

Predominant paternal inheritance pattern of light-energy processing adaptive traits in red and black spruce hybrids

John E. Major, Debby C. Barsi, Alex Mosseler, Om P. Rajora, and Moira Campbell

Abstract: Ecophysiological traits related to light-energy processing and freezing tolerance are important adaptive traits in plants. Our goal was to investigate the pattern of inheritance of these traits in hybrids using controlled intra- and inter-specific crosses of red spruce (*Picea rubens* Sarg.) (RS) and black spruce (*Picea mariana* (Mill.) BSP) (BS). Our initial working hypothesis was that expected hybrid index categories could be a predictor of adaptive traits. Species results of dark-adapted photochemical efficiency (F_v/F_m) and light-adapted light-energy processing traits, quantum yield (YLD), thermal dissipation efficiency (qN), and chlorophyll fluorescence (F_{pc}), were consistent with previously published open-pollinated, species provenance results. Initially, YLD, qN , and F_{pc} , showed an additive inheritance pattern, evident by average hybrid index 50 having a mid-parent value. Because of various crosstypes of the hybrid families, parental analysis, testing male, female, and interaction effects, and having three categories (pure RS, pure BS, and hybrid spruce), revealed significant male and nonsignificant female and interactive effects. Underlying the averaged additive results was a significant species-specific paternal inheritance pattern. Crosses with BS males had 13.7% higher YLD ($P = 0.001$), 15.4% lower qN ($P = 0.008$), and 43.0% higher F_{pc} ($P = 0.096$) than crosses with either RS or hybrid males. F_v/F_m showed a nonadditive or parental species pattern. Parental analysis of F_v/F_m showed significant male, female, and male \times female interaction effects, and further analysis supports a largely species-specific and paternally inherited trait. Freezing tolerance revealed a mixed model of inheritance dominated by species effects. Total dry mass was positively correlated with YLD, and negatively correlated with F_v/F_m and qN , suggesting a biological trade-off. We know of no other studies in trees demonstrating paternal inheritance of ecophysiological processes that affect adaptation and fitness.

Résumé : Les caractères écophysologiques associés à la tolérance au gel et à l'utilisation de la lumière comme source d'énergie sont des caractères adaptatifs importants chez les plantes. Les auteurs ont étudié le patron de transmission génétique de ces caractères chez des hybrides issus de croisements dirigés intraspécifiques et interspécifiques chez l'épinette rouge (*Picea rubens* Sarg.) (ÉR) et l'épinette noire (*Picea mariana* (Mill.) BSP) (ÉN). Selon l'hypothèse initiale de travail, les catégories espérées d'index pour l'identification des hybrides pourraient constituer un prédicteur de la valeur des caractères liés à l'adaptation. Les résultats pour les espèces concernant les caractères reliés à l'efficacité photochimique en l'absence de lumière (F_v/F_m) et à l'utilisation de la lumière comme source d'énergie, le rendement quantique (YLD), l'efficacité de dissipation de la chaleur (qN) et la fluorescence de la chlorophylle (F_{pc}) étaient congruents avec les résultats déjà publiés d'essais de provenances établis à partir de matériel issu de la pollinisation libre pour chaque espèce. Au départ, YLD, qN et F_{pc} affichaient un patron additif de transmission génétique, comme le démontrait l'index moyen d'identification des hybrides 50 qui avait une valeur intermédiaire entre celles des parents. En raison de la diversité des types de croisements ayant permis d'obtenir les familles hybrides, l'analyse parentale en testant les effets mâle, femelle et d'interaction, tout en considérant trois catégories de descendants (ÉR pure, ÉN pure et épinettes hybrides), a révélé des effets significatifs dus au parent mâle et des effets non significatifs dans le cas du parent femelle et de l'interaction. Sous-jacent aux résultats additifs moyens, un patron de transmission génétique paternel significatif spécifique à l'espèce a été remarqué. Les croisements impliquant des parents mâles d'ÉN avaient une valeur de YLD 13,7 % plus élevée ($P = 0,001$), une valeur de qN 15,4 % moins élevée ($P = 0,008$) et une valeur de F_{pc} 43,0 % plus élevée ($P = 0,096$) que celles des croisements impliquant aussi bien l'ÉR que des parents mâles hybrides. La variable F_v/F_m démontrait un patron non additif ou un patron typique des parents représentatifs des espèces. L'analyse parentale de la variable F_v/F_m a révélé qu'il y avait des effets significatifs mâle, femelle et d'interaction mâle \times femelle. Une analyse plus poussée supporte le concept d'un caractère transmis paternellement et largement spécifique à l'espèce. La tolérance au gel était expliquée par un modèle mixte de transmission dominé par les effets des espèces. La masse anhydre totale était corrélée positivement avec YLD et négativement avec F_v/F_m et qN , ce qui pourrait être un indice de compromis biologique. Les auteurs ne relèvent aucune autre

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étude qui mette en évidence chez les arbres un patron de transmission paternel des processus écophysologiques affectant l'adaptation et la santé.

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Introduction

Ecophysiological traits related to light-energy processing and freezing tolerance are important adaptive traits in plants (Lichtenthaler and Rinderle 1988; Schreiber et al. 1994). These traits can vary among and within species (Johnson et al. 1993; Demmig-Adams and Adams 1994; Major et al. 2003a). Red spruce (*Picea rubens* Sarg.) (RS), an important and characteristic component of late-successional forests of eastern Canada and the northeastern United States, has experienced a substantial decline over most of its geographic range. This decline has been associated with clearcut harvesting and shortened rotations (Korstian 1937; Leak and Smith 1996), and climate change (Hamburg and Cogbill 1988). It may also be related to hybridization with black spruce (*Picea mariana* (Mill.) BSP) (BS) (Major et al. 2003c), an early successional species (Viereck and Johnston 1990). Hybridization already confuses species' morphometric identities and complicates decisions on appropriate silvicultural management options for these otherwise ecologically distinct species (Manley 1971; Beyer 1999). Except in the southern part of its range, RS is largely sympatric with BS.

Morphologically (Manley 1971) and genetically (Eckert 1989; Bobola et al. 1992; Perron et al. 1995), RS and BS are similar and cross-compatible species (Gordon 1976; Manley and Ledig 1979). Interspecific crossability (0%–4%) was about seven times lower than that in intraspecific crosses and represents a substantial but imperfect reproductive barrier for maintaining the separation of the species (Major et al. 2005). Red spruce was found to have inherently greater photochemical efficiency and thermal dissipation efficiency, traits associated with shade tolerance, whereas BS was found to have greater quantum yield, aboveground productivity, and shoot:root ratio, traits associated with early successional species (Major et al. 2003a; Major et al. 2003b). Estimates of the extent of natural hybridization and introgression range from extensive (Morgenson and Farrar 1964; Bobola et al. 1996) to minor (Gordon 1976; Manley and Ledig 1979; Mosseler et al. 2000). Reproductive phenology is nearly identical; however, crossability and ecological barriers appear to be the most important in maintaining the separation of sympatric RS and BS (Major et al. 2005).

Very little is known about the inheritance pattern of light-energy processing and freezing-tolerance traits in plants, especially in conifers. Light-energy quenches into the three main components: photosynthesis (quantum yield), heat (thermal dissipation), and chlorophyll fluorescence (Lichtenthaler and Rinderle 1988; Schreiber et al. 1994). Chlorophyll fluorescence and quenching analyses have been used to quantify light-energy processing capabilities (Johnson et al. 1993; Major et al. 2003a) and as an indicator of freeze-induced injury, with results comparable with visible injury ratings (Adams and Perkins 1993; Perkins and

Adams 1995). An earlier study using open-pollinated seed found that RS had inherently higher photochemical efficiency (F_v/F_m), lower quantum yield (YLD), lower chlorophyll fluorescence (F_{pc}), higher thermal dissipation efficiency (qN), and lower freezing tolerance than BS (Major et al. 2003a). Controlled crosses, resulting in five hybrid index categories of various crosstypes and ranging from pure BS to pure RS, were used in this and a previous study that looked at biomass allocation (Major et al. 2003c). Our initial working hypothesis was that expected hybrid index could be a predictor of these adaptive traits as was the case for biomass allocation. Because of various crosstypes of the hybrid families, parental analysis, testing male, female, and interaction effects, and having three categories (pure RS, pure BS, and hybrid spruce), as were used in Major et al. (2003c, 2007), were also performed in this study. A recent study using the same controlled hybrid crosses grown under ambient and elevated CO₂ found that chloroplast pigment content showed significant male effect and nonsignificant female, and male × female interaction effect for chlorophyll content (Major et al. 2007). Our study objectives were to (i) investigate the inheritance pattern of light-energy processing and freezing-tolerance traits in hybrids of RS and BS using intra- and inter-specific controlled crosses of RS and BS by examining patterns by hybrid index and parental pedigree; and (ii) examine the relationship of key light-energy processing traits to morphometric traits.

Materials and methods

Original genetic material

Using a hybrid index system based on 19 leaf, twig, cone, and crown characteristics, parent trees were selected by Manley (1971, 1975) that approximated hybrid indices of 0, 25, 50, 75, and 100. The hybrid index represents the percentage of RS component estimated in the parent tree, with 0 denoting pure BS and 100 denoting pure RS. At least three different sets of parent trees were selected for controlled crosses performed over 3 years. These trees were located on or near the Acadia Research Forest, New Brunswick, Canada (46°N, 66°15'W).

Experimental genetic material

A series of controlled crosses using various parents were made to produce seedlots with expected hybrid indices: 0, 25, 50, 75, and 100, as described by Manley and Ledig (1979). Seeds stored from these crosses at the Atlantic Forestry Centre were used. Three crosses for each hybrid index (0, 25, 50, 75, and 100) for a total of 14 seedlots were used (one hybrid index 75 cross was missing because of low numbers) (Table 1).

Growth environment and morphological measurements

Seeds were sown in hydrated 36 mm Jiffy™ peat pellets (Jiffy Products (NB) Ltd.) (90 mL full volume) at the begin-

Table 1. Parentage and accession numbers of hybrid families.

| Hybrid crosstypes | Accession No. | ♀ parent × ♂ parent |
|-------------------------|-------------------|---------------------|
| Hybrid index 0 | | |
| 0-2 | 17-71 × 199-71 | 0 × 0 |
| 0-3 | 1-69 × BSM-69 | 0 × 0 |
| 0-4 | 49-71 × BSM-71 | 0 × 0 |
| Hybrid index 25 | | |
| 25-1 | 5-72 × 15-72 | 0 × 50 |
| 25-2 | 11-71 × 17-71 | 50 × 0 |
| 25-3 | 1035-72 × BSM-72 | 50 × 0 |
| Hybrid index 50 | | |
| 50-2 | BS3-9 × RS4-69 | 0 × 100 |
| 50-3 | 10-72 × BSM-72 | 100 × 0 |
| 50-4 | BS49-71 × RSM-71 | 0 × 100 |
| Hybrid index 75 | | |
| 75-1 | 23-71 × 25-71 | 75 × 75 |
| 75-2 | 52-71 × 11-71 | 100 × 50 |
| Hybrid index 100 | | |
| 100-1 | 1063-72 × 1064-72 | 100 × 100 |
| 100-3 | RS5-68 × RSM-68 | 100 × 100 |
| 100-5 | 1065-74 × RSM-74 | 100 × 100 |

Note: Hybrid index: 0, 0% red spruce; 25, 25% red spruce; 50, 50% red spruce; 75, 75% red spruce; 100, 100% red spruce, with the remaining proportion black spruce.

ning of June 1998. Each full-sib family was sown in two replicate trays containing 84 Jiffy peat pots per replicate, and the trays were placed randomly in a greenhouse compartment at the Canadian Forest Service - Atlantic Forestry Centre in Fredericton, New Brunswick. Seedlings were watered and fertilized twice weekly from 11 to 29 June with 11:41:8 (N:P:K) plus micronutrients at 50 ppm N (Plant Products Co. Ltd., Brampton, Ont.); from 1 July to 7 September with 20:8:20 (N:P:K) at 100 ppm N two or three times weekly; and from September 15 to October 28 with 8:20:30 (N:P:K) at 35 ppm N twice weekly. Temperature and relative humidity (RH) were set at 26 °C and 70%, respectively, from 1 to 29 June; 25 °C:18 °C and 60%:50% RH (day:night) from 1 July to 30 September; and 10 °C:5 °C to 30 November. Natural light was supplemented by sodium vapor lamps, with a 16 h light:8 h dark photoperiod starting from 8 September. Bud initiation began 18 September.

Seed germination averaged between 70% and 88%. At the beginning of December 1998 (day 191 after sowing), 10–20 seedlings from each seedlot (depending on seedling numbers) were randomly selected and prepared for dry mass measurements. Detailed seedling morphological data and methodology are described in Major et al. (2003c).

Low temperature exposure

Chlorophyll fluorescence measurements and low temperature exposure protocols followed that of Major et al. (2003a), and were made between 18 and 22 December 1998. Four seedlings were randomly selected from each of the 14 families. Intact needles were removed from the mid-

dle of the leader. Two sets of six needles were placed on clear adhesive tape oriented in the same direction and spaced evenly. One set was placed in one of two bags labeled for temperature exposure set at 5 °C (control), –20 °C, and –40 °C. The 5 °C set was measured immediately and was also used for the –20 °C temperature test.

All samples were placed immediately in a darkened growth chamber (Convion PG88, Winnipeg, Man.) set for 5 °C. A Superline –70 °C programmable freezer (Superline Refrigeration, Toronto, Ont.) equipped with an Omega temperature controller (Series CN-2010, Stamford, Conn.) was used to expose plant material to lower temperatures. The air temperature was monitored with a Campbell Scientific CR10 data logger (Edmonton, Alta.) and thermocouple probe for accuracy. The freezer was programmed to remain at 5 °C until early morning before automatic temperature ramping. Freezer air temperature was lowered at a rate of 5 °C/h to –20 °C and remained at –20 °C for 30 min. Samples were removed at the end of the 30 min and placed in the growth chamber set at 5 °C. The freezer was programmed to resume ramping until –40 °C and remained at –40 °C for 30 min before samples were removed and placed in a darkened 5 °C growth chamber for a period of 30 min to 1 h.

Chlorophyll fluorescence measurements

The three major deexcitation processes of absorbed light energy (E_{absorbed}) are indicated in the following equation (Lichtenthaler and Rinderle 1988):

$$[1] \quad E_{\text{absorbed}} = E_{\text{photochemistry}} + E_{\text{heat}} + E_{\text{fluorescence}}$$

Chlorophyll fluorescence was measured with a pulse amplitude modulated fluorometer (PAM-2000, Walz, Effeltrich, Germany). Thirty minutes before chlorophyll fluorescence measurements, needle samples were placed in a darkened growth chamber set at 20 °C. After the initial measurement of fluorescence (F_o , dark-adapted minimum fluorescence), needles were exposed to a saturating light ($>3000 \mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$) for 0.8 s to measure the maximal fluorescence of dark-adapted foliage (F_m). Dark-adapted photochemical efficiency ($F_v/F_m = (F_m - F_o)/F_m$), and variable fluorescence ($F_v = F_m - F_o$) were determined. Needle samples were then exposed to a continual actinic light level of $100 \mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ and exposed to saturating light every 20 s and light-adapted fluorescent traits were then measured. F_m' is maximal fluorescence when light-adapted foliage is exposed to a pulse of saturating light, and F_t is the fluorescence yield in actinic light. F_v' is the light-adapted variable fluorescence ($F_v' = F_m' - F_o'$), where F_o' is fluorescence after far-red light exposure (Schreiber et al. 1994). After 4 min of actinic light exposure with 20 s intervals of saturating light flashes, fluorescence, effective quantum yield, and quenching measurements had adjusted to the actinic light and reached a steady state. Quantum yield (YLD) of photosystem II was determined ($\text{YLD} = (F_m' - F_o')/F_m'$), and nonphotochemical quenching (qN) was calculated $qN = (F_m - F_m')/(F_m - F_o')$ according to Schreiber et al. (1994). Because needles could be of varying sizes, a relative measure of fluorescence was also required. Chlorophyll fluorescence was calculated as a per-

Table 2. Number of families in parental effect analysis.

| ♀ parent | ♂ parent | | | Total |
|--------------|--------------|--------|------------|-------|
| | Black spruce | Hybrid | Red spruce | |
| Black spruce | 3 | 1 | 2 | 6 |
| Hybrid | 2 | 1 | 0 | 3 |
| Red spruce | 1 | 1 | 3 | 5 |
| Total | 6 | 3 | 5 | 14 |

Note: Four replicates were measured for each family.

centage of total variable fluorescence ($F_{pc} = (F_{vfl}/F_{vall}) \times 100$) where F_{vfl} is $F_t - F_o'$ and F_{vall} is $F_m - F_o'$. Percent thermal dissipation efficiency was calculated (qN (%) = $qN \times 100$). Relative fluorescence after low-temperature exposure (LTE, -20°C and -40°C) was calculated $((F_t(\text{LTE}) - F_t(\text{control}))/F_t(\text{control}) \times 100)$ as in Major et al. (2003c). YLD, qN , and F_{pc} were used as the light-adapted relative measures for photochemistry, thermal dissipation, and fluorescence, respectively.

Statistical analyses

The data were normally distributed and subjected to analyses of variance (ANOVA). Hybrid index and family effects were considered fixed. Families were nested within hybrid index. The ANOVA model used was

$$[2] \quad Y_{ijk} = \mu + H_i + F_{j(i)} + e_{ijk}$$

where Y_{ijk} is the dependent seedling trait of the i th hybrid index of the j th family of the k th seedling, μ is the overall mean, H_i is the effect of the i th hybrid index ($i = 1-5$), $F_{j(i)}$ is the effect of the j th family nested within a i th hybrid index ($j = 1-3$), and e_{ijk} is the random error component. Differences between hybrid indices were determined using Tukey's mean separation test ($P \leq 0.05$).

Because of the various crosstypes of the hybrid families, parental analysis was also performed on the hybrid experimental data according to Major et al. (2003c, 2007). Female and male parents were grouped into three main parental classified categories for analysis: pure BS, pure RS, and hybrid spruce (includes hybrid indices 25, 50, and 75) (Table 2). Female and male categories were considered fixed. The ANOVA model used was

$$[3] \quad Y_{ijk} = \mu + F_i + M_j + FM_{ij} + e_{ijk}$$

where Y_{ijk} is the dependent seedling trait of the i th female, and j th male of the k th seedling, μ is the overall mean, F_i is the effect of the i th female category ($i = 1-3$), M_j is the effect of the j th male category ($j = 1-3$), FM_{ij} is the interaction effect of i th female and j th male categories, and e_{ijk} is the random error component. A male \times female interactive effect could not be perfectly calculated because of one missing cell combination, female hybrid \times male RS (Table 2); thus, the female \times male interaction had one less degree of freedom (4 to 3 df) and a type III ANOVA analysis was used. In describing the crosstypes (e.g., BS \times RS), the female parent always precedes the male parent.

A Tukey's mean separation test ($P \leq 0.05$) was used to determine significant differences between specific parental effects. Simple correlations were used to determine relation-

ships between key light-energy processing traits and seedling dry mass.

Results

Light-energy processing, hybrid index

Photochemical efficiency (F_v/F_m) had a significant hybrid index effect ($P < 0.001$) that was a two-tiered stratification among hybrid indexed seedlings, and had among the highest R^2 (0.721) of all the traits examined (Fig. 1A; Table 3). Seedlings with hybrid indices of 100, 75, and 50 had significantly higher F_v/F_m than seedlings with hybrid indices of 0 and 25 ($P < 0.001$). Light-adapted, light-energy processing traits, such as YLD, qN , and F_{pc} , appear on the surface to have an additive inheritance evident by hybrid 50 being always in the middle of the two species (Figs. 1B, 1C, and 1D). The highest to lowest ranking for YLD was as follows: hybrid indices 25, 0, 50, 100, and 75, ranging from 0.445 to 0.396 ($P = 0.025$) (Fig. 1B). Thermal dissipation efficiency ranged from 70.8% for BS (hybrid index 0) to 81.9% for RS (hybrid index 100) ($P = 0.001$) (Fig. 1C). Chlorophyll fluorescence ranged from 6.45% for BS to 4.2% for RS ($P = 0.020$) (Fig. 1D).

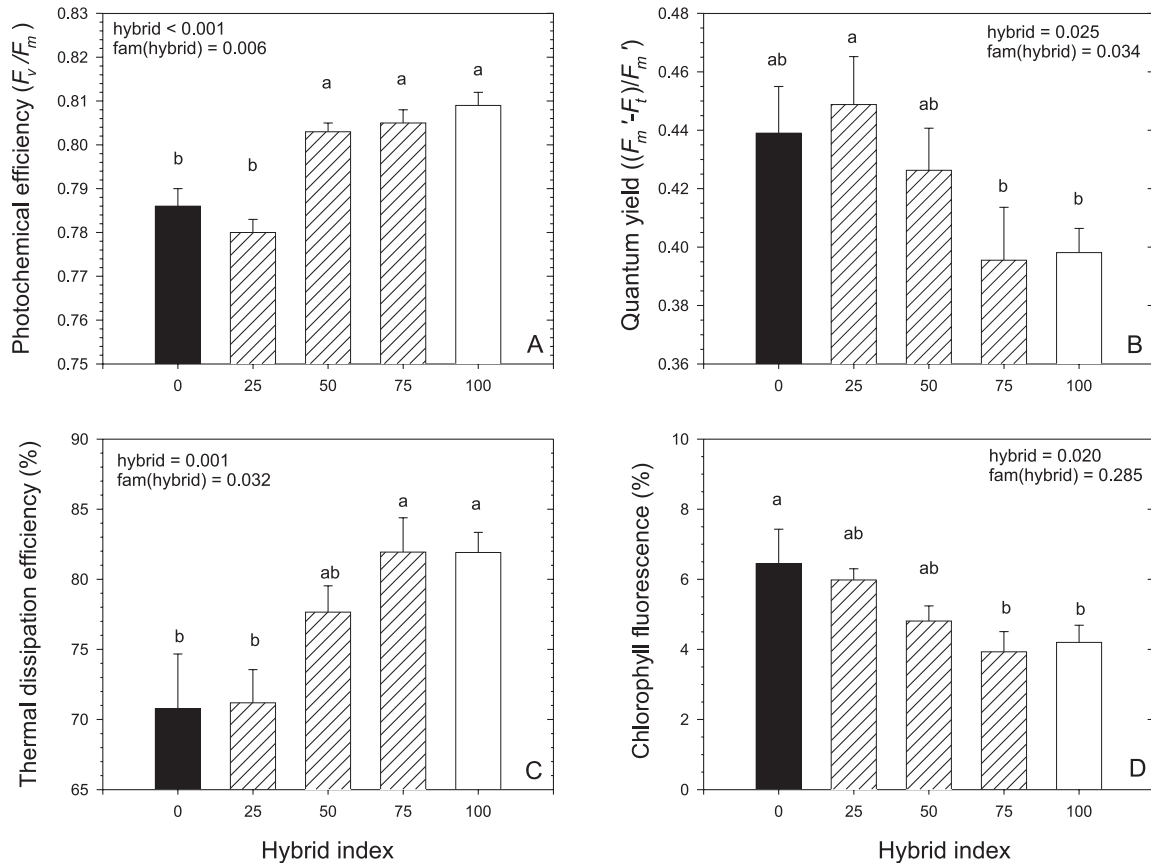
Light-energy processing, parental analyses

The ANOVA of YLD by parental effect showed a significant male effect ($P = 0.001$) (Fig. 2A), a nonsignificant female effect ($P = 0.949$) (Fig. 2B), and nonsignificant male \times female interaction ($P = 0.771$) (Table 4). Further analysis by male parent revealed that a cross with a BS male had 13.7% higher YLD ($P = 0.001$) than crosses with either RS or hybrid males (Fig. 2A). Analysis of male parents crossed with different female parents revealed (i) with BS as male, there was no significant female parent effect ($P = 0.338$) and the mean YLD was 0.455 (Fig. 2C); (ii) with hybrid as male, there was no significant female parent effect ($P = 0.811$) (Fig. 2D) and the mean YLD was 0.400; and (iii) with RS as male, crosses with BS or RS had no effect ($P = 0.773$) (Fig. 2E) and the mean YLD was 0.400.

The ANOVA of qN by parental effect showed a significant male effect ($P = 0.008$), nonsignificant female effect ($P = 0.331$), and nonsignificant male \times female interaction ($P = 0.914$) (Table 4). Overall analysis by male parent effect revealed that a cross with BS as a male parent resulted in a 15.4% lower qN ($P = 0.006$) than a cross with either RS or hybrid male (not shown). Analysis of male parents crossed with different female parents revealed (i) with BS as male, there was no significant female parent effect ($P = 0.802$) and the mean qN value was 0.701; (ii) with hybrid as male, there was no significant female parent effect ($P = 0.538$) and the mean qN value was 0.804; and (iii) with RS male, there was no significant female parent effect ($P = 0.452$) and the mean qN value was 0.813.

The ANOVA of F_{pc} by parental effect showed a significant male effect ($P = 0.096$), nonsignificant female effect ($P = 0.440$), and nonsignificant male \times female interaction ($P = 0.770$) (Table 4). Overall analysis by male parent effect revealed that a cross with BS as a male parent resulted in a 42% greater F_{pc} ($P = 0.006$) than a cross with either RS or hybrid male (not shown). Analysis of male parents crossed with different female parents revealed (i) with BS as male,

Fig. 1. Mean light-energy processing traits results by hybrid index: (A) dark-adapted photochemical efficiency (F_v/F_m), (B) quantum yield (YLD; $(F_m' - F_t)/F_m'$), (C) thermal dissipation efficiency (qN), and (D) chlorophyll fluorescence. The hybrid index values are the percentages of RS complement, with the remaining proportion BS. Indices 0 and 100 represent BS and RS, respectively. Source of variation and P values are from Table 3. Error bars are SEs. Bars with different letters are significantly different for the trait (Tukey's mean separation test, $P \leq 0.05$).



there was no significant female parent effect ($P = 0.696$) and the mean F_{pc} value was 6.22%; (ii) with hybrid as male, there was no significant female parent effect ($P = 0.419$) and the mean F_{pc} value was 4.35%; and (iii) with RS male, there was no significant female parent effect ($P = 0.568$) and the mean F_{pc} value was 4.36%.

Parental effect ANOVA for F_v/F_m showed significant male effect ($P = 0.035$), female effect ($P < 0.001$), and female \times male interaction ($P = 0.002$) (Table 4). Overall analysis of male parent effect showed that BS males had the lowest F_v/F_m , followed by hybrid males, which were significantly lower than RS male parents (Fig. 3A). Overall analysis of female parent effect showed a significantly higher F_v/F_m for RS compared with hybrid and BS females (Fig. 3B). Male and female interactions and analysis of each male parent crossed by female parents revealed (i) with BS as male, there was no female effect ($P = 0.128$) (Fig. 3C); (ii) with hybrid as male, BS female crosses had the lowest F_v/F_m compared with hybrid \times hybrid and RS \times hybrid crosses ($P < 0.001$) (Fig. 3D); and (iii) with RS as male, there was no female effect ($P = 0.438$) (Fig. 3E).

The F_v/F_m distribution is shown by parentage (Fig. 4). Of the three F_1 crosses (crosses with both RS and BS parents), two crosses had a RS male parent (50-2 and 50-4) (Table 1). These had significantly greater F_v/F_m than the cross with a BS male parent (50-3) ($P = 0.006$) (Fig. 4). The hybrid fe-

male \times BS male is made up of two crosses (25-2 and 25-3; both 50×0), and their responses were similar to those of BS \times BS crosses. The F_v/F_m of all non-BS \times BS crosses was not significantly different from the BS \times BS crosses ($P = 0.852$) (Fig. 4). The F_v/F_m of all non-RS \times RS crosses was not significantly different from the RS \times RS crosses ($P = 0.438$). The other parent \times hybrid cross, 25-1 segregated with the BS males, and 75-1 and 75-2 crosses segregated with the RS males.

Low temperature exposure, hybrid index

Relative fluorescence at -20°C exposure had a nonsignificant hybrid index effect ($P = 0.245$, not shown). Relative fluorescence at -40°C exposure had a significant hybrid index effect ($P < 0.001$) (Table 3). Hybrid indices 0, 25, and 50 had approximately 100% increase in relative fluorescence and were significantly lower than hybrid indices 75 and 100, which had an approximately 200% increase in relative fluorescence (not shown).

Low temperature exposure, parental analyses

Low temperature exposure (-40°C) relative fluorescence analysis resulted in significant male effect ($P = 0.003$), female effect ($P = 0.004$), and interaction ($P = 0.0011$) ($R^2 = 0.467$) (Table 4). Overall analysis of male parents showed that BS had significantly lower relative fluorescence com-

Table 3. ANOVA by hybrid index effect including source of variation (df), mean square values (MS), and P values for photochemical efficiency (F_v/F_m), quantum yield (YLD), thermal dissipation efficiency (qN), chlorophyll fluorescence (F_{pc}), and relative fluorescence at -40°C .

| Source of variation | df | F_v/F_m | | | YLD | | | qN | | | F_{pc} | | | Relative $F_t (-40^\circ\text{C})$ | | |
|---------------------|----|------------------------|--------|--|-----------------------|-------|--|------------------------|-------|--|----------|-------|--|------------------------------------|--------|--|
| | | MS | P | | MS | P | | MS | P | | MS | P | | MS | P | |
| Hybrid | 4 | 19.63 $\times 10^{-4}$ | <0.001 | | 6.19 $\times 10^{-3}$ | 0.025 | | 33.42 $\times 10^{-3}$ | 0.001 | | 13.23 | 0.020 | | 18.09 $\times 10^3$ | <0.001 | |
| Family (hybrid) | 9 | 2.37 $\times 10^{-4}$ | 0.006 | | 4.53 $\times 10^{-3}$ | 0.034 | | 14.06 $\times 10^{-3}$ | 0.032 | | 5.09 | 0.285 | | 6.42 $\times 10^3$ | 0.007 | |
| Error | 42 | 0.76 $\times 10^{-4}$ | | | 1.98 $\times 10^{-3}$ | | | 6.07 $\times 10^{-3}$ | | | 4.02 | | | 2.14 $\times 10^3$ | | |

pared with hybrid or RS, with values of 102%, 166%, and 155%, respectively (Fig. 5A). Overall analysis of female parents resulted in the following ranking from highest to lowest: RS, hybrid, and BS, with values of 173%, 130%, and 105%, respectively (Fig. 5B). Analysis of male parents crossed with different female parents revealed (i) with BS as male, no significant impact of female parent type on relative fluorescence ($P = 0.709$) (Fig. 5C); (ii) with hybrid as male, BS female crosses had lower relative fluorescence compared with hybrid \times hybrid and RS \times hybrid crosses ($P = 0.005$) (Fig. 5D); and (iii) with RS as male, BS female crosses had the lowest relative fluorescence, and RS female crosses had the highest relative fluorescence ($P = 0.033$) (Fig. 5E).

Chlorophyll fluorescence traits in relation to morphometric traits

Photochemical efficiency was negatively correlated to total dry mass ($P = 0.036$, $r = -0.902$) (Fig. 6A). The YLD was positively correlated to total dry mass and had the highest r value ($P = 0.003$, $r = 0.980$) (Fig. 6B). Thermal dissipation efficiency was negatively correlated to total dry mass ($P = 0.029$, $r = -0.915$) (Fig. 6C). The YLD was significantly and positively correlated to other aboveground biomass measures, including shoot, needle, and wood dry mass, and diameter but not height. Chlorophyll fluorescence was negatively and marginally significant in its relation to total dry mass ($P = 0.068$) (not shown). No light-energy processing traits were significantly correlated with root dry mass.

Discussion

Physiological response

The ratio F_v/F_m is a measure of photochemical efficiency of the photosystem II reaction center and the higher the F_v/F_m , the more efficient the plant is at trapping light energy in the pigment bed of photosystem II (Maxwell 1995). This trait is thought to enhance the ability of shade-tolerant species to trap light energy from periodic sun flecks under a forest canopy. The consistently higher F_v/F_m for RS compared with BS was consistent with open-pollinated species response and was not caused by stress or dormancy, as deduced in Major et al. (2003a).

Greater qN serves to dissipate excess light excitation energy in a photoprotective manner and measures the efficiency of heat dissipation at the photosystem II center (Maxwell and Johnson 2000). This trait was consistently lower for BS compared with RS across open-pollinated seed sources (Major et al. 2003a). The need for this photosynthetic control is to limit the deleterious effects caused by the photosynthetic apparatus absorbing more light than can be readily dissipated through photosynthesis (Maxwell 1995). This would appear to be a consistently desirable trait for shade-tolerant species adapted to late-successional ecology such as RS.

Although fluorescence is not the primary parameter of light-energy processing, it is an integral part and an alternative way of handling excess energy. Across RS and BS seed sources, there was a positive relationship between YLD and F_{pc} ($r = 0.757$), which was a consequence of a positive rela-

Fig. 2. Black, red, and hybrid spruce mean quantum yield (YLD) by parental analyses: (A) overall male effect, (B) overall female effect, (C) BS male, female effect, (D) hybrid spruce male, female effect, and (E) RS male, female effect. Hybrid spruce includes hybrid indices 25, 50, and 75. Error bars are SEs. Bars with different letters are significantly different (Tukey’s mean separation test, $P \leq 0.05$).

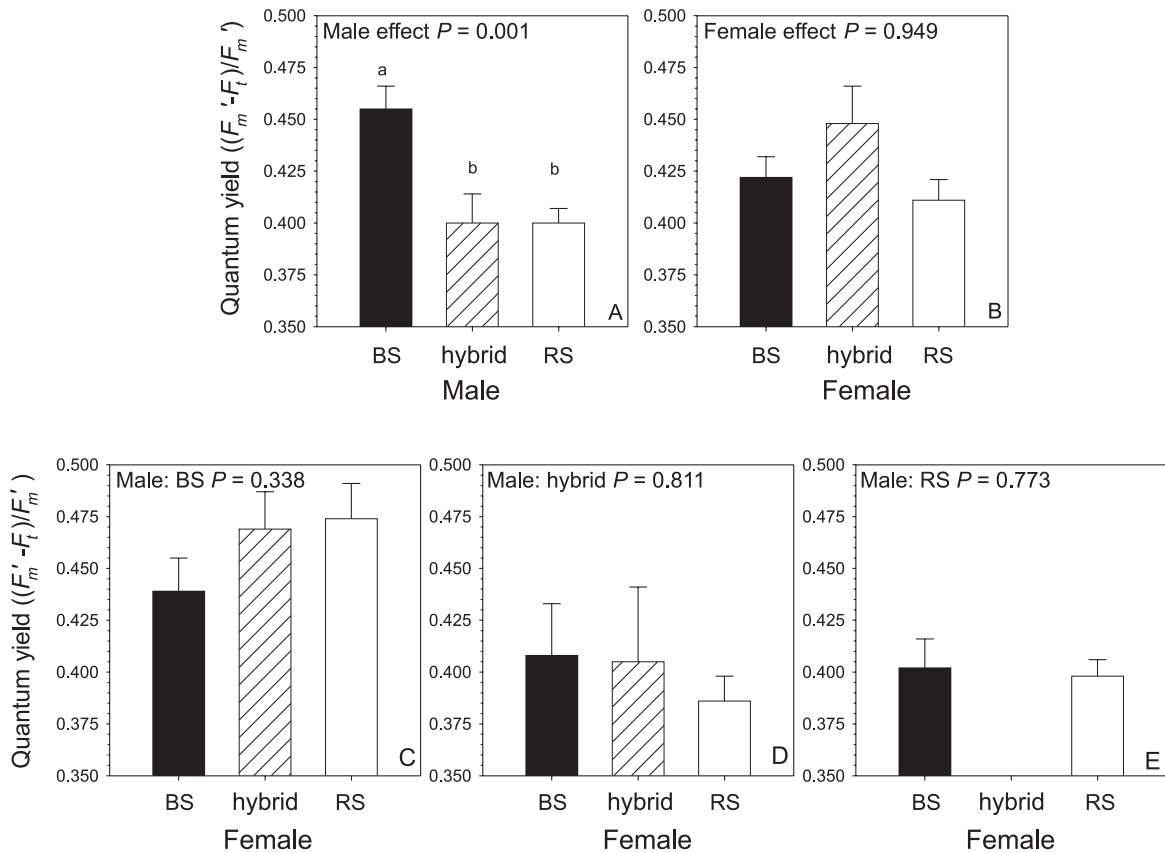


Table 4. ANOVA by parental category effect including source of variation, degrees of freedom (df), mean square values (MS), and P values for photochemical efficiency (F_v/F_m), quantum yield (YLD), thermal dissipation efficiency (qN), chlorophyll fluorescence (F_{pc}), and relative fluorescence at $-40\text{ }^{\circ}\text{C}$.

| Source of variation | df | F_v/F_m | | YLD | | qN | | F_{pc} | | Relative F_t ($-40\text{ }^{\circ}\text{C}$) | |
|----------------------|----|------------------------|--------|------------------------|-------|------------------------|-------|----------|-------|--|-------|
| | | MS | P | MS | P | MS | P | MS | P | MS | P |
| Male | 2 | 3.58×10^{-4} | 0.035 | 16.34×10^{-3} | 0.001 | 37.70×10^{-3} | 0.008 | 10.77 | 0.096 | 19.20×10^3 | 0.003 |
| Female | 2 | 10.82×10^{-4} | <0.001 | 0.11×10^{-3} | 0.949 | 6.85×10^{-3} | 0.331 | 3.65 | 0.440 | 18.20×10^3 | 0.004 |
| Male \times female | 3 | 5.91×10^{-4} | 0.002 | 1.60×10^{-3} | 0.771 | 1.70×10^{-3} | 0.914 | 1.65 | 0.770 | 11.89×10^3 | 0.011 |
| Error | 48 | 0.99×10^{-4} | | 2.07×10^{-3} | | 7.11×10^{-3} | | 4.37 | | 2.88×10^3 | |

tionship between YLD and F_m' (Major et al. 2003a). It appears that a higher F_m' results in a higher YLD and also a higher fluorescence (F_m' to F_t , $r = 0.924$); thus, the greater the F_v' the greater the energy available for both photosynthesis and fluorescence. However, the highest YLD is associated with the lowest qN and vice versa. This appears to be a biological tradeoff evidenced by strong species differences and populations of both species conforming to this relationship (Major et al. 2003a).

Freezing tolerance at $-20\text{ }^{\circ}\text{C}$ had high variation and no statistical differences among hybrid indices. This would be due to the averaging of three crosses of different parental combinations for what is considered a complex adaptive trait (Howe et al. 2003). The high variation of the hybrid crosses was also somewhat expected because of potentially large genetic differences between parental crosses and the subse-

quent heterosis effect (Mosseler 1990). Freezing tolerance has been found to be mainly additively inherited in Scots pine (*Pinus sylvestris* L.; Nilsson and Walfridsson 1990). However, freezing tolerance at the lowest test temperature ($-40\text{ }^{\circ}\text{C}$) showed a strong parental species effect. Although not statistically significant at $-20\text{ }^{\circ}\text{C}$ but significant at $-40\text{ }^{\circ}\text{C}$, RS has lower freezing tolerance than BS, which was expected as RS is documented to have had periodic winter injury (DeHayes et al. 1990) and lower freezing tolerance across seed sources compared with BS (Major et al. 2003a).

Inheritance pattern

The light-adapted traits, YLD, qN , and F_{pc} , of control-crossed RS and BS had values consistent with those reported for open-pollinated, species provenance results (Major et al.

Fig. 3. Black, red, and hybrid spruce dark-adapted mean photochemical efficiency by parental analyses: (A) overall male effect, (B) overall female effect, (C) BS male, female effect, (D) hybrid spruce male, female effect, and (E) RS male, female effect. Hybrid spruce includes hybrid indices 25, 50, and 75. Error bars are SEs. Bars with different letters are significantly different (Tukey's mean separation test, $P \leq 0.05$).

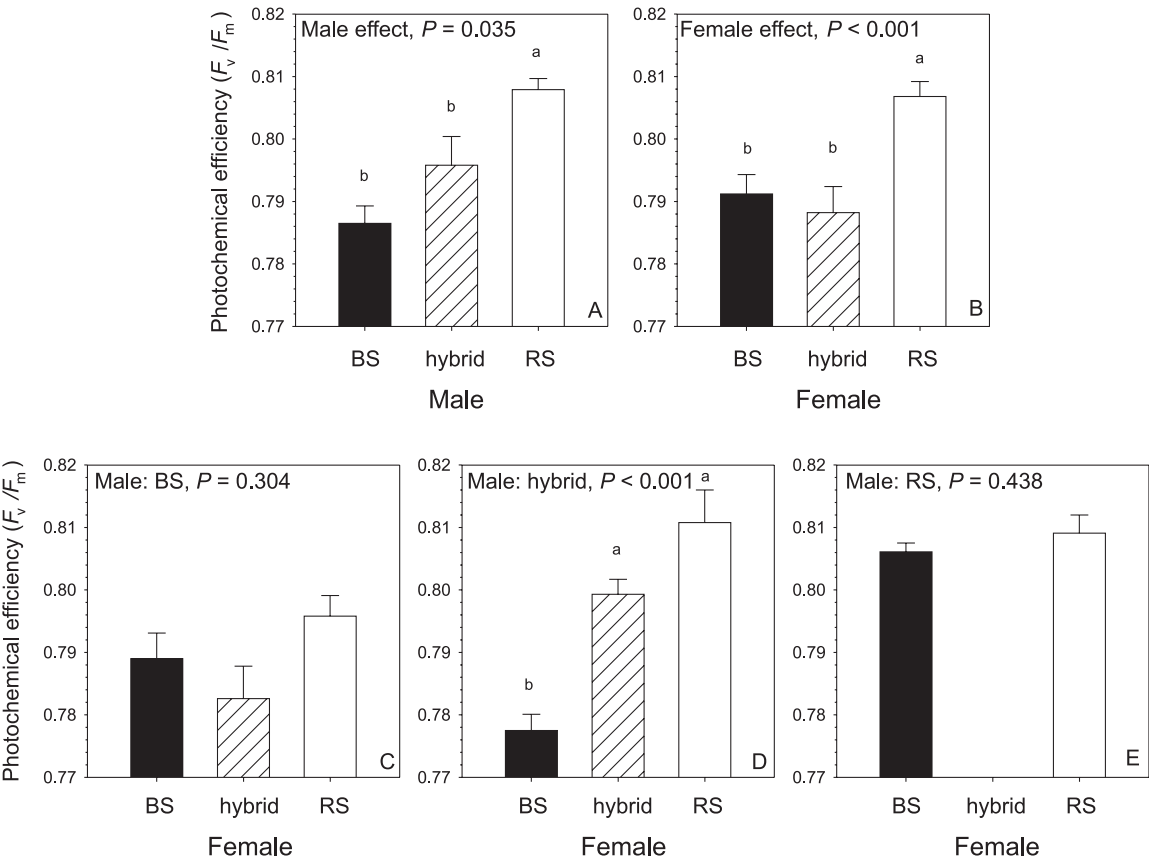
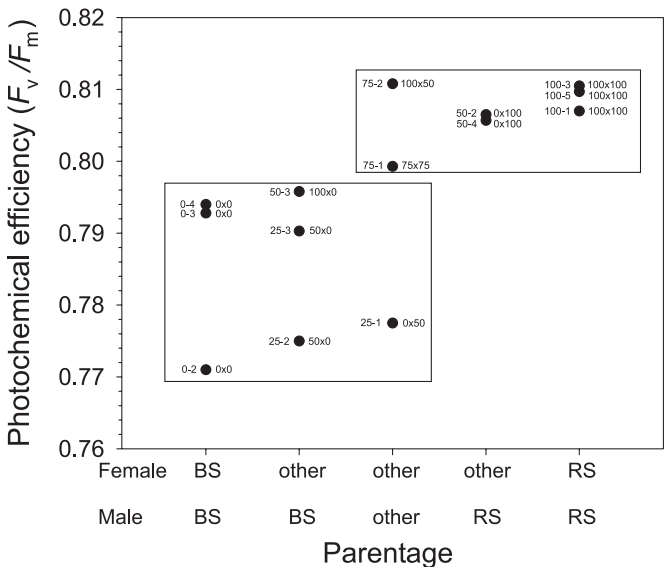


Fig. 4. Photochemical efficiency of controlled crosses presented by parentage, where parentage is classification as black spruce (BS), red spruce (RS), and other (hybrid).

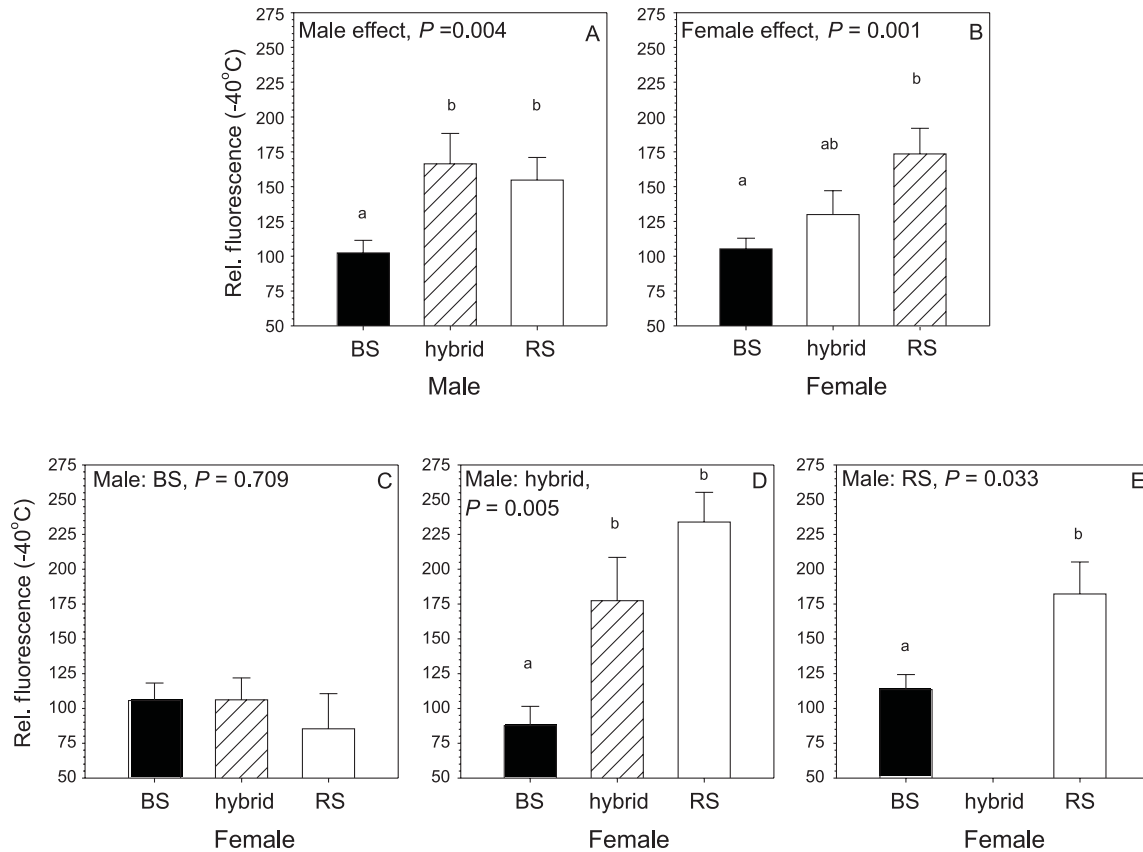


2003a). There was no evidence of negative heterosis of the hybrid 50 F_1 crosses of traits as found by Manley and Ledig (1979). They speculated that hybrid inferiority was an im-

portant mechanism of species separation. Initially, the results of these traits supported an additive hybrid inheritance model, evident by the mean hybrid index 50 having a mid-parent value. Parental analysis revealed significant male and nonsignificant female and interactive effects, which supports an underlying species-specific, paternal inheritance. Also, the ANOVA of F_1 hybrid index 50 by cross, showed dominance of the paternal parent, consistent with the species response. The paternal effect was also seen in the other hybrid \times species cross combinations. Thus, underlying the averaged additive results (crosses were produced various ways; see Table 1) was a significant paternal inheritance pattern. Consistent with these results, chloroplast pigment content results have also shown a significant male and non-significant female and male \times female interaction effects (Major et al. 2007). Crosses with BS males had 10.6% and 17.6% higher total chlorophyll content than crosses with hybrid and RS males in ambient and elevated CO_2 environments, respectively (Major et al. 2007). The higher YLD and chlorophyll content for BS is consistent with its ecological role as an early successional species (Viereck and Johnston 1990). A higher inherent energy processing ability would be advantageous in a competitive and high-light environment on exposed or highly disturbed sites.

There was a two-tiered F_v/F_m stratification among hybrid-indexed seedlings, indicating a species-specific or nonadditive inheritance. Parental analysis demonstrated a significant

Fig. 5. Black, red, and hybrid spruce mean relative fluorescence after exposure to -40°C by parental analyses: (A) overall male effect, (B) overall female effect, (C) BS male, female effect, (D) hybrid spruce male, female effect, and (E) RS male, female effect. Hybrid spruce includes hybrid indices 25, 50, and 75. Error bars are SEs. Bars with different letters are significantly different (Tukey's mean separation test, $P \leq 0.05$).



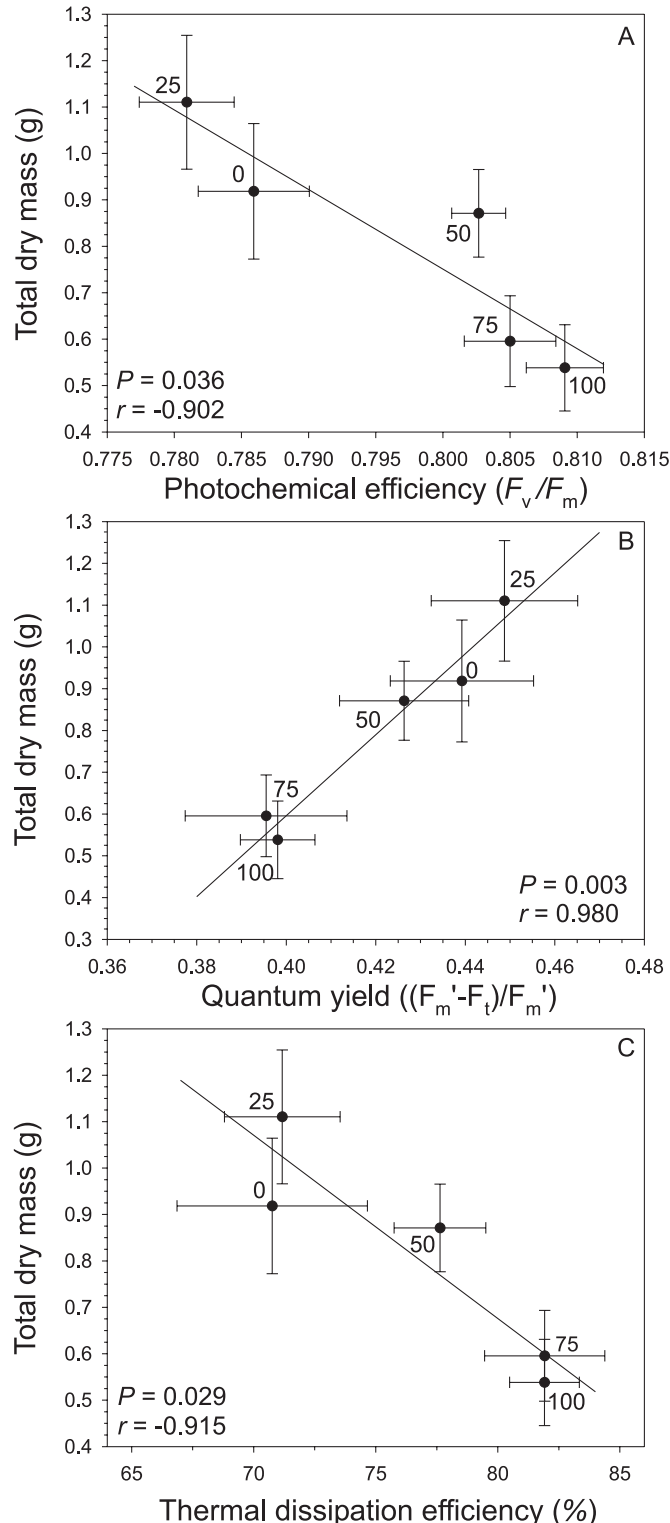
male and female effect, and also a male \times female interaction, which appears to be largely species specific, and paternally inherited. The evidence to support this is as follows: (i) analysis by species shows that hybrid index 100 crosses had significantly higher F_v/F_m than hybrid index 0 crosses ($P < 0.001$) consistent with that found across seed sources (Major et al. 2003a); (ii) an F_v/F_m analysis of F_1 crosses, hybrid index 50, by parents, showed paternal effect ($P = 0.006$) consistent with species results; (iii) the hybrid female \times BS male is made up of two crosses (25-2 and 25-3, both 50×0), and their responses were similar to that of BS; and (iv) analysis of all non-BS \times BS crosses was not significantly different from the BS \times BS crosses, and all non-RS \times RS crosses were not significantly different from the RS \times RS crosses. Thus, when RS and BS are crossed, photochemical efficiency appears to be largely paternally inherited.

The crosses with hybrid as the male continued to show segregation to either BS or RS values. Conserved paternal species effects are the most probable cause of segregation and the significant interaction seen. For instance, for F_v/F_m , the BS female \times hybrid male is made up of one cross (25-1, 0×50) and showed a species response similar to BS. This suggests that the paternal grandfather was most probably a BS and the paternal grandmother a RS. Also, the RS female \times hybrid male is also made up of one cross (75-2, 100×50) and showed a species response similar to RS. This suggests

that the paternal grandfather was most likely a RS and the paternal grandmother a BS. There remains only one other unexamined cross (75-1, 75×75), and its response is very similar to that of RS, indicating perhaps that the paternal parent or grandparent is a RS.

The above analyses are consistent with molecular genetics evidence for paternal inheritance of chloroplasts in spruce (Sutton et al. 1991) and other conifers (Neale et al. 1986; Wagner et al. 1987). The chloroplast genome is highly conserved (Palmer 1985). Approximately 100–120 genes are encoded in the chloroplast DNA (Clegg et al. 1994; Rochaix 1997); including genes for all the subunits of ribosomal RNA, transfer RNA, and various components of the photosynthetic apparatus (Clegg et al. 1994, Rochaix 1997). Proper chloroplast functioning requires not only products encoded in the chloroplast DNA but also other components of the photosynthetic apparatus, which are encoded in the nuclear genome (Clegg et al. 1994; Taiz and Zeiger 1997), synthesized in the cytosol, and transported into the chloroplast (Taiz and Zeiger 1997). In plants, maternal inheritance has been demonstrated for adaptive traits controlling male sterility (mostly cytoplasmic and mitochondrial based) (Dinnetz 1997) and seed development (Colombo et al. 1997). In trees, maternal inheritance of adaptive traits has been demonstrated in only a few instances in reciprocal controlled crossing studies based on variation in quantitative traits (Wilcox 1983; Perry 1976). Of course, other

Fig. 6. Mean total dry mass by mean hybrid index in relation to following light-energy processing traits: (A) dark-adapted photochemical efficiency, (B) quantum yield (YLD), and (C) thermal dissipation efficiency for hybrid indices 0, 25, 50, 75, and 100. The hybrid index values are the percentages of RS complement, with the remaining proportion BS. Indices 0 and 100 represent BS and RS, respectively.



adaptive traits in RS and BS hybrids have shown dominant species, additive, and mixed models of inheritance for complex traits such as height and shoot:root ratio (Major et al. 2003c), and freezing tolerance (current study). However, we know of no studies in trees demonstrating the importance of paternal inheritance (e.g., via chloroplasts) of ecophysiological processes that affect adaptation and fitness.

Relationship to productivity

The YLD trait is closely related to photosynthesis (Lichtenthaler and Rinderle 1988; Maxwell and Johnson 2000) and strongly correlated to total biomass productivity (Fig. 6B), suggesting the adaptive and fitness importance of this trait. Various tree biology research efforts have attempted to find the nature of the relationship between photosynthetic activity and growth. A number of studies have reported little or no significant positive correlation (Larson and Wellendorf 1990; Zhang et al. 1994; Pereira 1994), whereas others have reported positive correlations (Boltz et al. 1986; Major and Johnsen 1996; Johnsen et al. 1999). However, as discussed by Johnsen and Major (1995), genetic correlations between photosynthesis and growth in forest trees are complicated and depend on many factors, including the specific population being studied, the time(s) of year photosynthesis is measured, biomass allocation, and growing conditions. What is especially noteworthy is that this was seed material stored from a previously planted experiment and a robust relationship exists between the current 1-year-old mean seedling height growth and 22-year-old trees planted on two sites ($r = 0.918$ and 0.968) (Major et al. 2003c). Thus, it appears that light-energy processing traits have a lasting influence beyond 1-year-old seedlings.

In the RS–BS continuum of dry mass productivity, not only was there a positive YLD relationship, but there was also a strong negative relationship to F_v/F_m and qN . A higher F_v/F_m is perhaps an adaptation to low light levels, and appears to result in a biological tradeoff in lower YLD efficiency and greater photoprotection efficiency (higher qN) traits, consistent with some of those found for other shade-tolerant species (Johnson et al. 1993; Demmig-Adams and Adams 1994). As light levels increase, RS also appears to have a greater potential problem with photoinhibition (Major *unpublished*). Photosynthetic performance appears to be a more important trait for early successional species, whereas F_v/F_m and qN appear to be important traits for late-successional species. These biological adaptations also appear to result in lower overall dry mass productivity in RS. Because the tradeoff presents itself as a linear relationship and because BS and RS are situated at or near the extremes of this relationship, there appears to be support for natural selection pressure for these traits for their corresponding ecological niche. Nevertheless, as seen by the hybrid index 25 position, the selection pressure may not be overly strong or narrow and, through breeding, could be modified and (or) increased.

Hybridization

Hybridization between RS and BS has resulted in identification and forest management problems for these otherwise ecologically distinct species. The hybrid index approach is intensive (19 morphological traits) yet proved useful for dif-

ferentiating genotypes across a BS–RS continuum (Johnsen et al. 1998). The progenies' hybrid index values were highly heritable and strongly correlated with growth performance, clearly showing the robustness and applicability of the approach. Although RS and BS can hybridize, the level and role of introgression are likely to be minor under the characteristic habitat and ecological conditions of the parental species (Gordon 1976; Manley and Ledig 1979; Mosseler et al. 2000; Major et al. 2005). In contrast to field and laboratory observations based on morphological traits, reproductive traits, and crossability, molecular marker-based approaches (Bobola et al. 1996), some using only dominant markers (Perron and Bousquet 1997; Nkongolo et al. 2003), have suggested a significant introgression between BS and RS. In fact, Nkongolo et al. (2003) went so far as to state that there were "likely no reproductive barriers, nor ecological isolation" between these species, which is untenable (see Major et al. 2005). The reliability of some of the species-specific molecular markers reported in earlier studies is questionable, because these were not reproduced when tested against these intensive index evaluations. We emphasize that caution must be applied to interpretations based solely on molecular markers, because so far, they provide a limited (albeit still important) representation of the spruce genome, and available molecular data do not reflect ecophysiological or adaptive differences. However, the reliability of molecular markers in distinguishing between closely related BS and RS and their hybrids will only be confirmed when they unambiguously correspond to BS and RS species distinction based on the morphological and ecophysiological traits that determine the species identity of RS and BS. From this study, we now recognize that estimation of the hybrid index is not always enough to predict adaptive trait response. Knowing the parental pedigree also appears to be essential. Consequently, ecophysiological genetic measurements are more relevant means for assessing the adaptive trait under scrutiny until robust quantitative trait loci and genes controlling these adaptive traits are identified and mapped.

Petit et al. (1997) suggested that hybridization between sympatric early and late-successional species of oak (*Quercus* spp.) might be a step in the recolonization of the late-successional species in its northward migration. In advance of migration, the late-successional species would pollinate females of the early successional species to establish a hybrid complex. This hybrid complex would eventually be displaced by the late-successional species capitalizing on its adaptive traits (e.g., shade tolerance). It was suggested that interspecific hybridization in this direction would have greater success than hybridization in the opposite direction. Our data show a 56% higher seed set, with significantly more filled seed, for the BS \times RS than RS \times BS crosses (Major et al. 2005). A virtually identical reproductive phenology, differential crossability (Major et al. 2005), and predominant paternal inheritance of light-processing adaptive traits make this late-successional migration theory plausible for these spruces.

Conclusions

Pure species results of F_v/F_m , YLD, qN , and F_{pc} were consistent with previously published open-pollinated, species provenance results. Averaged light-adapted light-energy

processing traits, YLD, qN , and F_{pc} , showed an additive inheritance pattern. Parental analyses results of YLD, qN , and F_{pc} , showed significant male and nonsignificant female and interactive effects which supports an underlying species-specific, paternal inheritance. Although more complex, analysis of F_v/F_m showed support for a predominantly paternal inheritance. Any cross having BS as the male parent had significantly higher YLD and F_{pc} than those having either RS or hybrids as male parents. Furthermore, any cross having RS as the male parent had significantly higher F_v/F_m and qN . Freezing tolerance, as measured by relative fluorescence, showed a mixed model of inheritance dominated by species effects. Estimation of the hybrid index is not always enough to predict adaptive trait response. Knowing the parental pedigree appears to be essential. Total dry biomass is positively correlated with YLD and negatively correlated with F_v/F_m and qN . Photosynthetic performance appears to be a more important trait for early successional species, whereas photochemical efficiency and thermal dissipation efficiency appear to be important traits for late-successional species. We know of no other studies in trees demonstrating the importance of paternal inheritance (e.g., via chloroplasts) of ecophysiological processes that affect adaptation and fitness.

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