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Black spruce family growth performance under ambient and elevated atmospheric CO_2

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Application. Elevated CO_2 typically increases tree seedling growth and has also been shown to modify component physiological processes. A potential consequence is that trees selected for fast growth rate under current CO_2 concentrations may not necessarily be superior under future higher CO_2 concentrations. As many forest tree species are long lived and gains via tree breeding are relatively slow, incremental, and directional, it is prudent to investigate the potential for genotype × atmospheric CO_2 environment interactions.

Abstract. Seedlings from 20 families of black spruce (*Picea mariana* (Mill.) B.S.P.), representing a large range in field productivity, were subjected to a greenhouse retrospective test under ambient (409 ppm – year 1, 384 ppm – year 2) and high (686 ppm – year 1, 711 ppm – year 2) atmospheric CO_2 environments. After one and two growth cycles, seedling height and diameter growth significantly increased under elevated CO_2. At the end of the experiment, seedlings grown under high CO_2 had a mean above-ground dry weight of 48.77 g as compared to 26.36 g for seedlings grown under ambient atmospheric CO_2. Families were a significant source of variation for all growth parameters. Although the family × CO_2 environment interaction was not a statistically significant source of variation in the analysis of variance, the correlation between greenhouse and 15-year field height growth was weaker ($r = 0.29$, $p = 0.2177$) under elevated CO_2 compared to ambient CO_2 ($r = 0.51$, $p = 0.0223$) following the first growth cycle. However, following the second growth cycle, greenhouse-field correlations were similar between the two CO_2 environments (ambient CO_2: $r = 0.55$, $p = 0.0115$; elevated CO_2: $r = 0.56$, $p = 0.0101$). Thus, with this set of families, growth performance ranking after two years appears relatively stable under ambient and elevated CO_2.

Introduction

Genetic variation in the growth rate of forest trees is a function of genetic variation in component physiological processes. Potentially important component physiological processes include photosynthesis, water use efficiency, cold tolerance, and dry matter partitioning (Greenwood and Volkaert 1992).

Atmospheric CO_2 concentrations are predicted to approximately double during the next century. Elevated CO_2 typically increases tree seedling growth and has also been shown to modify component physiological processes including those listed above (Eamus and Jarvis 1989, Margolis and Vézina 1990, Johnsen 1993, Samuelson and Seiler 1994). A potential consequence is that trees selected for fast growth rate under current CO_2 concentrations may not necessarily be superior under future higher CO_2 concentrations. As northern conifers are long lived and gains via tree breeding are relatively slow, incremental, and directional, it is prudent to investigate the potential for genotype × atmospheric CO_2 environment interactions.

In retrospective genetic tests, genetic entry seedling performance is compared to older field trial performance of closely related trees usually to examine for the efficacy of early selection (Lambeth 1980, Carter et al. 1992). Here, we describe results from a retrospective greenhouse test conducted under current ambient (approximately 350 ppm) and elevated (approximately 700 ppm) atmospheric CO_2 concentrations. Results indicate that after two seasons of growth the black spruce families used here displayed relatively similar growth responses to the levels of atmospheric CO_2 anticipated to occur in the 21st century.

Materials and methods

Open-pollinated families were derived from plus tree selections in New Brunswick, Canada. Field tests were established by the New Brunswick Tree Improvement Council (NBTIC) on six sites in New Brunswick, Canada in 1979. A total of 154 families were planted in 10 replications of four-tree row plots at each location. Five-, 10- and 15-year height data were supplied by NBTIC.

Stored seed was used from 20 of 40 families used in an earlier retrospective test (Carter et al. 1992). These 40 families were stratified into three groups based on 10-year height growth rankings across all six sites; 7, 6, and 7 families were randomly selected from the upper, middle, and lower thirds, respectively (NBTIC identification numbers are shown in Table 1). Seeds were sown on May 18, 1994 onto 30 mm Jiffy Forestry Pellets (Jiffy Products Ltd., North Bay, Ontario).

All 20 families were established in a greenhouse experiment using a split-plot design with three blocks. Sown seed was established using 10 pellet-family-row plots per chamber. CO_2 treatments comprised main-plots and families were sub-plots.

To achieve different CO_2 treatments, two polyethylene chambers were constructed over each of three greenhouse benches with each bench considered a block. Each block had one chamber randomly designated as ambient and the other as elevated CO_2. Chamber design is as described in Johnsen

Table 1. Family code, New Brunswick Tree Improvement Cooperative (NBTIC) I.D., and second growth-cycle sub-sample sizes under ambient (A) and elevated (E) atmospheric CO_2.

		Second Growth-cycle Sub-sample n	
Family Code	NBTIC I.D.	A	E
A	1-08	5	13
B	1-12	8	11
C	1-24	4	14
D	1-26	9	15
E	1-29	10	13
F	1-37	10	18
G	1-49	12	11
H	1-67	8	15
I	4-21	7	12
J	4-32	6	13
K	4-33	11	14
L	4-45	6	10
M	4-46	7	9
N	4-49	6	15
O	4-56	9	8
P	4-68	9	11
Q	4-71	7	12
R	4-77	9	13
S	4-80	5	11
T	4-84	4	13

and Seiler (1996). Mean CO_2 concentrations (based on one measurement per chamber per hour) across all blocks for the ambient and elevated CO_2 treatments, respectively, were 410 ± 21(SD) ppm and 688 ± 55 ppm for the first growth cycle, 414 ± 22 ppm and 695 ± 45 ppm for the dormancy period between growth cycles, and 385 ± 53 ppm and 711 ± 68 ppm for the second growth cycle.

Following germination, seven sub-sample seedlings per family per chamber were transplanted into 7 L pots containing a 3:1 peatmoss:vermiculite mixture. The seven seedlings per family were then randomly redistributed within each chamber as non-contiguous plots. Seedlings were fertilized regularly with 20, 8, 20 N, P, K plus micronutrients (Plant Product Co. Ltd., Brampton, Ontario). Light was supplemented using sodium vapour lamps until July 19, 1994 (16 h photoperiod). Seedlings set bud in September and were measured for total height, diameter, branch number, and individual branch length in late October, 1994. Foliage was removed from four extra seedlings per chamber for determination of foliar N concentrations using standard Kjeldahl techniques.

Following this, from November 1, 1994 until January 16, 1995, minimum greenhouse temperatures were reduced to 5 °C for the purpose of permitting dormancy release. Due to warm autumn and early winter conditions, mean temperature across all chambers was 11.35 ± 2.74 °C. During December, an extra seedling from each chamber was moved to a heated greenhouse to examine time to budbreak which occurred within 21 days for all seedlings. During the dormancy period, watering was inadvertently discontinued from November 18 until January 6.

On January 16, greenhouse minimum temperature was raised to 25 °C to initiate the second growth cycle. Seedlings were fertilized as in 1994. Light was supplemented using sodium vapour lamps until June 16, 1995 (16 h photoperiod). Over the next several weeks it became apparent that lack of watering during the dormancy period resulted in significant mortality. In February, only healthy trees (less than 10% brown needles) were retained in the experiment. Seedlings were transferred across blocks within CO_2 treatments to facilitate near-equal family sub-sample sizes per block. Seedlings were grown through the summer of 1995 Following budset, trees were measured for total height and diameter on October 18. Above-ground biomass was harvested and dry weights were measured following drying to a constant weight at 65 °C. Foliage was sampled from four randomly selected seedlings per chamber for determination of foliar N concentrations using standard Kjeldahl techniques.

Seedling height, root collar diameter, branch number, mean branch length, and total branch length following the first growth cycle (family sub-sample n = 7 seedlings per block) and seedling height, root collar diameter and above-ground dry weight following the second growth cycle (family sub-sample n shown on Table 1) were analyzed via analysis of variance (ANOVA) using a split-plot design. Family means per CO_2 chamber were used for analysis. Pearson correlation coefficients (r) using family means, by CO_2 environment, were used to assess the relationship between greenhouse seedling traits and 5-, 10- and 15-year field height. To assess the potential influence that seedling mortality had on final results, correlations were examined between first year family means using all seedlings with first year family means based on only surviving seedlings.

Results

First growth cycle

Carbon dioxide environment was a significant ($p \leq 0.05$) source of variation for all growth parameters. Seedlings grown under elevated CO_2, compared to seedlings grown under ambient CO_2, were 20% greater in height, 16% greater in root collar diameter and had 78% more branches, 20% longer average branch length, and a 94% greater cumulative branch length. Seedlings grown under ambient CO_2 had a mean foliar N concentration (3.68%) significantly greater ($p = 0.0418$) than elevated CO_2-grown seedlings (3.49%).

There was also significant variation among families in all growth parameters measured ($P \leq 0.001$). Family height means ranged from 9.4 to 13.4 cm under ambient CO_2 and from 12.2 to 15.8 cm under elevated CO_2. Family × CO_2 environment was not a significant source of variation in height ($p = 0.8373$) or diameter ($p = 0.4171$).

Under ambient CO_2, greenhouse family mean height, branch number, and total branch length were significantly and positively correlated to family mean field height (Table 1). In contrast, correlations between family means from seedlings grown under elevated CO_2 and field family means were weaker and not statistically significant (Table 2). The relationships between family mean greenhouse seedling height with family mean 15-year field height, by CO_2 treatment, are shown in Figure 1A.

Second growth cycle

In February, only healthy trees were retained in the experiment resulting in 62% loss of ambient CO_2 trees and 40% loss in elevated CO_2 trees. Seedlings were transferred across blocks within CO_2 treatments to facilitate near-equal family sub-sample sizes per block. The resulting mean sub-sample size per family per block was 2.5 and 4.2 for ambient and elevated CO_2 grown seedlings, respectively. Overall sub-samples per family are shown in Table 1. A plot of first year family means using the full data set versus means calculated using only the surviving seedlings indicate that mortality had minimal effects on first year family ranking in both ambient and elevated CO_2 environments (Figure 2). On average, larger seedlings survived; mean first growth season dry weights of surviving seedlings were 0.65 and 0.73 g heavier than pre-mortality seedlings, for ambient and elevated seedlings, respectively.

At the end of the experiment, seedlings grown under elevated CO_2 were significantly larger than seedlings grown under ambient CO_2. Mean seedling height was 67.8 cm for elevated CO_2 grown seedlings versus 52.4 cm for ambient CO_2 grown seedlings ($p = 0.0202$). Mean seedling diameter was

Table 2. Pearson correlations (r) between greenhouse traits and field height, by CO_2 environment, for 20 open-pollinated black spruce families. P-values are shown in parentheses. P-values ≤ 0.05 are shown in bold.

		Field Trait		
Greenhouse Trait	CO_2 Environment	5-year height	10-year height	15-year height
Height (yr 1)	*Ambient*	0.51 (0.0225)	0.52 (0.0182)	0.51 (0.0223)
	Elevated	0.33 (0.1562)	0.24 (0.3083)	0.29 (0.2177)
Diameter (yr 1)	*Ambient*	0.28 (0.2394)	0.30 (0.1937)	0.29 (0.2143)
	Elevated	0.29 (0.2169)	0.19 (0.4196)	0.20 (0.3901)
No. of branches (yr 1)	*Ambient*	0.58 (0.0072)	0.46 (0.0414)	0.37 (0.0152)
	Elevated	0.40 (0.0766)	0.29 (0.2177)	0.25 (0.2858)
Avg. branch length (yr 1)	*Ambient*	0.11 (0.6381)	0.06 (0.7869)	0.03 (0.8893)
	Elevated	0.10 (0.6609)	0.01 (0.9775)	0.03 (0.9140)
Total branch length (yr 1)	*Ambient*	0.52 (0.0198)	0.41 (0.0696)	0.37 (0.1052)
	Elevated	0.36 (0.1239)	0.29 (0.2117)	0.25 (0.2858)
Height (yr 2)	*Ambient*	0.48 (0.0305)	0.55 (0.0129)	0.55 (0.0115)
	Elevated	0.32 (0.1735)	0.45 (0.0449)	0.56 (0.0101)
Diameter (yr 2)	*Ambient*	0.49 (0.0276)	0.42 (0.0637)	0.33 (0.1555)
	Elevated	0.50 (0.0255)	0.49 (0.0449)	0.44 (0.0540)
Top dry weight (yr 2)	*Ambient*	0.60 (0.0051)	0.46 (0.0417)	0.47 (0.0359)
	Elevated	0.55 (0.0123)	0.57 (0.0093)	0.61 (0.0046)

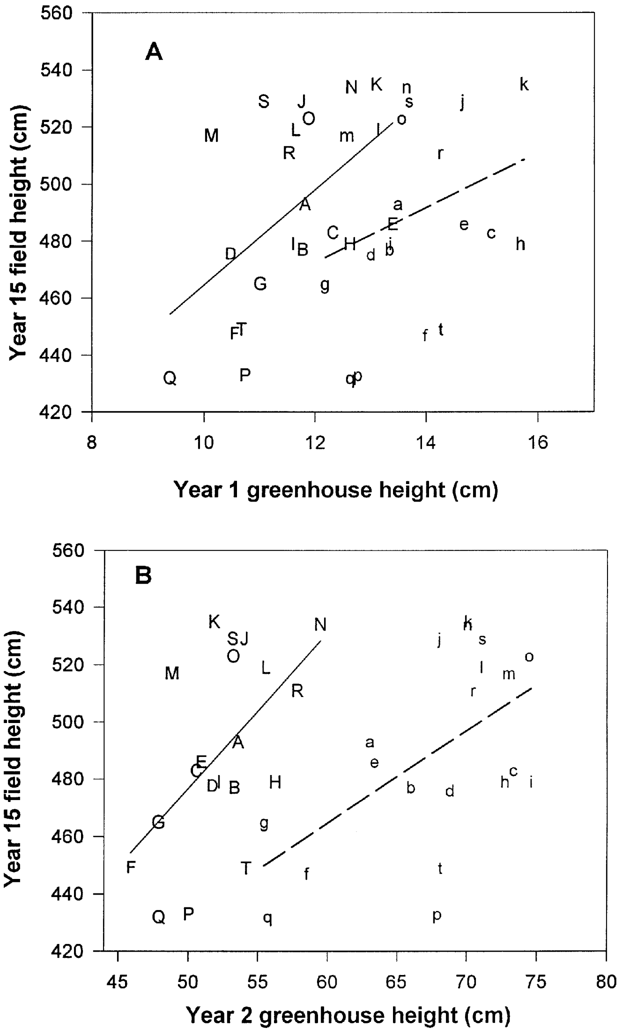

Figure 1. Relationship between 15-year field mean tree height with (*A*) first-year and (*B*) second-year greenhouse mean height for seedlings of 20 open-pollinated black spruce. Each letter represents a family mean after growth under ambient (upper case) or elevated (lower case) CO_2 with family codes as described in Table 1.

12.85 mm for elevated CO_2 grown seedlings versus 9.36 mm for ambient CO_2 grown seedlings ($p = 0.0114$). Mean seedling dry weight was 48.77 g for elevated CO_2 grown seedlings versus 26.35 g for ambient CO_2 grown seedlings ($p = 0.0202$). Seedlings grown under ambient CO_2 had a mean foliar N concentration 2.77% compared to 2.53% for elevated CO_2 grown seedlings although the difference was not statistically significant ($p = 0.3982$).

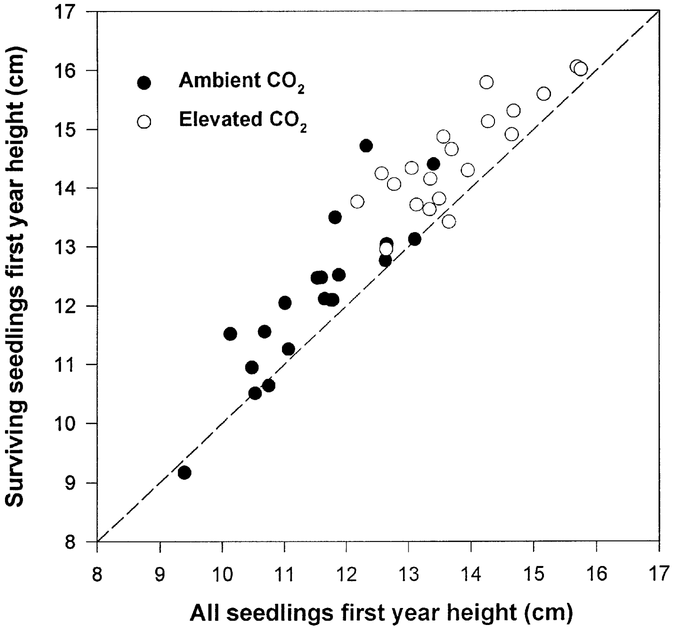

Figure 2. Relationship between first-year mean height based on all experimental seedlings versus first-year mean height based on surviving seedlings in year two for seedlings of 20 open-pollinated black spruce families grown under ambient CO_2 and elevated CO_2. Dashed line is a 1:1 ratio.

Families were a significant source of variation in height (p = 0.0133), diameter (p = 0.0093), and above-ground dry weight (p = 0.0103). Family above-ground dry weight means ranged from 20.69 g to 30.84 g under ambient CO_2 and from 35.10 to 62.14 g under elevated CO_2. Family × CO_2 environment was not a significant source of variaition with p = 0.7695, 0.1786, and 0.5895 for seedling height, diameter and above-ground dry weight , respectively.

Trends, magnitude and statistical significance of correlations between second year growth traits with field performance were similar between CO_2 environments (Table 1). The relationships between family final mean greenhouse seedling height with family mean 15-year field height, by CO_2 treatment, are shown in Figure 1B.

Discussion

After one growth cycle, family mean greenhouse height of seedlings grown under ambient CO_2 was significantly correlated to family means from trees measured after 5, 10, and 15 years of growth in the field. After two growth cycles, seedling growth under both CO_2 environments was similarly positively correlated with field performance. Carter et al. (1992) observed better correlations using a larger set of families (including the same ones used here). Sulzer et al. (1993) also found moderate correlations ($r = 0.48$) between greenhouse family mean height and 10-year field height using another series of NBTIC families. Early testing of black spruce is currently used operationally to aid in assortative mating among clones (Adams 1993).

If first growth cycle trends had continued and amplified over time, this would have suggested that the distinction between good and poor field performers was becoming clouded when seedlings were grown under elevated CO_2. However, correlations between seedling and field results became almost identical between the two CO_2 environments following the second growth cycle. Obviously, we are uncertain as to the ultimate influence of second growth cycle mortality on final results although first growth cycle results were similar regardless of whether all seedlings or only surviving seedlings were included in the analysis.

There is a growing body of literature where some form of genetic treatment (provenance, family or clone) has been tested for differential response to elevated CO_2. In a growth chamber study, differences in growth among northern Ontario, Canada provenances of black spruce were detected when seedlings were grown under 350 ppm CO_2 but not at 700 ppm CO_2 (Johnsen 1994). However, it has been more typical for such experiments to indicate no genotype × atmospheric CO_2 environment interaction (Cantin et al. 1993, Grulke et al. 1993, Johnsen 1993, Wang et al. 1994, Johnsen and Seiler 1996) or that the interaction was not fully expressed in growth but only in some component trait (Conroy et al. 1990, Murray et al. 1994, Wang et al. 1995).

As in the study presented here (except for the unplanned drought period), most of the experiments cited above have been conducted using conditions mostly non-limiting for growth. Thus, the seedlings typically did not experience stresses such as heat, cold, and drought that are common to field trees. Indeed, other climate change environmental factors may themselves, or in combination with elevated CO_2, interact with genotype in the future. Also, due to experimental limitations all genotype × atmospheric CO_2 interaction studies have been conducted with seedlings in growth chambers or greenhouses. These factors complicate the extrapolation of results to field growth situations and/or older trees. As in a study using diverse provenances of black spruce (Johnsen and Seiler 1996, Johnsen et al. 1996), some confidence is gained in the extrapolation of the results of this present study as greenhouse means were positively correlated with field performance of older trees of the same genetic entries.

Results of this study and most of the studies cited above suggest genotype × atmospheric CO_2 environment interactions might not be a large concern to tree breeders. Thus, CO_2 responses, per se, probably won't represent another factor to incorporate into selection programs. However, genetic variation in responses to abiotic conditions associated with potential climate change have been well documented (Flanagan and Johnsen 1995, Johnsen and Major 1995, Major and Johnsen 1996) and the ability to screen for stability across environments is improving with the development of technology. At the very least, broad genetic bases are still a prudent investment so that tree breeding programs can achieve satisfactory gains in future, potentially changing environments.

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