n, expressed on a leaf area basis, was approximately 12% lower than the hybrids and red spruce (Table 3). Again, SLA increased with hybrid index, and so, P_n on a unit weight basis was similar between pure black and pure red spruces. However, both putative F_1 and F_2 hybrids had approximately 8% higher P_n, expressed on a unit weight basis, than the two pure species. Unlike 1994, in 1996 there were statistically significant differences in needle conductance among hybrid index class types with g_{wv} increasing with hybrid index class.

Hybrid indices of 22-year-old trees generally conformed to expectation (Fig. 2) based on the original crosses. The relationship between expected hybrid index and phenotypic hybrid index was linear and strong on site 1 (Fig. 2A). On site 2, the same general relationship was found, but there was considerably more variability (Fig. 2B).

Discussion

Height growth patterns of 22-year-old trees indicate that genetic variation in height of this artificially produced population is additive. Tree growth rate declined linearly with increasing proportion of red spruce germplasm (increasing hybrid index). This is in contrast to expectations based on observed negative heterosis in photosynthetic traits (Manley and Ledig 1979), and for seedling growth (Manley 1975), conducted with other subsets of the same full-sib families studied here. Although these results do not support the existence of negative heterosis, they are in agreement with Morgenstern et al. (1981), Khalil (1987), and Fowler et al. (1988) who concluded that the fastest growing provenances in red spruce common garden studies were ones introgressed with black spruce.

Gas exchange results also do not indicate inferiority of red × black spruce hybrids. In their study, Manley and Ledig (1979) used 3-month-old greenhouse-grown seedlings and subjected them to an array of light and temperature treatments in a Plexiglas cuvette. Across all years and experimental conditions, Manley and Ledig (1979) observed that F_1 hybrids had P_n rates at least 30% lower than pure red and (or) black spruce seedlings. In 1994, in our study of mature trees, rates of P_n of hybrids were intermediate (leaf area basis) or the same as (leaf weight basis) those measured on black and red spruce. Similar field gas exchange sampling protocols, as used in the study presented here, have been successful in detecting small (5–10%) but consistent differences in photosynthesis among full-sib black spruce families (Johnsen and Major 1995; Major and Johnsen 1996) and larger differences among diverse provenances of black spruce (Johnsen et al. 1996); these trees were all of similar age to the trees studied here. In 1996, P_n results actually indicate a slight positive heterosis, in direct contrast with the clear hybrid inferiority that Manley and Ledig (1979) observed with seedlings of the same families.

Why do mature tree growth and gas exchange results differ from those obtained with seedlings? First, it is possible that some selection has occurred between germination and the mature tree stage in the plantations. If so, this would have happened before plantation establishment, as current plot occupancy is similar among the hybrid index types. Nursery mortality or even nursery culling may have resulted in early losses of unfit individuals. The result would have been an increase in the hybrid height and gas exchange means of the remaining population. It is also possible that hybrid inferiority might only occur in a transitory, seedling stage. Such maturation effects in photosynthetic traits have been inferred elsewhere (Hutchinson et al. 1990; Sandquist et al. 1993).

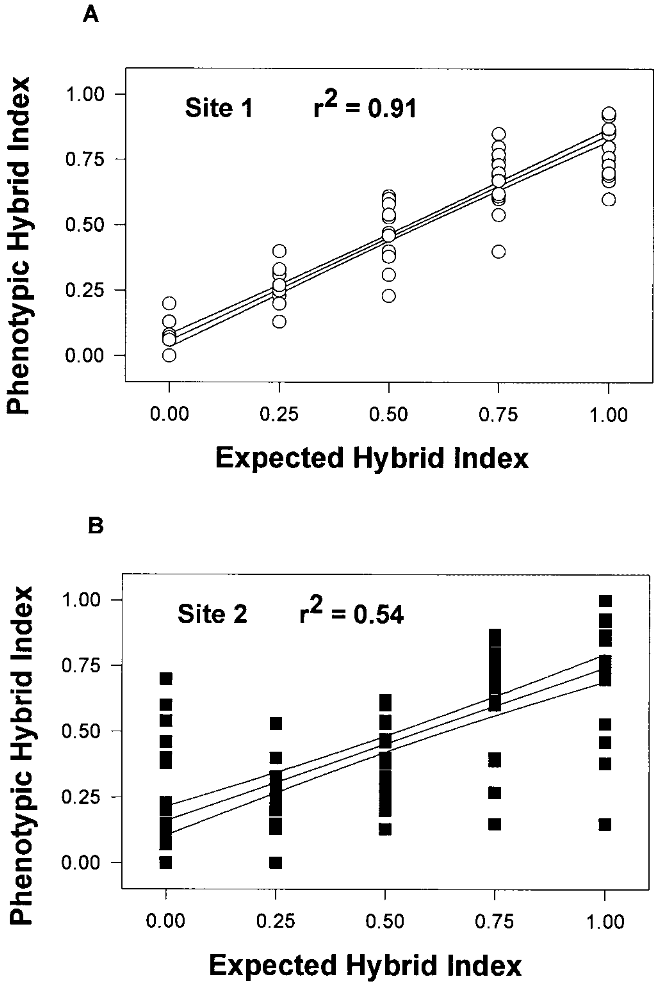
In both study years, red spruce foliage had a lower SLA than black spruce foliage. Differences in SLA can be due to many reasons including differences in starch and secondary compounds or anatomical differences (Lambers and Poorter 1992). In this study, red spruce had higher P_n than black spruce when expressed on a leaf area basis, but P_n rates were nearly identical when expressed on a leaf weight basis. Thus, it appears the lower SLA of red spruce, in this common garden experiment, was due to structural differences related to differences in photosynthetic tissue. SLA is often a plastic trait. For example, leaves that have developed in the understorey generally have a higher SLA than sun leaves (Kozlowski et al. 1991). Most species comparisons have been made in situ, which confounds environmental and genetic effects. However, Gower et al. (1993), in a study of five species using ≥25-year-old trees in a common garden study, reported large species variation in SLA, which was inversely related to leaf longevity. We are unaware of reports on the relative leaf longevity of red and black spruce.

In addition, sun species have been reported to have higher rates of P_n (at high light levels) than shade species (Bazzaz 1979). Red and black spruce differ greatly in their shade tolerance (Manley and Ledig 1979), but we observed no differences between the two species in mean P_n measured at saturated light (leaf weight basis). It is likely that differences in gas exchange would have been detected if gas exchange had been examined as a function of a large range in specific abiotic factors, such as light, as discussed above. Other potentially important environmental factors include vapor pressure deficit (Major and Johnsen 1996) and temperature (Vann et al. 1994).

The data clearly indicate that Manley (1975) was successful in producing a continuum of families and individuals ranging from pure black spruce to pure red spruce. Parents were originally selected based on an assortment of morphological characters that did not include tree size. The resulting pattern of tree size of the progeny, however, is well correlated with expected hybrid indices. Interestingly, the slopes of the regression line of height with expected hybrid index are almost identical on both sites indicating no genotype × environment (G × E) interaction. This lack of a G × E interaction result is probably due to both tests being planted in clearcut, open sites, conditions more favorable for the early-successional black spruce than the late-successional red spruce. Red spruce seedlings have been shown to grow faster than black spruce seedlings when each are grown under conditions of low light (Manley 1975). Considering the differences in shade tolerance between the species, growth rank changes may occur over time following crown closure (crown closure is occurring now on site 1).

The re-evaluation of hybrid indices of the progeny, although not in perfect accordance, also substantiates the precision of the initial selections and further validates the hybrid index approach. The relationship between expected hybrid index and phenotypic hybrid index is essentially the relationship between midparent and progeny performance. The slope of this relationship represents an estimate of heritability (Falconer 1981), which was 0.78 on site 1 and 0.58 on site 2. Thus, although no G × E interaction was evident in growth, the morphological distinction between red and black spruce is considerably more blurred on the lower productivity site (2) than on the higher productivity site (1). A close examination of the components of the hybrid index indicate that no one trait is responsible when hybrid index did not agree with expectations due to parentage. Individual tree morphological plasticity appears more strongly expressed on the lower productivity site, making visual distinction between the species more difficult. This again highlights the difficulty in differentiating these species under heterogeneous field conditions (also see Perron and Bousquet 1997).

Fig. 2. Relationship between expected hybrid index based on midparent means and individual progeny phenotypic hybrid index for 22-year-old trees grown on site 1 (A) and site 2 (B). Hybrid index 0 represents pure black spruce and hybrid index 1.00 represents pure red spruce, with a continuum between.



The results presented here have two main implications regarding understanding the status of red spruce, black spruce, and hybrid types across the landscape. First, the hybrid index approach proved generally useful for differentiating genotypes across a black spruce – red spruce continuum. However, as seen particularly on site 2, an individual tree's phenotype does not always correctly reflect its genotype. Hopefully, the continued development (Perron et al. 1995; Bobola et al. 1996*b*) and application of molecular genetic tools, such as microsatellite markers, will produce more reliable estimates of the levels of hybridization and (or) introgression across the landscape. Second, at least with trees past the seedling stage, negative heterosis appears to be of little importance as an isolating barrier between red and black spruce. Crossability and ecological barriers appear more important in maintaining the segregation of the species (Manley 1975; Gordon 1976).

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