

Growth and allocation of *Picea rubens*, *Picea mariana*, and their hybrids under ambient and elevated CO₂

John E. Major, Alex Mosseler, Kurt H. Johnsen, Moira Campbell, and John Malcolm

Abstract: Red spruce (RS; *Picea rubens* Sarg.) – black spruce (BS; *Picea mariana* (Mill.) B.S.P.) controlled crosses (100%, 75%, 50%, 25%, and 0% RS, balance BS) showed increasingly greater height with increasing proportion of BS in each successive year. Height growth of 4-year-old ambient CO₂ (aCO₂) grown trees was highly correlated with height of 22-year-old field-grown trees of the same or similar crosses. Bud flush was earliest in BS and declined linearly with increasing proportion of RS with no significant CO₂ effect. Percent stem (stem + branches) mass increased under elevated CO₂ (eCO₂), a quarter of which was due to ontogeny. Conversely, percent needle mass had a significant negative relationship with increasing tree size, and there was a CO₂ × tree size interaction. Shoot-to-root ratio was greatest for BS, whereas RS had among the lowest. Hybrid index (HI) 50 had the greatest root mass allocation, lowest shoot-to-root ratio, and among the greatest total mass under eCO₂. Growth efficiency increased with tree size and eCO₂ but decreased with HI. Percent total biomass stimulation under eCO₂ was lowest for BS at 6.5%, greatest for HI 50 at 20.3%, and RS had 17.5%.

Key words: allocation, black spruce, elevated CO₂, growth, fitness, interspecific hybridization, red spruce.

Résumé : Des croisements dirigés entre l'épinette rouge (ER; *Picea rubens* Sarg.) et l'épinette noire (EN; *Picea mariana* (Mill.) B.S.P.) (composés à 100, 75, 50, 25 et 0 % de ER et le reste de EN) ont produit des individus dont la hauteur augmentait avec la proportion grandissante de EN pour chaque année consécutive. La croissance en hauteur à 4 ans des arbres sous des conditions de CO₂ ambiant (aCO₂) était fortement corrélée à la hauteur à 22 ans d'arbres ayant poussé au champ et provenant des mêmes croisements ou de croisements similaires. Le débourrement des bourgeons était le plus hâtif chez EN et déclinait linéairement avec une proportion grandissante de ER, sans effet significatif du CO₂. La masse de la tige (tige + branches) en pourcentage augmentait sous des conditions d'enrichissement en CO₂ (eCO₂) et le quart de cette augmentation était dû à l'ontogénie. À l'inverse, il y avait une relation négative significative entre la masse des aiguilles en pourcentage et l'augmentation de la taille des arbres, ainsi qu'une interaction entre le CO₂ et la taille des arbres. Le rapport entre la tige et les racines de EN était le plus élevé, alors que celui de ER était parmi les plus faibles. Les individus avec l'indice d'hybridation (IH) 50 allouaient le plus de masse aux racines, avaient le rapport le plus faible entre la tige et les racines et parmi la plus grande masse totale sous des conditions d'eCO₂. L'efficacité de croissance augmentait avec la taille des arbres et l'eCO₂, mais elle diminuait avec l'IH. Le pourcentage de la biomasse totale attribuable à la stimulation par l'eCO₂ était le plus faible chez EN avec 6,5 %, le plus élevé chez les individus d'IH 50 avec 20,3 % et atteignait 17,5 % chez ER. [Traduit par la Rédaction]

Mots-clés : allocation, épinette noire, enrichissement en CO₂, croissance, aptitude, hybridation interspécifique, épinette rouge.

Introduction

Red spruce (RS; *Picea rubens* (Sarg.)) is an important and characteristic component of the late-successional forest of the Acadian Forest Region in eastern Canada and the northeastern United States. Mature trees can reach heights of up to 35 m and diameter at breast height (DBH) of up to 1.3 m; RS grows best in a cool, moist climate and is classified as very shade tolerant (Blum 1990). RS has experienced a substantial decline over most of its geographic range associated with excessive harvesting (Korstian 1937; Leak and Smith 1996), acidic deposition and associated winter injury (DeHayes et al. 1990), and climate warming (McLaughlin et al. 1987; Hamburg and Cogbill 1988). The decline may also be related to hybridization and introgression with black spruce (BS; *Picea mariana* (Mill.) B.S.P.) (Major et al. 2003c), an early to mid-successional species in terms of relative shade tolerance and adaptations for postfire regeneration (Viereck and Johnston 1990). BS is a transcontinental species, tolerant of very many abiotic

stressors (Farrar 1995), and mature trees can reach heights of 30 m and DBH of 1 m. Only in the southern part of its range is RS largely sympatric with the transcontinental BS. The two species occupy ecologically distinct niches due to a suite of species-specific traits such as dry-matter partitioning, light-energy processing, and chlorophyll pigment concentration (Major et al. 2003a, 2003b, 2003c, 2007; Barsi et al. 2009). Estimates of the extent of natural hybridization and introgression range from extensive (Morgenstern and Farrar 1964; Manley 1972; Bobola et al. 1996) to minor (Gordon 1976; Manley and Ledig 1979; Mosseler et al. 2000). Hybridization and introgression can confuse species' morphological identities and complicate decisions on potential silvicultural management options for these otherwise successional and ecologically distinct species (Manley 1972; Beylor 1999). Benchmark empirical information is lacking on the comparative species-hybrid response to climate change factors.

Paleoecological reconstructions support the conclusion that species migrate singly rather than as intact plant communities

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(Peters 1990). Pollen cores in a number of interior Maine locations show that RS has only been in abundance for the last 1000 years, during which time, the climate has been relatively cool and wet compared with the previous 1000–7000 years when it was warmer and dryer, favoring pines (*Pinus* spp.) instead of spruces (Schauffler and Jacobson 2002; Lindbladh et al. 2003). A predicted doubling of atmospheric carbon dioxide (CO₂) concentrations during this century may change species' competitive relationships and result in novel species mixes with no modern counterparts (Tjoelker et al. 1998a, 1998b; Dawes et al. 2011). Elevated CO₂ typically increases tree growth and has also been shown to modify component physiological processes, including net assimilation (A), water-use efficiency, cold tolerance, and dry-matter partitioning (Greenwood and Volkaert 1992; Johnsen 1993; Samuelson and Seiler 1994; Major et al. 2014). As northern conifers are long lived, it is thought to be prudent to investigate atmospheric CO₂ interactions with species and their hybrids.

A number of studies have hypothesized and reported that faster growing species have greater absolute and relative growth responses to elevated CO₂ (eCO₂) than inherently slower growing species (Atkin et al. 1999; Poorter and Navas 2003), although this was not seen by others (Tjoelker et al. 1998b; Ghannoum et al. 2010). BS has greater juvenile growth rates (Johnsen et al. 1998; Major et al. 2003b) and a higher quantum yield than RS (Major et al. 2003a). Furthermore, a controlled-cross study showed that BS had greater total chlorophyll concentration and needle nitrogen concentration than RS under aCO₂ and eCO₂ (Major et al. 2007) and across varying light × soil moisture conditions (Barsi et al. 2009). Numerous observations and meta-analyses of 74 total biomass response ratios of eCO₂ to aCO₂ comparing shade-tolerant and -intolerant tree species revealed that response ratios differed widely and significantly and that shade-tolerant species had greater response ratios than shade-intolerant species (Bazzaz et al. 1990; Kerstiens 2001; Ellsworth et al. 2012).

How species–hybrid biomass traits interact with eCO₂ will contribute to their relative fitness under eCO₂ and may have important ecological implications for community structure. Our goal was to quantify and compare biomass, components of growth, and allocation parameters from a RS–BS hybrid complex under aCO₂ and eCO₂ environments, using seedlings from an intra- and inter-specific controlled-cross experiment. Because this study used the same seed lots from the same controlled crosses as in Johnsen et al. (1998), we examined the relationship between our seedling results and 22-year field tree performance. Our specific objectives were to (i) determine species and hybrid variation in biomass, components of growth, and allocation parameters, (ii) examine how these traits respond to and interact with eCO₂, and (iii) examine how these traits relate to additional productivity results.

Materials and methods

Controlled-cross experiment

Using a hybrid index (HI) system based on 19 needle, 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 HI estimates the percentage of the RS component in the parent tree, with 0 denoting pure BS and 100 denoting pure RS. This HI appears to clearly distinguish the species and their degree of hybridity as it is highly heritable (Johnsen et al. 1998). At least three different sets of parent trees were selected for controlled crosses performed over a 3-year period. These parent trees were located in or near the Acadia Research Forest near Fredericton, New Brunswick, Canada (46°N, 66°15'W). A series of controlled crosses using various parents were made to produce F₁ seedlots with expected hybrid indices 0, 25, 50, 75, and 100, as described by Manley and Ledig (1979). Stored seeds from these crosses were used to produce seedlings. These seedlings were some of the same

Table 1. Parentage and accession numbers of hybrid families.

Hybrid crosstypes	Accession no.	Hybrid index of ♀ parent × ♂ parent
Hybrid index 0		
0-1	5-71×5-72	0×0
0-2	17-71×199-71	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	BS3-69×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
75-6	31-70×1-70	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 denotes 0% red spruce, hybrid index 100 denotes 100% red spruce, and hybrid indices 25, 50, and 75 represent the percentage of red spruce, with the remaining proportions being black spruce.

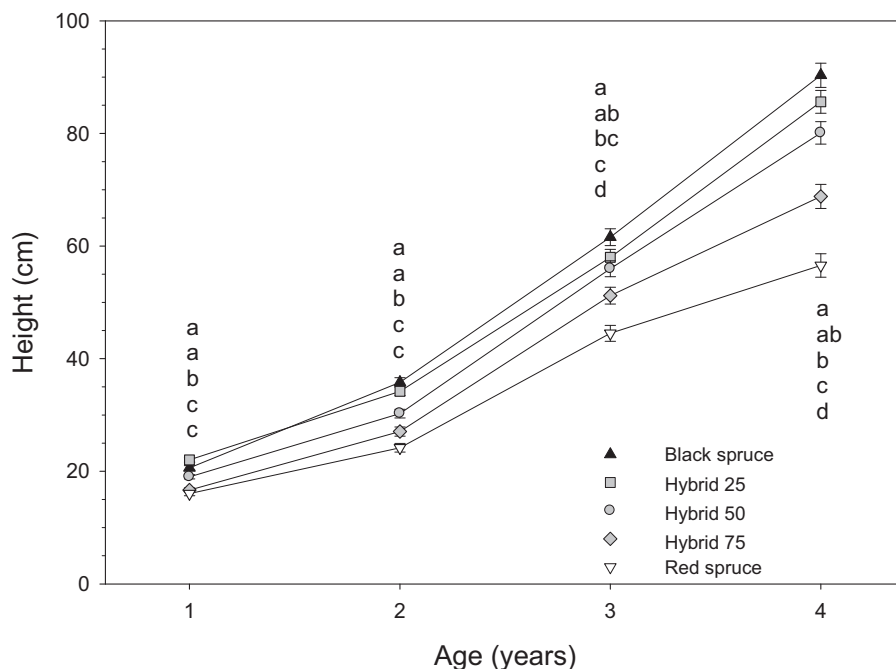
hybrid families used by Manley to establish a RS, BS, and hybrid experiment on two common-garden sites at the Acadia Research Forest in 1974 (Johnsen et al. 1998). Three crosses for each HI (0, 25, 50, 75, and 100), for a total of 15 families, were used in our study (Table 1).

Growth conditions

Seeds were sown in hydrated 36-mm Jiffy peat pellets (Jiffy Products Ltd, Lincoln, New Brunswick) (90 mL full volume) in June 1998. Trays were randomly located across greenhouse benches at the Canadian Forest Service – Atlantic Forestry Centre (CFS–AFC) in Fredericton, New Brunswick, Canada (45°52'N, 66°31'W). First-year growth was under aCO₂ conditions and was described in Major et al. (2003b, 2003c). Average seedlot germination was 70%–88%. Seedlings were stored on site overwinter in cold storage at –5.0 °C. Six (three per CO₂ treatment) 2.15 × 4.30 × 2.5 m specially constructed chambers, located at the CFS–AFC greenhouses and covered with 4 mm polyethylene greenhouse film with air cooling and outside air exchanger, were used to grow seedlings planted in 25 cm diameter × 30 cm deep felt root control bags (Root Control Inc., Oklahoma City, Oklahoma). These chambers were monitored and maintained to be the same as outside ambient temperatures and relative humidity conditions at either 360 or 720 ppm of CO₂, representing aCO₂ and eCO₂ levels, respectively, using a greenhouse monitoring system, calibrated monthly, that dosed the chambers when they fell below the targeted CO₂ values (Major et al. 2007). Light levels were measured outside and inside chambers using quantum sensors LI-190SA (LI-COR, Lincoln, Nebraska). Using the light extinction method detailed by Parent and Messier (1996), light levels were approximately 70% of outside ambient conditions.

Four seedlings for each of the 15 controlled-cross seedlots were randomly established in each greenhouse chamber in May 1999. Seedlings were watered once weekly and fertilized once weekly in May with 11:41:8 (N:P:K) plus micronutrients at 50 ppm N (Plant Products Co. Ltd., Brampton, Ontario), from June to the end of August with 20:8:20 at 100 ppm N, and from September to the end of October with 8:20:30 at 35 ppm N. Soil moisture conditions were set between –0.1 and –0.5 MPa, calculated from a soil retention curve, measured using a soil moisture probe (CS615, Campbell

Fig. 1. Total height increment (mean \pm SE) over 4 years under aCO₂ and eCO₂ environments by hybrid indices (HI) 0, 25, 50, 75, and 100, where 0 and 100 represent BS and RS, respectively. Hybrids 25, 50, and 75 represent the percentage of RS, with the remaining proportion being BS. Different letters show significant hybrid index difference using Tukey's mean separation test ($P = 0.05$). CO₂ treatments started at year 1.



Scientific, Logan, Utah) put in each chamber, and recorded with a datalogger (CR10X, Campbell Scientific).

Sampling, measurements, and parameter estimation

At the end of each growing season, all seedlings of the 15 seedlots from each treatment were measured for height. Bud flush index was determined on the terminal and any lateral buds on 4 May at the beginning of the 4th year (3rd year of treatment). The buds were categorized as follows: 1, no activity; 2, bud swelling; 3, budbreak (see green foliage); and 4, flushing. Free growth generally ceases in spruces 5–10 years following germination (von Wühlisch and Muhs 1986). Four-year-old spruce seedlings are at a life-cycle phase in which they are transitioning from free to fixed growth and may exhibit proleptic free growth following expansion and growth of the winter bud; a summer bud is formed that rests temporarily, followed by another period of shoot growth. We quantified late-summer shoot activity using a bud set activity index, determined on the main and lateral shoots on 21 August of the 4th year. Shoot activity was categorized as follows: 5, the initial bud formed that summer was set; 6, needle primordia in the initial bud formed that summer were clearly visible, indicating that the second bud was breaking; 7, following budbreak of the initial bud, shoot growth was active; and 8, the second bud had set. After 3 years of treatment, all seedlings were measured for total height, root-collar diameter (RCD), 4th-year growth increment, and needle, stem (stem + branches), and root biomass. Biomass was measured after oven drying the seedling components at 65 °C for 48 h. From the morphometric data, the following parameters were determined: percent needle, stem, and roots; shoot-to-root ratio; and growth efficiency (GE) = stem biomass / needle biomass.

Statistical analyses

The data were subjected to analyses of variance (ANOVA). Greenhouse chamber (block), HI, family, and CO₂ level were considered fixed effects. Families were nested within HI. The ANOVA model used was as follows:

$$(1) \quad Y_{ijklm} = \mu + G_i + H_j + F_{k(j)} + C_l + HC_{jl} + e_{ijklm}$$

where Y_{ijklm} is the dependent seedling trait of greenhouse chamber i of the j th HI, of the k th family, of the l th CO₂ treatment, of the m th seedling; μ is the overall mean; G_i is the effect of greenhouse chamber i ($i = 1, 2, 3$); H_j is the effect of the j th HI ($j = 1, 2, 3, 4, 5$); $F_{k(j)}$ is the effect of the k th family nested within the j th HI ($k = 1, 2, 3$); C_l is the effect of the l th CO₂ treatment ($l = 1, 2$); HC_{jl} is the interaction effect of the j th HI and l th CO₂ treatment; and e_{ijklm} is the random error component. Effects were considered statistically significant at the $P = 0.05$ level, although individual P values are provided so that readers can make their own interpretations. The data satisfied normality and equality of variance assumptions. The general linear model from SYSTAT (Chicago, Illinois) was used for analysis.

Treatment effects on biomass allocation changes were then assessed using analysis of covariance (ANCOVA). In these analyses, three sources of variation were studied: (i) covariate (i.e., total height), (ii) independent effect (i.e., CO₂), and (iii) independent effect \times covariate. This analysis differentiates the changes in allocation ratios determined by ANOVA (eq. 1) from changes in allocation due to changes in tree size. The ANCOVA CO₂ results will thus have been corrected for tree size. The analyses were done based on the following model:

$$(2) \quad Y_{ij} = B_0 + B_{0i} + B_1 X_{ij} + B_{1i} X_{ij} + e_{ij}$$

where Y_{ij} is the dependent trait of the j th plant of the i th site or species, B_0 and B_1 are average regression coefficients, B_{0i} and B_{1i} are the site- or species-specific coefficients, X_{ij} is the independent variable, and e_{ij} is the error term. Results were considered statistically significant at $\alpha = 0.10$, although individual P values are provided for all traits so that readers can make their own interpretations. The general linear model from SYSTAT was used for analysis.

Table 2. Yearly height ANOVAs, including source of variation, degrees of freedom (df), mean square values (MS), and *P* values.

Source of variation	df	Total height (cm)									
		Year 1		Year 2		Year 3		Year 4		Current (year 4) growth (cm)	
		MS	<i>P</i> value	MS	<i>P</i> value	MS	<i>P</i> value	MS	<i>P</i> value	MS	<i>P</i> value
Block	2	9.9	0.371	142.3	0.003	6.7	0.222	23.0	0.922	6.63×10 ³	0.524
Hybrid (H)	4	446.3	<0.001	1582.3	<0.001	2968.1	<0.001	11973.2	<0.001	34.16×10 ³	<0.001
CO ₂	1	1.8	0.576	70.7	0.192	11.6	0.774	784.5	0.096	6.06×10 ³	<0.001
Hybrid × CO ₂	4	20.3	0.993	14.9	0.837	78.1	0.694	85.9	0.875	130.9×10 ³	0.670
Family (hybrid)	10	133.4	<0.001	130.9	<0.001	811.8	<0.001	1992.7	<0.001	2.01×10 ³	<0.001
Error	319	10.0		41.3		140.4		282.1		8.12×10 ³	

Note: *P* values < 0.05 are in bold type.

Results

Height growth and diameter

First-year height, prior to CO₂ treatment, was significant for HI and families nested within HI (Fig. 1; Table 2). There was one rank change among hybrid indices in the 2nd year, 25 and 0. There were no significant CO₂ or HI × CO₂ effects on 2nd-year heights. There were no significant CO₂ or hybrid × CO₂ effects on 3rd-year height and no rank change. Generally, eCO₂ heights were equal to or greater than aCO₂ heights for 2nd- and 3rd-year heights. Fourth-year heights also displayed no change in ranking (Fig. 2A). Across all hybrid indices, seedlings grown under eCO₂ were 3.1 cm taller than seedlings grown under aCO₂ after 4 years of growth. Fourth-year RCD was significant for HI and CO₂ effect, with RCD of 13.1 and 11.4 mm for eCO₂ and aCO₂, respectively (Table 3; Fig. 2B). There was no significant CO₂ × HI effect for RCD. Fourth-year height growth increment, in descending order, was 28.3, 27.0, 24.7, 17.9, and 12.9 cm, for hybrid indices 0, 25, 50, 75, and 100, respectively (not shown). There was no significant HI × CO₂ effect but a significant CO₂ effect on 4th-year height growth increment, with height growth increments of 23.8 and 20.5 cm for eCO₂ and aCO₂, respectively.

Phenology

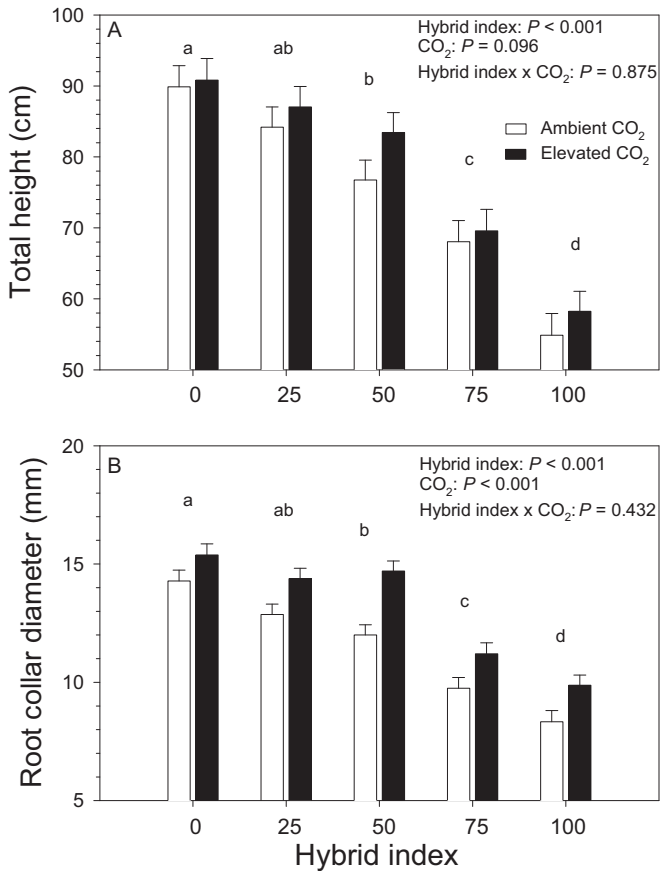
We present the lateral bud flush index, which typically mirrored patterns of terminal shoots, but we indicate where differences were observed between lateral and terminal shoot activity. Lateral bud flush index was significant for HI effect (Fig. 3A; Table 3). There were no significant CO₂ or HI × CO₂ effects for bud flush index.

Lateral bud set activity index was significant for HI and CO₂ treatment. Lateral bud set index had a two-tiered effect and was greatest for hybrid indices 0, 25, and 50 compared with hybrid indices 75 and 100 (Fig. 3B; Table 3). For aCO₂ and eCO₂, the lateral bud set activity index was 6.25 and 5.86, respectively. Terminal bud set activity index showed the same trends as lateral bud set activity index, but these were not significant.

Biomass

At 4 years old, total biomass was significant for HI (*P* < 0.001; Table 4). Overall average biomass for eCO₂ and aCO₂ was 80.5 and 70.2 g, respectively. Root and stem biomass showed significant CO₂ effect (*P* = 0.004 and 0.001, respectively), whereas needle biomass did not (*P* = 0.065) (Tables 4, 5). Seedlings grown under eCO₂ had a final total biomass 15.5% greater than seedlings grown under aCO₂. By HI, the percentage increase in total biomass from aCO₂ to eCO₂ was lowest for BS at only 6.5%, 16% for HI 25, and greatest for HI 50 at 20.3%, whereas HI 75 and RS had moderate increases of 17.3% and 17.5%, respectively. Compared with aCO₂, and in descending order, eCO₂ treatment increased stem, root, and needle biomass by 21.5% (*P* < 0.001), 14.1% (*P* < 0.001), and 8.6% (*P* = 0.821), respectively. For needle biomass by HI, Tukey analysis showed a two-tiered effect, with hybrid indices 0, 25, and 50 significantly greater than hybrid indices 75 and 100. Root biomass displayed a two-tiered pattern similar to needle biomass.

Fig. 2. Year 4 (A) total height (mean ± SE) and (B) root collar diameter under ambient CO₂ and elevated CO₂ treatments by hybrid indices 0, 25, 50, 75, and 100, where 0 and 100 represent BS and RS, respectively. Hybrids 25, 50, and 75 represent the percentage of RS, with the remaining proportion being BS. Different letters show significant hybrid index difference using Tukey's mean separation test (*P* = 0.05).



Allocation

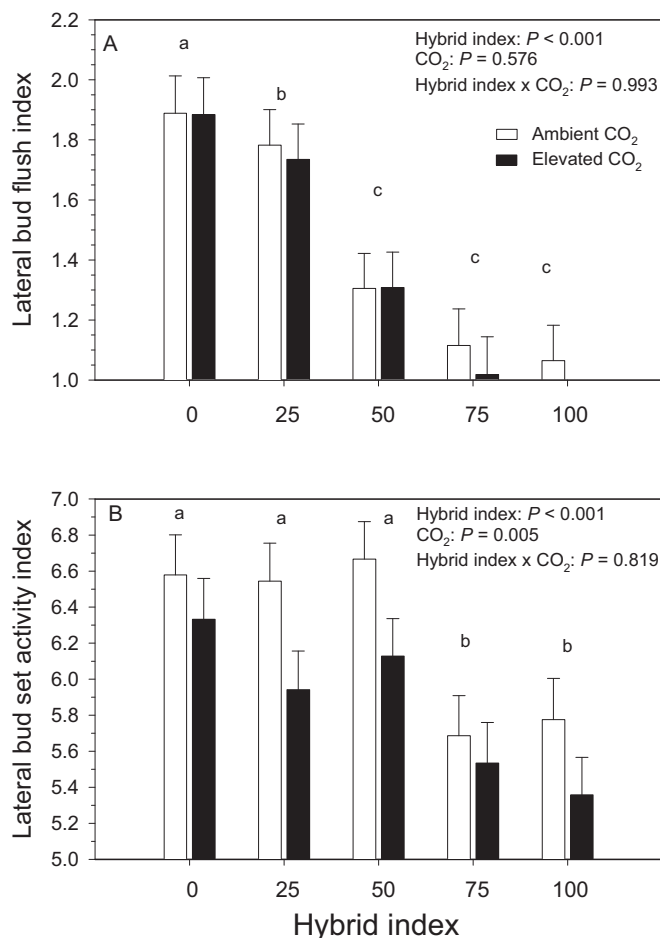
Percent needle mass was, on average, 34.6 for hybrid indices 0, 25, and 50 and averaged 38% for hybrid indices 75 and 100, respectively (Fig. 4A; Table 6). Percent needle mass for eCO₂ and aCO₂ treatments was 34.9% and 37.3%, respectively (*P* < 0.001). Percent stem mass was significant for HI (Fig. 4B), with stem biomasses of 44.1% and 41.8% for eCO₂ and aCO₂, respectively. Percent root mass HI ranking was very different than the percent rankings above (Fig. 4C).

Table 3. Year 4 diameter and phenology ANOVAs, including source of variation, degrees of freedom (df), mean square values (MS), and *P* values.

Source of variation	df	Root collar diameter		Lateral bud flush index		Lateral bud set activity index	
		MS	<i>P</i> value	MS	<i>P</i> value	MS	<i>P</i> value
Block	2	22.8	0.033	5.2	<0.001	1.5	0.378
Hybrid (H)	4	364.1	<0.001	10.3	<0.001	12.1	<0.001
CO ₂	1	227.2	<0.001	0.2	0.576	12.6	0.005
Hybrid × CO ₂	4	6.4	0.432	<0.1	0.993	0.6	0.819
Family (hybrid)	10	34.6	<0.001	2.2	<0.001	6.3	<0.001
Error	319	6.6		0.5		1.6	

Note: *P* values < 0.05 are in bold type.

Fig. 3. (A) Lateral bud flush index (mean ± SE) and (B) lateral bud set activity index under ambient CO₂ and elevated CO₂ treatments by hybrid indices 0, 25, 50, 75, and 100, where 0 and 100 represent BS and RS, respectively. Hybrids 25, 50, and 75 represent the percentage of RS, with the remaining proportion being BS. Different letters show significant hybrid index difference using Tukey's mean separation test (*P* = 0.05).



Shoot-to-root ratio was significant for HI but not CO₂ treatment (Fig. 5A; Table 6). Shoot-to-root ratio in descending order was as follows: hybrid indices 0, 25, 75, 100, and 50. Overall GE was significant for HI (*P* < 0.001; Fig. 5B), with GI of 1.91 and 1.74 for eCO₂ and aCO₂, respectively (*P* < 0.001).

Relationships to other productivity results

The relationship of HI means between 4- and 22-year-old tree heights under aCO₂ (Johnsen et al. 1998) were *R*² = 0.855 (*P* = 0.024) and *R*² = 0.832 (*P* = 0.031) for sites 1 and 2, respectively (Fig. 6).

Covariate analysis of percent stem mass using total height (year 4) as covariate and testing for CO₂ effect showed no significant CO₂ × total height interactive effect (*P* = 0.227). Further analysis showed a significant CO₂ effect (*P* = 0.009) and positive total height effect (*P* < 0.001) (*R*² = 0.919; Fig. 7A). Covariate analysis of percent needle mass using total height as covariate and testing for CO₂ effect showed a significant CO₂ × total height interactive effect (*P* = 0.030). There was a negative relationship between the proportion of total biomass in needles and total seedling height relationship, but the slope was less steep for seedlings grown under aCO₂ than those grown under eCO₂ (*R*² = 0.939; Fig. 7B). There was no tree size covariate effect on percent root mass. Covariate analysis of GE using total height as covariate and testing for CO₂ effect showed a significant CO₂ × total height interactive effect (*P* = 0.073). The result is a positive relationship, with total height with eCO₂ showing a greater slope than aCO₂ (*R*² = 0.942; Fig. 7C).

Discussion

Biomass and height growth

Except for a minor rank change from year 1 to year 2, each yearly height measurement showed increasingly greater total height for hybrid indices with increasing proportion of BS, indicating height of this artificially produced population is strongly additive and affected by species contribution. Additive quantitative traits such as height can be influenced by many hundreds of genes, each contributing small amounts to growth differences (Eckert et al. 2009, 2013; Palle et al. 2013). The data clearly indicated that Manley (1975) was successful in producing a continuum of families and individuals ranging from pure BS to pure RS. Note also that parents were originally selected based on an assortment of morphological characters that did not include tree size. The resulting pattern of tree size in progeny, however, is well correlated with expected hybrid indices. The 4-year-old trees in our study were derived from stored seed from the same controlled crosses conducted by Manley (1975) to establish a hybrid continuum between RS and BS. These full-sib families were used to establish a common-garden experiment on two sites at the Acadia Research Forest in 1974. Using results from measurements from 22-year-old mature trees (Johnsen et al. 1998), we found a strong age-to-age correlation between the height (4th year) of our seedlings grown under aCO₂ and the 22-year-old trees using data from both field sites. Both seedlings and 22-year-old trees displayed a similar linear decline in height with increasing proportion of RS (increasing HI). BS is generally considered an early to mid-successional species based on shade tolerance, and thus rapid early height growth appears to confer a competitive advantage for survival and fitness. RS is a late-successional species that often establishes in an understory and then uses canopy gaps, and as such, early height growth does not appear to be a competitive imperative for fitness. It would appear that fitness traits under low light conditions such as greater light-energy processing traits and lower shoot-to-root ratio than BS are a greater competitive advantage for RS (Major et al. 2003a, 2003c).

Table 4. Year 4 needle, stem, and root biomass (mean± SE) by hybrid index and CO₂ treatments.

	Hybrid index 0		Hybrid index 25		Hybrid index 50		Hybrid index 75		Hybrid index 100	
	aCO ₂	eCO ₂	aCO ₂	eCO ₂	aCO ₂	eCO ₂	aCO ₂	eCO ₂	aCO ₂	eCO ₂
Needle (g)	34.9±2.0	35.0±2.0	29.9±1.9	32.7±1.9	28.3±1.9	32.7±1.9	18.9±2.0	20.9±2.0	15.2±2.0	17.4±1.9
Stem (g)	43.6±3.0	49.1±3.0	39.1±2.8	47.9±2.8	33.6±2.8	44.9±2.8	20.5±3.0	24.9±3.0	16.4±3.1	19.7±2.8
Root (g)	17.8±1.1	20.3±1.1	18.1±1.0	19.7±1.0	17.0±1.0	19.6±1.0	10.1±1.1	11.5±1.1	8.2±1.1	9.8±1.0
Total (g)	96.5±5.8	102.8±5.9	86.4±5.5	100.4±5.5	78.9±5.4	95.0±5.5	49.6±5.8	57.3±5.8	39.8±5.9	46.9±5.4

Note: Hybrid index 0 denotes 0% red spruce, hybrid index 100 denotes 100% red spruce, and hybrid indices 25, 50, and 75 represent the percentage of red spruce, with the remaining proportions being black spruce. aCO₂, ambient CO₂; eCO₂, elevated CO₂.

Table 5. Year 4 biomass ANOVAs, including source of variation, degrees of freedom (df), mean square values (MS), and P values.

Source of variation	df	Total biomass (g)		Needle biomass (g)		Stem biomass (g)		Root biomass (g)	
		MS	P value	MS	P value	MS	P value	MS	P value
Block	2	839.5	0.444	513.4	0.017	266.3	0.384	94.2	0.076
Hybrid (H)	4	40571.9	<0.001	4115.9	<0.001	10442.1	<0.001	1534.1	<0.001
CO ₂	1	8453.6	0.004	424.8	0.065	3601.4	<0.001	303.4	0.004
Hybrid × CO ₂	4	330.7	0.864	40.4	0.861	185.2	0.615	5.6	0.961
Family (hybrid)	10	6255.8	<0.001	681.9	<0.001	1588.0	<0.001	171.5	<0.001
Error	319	1030.5		123.9		277.6		36.3	

Note: P values < 0.05 are in bold type.

Although height rank among hybrid indices remains the same with age, there are significant relative changes with age. Height growth differences between BS and RS were 28%, 60%, and 35% for 1-, 4-, and 22-year-old trees (Johnsen et al. 1998), respectively, in favor of BS. Thus, there was a relative widening and then reduction of the differences between species with age. In a recent growth remeasurement of the study used by Johnsen et al. (1998), these now 41-year-old trees, across the hybrid continuum, have doubled their overall heights since the 22-year assessment (J.E. Major, A. Mosseler, K. Johnsen, M. Campbell, and J. Malcolm, unpublished). Pure BS is now only, on average, 4% taller than pure RS. This shift in rank with stand development was hypothesized by Johnsen et al. (1998) due to species differences in shade tolerance.

At ages 1 year (Major et al. 2003b), 4 years (this study), and 22 years (Johnsen et al. 1998), growth was greater with decreasing HI predominately in a linear fashion, indicating that genetic effects were additive with only minor positive or negative heterosis. These results are in contrast to the severe negative heterosis hypothesis presented by Manley and Ledig (1979). Their research, from the same set of controlled full-sib RS-BS crosses used in this study and in Johnsen et al. (1998), showed that interspecific hybrids were inferior in growth and assimilation (A) relative to both parent species. They hypothesized that it was the competitive inferiority of the hybrids under a number of environmental conditions (temperature and light) that kept the species from introgressing and thus kept the two species distinct. In the summer of 1994, when the trees were 20 years old, we found that intermediate HI classes had intermediate A (leaf area basis) or the same A (leaf mass basis) as those measured on pure BS or RS (Johnsen et al. 1998). In 1996, gas exchange was remeasured, and A of hybrids displayed a slightly positive heterosis, which again is in direct contrast to the severe hybrid inferiority presented by Manley and Ledig (1979). Interestingly, by age 41 years, there was a positive heterosis in growth (J.E. Major, A. Mosseler, K. Johnsen, M. Campbell, and J. Malcolm, unpublished).

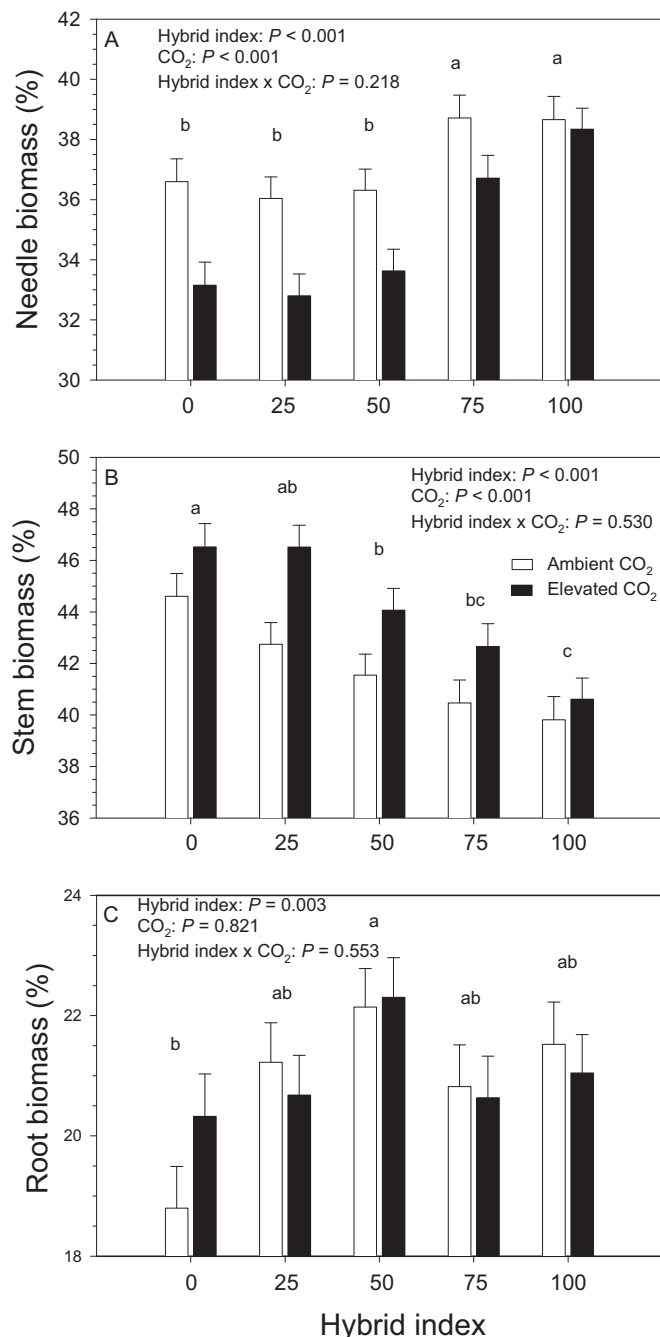
Furthermore, we have actually observed positive heterosis in seedling gas exchange. There were significant HI, CO₂, and HI × CO₂ interactions for assimilation of the 4-year-old trees growing in aCO₂ and eCO₂ (Major et al. 2014). Although assimilation under aCO₂ was a near-linear decline from BS to RS, assimilation under eCO₂ showed that HI 50 had the greatest values compared with the other four hybrid indices, including pure BS and RS, for a

positive heterosis averaging 45% measured at both 360 and 720 ppm (Major et al. 2014). This positive heterosis was due to HI 50 having the least assimilation downregulation but also the most positive total mass response to eCO₂ (more on this below).

Height and RCD were enhanced under eCO₂, consistent with other spruce findings (Marfo and Dang 2009). In terms of total biomass, we found an average 15.5% stimulation from aCO₂ to eCO₂, which is near the average seen in a meta-analysis of tree species (Ainsworth and Long 2005). Although there was not a significant CO₂ × HI interaction for total biomass, perhaps due to a significant family nested within HI effect, there was a significant relationship between A₃₆₀ (assimilation at aCO₂, 360 ppm) and aCO₂ total biomass among indices, but none was found between A₇₂₀ and eCO₂ total biomass (Major et al. 2014). As per the sink-source theory (Paul and Foyer 2001; Ainsworth et al. 2004; Fatichi et al. 2014), we examined the change in total biomass in response to eCO₂. Interestingly, we found that the percent increase in biomass was related to percent assimilation downregulation. Inversely, the relative (%) increase in A due to growth under eCO₂ treatment versus aCO₂ treatment after 3 years was 10.8%, 57.8%, 74.1%, 69.8%, and 58.7% for hybrid indices 0 (BS), 25, 50, 75, and 100 (RS), respectively, and there was a very strong, positive linear relationship among hybrid indices of percent biomass stimulation and percent assimilation increase (R² = 0.931; P = 0.008; Major et al. 2014). In another experiment in which three provenances each of BS and RS were used, eCO₂ resulted in a total biomass enhancement of 10.8% and 18.0% for BS and RS, respectively (Major et al. 2015). In another study examining four spruces (white (*Picea glauca* (Moench) Voss), black, red, and Norway (*Picea abies* (L.) Karst.)) in response to eCO₂, we found a similar total biomass increase for BS and RS of 5% and 17%, respectively (J.E. Major, A. Mosseler, K. Johnsen, M. Campbell, and J. Malcolm, unpublished). Although the assimilation downregulation (A_{dr}) effect in response to elevated CO₂ has been observed and A_{dr} has been related to a degree of increase in nonstructural carbohydrate (Centritto and Jarvis 1999; Griffin et al. 2000; Rogers and Ellsworth 2002), this is the first instance to our knowledge of a direct relationship among species-hybrids between A stimulation, change in assimilation, and biomass growth stimulation in relation to eCO₂.

It has been postulated that faster growing species will display a greater growth response to eCO₂ than slower growing species (Atkin et al. 1999; Poorter and Navas 2003). Our results, similar to those of others (Tjoelker et al. 1998b; Ghannoum et al. 2010), do

Fig. 4. (A) Percent needle mass (mean \pm SE), (B) percent stem mass, and (C) percent root mass under ambient CO₂ and elevated CO₂ treatments by hybrid indices 0, 25, 50, 75, and 100, where 0 and 100 represent BS and RS, respectively. Hybrids 25, 50, and 75 represent the percentage of RS, with the remaining proportion being BS. Different letters show significant hybrid index difference using Tukey's mean separation test ($P = 0.05$).



not support this theory: RS, which has among the slowest early growth rates compared with other spruces (Johnsen et al. 1998; Bauer et al. 2001; Major et al. 2003b, 2015), showed greater relative biomass increase than faster growing BS. Others have hypothesized that shade-tolerant trees respond to eCO₂ more than shade-intolerant trees. In a meta-analysis of 74 total biomass response ratios (eCO₂ to aCO₂) among woody seedlings and saplings, the response ratios were significantly greater for shade-tolerant species than for shade-intolerant species (Kerstiens 2001). This

phenomenon has subsequently been observed in physiological comparisons in long-term free-air CO₂ enrichment experiments (Mohan et al. 2007; Ellsworth et al. 2012). One potential explanation is that this response is an experimental artifact due to limitations to growth (insufficient soil volume, fertilization, or water stress) experienced by the typically faster growing shade-intolerant species. However, Kerstiens (2001) found that in five of 17 studies, shade-tolerant species had greater response ratios and grew as large as or larger than their less shade-tolerant counterparts. In our own study, our experiment was fertilized and watered on a regular basis with foliage N between 2.0% and 2.3%, well above normal field values (Major et al. 2007), and we observed no root binding as we used large felt root bags (25 cm diameter \times 30 cm deep), which were more than adequate for 4-year-old, generally slow-growing, northern conifers. Also, our hybrid experiment results showed no relationship between downregulation and total biomass under eCO₂ ($P = 0.489$) (Major et al. 2014). In fact, HI 50, with the lowest downregulation, had among the greatest total biomass under eCO₂. Thus, our findings are consistent with the hypothesis linking shade tolerance to response to elevated CO₂. The exact physiological or structural causes of our observed species differences in growth stimulation by eCO₂ remain uncertain but may be related to an ability to increase photosynthetic carbon capture even under different light conditions (Kerstiens 2001; Ellsworth et al. 2012).

Phenology

Bud flush occurred earlier in BS and was delayed linearly with increasing proportion of RS. In assessing seed germination of BS, RS, and their hybrids, the greater the BS content is, the earlier the germination is (Major et al. 2003c), a mirroring of these 4-year-old bud flush index results. In an assessment of vegetative and reproductive phenology in the spring of 2000 of the then 28-year-old mature BS–RS hybrid study, RS had a slightly and sometimes significantly greater bud flush index than hybrids, which were greater than BS, in direct contrast to our early age phenology and germination assessments (Major et al. 2003c, 2005). In this current study, there was no CO₂ effect on bud flush index, similar to findings for Sitka spruce (*Picea sitchensis* (Bong.) Carr.; Centritto et al. 1999) and BS (Johnsen and Seiler 1996; Bigras and Bertrand 2006). However, in a study in which we examined BS and RS provenances, we observed a moderate effect of eCO₂ ($P = 0.077$), as well as a significant species \times CO₂ treatment effect ($P = 0.040$) (Major et al. 2015).

Greater bud set activity index reflects a stronger propensity toward free growth, in this case proleptic free growth (von Wühlisch and Muhs 1986). Bud set activity index results display a two-tiered pattern, with BS, HI 25, and HI 50 showing greater values than RS and HI 75. BS has been shown in its early juvenile years to be capable of free growth when conditions are favorable (Pollard and Logan 1974). RS displayed little evidence of free growth, indicating that it transitions to pure fixed growth very early in its life cycle. Contrary to our expectations, aCO₂-grown seedlings had greater bud set activity index in late summer than eCO₂-grown seedlings, which was also observed in BS by Bigras and Bertrand (2006). This was also evident in a study using RS and BS provenances (Major et al. 2015). We envisaged that growth enhancement from increased atmospheric CO₂ would prolong shoot activity. However, the earlier bud set in eCO₂ may be explained by accelerated ontogenetic development, which was also found to suppress lammas growth in Sitka spruce (Centritto et al. 1999).

Allocation

Elevated CO₂ resulted in greater percent stem partitioning across all hybrid indices. Was this a direct CO₂ effect or was this due to increased tree size as observed by Pajtik et al. (2011)? Using the conventional ANOVA (eq. 1), percent stem allocation across

Table 6. Year 4 dry-matter partitioning ANOVAs, including source of variation, degrees of freedom (df), mean square values (MS), and *P* values.

Source of variation	df	Needle biomass (%)		Stem biomass (%)		Root biomass (%)		Shoot-to-root ratio		Growth efficiency (g·g ⁻¹)	
		MS	<i>P</i> value	MS	<i>P</i> value	MS	<i>P</i> value	MS	<i>P</i> value	MS	<i>P</i> value
Block	2	141.0	<0.001	186.1	<0.001	216.5	<0.001	12.9	<0.001	0.91	<0.001
Hybrid (H)	4	226.0	<0.001	301.0	<0.001	59.3	0.003	3.8	<0.001	1.43	<0.001
CO ₂	1	442.8	<0.001	407.2	<0.001	0.8	0.821	0.1	0.742	2.44	<0.001
Hybrid × CO ₂	4	25.5	0.218	19.1	0.530	11.2	0.553	0.7	0.504	0.18	0.148
Family (hybrid)	10	71.6	<0.001	86.5	<0.001	26.0	0.066	1.4	0.056	0.39	<0.001
Error	319	17.6		24.1		14.7		0.8		0.10	

Note: *P* values < 0.05 are in bold type.

Fig. 5. (A) Shoot-to-root ratio (mean ± SE) and (B) growth efficiency under ambient CO₂ and elevated CO₂ treatments by hybrid indices 0, 25, 50, 75, and 100, where 0 and 100 represent BS and RS, respectively. Hybrids 25, 50, and 75 represent the percentage of RS, with the remaining proportion being BS. Different letters show significant hybrid index difference using Tukey's mean separation test (*P* = 0.05).

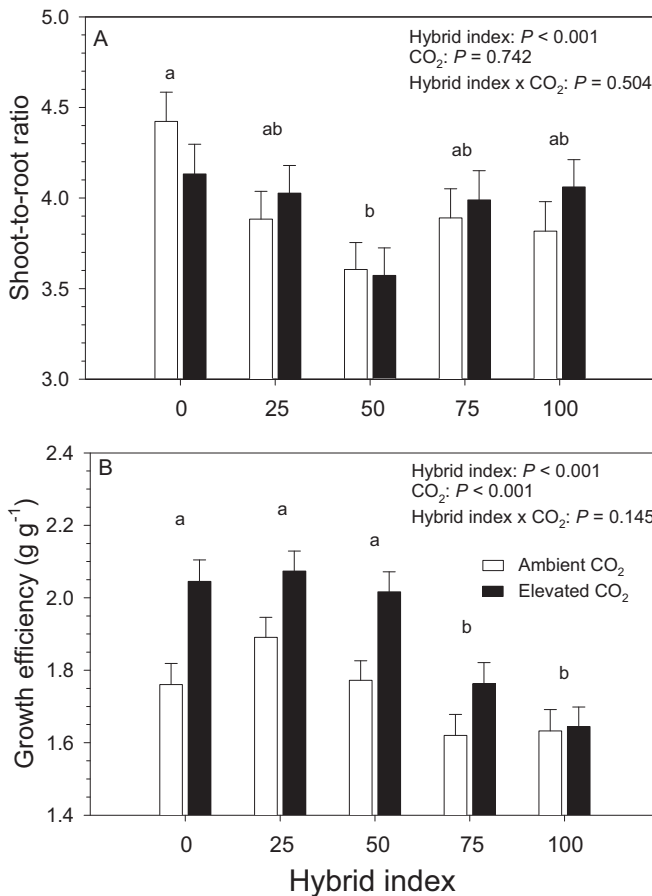
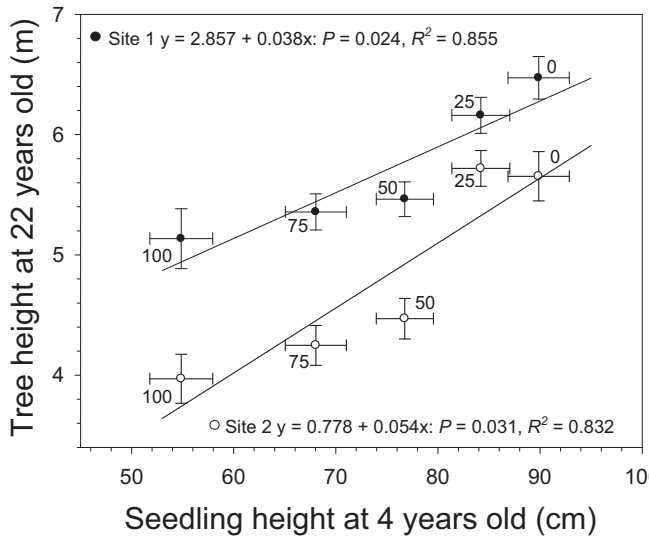


Fig. 6. Seedling total height at age 4 years under ambient conditions in relation to tree height at age 22 years on two sites by hybrid indices 0, 25, 50, 75, and 100, where 0 and 100 represent BS and RS, respectively. Hybrids 25, 50, and 75 represent the percentage of RS, with the remaining proportion being BS.



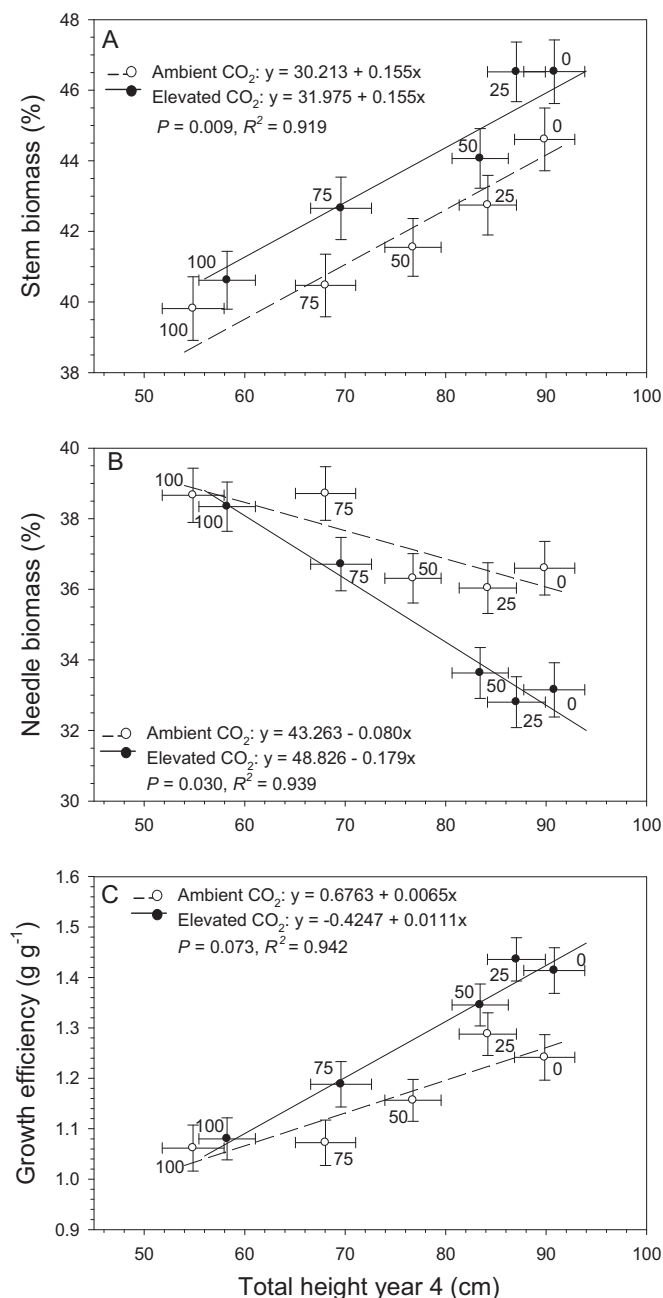
analysis, we found a significant tree size × CO₂ treatment interaction (Fig. 7B). Thus, the percent total seedling biomass made up by needles decreased with tree size depending on CO₂ treatment. The aCO₂ negative slope was approximately half that of eCO₂. Thus, at tree sizes ca. 55 cm, there was a small percent needle biomass difference between eCO₂ and aCO₂, but as tree size increased, the differences became progressively greater and steeper under eCO₂. As a resource becomes more available, in this case CO₂, the organ that obtains it generally is reduced, as is seen in roots with water (Major et al. 2012) and nutrients (Ostonen et al. 2005). In our study, a doubling of atmospheric CO₂ resulted in a decline in needle mass. Stems, which benefited from the reduction in needle mass, showed an increase in relative stem mass. Root percent mass did not see a change from the doubling of CO₂.

Growth efficiency, a measure of total stem mass in relation to total needle biomass, increased with increasing tree size, and there was a significant tree size × CO₂ treatment interaction, and thus, the magnitude of the GE increase with seedling size was dependent on CO₂ treatment (Fig. 7B). Elevated CO₂ had almost twice the GE slope as aCO₂. At tree size ca. 55 cm, there was a small difference in GE, but as the tree size increased, the differences became progressively greater for eCO₂ compared with aCO₂. Greater GE, whether by HI, tree size, or eCO₂, is often a result of greater net photosynthesis and greater partitioning to stem relative to roots or needles (Major et al. 2013).

hybrid indices increased, on average, from 41.8% to 44.1% for aCO₂ and eCO₂, respectively. Correcting for tree size (eq. 2), the CO₂ effect decreased from 2.3% to a 1.8% increase, thus a true CO₂ effect was present and not just an artifact of accelerated ontogeny. Similar results were found using 12 populations of RS and BS (Major et al. 2015).

Needle biomass increased from aCO₂ to eCO₂; however, percent needle mass allocation across hybrid indices decreased with tree height, an effect also noted by Claveau et al. (2005). Our decrease was 37.3% to 34.9% using conventional analysis. Using covariate

Fig. 7. Relationship of (A) stem mass (%), (B) needle mass (%), and (C) growth efficiency to total height at year 4 under ambient CO_2 and elevated CO_2 for hybrid indices 0, 25, 50, 75, and 100, where 0 and 100 represent BS and RS, respectively. Hybrids 25, 50, and 75 represent the percentage of RS, with the remaining proportion being BS.



Shoot-to-root ratio was greatest for BS, as expected for a species adapted to a highly competitive early successional environment (Major et al. 2003b, 2003c), whereas RS had among the lowest shoot-to-root ratios, as might be expected for a late-successional species. Surprisingly, HI 50 had not only among the greatest aboveground growth and belowground growth, but also the lowest shoot-to-root ratio, combining what could be considered the best of both species. Similar to Tjoelker et al. (1998b), we found that there was no consistent CO_2 effect on shoot-to-root ratio, which they observed across five boreal species. However, the response of the two species in the present study was the same as that

found for the 12 population BS–RS provenance study (Major et al. 2015). Not only did BS have a greater shoot-to-root ratio than RS, but the BS shoot-to-root ratio decreased with eCO_2 and RS shoot-to-root ratio increased with eCO_2 (species \times CO_2 interaction, $P = 0.028$; Major et al. 2015). This was also found by others for BS (Bigras and Bertrand 2006; Marfo and Dang 2009). These results indicate that “excess” resources were shifted to secondary early growth imperatives: eCO_2 allows more belowground growth for early successional BS but also allows more aboveground growth for late-successional RS.

Interestingly, the pure BS–RS hybrid (HI 50) responded even better than either BS or RS to eCO_2 by growing more, downregulating the least, having the greatest root allocation, and thus having the lowest shoot-to-root ratio. On average, productivity of HI 50 became progressively better than either parent species up to age 41 years. This positive hybrid response may result from an increase in heterozygous alleles resulting from interspecific crossing. Increased heterogeneity may give an organism a greater range of genetic diversity for improved adaptation potential. Does this mean that there is an increasing role for interspecific hybrids in an eCO_2 world, perhaps allowing gene flow or increased introgression for improved adaptation to a new environment? Although BS and RS hybridize in nature, there are strong ecological barriers to such hybridization to maintain species fitness (Major et al. 2005). However, “gene leakage” into other species may be a way to introduce novel genes into a species, especially useful during times of environmental change.

Conclusions

In summary, there was increasingly greater height with increasing proportion of BS in each successive year, indicating that height of this artificially produced population is strongly additive and affected by species contribution, with only minor positive or negative heterosis, in contrast to severe negative heterosis described by Manley and Ledig (1979). Height growth of 4-year-old seedlings grown under aCO_2 was highly correlated with height of 22-year-old field-grown trees of the same or similar crosses. Height growth differences between BS and RS were 28%, 60%, and 35% for 1-, 4-, and 22-year-old trees, respectively, in favor of BS, but these differences had narrowed considerably by age 41 years. This shift in rank with stand development is likely due to the species differences in shade tolerance. Bud flush was earliest in BS and declined linearly with increasing proportion of RS with no significant CO_2 effect. Percent stem mass increased under eCO_2 , a quarter of which was due to ontogeny. Conversely, percent needle mass had a significant negative relationship with increasing tree size, and there was a $\text{CO}_2 \times$ tree size interaction. Shoot-to-root ratio was greatest for BS, whereas RS had among the lowest. HI 50 had the greatest root mass allocation, lowest shoot-to-root ratio, and among the greatest total mass under eCO_2 . Growth efficiency increased with tree size and eCO_2 but decreased with HI. Contrary to the theory that faster growing trees would have a competitive advantage in eCO_2 , percent total biomass enhancement to eCO_2 was lowest for BS at 6.5%, greatest for HI 50 at 20.3%, and RS had 17.5%. This agrees with observations and the hypothesis that shade-tolerant species display a greater relative total biomass response to eCO_2 than shade-intolerant species.

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