

# Effect of Seed Size on Seedling Growth Response to Elevated CO<sub>2</sub> in *Picea abies* and *Picea rubens*

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**Abstract:** Several previous studies have observed that species and individuals with large seeds respond more positively to elevated CO<sub>2</sub> than those with small seeds. We explored the reasons for this pattern by examining the relationship between seed size and CO<sub>2</sub> response in *Picea abies* and *P. rubens* using growth analysis. The large seeded species (*P. abies*) responded more positively to elevated CO<sub>2</sub> than the small seeded species (*P. rubens*). At the intraspecific level, *P. abies* individuals from large seeds responded more positively to elevated CO<sub>2</sub> than individuals from small seeds, however, there was no significant intraspecific variation in CO<sub>2</sub> response in *P. rubens*. The greater CO<sub>2</sub> response of plants from large seeds was not simply the result of a larger starting capital compounded at the same rate as in plants from small seeds. Elevated CO<sub>2</sub> increased relative growth rate to a greater extent in individuals from large seeds. This effect appears to be related to differences in time of establishment, source to sink ratio and nutrient availability with seed size. These results are significant not only in understanding the potential effect of rising atmospheric CO<sub>2</sub> concentrations on plant populations, but also in understanding the factors affecting plant success at current atmospheric CO<sub>2</sub> levels due to the elevation of CO<sub>2</sub> within the litter layer that occurs at many germination sites.

**Key words:** Functional group, seed size, elevated CO<sub>2</sub>, growth analysis, source:sink ratio, nitrogen availability.

## Introduction

Due to rising atmospheric carbon dioxide concentrations, the effects of CO<sub>2</sub> on the growth of plants have been studied from a wide range of perspectives (Körner, 2000). These studies have shown that, largely because of its positive effects on photosynthetic rate and water use efficiency, elevated CO<sub>2</sub> generally has positive effects on growth. The magnitude of these effects, however, can vary widely depending upon species, genotype and environmental conditions (Jablonski et al., 2002; Poorter and Navas, 2003). To understand and predict these ef-

fects, we require knowledge of how and why response to elevated CO<sub>2</sub> varies so widely, both within and among species. Attempts to explain this variation have focussed on classifying plants into functional groups based on physiological or morphological differences, and then comparing the CO<sub>2</sub> response of these functional groups. Some of the functional groups studied include: shade tolerant versus shade intolerant plants (Rocheffort and Bazzaz, 1992; Bazzaz et al., 1993; Kubiske and Pregitzer, 1996), C3 versus C4 plants (Hand et al., 1993; Dippert et al., 1995; Tissue et al., 1995; Hamerlynck et al., 1997), fast- versus slow-growing plants (Diaz et al., 1993; Hunt et al., 1995), early versus late successional plants (Rocheffort and Bazzaz, 1992; Bazzaz et al., 1993) and stress tolerant versus stress intolerant plants (Bunce, 1992; Bazzaz et al., 1993; Kinney and Lindroth, 1997). These efforts have been partially successful, however, much unexplained variation remains (Poorter and Navas, 2003).

One factor that may be important in explaining differences in CO<sub>2</sub> response among germinating seedlings is variation in seed size. It is well established that seed size has marked effects on seedling establishment (e.g., Tripathi and Khan, 1990; Seiwa and Kikuzawa, 1991; Gross, 1984; Leishman and Westoby, 1994a, b; Metcalfe and Grubb, 1997), and four previous studies report that response to elevated CO<sub>2</sub> varies with seed size. Bazzaz and Miao (1993) found that seedlings of large-seeded deciduous tree species responded more positively to elevated CO<sub>2</sub> than those of small-seeded species. The same pattern was observed at the intraspecific level in the deciduous tree, *Quercus rubra* (Miao, 1995) and in the grass, *Bromus erectus* (Steinger et al., 2000). Similarly, Khurana and Singh (2004) found that response to elevated CO<sub>2</sub> was positively correlated with seed size at both the intra- and interspecific level in seedlings of five tropical trees. In all four of these studies, the greater CO<sub>2</sub> response of seedlings from large seeds was attributed to the greater reserves of large seeds and the impact this has on establishment. It is argued that greater reserves allow the seedlings to more effectively utilize the additional CO<sub>2</sub>. However, the mechanistic basis for this effect is not yet clear.

One means of exploring the mechanistic basis for differences among plants in their response to elevated CO<sub>2</sub> is through plant growth analysis (e.g., Hunt et al., 1995). Plant size at any given time ( $W_t$ ) is determined by the initial size ( $W_0$ ) (i.e., seed size in this case), the average relative growth rate over the time

interval in question ( $R$ ), and length of time elapsed ( $t$ ) as shown in the following equation.

$$W_t = W_0 \cdot R \cdot t \quad (1)$$

If we assume that seed size does not affect relative growth rate and that elapsed time is constant, it is clear from this equation that plants that grow from large seeds will have a greater final size than plants from small seeds due to the compounding of the initial size differences over time. If we now assume that elevated CO<sub>2</sub> increases relative growth rate in plants from small and large seeds to the same extent, and multiply the initial sizes by the relative growth rate at low versus high CO<sub>2</sub>, we find that, due to the compounding of the initial differences in starting capital, the difference in size between plants grown at low versus high CO<sub>2</sub> will be larger for plants from large seeds than for plants from small seeds. In other words, we do not need to assume any physiological difference between individuals from large and small seeds to explain the greater absolute response of individuals from large seeds to increases in the level of CO<sub>2</sub>. Similarly, if we assume that establishment time is shorter for seedlings from large seeds than for seedlings from small seeds due to the availability of growth reserves, the amount of time available for photosynthesis at either ambient or elevated CO<sub>2</sub> (i.e., elapsed time in the above equation) will be longer for seedlings from large seeds. This means that, again, even though elevated CO<sub>2</sub> may have identical effects on relative growth rate in seedlings from small and large seeds, seedlings from large seeds will have a greater absolute response to elevated CO<sub>2</sub>. Of course, it is entirely possible that the effect of elevated CO<sub>2</sub> on relative growth rate will indeed differ depending upon seed size. One likely explanation for such an effect is a difference in nutrient availability with seed size. Increases in nutrient availability and, in particular, nitrogen availability are known to have a positive impact on the response of plants to elevated CO<sub>2</sub> (Bazzaz et al., 1993; Johnsen, 1993; Thomas et al., 1994; Prior et al., 1997; Tissue et al., 1997). Seed size will, of course, affect nutrient content of the seed and often has marked effects on nutrient concentrations as well (Grubb, 1998; Fenner, 2004).

The present study examines the interactive effects of seed size and CO<sub>2</sub> concentration on early seedling growth of *Picea abies* (L.) Karst. and *Picea rubens* Sarg. These two species differ markedly in seed size and both species exhibit substantial intraspecific variation in seed size, allowing us to test the hypothesis that seedlings from large seeds respond more positively to elevated CO<sub>2</sub>. We used growth analysis to examine the basis for differences in the response and, in particular, determine if the observed differences were due to differences in starting capital, establishment time or relative growth rate. We also examined how nitrogen concentration and total content varied with seed size.

In addition to helping us to understand how plant communities may change in a future high CO<sub>2</sub> world, this study is relevant to an understanding of the factors controlling the establishment of plants under current conditions (Bazzaz and Williams, 1991). Due to decomposition and root respiration, CO<sub>2</sub> levels in the soil generally range between 1500 and 6500  $\mu\text{L L}^{-1}$  and can reach levels in excess of 100 000  $\mu\text{L L}^{-1}$  under conditions of poor aeration (Russell, 1973). Above the soil surface, CO<sub>2</sub> levels decline rapidly, but within a few centimetres of the

soil surface, levels can be substantially higher than those in the general atmosphere (Fuller, 1948; Sparling and Alt, 1965; Schwartz and Bazzaz, 1973; Bazzaz and Williams, 1991). This means that, at the site of germination and early growth for many species, present-day CO<sub>2</sub> levels are equal to or greater than the levels predicted for the general atmosphere by the end of this century.

## Materials and Methods

### Plant culture

Seeds of *Picea abies* (seed lot 3699) and *Picea rubens* (seed lot 3556) were obtained from the Nova Scotia Department of Lands and Forest, Tree Breeding Center, Debert, Nova Scotia. Seeds were weighed individually, dusted with fungicide (50% Captan and 50% Benlate) and stratified at 4°C for 4 weeks in petri dishes lined with moistened Kimwipes and sealed with Parafilm. Seeds were then placed in controlled environment chambers at 17°C, with no light, to germinate; germination took place in 10 days for *Picea abies* and 12 days for *Picea rubens*. Individual seeds with visible and healthy radicles were transplanted into one of eight 72-cell flats. The 53 × 27 cm flats were made up of 12 blocks of 6 cells. Each cell had a volume of 74 cm<sup>3</sup>. The substrate was a 1:1 mixture of autoclaved sand and peat. Approximately 0.16 g of a 14-14-14 slow release fertilizer from Plant Products Co. Ltd. was placed in each cell. The flats were placed inside one of eight 56-L glass-sided tanks (32 cm tall by 32 cm wide and 60 cm in length). The tanks were placed into one of two growth chambers (Conviron E15) and grown for 98 days. The growth chambers were programmed for a 14/10 h light/dark cycle at 100  $\mu\text{mol m}^{-2} \text{sec}^{-1}$  for the first week and then reprogrammed to 170 ± 15  $\mu\text{mol m}^{-2} \text{sec}^{-1}$  for the remaining 13 weeks. The light levels were chosen to be representative of those experienced by seeds germinating in a forest gap beneath an herbaceous canopy (e.g., see Pages and Michalet, 2003). The coinciding temperature cycle was 15/10 ± 0.5°C. Plants were watered from below when the surface of the substrate was visibly dry.

### Carbon dioxide fumigation system

A flow-through fumigation system was used to subject seedlings to the appropriate CO<sub>2</sub> level. The glass tanks in which the individual flats were placed served as the fumigation chambers. Air for the fumigation system was drawn from outside the building, at ~2 m above ground level. Unmodified air was used to fumigate the ambient CO<sub>2</sub> chambers. For the elevated CO<sub>2</sub> treatment, pure CO<sub>2</sub> from a pressure regulated source was bled into the ambient air stream using needle valves and allowed to mix in a 2-L reservoir prior to being delivered to the fumigation chambers. Air for both treatments was pressure-regulated and the flow for the individual fumigation chambers was controlled with needle valves to achieve a flow rate of 4 L min<sup>-1</sup>. The carbon dioxide level of the air entering the fumigation chambers was checked periodically using a Li-Cor 6200 infrared gas analyzer. Spot checks of the CO<sub>2</sub> level in the air flowing out of the fumigation chambers revealed that respiration/photosynthesis had no significant effect on the level of CO<sub>2</sub> within the chambers.

### Experimental design and measurements

Two separate experiments were run concurrently; the first involved harvesting plants at 4-week intervals for growth analysis, while the second involved a single harvest at the end of a 98-day growth period. In both experiments, plants were grown at one of two CO<sub>2</sub> levels (380 versus 1400 µL L<sup>-1</sup>). In the second experiment, seeds were classified into one of three size classes (large, medium or small). The large and the small seed size classes were defined by the first and fourth quartiles of the population of seed sizes, while the medium size class included seeds from the second and third quartiles. Seeds were planted in an alternating pattern in the flats such that both species were regularly distributed throughout all eight flats. Within each flat, every second block of six plants was reserved for the final harvest, while the rest were used for growth analysis. For the final harvest, there was one large, one small and one medium seed from each species, arranged in a semi-random distribution pattern within each block of six plants. That is, the alternating pattern of species was maintained, but the size categories were randomly placed in one of the three designated locations for each species within the block of cells. Within each of the two growth chambers, the four flats were assigned to one of the two carbon dioxide treatments.

To assess differences in time of establishment in experiment I, the number of plants that had emerged leaves versus those in which the leaves were still contained in the seed coat was determined on day 14. At time of harvest in both experiments, total leaf area was determined using a Li-Cor 3100 area meter calibrated to the most sensitive resolution (10<sup>-3</sup> ± 1.5%). Needles, roots and stems were placed in an oven to dry at 50°C for one week and dry weights were determined to the nearest 10<sup>-5</sup> g.

### Nitrogen determinations

Nitrogen concentration for seeds in the small, medium and large size classes described above was determined for both species using a LECO CHN analyzer. As the analyzer required a sample size of 250 mg, each sample consisted of multiple seeds from a particular size category and species. There were four replications (seed lots) per species for the small and large size categories and eight replications for the medium size category. Total nitrogen content per seed was calculated as the product of nitrogen concentration and the mean seed weight for each size category and species combination.

### Statistical analysis

Differences between species in the number of individuals that were established (i.e., had leaves exposed to light) at day 14 was examined by means of a two-by-two contingency table. The effect of intraspecific variation in seed size on establishment was examined by determining if the mean seed size of established versus nonestablished plants at day 14 differed significantly, using a one way analysis of variance.

Where possible (i.e., when the assumptions of normality were met), the analyses for total mass were conducted on both the untransformed weights and the natural logarithm transformed values. The first analysis tests the significance of differences between treatment means, while the second analysis

compares the ratio of one mean to another. A significant difference between logarithm transformed means also means that the average relative growth rates of the respective treatments differ (Poorter and Navas, 2003).

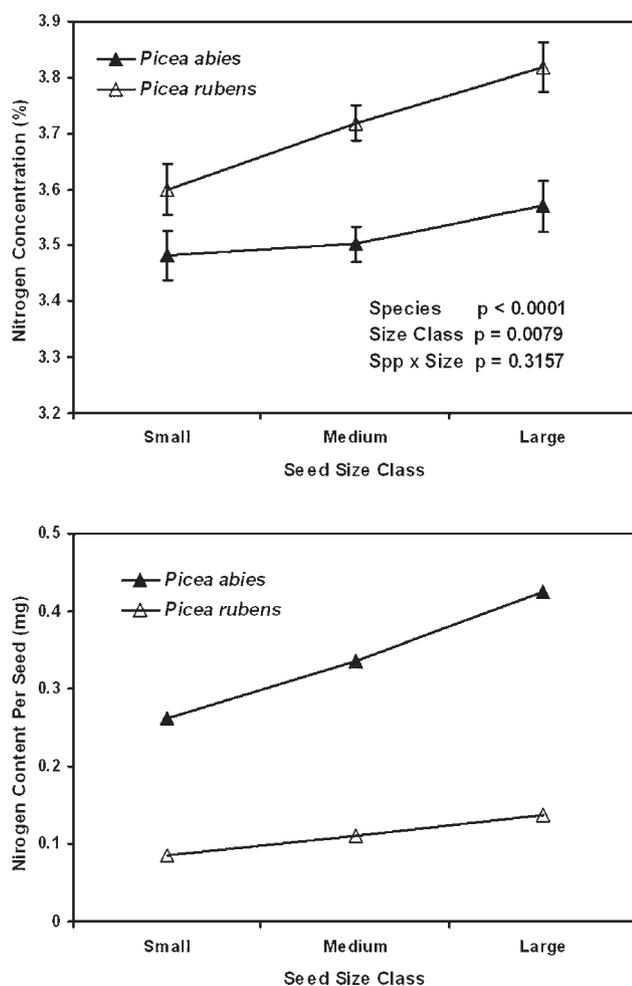
Analysis of variance was used to examine the effects of CO<sub>2</sub>, species and day of harvest on total weight in experiment I. A split plot design was employed, with growth chamber as a blocking factor, fumigation chamber (i.e., individual flats) as the main plots and each block of six plants within the flats as the subplots. It was not possible to analyze the untransformed total weight in this analysis as it was not normally distributed across harvests, so the analysis was only conducted on the transformed values. Species, CO<sub>2</sub> and day of harvest were considered fixed effects, while growth chamber, flats and blocks were considered random effects. The CO<sub>2</sub> effect was tested using the main plot error term, while species, day of harvest and the various interactions were tested using the residual error term.

To examine the effects of intraspecific variation on seed size in experiment I, a similar model to the one described above was used, except separate analyses were conducted for each species, and seed weight of the individual plants was entered as a covariate in the analysis. We also included the interactions among seed weight, CO<sub>2</sub> and day of harvest as effects in the model. We analyzed both the transformed and the untransformed data, but as the untransformed data were not normally distributed across harvests, separate analyses were conducted for each harvest date.

Average relative growth rate for each time interval in experiment I (i.e., days 0–28, 28–56 and 56–84) was calculated using an analysis of covariance model, to regress log transformed total weight against time for each consecutive pair of harvests. In these analyses, growth chamber, CO<sub>2</sub> and species were categorical independent variables and day of harvest (time) was the covariate. The slope of the log weight versus time relationship in this analysis is relative growth rate, and significant time × CO<sub>2</sub> and time × species interactions represent significant differences in relative growth rate for CO<sub>2</sub> and species, respectively.

In experiment II, analysis of variance was used to examine the effects of CO<sub>2</sub>, species and seed size class (small, medium or large) on total weight, leaf area ratio and root:shoot ratio. A split plot design, as described above, was used to test for differences between treatments, plots and subplots. The analysis was conducted on both the untransformed total weight and on the log transformed total weight as both variables were normally distributed. Average relative growth rate over the entire experimental period for each individual plant was calculated as (log total weight – log seed weight)/length of experiment. Differences in relative growth rate among treatments were then examined by analysis of variance as described above for the other variables.

Differences among size classes and between species in seed nitrogen concentration were examined by means of a two-way factorial analysis of variance. Both seed size class and species were considered fixed effects.



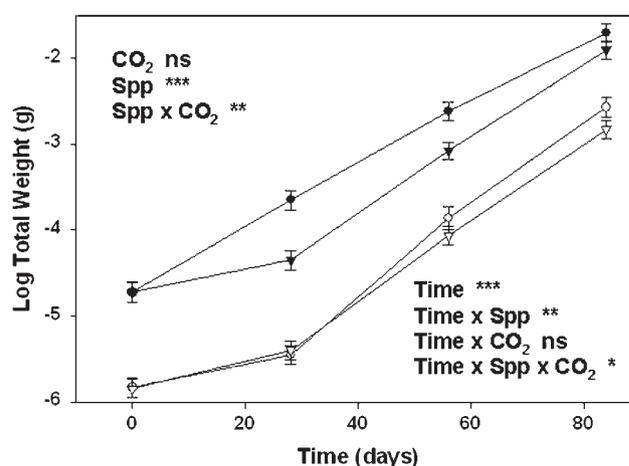
**Fig. 1** Nitrogen concentration (% dry weight) and total nitrogen content (mg) in small, medium and large seeds of *Picea abies* (closed symbols) and *P. rubens* (open symbols). The p values represent the level of significance for the effect of species, size class and their interaction for differences in nitrogen concentration. Error bars are  $\pm$  one standard error. Total nitrogen content was calculated as the product of nitrogen concentration and mean seed size for each size category.

## Results

### Interspecific variation

The two species differed markedly in seed size. Averaged across all harvests and over both experiments, the mean ( $\pm$  standard deviation) seed weight of *Picea abies* was  $9.7 \pm 2.1$  mg compared to  $3.0 \pm 0.6$  mg for *P. rubens*. *Picea rubens* had a higher nitrogen concentration in the seeds, but a lower total nitrogen content per seed than *P. abies* (Fig. 1). By day 14, all of the *P. abies* individuals were established and had leaves exposed to the light, whereas only 64% of the *P. rubens* individuals were established ( $\text{Chi}^2 = 10.564$ , 1 df,  $p < 0.05$ ).

Differences in seed size between the two species were reflected in subsequent plant size in experiment I. *Picea abies* was always larger than *P. rubens*, regardless of harvest date (Fig. 2). However, the magnitude of the difference between the two species declined over time. Elevated CO<sub>2</sub> increased plant size in *P. abies* but not in *P. rubens*. The beneficial effect of CO<sub>2</sub> on



**Fig. 2** Total weight (log transformed) of *Picea abies* (closed symbols) and *P. rubens* (open symbols) grown at low (triangles) versus high (circles) carbon dioxide and harvested on day 28, 56 or 84 in experiment I. Plant size on day 0 represents seed size. Error bars are  $\pm$  one standard error. A split plot ANOVA was used to determine whether species (Spp), carbon dioxide level (CO<sub>2</sub>), time of harvest (Time), and their interactions were significant. Effects followed by ns, or one, two or three asterisks were not significant, or significant at the 0.05, 0.01 or 0.001 levels of probability, respectively.

plant size in *P. abies* was most evident on days 28 and 56. Given that the analyses described above were conducted on the log transformed weights (Fig. 2), the results represent not only absolute differences among means, but also relative differences or differences in the ratio of one mean to another. The CO<sub>2</sub> enhancement ratios for *P. abies* on days 28, 56 and 84 were 2.00, 1.59 and 1.22, respectively, compared to 0.94, 1.22 and 1.30 for *P. rubens*. It should be noted that, on day 28, when the difference between the two species in their response to CO<sub>2</sub> was at its maximum, elevated CO<sub>2</sub> resulted in a 100% increase in growth in *P. abies* compared to no enhancement of growth in *P. rubens*.

The differences observed between species and between CO<sub>2</sub> levels in total weight in experiment II (Table 1) were roughly similar to those observed in experiment I. *Picea abies* was again larger than *P. rubens*, but elevated CO<sub>2</sub> increased size in both species, although the effect was more marked in the case of *P. abies*. The main effects of both species and CO<sub>2</sub> level were significant, regardless of whether total weight or log transformed weight was used as the dependent variable (Table 2). However, the interaction between species and CO<sub>2</sub> was only significant in the case of total weight, indicating that although elevated CO<sub>2</sub> increased absolute size to a greater extent in *P. abies* than in *P. rubens*, the CO<sub>2</sub> enhancement ratios of the two species were not significantly different by the time plants were harvested in experiment II (day 98). The CO<sub>2</sub> enhancement ratio of *P. abies* was 1.52, compared to 1.50 for *P. rubens*. In other words, elevated CO<sub>2</sub> resulted in about a 50% increase in growth in both species.

Averaged across the entire experimental period in experiment II, relative growth rate was higher in *P. rubens* than in *P. abies* and higher at elevated CO<sub>2</sub> than at ambient CO<sub>2</sub> (Tables 2, 3). In experiment I, where relative growth rate was calculated for three separate time periods, there were significant differences

**Table 1** Mean ( $\pm$  one standard error) total weight, relative growth rate, leaf area ratio and root:shoot ratio of *Picea abies* and *P. rubens* individuals selected from one of three seed size classes and grown at either 350 or 1400 ppm CO<sub>2</sub> for 98 days in experiment II

Species	CO <sub>2</sub> level	Size class	Total weight (g)	Relative growth rate (g g <sup>-1</sup> d <sup>-1</sup> )	Leaf area ratio (cm <sup>2</sup> g <sup>-1</sup> )	Root:shoot ratio (g g <sup>-1</sup> )
<i>P. abies</i>	low	small	0.158 $\pm$ 0.017	0.0301 $\pm$ 0.0012	36.8 $\pm$ 2.1	0.355 $\pm$ 0.036
		medium	0.193 $\pm$ 0.017	0.0288 $\pm$ 0.0012	41.6 $\pm$ 2.0	0.345 $\pm$ 0.035
		large	0.203 $\pm$ 0.017	0.0279 $\pm$ 0.0012	38.2 $\pm$ 2.2	0.381 $\pm$ 0.037
	high	small	0.247 $\pm$ 0.015	0.0348 $\pm$ 0.0011	30.5 $\pm$ 1.9	0.294 $\pm$ 0.032
		medium	0.318 $\pm$ 0.016	0.0350 $\pm$ 0.0012	30.3 $\pm$ 2.0	0.290 $\pm$ 0.035
		large	0.274 $\pm$ 0.015	0.0312 $\pm$ 0.0011	29.5 $\pm$ 1.9	0.336 $\pm$ 0.032
<i>P. rubens</i>	low	small	0.078 $\pm$ 0.015	0.0346 $\pm$ 0.0011	43.9 $\pm$ 1.9	0.216 $\pm$ 0.033
		medium	0.080 $\pm$ 0.017	0.0332 $\pm$ 0.0012	45.8 $\pm$ 2.1	0.253 $\pm$ 0.037
		large	0.110 $\pm$ 0.018	0.0338 $\pm$ 0.0013	43.2 $\pm$ 2.3	0.190 $\pm$ 0.040
	high	small	0.111 $\pm$ 0.016	0.0374 $\pm$ 0.012	32.2 $\pm$ 2.0	0.275 $\pm$ 0.035
		medium	0.140 $\pm$ 0.015	0.0382 $\pm$ 0.011	32.4 $\pm$ 1.9	0.269 $\pm$ 0.033
		large	0.138 $\pm$ 0.015	0.0359 $\pm$ 0.0011	31.1 $\pm$ 1.9	0.245 $\pm$ 0.033

**Table 2** Analysis of variance table for total weight, log transformed total weight, relative growth rate, leaf area ratio and root:shoot ratio as affected by species, carbon dioxide level and seed size class in experiment II. Sum of squares followed by one, two or three asterisks were significant at the 0.05, 0.01 or 0.001 levels of probability, respectively

Source of variation	df	Total weight	Log total weight	Relative growth rate	Leaf area ratio	Root:shoot ratio
		Sum of squares				
Chamber	1	0.2457**	10.799**	0.001084**	136	0.938**
CO <sub>2</sub>	1	0.2454**	8.239**	0.000817**	6857***	0.009
Error A	5	0.0457	1.407	0.000185	619	0.177
Species	1	0.9481***	34.852***	0.001111***	744*	0.480***
Species $\times$ CO <sub>2</sub>	1	0.0406**	0.233	0.000029	181	0.137
Size class	2	0.0534**	2.218**	0.000218*	145	0.002
Size $\times$ CO <sub>2</sub>	2	0.0149	0.690	0.000067	97	0.003
Size $\times$ species	2	0.0168	0.182	0.000035	15	0.075
Size $\times$ CO <sub>2</sub> $\times$ species	2	0.0011	0.037	0.000001	26	0.006
Error B	227	1.1316	52.609	0.00573	17743	5.259

between species and CO<sub>2</sub> treatments in the first time period (days 0–28) only (Table 3). Elevated CO<sub>2</sub> significantly increased relative growth rate in *P. abies* but not in *P. rubens*.

Allocation patterns were affected by both species and CO<sub>2</sub> level (Tables 2, 3). *Picea abies* had a lower leaf area ratio and a higher root:shoot ratio than *P. rubens*. Elevated CO<sub>2</sub> decreased the leaf area ratio in both species while it decreased the root:shoot ratio in *P. abies* and increased it in *P. rubens*.

#### Intraspecific variation

There was substantial intraspecific variation in seed size in both species. Seed weight in *P. abies* ranged between 4.8 and 17.1 mg, while in *P. rubens*, it ranged between 1.2 and 4.8 mg (i.e., a 3–4-fold variation in seed weight within both species). In experiment II the mean seed weights ( $\pm$  standard deviation) for the three seed size classes were: 7.5  $\pm$  0.9, 9.6  $\pm$  0.5 and 11.9  $\pm$  1.1 mg for *P. abies* and 2.4  $\pm$  0.3, 3.0  $\pm$  0.1 and 3.6  $\pm$  0.3 for *P. rubens*. Nitrogen concentration in the seeds and nitrogen content per seed increased with seed size in both species

(Fig. 1). In experiment I, there was no difference in mean seed size of established (3.0  $\pm$  0.1) versus nonestablished (2.9  $\pm$  0.2 mg) *P. rubens* seedlings at day 14 ( $p = 0.453$ ).

In experiment I, intraspecific variation in seed size affected plant size only in *P. abies* grown at elevated CO<sub>2</sub> on days 28 and 56 (Fig. 3). On both days, individuals from large seeds responded more positively to elevated CO<sub>2</sub> than individuals from small seeds. Similar results were obtained when the results were log transformed. An analysis of covariance for the relationship between log transformed total weight and seed size, as affected by level of CO<sub>2</sub> and time of harvest in *P. abies*, revealed a significant three-way interaction between seed size, CO<sub>2</sub> and time. However, a similar analysis for *P. rubens* indicated that there was no significant relationship between log transformed total weight and seed size, regardless of day of harvest or level of CO<sub>2</sub>. This indicates that individuals from large seeds in *P. abies* not only had a greater absolute response to CO<sub>2</sub> but also a greater relative response. Using the values predicted by the calculated regressions (Fig. 3), the CO<sub>2</sub> enhancement ratio of a relatively small *P. abies* seed (7.5 mg) on

**Table 3** Relative growth rate of *Picea abies* and *P. rubens* as affected by level of carbon dioxide for three separate time intervals in experiment I. The sum of squares for each effect is presented below the treatment means. Values in parentheses after each effect are the degrees of freedom. Sum of squares followed by an asterisk indicate an effect significant at the 0.05 level of probability

	Time period		
	0–28 days	28–56 days	56–84 days
<b>Treatment</b>	Mean relative growth rate ± std error		
<i>Picea abies</i>			
Low CO <sub>2</sub>	0.0137 ± 0.0061	0.0454 ± 0.0058	0.0420 ± 0.0050
High CO <sub>2</sub>	0.0386 ± 0.0058	0.0369 ± 0.0058	0.0325 ± 0.0050
<i>Picea rubens</i>			
Low CO <sub>2</sub>	0.0163 ± 0.0054	0.0475 ± 0.0058	0.0440 ± 0.0051
High CO <sub>2</sub>	0.0137 ± 0.0056	0.0548 ± 0.0063	0.0446 ± 0.0060
<b>Source of variation</b>	Sum of squares		
Species (1)	0.5250	0.3778	0.1838
CO <sub>2</sub> (1)	0.4658	0.0068	0.1329
Species × CO <sub>2</sub> (1)	0.7651*	0.2530	0.1054
Error (74)	9.4384	10.3318	8.5750

day 28 was calculated to be 1.26, compared to 2.40 for a relatively large seed (12.0 mg). The corresponding values for day 56 were 1.24 and 2.00 for small and large seeds, respectively.

In experiment II, individuals from large seeds in both species had a greater final size than individuals from small seeds (Tables 1, 2). The same results were obtained regardless of whether the absolute difference between size categories or the ratio of one size category to another was used as the dependent variable. Individuals from small seeds had a greater relative growth rate than individuals from large seeds. There were no differences in leaf area ratio or root:shoot ratio among size categories. Size category had no impact on response to elevated CO<sub>2</sub>.

## Discussion

The larger-seeded *Picea abies* responded more positively to elevated CO<sub>2</sub> than the smaller-seeded *P. rubens*, and at the intraspecific level, *P. abies* individuals from large seeds responded more positively to elevated CO<sub>2</sub> than individuals from small seeds. These results provide partial support for the conclusions of previous studies (Bazzaz and Miao, 1993; Miao, 1995; Steinger et al., 2000; Khurana and Singh, 2004). However, the fact that the same pattern was not observed at the intraspecific level in *P. rubens* suggests that this pattern is not universal. It might be argued that the lack of response in *P. rubens* was simply an artefact resulting from the lack of seed size variation in *P. rubens*. It is certainly true that the absolute range in seed size in *P. rubens* (4.8–1.2 = 3.6 mg) was much less than that observed in *P. abies* (17.1–4.8 = 12.3 mg). However, on a relative scale, there was as much variation in seed size in *P. rubens* (4.8/1.2 = 4.0) as there was in *P. abies* (17.1/4.8 = 3.6). As it seems logical to assume that it is variation in seed size relative to what is normal or typical for a species that is relevant, it would appear that one cannot simply dismiss the lack of response in *P. rubens*. It should also be noted that there was sufficient intraspecific variation in both species to have a significant effect on final biomass.

In part, the greater responsiveness of plants from large seeds to elevated CO<sub>2</sub> may be a simple consequence of a larger starting capital that is compounded at the same rate as in plants from small seeds. The results of experiment II are in accord with this hypothesis, in that the larger seeded *P. abies* responded more positively to elevated CO<sub>2</sub> than *P. rubens*, but there was no difference in enhancement ratio or the effect of CO<sub>2</sub> on average relative growth rate between the two species. However, neither the interspecific variation in CO<sub>2</sub> responsiveness nor the intraspecific variation in the case of *P. abies* was in accord with this hypothesis in experiment I. In reality, the CO<sub>2</sub> enhancement ratio of *P. abies* was substantially greater than that of *P. rubens* on both days 28 and 56. Similarly, the CO<sub>2</sub> enhancement ratio of *P. abies* individuals from large seeds was greater than that of individuals from small seeds on days 28 and 56. It was also demonstrated that elevated CO<sub>2</sub> had a greater effect on relative growth rate in *P. abies* than in *P. rubens* on day 28.

Although there was evidence that seed size affected both CO<sub>2</sub> enhancement ratio and the effect of CO<sub>2</sub> on relative growth rate, it was also clear that these effects were restricted to the early stages of the experiment. The magnitude of all these effects dwindled over time such that the relative growth rate of the two species responded similarly to CO<sub>2</sub> by day 56, and there were no significant differences in the enhancement ratios of the two species or of different size classes within species by days 84 in experiment I or 98 in experiment II. The transient nature of the “seed size” effect on CO<sub>2</sub> response is perhaps to be expected. If the differences between species or among size classes is truly a consequence of differences in seed reserves, you would not expect the difference in relative growth rate to persist long after the initial reserves are utilized. However, this does not mean that these transient effects are necessarily trivial with regard to long-term success. How important these transient effects are will depend upon what stage mortality normally occurs in the field. Although initial differences in size can be outgrown in a noncompetitive environment and under the more or less optimal conditions of the laboratory, relatively small differences in plant size and

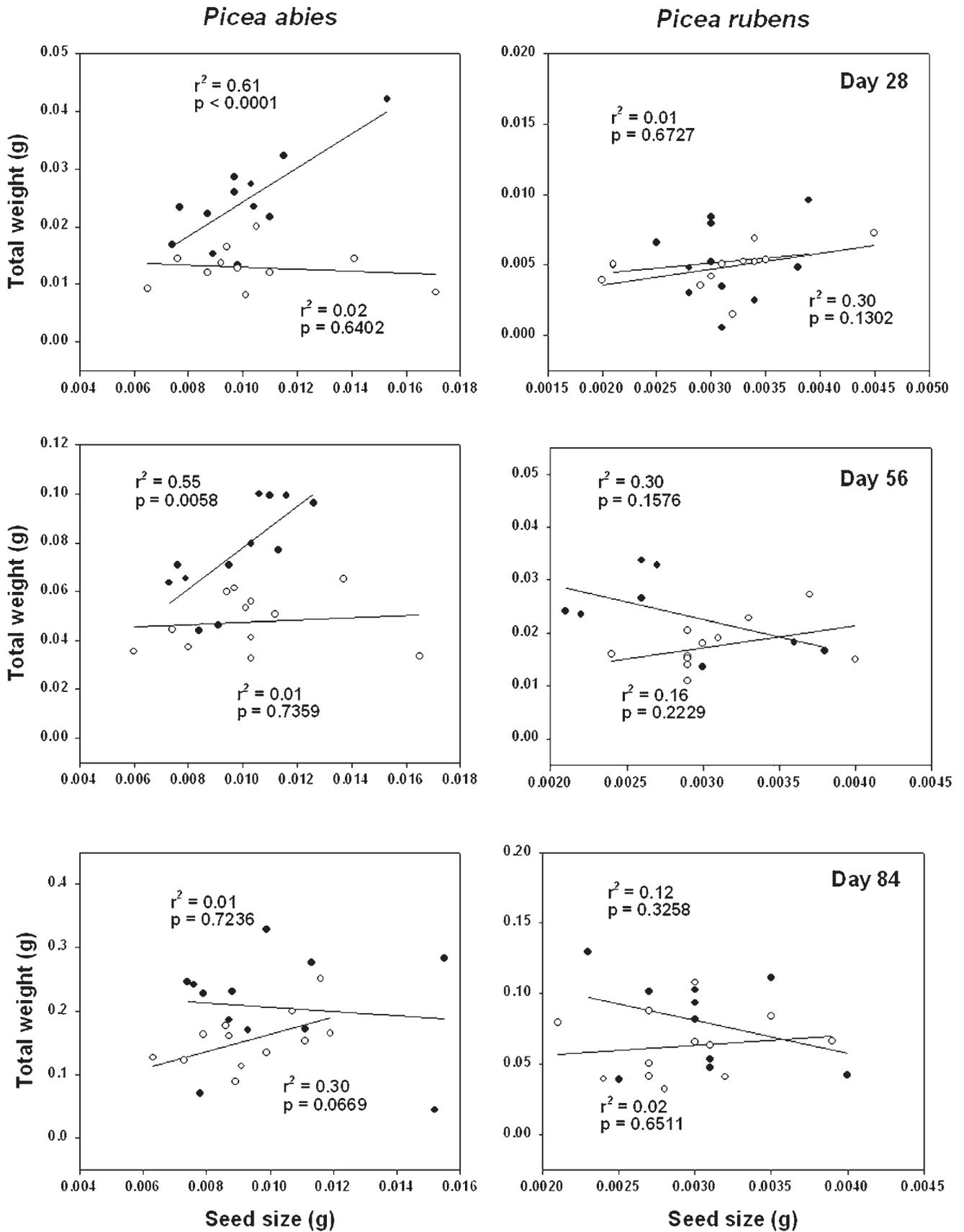


Fig. 3 Relationship between total weight and seed size in *Picea abies* and *P. rubens* grown at low (open symbols) versus high (closed symbols) carbon dioxide and harvested on day 28, 56 or 84 in experiment I.

Lines are linear regressions for each species  $\times$  CO<sub>2</sub> combination.

growth often markedly affect survivorship under conditions of stress or competition (Harper, 1977). Since, in the majority of plant species, most mortality occurs in the very early stages of growth (Harper, 1977), the transient differences in growth rate observed here could have a marked impact on selection.

Given that the relative growth rate of individuals from large seeds may respond more positively to elevated CO<sub>2</sub> than that of individuals from small seeds, the question arises as to why this should be the case. Part of the answer may lie in how timing of establishment varies with seed size. In calculating relative growth rate in this study, we assumed that the time intervals over which growth occurred were identical for the two species and for seeds of various sizes. In reality, we know that the larger seed reserves of *P. abies* allowed this species to establish more quickly and unfold its leaves earlier than *P. rubens*. As elevated CO<sub>2</sub> cannot have any impact on photosynthesis until the leaves are exposed to light, this means that *P. abies* was exposed to the positive effect of elevated CO<sub>2</sub> on photosynthesis for a longer period of time than *P. rubens*. If this hypothesis is correct, the effect of species on the response of relative growth rate to CO<sub>2</sub> should be limited to the first time interval, as all seedlings had unfolded their leaves by day 28. This was indeed what we observed. However, differences in time of establishment cannot explain all of the effects of seed size, as intraspecific variation in seed size had no effect on time of establishment.

The effect of intraspecific variation in seed size on CO<sub>2</sub> responsiveness may be related to nutrient availability. At the intraspecific level, increases in seed size increased both nitrogen concentration and total nitrogen content of the seeds. Increases in nitrogen availability have been shown to enhance response to elevated CO<sub>2</sub> in numerous studies (e.g., Bazzaz et al., 1993; Johnsen, 1993; Thomas et al., 1994; Prior et al., 1997; Tissue et al., 1997). Meta-analyses have shown that nutrient availability is one of the most important environmental factors controlling the response of relative growth rate to elevated CO<sub>2</sub> in a wide range of species (Poorter and Navas, 2003). This, of course, raises the question of why intraspecific variation in seed size only affected the response to CO<sub>2</sub> in *P. abies* and not in *P. rubens*. This may be related to the different nitrogen concentrations found in the seeds of the two species. The lower nitrogen concentration in the seeds of *Picea abies* would mean that seedlings of this species are more likely to be limited by nitrogen availability. Therefore, the increase in nitrogen concentration and content with seed size is more likely to have an impact upon this species. In this regard, it should be noted that the root:shoot ratio of *P. abies* seedlings was higher than those of *P. rubens*, supporting the suggestion that *P. abies* was more limited by nutrients than *P. rubens*.

Although differences in nutrient availability may help explain why the relative growth rate of seedlings from larger seeds may be more responsive to elevated CO<sub>2</sub> than that of seedlings from small seeds, it may not be the only explanation. As demonstrated in the present study, plants grown from large seeds often have a lower relative growth rate than plants grown from small seeds when both are grown under conditions of low stress (Grime and Hunt, 1975; Maranon and Grubb, 1993). This effect is largely due to the fact that large seeds typically allocate a greater proportion of their resources to constructing sturdy, high-density tissue, reducing the resources avail-

able for immediate resource acquisition and further growth (Wright and Westoby, 1999). The construction of "sturdy" tissue is thought to be one of the major reasons why large seeds are more resistant to stresses such as drought and burial by litter (Leishman et al., 2000). However, allocation of resources to support functions also imposes a significant respiratory burden on the seedling and reduces the resources available to develop the photosynthetic surface area of the plant. In other words, the seedling will have a low leaf area ratio and, therefore, a relatively low source:sink ratio compared to a seedling germinating from a small seed. Studies that have examined the effect of variation in source to sink ratio on CO<sub>2</sub> response have shown that plants with a high proportion of energy sources (e.g., photosynthetic leaves) to energy requiring sinks (e.g., respiring roots or storage organs) do not respond as well to elevated CO<sub>2</sub> as plants with a low source to sink ratio (e.g., Clough et al., 1981; Peet, 1984; Reekie et al., 1998). In this regard, it should be noted that *P. abies* had a lower leaf area ratio than *P. rubens* as expected, given the difference in seed size. Further, at the intraspecific level, increasing seed size decreased leaf area ratio in experiment I (data not presented) at the same time that seed size increased the relative growth rate response to elevated CO<sub>2</sub>.

Although further work is needed at both the inter- and intraspecific levels with a broader range of species, the pattern that seems to be emerging from this and previous studies is that plants from large seeds do respond more positively to elevated CO<sub>2</sub>. There appear to be three different reasons for this effect: 1) the compounding of the initial differences in size over time, 2) the earlier establishment of seedlings from large seeds and the effect this has on the length of exposure to elevated CO<sub>2</sub>, and 3) a more positive effect of elevated CO<sub>2</sub> on relative growth rate of seedlings from large seeds compared to those from small seeds that probably results from differences in nutrient availability and source to sink ratio.

The conclusion that seed size affects response to elevated CO<sub>2</sub> has important implications, not only for understanding how rising atmospheric CO<sub>2</sub> levels will affect plants, but also for understanding the forces that act on germinating seeds at current atmospheric CO<sub>2</sub> levels. It is generally believed that competition, shade, drought, nutrient deprivation, burial and herbivory are the major selective forces acting on seed size during the establishment phase of plant growth (Leishman et al., 2000). However, we have shown that the increase in CO<sub>2</sub> concentration that one would expect to find at the site of germination in many communities can result in as much as a 100% increase in plant size in individuals from large seeds compared to no significant increase in individuals from small seeds. These are not trivial effects and have the potential to alter the relative success of seedlings germinating from small versus large seeds. A comprehensive understanding of why seed size varies among habitats will require not only knowledge of how light, water, nutrients and herbivory vary, but also how CO<sub>2</sub> varies among these habitats.

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