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Elevated CO₂ will not select for enhanced growth in white spruce despite genotypic variation in response

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Abstract

The effect of elevated atmospheric CO_2 on plant growth has been well-documented in the literature. However, few studies have quantified intra-specific genetic variation in growth response, and the potential for natural and artificial selection to act upon this variation. This study examined intra-specific variation in growth response to elevated CO_2 in 29 genotypes of white spruce (*Picea glauca*), a widely distributed and economically important species of the boreal forest region in North America. Trees were exposed to either ambient $(370 \,\mu L \,L^{-1})$ or twice-ambient CO_2 (740 $\mu L \,L^{-1}$). The opportunity for selection (i.e. the relative variation in fitness) was determined at low and high CO_2 levels with size as a measure of fitness and heritability of this variation determined. There was considerable variation among the genotypes in size and response to elevated CO_2 . The increase in mass at elevated CO_2 ranged from 23% to 108% depending upon genotype. In spite of this variation, the genetic correlation between the two environments approached unity, as genotype variance was much greater than the genotype × CO_2 variance. Elevated CO_2 had no effect on heritability of the size-related traits we examined, and either had no effect on opportunity for selection, or decreased it. We conclude that selection at elevated atmospheric CO_2 is unlikely to increase mean plant size in white spruce beyond that observed for present day populations grown at elevated CO_2 , despite the substantial genetic variation in CO_2 response displayed by this species.

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Zusammenfassung

Der Effekt einer Erhöhung des atmosphärischen CO₂-Gehaltes auf das Pflanzenwachstum ist in der Literatur gut dokumentiert. Es gibt jedoch wenige Untersuchungen, die eine intraspezifische genetische Variation in der Wachstumsreaktion und die Möglichkeiten einer artifiziellen oder natürlichen Selektion, die auf dieser Variation beruht, quantifizieren. Diese Untersuchung betrachtete die intraspezifische Variation in der Wachstumsreaktion auf erhöhte CO₂-Gehalte von 29 Genotypen der Weißfichte (*Picea glauca*), einer weit verbreiteten und ökonomisch bedeutenden Art der borealen Waldregion in Nordamerika. Die Bäume wurden einer Umgebung mit einfachem (370 iL L⁻¹) oder doppeltem (740 iL L⁻¹) CO₂ ausgesetzt. Die Möglichkeit einer Selektion (d. h. die relative Fitness) wurde für die niedrigen und hohen CO₂-Gehalte bestimmt, indem die Größe als ein Maß für die Fitness und die Erblichkeit dieser Variation bestimmt wurde. Es gab eine beträchtliche Variation zwischen den Genotypen in der

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Größe und der Reaktion auf erhöhte CO₂-Gehalte. Die Massenzunahme bei erhöhten CO₂-Gehalten variierte zwischen 23% und 108% in Abhängigkeit vom Genotyp. Trotz dieser Variation näherte sich die genetische Korrelation zwischen den beiden Umwelten der Eins, weil die Genotypenvarianz sehr viel größer als die Varianz Genotyp \times CO₂ war. Ein erhöhter CO₂-Gehalt hatte keinen Effekt auf die Erblichkeit der untersuchten größenbezogenen Eigenschaften, und hatte entweder keinen Effekt auf die Möglichkeit einer Selektion oder verminderte ihn. Wir schließen daraus, dass es unwahrscheinlich ist, dass eine Selektion bei erhöhtem CO₂-Gehalt die mittlere Baumgröße der Weißfichte über diejenige der heutigen Populationen, die bei erhöhtem CO₂-Gehalt wachsen, erhöht, obwohl es eine deutliche genetische Variation in der Reaktion auf CO₂ bei dieser Art gibt. (C) 2008 Gesellschaft für Ökologie. Published by Elsevier GmbH. All rights reserved.

Keywords: Adaptation; Global change; Carbon sequestration; Genotype × environment interactions; Boreal forest; *Picea glauca* (Moench (Voss))

Introduction

Over the past century, the concentration of atmospheric CO₂ has increased by about $100 \,\mu L \,L^{-1}$. The current concentration is higher than at any time in the past 650,000 years (IPCC, 2007), and it is predicted that CO_2 levels will double before the end of this century (Cao & Woodward, 1998). This increase in atmospheric CO₂ will likely have a profound impact on plant function and distribution, ultimately affecting populations, communities, and the biosphere as a whole (Körner, 2000; Norby, 2004). Although a considerable amount is now known about the effects of elevated CO₂ on plant function, interactions among plants, and interactions between plants and other organisms, relatively little is understood about the extent to which elevated CO₂ may influence plant evolution (e.g. Lau, Shaw, Reich, Shaw, & Tiffin, 2007; Steinger, Stephan, & Schmid, 2007; Wieneke, Prati, Brandl, Stöcklin, & Auge, 2004). However, previous studies have demonstrated that there is substantial genetic variation in response to CO_2 in at least some species (e.g. Lindroth, Roth, & Nordheim, 2001; Wang, Lechowicz, & Potvin, 1994). In fact, based on the available evidence it has been suggested that there may be as much variation in CO₂ response within—as there is among—species (Thomas & Jasienski, 1996).

Understanding within-species variation in response to CO_2 is critical for developing models and predictions of plant population and community responses to future environments (Bazzaz, Jasienski, Thomas, & Wayne, 1995; Steinger et al., 2007; Ward & Kelly, 2004). If the large differences in CO_2 response that have been observed within some species are heritable, current predictions of how elevated CO_2 may impact future growth and productivity could be in serious error. The actual growth response may be much greater than estimates based on the response of current populations if selection favours genotypes that respond more favourably to elevated CO_2 . In turn, this will impact our estimates of the amount of carbon that will be

sequestered by ecosystems and to what extent the biosphere will buffer atmospheric CO_2 levels. Furthermore, the question of whether an enhanced growth response to CO_2 can be selected for is important to plant breeders attempting to maximize yield in a future high CO_2 world.

In spite of its crucial importance, few studies have quantified intra-specific genetic variation in CO₂ response and only a small fraction of these examined trees (see literature reviewed in Lau et al., 2007). Forests contain about 80% of the total above-ground carbon (Dixon et al., 1994); as much of this carbon is stored in wood which decomposes slowly, woody plants play a critical role in carbon sequestration. It is also known that tree species exhibit a greater photosynthetic enhancement to elevated CO₂ than other plants (Ainsworth & Rogers, 2007), and so it is particularly important that we understand to what extent selection at elevated CO₂ may enhance the carbon uptake of woody species.

Most studies examining intra-specific genetic variation in CO₂ response have utilized a relatively small number of genotypes or genetic lines, making it difficult to draw general conclusions regarding the extent and importance of this variation. Further, with only a few exceptions (Bazzaz et al., 1995; Lau et al., 2007; Steinger et al., 2007; Ward & Kelly, 2004; Wieneke et al., 2004), the extent to which this variation may be acted upon by selection has not been quantified, and all of these studies deal with herbaceous plants. The presence of significant genotype \times environment interactions in response to CO_2 does not necessarily mean that natural selection can effectively act on this variation. If this variation accounts for only a small proportion of the total variance in performance, or does not change the performance rankings of the genotypes, it will have little impact on the genetic composition of the population (Lynch & Walsh, 1998).

Picea glauca (Moench) Voss (white spruce) is a common conifer species in the boreal forest region of North America (Grossnickle, 2000). It can be found in a

wide range of sites differing in soil fertility, moisture availability and temperature regime. As a source of pulp and lumber, white spruce plays an important role in the Canadian forest industry, and is used in tree breeding and selection programs for reforestation. The boreal forest has played a critical role in global carbon storage in the recent past and has the potential to continue to play an important role in the future (Hyvönen et al., 2007).

Our objectives in the present study were twofold: (1) determine the extent to which genotypes of white spruce differ in their growth response to elevated CO_2 (i.e. the extent to which elevated CO_2 increases growth), and (2) determine if this genotypic variation can be acted upon by natural or artificial selection. To achieve these objectives, we grew 29 clonal lines of white spruce at two levels of CO₂ and assessed effects on three sizerelated traits: height, diameter and total biomass. We addressed the first objective by adopting the approach used by the majority of previous studies and tested for the presence of genotype \times environment interactions. To address the second objective, we used the tools of quantitative genetics to determine the genetic correlation between performance at low versus high CO_2 , and the range of phenotypic variation and its heritability in these two environments.

Materials and methods

Twenty-nine white spruce (P. glauca (Moench)Voss) genotypes were obtained from the New Brunswick Tree Improvement Council breeding population. Male and female parents were collected from various locations in the province of New Brunswick, Canada and the adjacent state of Maine, USA (see Appendix A: Table A1). One individual offspring from each cross was cultured using somatic embryogenesis (Park, Pond, & Bonga, 1994). Prior to propagation, all genotypes were grown in a common garden and experienced the same environment. Further, the somatic cells used to clone the genotypes received identical "provisioning" in that the artificial embryogenesis procedure was the same for each genotype. Therefore, maternal effects in this experiment should be minimal and any differences among clones can be largely attributed to genetic differences.

Two-to-three cm-sized germinants were transplanted to multipots (67 cavities per tray, volume 57 mL) (Can-Am Division, Ropak Canada Inc., Springhill, NS) filled with a peat-based potting soil in April 2005. In August 2005, plants were placed in an outside nursery to harden and over-winter naturally. Individuals were transplanted to plastic 2.83 L pots filled with a commercial potting mixture of peat and vermiculite on 8–10 May 2006. Due to variation in culturing success, number of individuals per genotype varied between 12 and 35 (see Appendix A: Table A1).

Four glasshouse compartments at the K.C. Irving Environmental Centre, Acadia University (Wolfville, NS) were used to impose CO₂ treatments. Two compartments were maintained at ambient $(370 \,\mu L \, L^{-1})$ and two at elevated CO_2 (740 µL L⁻¹). Each compartment $(3.58 \text{ m} \times 4.27 \text{ m})$ faced south, had an independent environmental control system (Argus Controls, White Rock. BC) and was cooled by a geothermal heat exchange unit that provided excellent temperature control (+0.5 °C). External air temperature was monitored in real time and used as the temperature set point for the compartments. Relative humidity was maintained at a minimum level of 65%. Carbon dioxide levels were adjusted by injecting pure CO₂ using a solenoid valve controlled by an adaptive control loop (Argus Controls, White Rock, BC). Plants received natural light levels and photoperiod. The experiment was conducted between 10 May and 23 September 2006.

Individuals of each genotype were randomly assigned to CO₂ level, glasshouse compartment and position within compartments. To avoid confounding potential differences among compartments with CO₂ treatment, plants together with their corresponding CO₂ treatment were rotated among compartments nine times over the course of the experiment. All plants spent approximately equal amounts of time in each of the four compartments. Pots were fertilized at 2-week intervals with water-soluble 20:20:20 (N:P:K) fertilizer with micronutrients (Plant Prod, Brampton, ON) at a concentration of 100 ppm N. Plant performance was assessed by measuring final plant height (+0.1 cm), basal stem diameter (± 0.01 mm) with an electronic calliper (Fisher Scientific Company, Ottawa, ON) and total mass, including roots, after drying at 50 °C to a constant weight (+0.001 g).

To determine if differences among genotypes in performance measured under greenhouse conditions were reflective of performance in the field, we used data on the performance of 19 of the 29 genotypes that were available from a previous experiment. The 19 genotypes were planted in field tests established in 2000 and 2002 at 6 different sites across New Brunswick and Nova Scotia (4 in 2000, 2 in 2002) in which over 600 genotypes were evaluated. At each site, the genotypes were replicated 10 times as single-tree plots in a randomized block design at $2 \text{ m} \times 2 \text{ m}$ spacing. Height growth was measured to the nearest centimetre after 5 field-growing seasons. The genotype and overall site averages were combined for each test year. Genotype averages were calculated and expressed in terms of standard deviations of the overall mean.

A split plot analysis of variance using the general linear model (GLM) procedure of SAS version 9.1 was performed to examine the effects of CO_2 , genotype, and

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their interaction on the dependent variables in the greenhouse experiment. Main plots were the groups of plants rotated among glasshouse compartments. Carbon dioxide level was a fixed factor in the analysis, while genotype and main plots were considered random. The expected mean square option of the GLM procedure was used to determine appropriate error terms for testing effects in this mixed model. To correct for potential differences between CO₂ treatments in initial plant size, plant height at the start of the experiment was used as a covariate (nested within genotypes) in analyses for final biomass and plant height, and initial diameter was used as a covariate in the analysis of final diameter. Normal probability plots of the residuals were examined to determine if data were normally distributed. The natural logarithm and square root transformations were used for the biomass and height data, respectively.

Phenotypic correlations between the three different measures of performance (biomass, height and diameter) and between performances at low versus high CO_2 were assessed by computing Pearson's product moment correlation using genotype means. The correlation between performance in the glasshouse and field was similarly determined using means for the 19 genotypes involved in both experiments. To assess the extent to which there were changes in genotype ranking between CO_2 levels, the original data were ranked transformed and a two-way analysis of variance performed to determine if there was a significant interaction between CO_2 and genotype (Inman, 1974; Thomas & Bazzaz, 1993).

The mixed model procedure (MIXED) of SAS was used to estimate broad sense heritability for each dependent variable measured in the glasshouse experiment. Separate analyses were conducted for each CO_2 level with genotype, as the only effect in the model. Broad sense heritability (H^2) was calculated as genotype variance divided by total variance. The "opportunity for selection" (I) which is a measure of relative variation in fitness (Arnold & Wade, 1984), was calculated as the square of the coefficient of variation for all individuals, regardless of genotype, at a given CO₂ level. "Response to selection" (R) is the expected rate of increase in fitness (Arnold & Wade, 1984) and was calculated as the product of H^2 and I. This analysis assumed fitness was related to size. As tree breeders select directly upon size (Mullin and Park, 1994), this is a valid assumption for artificial selection. Studies of natural selection (e.g. Steinger et al., 2007) have also used size as a measure of fitness because it is an important determinant of survival and reproductive output (e.g. Dolezal, Srutek, Hara, Sumida, & Penttila, 2006). However, size is not necessarily closely correlated with fitness in all species (e.g. Bazzaz et al., 1995), therefore our estimate of R may overestimate the extent to which natural selection may act upon size. A bootstrap procedure

(Efron & Tibshirani, 1993) conducted in SAS with the author's code was used to estimate the standard error and hence, confidence intervals, for H^2 , I and R. The original data set was randomly sampled with replacement within genotypes/treatments to get 1000 data sets with the same number of replicates for each genotype/ treatment combination as the original data set. H^2 , I and R were then calculated as described above for each data set. The standard deviation of these 1000 samples was taken as the standard error of the original estimate.

The genetic correlation between performance at the two CO_2 levels was calculated by dividing the phenotypic correlation (see above) by the square root of the product of clonal heritability in the two CO_2 environments (Burdon, 1977). Clonal heritability is used for selection of clones (i.e. genets) by tree breeders in contrast to broad sense heritability which is used for individual tree (i.e. ramet) selection. Clonal heritability was calculated as the variance component for line divided by the sum of the component for line and the component for residual variance divided by the line expected mean square (Mullin & Park, 1992).

Results

The genotypes examined in this study differed markedly in performance regardless of trait used to assess size (Table 1). Biomass of genotypes grown at low CO_2 ranged from 1.65 to 5.76 g (Fig. 1), while height ranged from 9.7 to 25.5 cm and stem diameter from 3.8 to 6.9 mm (see Appendix A: Figs. A1 and A2). Genotype ranking with regard to performance at low CO₂ varied somewhat depending upon trait, but in general, genotypes that performed well in terms of one measure usually did well when assessed with another; Pearson's correlations varied from 0.684 to 0.786 (Table 2). Performance of genotypes within the glasshouse at low CO_2 was also correlated with performance in the field; Pearson's correlations varying from 0.371 to 0.596 (Table 2). Correlations between glasshouse and field were strongest when height was used to assess size.

The effect of elevated CO_2 on biomass, height and diameter varied among genotypes (Table 1). Depending upon genotype, the increase in biomass at elevated CO_2 as a percentage of that at ambient CO_2 ranged from 23% to 108% (Fig. 1, Table 1), while increases in height ranged from 4% to 48%. In the case of stem diameter, the effect of elevated CO_2 varied from a non-significant decrease of 6% to an increase of 32% depending upon genotype.

There were a number of apparent crossovers in the ranking of genotypes between CO_2 levels regardless of which measure of performance was used. Most of the genotypes experienced relatively minor shifts in rank

Source of variation	Total biomass			Stem height			Stem diameter		
	Sum of squares	df	<i>p</i> -Value	Sum of squares	df	<i>p</i> -Value	Sum of squares	df	<i>p</i> -Value
CO ₂	37.8	1	0.0909	40.6	1	0.3904	66.0	1	0.0254
Main plot error	7.9	2		68.7	2		3.5	2	
Genotype	167.0	28	< 0.0001	349.1	28	< 0.0001	370.4	28	< 0.0001
Genotype \times CO ₂	3.7	28	0.0061	11.6	28	0.0044	31.4	28	0.0299
Covariate (Genotype)	96.6	29	< 0.0001	65.3	29	< 0.0001	229.0	29	< 0.0001
Residual error	51.3	701		156.0	705		501.7	706	

Table 1. Analysis of variance results for the effects of atmospheric CO_2 and genotype on the growth of white spruce

To achieve normality, biomass and stem height data were transformed prior to analysis by taking either the natural logarithm (biomass) or square root (height) of the original values. The probability (p) values in bold represent effects that were significant at p < 0.05.



Fig. 1. The effect of ambient versus elevated CO_2 on total biomass of 29 genotypes of white spruce. See Appendix A: Table A1 for key to genotype designations.

however a few genotypes exhibited more substantial shifts (Fig. 1). For example, genotype U was ranked eighth in total biomass at ambient CO_2 , but rose to number one at elevated CO_2 . In spite of these individual shifts in rank, the overall phenotypic correlation between performances at low versus high CO_2 was high for all three measures of size with Pearson's correlation

coefficient ranging between 0.745 and 0.931 (Table 2). The two-way analysis of variance on ranks revealed no significant change in genotype ranking between CO_2 levels for biomass (p = 0.3547 for the $CO_2 \times$ genotype interaction), height (p = 0.1559), or stem diameter (p = 0.3705). The correlations between performance at high CO_2 in the glasshouse and performance in the field were similar to, or higher than, the correlations between performance in the field and that at ambient CO_2 in the glasshouse (Table 2).

Broad sense heritability (H^2) for the three traits varied between 0.306 and 0.552 (Table 3). The values for stem diameter were lower than those for either biomass or height. Based upon the overlap in confidence intervals, there were no significant differences between CO₂ levels in H^2 for any of these traits. Clonal heritability ranged between 0.859 and 0.945. The opportunity for selection (I) varied between 0.042 and 0.261 depending upon trait; values were higher for biomass than for either height or stem diameter. Elevated CO_2 significantly decreased I when biomass was used as a measure of fitness, but had no effect when either height or stem diameter were used. Response to selection (R) varied between 0.016 and 0.124. The highest values were observed for biomass and the lowest for stem diameter. There were no significant differences between CO_2 levels in R for any of the three traits examined. The genetic correlation between performance at low and high CO2 approached 1.0 for biomass and height and was slightly lower, but still very high, for stem diameter (Table 3).

Discussion

Similar to previous studies (e.g. Bazzaz et al., 1995; Lindroth et al., 2001; Moya, Ziska, Namuco, & Olszyk, 1998; Steinger, Lavigne, Birrer, Groppe, & Schmid, 1997; Volk & Körner, 2001), we found significant genotype \times CO₂ interactions for size related traits. In

(LC) or high (HC	(0,0,0) CO ₂ , and with perfor	imance of the same gen	otypes grown on a rang	ge of different field sites	at ambient CO_2 levels)cs gi uwil ill a glassilu	use at citile1 10 w
	Biomass LC	Biomass HC	Height LC	Height HC	Diameter LC	Diameter HC	Field growth
Biomass LC	1.000						
Biomass HC	0.925 (< 0.0001)	1.000					
Height LC	0.786 (< 0.0001)	0.770 (< 0.0001)	1.000				
Height HC	0.774 (< 0.0001)	0.825 (< 0.0001)	0.931 (< 0.0001)	1.000			
Diameter LC	0.684 (< 0.0001)	0.696 (< 0.0001)	0.764 (< 0.0001)	0.767 (< 0.0001)	1.000		
Diameter HC	0.502 (0.0055)	0.605(0.0005)	0.586(0.0008)	0.651 (0.0001)	0.745 (< 0.0001)	1.000	
Field growth	$0.371 \ (0.1183)$	0.540(0.0169)	$0.596\ (0.0070)$	0.650(0.0026)	0.519 (0.0227)	0.521 (0.0220)	1.000
Pearson correlation	coefficients were calculate	d using genotype means (n)	i = 29 for correlations amo	ong traits measured only in	the glasshouse, and $n = 1$	9 for correlations involv	ving growth in the
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our study, the range among genotypes in CO_2 response was substantial, with anywhere from a 23% to a 108% increase in biomass at elevated CO_2 . Several studies report a similar range in response; for example, Wang, Curtis, Pregitzer, and Zak (2000) found anywhere from a 29% reduction to a 94% increase in *Populus tremuloides*. In red maple, the values ranged from no significant increase, to a 93% increase in biomass at elevated CO_2 (Mohan, Clark, & Schlesinger, 2004). In our study we observed a number of apparent crossovers in the ranking of genotypes at low versus high CO_2 . Results from previous studies have been mixed, some studies reporting changes in the ranking (Bazzaz et al., 1995; Curtis, Snow, & Miller, 1994; Wang et al., 2000), while others have not (Wang et al., 1994).

It has been suggested that genetic variation in response to elevated CO₂ may allow selection to occur that will enhance the response of the population over time (e.g. Bazzaz et al., 1995; Curtis et al., 1994; Lindroth et al., 2001; Roumet, Laurent, Canivenc, & Roy, 2002). In spite of the genotype \times CO₂ interactions we observed, genetic correlations between performances at the two CO_2 levels were very high regardless which trait was used to measure performance. In other words, genotypes that were successful at ambient CO₂ were equally successful at elevated CO₂. The apparent disconnect between these two approaches of examining genetic variation in CO₂ response arises because simply testing for the presence of genotype × environment interactions does not put this variation into perspective relative to other sources of variation which may influence selection. Although there were significant genotype \times CO₂ interactions, the magnitude of these effects was dwarfed by the large differences in size among genotypes. As a result, genotype ranking did not differ significantly between CO₂ levels. This was shown by the analysis of variance conducted on ranks, indicating that the apparent "differences" in ranks that were observed in the interaction diagrams (Fig. 1, see Appendix A: Figs. A1 and A2) were simply chance reversals.

Even if the same genotypes are favoured by selection at ambient and elevated CO_2 , it is possible there could be differences in selection between CO_2 levels if either the opportunity for selection or the heritability of the traits differed between environments. This is reflected in Fisher's Fundamental Theorem, where response to selection is product of the opportunity for selection and heritability of the trait (Lynch & Walsh, 1998). As heritability is calculated as the ratio of genetic to total phenotypic variance, its value is highly dependent upon the environment in which it is measured. In general, it has been proposed that environmental variance should be lower in more favourable environments (Blum, 1988). Given that elevated CO_2 enhances growth, it could be argued that it will lower environmental variance.

	Total biomass		Stem height		Stem diameter	
	LC	НС	LC	НС	LC	HC
Genotype variance	0.2103	0.2332	0.3795	0.5238	0.4171	0.5311
Error variance	0.2339	0.1917	0.3077	0.4719	0.9459	1.0436
Broad-sense heritability (H^2)	0.473	0.549	0.552	0.526	0.306	0.337
Upper CL	(0.402)	(0.464)	(0.498)	(0.463)	(0.231)	(0.253)
Lower CL	(0.545)	(0.634)	(0.606)	(0.589)	(0.381)	(0.422)
Opportunity for selection (I)	0.261	0.138	0.042	0.050	0.052	0.048
Upper CL	(0.212)	(0.109)	(0.038)	(0.046)	(0.046)	(0.041)
Lower CL	(0.310)	(0.166)	(0.046)	(0.054)	(0.058)	(0.055)
Response to selection (R)	0.124	0.076	0.023	0.026	0.016	0.016
Upper CL	(0.097)	(0.053)	(0.019)	(0.022)	(0.011)	(0.011)
Lower CL	(0.151)	(0.098)	(0.027)	(0.031)	(0.021)	(0.022)
Clonal mean heritability	0.925	0.942	0.945	0.938	0.859	0.873
Genetic correlation	0.9	991	0.9	990	0.8	360

Table 3. Summary of variance components, heritability, opportunity for selection and response to selection for plants grown at high (HC) versus low (LC) CO_2 for three growth related traits

To achieve normality, biomass and stem height data were transformed prior to analysis by taking the natural logarithm (biomass) or the square root (height) of the original values. The values in parentheses below the H^2 , *I* and *R* values are the upper and lower 95% confidence limits for these values. The calculated genetic correlations between genotype means at high versus low CO₂ are provided for each trait.

Alternatively, elevated CO_2 is a novel environment and as such, may act as a stress and increase environmental variance. Thus, it is possible to argue that elevated CO_2 could either increase or decrease heritability. The opportunity for selection may also change at elevated CO_2 . It has been argued that in novel environments, there may be higher levels of genetic variation in particular traits if the genetic trade-offs among traits that constrain variation are altered under the new environmental conditions (Hoffmann & Merilä, 1999).

The above hypotheses were developed and tested using studies of inheritance in which narrow sense heritability is determined, in contrast to the present study which determined broad sense heritability. However, these hypotheses illustrate that it is important to at least consider the possibility that level of CO₂ may affect either the range or heritability of phenotypic variation. The calculation of broad sense heritability in the present study allows us to explore this possibility. We found elevated CO_2 decreased I when biomass was used as a measure of fitness, but had no significant effect on H^2 for any trait. However, R was similar in the two CO_2 environments for all three traits as the decrease in I in the case of biomass, was compensated for by a nonsignificant increase in H^2 at elevated CO₂. It would appear that for the population of white spruce we studied, elevated CO₂ is unlikely to affect response to selection under the conditions examined.

Our conclusions are constrained by the fact that this study was based on a relatively short-term glasshouse

experiment with very young trees. However, seedling growth is often correlated with subsequent performance, and early growth under glasshouse conditions is a useful selection criterion in tree breeding programs (Mullin & Park, 1994). In the present case, ranking of genotypes between glasshouse and field was similar, especially when the same measure of performance (i.e. height) was used to compare growth in both environments. These correlations are even more impressive when you consider that in the field, plants were grown over a range of sites differing in soil and climate. This suggests our conclusions regarding the relative performance of genotypes are robust. The fact that growth in the field was as closely correlated with growth at elevated CO₂ as it was in ambient CO_2 further confirms that CO_2 concentration is unlikely to have an effect on the relative performance of genotypes. It is also important to remember that response to elevated CO_2 is greatest early in growth (Poorter & Navas, 2003), and the highly controlled conditions in this glasshouse experiment maximized our ability to detect differences among genotypes in CO_2 response. Therefore, it is unlikely that selection for an enhanced growth response to elevated CO₂ will be any stronger in a much more variable field environment with older, less responsive plants.

The few previous studies that have quantified the extent that selection may act on genetic variation in response to elevated CO_2 have had varying results. A study examining the response of the perennial herb,

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Sanguisorba minor, to 6 years of growth at ambient versus elevated CO_2 found that genetic lines with increased leaf production were selected for at elevated CO_2 (Wieneke et al., 2004). Bazzaz et al. (1995), Steinger et al. (2007), Ward, Antonovics, Thomas, & Strain, (2000) working with *Abutilon theophrasti*, *Arabidopsis thaliana* and *Bromus erectus*, respectively, all concluded there would be selection for specific genotypes at elevated CO_2 , but the genotypes favoured at elevated CO_2 would not have enhanced productivity. On the other hand, Lau et al. (2007), also working with *A. thaliana*, concluded that elevated CO_2 would have little evolutionary impact.

In conclusion, neither natural nor artificial selection is likely to enhance the growth response of white spruce to elevated CO₂ despite the fact there were significant genotype \times CO₂ interactions for growth related traits in this species. As woody tissue serves as a long-term carbon store, determining whether such selection will take place in tree species is critical in evaluating to what extent the biosphere will serve as a carbon sink. It is also critical from the point of view of tree breeding and reforestation programs as trees planted today will be exposed to CO₂ levels substantially higher than present day levels before maturation. Given the importance of this question and evidence from previous studies that elevated CO₂ can have an impact on selection in at least some herbaceous species, there is a need for future studies of this issue using a variety of different tree species and environmental conditions.

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Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.baae.2008.08.005

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