THE EFFECTS OF SEED SIZE ON SEEDLING GROWTH RESPONSE TO ELEVATED CO_2 IN FOUR CONIFER SPECIES

by

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Thesis

submitted in partial fulfillment of the requirements

for the Degree of Master of Science (Biology)

Acadia University

Fall Convocation 2001

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ABSTRACT

1. Four species of conifer were studied at both the interspecific and intraspecific levels to examine how variation in seed size affects early seedling growth response to elevated CO_2.
2. Elevated CO_2 affected the large-seeded *Picea abies* more positively than the small seeded *Picea rubens*. Differences in growth responses of the *Picea* were likely due to differences in relative growth rate (RGR) within the first two weeks of growth. Elevated CO_2 did not affect the growth of the *Pinus* species.
3. Intraspecific variation in seed size did affect growth, but there was no evidence of intraspecific differences in seed size on growth response to elevated CO_2 in any of the four species studied.
4. Contrasting growth responses to elevated CO_2 of seeds of different size, at the interspecific and intraspecific levels, are explained in terms of seed reserves and the ratio of energy to mineral nutrient reserves within the seed. Specifically, if energy reserves are increased to a greater extent than mineral nutrients as seed size increases, the CO_2 response will decrease.

ACKNOWLEDGMENTS

I would like to thank Edward G. Reekie for the time and energy he has spent advising and helping with everything from experimental design to polishing and revising the manuscripts. I would also like to thank Julie Appleby, Rosie Taylor, Julie Black, and Dave Hayman for their help in throwing and catching needles during the harvests, as well as, Caryn Thompson from Statistical Consulting Services for her help with the development and refinement of the models and Krystyna Palczynski for generously lending me some much needed equipment. The Natural Science and Engineering Research Council of Canada provided financial support for the project.

INTRODUCTION

The effects of CO_2 on the growth of plants have been studied from a wide range of perspectives. Generally, increased CO_2 causes plants to be larger in total weight (Bazzaz & Miao 1993; Cantin *et al.* 1997; Johnsen 1993; Samuelson & Seiler 1994). The response to elevated CO_2 varies dramatically however, depending on many factors. In particular, there is tremendous interspecific variation in response to elevated CO_2 ranging from no response, to an increase of 50% or more growth. This makes it very difficult to predict how changes in atmospheric CO_2 may impact the productivity and composition of plant communities in the future without knowing the CO_2 response of all the individual species in a community. Given the large number of species in most natural communities and the large number of different communities, the prospect of examining the CO_2 response of all these species is daunting. Therefore, attempts have been made to classify species into functionally similar groups based on physiological or morphological differences between species (Poorter *et al.* 1996) and to establish relationships between these functional groups and their response to CO_2. If most of the interspecific variation in CO_2 response can be explained by a species' functional group, then it may be possible to make predictions about the effect of increasing CO_2 on community structure and productivity without conducting experiments on all the individual species. Some of these functional groups include: shade tolerant versus shade intolerant plants (Bazzaz *et al.* 1993; Kubiske & Pregitzer 1996; Rochefort & Bazzaz 1992), C3 versus C4 plants (Dippery *et al.* 1995; Hamerlynck *et al.* 1997; Hand *et al.* 1993; Poorter 1993; Tissue *et al.* 1995), fast versus slow growing plants (Diaz *et al.* 1993; Hunt *et al.* 1995; Poorter 1993), early versus late successional plants (Bazzaz *et al.* 1993; Rochefort & Bazzaz 1992) and stress tolerant versus stress intolerant plants (Bazzaz *et al.* 1993; Bunce 1992; Kinney & Lindroth 1997). These efforts have only been partially successful; some functional groups, such as C3 versus C4 plants, are important in explaining interspecific variation. However, much unexplained variation remains.

Seed size is another potential functional group that should be studied more thoroughly to further understand how and why some plant types respond positively to elevated CO_2 whereas others do not. Seed size has been linked to many ecological and physiological processes, which may affect the way plants respond to CO_2 under different growing conditions. The effect of seed size variation, within and among species, on the germination, emergence and early growth of species has been well studied. Increased seed size decreases the time required to germinate (Tripathi & Khan 1990), increases percent emergence (Gross 1984; Leishman & Westoby 1994b), and allows plants to unfold their first set of true leaves earlier (Seiwa & Kikuzawa 1991). Shade tolerance (Leishman & Westoby 1994a), and resistance to burial and desiccation (Metcalfe & Grubb 1997) has also been related to seed size. Large seeded species tend to exhibit lower rates of seedling mortality and survive longer (Jurado & Westoby 1992; Walters & Reich 2000). Seedlings from larger seeds have also been shown to have lower relative growth rates (RGR) in both interspecific and intraspecific comparisons (Jurado & Westoby 1992; Maranon & Grubb 1993; Reich *et al.* 1998; Walters *et al.* 1993). Large-seeded species also tend to have a lower leaf area ratio (LAR) compared to species with smaller seeds (Reich *et al.* 1998). Net assimilation rate (NAR) is generally higher for smaller seeded boreal tree species (Reich *et al.* 1998).

Increasing the CO_2 level alters many of the same aspects of plant growth that are also affected by changes in seed size. Increased CO_2 has been shown to alter NAR, LAR and RGR. NAR is generally increased by elevated CO_2 (Tjoelker *et al.* 1998; Townend 1993; Tschaplinski *et al.* 1995), while LAR is generally lower in elevated CO_2 treatments (Tjoelker *et al.* 1998). Elevated CO_2 has been shown to increase RGR early in the growth of both evergreen and deciduous seedlings (Hoddinott & Scott 1996; Hunt *et al.* 1995; Mortensen 1994; Tissue *et al.* 1997; Tschaplinski *et al.* 1995). Elevated CO_2, much like seed size, has also been shown have the strongest effects in early plant growth (Bazzaz *et al.* 1993; Hunt *et al.* 1995).

Whether the impact of CO_2 concentrations on plant growth varies with seed size has not been clearly established. An early study found that seedlings of large seeded deciduous tree species responded more positively to increasing CO_2 than did those of small seeded species (Bazzaz *et al.* 1993). This experiment led to a further study with *Quercus rubra* to examine the effects of intraspecific variation in seed size on seedling growth response to elevated CO_2 (Miao 1995). This study also found that larger seeds responded more positively to elevated CO_2 than smaller seeds. A similar study examined intraspecific variation in *Bromus erectus* and again showed that larger seeds responded more positively to elevated CO_2 (Steinger *et al.* 2000).

This study was designed to evaluate the early seedling growth of four conifer species in two genera, *Picea abies*, *Picea rubens*, *Pinus strobus* and *Pinus resinosa*, to determine how interspecific and intraspecific differences in seed size affect plant response to elevated CO_2. Using growth analysis we will determine how plant growth of *Pinus* and *Picea* species respond to both seed size and CO_2 and determine if: 1) smaller seeds react more positively, 2) larger seeds react more positively, or 3) CO_2 response is independent of seed size.

MATERIALS AND METHODS

Two consecutive experiments were conducted. The first examined *Picea abies* (Norway Spruce) and *Picea rubens* (Red Spruce), and the second examined *Pinus strobus* (White Pine) and *Pinus resinosa* (Red Pine). Seeds of *Picea abies* (seed lot 3699), *Picea rubens* (seed lot 3556), *Pinus resinosa* (seed lot 2548) and *Pinus strobus* (no seed lot identification provided) were obtained from the Nova Scotia Department of Lands and Forest, Tree Breeding Centre, Debert, Nova Scotia. These Species were selected for the study because they are common species found in Nova Scotia and exhibited initial seed weights which varied dramatically both interspecifically and intraspecifically.

Plant Culture:

Seeds were weighed individually and dusted with fungicide (50% Captan and 50% Benlate). The seeds were stratified at 4 °C for four weeks in numbered petri dishes that were lined with moistened Kimwipes and sealed with Parafilm. The *Picea* seeds were then placed in Conviron growth chambers at 17 °C, with no light, to germinate; 10 days for *Picea abies* and 12 days for *Picea rubens*. The *Pinus* seeds were germinated under the same light and temperature regime, but the *Pinus resinosa* were germinated for 17 days whereas the seeds of *Pinus strobus* were germinated for 20 days. Individual seeds with visible and healthy radicles were transplanted into one of eight 72-cell trays. The 53 x 27 cm trays were made up of twelve blocks of 6 cells. Each cell had a volume of 74 cm^3. The substrate in the cells was a 1:1 mixture of autoclaved sand and peat. Approximately 0.16 g of a 14-14-14 slow release planter fertilizer, equivalent to 40 mg

m^{-2} $year^{-1}$, from Plant Products Co. Ltd. was placed in each cell. The trays 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 hour light/dark cycle at 100 μmol cm^{-2} sec^{-1} for the first week and then reprogrammed to 170 ± 15 μmol cm^{-2} sec^{-1} for the remaining 13 weeks. The lighting was a mixture of incandescent and fluorescent. The coinciding temperature cycle was 15/10 ± 0.5 °C. Chamber light and temperature regimes were chosen to emulate average growing conditions under a closed canopy during spring and early summer. Light levels are approximately 10% of full sunlight on a clear cloudless day, while the temperatures approximate mean seasonal temperatures in Nova Scotia Canada between April and June. Plants were watered by filling the bottom of the tanks with water so that it could be absorbed into the cells. More water was added to the tanks when the surface of the substrate was visibly dry.

Experimental Design:

The seeds were arranged in an alternating pattern such that both species were regularly distributed throughout all eight trays. Within each tray, every second block of six plants was designated for final harvest while the rest were designated as weekly harvests for growth analysis (Fig. 1b). Prior to germination, the seeds were divided into three size categories: large, medium and small. The large and the small seed sizes were defined by the first and fourth quartiles of a normally distributed population of seeds sizes, while the medium size class included seeds from the second and third quartile. Only medium seeds were planted for the weekly harvests, for the growth analysis, but all three seed size classes were planted for the final harvest. For the final harvest, there was one large, one small and one medium seed from each species arranged in a semi-random distribution in 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 (Fig. 1c). Within each chamber, the four trays were assigned one of two carbon dioxide treatments, ambient (≈350 ppm) or elevated (1400 ppm), such that there were two ambient and two elevated carbon dioxide treatments per chamber. These CO_2 levels were chosen because they represent the naturally occurring ranges of CO_2 concentration at the soil surface that a young plant may experience during germination and early growth. The low CO_2 treatment is representative of a CO_2 concentration at the soil surface of a bare mineral soil while the elevated CO_2 treatment was representative of a CO_2 concentration within a deep litter layer.

Air Circulatory System:

A flow through fumigation system was used to subject seedlings to the appropriate CO_2 levels in the eight 56 L glass-sided tanks. The top edges of the tanks were lined with closed cell foam rubber so that plexiglass lids placed on top of the tanks and clamped down using elastic cords provided a leak resistant seal. A 50 cm long section of 0.7 cm diameter Tygon tubing was attached to the outflow hole (a 0.7 cm hole drilled in the corner of the lid opposite the air inflow) to reduce back diffusion of air into the tanks. Ambient air was drawn from outside the building, at ≈2 m above the ground. The ambient air was pressure regulated as it entered the building and the flow rate going into the tanks was controlled with needle valves. To supplement the ambient CO_2 levels in the elevated CO_2 treatment, pressure regulated CO_2 was bled into the ambient air using needle valves, and allowed to mix thoroughly in 2L reservoirs. The flow rate of both the ambient and elevated CO_2 treatments was 4 L min^{-1} upon entering the tanks. The carbon dioxide levels were checked immediately before entering the growth tanks, using either a Li-Cor 6200 infra-red gas analyzer or a NOVA Analytical Systems CO_2 gas analyzer, model 421P. These measurements were taken 3 times per week. Adjustments to CO_2 levels were made if necessary. Carbon dioxide levels leaving the tanks were checked periodically to ensure that CO_2 levels inside the tanks did not increase above set levels due to respiration.

Experimental Measurements:

Harvests were done biweekly for the growth analysis. One block of six plants, containing three plants of each species, was chosen at random from the designated biweekly harvest blocks in each tank (Fig. 1b). Plants were harvested and separated into roots, stems and needles. The last whorl of needles, those that were just emerging and were too small or delicate for leaf area measurements, were included with the stems. The needles were counted and the cumulative area of the needles determined using a Li-Cor 3100 area meter calibrated to the most sensitive resolution (10^{-3} cm^2 ± 1.5%). The needles, roots and stems were then placed in an oven to dry at 50 °C for one week. Dry weights for the roots, stems and needles were measured to 10^{-5} g. The final harvest was done in week 14 of the experiment over a two-day period. The same sampling procedure was followed for the final harvest as for the biweekly harvests.

Statistical Analysis:

Analyses for *Picea* and *Pinus* were conducted separately because they were designed as two separate trials. A split plot design was used to test the effects of CO_2 and species at the final harvest, with trays as the main plot and each block of six plants within the trays as the sub plots. Species and CO_2 were considered fixed effects while trays and blocks were random effects. The CO_2 effect was tested using the main plot error term while the species and the interactions between species and CO_2 were tested using the residual error term. To examine the effects of intraspecific variation in seed size, seed weight was entered as a covariate in the analysis prior to the other treatment effects. We also included the interactions between seed weight and CO_2 and between seed weight and species as effects in the model. To determine whether the covariate and its interactions were explaining additional variation, by including the covariate interactions, or simply redistributing the variance from one term to another, an extra sum of squares F-test was used (Myers 1990).

Relative growth rate (RGR), net assimilation rate (NAR) and leaf area ratio (LAR) of the biweekly harvest data were calculated separately for each species x CO_2 combination using the Hunt and Parsons (1974) growth analysis program. More detailed explanations of RGR, NAR and LAR are provided in Appendix A. The RGR of the species was found to be constant throughout the entire growing period for each CO_2 x species combination. To test the significance of treatments on RGR, a model similar to the one above was used except that time of harvest was used as a covariate instead of seed size, and the data were natural log transformed prior to analysis. The slope of the weight versus time curve was equivalent to RGR because the curve was linear and the slope of the curve was constant. Significant time x CO_2 and time x species interactions in the model represent significant differences in RGR for CO_2 and species, respectively.

RESULTS

Seed Size Differences:

There were large differences in seed weight at both the interspecific and intraspecific levels in both *Picea* and *Pinus* (Table 1). The average seed weight of *Picea abies* was 9.6 mg but it ranged between 4.6 mg and 17.1 mg, *Picea rubens* had an average seed weight of 3.0 mg while it ranged between 1.2 mg and 4.8 mg. The average seed weight of *Pinus strobus* was 17.8 mg with a range of 7.3 mg to 33.6 mg, while for *Pinus resinosa*, the mean was 8.4 mg and ranged from 5.3 mg to 12.3 mg.

Picea Final Harvest:

Picea abies had a significantly larger total weight than *Picea rubens* (Tables 2 and 3) after 98 days. The elevated CO_2 treatment significantly increased total weight and stem length, but decreased leaf area ratio (LAR). *Picea abies* responded more positively to elevated CO_2 with respect to total weight than *Picea rubens*. The enhancement ratio for total plant weight due to elevated CO_2 was 1.62 for *Picea abies* and 1.32 for *Picea rubens* (Table 3). The root to shoot ratio (RSR) of *Picea abies* decreased in the elevated CO_2 treatment while the RSR of *Picea rubens* increased at elevated CO_2. Within species, large seeded individuals had higher total weight, LAR and stem height (Table 2). The extra sums of squares F-test revealed that the effects of CO_2 did not vary with seed size at the intraspecific level (Table 4).

Pinus Final Harvest:

Pinus resinosa had a greater total weight and a larger LAR than *Pinus strobus* (Tables 2 and 5). Elevated CO_2 significantly decreased LAR but did not affect plant dry weight, stem height or RSR. There was no evidence that the species responded differently to elevated CO_2 under these experimental conditions. At the intraspecific level of seed size variation, large seeded individuals had higher total weight, stem height, and RSR and lower leaf area ratio regardless of species or CO_2 treatment. The extra sums of squares F-test revealed that the effects of CO_2 did not vary with seed size at the intraspecific level. The mean squared values from the extra sum of squares F-test, comparing the model with only seed size as a covariate to the model with initial weight as well as the seed size interactions with species and CO_2 (Table 4).

Picea Growth Analysis:

Both *Picea* species grew exponentially over the entire growing period (Fig. 2). *Picea abies* was significantly larger than *Picea rubens* over the entire growing period even though *Picea rubens* had a higher relative growth rate than *Picea abies* (Table 1). There was no difference in RGR between 1400 ppm and 350 ppm CO_2 treatments (Table 1, Fig. 2). However, examination of the total weight growth curves for *Picea abies* and to a lesser extent in *Picea rubens*, reveals that there were differences in total weight between 1400 ppm and 350 ppm CO_2 treatments at various times (Fig. 2). The net assimilation rate (NAR) of both *Picea* species increased over time (Fig. 3). The NAR of the 1400 ppm CO_2 treatments were higher than the NAR of the 350 ppm CO_2 treatments for both *Picea* species but they were only significant in *Picea rubens* at specific times. The LAR of both *Picea* species decreased over time (Fig. 4). The LAR was higher in the 350 ppm CO_2 treatment for both species except on the final biweekly harvest date.

Pinus Growth Analysis:

Both Pinus species grew exponentially over the entire growing period (Fig. 5). *Pinus strobus* was larger than *Pinus resinosa* initially but the weight difference between the species was reduced over time. *Pinus resinosa* had a higher relative growth rate than *Pinus strobus* (Table 1). The net assimilation rates of *Pinus strobus* and *Pinus resinosa* did not differ dramatically but there were differences in response to CO_2 treatments over time although no consistent pattern of variation was observed (Fig. 6). The leaf area ratio of *Pinus resinosa* and *Pinus strobus* was highly variable over time. There were significant differences in LAR with respect to CO_2; however these differences were either positive or negative depending on the harvest time (Fig. 7).

DISCUSSION

The larger seeded *Picea abies* responded more positively to elevated CO_2 for total biomass than the smaller seeded *Picea rubens* at the final harvest (Table 2). However, in the growth analysis, CO_2 did not significanly affect RGR in either species (Table 1). Elevated CO_2 did increase NAR (Fig. 3) and decrease LAR (Fig. 4) in both species. Due to the functional relationship between RGR, NAR and LAR, increasing NAR slightly and decreasing LAR slightly will result in no significant change in RGR as was observed.

The apparent discrepancy between the growth analysis and the final harvest results has two possible explanations. One possibility is that elevated CO_2 results in a non-significant increase in RGR. When these insignificant increases are compounded over time, they may have a significant positive effect on final plant size. This was clearly not the case, since in both species the RGR of the ambient CO_2 treatment was numerically higher than the elevated CO_2 treatment (Table 4). The lower RGR for the elevated CO_2 treatment was due primarily to the negative effects of a consistently lower LAR (Fig. 4), compared to the more transient and less positive effect of elevated CO_2 on NAR (Fig. 3). The other possibility is that the effect of elevated CO_2 on RGR occurred prior to the first harvest date, i.e. day 14 of the experiment. This is probably the case since the weight of the elevated CO_2 plants was higher over the entire growing period and the differences appeared to be greater for *Picea abies* than *Picea rubens* (Fig. 2). Since the seeds were assigned to CO_2 treatments randomly and there was no difference in initial seed weight between CO_2 treatments, the effects of CO_2 on RGR that resulted in greater biomass at the time of the first harvest, must have occurred between the time of planting and the first harvest, on day 14. Therefore, the larger seeded *Picea abies* must have responded more positively to elevated CO_2 during the initial stages of growth in order to achieve a proportionally greater biomass at day 14 and at the final harvest (Table 3).

This interspecific variation in growth response to elevated CO_2 was not observed at the intraspecific level of seed size variation. Initial seed size had a significant positive effect on the growth of the *Picea*, such that larger seeds produced larger plants (Table 2). However, there was no evidence of increased growth response to elevated CO_2 in larger seeds within either of the *Picea* species. One possible explanation for this lack of response is that there was simply not enough within species variation in seed size to allow these effects to be detected. I do not believe this is true in the present study since there were large differences in seed size both within and between species (Table 1). Furthermore, within species variation in seed size produced noticeable growth differences (Table 2).

There were no significant effects of CO_2 on either of the *Pinus* species. The lack of significant CO_2 response in the *Pinus* species was unexpected and may be the result of a general lack of responsiveness to elevated CO_2 because of species-specific growth characteristics or limiting growing conditions. Studies using other *Pinus* species, namely *Pinus banksiana*, *Pinus taeda* and *Pinus palustris*, have found significant responses to elevated CO_2 (Tissue *et al.* 1997; Cantin *et al.* 1997; Gebauer *et al.* 1996; Prior *et al.* 1997). Therefore, the lack of response to CO_2 in my study was probably not due to the growth characteristics of the *Pinus* species tested. There was, however, high mortality in both *Pinus* species, 38% in *Pinus resinosa* and 28% in *Pinus strobus*, most of which can be attributed to disease. The reservoir of water in combination with the 1:1 mixture of peat and sand kept the substrate continually moist. These growing conditions were apparently conducive to the development of root diseases to which these *Pinus* species were very susceptible. These growing conditions may have adversely affected growth of the seedlings that did survive and may account for the erratic CO_2 response. Since mortality in the *Pinus* species was so high and the stress on the remaining plants had unknown effects on CO_2 enhancement, all of the results for the two *Pinus* species will be discounted and will not be discussed further.

Seed weight has been repeatedly shown to be important in the early establishment phase of plant growth (Jurado & Westoby 1992; Leishman & Westoby 1994b; Walters *et al.* 2000; Wang *et al.* 1994). The beneficial effects of CO_2 on plant growth have also been shown to be most obvious in the establishment phase (Bazzaz *et al.* 1989; Hunt *et al.* 1995; Tjoelker *et al.* 1998). This suggests that there is potential for strong interaction between the effects of seed size and CO_2 on early plant growth. However, the question of how seed size and CO_2 interact to alter growth in *Picea* species remains unresolved due to the contrasting effects at the interspecific and intraspecific levels of seed size variation. One possible explanation for these contrasting effects is that the differences at the interspecific level may have nothing to do with seed size at all. *Picea abies* may be responding more positively to elevated CO_2 than *Picea rubens* for reasons other than differences in seed size. However, the fact that the CO_2 effects on *Picea* occur early in the growth of the plants, when seed size is also affecting growth, lends some support to the idea that CO_2 could be interacting with seed size. Further, the growth enhancement of the larger seeded *Picea abies* over the smaller seeded *Picea rubens* (Table 3) is in agreement with the earlier study which examined the effects of seed size on the CO_2 response of a number of deciduous tree species (Bazzaz *et al.* 1993) and with studies at the intraspecific level in both *Quercus rubra* (Miao 1995) and *Bromus erectus* (Steinger *et al.* 2000).

A possible explanation for the contrasting results in the present study may be found in the underlying explanation for why seed size affects CO_2 response. Previous studies argued that seedlings from larger seeds responded more positively to elevated CO_2 than those from smaller seeds because of greater resource availability. This allows plants grown from larger seeds to take greater advantage of elevated CO_2 in the early stages of growth (Bazzaz *et al.* 1993; Miao 1995; Steinger *et al.* 2000). However, if you examine the literature on effect of resource availability on response to CO_2 you find contrasting results depending on the limiting resource. Studies relating light availability and CO_2 responsiveness suggest that plants grown in low light, which limits energy availability, respond more positively to elevated CO_2 than plants grown in high light (Bazzaz *et al.* 1993). Also relevant to the question of how energy availability affects response to CO_2 are the studies that examine the effect of variation in source to sink ratio on CO_2 response (Fig. 8). The source to sink ratio in a plant can be varied by removing leaves (i.e., the source of fixed carbon or energy) or by removing active sinks for fixed carbon, such as developing fruits. Variation in source to sink ratio can also be achieved by comparing species or genotypes that have genetic differences in the size of active sinks relative to the photosynthetic apparatus. Both types of studies have demonstrated that plants with a low source to sink ratio or low energy availability respond more positively to elevated CO_2 than those with a high source to sink ratio. (Clough *et al.* 1981; Peet 1984; Reekie *et al.* 1998). On the other hand, studies looking at mineral nutrients (most commonly nitrogen), have found that plants grown at low nutrient availability tend to respond poorly to elevated CO_2 while those grown at high nutrient availability tend to respond more positively (Bazzaz *et al.* 1993; Johnsen 1993; Prior *et al.* 1997; Thomas *et al.* 1994; Tissue *et al.* 1997). For example, *Pinus taeda*, grown in a number of nutrient regimes and at ambient or elevated CO_2, showed less response to elevated CO_2 at the lowest concentrations of nutrient availability than at high nutrient availability (Tissue *et al.* 1997). The CO_2 response of plants limited by different resources seems to simply reflect Leibig's law of the minimum. Elevated CO_2 increases photosynthesis and therefore increases the availability of energy in the form of fixed carbon. Therefore, if energy is a limiting resource, and nutrient availability is not, a plant will respond positively to elevated CO_2 because of its increased ability to produce carbohydrates. On the other hand, if mineral nutrients are limiting, the plant will respond poorly to elevated CO_2 because the increased availability of fixed carbon does not alleviate the nutrient deficiency.

Seeds supply two types of reserves for early plant growth: (i) energy in the form of carbohydrates and oils and (ii) mineral nutrients. How plants with differing ratios of energy to mineral reserves in their seeds respond to elevated CO_2 is a topic that has not been studied. However, if we extrapolate from the studies in which energy and nutrient availability have been manipulated by controlling the environment, it stands to reason that plants grown from seeds that have a relatively high concentration of mineral nutrients, compared to energy reserves will respond more positively in early growth stages to elevated CO_2 than those with relatively low concentrations of mineral nutrients and high energy reserves (Fig. 8).

If variation in seed size does not affect the ratio of energy to mineral reserves in the seed, increasing seed size will increase the availability of both energy and mineral nutrients at the same time. The effects of seed size on CO_2 response will then depend on the relative size of the energy and mineral nutrient reserves in the seed. If the mineral reserves are relatively large, then increasing seed size should enhance the CO_2 response. On the other hand, if energy reserves are relatively large, increasing seed size should decrease CO_2 response. If increasing seed size changes the ratio of energy to mineral reserves, then other patterns may emerge. If by increasing seed size, the relative amount of nutrients increases to a greater extent than energy, the source sink ratio of the plant will be decreased and the CO_2 response will be greater. On the other hand, if by increasing seed size, the energy stores increases to a greater extent than the mineral reserves, the source sink ratio of the plant will be increased and the CO_2 response will be less. Therefore, at any level of seed size variation, whether it is interspecific or intraspecific, if the ratio of mineral nutrients to energy is altered with increasing seed size, the CO_2 response will be altered accordingly.

Although mineral nutrients and energy stores were not measured directly, there were indications that the ratio of mineral to energy reserves within the seed changed with seed size. The RSR of a plant often varies with availability of mineral nutrients. Plants grown in low nutrient soil, as well as responding poorly to elevated CO_2, generally have higher root shoot ratios than plants grown in high nutrient soil (Chapin 1991; Ericsson 1995; Larigauderie *et al.* 1994; Pettersson *et al.* 1993). If nutrients are limiting, a greater allocation to root biomass occurs which will improve nutrient uptake and maintain growth. In our study, there were no interspecific differences in RSR between Picea species or CO_2 treatments (Table 2). At the intraspecific level of seed size variation however, larger seeds had a higher RSR than the smaller seeds as indicated by the significantly positive slope due to initial seed size (Table 2). This could explain why there was no CO_2 response at the intraspecific level of seed size variation. Since increasing RSR is an indication of decreasing nutrient availability, it suggests that the ratio of mineral nutrients to energy reserves within the seeds decreased with increasing seed size. If this were the case, this would tend to counteract any positive effects of increasing seed size on nutrient availability and therefore any positive effects of seed size on CO_2 response.

CONCLUSION

Seed size, being a relatively easy to measure indicator of resource availability within seeds, may not be the best measure of a plants potential response to elevated CO_2 early in growth. In order to understand why some plants respond positively to CO_2 and others do not, a better understanding of what sort of resources are stored within the seeds is needed. Further, an understanding of how the ratio of carbon to nutrient reserves in the seed vary in relation to seed size may be needed to explain why there may or may not be a more positive growth response of larger seeds to elevated CO_2. In order to arrive at a clear understanding of how CO_2 affects early growth in *Picea* and *Pinus*, the mineral and carbon reserve status within the seeds will have to be examined more closely in conjunction with seed size.

Table 1. Mean and standard error of initial weight (seed size) and relative growth rates of both *Pinus* and *Picea* species at 1400 ppm or 350 ppm CO_2. Estimates followed by the same letter were not significantly different at the 0.05 probability level. Analyses for the *Picea* and *Pinus* species were done separately and cannot be compared directly.

Species	Average Initial Weight (mg)	Relative Growth Rate ($g\ g^{-1}\ day^{-1}$)	
		350 ppm	1400 ppm
Picea abies	9.6 ± 1.9	0.0409 a	0.0386 a
Picea rubens	3.0 ± 0.6	0.0455 b	0.0449 b
Pinus strobus	17.8 ± 5.0	0.0298 c	0.0293 c
Pinus resinosa	8.4 ± 1.4	0.0410 d	0.0404 d

Table 2. Slopes, calculated mean square values and significance levels from analysis of covariance model for *Picea* and *Pinus* species. Significance levels are: $P<0.001$ ***, $P<0.01$ **, $P<0.05$ *, $P>0.05$ ns. The extra sum of squares F test revealed no significant interactions between initial weight and either species or CO_2 indicating that neither species nor CO_2 had any effect on the slopes of the relationship between final weight and seed weight.

Genus	Response	Slope	Mean Square Values				
			Initial Weight (Seed Size)	Species	CO_2	CO_2 x Species	Chamber
Picea	Total Weight (g)	6.99	0.889 ***	0.060 **	0.232 ***	0.036 **	0.271 ***
	RSR	9.21	0.534 ***	0.007 ns	0.006 ns	0.108 *	0.871 ***
	LAR ($cm^2\ g^{-1}$)	-92.5	364.6 **	91.02 ns	2638.8 ***	114.0 ns	33067 ***
	Stem length (cm)	82.16	120.7 ***	8.68 ns	20.60 *	0.012 ns	259.8 ***
Pinus	Total Weight (g)	5.75	0.097 ***	0.010 *	0.006 ns	0.002 ns	0.029 ***
	RSR	1.178	0.110 ***	0.054 **	0.003 ns	0.000 ns	0.668 ***
	LAR ($cm^2\ g^{-1}$)	-166.36	1587.6 ***	560.2 **	473.9 **	35.33 ns	2053.0 ***
	Stem Length (cm)	54.24	8.61 **	0.871 ns	1.154 ns	1.152 ns	5.746 **

Table 3. Enhancement ratios for two *Picea* species grown at either 1400 ppm or 350 ppm CO_2. Values of growth measures for 1400 ppm and 350 ppm CO_2 are means from the final harvest. A significant species x CO_2 interaction indicates interspecific differences in growth response to elevated CO_2. Significance values are: $P<0.001$ ***, $P<0.01$ **, $P<0.05$ *, and $P>0.05$ ns.

	Picea abies			*Picea rubens*			
Measurement	350	1400	Enhancement Ratio	350	1400	Enhancement Ratio	Species x CO_2
Total weight (g)	0.159	0.258	1.62	0.116	0.153	1.32	**
RSR (g g^{-1})	0.318	0.276	0.87	0.260	0.294	1.13	*
LAR (cm^2 g^{-1})	25.19	20.17	0.80	29.16	21.47	0.74	ns
Stem Length (cm)	6.422	7.158	1.12	5.510	6.159	1.12	ns

Table 4. Extra sum of squares F-test for *Picea* and *Pinus* species comparing the model with only seed size as a covariate, to the model with seed size as a covariate as well as the interactions between seed size and species and seed size and CO_2. Significance was evaluated at the $P<0.05$ level.

Species	Response	Model SS with extra interactions	Model SS without extra interactions	Extra df	Model MSE with extra interactions	F-Value*	Significance
Picea	Total Weight (g)	1.643	1.634	2	0.0049	0.93	ns
	RSR (g g^{-1})	2.077	2.00	2	0.022	1.74	ns
	LAR (cm^2 g^{-1})	37637	37602	2	39.843	0.44	ns
	Stem Length (cm)	484.2	467.3	2	3.022	2.8	ns
Pinus	Total Weight (g)	0.226	0.220	2	0.0017	1.98	ns
	RSR (g g^{-1})	1.298	1.272	2	0.0067	1.93	ns
	LAR (cm^2 g^{-1})	7320.4	7317.5	2	57.7	0.02	ns
	Stem Length (cm)	28.02	26.37	2	0.68	1.21	ns

* F-Value calculated:

$$\text{Difference of models MS} = \frac{(\text{Model SS with interactions} - \text{Model SS without interactions})}{\text{Extra df due to interactions}}$$

$$\text{Difference of models F} = \frac{\text{Difference of models MS}}{\text{Model with interactions MSE}}$$

Table 5. Enhancement ratios for two *Pinus* species grown at either 1400 ppm or 350 ppm CO_2. Values of growth measures for 1400 ppm and 350 ppm CO_2 are means from the final harvest experiment. A significant species x CO_2 interaction indicates interspecific differences in growth response to elevated CO_2. Significance values are: P<0.001 ***, P<0.01 **, P<0.05 *, and P>0.05 ns.

	Pinus strobus			*Pinus resinosa*			Species x CO_2
Measurement	350	1400	Enhancement Ratio	350	1400	Enhancement Ratio	
Total weight (g)	0.103	0.123	1.19	0.139	0.144	1.04	ns
RSR (g g^{-1})	0.331	0.353	1.07	0.274	0.282	1.03	ns
LAR (cm^2 g^{-1})	47.70	45.96	0.96	54.86	51.37	0.94	ns
Stem Length (cm)	4.079	4.476	1.10	4.554	4.545	1.00	ns

Figure 1. A) Schematic diagram of tray arrangement within a growth chamber. B) The distribution of the final harvest block (dark gray) and the growth analysis blocks (light gray) within a tray. C) The alternating species distribution of randomly located large, medium and small seeds within a final harvest block (dark gray) and the medium seeds in the growth analysis block (light gray).

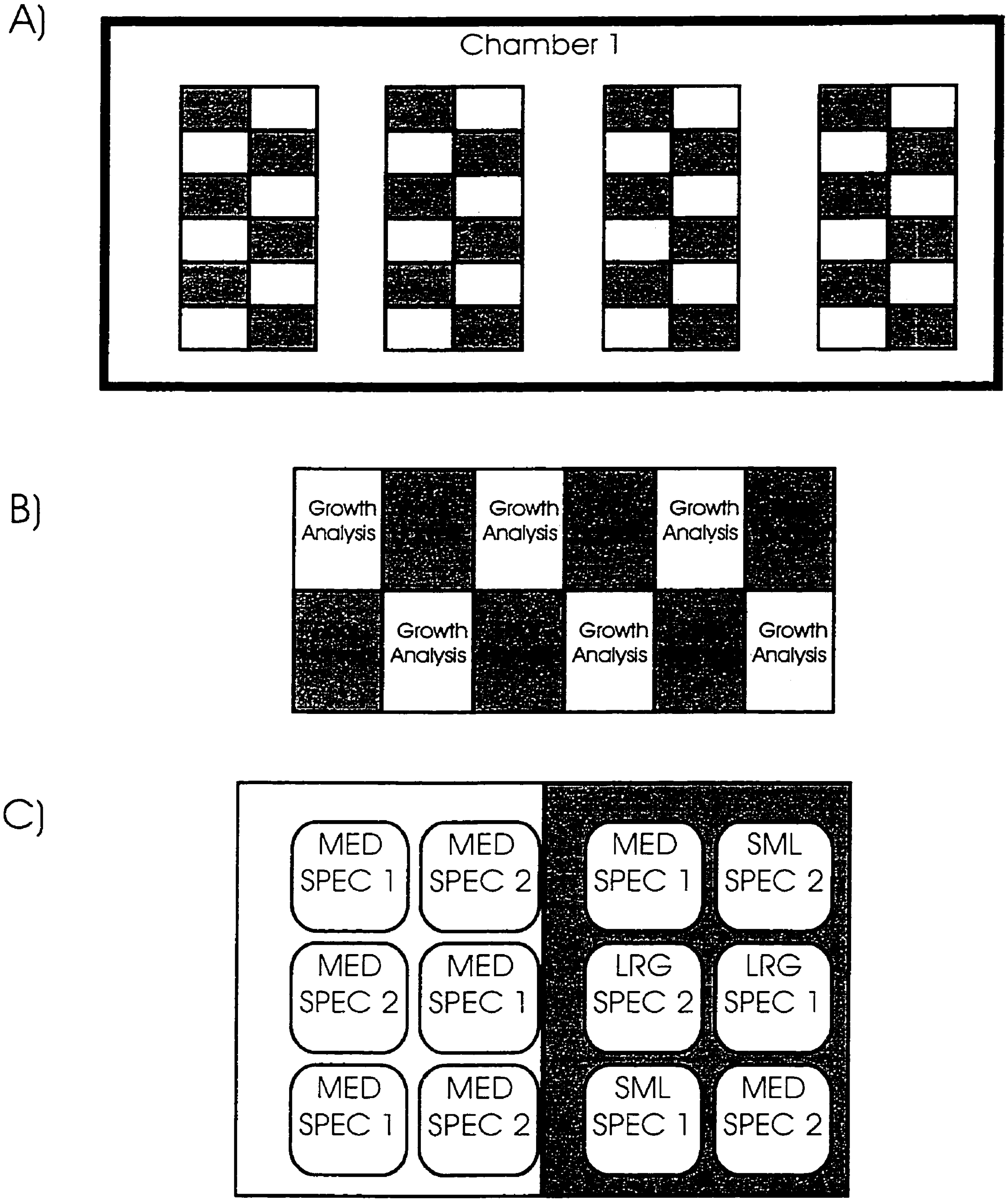
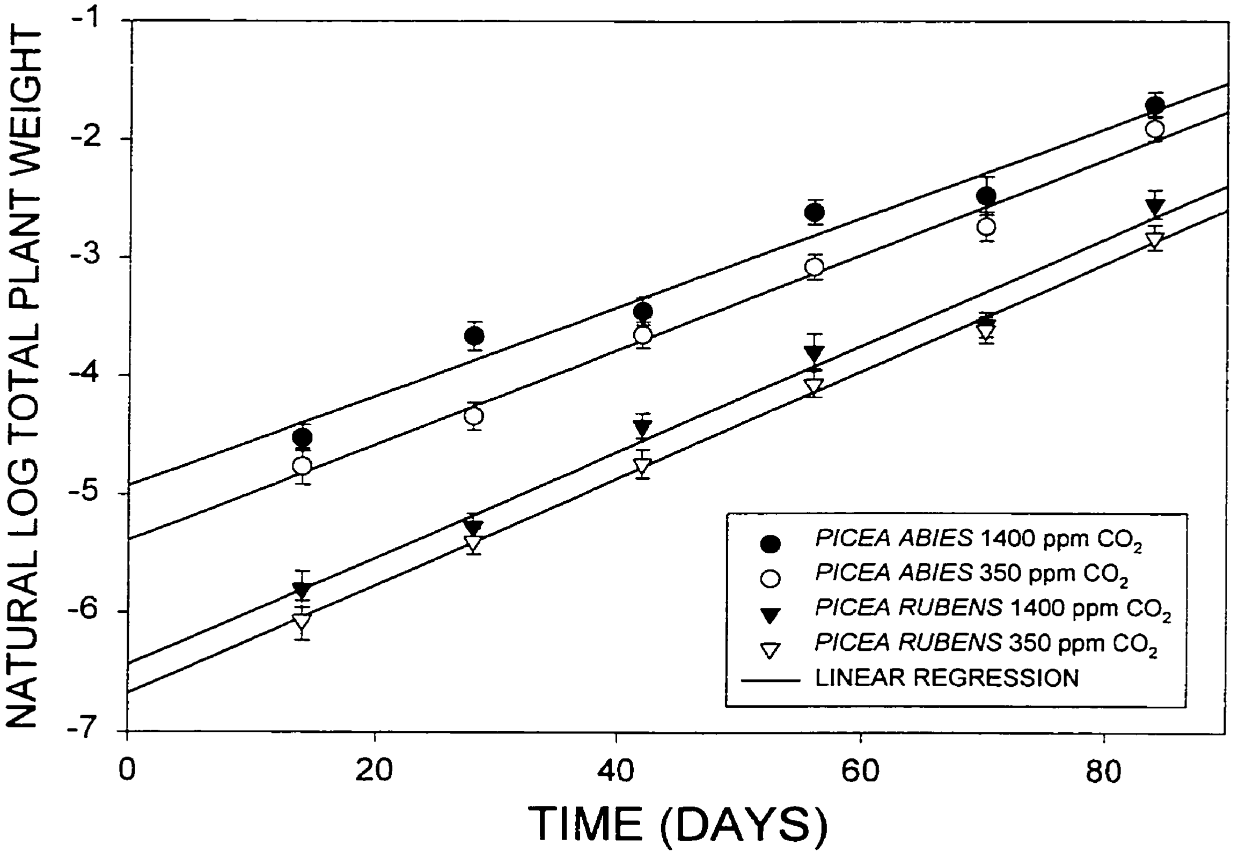

A)
Chamber 1
B)
Growth Analysis
Growth Analysis
Growth Analysis
Growth Analysis
Growth Analysis
Growth Analysis
C)
MED SPEC 1
MED SPEC 2
MED SPEC 1
SML SPEC 2
MED SPEC 2
MED SPEC 1
LRG SPEC 2
LRG SPEC 1
MED SPEC 1
MED SPEC 2
SML SPEC 1
MED SPEC 2

Figure 2. Scatter plots of natural log transformed total plant dry weight against time of harvest in *Picea abies* (circles) and *Picea rubens* (triangles) at either 1400 ppm (closed symbols) or 350 ppm (open symbols) CO_2. Each point represents the mean of all plants within a species x CO_2 combination while the error values are plus or minus one standard error. Lines are linear regressions for each species x CO_2 combination.



NATURAL LOG TOTAL PLANT WEIGHT
-1
-2
-3
-4
-5
-6
-7
0
20
40
60
80
TIME (DAYS)
● PICEA ABIES 1400 ppm CO2
○ PICEA ABIES 350 ppm CO2
▼ PICEA RUBENS 1400 ppm CO2
▽ PICEA RUBENS 350 ppm CO2
LINEAR REGRESSION

Figure 3. Fitted NAR (g cm^{-2} day^{-1}) over time in *Picea abies* and *Picea rubens* grown at either 1400 ppm (closed circles) or 350 ppm (open circles) CO_2. Means and 95% confidence limits for each CO_2 x species combination were calculated using the Hunt & Parsons (1974) growth analysis program.

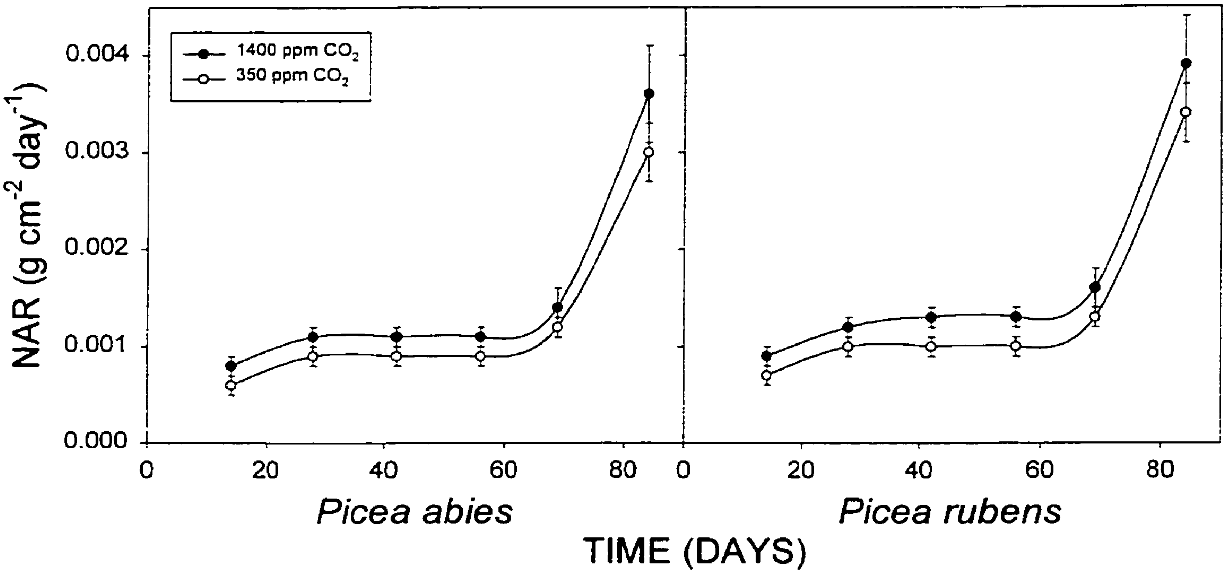

1400 ppm CO_2
350 ppm CO_2
NAR (g cm^{-2} day^{-1})
0.000
0.001
0.002
0.003
0.004
0
20
40
60
80
0
20
40
60
80
Picea abies
Picea rubens
TIME (DAYS)

Figure 4. Fitted LAR (cm^2 g^{-1}) over time in *Picea abies* and *Picea rubens* grown at either 1400 ppm (closed circles) or 350 ppm (open circles) CO_2. Means and 95% confidence limits for each CO_2 x species combination were calculated using the Hunt & Parsons (1974) growth analysis program.

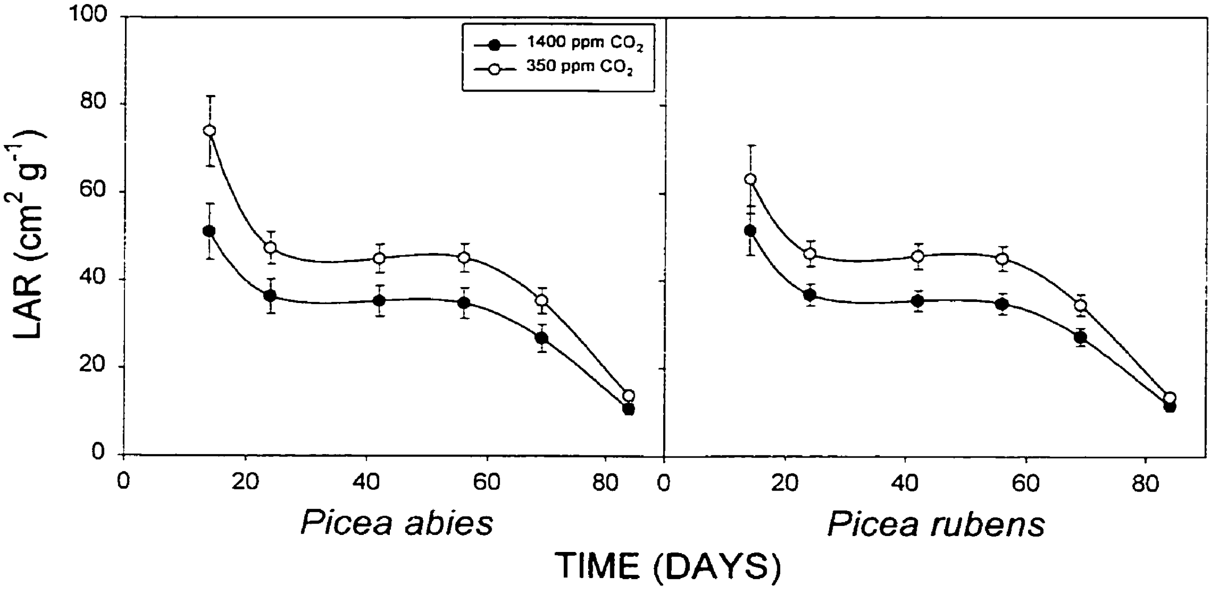

100
80
60
40
20
0
LAR (cm^2 g^{-1})
1400 ppm CO$_2$
350 ppm CO$_2$
0
20
40
60
80
Picea abies
Picea rubens
TIME (DAYS)

Figure 5. Scatter plots of natural log transformed total plant dry weight against time of harvest in *Pinus resinosa* (circles) and *Pinus strobus* (triangles) at either 1400 ppm (closed symbols) or 350 ppm (open symbols) CO_2. Each point represents the mean of all plants within a species x CO_2 combination while the error values are plus or minus one standard error. Lines are linear regressions for each species x CO_2 combination.

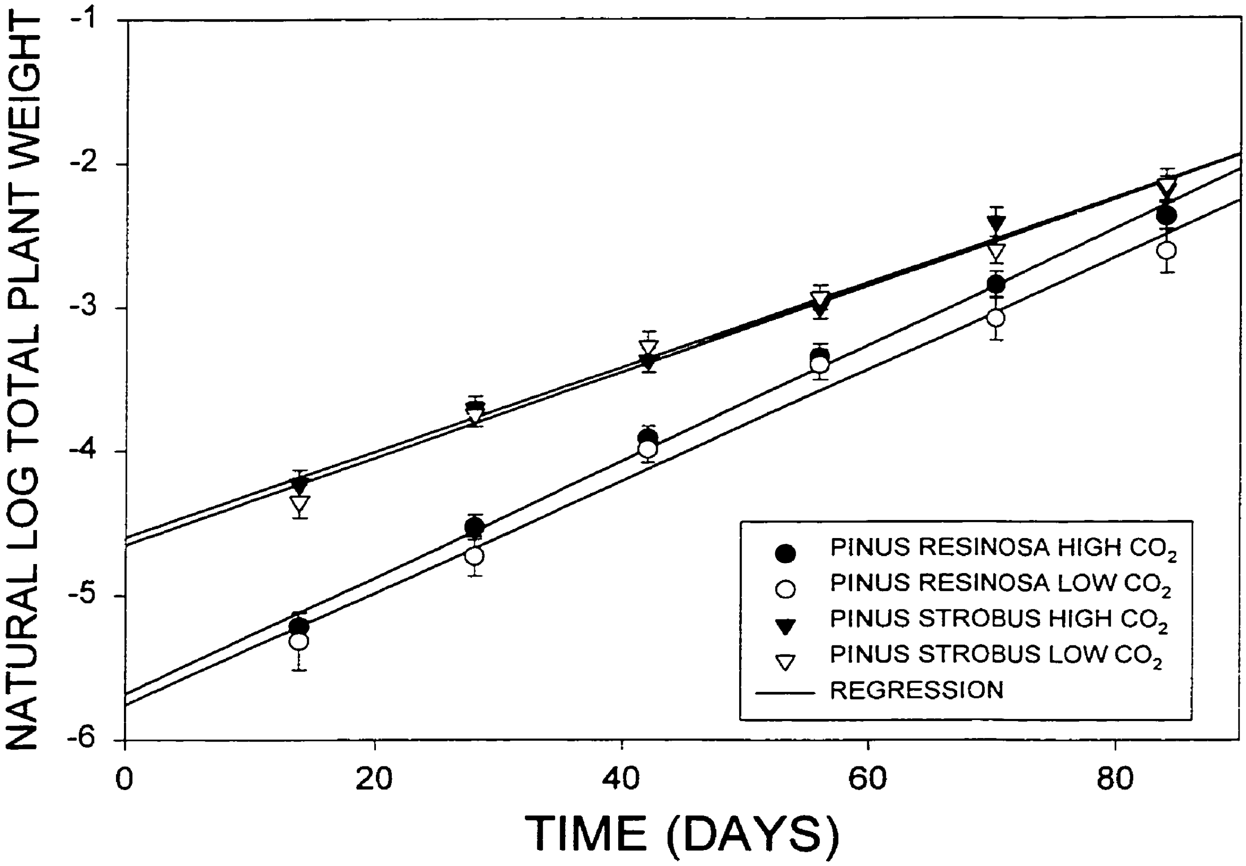

NATURAL LOG TOTAL PLANT WEIGHT
-1
-2
-3
-4
-5
-6
0
20
40
60
80
TIME (DAYS)
● PINUS RESINOSA HIGH CO_2
○ PINUS RESINOSA LOW CO_2
▼ PINUS STROBUS HIGH CO_2
▽ PINUS STROBUS LOW CO_2
—— REGRESSION

Figure 6. Fitted NAR (g cm^{-2} day^{-1}) over time in *Pinus strobus* and *Pinus resinosa* grown at either 1400 ppm (closed circles) or 350 ppm (open circles) carbon dioxide. Means and 95% confidence limits for each CO_2 x species combination were calculated using the Hunt & Parsons (1974) growth analysis program.

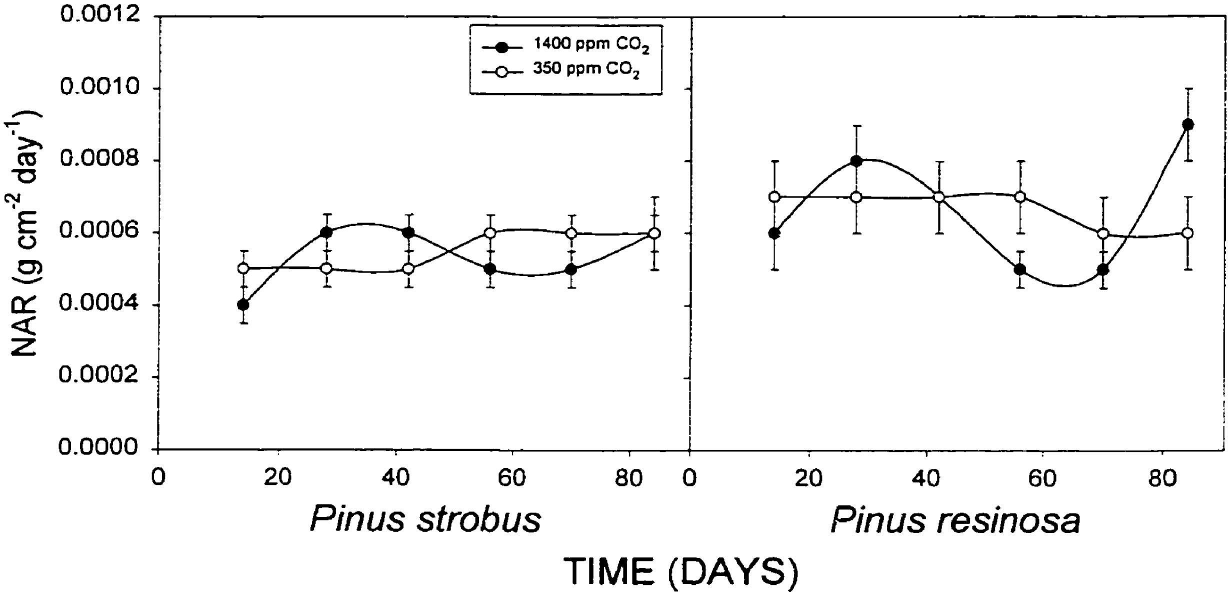

0.0012
0.0010
0.0008
0.0006
0.0004
0.0002
0.0000
NAR (g cm^{-2} day^{-1})
● 1400 ppm CO_2
○ 350 ppm CO_2
0
20
40
60
80
Pinus strobus
Pinus resinosa
TIME (DAYS)

Figure 7. Fitted LAR (cm^2 g^{-1}) over time in *Pinus strobus* and *Pinus resinosa* grown at either 1400 ppm (closed circles) and 350 ppm (open circles) CO_2. Means and 95% confidence limits for each CO_2 x species combination were calculated using the Hunt & Parsons (1974) growth analysis program.

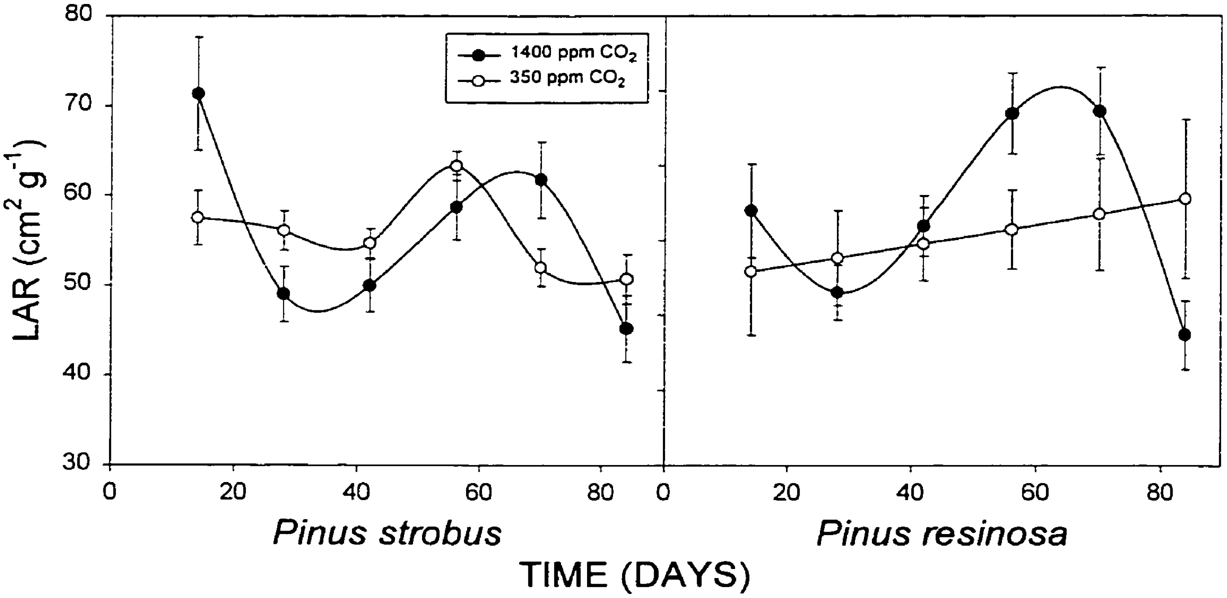
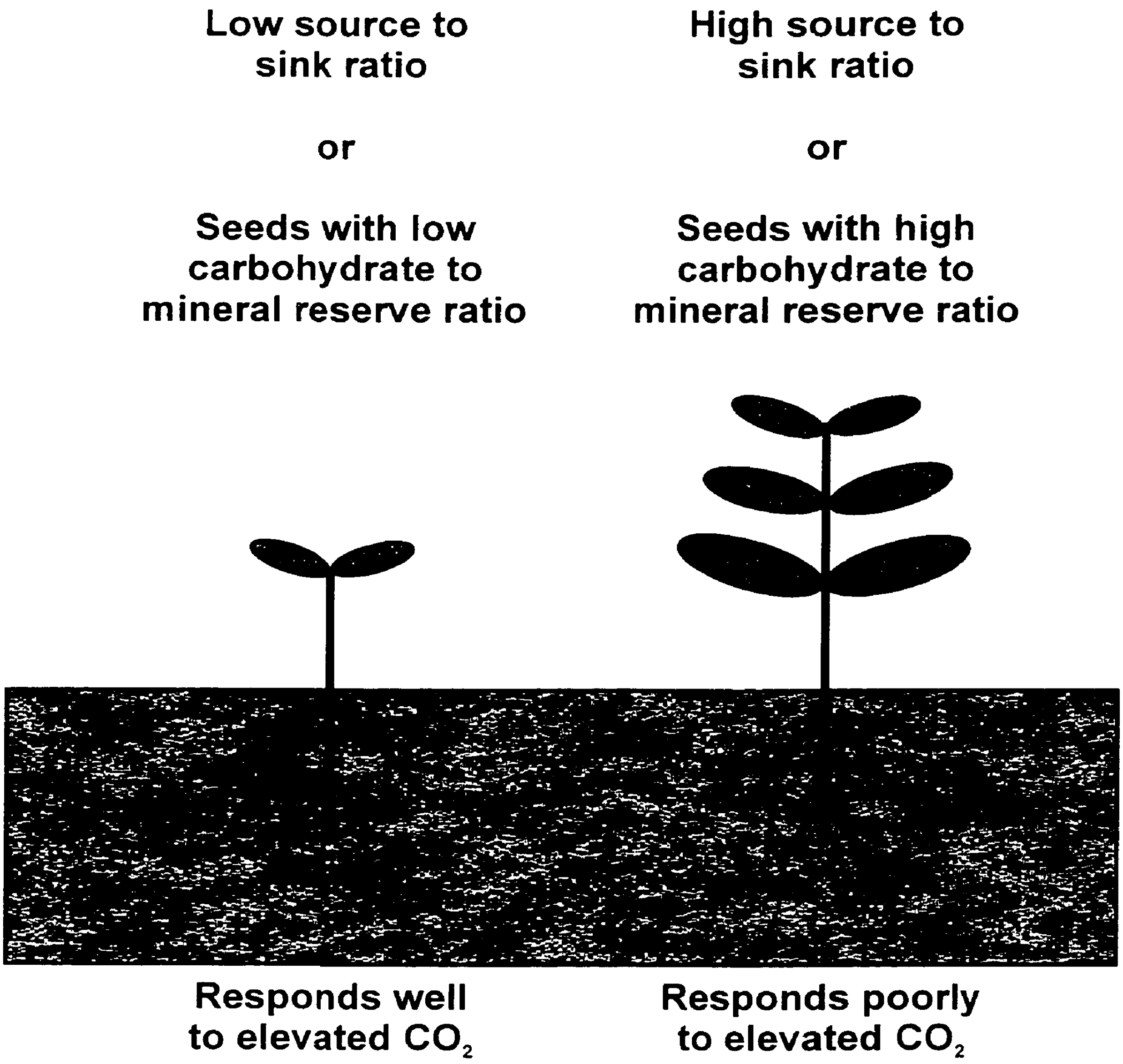

80
70
60
50
40
30
LAR (cm^2 g^{-1})
1400 ppm CO$_2$
350 ppm CO$_2$
0
20
40
60
80
0
20
40
60
80
Pinus strobus
Pinus resinosa
TIME (DAYS)

Figure 8. Schematic diagram of a plant with a low source to sink ratio, which responds well to elevated CO_2 and is equivalent to a seed with low carbohydrate to mineral reserve ratio. This is in contrast to a plant with high source to sink ratio that responds poorly to elevated CO_2 and is equivalent to a seed with high carbohydrate to mineral reserve ratio.


Low source to sink ratio
or
Seeds with low carbohydrate to mineral reserve ratio
High source to sink ratio
or
Seeds with high carbohydrate to mineral reserve ratio
Responds well to elevated CO_2
Responds poorly to elevated CO_2

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APPENDIX A

The quantitative analysis of plant growth can be broken in to three basic components: relative growth rate (RGR), net assimilation rate (NAR), and leaf area ratio (LAR). These three components are mathematically related to each other by a simple equation:

(1) $RGR = NAR \times LAR$

RGR ($g\ g^{-1}\ day^{-1}$) is a measure of plant biomass production per unit of plant biomass (W) per unit of time (t) and is calculated from the slope of the natural log transformed weight versus time curve. Therefore, the RGR can be calculated at any time by the following equation:

(2) $RGR = dW/dt \times 1/W$

NAR ($g\ cm^{-2}\ day^{-1}$) is a descriptive measure of plants ability to increase in biomass (W) per unit of leaf area (A) per unit of time (t) and can be calculated by the following equation:

(3) $NAR = dW/dt \times 1/A$

LAR ($cm^2\ g^{-1}$) is simply the ratio of a plants leaf area (A) to a plants weight (W) and is calculated by the following equation:

(4) $LAR = A/W$

For further explanation on growth analysis, refer to Beadle (1985) for a complete description.