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Physiological and growth responses of eleven Ontario provenances of one-year old Picea glauca seedlings to elevated CO2 concentrations

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NOTE TO USERS

Physiological and growth responses of eleven Ontario provenances of one-year old *Picea glauca* seedlings to elevated CO_2 concentrations.

by

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ABSTRACT

Jodi M. Maepea. 2004. Physiological and growth responses of eleven Ontario provenances of one-year old *Picea glauca* seedlings to elevated CO_2 concentrations. 93 pp.

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To study the physiological and growth responses of eleven Ontario provenances of white spruce [*Picea glauca* (Moench) Voss] to elevated atmospheric CO_2 concentrations, one-year old seedlings were grown in three greenhouses under CO_2 concentrations of ambient, 530 ppm, and 700 ppm for a period 90 days. The following physiological traits were measured after 60 and 90 days of treatment: net assimilation rate (A), stomatal conductance (g_s), transpiration (E), water-use efficiency (WUE), and intercellular to ambient CO_2 concentration ratio (C_i/C_a). Shoot, root, and total biomass and biomass allocation were also measured after 60 and 90 days of treatment. Height and root collar diameter (RCD) data were collected after 0, 30, 60, and 90 days of treatment.

Net CO_2 assimilation was significantly enhanced by elevated [CO_2] after 60 and 90 days of treatment despite reductions in photosynthetic capacity. Elevated [CO_2] also enhanced WUE and decreased g_s and E after 60 days of treatment. There was significant down-regulation of photosynthesis in response to CO_2 concentration elevation. Elevated [CO_2] significantly reduced Rubisco carboxylation efficiency (V_{cmax}), RuBP regeneration capacity (J_{max}), and triose phosphate utilisation (TPU), but had no significant effect on leaf respiration (R_d) or CO_2 compensation point. Despite its substantial influence on gas exchange, CO_2 concentration did not significantly affect seedling biomass, biomass allocation, height, or RCD. No significant CO_2 x provenance interactions were found in the gas exchange measurements. Provenance had a significant effect on the height and RCD of the white spruce seedlings. Strong correlations existed between gas exchange and monthly climate variables for the 11 Ontario provenances of white spruce at ambient and elevated CO_2 concentrations, suggesting adaptation of individual provenances to local climate.

The implications of CO_2 response of diverse sources of white spruce seedlings on tree improvement programs will probably be minimal. Seed sources of white spruce in Ontario selected for superior growth characteristics in the present climate will probably perform well in the predicted future climate.

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ACKNOWLEDGEMENTS

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INTRODUCTION

Over the past 100 years, the concentration of atmospheric [CO_2] has increased from 270 ppm (parts per million) to approximately 360 ppm. Based on an estimated current annual increase of 1 to 2 ppm, a doubling of the CO_2 concentration in the atmosphere will occur by 2075 (IPCC 1996). Elevated [CO_2] and its associated changes in future climate, predominantly temperature and precipitation regimes, are predicted to have a dramatic impact on the structure, distribution, productivity and health of boreal forest tree species through its influence on tree ecophysiological processes. Climate change will most likely modify the composition of plant communities in the boreal forest due to the predicted northward migration of species following latitudinal and altitudinal shifts in climate regimes. If the predicted changes in climate become reality, recently established trees will experience different atmospheric CO_2 concentrations and climatic conditions during their rotation (Parker *et al.* 2000). As a result, existing stands will potentially be less adapted to the future environmental conditions. Therefore, it will be essential that present and future planting stock represent widely adapted populations and seed sources to ensure the long-term regeneration success (Ledig and Kitzmiller 1992). Understanding and recognizing the potential effects of climate change on forests will allow resource managers to modify forest management planning strategies and policies to adequately encompass the possibility of climate variability and change and to minimize negative impacts on the health, sustainability, biodiversity, and socioeconomic benefits of the boreal forest.

Physiological and growth response mechanisms of tree seedlings may be profoundly affected by the genetic variability within a species (Houpis *et al.* 1999 and Centritto and Jarvis 1999). Genotypic variation in the physiology and growth of a tree species can enhance its ability to adapt and acclimate to diverse environmental

conditions. Understanding how specific white spruce seed sources respond to elevated [CO_2] in terms of physiology and growth is necessary to predict the projected impact of climate change on the boreal forest.

This study investigated provenance variation in physiological and growth response of one-year old white spruce seedlings from eleven Ontario provenances to elevated concentrations of CO_2 for one growing season. The objectives of this study were:

1) To investigate the effects of elevated CO_2 concentrations on the physiology (photosynthesis, stomatal conductance, transpiration, water-use efficiency, and intercellular to ambient CO_2 concentration ratio), biomass, and biomass allocation of one-year old white spruce seedlings.
2) To determine if one-year old white spruce seedlings will exhibit short-term photosynthetic acclimation under elevated CO_2 concentrations after one growing season.
3) To examine genetic variation among the eleven Ontario seed sources of white spruce in the above responses.
4) To determine if the physiological processes and growth response of young white spruce from distinct genetic sources represent adaptations to local climate conditions.

To test these objectives, one-year old white spruce seedlings from eleven Ontario provenances were grown in greenhouses at Lakehead University and maintained in ambient, 530, and 700 ppm CO_2 concentrations for a period of 90 days. Changes in physiology were determined by measuring net assimilation (A), stomatal conductance (g_s), transpiration (E), water-use efficiency (WUE), and intercellular to ambient CO_2

concentration ratio (C_i/C_a) at both ambient and growth CO_2 concentrations after 60 and 90 days of treatment. Shoot, root, and total biomass and biomass allocation were also measured after 60 and 90 days of treatment. Height and root collar diameter (RCD) data were collected after 0, 30, 60, and 90 days of treatment.

The findings of this study will provide fundamental information on the appropriate selection and mechanisms of adaptation of superior seed sources of white spruce that will best be able to maximize the utilization of increasing levels of atmospheric [CO_2]. As seed produced from tree improvement programs is being increasingly used in forest regeneration, the information obtained in this project could be applied to white spruce tree improvement programs promoting CO_2 responsive seed sources and ensure successful establishment of white spruce plantations adapted to future local climate conditions.

LITERATURE REVIEW

CLIMATE CHANGE

Over the past 100 years, the concentration of CO_2 in the earth's atmosphere has increased from 270 ppm (parts per million) to approximately 360 ppm. Based on an estimated current annual increase of 1 to 2 ppm, a doubling of the CO_2 concentration in the atmosphere will occur by 2075, which is predicted to increase global air temperatures by 1 to 3.5°C with more extreme increases occurring at higher latitudes (IPCC 1996). The Canadian General Circulation Model (GCM) (Boer *et al.* 1992), a computer model used to predict future climate conditions, anticipates that a doubling of atmospheric [CO_2] will result in a growing season air temperature increase of 3 to 5°C in Ontario. The Canadian GCM also projects up to a 10 % reduction in growing season precipitation in the region (Parker *et al.* 2000).

The boreal forest accounts for 74% of the forested land area in Canada (Forestry Canada 1991). It sustains the economies of hundreds of communities across the country, improves air and water quality, and provides habitat for countless species of plants and animals. Furthermore, the longevity of boreal forest trees species plays an important role in moderating our climate and providing a sink for atmospheric carbon dioxide.

The effects of climate change, predominantly the effects of elevated atmospheric [CO_2] and its associated changes in temperature and precipitation, will be critical to the structure, function, distribution, and sustainability of the boreal forest. Climate change will most likely modify the composition of plant communities in the boreal forest due to the predicted migration of species following latitudinal and altitudinal shifts in

temperature regimes. Increased temperatures and changes in precipitation regimes are predicted to initiate a northward migration of Great Lakes St. Lawrence forest species, as much as 500 km within the next 100 years, into the southern limits of the boreal forest, as some of the more poorly adapted boreal species decline (Parker *et al.* 2000).

The environmental conditions in the boreal forest change over time periods ranging from days to centuries. Trees respond to long-term climate and successional changes through adaptation and to short-term fluctuations in environmental conditions through acclimation (Margolis and Brand 1990). If the predicted changes in climate become reality, recently established trees being used to regenerate harvested sites will be exposed to different climatic conditions at the time of rotation. As a result, existing stands will potentially be less adapted to the future climatic conditions. Therefore, it will be essential that present and future planting stock represent widely adapted populations and seed sources to ensure the long-term regeneration success and site adaptation (Ledig and Kitzmiller 1992).

CO_2 AND PHYSIOLOGICAL RESPONSE

Physiological responses to elevated atmospheric [CO_2] may be evident over a range of spatial and temporal scales and are dependent on the level of hierarchical biological organization. Acclimation in trees and forests may occur over a time period of seconds to days. At the cellular scale, responses in enzyme activation, carboxylation and cellular transport may occur within seconds to minutes. At the tissue scale, processes including assimilation, partitioning, transpiration, and stomatal conductance may be influenced over a period of hours to days. Seedling growth processes such as carbon

allocation, nutrient uptake, and root/shoot dynamics may respond to an increase in elevated [CO_2] over a period of weeks to months (Eamus and Jarvis 1989). In trees and forests, genetic adaptation may occur over centuries. Long-term effects (hundreds of years) over entire forests or ecosystems are difficult to investigate because most experiments are often limited by feasibility, technological and financial resources.

It is widely accepted that an increase in elevated atmospheric [CO_2] will affect the photosynthetic processes of boreal forest trees (Parker *et al.* 2000). Photosynthesis is the primary physiological process responsible for the survival and growth of plants (Lambers *et al.* 1998). It is for this reason that photosynthesis has received more attention by researchers than any other physiological process.

Photosynthesis involves a series of complex chemical and physiological processes within the leaves of plants in which light energy is captured by photosynthetic pigments in leaves and biomass is produced. The first series of interactions are the light reactions. Photons of light of photosynthetically active radiation (400 – 700 nm) are absorbed by photosynthetic pigments located in the thylakoid membranes of chloroplasts within the leaf mesophyll. The energy absorbed from the photons is transferred to an electron, which is passed through the electron transport chain, where it is used to convert ADP to ATP. The splitting of water within the lumen of the thylakoid interior produces oxygen and hydrogen ions, thus producing an electrochemical gradient between the lumen and the surrounding stroma. The ATPase enzyme, located within the thylakoid membrane, couples the pumping of protons across the gradient out of the lumen into the stroma with the addition of a phosphate group to ADP to produce ATP. NADPH is also produced (Lambers *et al.* 1998).

The products of the light reactions of photosynthesis (ATP and NADPH) are employed in the second series of reactions, collectively referred to as the Calvin Cycle. The Calvin cycle enzyme Ribulose-1,5-bisphosphate carboxylase/oxygenase (Rubisco) catalyzes the binding of a CO_2 molecule to Ribulose-1,5-bisphosphate (RuBP) thus producing phosphoglyceric acid (PGA). The energy carriers, ATP and NADPH are used to reduce PGA to glyceraldehyde-3-phosphate (G3P). The G3P compounds are either 1) used in the regeneration of RuBP, 2) exported to the cytosol in exchange for inorganic phosphate, or 3) used in the production of starch to be stored in the chloroplast (Lambers *et al.* 1998).

Atmospheric CO_2 concentration is an environmental factor that can limit the efficiency and chemical yield of photosynthetic activity in C_3 plants, including conifers (Teskey *et al.* 1994). Under unlimited supplies of water and nutrients, conifers exposed to short-term elevations of atmospheric CO_2 exhibit increased photosynthetic activity (Eamus and Jarvis 1989, Ceulemans and Mousseau 1994, Curtis and Wang 1998). CO_2 and O_2 compete for active sites on Rubisco and the ratio of carboxylation and oxygenation of RuBP is dependent on the relative concentrations of CO_2 and O_2. Carboxylation produces two C_3 molecules (PGA), whereas oxygenation produces only one PGA and one C_2, thus leading to a substantial photorespiratory loss of carbon from a plant (Lambers *et al.* 1998). An increase in the concentration of atmospheric CO_2 will increase the carboxylation of RuBP over oxygenation, thus leading to enhanced rates of net photosynthesis and tree growth through reduced photorespiration. On average, studies conducted with conifers have shown a 40% short-term increase in photosynthetic rate in response to elevated [CO_2]. However, there is considerable variation between

studies in experimental conditions including species investigated, age of trees, duration of study, and CO_2 concentrations used (Ceulemans and Mousseau 1994).

Evidence suggests that the response of photosynthetic processes to an increase in ambient CO_2 is not constant through time. Multiple studies have concluded that exposure to long-term elevations of atmospheric [CO_2] may result in the photosynthetic down-regulation of many tree species (Long *et al.* 1993, Sage 1994, Drake *et al.* 1997, Gunderson and Wullschleger 1994). This down-regulation in photosynthetic activity may be attributed to the reduction of the amount and activity of Rubisco, the enzyme which catalyzes the carboxylation of RuBP and subsequent synthesis of carbohydrates. Two mechanisms have been hypothesized to be the cause of reduced Rubisco in the leaves of trees exposed to long-term elevated atmospheric [CO_2].

The first hypothesis is that there is a sugar-sensing mechanism which regulates Rubisco concentration via feedback control. The increased concentration of hexose sugars in leaf cells associated with increased rates of photosynthesis under elevated atmospheric [CO_2], is sensed by the hexokinase enzyme. The hexokinase enzyme signals the suppression of Rubisco gene expression, thus decreasing the concentration of the catalyzing enzyme (Lambers *et al.* 1998). Tissue *et al.* (1999), Griffin *et al.* (2000), and Rogers and Ellsworth (2002) support this hypothesis with their results of photosynthetic acclimation of pine species to long-term exposure to elevated [CO_2]. Furthermore, Rogers and Ellsworth (2002) found that Rubisco levels were significantly lower in one-year old needles compared to current-year needles.

The second mechanism of photosynthetic acclimation hypothesized involves a non-selective reduction in nitrogen content in the leaves to maximize nitrogen use in other growing organs in the tree. Because as much as 25 % of leaf nitrogen can be invested in the production of Rubisco, a decrease in nitrogen content would lead to decreased levels of the enzyme (Long *et al.* 1993). Stitt and Krapp (1999) and Curtis *et al.* (2000) support the mechanism of maintaining a nitrogen balance between source and sink within the plant by decreasing leaf N.

In conditions where rooting volume and nutrient availability are limiting, acclimation of the photosynthetic response to elevated [CO_2] is more prevalent. Thomas and Strain (1991) and Arp (1991) found that pot sizes that constrained root growth and volume accentuated the acclimation response. It is also widely accepted that nutrient deficiency, as a result of low amounts of nutrient additions to pots or nutrient poor soils, will enhance the acclimation of photosynthesis to elevated [CO_2] (Ceulemans and Mousseau 1994).

Stomatal conductance (g_s) regulates the diffusion of CO_2 and water vapour through the stomata. It is the controlling mechanism of plant water relations and is driven by both the vapour pressure gradient between leaf and air and soil moisture content (Lambers *et al.* 1998). Several mechanisms have evolved that control g_s. Each mechanism is stimulated by a particular environmental or physiological factor, which may include leaf water potential, irradiance, atmospheric vapour pressure deficit, and internal CO_2 concentration (Lambers *et al.* 1998).

Stomata respond to changes in internal CO_2 concentration (C_i) (Mott 1988). Thus, an increase in C_i results in a reduction in g_s. Stomatal response to C_i as a

mechanism for the reduction in g_s under elevated [CO_2] is well documented over a range of herbaceous plant and tree species (Paoletti and Gellini 1993, Sage 1994, Lewis *et al.* 2002, Ceulemans and Mousseau 1994, and Curtis and Wang 1998). However, considerable variation exists between the wide range of species investigated. Herbaceous plants have demonstrated the greatest reductions in g_s, followed by deciduous trees, and finally conifer trees (Saxe *et al.* 1998). Furthermore, tree age is also thought to affect the degree of reduction in g_s, with responses from younger conifer seedlings being more prominent than those shown by older trees (Medlyn *et al.* 2001).

A majority of the studies to date have also reported that long-term stomatal density decrease in response to elevated atmospheric [CO_2] (Eamus and Jarvis 1989, Woodward and Bazzaz 1988). A stomatal density reduction of 40% was reported by Woodward (1987) in tree species over a 200 year period associated with increases in elevated atmospheric [CO_2].

Despite the overwhelming evidence suggesting a reduction in g_s under enriched CO_2 concentrations, Gunderson *et al.* (1993) found no clear response of *Quercus alba* L. and *Liriodendron tulipifera* L. to [CO_2] treatment. Results reported for stomatal activity in *Prunus persica* L. by Centritto *et al.* (2002) also conflict with the general view of reduced g_s under elevated [CO_2].

Reductions in both stomatal conductance and stomatal density decrease leaf-level transpiration (E). Thus, under elevated atmospheric [CO_2], a reduction in E, combined with an increase in net photosynthetic rate consequently results in an increase in water use efficiency (WUE). Increased WUE has been reported in many studies for well-

watered plants (Eamus and Jarvis 1989). It has been suggested that enhanced WUE may be beneficial to plants exposed to drought (Norby and O'Neill 1989). However, whole-plant E may not be reduced as increased total transpiring leaf area may compensate for leaf-level stomatal effects (Ceulemans and Mousseau 1994, Eamus and Jarvis 1989). Le Thiec and Dixon (1996) reported increased WUE in young Norway spruce [*Picea abies* (L.) Karst.] and red oak [*Quercus rubra* L.] grown at elevated [CO_2]; however, under drought conditions, increases in WUE were less pronounced in red oak. Guehl *et al.* (1994) also reported minimal differences in WUE in drought stressed sessile oak [*Quercus petraea* (Mattuschka) Liebl.)] grown at ambient and elevated CO_2 concentrations.

CO_2 AND BIOMASS, BIOMASS ALLOCATION, AND GROWTH RESPONSE

Seedlings exhibit enhanced rates of growth due to increased carbon uptake (Centritto *et al.* 1999). Under a doubling of CO_2 concentration, Eamus and Jarvis (1989) and Curtis and Wang (1998) reported on average a 30 – 40% increase in plant dry mass across a range of herbaceous and tree species. However, initial growth enhancement may not be sustained over time, possibly as a result of photosynthetic down regulation or shifts in biomass allocation and ontogeny (Ward and Strain 1999 and Tjoelker *et al.* 1998).

The primary effect of elevated [CO_2] is to accelerate all aspects of tree seedling growth with no difference in the proportion of biomass allocated to the root or shoot. However, studies have concluded that elevated CO_2 results in increased biomass allocation to roots over shoot in tree seedlings where soil nitrogen is limiting (Eamus and Jarvis 1989 and Ceulemans and Mousseau 1994). The preferential investment in root

over shoot biomass, suggests a mechanism to allocate biomass to structures of the plant that are involved in the acquisition of limiting resources. Conversely, Curtis and Wang (1998) found no such shift in biomass allocation in their meta-analysis of elevated CO_2 effects on plant mass. More research is required to better understand the mechanisms involved in biomass allocation under elevated CO_2.

WHITE SPRUCE – SILVICS AND GENETIC VARIATION

White spruce [*Picea glauca* (Moench) Voss] is an important boreal forest tree species to the forest industry in Canada. It is harvested extensively for commercial pulpwood and lumber. White spruce has a wide range of natural distribution extending from the Atlantic coast through to the Great Lakes and central Canada and west to British Columbia and Alaska. This species has been found as far south as northern Montana and South Dakota (Morgenstern 1996).

White spruce is able to grow on a diversity of sites, but grows best on upland sites with well-drained, moist soils. Although white spruce is able to tolerate a wide range of nutrient and moisture levels, it is generally more demanding than other commonly associated conifer species (Nienstaedt and Zasada 1990). Soil moisture, in combination with increased soil fertility will improve growth and yield of this species (Sutton 1969).

Diverse environmental and climate conditions (i.e. minimum and maximum temperatures, annual precipitation levels, and length of growing season) can cause natural selection to create genetic variation related to the environment of origin over a species' range. The physiological and morphological attributes of white spruce are highly

variable over its range, as a result of ecological and climatic differences, population sizes, and mating systems (Morgenstern 1996).

The relative economic importance of white spruce has provoked extensive research concerning its geographic variation. Provenance tests are designed to identify phenotypic differences among different geographic origins of a species. As the phenotypic response trait of a tree is a product of its genetic make-up and its surrounding environment, by growing different provenances in a common test environment, environmental variation can be minimized and any variation in the physical appearance or performance of an individual tree could be attributed to genetic variation (Joyce *et al.* 2001).

The results of white spruce provenance studies conducted in 1950 in Petawawa, Ontario revealed geographic variation in morphological, physiological, and genetic response mechanisms including height growth, seedling phenology, branch, root and needle length, number of stomata, and branch pubescence (Nienstaedt and Teich 1972). High inter-tree variation within stands and low inter-stand variation in height growth and phenology were also reported by Dhir (1976) and Pollard and Ying (1979) in studies conducted in the Ottawa Valley and in southeastern Ontario. In a more recent study involving 23 Ontario provenances of white spruce grown at four sites across the province, Lesser *et al.* (2004) reported significant differences in height growth among test sites and between stands in southern Ontario.

Although geographic variation is the predominant source of variation in white spruce, the existence of ecotypic variation has been reported in part of its range where height growth has shown adaptation to limestone and granitic soils (Teich and Holst

1974). However, Lesser *et al.* (2004) offered little support to the existence of limestone ecotypes as they concluded that variation in growth between stands and test sites could not be attributed to the bedrock origin of the provenances studied.

Coursolle *et al.* (1997) investigated clinal variation in phenological response of white spruce and found that initiation of spring budbreak and shoot growth was correlated with the latitude of the seed source. A gradual progression of earlier budbreak corresponding to spring temperatures was observed in sources that ranged from north to south. As well, northern compared to southern provenances of white spruce initiated spring shoot growth at lower temperatures.

Despite the numerous studies that have examined geographic variation in various morphological and phenological attributes of white spruce, few studies have investigated genetic variation in physiological traits. However, white spruce has exhibited genetic variation in its short-term physiological response to a gradient of environmental conditions (Grossnickle 2000). Binder and Fielder (1996) found that white spruce populations from northern, compared to southern latitudes, exhibited a more rapid decrease in their photosynthetic capacity in response to a decline in fall photoperiods and temperatures.

Despite these findings, more research is needed in defining the ecophysiological response of white spruce to diverse site conditions within its geographic range. Such research will provide a better understanding of the biological foundation for the adaptability of white spruce to its environment.

GEOGRAPHIC VARIATION AND ITS IMPLICATIONS FOR SILVICULTURE

Geographic variation in a tree species can have a profound effect on the success of a silviculture program when seedlings are grown and used for artificial regeneration purposes (Grossnickle 2000). Due to its relative economic importance, white spruce is a common species used in artificial regeneration programs throughout Ontario. Tree improvement programs are an intensive silvicultural tool that can enhance the genetic potential of trees used in forest plantations (Joyce *et al.* 2001). A successful tree improvement program consists of the following components: 1) identification of the species, the variability of the species within its range, and the land base for which it will be used in, 2) selection of desired traits to be improved, 3) production of improved individuals for regeneration purposes in large quantities, and 4) development and maintenance of a broad genetic base population (Zobel and Talbert 1984).

As part of the initial establishment of a tree improvement program, careful consideration is taken in the selection of the genetic source used to produce improved individuals. Seed zones are geographic subdivisions of a species range based on genetic and ecological criteria (Morgenstern 1996). Their purpose is to regulate how individual genetic sources are collected and used within a defined geographic region and ensure that seedlots selected for seedling production be ecologically suited to site environmental conditions over the entire rotation (Grossnickle 2000).

Tree breeding is a component of the tree improvement strategy in which the control of tree parentage is combined with other silvicultural activities to increase regeneration productivity and long-term adaptability (Grossnickle 2000). Traditionally, the primary objective of breeding in tree improvement programs is to promote improved volume

growth, stem quality, branching characteristics, wood quality, and disease and insect resistance. However, given the uncertainty surrounding the progressive northward shift of current climate conditions, it will be critical for tree breeding programs to promote seed sources with improved capability to tolerate environmental stresses and respond to elevated atmospheric [CO_2] (Wang *et al.* 1995). Thus, seeds selected to be produced in large quantities in tree improvement seed orchards, must represent widely adapted populations and diverse seed sources that will foster the migration of white spruce from its current to future range (Ledig and Kitzmiller 1992).

CO_2 x PROVENANACE INTERACTION

The morphological, phenological, and physiological response of tree seedlings to elevated [CO_2] may be profoundly affected by the genetic variability within a species (Curtis and Wang 1998). Genetic variation within a species in response to increasing atmospheric [CO_2] may profoundly affect future performance among genotypes within natural populations and the composition and structure of future forest communities. Genetic variation not only exists at the species level, but is also evident at the provenance, family and individual levels. Furthermore, woody species vary in intraspecific response to elevated [CO_2] - intraspecific variation may be enhanced in one species while suppressed in another. (Thompson 1998). Literature exists for a wide range of both conifer and hardwoods species that documents the effects of genetic variation among and within populations in the response to elevated [CO_2] and have reported varying conclusions.

Houpis *et al.* (1999) found substantial among-provenance variation in the growth response (height, diameter, and stem volume) of California ponderosa pine [*Pinus ponderosa* Dougl. ex P. Laws] seedlings subjected to CO_2 concentrations of 350, 525 and 700 $\mu mol\ mol^{-1}CO_2$. Centritto and Jarvis (1999) found that Sitka spruce [*Picea sitchensis* (Bong.) Carr.] seedlings exhibited a significant CO_2 x provenance interaction in nitrogen use efficiency (NUE). Seedlings from more southerly provenances had higher initial NUE than those from more northerly provenances when grown under elevated CO_2 concentration, which was reflected in the initially higher growth rates under elevated [CO_2] (Centritto *et al.* 1999).

Wang *et al.* (1995) grew families of black spruce [*Picea mariana* (Mill.) B.S.P.] seedlings from Quebec for two growing seasons under simulations of atmospheric CO_2 concentration, temperature, and nitrogen availability of present and predicted future climates. After the first growing season, there was little CO_2 x genotype interaction in the response of the seedlings, however performance appeared to have been strongly influenced by maternal variation in seed mass (Wang *et al.* 1994). Conversely, second year results indicated significant family differences in seedling response between the simulated climates. Although second year results implied that superior black spruce seed trees may be selected for a future climate, initial seed mass was again an evident source of variation in seedling performance.

Johnsen and Seiler (1996) reported opposing results to those found in the second year of the study conducted by Wang *et al.* (1995) with six diverse black spruce provenances whose origins spanned across Canada. They concluded that the provenances

responded similarly in growth, biomass partitioning, shoot phenology, and gas exchange under both ambient and double ambient atmospheric CO_2 concentration.
Similar findings were again reported by Johnsen and Major (1998) who concluded in a separate study that the CO_2 x family interaction did not prove to be significant source of variation in seedling height and diameter after two growing seasons for 20 families of black spruce from New Brunswick.

Wang *et al.* (2000) reported a significant CO_2 x genotpye interaction in the photosynthetic activity of six genotypes of trembling aspen [*Populus tremuloides* Michx.] grown under ambient and double ambient CO_2 concentrations. Net CO_2 assimilation, stomatal conductance, and biomass responses varied between the genotypes at elevated CO_2, however the degree of variation was dependent on soil N and water availability.

Cantin *et al.* (1997) investigated the response of 15 maternal families of jack pine [*Pinus banksiana* Lamb.] seedlings exposed to an elevated CO_2, temperature, and nitrogen availability environment. With the exception of seedling WUE, their findings failed to detect a significant CO_2 x genotype interaction effect on height growth, biomass, biomass allocation, or net assimilation rates after one growing season in the predicted future climate conditions.

MATERIALS AND METHODS

EXPERIMENT DESIGN

The experiment design was a split plot design. The two treatment factors were CO_2 concentration and provenance. The CO_2 concentrations were ambient (350 ppm), 525 ppm (1.5x ambient), and 700 ppm (2x ambient). The two elevated [CO_2] treatments (525 and 700 ppm) were randomly assigned to two greenhouses capable of increasing CO_2 concentration. Due to the limited number of greenhouses equipped with [CO_2] monitoring equipment at the Lakehead University Greenhouse, replication of the [CO_2] treatments was not possible.

The [CO_2] treatments represented the main plots and the benches nested within the greenhouses represented the sub-plots. Two blocks (benches) were set up within each greenhouse ([CO_2] treatment). Each block contained 6 replicates of the 11 provenances of white spruce. Thirty-six seedlings (3 [CO_2] x 2 blocks x 6 replicates) from each provenance were randomly selected and assigned a [CO_2] treatment. Two rows of border seedlings surrounded the test seedlings acted to minimize any edge effects on test seedlings. The greenhouse set-up of the project is illustrated in Figure 1.

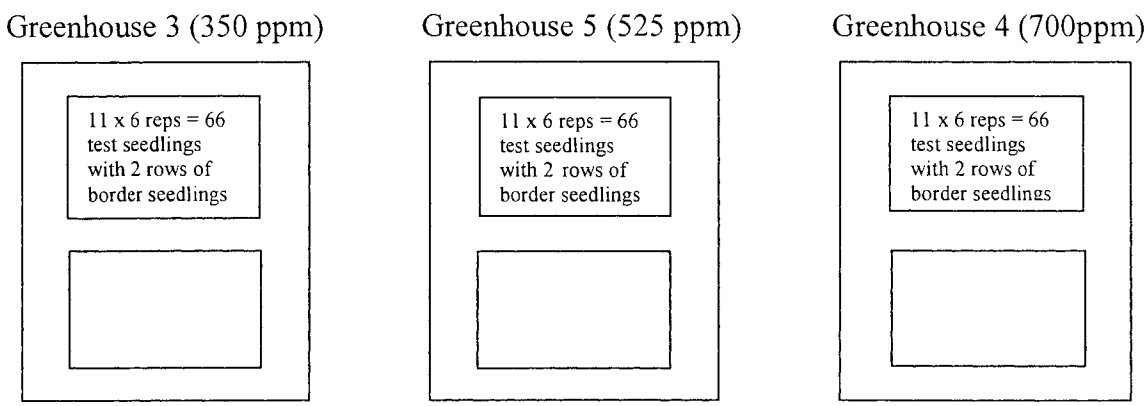

Figure 1. Greenhouse set-up of project.

PLANT MATERIAL

White spruce seeds from 11 sources of different geographic origin in Ontario (Figure 2) were obtained from Dale Simpson of the Canadian Forest Service in the fall of 2001. The location and elevation of the 11 seed sources are summarized in Table 1. Temperature and precipitation data were obtained from McKenny (2004) for each of the 11 provenances (APPENDIX I).

Table 1. Seed source origins of 11 Ontario white spruce provenances.

Seed Source	Source	Source ID	Location	Latitude (°N)	Longitude (°W)	Elevation (m)
78	OFRI	7	Bentinck	44.17	81.00	305
1	OFRI	22	Cornwall	45.07	74.83	80
17	CFS	8032	Antrim	45.32	76.18	121
58	CFS	8166	Sinclair Twp.	45.47	79.08	370
86	OFRI	74	Proctor	46.33	82.50	249
121	CFS	8087	Pigeon River	48.02	89.65	306
124	CFS	8088	Shebandowan	48.62	90.18	459
96	CFS	8067	Strathearn	48.72	85.87	335
112	LU	2001.3	Mountain Bay	48.91	87.77	195
81	CFS	8053	Fraserdale	49.03	81.58	215
113	CFS	8078	Auden	50.15	87.88	335

OFRI – Ontario Forest Research Institute
CFS – Canadian Forest Service
LU – Lakehead University

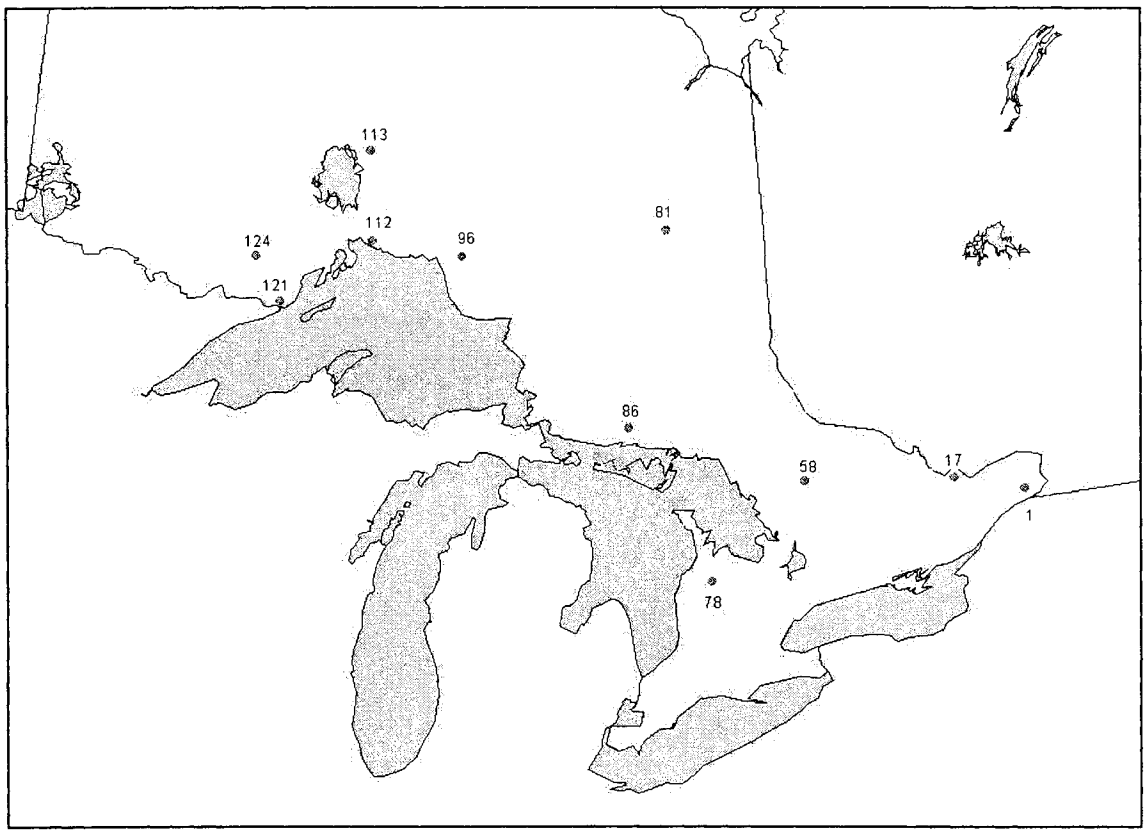

Figure 2. Map of eleven white spruce seed sources in Ontario.

Seeds from the 11 provenances were obtained and stratified at approximately 3°C for two weeks starting December 24, 2001. Seeds were sown in 140 mL Jiffy containers at the Lakehead University Greenhouse. Day/night temperatures were set at 30/20°C for the germination phase, which lasted 3 weeks.

When the germination phase was complete, a thermoperiod of 22/12°C was maintained throughout the remainder of the first season of growth. Photoperiod was set at 16 hours with a minimum irradiance level of 900 μmol m^{-2} s^{-1}. All seedlings were watered to saturation once a day. For the first 8 weeks after the germination phase, seedlings were fertilized with a starter fertilizer (11-41-8) progressing from 50 to 100 ppm nitrogen to promote root growth. The fertilizing regime then switched to alternating

20-20-20 and 20-8-20 at 100 ppm nitrogen, which was progressively increased to 175 ppm by the end of the first growing season as per the direction of Jenny Millson, a representative of Millson Nurseries.

Procedures for hardening off of the seedlings started on May 20, 2002. An 8-20-30 fertilizer was applied to the seedlings to promote hardening off. The seedlings were then blacked-out for 14 hours/day for a two-week period. During the hardening off phase, the seedlings were transferred from the greenhouse to an outdoor shade house where they remained until January 2003. The seedlings did not break bud during the summer months in the outdoor shade house. They were watered naturally by precipitation and did not receive any fertilization treatments in the shade house.

GROWTH CONDITIONS AND CO_2 TREATMENTS

In January 2003, the frozen seedlings were obtained from the outdoor shade house and thawed in the Lakehead University Greenhouses at ambient [CO_2] at a 10/ 5°C thermoperiod for 7 days and then a 14/7°C thermoperiod for 2 days. After thawing, the Jiffy seedlings were transplanted into 1.4 dm^3 (10.7 x 10.7 x 12.5cm) pots containing a mixture of peat and vermiculite (50/50, v/v). A starter fertilizer (11-41-8) at 100ppm nitrogen was applied to all seedlings upon commencement of the treatment period to stimulate root growth.

The seedlings were randomly assigned to one of three ARGUS® Computerized Environment Control greenhouses (Vancouver, Canada) at Lakehead University. The CO_2 concentrations within each greenhouse were set at ambient (approximately 360 ppm), 525 ppm, and 700 ppm respectively for a treatment period of 90 days. The

thermoperiod was set at 22/12°C throughout the treatment period. A two-hour temperature ramping period in the morning and evening was established to coincide with photoperiod. Photoperiod was set at 16 hours at an irradiance level of 900 μmol m^{-2} s^{-1}. During overcast conditions and early morning and late evening hours, supplementary lighting was provided with high-pressure sodium lamps to maintain the photoperiod and irradiance levels. Relative humidity was set at 50%. All seedlings were watered to saturation every 3 days. All seedlings were fertilized according to the direction of Jenny Millson from Millson Tree Nursery once a week with alternating 20-20-20 and 20-8-20 at 150ppm nitrogen. The electro-conductivity of the fertilizer solution was tested to ensure that a uniform concentration was being applied to all seedlings.

Environmental conditions in the greenhouses were monitored and controlled continuously using the ARGUS® computerized environment control system (Vancouver, Canada). The actual average CO_2 concentrations for the duration of the treatment period were 337.5 +/- 16ppm, 545 +/-34ppm, and 687+/- 34ppm, respectively for the three CO_2 treatments. The average temperatures for the three greenhouses were 16.9, 16.7, and 16.1°C, respectively (APPENDIX II).

GAS EXCHANGE MEASUREMENTS

Around the 60th and 90th day of treatment, 3 trees per provenance, block, and CO_2 treatment were randomly selected for gas exchange measurements. A PP-Systems CIRAS-1 gas exchange system with a Parkinson's Leaf Cuvette for conifers (PP-Systems, Haverhill, MA, USA) was used for the measurements. The CO_2 concentration within the chamber was set to correspond with the growth concentration. Additionally, to evaluate

photosynthetic down-regulation, the gas exchange of seedlings subjected to the elevated [CO_2] treatments was also measured under ambient [CO_2]. PAR flux density and air temperature within the chamber were set at 800 μmol m^{-2} s^{-1} and 22.0°C, respectively. Gas exchange measurements were taken between 9:00 a.m. and 4:30 p.m. to minimize the effect of diurnal variation in leaf gas exchange rates. Each measurement period took approximately 4 days. The order of measurements among blocks and CO_2 treatments was randomized, however for operational efficiency, seedlings within the same block were sampled consecutively. Gas exchange measurements were taken on the current-year needles of the terminal shoot. The following parameters were measured and/or calculated using the equations from von Caemmerer and Farquhar (1981): net CO_2 assimilation (A), stomatal conductance (g_s), leaf transpiration rate (E), and the intercellular to leaf surface CO_2 concentration ratio (C_i/C_a). The projected leaf area for each sample was determined using the Regent WinSeedle® leaf analysis system (Regent Instruments Inc., Quebec City, Quebec, Canada). Photosynthetic water use efficiency (PWUE) was calculated as A/E. Additionally, the response of A to C_i (A/C_i curve) was measured at six CO_2 concentrations ranging from 50 to 900 ppm CO_2 concentration for six randomly selected seedlings per CO_2 treatment. PAR flux density and air temperature within the chamber were set at 800 μmol m^{-2} s^{-1} and 22.0°C, respectively. Using the mechanistic A/Ci curve analysis program in the software package, Photosyn Assistant Ver. 1.1.2 (Dundee Scientific, UK), parameters of Rubisco carboxylation efficiency (V_{cmax}), RuBP regeneration capacity (J_{max}), leaf respiration (R_d), and triose-phosphate utilization (TPU) were estimated that best fit the model proposed by Farquhar *et al.* (1980).

HEIGHT, ROOT COLLAR DIAMETER (RCD), AND BIOMASS MEASUREMENTS

On Day 1, 30, 60, and 90 of the treatment period, seedling height and RCD were measured. Seedling height was measured from the root collar to the base of the terminal bud. RCD was measured using a pair of digital calipers.

At the end of each gas exchange measurement period, the seedlings used for gas exchange measurements were harvested and dried for 48 hours at a temperature of 70°C. Oven-dry weights (ODW) of the shoot and root for each seedling were measured. The portion of the seedling shoot used to calculate projected leaf area for the gas exchange measurements was dried and weighed separately and added to the shoot ODW. Total seedling ODW and the shoot to root ratio (S:R) were then calculated.

STATISTICAL ANALYSIS

The linear model for the experiment was:

$$Y_{ijklm} = \mu + C_i + B_{(i)j} + \partial_{(ij)} + P_k + CP_{ik} + BP_{(i)jk} + \varepsilon_{(ijk)l}$$

$i = 1, 2, 3;\ j = 2;\ k = 1, 2,....11;\ l = 1, 2,3$

where:

Y_{ijkl} = a particular morphological or physiological variable of the l^{th} seedling in the k^{th} provenance in the j^{th} block within the i^{th} greenhouse/CO_2 concentration

μ = the overall mean

C_i = the fixed effect of the i^{th} greenhouse/CO_2 concentration

$B_{(i)j}$ = the random effect of the j^{th} block (bench) within the i^{th} greenhouse/CO_2 concentration, assumed to be identically and independently distributed as $N(0, \sigma^2)$

$\partial_{(ij)}$ = the restriction error due to the restriction on the randomization of the 11 provenances in the j^{th} block. The $\partial_{(ij)}$'s are assumed to be identically and independently distributed as $N(0, \sigma^2)$

P_k = the random effect of the k^{th} provenance.

CP_{ik} = the interaction effect of the i^{th} greenhouse/CO_2 concentration with the k^{th} provenance.

$BP_{i(j)k}$ = the interaction effect of the j^{th} block within the i^{th} greenhouse/CO_2 concentration with the k^{th} provenance.

$\varepsilon_{(ijk)l}$ = the random effect of the l^{th} seedling in the ijk^{th} treatment combination. The $\varepsilon_{(ijkl)}$'s are assumed to be identically and independently distributed as $N(0, \sigma^2)$.

The EMS table associated with the above model is given in Table 2 which assumes the greenhouse/CO_2 concentration effect is fixed while block, provenance, and replicate are random. Tests of the five null hypotheses associated with the linear model are presented in Table 3.

Table 2. The EMS table associated with the ANOVA.

Source	3 F i	2 R j	11 R K	3 R l	df	EMS
C_i	0	1	11	3	2	$\sigma^2 + 33\sigma^2_{\partial} + 33\sigma^2_B + 33\phi(C)$
$B_{(i)j}$	1	1	11	3	3	$\sigma^2 + 33\sigma^2_{\partial} + 33\sigma^2_B$
$\partial_{(ij)}$	1	1	11	3	0	$\sigma^2 + 33\sigma^2_{\partial}$
P_k	3	2	1	3	10	$\sigma^2 + 3\sigma^2_{BP} + 18\sigma^2_P$
CP_{ik}	0	2	1	3	20	$\sigma^2 + 3\sigma^2_{BP} + 6\sigma^2_{CP}$
$BP_{(i)jk}$	1	1	1	3	30	$\sigma^2 + 3\sigma^2_{BP}$
$\varepsilon_{(ijk)l}$	1	1	1	1	132	σ^2
Total					197	

Table 3. Tests of null hypotheses associated with the ANOVA.

Hypothesis	Test Statistic	Reference Distribution
C = 0	MS(C)/MS(B)	F(2, 3)
B = 0	No test	-
∂ = 0	No test	-
P = 0	MS(P)/MS(BP)	F(10,30)
CP = 0	MS(CP)/MS(BP)	F(20,30)

Statistical analysis was performed using SAS/STAT® 8.2 (SAS Inc. 1989). The GLM procedure combined with the RANDOM/test statement was used to analyze the gas exchange parameters as a mixed model ANOVA. TEST statements that tested [CO_2] against block and provenance against the block*provenance interaction were incorporated into the model to account for the nonstandard error structure of the split-plot design. When ANOVA showed a significant effect ($p<0.05$), multiple comparisons were performed using Bonferroni t-tests. The PROC VARCOMP procedure was used to estimate the contribution of each of the random effects (block, provenance, CO_2*provenance effects) to the variance of the dependent variable.

As part of the analysis of photosynthetic acclimation, parameter means from the response of A to C_i (V_{cmax}, J_{max}, TPU, R_d, and CO_2 compensation point) for the six seedlings per CO_2 treatment were compared using one-way ANOVA. When one-way ANOVA showed a significant effect ($p<0.05$), multiple comparisons were performed using Bonferroni t-tests.

Multiple linear regressions were performed to determine the relationships between gas exchange, biomass, and morphological measurements and the 70 climate variables associated with each of the 11 provenances ($p<0.05$).

RESULTS

GAS EXCHANGE MEASUREMENTS AT DAY 60

After 60 days of treatment, CO_2 elevation resulted in a significant increase in CO_2 assimilation rate (A)(Table 4). Increases of 22.5 and 41.3% respectively were observed from seedlings grown at 700 ppm CO_2 compared to those grown at 530 and 360 ppm CO_2 (Figure 3a). CO_2 assimilation rates of seedlings grown at 530 ppm were 24.3% higher than those of seedlings grown under ambient CO_2. No provenance effects or CO_2*P interaction effects were found to significantly influence A after 60 days of treatment. Despite its insignificance, there was considerable variation between provenances. Net A of seedlings from P58 (Sinclair Twp.) was noticeably greater than that of P96 (Strathearn) and P112 (Mountain Bay).

When measured under ambient [CO_2], A was significantly lower in seedlings grown under elevated [CO_2] than ambient [CO_2] (Table 5, Figure 4). When provenance data were pooled, mean A of seedlings grown at 700 and 530 ppm [CO_2] were 53.8 and 38.8% less than that of seedlings grown at ambient [CO_2], measured at ambient [CO_2], respectively (Figure 4). No CO_2*P interaction or provenance effects were found to have significantly influenced A when measured at common CO_2 concentration.

When provenance data were pooled, the A/C_i curves showed a reduction in photosynthetic capacity in seedlings grown in both elevated [CO_2] treatments of 530 and 700 ppm as compared to the ambient [CO_2] treatment (Figure 5). Slopes of the A/C_i curves from the elevated [CO_2] treatments were also noticeably less than that of the ambient [CO_2] treatment. However, little variation existed between the CO_2 compensation points for the three CO_2 concentrations, which ranged between 111 to

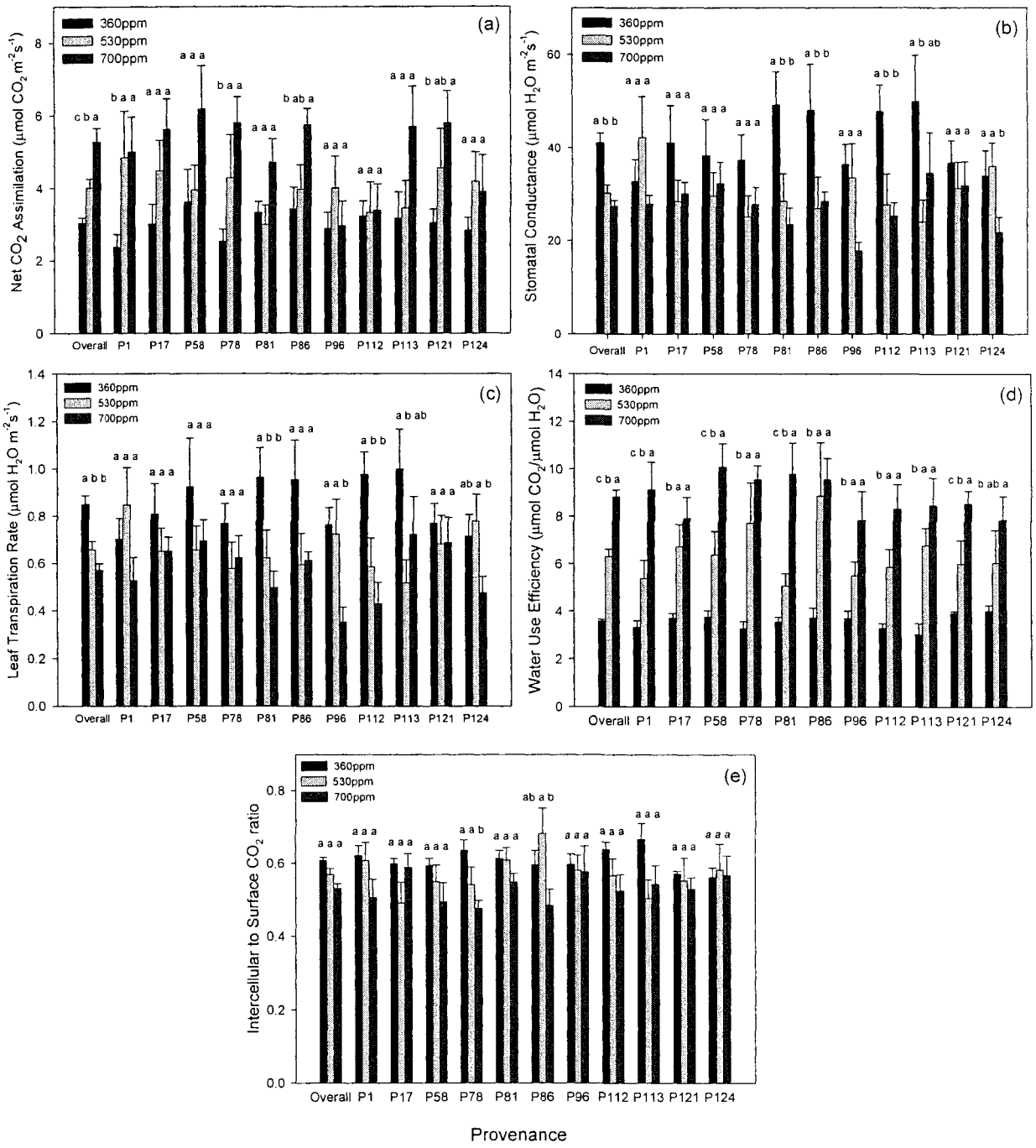

Figure 3. Mean (± 1 SE) net CO_2 assimilation (a), stomatal conductance (b), needle transpiration (c), water use efficiency (d), and intercellular to needle surface CO_2 concentration ratio (e) for 11 provenances of white spruce seedlings after 60 days of exposure to ambient, 530 ppm, and 700 ppm CO_2 concentrations. Different letters above bars indicate significant differences ($p<0.05$) among the three CO_2 concentrations.

Table 4. Summary of ANOVA of a split plot design with P-values for gas exchange measurements from 11 Ontario provenances of one-year old white spruce seedlings exposed to ambient, 530, and 700 ppm [CO_2] after 60 days of treatment.

		A		g_s		C_i/C_a		E		WUE	
Source	df	MS	P	MS	P	MS	P	MS	P	MS	P
C	2	74.48	0.003*	3955	0.02*	0.120	4.60	1.35	0.04*	481.88	0.007*
B	3	0.97	0.76	238	0.19	0.027	0.03*	0.13	0.08	11.80	0.12
δ	0	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A
P	10	4.14	0.13	97	0.73	0.004	0.55	0.03	0.77	4.24	0.67
CP	20	3.13	0.27	256	0.07	0.014	0.07	0.09	0.07	3.77	0.82
BP	30	2.47	0.93	142	0.95	0.008	0.86	0.05	0.93	5.62	0.40
Error	132	3.91		256		0.011		0.08		5.31	

Note: 1) A= Assimilation rate, g_s=stomatal conductance, E=leaf transpiration rate, WUE=water use efficiency, C_i/C_a =intercellular to leaf surface CO_2 concentration ratio
2) * indicates significant difference at $p \leq 0.05$

Table 5. Summary of ANOVA of a split plot design with P-values for CO_2 assimilation measurements from ambient and elevated CO_2 concentrations at ambient [CO_2] at Day 60 of the treatment period. Data from different provenances were pooled.

		A	
Source	df	MS	P
C	2	46.83	0.01*
B(C)	3	1.56	0.10
δ	0	N/A	N/A
P	10	0.23	0.96
CP	20	1.12	0.12
BP	30	0.70	0.99
Error	132	1.71	

Note: 1) A= Assimilation rate
2) * indicates significant difference at $p \leq 0.05$

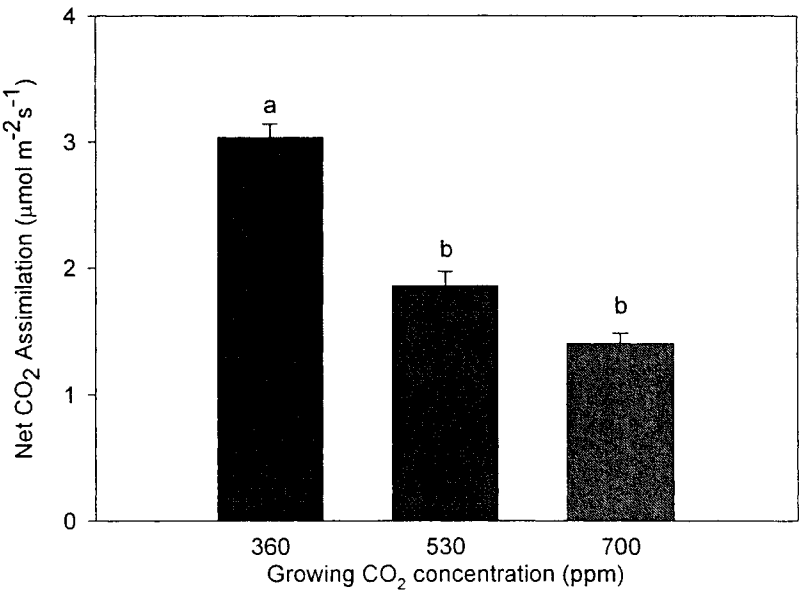

Figure 4. Net CO_2 assimilation rates of seedlings grown under ambient and elevated CO_2 concentrations measured at ambient CO_2 after 60 days of treatment (different letters located above each of the bars denotes a significant difference at p=0.05). Data from different provenances were pooled.

118 ppm [CO_2] (Table 6). Elevated [CO_2] treatment resulted in a decrease in the maximum Rubisco carboxylation efficiency (V_{cmax}), RuBP regeneration capacity (J_{max}), and triose-phosphate utilization (TPU) (Table 6). V_{cmax} of seedlings grown at 700 and 530 ppm [CO_2] were 24.0 and 21.9% less than that of seedlings grown at ambient [CO_2] respectively (Table 6). J_{max} of seedlings grown at 700 and 530 ppm [CO_2] was 28.2 and 25.1% less than that of seedlings grown at ambient [CO_2] respectively and TPU at 700 and 530 ppm [CO_2] was 34.5 and 34.0% less than that at ambient [CO_2] respectively (Table 6). Growth CO_2 concentration did not have a significant effect on R_d (Table 6).

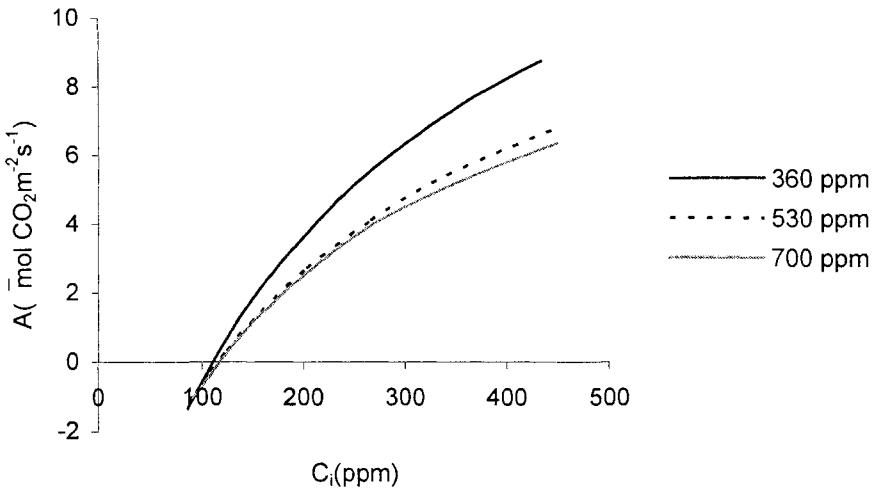

Figure 5. A/C_i curves measured at a range of ambient CO_2 concentration from 50 to 900 ppm for seedlings exposed to ambient (360ppm), 530 ppm, and 700 ppm [CO_2] after 60 days of treatment. PAR flux density and air temperature within the chamber were set at 800 μmol m^{-2} s^{-1} and 22.0°C, respectively.

Table 6. Gas exchange parameter means ± SE calculated from A/C_i curves with P-values for one-year old white spruce seedlings exposed to ambient, 530, and 700 ppm [CO_2] after 60 days of treatment.

	[CO_2] Treatment			P-value
Parameter	Ambient	530 ppm	700 ppm	
V_{cmax} (μmol $m^{-2}s^{-1}$)	23.7(1.33)[a]	18.5 (1.62)[b]	18.0(1.68)[b]	0.001*
J_{max} (μmol $m^{-2}s^{-1}$)	76.0(2.06)[a]	56.9(2.16)[b]	54.6(2.79)[b]	0.000*
R_d (μmol $m^{-2}s^{-1}$)	4.58(0.44)[a]	3.78(0.17)[a]	3.73(0.52)[a]	0.114
TPU (μmol $m^{-2}s^{-1}$)	5.21(0.29)[a]	3.44(0.57)[b]	3.41(0.54)[b]	0.000*
CO_2 Compensation Point (ppm)	111(2.7)[a]	116(2.1)[a]	118(3.8)[a]	0.230

Note: 1) Different letters beside treatment means indicate significant differences between CO_2 concentrations ($p \leq 0.05$).
2) * indicates significant difference at $p \leq 0.05$

Stomatal conductance was significantly decreased by [CO_2] elevation (Table 4). Mean g_s was 27.6 % and 35.8% lower in seedlings grown at 530 and 700 ppm CO_2 than at ambient [CO_2] respectively. Stomatal conductance did not vary significantly between the two elevated CO_2 treatments ($p>0.05$) (Figure 3b). Despite the significant effect on g_s, the differences in intercellular to leaf surface CO_2 concentration ratios (C_i/C_a) between

different CO_2 treatments were statistically not significant ($p>0.05$)(Table 4). However, C_i/C_a generally decreased with the growth CO_2 concentration (0.61, 0.57 and 0.52 for ambient, 530 ppm, and 700 ppm [CO_2] respectively). No provenance or CO_2*P effects were found to significantly influence C_i/C_a after 60 days of treatment.

Elevated CO_2 concentration significantly reduced leaf transpiration rate (E) (Table 4). Mean E was 22.6 and 33.0% lower respectively for seedlings grown at 525 and 700ppm than those grown at ambient [CO_2] (Figure 3c). However, the difference between 525 and 700 ppm [CO_2] (13.0%) was not significant statistically. No provenance effects or CO_2*P interaction effects were found to significantly influence E ($p>0.05$).

In contrast to E, significant increases in WUE of 44.4% and 60.6% were associated with the 530 and 700 ppm [CO_2] treatments respectively compared to WUE under ambient [CO_2] (Figure 3d). Mean WUE of seedlings grown under 700 ppm [CO_2] was 29.1% higher than those of seedlings grown under 530 ppm [CO_2]. Provenance or CO_2*P did not have a significant effect on WUE after 60 days of treatment.

GAS EXCHANGE MEASUREMENTS AT DAY 90

In concurrence with the results at Day 60, split-plot ANOVA indicated significant variance in the response of CO_2 assimilation rate to CO_2 concentration after 90 days of treatment (Table 7). CO_2 assimilation at 530 and 700 ppm [CO_2] was 58.5 and 69.0% greater than at ambient CO_2 concentration (Figure 6a). Furthermore, there was a 25.4 % increase in A in seedlings grown in 700 ppm compared to 530 ppm CO_2 concentration. No significant provenance or CO_2*P interaction differences were observed for A at Day 90 of the treatment period.

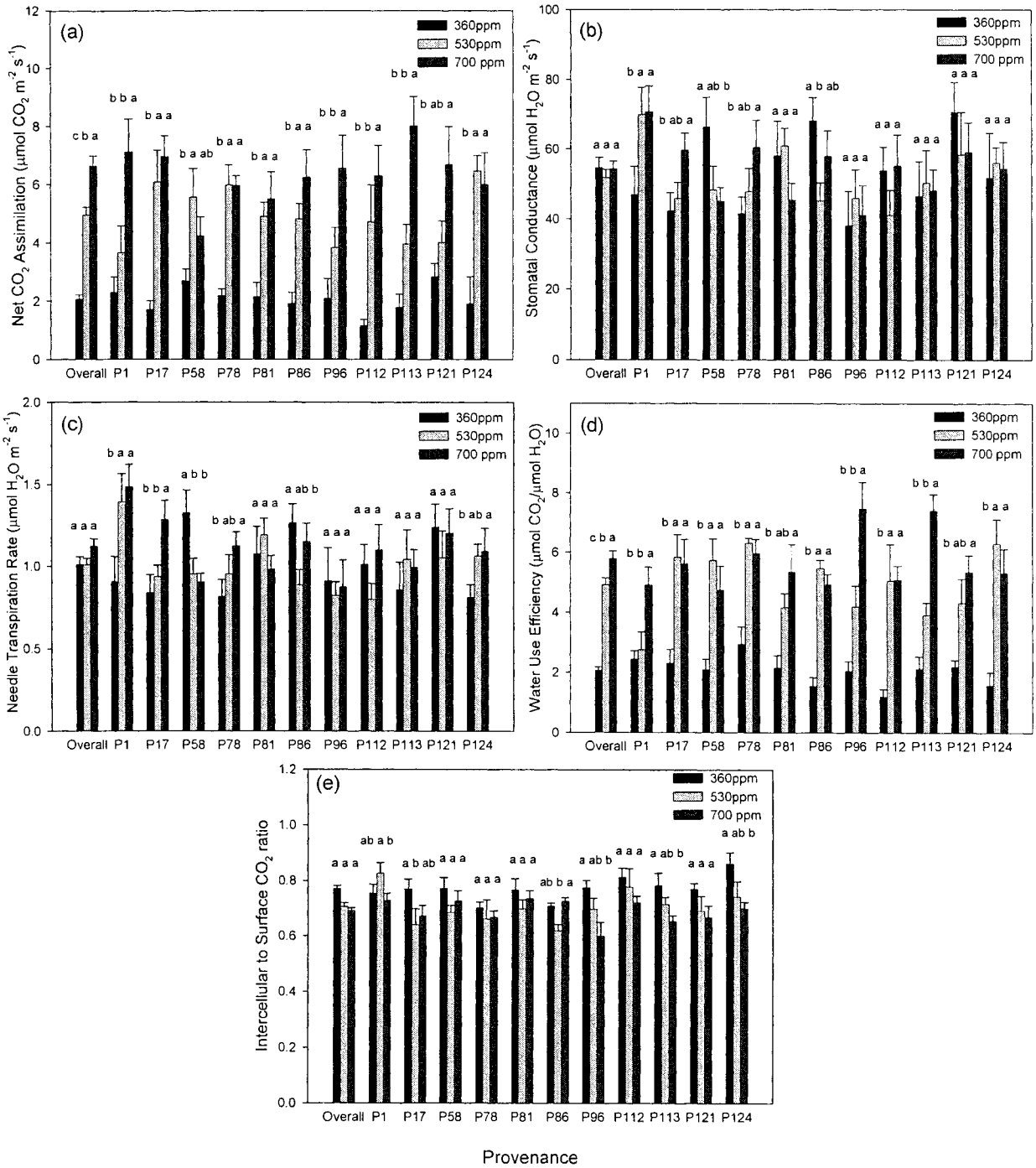

Figure 6. Mean (± 1 SE) net CO_2 assimilation (a), stomatal conductance (b), needle transpiration (c), water use efficiency (d), and intercellular to needle surface CO_2 concentration ratio (e) for 11 provenances of white spruce seedlings after 90 days of exposure to ambient, 530 ppm, and 700 ppm CO_2 concentrations. Different letters above bars indicate significant differences ($p<0.05$) among the three CO_2 concentrations.

Table 7. Summary of split-plot ANOVA with P-values for gas exchange measurements from 11 Ontario provenances of one-year old white spruce seedlings exposed to ambient, 530, and 700 ppm CO_2 after 90 days of treatment.

		A		g_s		Ci/Ca		E		WUE	
Source	df	MS	P	MS	P	MS	P	MS	P	MS	P
C	2	345.43	0.009*	180.5	0.56	0.109	0.08	0.32	0.28	255.54	0.014*
B(C)	3	10.41	0.052	257.5	0.56	0.016	0.19	0.16	0.32	10.73	0.004*
δ	0	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A
P	10	2.60	0.70	538.8	0.21	0.019	0.07	0.21	0.16	3.89	0.07
CP	20	5.78	0.12	504.6	0.22	0.011	0.40	0.21	0.12	5.72	0.004*
BP	30	3.60	0.83	370.7	0.17	0.010	0.28	0.13	0.13	1.93	0.91
Error	132	4.89		287.8		0.008		0.10		2.95	

Note: 1) A= Assimilation rate, g_s=stomatal conductance, E=leaf transpiration rate, WUE=water use efficiency, C_i/C_a =Intercellular to leaf surface CO_2 concentration ratio
2) * indicates significant difference at $p \leq 0.05$

After 90 days of treatment, growth CO_2 concentration was a source of variation when A of all seedlings was measured at ambient [CO_2] (Table 8). When provenance data were pooled, mean A of seedlings grown at 530 and 700 ppm [CO_2] was 22.3 and 35.0% less than those of seedlings grown at ambient [CO_2], respectively (Figure 7), which indicated that the seedlings from the elevated [CO_2] treatments continued to experience a loss of photosynthetic capacity from Day 60 to 90 when measured at ambient [CO_2]. No CO_2*P or provenance effects were found to significantly influence A when measured at common CO_2 concentration.

After 90 days of treatment, analysis of the A/C_i curves indicated a similar reduction in photosynthetic capacity in seedlings grown in both elevated [CO_2] treatments of 530 and 700 ppm as that shown after 60 days of treatment (Figure 8). Slopes of the A/C_i curves from the elevated [CO_2] treatments were again noticeably less than that of the ambient [CO_2] treatment.

Table 8. Summary of split-plot ANOVA with F-values for CO_2 assimilation measurements from ambient and elevated CO_2 concentrations measured at ambient CO_2 at Day 90 of the treatment period. Data from different provenances were pooled.

		A	
Source	df	MS	F
C	2	25.81	0.03*
B(C)	3	1.91	0.31
δ	0	N/A	N/A
P	10	1.98	0.28
CP	20	1.00	0.84
BP	30	1.54	0.86
Error	132	2.16	

Note: 1) A= Assimilation rate
2) * indicates significant difference at $p \leq 0.05$

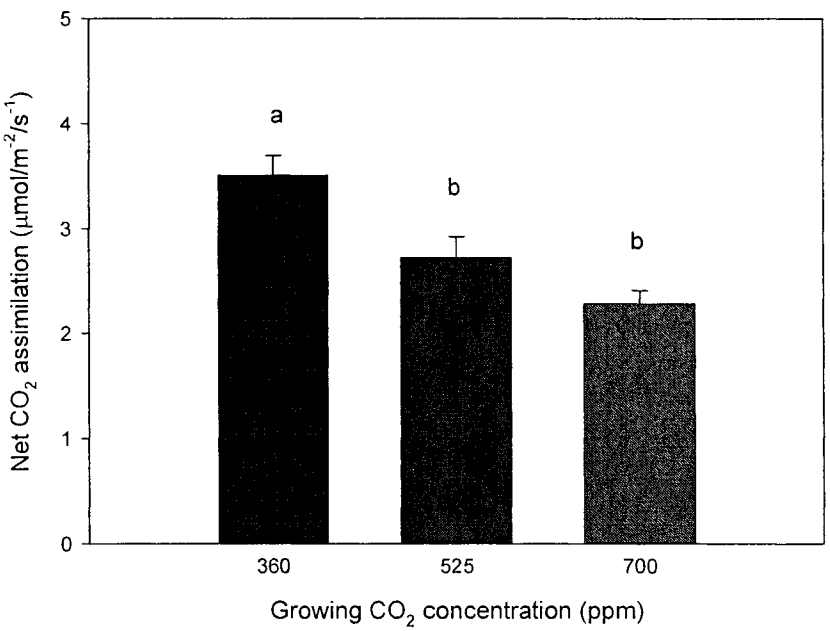

Figure 7. Net CO_2 assimilation rates of seedlings grown at ambient and elevated CO_2 concentrations measured at ambient CO_2 after 90 days of treatment (different letters located above each of the bars denotes a significant difference at p=0.05). Data from different provenances were pooled.

Elevated [CO_2] treatment reduced maximum V_{cmax}, J_{max}, and TPU (Table 9). V_{cmax} of seedlings grown at 700 and 530 ppm [CO_2] were 20.1 and 12.6% less than that

of seedlings grown at ambient [CO_2] respectively (Table 9). J_{max} of seedlings grown at 700 and 530 ppm [CO_2] was 14.0 and 11.4% less than that of seedlings grown at ambient [CO_2] respectively and TPU at 700 and 530 ppm [CO_2] was 18.5% less and 28.1% more than that at ambient [CO_2] respectively (Table 9). Growth CO_2 concentration did not have a significant effect on R_d which was 5.17, 5.07, and 4.47 μmol CO_2 $m^{-2}s^{-1}$ for ambient, 530, and 700 ppm [CO_2] respectively (Table 9). Similarly, the [CO_2] effect was not statistically significant on the CO_2 compensation points which were 146, 163, and 157 ppm C_i for ambient, 530, and 700 ppm [CO_2] respectively (Table 9).

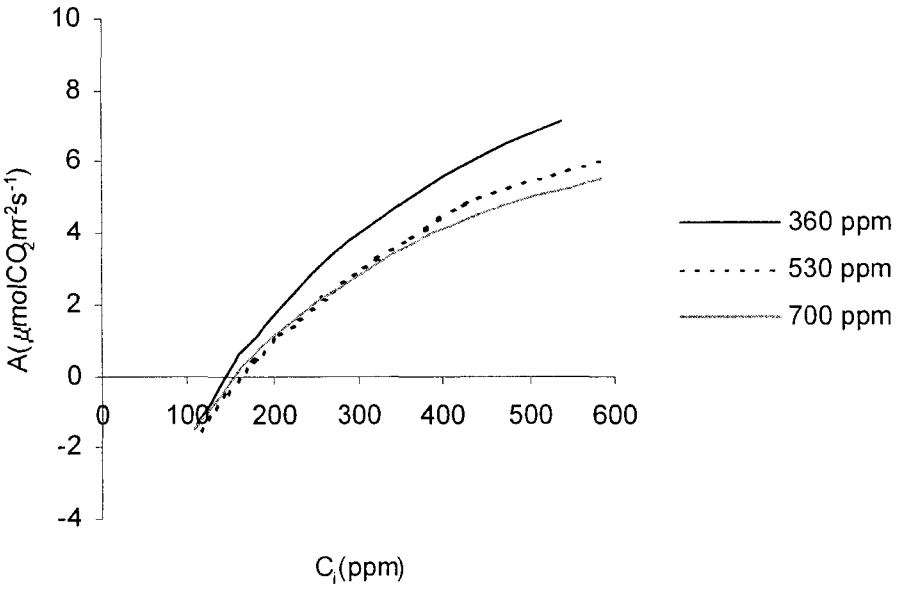

Figure 8. A/C_i curves measured at a range of ambient CO_2 concentration from 50 to 900 ppm for seedlings exposed to ambient, 530 and 700 ppm [CO_2] after 90 days of treatment. PAR flux density and air temperature within the chamber were set at 800 μmol m^{-2} s^{-1} and 22.0°C, respectively.

Table 9. Gas exchange parameter means ± SE calculated from A/C_i curves with P-values for one-year old white spruce seedlings exposed to ambient, 530, and 700 ppm [CO_2] after 90 days of treatment.

	[CO_2] Treatment			P-value
Parameter	Ambient	530 ppm	700 ppm	
V_{cmax} (μmol $m^{-2}s^{-1}$)	19.9(1.00)[a]	17.4(1.52)[b]	15.9(1.43)[b]	0.003*
J_{max} (μmol $m^{-2}s^{-1}$)	63.3(2.57)[a]	56.7(2.68)[b]	54.4(1.86)[b]	0.001*
R_d (μmol $m^{-2}s^{-1}$)	5.17(0.26)[a]	5.07(0.39)[a]	4.47(0.37)[a]	0.325
TPU (μmol $m^{-2}s^{-1}$)	3.95(0.17)[b]	5.5(0.27)[a]	3.22(0.21)[b]	0.038*
CO_2 Compensation Point (ppm)	146(4.9)[a]	163(4.0)[a]	157(5.5)[a]	0.071

Note: 1) Different letters beside treatment means indicate significant differences between CO_2 concentrations ($p \leq 0.05$).
2) * indicates significant difference at $p \leq 0.05$

The effects of elevated [CO_2] on g_s and E became statistically insignificant ($p<0.05$)(Table 7) after 90 days of treatment. Mean stomatal conductance under ambient [CO_2] conditions was only 1.8 % greater than g_s under 530 ppm CO_2 and 4.5 % less than g_s under 700 ppm CO_2. Mean needle transpiration rate at ambient [CO_2] was only 1% greater than E under 530 ppm and 9.8% less than E under 700 ppm (Figure 6c). No significant provenance or CO_2*P interaction differences were observed for g_s and E after 90 days of treatment.

Comparable to Day 60, the intercellular to needle surface CO_2 concentration was also not significantly affected by elevated levels of [CO_2], provenance and CO_2*P (Table 7). Seedling exposed to elevated concentrations of CO_2 exhibited a trend toward lower C_i/C_a values, however it was not statistically significant. C_i/C_a was 0.77, 0.71, and 0.69 under ambient, 530 ppm and 700 ppm CO_2 concentration respectively.

At the conclusion of the treatment period, split-plot ANOVA indicated that CO_2*P had a significant effect on WUE (Table 7). All provenances demonstrated an increase in WUE under 530 ppm and 700 ppm CO_2 compared to the ambient level.

Between the two elevated CO_2 treatments, provenances 1, 96, and 113 exhibited sharp increases in WUE in response to exposure to 700 ppm [CO_2] (Figure 9).

As observed on Day 60, split-plot ANOVA also indicated distinct variation in WUE of seedlings exposed to elevated CO_2 concentration (Table 7). WUE of seedlings under 700 ppm CO_2 was 17.5 and 65.2% greater than that under 525 ppm and ambient CO_2 concentrations respectively (Figure 6d). A 57.9% increase in WUE was observed at 530 ppm compared to ambient CO_2 concentration. No significant provenance differences were observed for WUE at Day 90 of the treatment period.

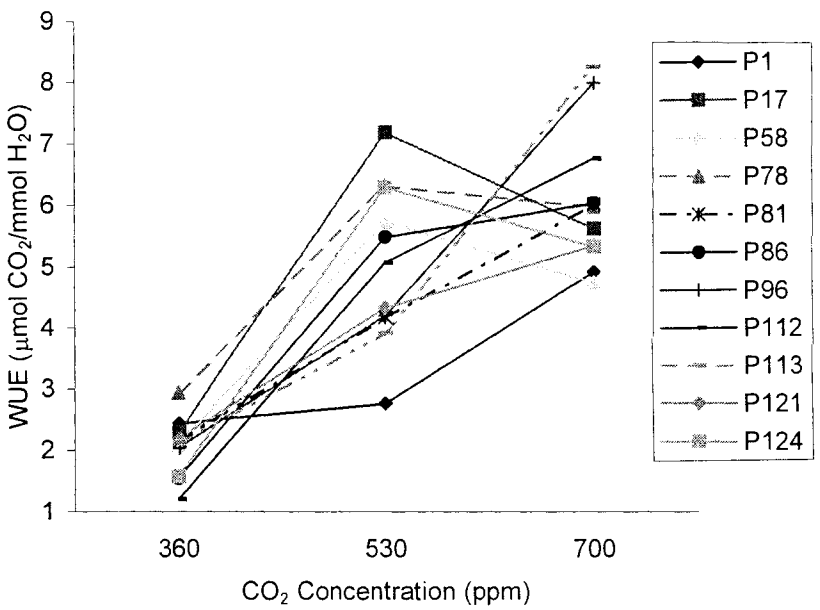

Figure 9. Comparison of water use efficiency of 11 Ontario provenances of one-year old white spruce seedlings after 90 days of exposure to ambient, 530 ppm, and 700 ppm CO_2 concentrations.

BIOMASS MEASUREMENTS

After 60 days of treatment, the CO_2*P interaction effect resulted in substantial variability in mean shoot, root, and total seedling dry masses (Table 10). Mean shoot,

root, and total dry masses from P17 (Antrim), P58 (Sinclair Twp.), and P121 (Pigeon River) were higher in the elevated [CO_2] treatments compared to those observed at ambient [CO_2] (Figure 10a and b). CO_2*P, however, did not significantly influence shoot to root ratios at Day 60.

After 60 days of treatment, provenance had a significant effect on mean shoot dry mass and total dry mass of the seedlings (Table 10). Among-provenance multiple comparison results indicated that seedlings from P81 (Fraserdale) and P112 (Mountain Bay) had significantly greater mean shoot and total dry masses than seedlings from P121 (Pigeon River) and P58 (Sinclair Twp.) (Figure 10a and b). Elevated CO_2 concentration did not significantly influence any of the biomass measurements after 60 days of treatment.

Table 10. Summary of split plot ANOVA with P-values for biomass measurements from 11 Ontario provenances of one-year old white spruce seedlings exposed to ambient, 530, and 700 ppm [CO_2] at Day 60 of the treatment period.

		SM		RM		TM		S:R	
Source	Df	MS	P	MS	P	MS	P	MS	P
C	2	1.35	0.07	0.15	0.14	2.31	0.07	2.00	0.43
B(C)	3	0.18	0.68	0.04	0.51	0.32	0.64	1.73	0.12
δ	0	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A
P	10	0.98	0.02*	0.09	0.09	1.61	0.01*	0.38	0.90
CP	20	1.22	0.001*	0.11	0.02*	1.88	0.001*	1.28	0.14
BP	30	0.35	0.99	0.05	0.95	0.56	0.98	0.83	0.53
Error	132	0.73		0.08		1.11		0.86	

Note: 1) SM= shoot dry mass, RM=root dry mass, TM=total seedling dry mass, S:R=shoot to root ratio
2) * indicates significant difference at $p \leq 0.05$

The provenance effect could explain 14.7 and 12.8% of the variation in shoot and total dry mass measurements after 60 days of treatment respectively (Table 11). The percent variation that could be attributed to CO_2*P was 11.1, 6.7, and 11.7 for shoot dry mass, root dry mass, and total dry mass respectively.

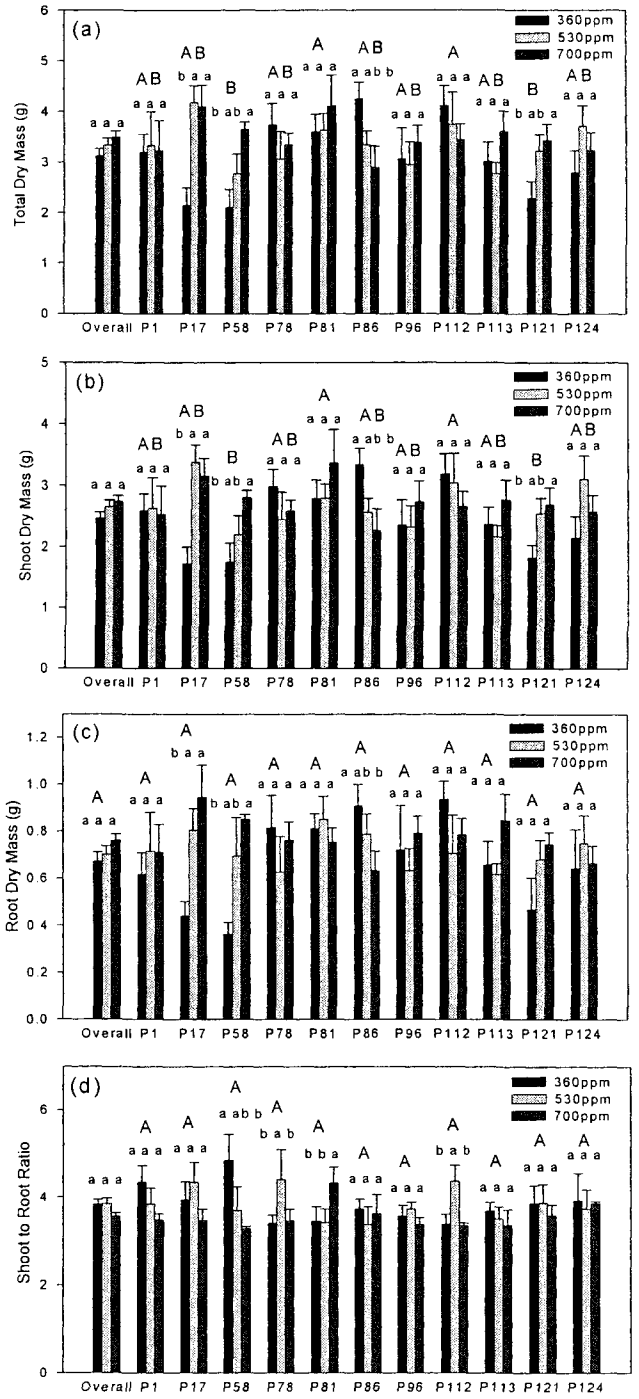

Figure 10. Mean (± 1 SE) total dry mass (a), shoot dry mass (b), root dry mass (c), and shoot to root ratio (d) for 11 Ontario provenances of one-year old white spruce seedlings after 60 days of exposure to ambient, 530 ppm, and 700 ppm [CO_2]. Different upper and lower-case letters above the bars indicate significant differences ($p<0.05$) in means among provenances and CO_2 concentrations respectively.

Table 11. P-values and percent variance (%VAR) explained by the random variables for biomass measurements of one-year old white spruce seedlings from 11 Ontario provenances exposed to ambient, 530 ppm and 700 ppm [CO_2] measured at Day 60 of the treatment period.

		SM		RM		TM		S:R	
Source	Df	P-value	%VAR	P-value	%VAR	P-value	%VAR	P-value	%VAR
B(C)	3	0.675	0.0	0.509	0.0	0.635	0.0	0.122	2.2
P	10	0.015	14.7	0.390	0.0	0.012	12.8	0.902	0.0
CP	20	0.001	11.1	0.018	6.7	0.001	11.7	0.137	2.6
BP	30	0.988	0.0	0.946	0.0	0.985	0.0	0.530	0.0
Error	132		88.9		93.3		88.3		95.2

Note: 1) SM= shoot dry mass, RM=root dry mass, TM=total seedling dry mass, S:R=shoot to root ratio
2) * indicates significant difference at $p \leq 0.05$

The CO_2*P interaction effect on mean shoot, root, and total seedling dry mass in response to [CO_2] elevation observed at Day 60 did not persist to Day 90 of the treatment period, nor was it present among the shoot to root ratio observations (Table 12). The provenance effect continued to contribute to the significant variation in shoot dry mass and total dry mass at the end of the treatment period (Table 12). Provenance accounted for 11.9 and 8.3% of the variation observed in the shoot and total dry mass measurements at Day 90 (Table 13). Seedlings from P86 (Fraserdale), P17 (Antrim), and P1 (Cornwall) exhibited significantly greater shoot dry masses than seedlings from P113 (Auden) (Figure 11b). In terms of total dry mass, significantly higher values were observed in seedlings from P86 (Fraserdale) and P17 (Antrim) than from P113 (Auden).

The provenance effect became statistically significant on the shoot to root ratio after 90 days of treatment (Table 12). Shoot to root ratios from seedlings from P86 and P1 (Cornwall) were significantly greater than those from P96 (Strathearn) and P124 (Shebandowan) (Figure 11d). Provenance was able to explain 5.9% of the total variation observed in the shoot to root ratios (Table 13). Blocks within the CO_2 greenhouses also

had a significant effect on the shoot to root ratio and explained 8.7% of the total variation observed in the shoot to root ratios at Day 90 of the treatment period (Tables 12 and 13). However, as at Day 60, variation in response to elevated [CO_2] treatment was not significant for shoot to root ratio or shoot, root and total dry mass after 90 days of treatment (Table 12).

HEIGHT AND ROOT COLLAR DIAMETER (RCD) MEASUREMENTS

Split-plot ANOVA on height measurements indicated a significant provenance effect on heights at Day 0, 30, 60, and 90 (Table 14). Heights of one-year old seedlings from P112 (Mountain Bay) were substantially higher than the other 10 provenances over the entire course of the treatment period but not significantly different from P81 (Fraserdale) and P86 (Proctor)(Figure 12). Through the first 60 days of the treatment period, the lowest heights were observed from seedlings from P58 (Sinclair Twp.) and 78 (Bentinck). After 90 days, heights of seedlings from P113 (Auden) and 121 (Pigeon River) were lower than those of the other 9 provenances (Figure 12d).

Provenance contributed 17.3 and 17.0% to the variation in height measurements taken at Day 0 and 30 of the treatment period respectively (Table 15). However, as the experiment progressed, variation attributed to provenance decreased to 10.5 and 8.5% at Day 60 and 90 of the treatment period respectively. CO_2*P, which proved to have a significant effect on seedling height at Day 30 and 60, explained 6.4 and 10.8% of the variation in the height measurements taken at these times (Table 15).

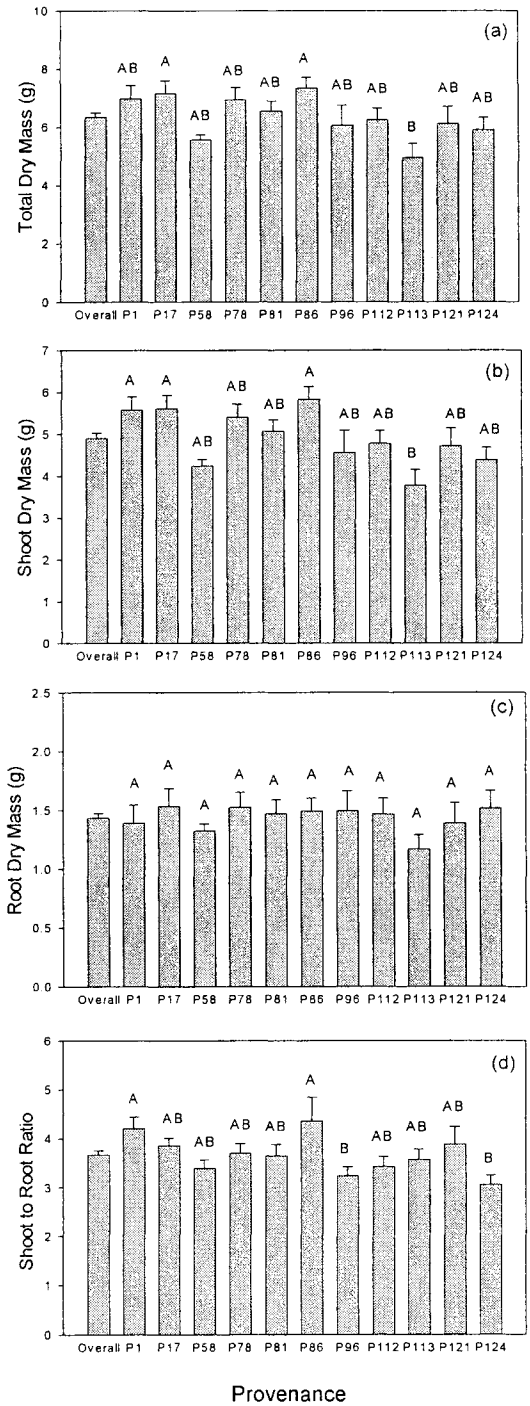

Figure 11. Mean (± 1 SE) total dry mass (a), shoot dry mass (b), root dry mass (c), and shoot to root ratio (d) for 11 Ontario provenances of one-year old white spruce seedlings after 90 days of exposure to ambient, 530 ppm, and 700 ppm [CO_2]. Different letters above the bars indicate significant differences ($p<0.05$) among provenances.

Table 12. Summary of split-plot ANOVA with P-values for biomass measurements from 11 Ontario provenances of one-year old white spruce seedlings exposed to ambient, 530, and 700 ppm CO_2 at Day 90 of the treatment period.

		SM		RM		TM		S:R	
Source	Df	MS	P	MS	P	MS	P	MS	P
C	2	2.29	0.33	0.3	0.63	3.34	0.50	2.64	0.56
B(C)	3	1.39	0.49	0.5	0.15	2.69	0.49	3.68	0.01*
δ	0	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A
P	10	7.61	0.01*	0.3	0.47	9.48	0.01*	1.96	0.03*
CP	20	2.46	0.17	0.2	0.64	3.71	0.36	0.92	0.36
BP	30	1.69	0.77	0.3	0.60	3.25	0.60	0.81	0.34
Error	132	2.14		0.3		3.57		0.74	

Note: 1) SM= shoot dry mass, RM=root dry mass, TM=total seedling dry mass, S:R=shoot to root ratio
2) * indicates significant difference at $p \leq 0.05$

Table 13. P-values and percent variance (%VAR) explained by the random variables for biomass measurements of one-year old white spruce seedlings from 11 Ontario provenances exposed to ambient, 530 ppm and 700 ppm CO_2 measured at Day 90 of the treatment period.

		SM		RM		TM		S:R	
Source	Df	P-value	%VAR	P-value	%VAR	P-value	%VAR	P-value	%VAR
B(C)	3	0.491	0.0	0.152	3.6	0.488	0.0	0.009	8.7
P	10	0.012	11.9	0.473	0.0	0.011	8.3	0.030	5.9
CP	20	0.001	2.9	0.646	0.0	0.362	0.0	0.360	1.4
BP	30	0.771	0.0	0.599	0.0	0.604	0.0	0.357	3.7
Error	132		85.2		96.4		91.7		80.3

Note: 1) SM= shoot dry mass, RM=root dry mass, TM=total seedling dry mass, S:R=shoot to root ratio
2) * indicates significant difference at $p \leq 0.05$

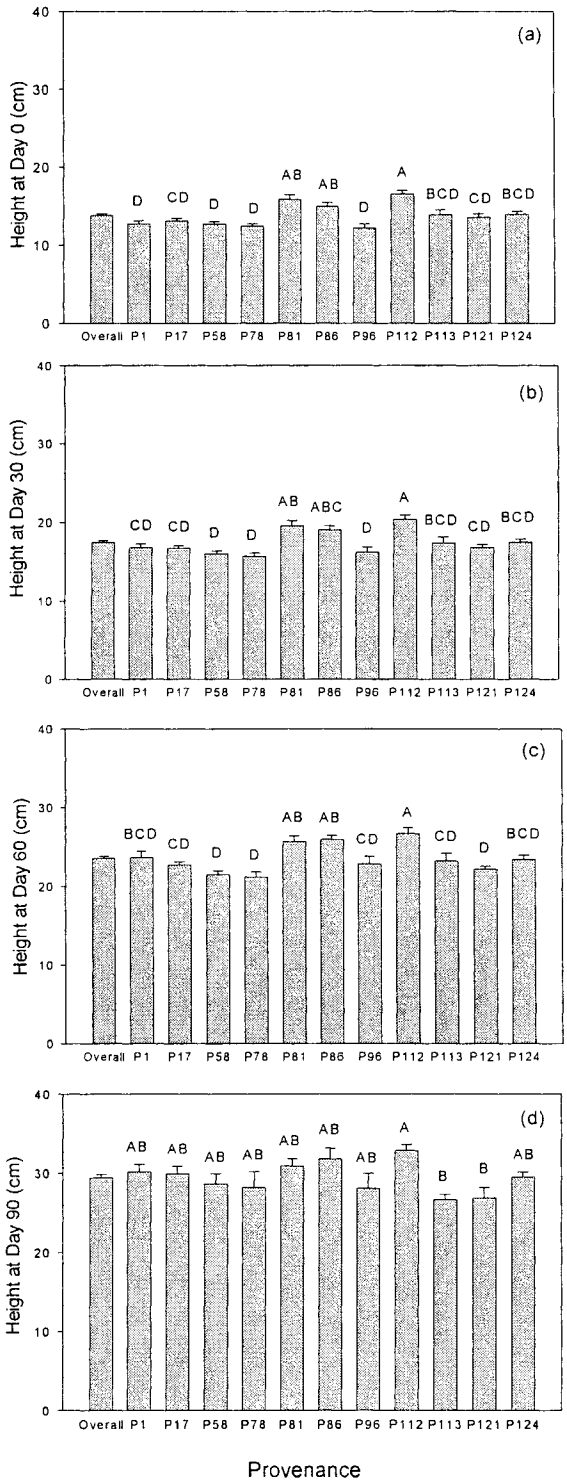

Figure 12. Mean (± 1 SE) heights for 11 provenances of one-year old white spruce seedlings after 0 (a), 30 (b), 60 (c) and 90 (d) days of exposure to ambient, 530 ppm, and 700 ppm CO_2 concentrations. Different letters above bars indicate significant differences ($p<0.05$) among provenances.

Table 14. Summary of split-plot ANOVA with P-values for height measurements from 11 Ontario provenances of one-year old white spruce seedlings exposed to ambient, 530, and 700 ppm CO_2 at Day 0, 30, 60, and 90 of the treatment period.

		HTO		HT30		HT60		HT90	
Source	Df	MS	P	MS	P	MS	P	MS	P
C	2	7.05	0.09	8.05	0.09	43.64	0.16	132.7	0.25
B(C)	3	1.17	0.92	1.42	0.90	12.03	0.55	57.3	0.02*
δ	0	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A
P	10	71.25	0.001*	88.40	0.001*	124.5	0.001*	74.9	0.004*
CP	20	10.41	0.21	17.50	0.02*	43.75	0.008*	23.1	0.16
BP	30	7.56	0.55	7.32	0.76	16.71	0.48	15.5	0.98
Error	132	7.99		9.14		16.87		29.8	

Note: 1) HTO height at Day 0, HT30=Height at Day 30, HT60=Height at Day 60, HT90=Height at Day 90
2) * indicates significant difference at $p \leq 0.05$

Table 15. P-values and percent variance (%VAR) explained by the random variables for the heights of one-year old white spruce seedlings from 11 Ontario provenances exposed to ambient, 530 ppm and 700 ppm CO2 measured at Day 0, 30, 60, and 90 of the treatment period.

		HTO		HT30		HT60		HT90	
Source	Df	P-value	%VAR	P-value	%VAR	P-value	%VAR	P-value	%VAR
B(C)	3	0.926	0.0	0.900	0.0	0.548	0.0	0.023	3.1
P	10	0.000	17.3	0.000	17.0	0.000	10.5	0.000	8.5
CP	20	0.209	2.3	0.015	6.4	0.008	10.8	0.157	0.0
BP	30	0.551	0.0	0.765	0.0	0.485	0.0	0.980	0.0
Error	132		80.4		76.6		78.7		88.4

Note: 1) HTO=Height at Day 0, HT30=Height at Day 30, HT60=Height at Day 60, HT90=Height at Day 90
2) * indicates significant difference at $p \leq 0.05$

Results of the split-plot ANOVA on root collar diameter measurements indicated that provenance effects were significant at Day 0, 30, and 60, but not at Day 90 (Table 16). All other factor effects were not significant on RCD throughout the course of the treatment period. Root collar diameters from provenances 78, 112, 86 were generally greater than the other provenances. Provenances 113, 96, and 58 generally had the lowest recorded RCDs throughout the 90-day treatment period (Figure 13).

Table 16. Summary of split-plot ANOVA with P-values for root collar diameter measurements from 11 Ontario provenances of one-year old white spruce seedlings exposed to ambient, 530, and 700 ppm CO_2 at Day 0, 30, 60, and 90 of the treatment period.

		RCDO		RCD30		RCD60		RCD90	
Source	Df	MS	P	MS	P	MS	P	MS	P
C	2	0.74	0.32	1.68	0.07	0.99	0.35	0.01	0.98
B(C)	3	0.43	0.51	0.11	0.74	0.64	0.13	0.54	0.35
δ	0	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A
P	10	2.17	0.002*	1.46	0.001*	1.14	0.003*	0.91	0.09
CP	20	0.77	0.19	0.41	0.15	0.54	0.09	0.58	0.32
BP	30	0.55	0.51	0.27	0.09	0.32	0.42	0.48	0.58
Error	132	0.57		0.19		0.31		0.52	

Note: 1) RCDO=RCD at Day 0, RCD30=RCD at Day 30, RCD60=RCD at Day 60 RCD90=RCD at Day 90
2) * indicates significant difference at $p \leq 0.05$

The components of variation attributed to provenance were 6.1, 11.7, 4.4 and 1.6% of the total variance in measurements taken at Day 0, 30, 60 and 90 respectively (Table 17). Despite its insignificance on RCD, CO_2*P explained 2.8, 5.3, 6.6 and 5.0% of the variance in the RCD measurements taken at Day 0, 30, 60, and 90 respectively.

Table 17. P-values and percent variance (%VAR) explained by the random variables for the root collar diameters of one-year old white spruce seedlings from 11 Ontario provenances exposed to ambient, 530 ppm and 700 ppm CO_2 measured at Day 0, 30, 60, and 90 of the treatment period.

		RCDO		RCD30		RCD60		RCD90	
Source	Df	P-value	%VAR	P-value	%VAR	P-value	%VAR	P-value	%VAR
B(C)	3	0.511	0.0	0.740	0.0	0.131	1.3	0.353	0.0
P	10	0.002	6.1	0.000	11.7	0.003	4.4	0.086	1.6
CP	20	0.197	2.8	0.149	5.3	0.089	6.6	0.319	5.0
BP	30	0.513	0.0	0.088	4.2	0.417	0.0	0.583	0.0
Error	132		91.1		78.8		87.7		93.4

Note: 1) RCDO=RCD at Day 0, RCD1=RCD at Day 30, RCD2=RCD at Day 60 RCD3=RCD at Day 90
2) * indicates significant difference at $p \leq 0.05$

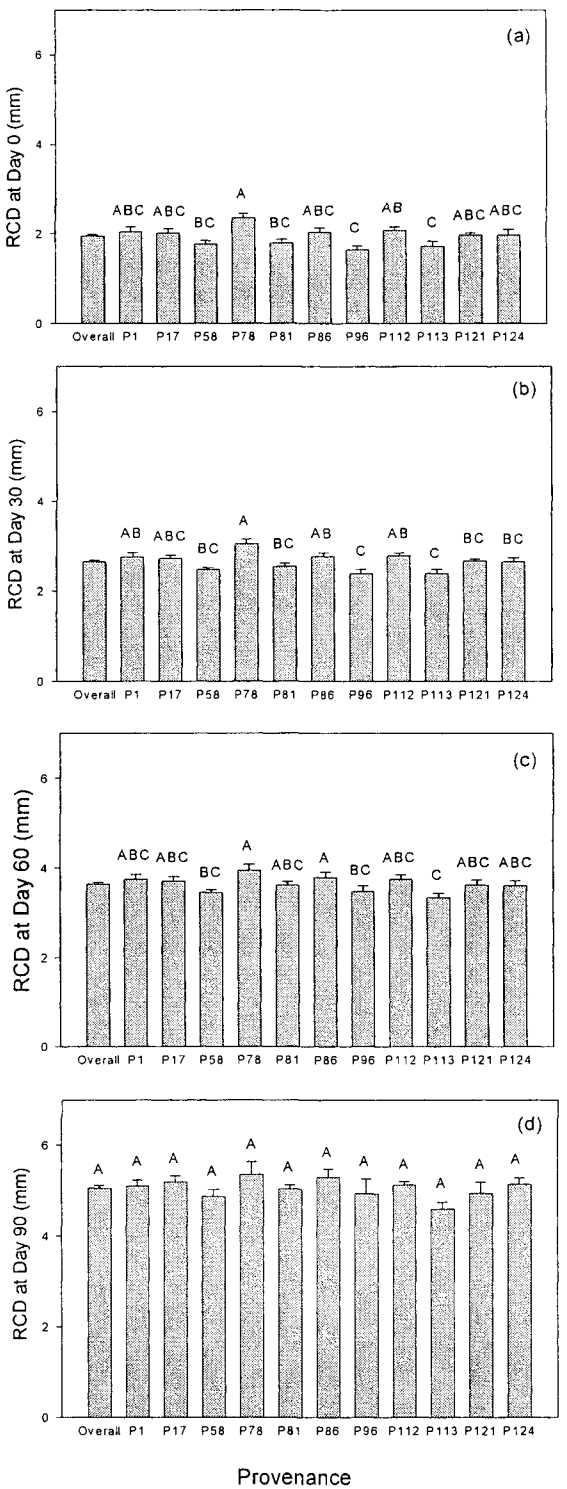

Figure 13. Mean (± 1 SE) root collar diameters for 11 provenances of one-year old white spruce seedlings after 0 (a), 30 (b), 60 (c) and 90 (d) days of exposure to ambient, 530 ppm, and 700 ppm CO_2 concentrations. Different letters above bars indicate significant differences ($p<0.05$) among provenances.

RELATIONSHIPS BETWEEN GAS EXCHANGE, BIOMASS, AND GROWTH MEASUREMENTS AND CLIMATE VARIABLES

Monthly maximum temperature and precipitation between April and November and growing season mean temperature and total precipitation appeared to be the strongest predictors of net CO_2 assimilation, stomatal conductance, needle transpiration and water use efficiency rates at Day 60 of the treatment period (Table 18). However, the results of the multiple regressions indicated that the ability of the climate variables to predict the above gas exchange parameters appeared to decline under 700 ppm CO_2 concentration. With the exception of late summer precipitation levels at 700 ppm CO_2 concentration, none of the climate variables appeared to account for the variation in intercellular to needle surface CO_2 concentration ratio at $p<0.05$.

After 90 days of treatment, monthly precipitation, monthly maximum temperature, and mean temperature during the wet and dry quarters of the year accounted for a high percentage of the variation expressed by net CO_2 assimilation, needle transpiration, and water-use efficiency, regardless of CO_2 concentration (Table 19). Similar to the observations made at Day 60, variation in the intercellular to needle surface CO_2 concentration ratio was poorly explained by the climate variables, with the exception of June and August precipitation levels under ambient CO_2 concentration.

Table 18. R^2 values from multiple regressions between gas exchange measurements and climate variables from 11 Ontario provenances of one-year old white spruce seedlings after 60 days of exposure to ambient, 530 ppm and 700 ppm CO_2.

	360 ppm		530 ppm		700 ppm	
Parameter	Climate Variable(s)	R^2	Climate Variable(s)	R^2	Climate Variable(s)	R^2
A	TPrecipP4 NovPrecip	0.851	MayMaxTemp JunMaxTemp	0.851	AprPrecip	0.528
g_s	AprMaxTemp AprPrecip AugPrecip	0.786	MTempWetQ AugPrecip	0.623	None($p<0.05$)	0.000
E	TPrecipP3 MayMaxTemp JunMaxTemp AprPrecip	0.940	MTempP3	0.575	None($p<0.05$)	0.000
WUE	Isotherm TPrecipP4	0.798	PrecipSeas PrecipWarmQ TPrecipP1 MarMaxTemp	0.993	OctPrecip NovPrecip	0.840
C_i/C_a	None($p<0.05$)	0.000	None($p<0.05$)	0.000	PrecipWarmQ AugPrecip	0.754

Note: Descriptions of climate variable codes can be found in APPENDIX II.

Table 19. R^2 values from multiple regressions between gas exchange measurements and climate variables from 11 Ontario provenances of one-year old white spruce seedlings after 90 days of exposure to ambient, 530 ppm and 700 ppm CO_2.

	360 ppm		530 ppm		700 ppm	
Parameter	Climate Variable(s)	R^2	Climate Variable(s)	R^2	Climate Variable(s)	R^2
A	PrecipDP SepPrecip OctPrecip	0.789	MtempWetQ MayPrecip SepPrecip DecPrecip	0.922	TempSeas MTempDryQ MayPrecip	0.931
g_s	None($p<0.05$)	0.000	JulyMinTemp JulyPrecip	0.738	DiurnRan PrecipSeas AugMaxTemp	0.885
E	PrecipWetQ SepPrecip	0.641	MTempWarmQ	0.690	JunMaxTemp AugMaxTemp	0.876
WUE	MTempDryQ TempRanP3 MarMaxTemp	0.906	MtempWetQ PrecipWP MayPrecip DecPrecip	0.926	MayPrecip	0.423
C_i/C_a	JunPrecip AugPrecip	0.802	None($p<0.05$)	0.000	None($p<0.05$)	0.000

Note: Descriptions of climate variable codes can be found in APPENDIX II.

In terms of biomass, multiple regression analysis indicated that climate variables associated with spring and fall precipitation levels were the most frequent predictors of total seedling dry mass and shoot to root ratio after 60 days of treatment (Table 20). No climate variables were able to predict total biomass at 700 ppm CO_2 or shoot to root ratio at 360 and 530 ppm CO_2 concentration ($p<0.05$).

Table 20. R^2 values from multiple regressions between biomass measurements and climate variables from 11 Ontario provenances of one-year old white spruce seedlings after 60 days of exposure to ambient, 530 ppm and 700 ppm CO_2.

	360 ppm		530 ppm		700 ppm	
Parameter	Climate Variable(s)	R^2	Climate Variable(s)	R^2	Climate Variable(s)	R^2
Total Dry Mass	AugMinTemp MarPrecip SepPrecip	0.840	PrecipWP	0.448	None($p<0.05$)	0.000
Shoot:Root	None($p<0.05$)	0.000	None($p<0.05$)	0.000	MTempDryQ MarPrecip	0.713

Note: Descriptions of climate variable codes can be found in APPENDIX II.

At the conclusion of the treatment period, a wide range of climate variables were able to account for a minimum of 62.9% to a maximum of 91.0% of the variation expressed in total seedling biomass and shoot to root ratio over the three CO_2 treatment levels (Table 21). Monthly precipitation, seasonal mean temperatures, and fall minimum and maximum temperatures were the dominant variables associated with the biomass parameters at Day 90 of the treatment period.

Table 21. R^2 values from multiple regressions between biomass measurements and climate variables from 11 Ontario provenances of one-year old white spruce seedlings after 90 days of exposure to ambient, 530 ppm and 700 ppm CO_2.

	360 ppm		530 ppm		700 ppm	
Parameter	Climate Variable(s)	R^2	Climate Variable(s)	R^2	Climate Variable(s)	R^2
Total Dry Mass	MTempDryQ AnnPrecip Marprecip	0.887	TPrecipP4 NovMaxTemp NovPrecip	0.887	OctMinTemp	0.629
Shoot:Root	DayStart MTempP3	0.762	MtempWarmQ AprPrecip MayPrecip	0.864	NovMinTemp FebPrecip NovPrecip	0.910

Note: Descriptions of climate variable codes can be found in APPENDIX II.

Compared to the gas exchange and biomass measurements, climate variables were relatively weaker in predicting the growth measurements of height and root collar diameter at Day 0, 30, 60, and 90 (Table 22), which is evident in the absence and/or lower R^2 values ($p<0.05$). Only 5 out of the 11 mean provenance height measurements over the three CO_2 concentrations taken from Day 0 to 90 could be predicted by the 70 available climate variables used in the model. Over the three CO_2 concentrations, root collar diameter was predicted predominantly by monthly minimum temperature, diurnal temperature range, and monthly precipitation levels (Table 22).

Table 22. R^2 values from multiple regressions between height and root collar diameter (RCD) measurements and climate variables from 11 Ontario provenances of one-year old white spruce seedlings after 0, 30, 60 and 90 days of exposure to ambient, 530 ppm and 700 ppm CO_2.

	360 ppm		530 ppm		700 ppm	
Parameter	Climate Variable(s)	R^2	Climate Variable(s)	R^2	Climate Variable(s)	R^2
Original Height	None($p<0.05$)	0.000	MayMaxTemp	0.513	None($p<0.05$)	0.000
Height Day 30	None($p<0.05$)	0.000	MayMaxTemp	0.427	None($p<0.05$)	0.000
Height Day 60	Isotherm TempRanP3	0.621	JanPrecip	0.417	MTempWetQ SepPrecip	0.633
Height Day 90	None($p<0.05$)	0.000	None($p<0.05$)	0.000	None($p<0.05$)	0.000
Original RCD	JulMaxTemp AugMaxtemp	0.855	FebMinTemp	0.423	DayEnd	0.573
RCD Day30	DiurnRan	0.385	PrecipDP DecMinTemp	0.782	DayEnd JunMinTemp MayPrecip	0.975
RCD Day 60	DiurnRan	0.375	PrecipSeas PrecipWarmQ JunMinTemp OctMinTemp	0.967	MTempDryQ SepPrecip	0.810
RCD Day 90	TempRan	0.478	DiurnRan JulyPrecip	0.677	MarPrecip NovPrecip	0.568

Note: Descriptions of climate variable codes can be found in APPENDIX II.

DISCUSSION

Net CO_2 assimilation of one-year old white spruce seedlings was enhanced at elevated CO_2 concentrations, despite reductions in photosynthetic capacity. Net A was enhanced by 41 and 69 % after 60 and 90 days in seedlings exposed to 700 ppm as compared to ambient CO_2 concentration. Mean increases in A fall within the range reported by many authors. In a meta-analysis of [CO_2] responses in trees, Curtis and Wang (1998) reported a 54 % increase in A in studies that were conducted in greenhouses with pot sizes less than 2.4L. Gunderson and Wullschleger (1994) estimated an overall increase in tree photosynthetic activity of 44% in response to high atmospheric [CO_2] levels.

The increase in net photosynthesis may largely be explained by an increase in carboxylation efficiency of ribulose-1,5-biphosphate carboxylase/oxygenase (Rubisco). Under ambient atmospheric CO_2 concentrations, Rubisco is CO_2 substrate-limited. As [CO_2] partial pressure increases, the ratio of oxygenation to carboxylation decreases, thereby increasing net photosynthesis over photorespiration (Lambers *et al.* 1998).

In this study, photosynthetic measurements were taken on current year growth. Jach and Ceulemans (1999 and 2000) reported that significant variation existed in the response of A to elevated CO_2 concentrations in *Pinus sylvestris* L. due to needle age (current year versus one-year old foliage), seedling age, and time of growing season. Thus, the results of this study should not be extended to all needle ages, tree ages, or all times of the growing season.

When measured under ambient CO_2 concentration, mean A of the current foliage of one-year old white spruce seedlings grown at elevated [CO_2] was lower than that of

seedlings grown at ambient [CO_2], which suggests that the seedlings in the present study exhibited photosynthetic down-regulation as a result of exposure to 530 and 700 ppm CO_2 concentrations after both 60 and 90 days of treatment. Down-regulation has been reported in some, but not all studies investigating the effects of elevated concentrations of CO_2 on trees. These results agree those found by Centritto and Jarvis (1999) for *Picea sitchensis* (Bong.) Carr. and Tissue *et al.* (1999) for *Pinus ponderosa* Dougl. ex P. & C. Laws.

A reduction in photosynthetic capacity in elevated [CO_2] may be associated with reductions in Rubisco activity and regeneration capacity (Sage 1994). Analysis of the A/Ci response curves after both 60 and 90 days of treatment revealed that the photosynthetic down-regulation observed in this study involved a reduction in the initial slope (Rubisco carboxylation efficiency (V_{cmax})), plateau of the curve (RuBP regeneration capacity (J_{max})), and regeneration of inorganic phosphate for the Calvin cycle (TPU). The results of this study agree with those reported by Long *et al.* (1993), Gunderson and Wullschleger (1994), Tissue *et al.* (1999) and Rey and Jarvis (1998) for a variety of tree species. However, these results contrast those reported by Arp and Drake (1991), Kellomaki and Wang (1996) and Teskey (1995) who found no change in photosynthetic capacity of several tree species as a result of enhanced [CO_2].

It has been proposed by Sage (1994) that acclimation to elevated CO_2 would involve a reduction in the investment in Rubisco and an increase in RuBP regeneration capacity. The results of this study do not support this theory as J_{max} decreased in the elevated CO_2 treatments, thus indicating no reallocation of N away from Rubisco towards RuBP regeneration. Incorporation of foliar nitrogen, starch, soluble sugars and

chlorophyll analysis would have provided further understanding of the basic mechanisms underlying the photosynthetic down-regulation observed in the white spruce seedlings.

Photosynthetic down-regulation is most prominent in plants under nutrient and rooting volume limitations and/or reduced sink strength (Sage 1994 and Ward and Strain 1999). When the seedlings were extracted from the pots after the gas exchange measurements, rooting volume did not appear to be limited by pot size. However, the fertilization regime may not have been adequate for the seedlings exposed to elevated CO_2 concentrations, thus resulting in photosynthetic down-regulation.

The elevated [CO_2] treatments of 530 ppm and 700ppm significantly reduced stomatal conductance by 27.6 and 36% respectively after 60 days. However, this effect was not present after 90 days of treatment. The reductions in g_s under elevated [CO_2] after 60 days are in concurrence with the 10 to 60% reduction in g_s in trees grown under elevated [CO_2] reported by Eamus and Jarvis (1989). However, in a meta-analysis of 48 studies, Curtis and Wang (1998) reported an insignificant 11% reduction in g_s in response to elevated [CO_2]. Similarily, Roberntz and Stockfors (2002) reported that elevated [CO_2] had no significant effect on g_s in current year growth of 30-year-old *Picea abies* (L.) Karst. However, tree and leaf age has been reported to affect the degree of reduction in g_s, with responses from younger conifer seedlings and foliage being more prominent than those shown by older trees (Medlyn *et al.* 2001, Jach and Ceulemans 2000).

A possible mechanism for the reduction in g_s in seedlings grown in a high-[CO_2] environment is an initial acclimatory response of stomata to increased C_i. Stomatal conductance appears to regulate CO_2 uptake as to maintain C_i as a constant proportion of

C_a (Farquhar and Sharkey 1982 and Drake *et al.* 1997). Although statistically insignificant, C_i/C_a tended to be lower in needles of seedlings grown at elevated [CO_2], suggesting evidence of stomatal acclimation to elevated [CO_2] in young white spruce.

The reduction in g_s demonstrated by the white spruce seedlings grown in elevated CO_2 concentrations may have important implications for the ability of white spruce seedlings to survive in a future climate in which the incidence of drought is predicted to increase. After 60 days of treatment, the acclimatory response of stomata to higher C_i may suggest that the stomata of white spruce seedlings will be more conservative in a future climate (Sage 1994).

A reduction in stomatal conductance decreased leaf-level transpiration (E) for one-year white spruce seedlings exposed to elevated CO_2 concentrations. A reduction in E, combined with an increase in net photosynthetic rate consequently resulted in an increase in water use efficiency (WUE), defined as the ratio between instantaneous rates of photosynthesis and transpiration. Increased WUE has been reported by Eamus and Jarvis (1989) for many species of well-watered plants. Young *Picea abies* and *Picea mariana* (Mill.) BSP seedlings have been reported to demonstrate increased WUE under elevated [CO_2] by Le Thiec and Dixon (1996), Egli *et al.* (1998), and Wang *et al.* (1995).

With the exception of A, CO_2 concentration did not have a significant effect on the gas exchange of young white spruce after 90 days of treatment. Stomatal conductance, E, and WUE were not affected by elevated [CO_2] in the final measurements. The lack of a significant response from g_s, E, and therefore, WUE to elevated [CO_2] after 90 days of treatment compared to that after 60 days of treatment may

be indicative of an acclimatory response of the photosynthetic components of the white spruce seedlings to increased [CO_2] over time (Sage 1994 and Ward and Strain 1999).

Exposure to elevated [CO_2] did not significantly increase biomass production and growth of young white spruce after 60 or 90 days of treatment. This result is in disagreement with the average biomass gains of 29% and 40% across a range of tree species reported by Curtis and Wang (1998) and Eamus and Jarvis (1989) respectively, and with the gains reported for other spruce species (Centritto *et al.* 1999, Johnsen and Major 1998, Johnsen and Seiler 1996, and Campagna and Margolis 1989). Possible causes for the lack of biomass gain in the seedlings may have been inadequate N supply in the soil (Wang *et al.* 1998) or the relative short length of the treatment period. Elevated CO_2 concentration also did not significantly affect biomass allocation after 90 days of treatment. This finding agrees with the majority of the studies which have shown that elevated [CO_2] rarely alters the allocation of biomass between roots and shoots (Ward and Strain 1999, Curtis and Wang 1998).

Provenance did not influence the gas exchange of the one-year old white spruce seedlings after 90 days of treatment. The lack of a provenance effect on the gas exchange variables agrees with the results reported by Houpis *et al.* (1999) for *Pinus ponderosa* and by Liu (2001) for *Populus tremuloides* Michx. The duration of the treatment period may not have provided sufficient time for the provenance effect to have been expressed in the physiological variables. Conclusive evidence for a provenance effect on the gas exchange processes of white spruce may require a longer-term study that spans two or more growing seasons.

In contrast to the gas exchange processes, substantial variation in the biomass and the heights and RCDs of the white spruce seedlings existed between provenances. Genotypic variation in biomass, height, and diameter in white spruce has also been reported by Nienstaedt and Teich (1972), Morgestern (1996), and Morgenstern and Copis (1999). The largest seedlings which originated from P112 – Mountain Bay, P86 – Proctor, and P17 – Antrim did not appear to have any geographical relationship in terms of latitude, longitude, or elevation. The consistently smallest seedlings originated from the northernmost provenance in the study; P113 (Auden). Of all the provenances included in the study, the local climate associated with P113 was represented by the lowest mean annual temperature and precipitation levels, the shortest growing season, and the largest annual and diurnal temperature ranges (McKenney 2004). The overall weak performance of P113 suggests that the extreme climate conditions in the Auden area had a considerable effect on the productivity of seedlings from that provenance in comparison to seedlings from the other provenances when grown in a common environment.

The CO_2*P interaction did not appear to significantly influence net assimilation, stomatal conductance, transpiration, or the atmospheric to intercellular CO_2 concentration ratio of one-year old white spruce seedlings after 60 and 90 days of treatment. A lack of significance of the CO_2*P interaction on gas exchange of one-year old white spruce seedlings agrees with the findings of Liu (2001) for young *Populus tremuloides* and Johnsen and Seiler (1996) for *Picea mariana.* Results from the present study disagree with those reported by Wang *et al.* (2000) and Centritto and Jarvis (1999) who found a significant CO_2*genotype interaction in photosynthetic responses of *Populus tremuloides*

and *Picea sitchensis* respectively. There was a between-provenance difference in response of WUE to elevated [CO_2] after 90 days of treatment. This may suggest the seedlings originating from provenances demonstrating higher WUE, P113 and P96, may perform better in a future climate in which the incidence of drought is predicted to increase (Medlyn *et. al.* 2001) .

Wang *et al.* (1994) conducted a similar study investigating CO_2*provenance interactions on the physiology and growth of *Picea mariana.* Although they reported no significant CO_2*provenance interactions after one growing season, several traits were significantly affected by the interaction after a second growing season (Wang *et al.* 1995). Thus, a study spanning two or more growing seasons is warranted in order to further understand the CO_2*provenance interaction on the physiology of young white spruce.

There were strong correlations between the gas exchange variables (A, g_s, E, and WUE) and monthly climate variables for the 11 Ontario provenances of white spruce at ambient and elevated CO_2 concentrations after 60 and 90 days of treatment. These findings agree with those reported by Liu (2001) for four provenances of *Populus tremuloides* from northwestern Ontario.

The results show that local climates associated with the 11 provenances can be used to predict physiological processes of white spruce seedlings under ambient and elevated CO_2 concentrations. Furthermore, these results suggest that the physiological processes occurring within white spruce from genetically distinct sources are sensitive to changes in local precipitation and temperature levels and have adapted to local climates.

Local climate was able to explain more variation in A, g_s, and E at 700 ppm CO_2 after 90 days of treatment compared to that after 60 days of treatment. The higher correlation between these gas exchange parameters and the local climate after 90 days of treatment may suggest that the extended period of exposure permitted physiological adjustment in the seedlings to a doubling of [CO_2]. However, after both 60 and 90 days of treatment, local climate was able to predict physiological processes of white spruce seedlings grown in 530 and 700 ppm CO_2 concentration to a noticeably lesser extent than those grown at ambient [CO_2]. This may suggest that there is strong interactive effect between the environmental conditions of temperature, precipitation and atmospheric CO_2 concentration on white spruce physiology. Future studies that incorporate temperature and precipitation conditions that simulate those predicted in a future climate, in addition to elevated [CO_2], may improve the ability of local climates to predict physiological processes in high atmospheric [CO_2] environments.

During the last week of the treatment period (mid-March), the stability of [CO_2] levels within the CO_2-enriched greenhouses (predominantly the 2x ambient [CO_2] greenhouse) became a concern. During this time, outdoor temperatures began to exceed 0^oC. Increased outdoor temperatures, combined with the solar heat absorbed by the greenhouse glass during sunny afternoons quickly increased internal temperatures, thus forcing the greenhouse vents to open to cool the greenhouses in order to maintain target day-time air temperatures. As a result of the venting, the CO_2-enriched greenhouses experienced sporadic, rapid losses of [CO_2] to the outside air during the afternoon hours, despite maximum output rates of [CO_2] enhancement. To avoid this problem in future CO_2 experiments housed in the Lakehead University Greenhouses, it is recommended

that experiments be scheduled to coincide with the coldest months of the year (December through February). During this time period, outdoor temperatures are low enough to minimize greenhouse venting (cooling) and thus the sporadic short-term loss of [CO_2] from the CO_2-enriched greenhouses. If an experiment needs to be conducted during the warmer months of the year or an extended treatment period is desired, the establishment of an additional supply of CO_2 for the 2x ambient [CO_2] greenhouse is recommended to maintain target concentrations during greenhouse venting (cooling) periods. Another possible solution, however more costly, would be to install growth chambers capable of enriching CO_2 concentration at Lakehead University. The use of growth chambers would ultimately provide a researcher with maximum control of environmental conditions including air temperature, CO_2 concentration, light intensity, and humidity.

The ability of the physiological and growth processes of white spruce to acclimate to local climate conditions will be critical as the predicted changes in atmospheric CO_2 concentrations and temperature and precipitation regimes associated with global warming manifest themselves across Ontario. Based on the findings of this study, white spruce tree improvement programs will not need to promote CO_2 responsive genotypes in order to ensure successful establishment of white spruce plantations adapted to future local climate conditions. Seed sources of white spruce in Ontario selected for superior growth characteristics in the present climate should perform well in the predicted future climate.

The findings of this study must be interpreted with caution because they only pertain to a short-term greenhouse experiment. Further studies that integrate the simulation of the future climate and resource availability conditions (temperature, precipitation, nitrogen availability) associated with elevated atmospheric [CO_2] are

warranted in order to obtain a better understanding of the response white spruce provenances in Ontario to climate change.

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APPENDICES

APPENDIX I

Table A.1. Climate variables (1961-1990 normals) associated with the 11 white spruce provenances obtained from Dan McKenny, Canadian Forest Serivce (2004).

Provenance	long	lat	elev	diurnran	isotherm	tempseas	maxtempwp	mintempcp	tempanran	mtempwetq	mtempdryq	mtempwarmq	mtempcoldq
1	-74.83	45.07	80	10.1	0.24	4.02	26.4	-14.8	41.1	18.1	-7.1	19.2	-8.6
17	-76.18	45.32	121	10.8	0.25	4.09	26.4	-16.3	42.7	17.7	-7.9	18.8	-9.5
58	-79.08	45.47	370	11.6	0.28	3.93	24.6	-17.5	42.1	6	-3.8	16.5	-10.3
78	-81	44.17	305	9.4	0.26	3.53	25.1	-11.3	36.4	-2.9	4.8	18	-6.1
81	-81.58	49.03	215	12.6	0.26	4.72	24.1	-24.8	49	14.5	-8.1	15.7	-16.2
86	-82.5	46.33	249	10.4	0.25	3.95	24.8	-16.2	41	6.8	-8.6	17.3	-9.7
96	-85.87	48.72	335	11.6	0.26	4.4	23.8	-21.2	44.9	14.8	-6.4	15.8	-13.9
112	-87.77	48.91	195	11.1	0.25	4.22	22.2	-22.1	44.3	10.5	-6.4	14.8	-13.7
113	-87.88	50.15	335	13.3	0.25	4.94	23.9	-28.2	52.1	13.5	-16.5	14.9	-18.4
121	-89.65	48.02	306	12.4	0.27	4.15	24.6	-20.6	45.3	14.9	-4.9	15.9	-12.5
124	-90.18	48.62	459	13.2	0.27	4.53	24.2	-24.5	48.7	14	-13.3	15.3	-15.4

Provenance	annprecip	precipwp	precipdp	precipseas	precipwettq	precipdryq	precipwarmq	precipcoldq	daystart	dayend	daygrow	tprecipp1	tprecipp2
1	941	98	59	17	278	182	267	206	106	317	212	187.8	102.5
17	862	81	57	13	238	176	234	193	108	313	206	179.4	100.3
58	1118	116	72	16	328	223	278	277	115	302	188	227.6	117.8
78	1041	109	67	16	309	212	256	288	108	323	216	229	101.2
81	831	97	41	27	277	141	272	157	129	296	168	145.7	109.6
86	884	100	50	20	278	171	219	191	113	310	198	172.2	100
96	847	94	48	22	264	149	255	178	122	295	174	150.5	100.9
112	808	93	36	28	263	124	254	157	126	299	174	128	107.7
113	739	96	28	41	276	97	272	105	133	290	158	116	110.4
121	797	97	32	32	276	128	270	135	125	296	172	131.9	110.9
124	796	100	33	38	290	120	289	120	130	292	163	129.8	121.3

Provenance	tprecipp3	tprecipp4	ggdp3	annmtemp	annmintemp	annmaxtemp	mtempp3	tempranp3	janmintemp	febmintemp	marmintemp	aprmintemp
1	586.7	484.2	1885	6.04	1.01	11.08	14.25	28.08	-14.76	-13.74	-7.1	0.78
17	514.2	413.9	1788	5.45	0.05	10.86	14.08	27.99	-16.27	-15.39	-8.15	-0.24
58	582.5	464.7	1427	3.91	-1.9	9.71	13.02	26.32	-17.53	-16.76	-10.57	-2.35
78	608	506.8	1723	6.32	1.61	11.02	13.32	26.74	-10.89	-11.33	-6.65	0.22
81	469.9	360.4	1205	0.88	-5.43	7.19	12.62	25.86	-24.85	-23.71	-16.36	-5.96
86	515.2	415.3	1547	4.48	-0.73	9.69	13.19	26.54	-16.21	-16.2	-9.97	-1.4
96	472.7	371.8	1252	1.78	-4.01	7.56	12.64	25.7	-20.79	-21.16	-13.81	-4.1
112	475.3	367.6	1128	1.64	-3.93	7.21	11.89	23.95	-22.08	-20.06	-13.22	-4.52
113	445.7	335.3	1037	-0.65	-7.28	5.98	12	25.83	-28.21	-25.73	-18.74	-7.85
121	482.8	371.9	1270	2.54	-3.66	8.75	12.8	26.51	-20.62	-18.2	-11.8	-3.99
124	487.3	365.9	1149	0.98	-5.65	7.6	12.47	26.11	-24.45	-21.73	-14.92	-5.67

Provenance	maymintemp	junmintemp	julmintemp	augmintemp	sepmintemp	octmintemp	novmintemp	decmintemp	janmaxtemp	febmaxtemp	marmaxtemp
1	6.96	11.92	14.84	13.68	9.21	3.45	-2.09	-11.08	-5.28	-4	2.17
17	6.18	11.14	14	12.86	8.38	2.7	-2.77	-11.9	-5.88	-4.16	2.2
58	3.85	8.47	11.25	10.5	6.6	1.17	-4.24	-13.21	-6.19	-4.34	1.67
78	5.54	10.4	13.47	12.96	9.52	4.18	-0.62	-7.43	-3.39	-2.85	2.28
81	1.71	6.94	10.42	9.18	5.02	0.19	-7.7	-19.99	-11.57	-8.61	-1.3
86	4.69	9.44	12.94	12.05	8.16	2.82	-3.31	-11.8	-5.91	-4.73	1.17
96	3.05	7.85	11.28	10.23	5.92	0.03	-8.05	-18.55	-8.57	-7.07	-0.61
112	2.11	6.68	10.16	9.93	5.82	0.91	-6.37	-16.52	-9.9	-7.08	-0.64
113	0.47	5.96	9.67	8.02	3.2	-1.48	-10.5	-22.21	-13.92	-9.77	-2.36
121	2.14	6.84	10.62	9.49	4.81	0.11	-6.93	-16.37	-8.37	-5.24	0.68
124	1.12	6.24	9.58	8.25	3.7	-0.97	-9.15	-19.76	-11.22	-6.99	-0.38

Provenance	aprmaxtemp	maymaxtemp	junmaxtemp	julmaxtemp	augmaxtemp	sepmaxtemp	octmaxtemp	novmaxtemp	decmaxtemp	janprecip	febprecip
1	11.06	18.57	23.31	26.37	24.86	19.94	13.16	5.45	-2.65	60.7	59.41
17	10.93	18.57	23.41	26.38	24.82	19.66	12.8	4.75	-3.17	57.88	56.52
58	9.75	17.46	21.8	24.58	22.67	17.84	11.38	3.52	-3.56	97.44	71.53
78	10.18	17.27	22.22	25.11	23.86	19.6	12.96	5.87	-0.9	102.81	76.46
81	7.21	15.77	21.42	24.14	22.12	16.13	9.4	0.38	-8.76	54.26	40.87
86	9.43	16.93	21.51	24.78	22.92	18.01	11.47	3.74	-3.08	61.21	49.98
96	8.06	16.38	20.3	23.78	21.58	15.76	8.53	-0.01	-7.39	65.27	48.57
112	7.34	14.66	18.77	22.17	21.17	15.64	9.49	1.37	-6.52	55.87	36.14
113	6.57	15.2	20.14	23.88	21.46	14.7	7.77	-1.36	-10.49	34.23	27.6
121	9.12	16.85	20.77	24.64	22.87	16.96	10.63	2	-5.95	49.48	32.08
124	8.3	16.74	21.07	24.25	22.4	16.1	9.38	-0.17	-8.3	43.36	32.87

Provenance	marprecip	aprprecip	mayprecip	junprecip	julprecip	augprecip	sepprecip	octprecip	novprecip	decprecip
1	61.87	74.18	72.29	83.09	86.18	98.09	93.72	78.26	87.57	85.87
17	61.28	65.39	73.67	76.87	78.08	79.28	80.62	73.16	80.04	78.98
58	75.42	75.68	84.58	90.42	85.42	102.19	111.05	100.31	116.19	108.12
78	70.18	66.85	74.5	81.49	76.1	98.79	98.52	89.03	96.98	109.19
81	53.73	45.93	68.92	86.42	96.87	88.48	91.84	71	70.73	61.59
86	60.1	62.71	73.15	69.92	65.47	83.13	100.49	87.76	90.22	79.56
96	52.36	47.73	67.23	84.66	84.12	85.85	94.28	80.95	71.85	63.72
112	44.52	43.83	67.61	87.93	78.26	87.8	93.08	82.34	66.35	64.6
113	34.93	42.95	62.18	88.03	96.12	87.81	91.63	71.61	58.7	43.3
121	46.74	49.05	70.97	91.38	86.94	91.65	97	69.42	59.33	52.96
124	43.85	44.27	73.3	96.2	100.39	92.61	96.6	72.23	55.83	44

APPENDIX II

Table A.2. Daily averages of CO_2 concnetration, and air temperature for the three greenhouses used for 90 days of treatment.

Day	GH3 - CO_2 (ppm)	GH4 - CO_2 (ppm)	GH5 - CO_2 (ppm)	GH3 - TEMP (°C)	GH4 - TEMP (°C)	GH5 - TEMP (°C)
1	335.8	693.6	585.9	17.0	16.0	16.6
2	335.4	698.9	555.0	16.9	16.2	16.7
3	352.9	698.2	552.5	16.6	16.2	16.9
4	356.7	717.8	562.0	16.8	16.2	16.7
5	358.1	716.1	543.3	16.6	15.8	16.6
6	359.1	696.9	556.2	17.0	16.3	16.5
7	351.8	691.4	540.6	16.6	16.2	16.7
8	338.3	671.3	523.0	16.6	17.0	16.4
9	341.1	686.3	515.1	16.6	16.0	16.7
10	335.7	686.5	506.8	16.7	15.8	16.6
11	335.4	686.3	520.8	18.0	17.3	18.0
12	337.9	695.7	548.6	17.1	16.0	16.9
13	339.0	701.7	537.9	16.7	15.9	16.6
14	343.0	690.9	547.1	16.9	15.8	16.8
15	338.2	696.9	548.0	16.7	16.0	16.7
16	334.5	685.3	524.8	17.2	15.9	16.7
17	336.4	691.2	563.9	16.8	15.9	16.6
18	327.2	690.0	524.3	16.7	15.7	16.5
19	324.6	671.0	504.4	17.0	16.0	16.7
20	331.8	678.1	504.5	16.8	16.0	16.8
21	333.5	678.3	502.9	16.8	15.9	16.8
22	333.5	681.1	504.5	16.6	15.9	16.5
23	336.0	689.8	519.0	17.0	16.0	16.8
24	340.5	696.7	573.6	16.9	16.0	16.7
25	333.7	690.4	518.8	16.9	15.8	16.5
26	331.5	685.3	556.5	16.7	15.9	16.7
27	344.5	690.4	522.0	16.8	15.9	16.6
28	341.7	675.3	501.0	17.1	16.3	16.8
29	338.8	668.1	527.9	17.0	16.1	16.7
30	330.2	665.3	554.4	16.9	16.2	16.8
31	349.3	684.0	584.3	16.6	16.8	16.9
32	351.2	701.9	562.5	16.4	16.0	16.6
33	351.0	678.0	549.2	16.7	16.1	16.8
34	352.6	687.0	570.9	16.7	15.8	16.8
35	339.0	645.3	515.6	16.8	16.0	16.5
36	349.9	655.1	513.9	16.6	16.2	16.8
37	339.5	672.6	509.7	16.8	16.1	16.6
38	327.3	681.1	518.3	17.1	15.9	17.0
39	319.4	661.7	517.9	16.9	15.9	16.7
40	316.8	662.1	530.8	16.6	15.9	16.7
41	319.8	652.1	557.6	16.7	15.9	16.7
42	319.4	651.5	547.7	17.1	15.9	16.7
43	320.6	658.0	567.4	16.8	16.1	16.7
44	325.5	665.0	536.1	17.0	16.0	16.5
45	330.8	679.7	561.6	17.2	15.9	16.6
46	335.8	693.6	585.9	17.0	16.0	16.6

47	335.4	698.9	555.0	16.9	16.2	16.7
48	345.9	683.8	572.5	17.3	16.8	16.7
49	338.3	688.4	561.0	17.1	16.0	16.7
50	330.6	656.8	533.3	17.6	16.9	16.7
51	337.3	698.1	537.8	16.5	16.0	16.7
52	332.9	697.5	548.8	16.8	16.2	16.7
53	331.0	671.3	561.0	17.2	16.1	16.6
54	330.8	688.8	558.2	17.0	16.0	16.6
55	336.1	702.8	567.8	17.0	16.1	16.7
56	337.8	706.1	566.4	16.9	16.2	16.3
57	346.3	697.8	571.3	17.1	16.5	16.7
58	342.9	684.5	568.9	17.6	17.1	17.3
59	333.9	686.7	572.4	17.1	16.8	16.9
60	339.8	711.5	579.0	16.6	16.2	16.6
61	321.7	701.3	546.1	16.6	16.0	16.6
62	327.4	714.2	565.6	16.7	15.8	16.5
63	325.5	703.0	548.1	17.3	16.1	16.7
64	341.0	699.1	563.5	17.3	16.5	16.4
65	363.0	707.2	578.5	16.8	16.1	16.7
66	332.5	707.6	560.7	17.4	16.5	16.8
67	325.1	698.8	550.7	17.3	16.2	16.5
68	321.0	702.4	540.6	16.8	15.9	16.4
69	327.3	688.8	550.9	17.4	16.6	16.6
70	327.3	712.6	566.0	17.0	16.0	16.6
71	331.2	699.9	553.1	17.0	16.4	16.5
72	338.3	698.7	562.0	17.3	16.6	16.9
73	358.1	716.1	543.3	16.6	15.8	16.6
74	359.1	696.9	556.2	17.0	16.3	16.5
75	351.8	691.4	540.6	16.6	16.2	16.7
76	316.8	662.1	530.8	16.6	15.9	16.7
77	319.8	652.1	557.6	16.7	15.9	16.7
78	319.4	651.5	547.7	17.1	15.9	16.7
79	338.8	668.1	527.9	17.0	16.1	16.7
80	330.2	665.3	554.4	16.9	16.2	16.8
81	349.3	684.0	584.3	16.6	16.8	16.9
82	333.5	681.1	504.5	16.6	15.9	16.5
83	336.0	689.8	519.0	17.0	16.0	16.8
84	340.5	696.7	573.6	16.9	16.0	16.7
85	338.3	671.3	523.0	16.6	17.0	16.4
86	341.1	686.3	515.1	16.6	16.0	16.7
87	335.7	686.5	506.8	16.7	15.8	16.6
88	335.4	686.3	520.8	18.0	17.3	18.0
89	341.0	699.1	563.5	17.3	16.5	16.4
90	363.0	707.2	578.5	16.8	16.1	16.7
Mean	336.5	687.3	545.6	16.9	16.1	16.7
Std dev.	15.8	34.3	33.7	4.9	4.8	4.5

APPENDIX III

Table A.3. Climate variable codes and definitions.

Code	Definition	Unit
diurnran	Mean diurnal range	C°
isotherm	Isothermality 2/7	
tempseas	Temperature Seasonality	C°
tempanran	Annual temperature range	C°
mtempwetq	Mean temperature in wettest quarter	C°
mtempdryq	Mean temperature in driest quarter	C°
mtempwarmq	Mean temperature in warmest quarter	C°
annprecip	Annual precipitation	mm
precipwp	Precipitation in wettest period	mm
precipdp	Precipitation in driest period	mm
precipseas	Precipitation seasonality (c of v)	mm
precipwetq	Precipitation in wettest quarter	mm
precipwarmq	Precipitation in warmest quarter	mm
dayend	Julian day number of end of growing season	Julian day
tprecipp1	Total precipitation for period 1	mm
tprecipp4	Total precipitation for period 4	mm
mtempp3	Mean temperature for period 3	mm
tempranp3	Temperature range for period 3	mm
febmintemp	February minimum temperature	C°
junmintemp	June minimum temperature	C°
julmintemp	July minimum temperature	C°
augmintemp	August minimum temperature	C°
octmintemp	October minimum temperature	C°
novmintemp	November minimum temperature	C°
decmintemp	December minimum temperature	C°
marmaxtemp	March maximum temperature	C°
aprmaxtemp	April maximum temperature	C°
maymaxtemp	May maximum temperature	C°
junmaxtemp	June maximum temperature	C°
julmaxtemp	July maximum temperature	C°
augmaxtemp	August maximum temperature	C°
novmaxtemp	November maximum temperature	C°
janprecip	January precipitation	mm
febprecip	February precipitation	mm
marprecip	March precipitation	mm
aprprecip	April precipitation	mm
mayprecip	May precipitation	mm
junprecip	June precipitation	mm
julprecip	July precipitation	mm
augprecip	August precipitation	mm
sepprecip	September precipitation	mm
octprecip	October precipitation	mm
novprecip	November precipitation	mm
decprecip	December precipitation	mm

APPENDIX IV

Table A.4. Net CO_2 assimilation (A)(mmol CO_2 m^{-2} s^{-1}), stomatal conductance (g_s)(mmol H_2O m^{-2} s^{-1}), intercellular to surface CO_2 ratio, leaf transpiration rate (mmol H_20 m^{-2} s^{-1}), and water-use efficiency (WUE) (mmol CO_2/H_2O) means and standard deviations for ambientand elevated CO_2 treatments after 60 days of treatment.

Provenance		Ambient CO2		530 ppm CO2		700 ppm CO2	
		Mean	SE	Mean	SE	Mean	SE
Overall	A	3.03	0.15	4	0.26	5.28	0.38
	g_s	41.82	2.19	30.29	1.8	27.38	1.28
	E	0.85	0.04	0.66	0.04	0.57	0.03
	WUE	3.57	0.09	6.42	0.36	9.18	0.42
	C_i/C_a	0.61	0.01	0.57	0.02	0.53	0.02
1	A	2.37	0.35	4.85	1.28	5.01	0.96
	g_s	32.77	4.73	42.19	8.78	27.84	1.95
	E	0.7	0.09	0.85	0.16	0.53	0.1
	WUE	3.33	0.28	5.38	0.77	9.12	1.18
	C_i/C_a	0.62	0.03	0.61	0.05	0.51	0.05
17	A	3	0.56	4.48	0.85	5.62	0.84
	g_s	41.02	8.02	28.41	4.68	30.07	2.51
	E	0.81	0.13	0.65	0.1	0.65	0.06
	WUE	3.7	0.21	6.73	0.94	9.09	1.86
	C_i/C_a	0.6	0.01	0.49	0.06	0.59	0.04
58	A	3.62	0.9	3.94	0.69	9.42	2.87
	g_s	46.91	11.58	29.58	5.08	32.27	4.68
	E	0.92	0.21	0.66	0.1	0.69	0.09
	WUE	3.77	0.26	6.37	1	12.82	2.91
	C_i/C_a	0.59	0.02	0.55	0.05	0.49	0.05
78	A	2.52	0.35	4.29	1.19	5.8	0.72
	g_s	37.32	5.51	25.1	4.56	27.79	3.69
	E	0.77	0.08	0.58	0.11	0.62	0.09
	WUE	3.26	0.3	8.05	2	9.56	0.59
	C_i/C_a	0.64	0.03	0.54	0.05	0.48	0.02
81	A	3.32	0.3	2.98	0.55	4.71	0.66
	g_s	49.13	7.09	28.51	5.94	23.44	3.63
	E	0.96	0.13	0.62	0.12	0.5	0.07
	WUE	3.56	0.18	5.07	0.52	9.79	1.32
	C_i/C_a	0.61	0.02	0.61	0.03	0.55	0.02
86	A	3.42	0.61	3.97	0.68	5.75	0.45
	g_s	48.01	9.93	26.89	6.8	28.45	1.98
	E	0.96	0.17	0.59	0.13	0.61	0.04
	WUE	3.72	0.42	8.87	2.2	9.55	0.91
	C_i/C_a	0.6	0.04	0.68	0.07	0.48	0.04

96	A	2.87	0.46	4.01	0.88	2.95	0.69
	g_s	36.39	4.36	33.54	7.36	17.79	1.84
	E	0.76	0.07	0.72	0.15	0.35	0.06
	WUE	3.69	0.32	5.48	0.61	7.83	1.23
	C_i/C_a	0.6	0.03	0.58	0.04	0.58	0.07
112	A	3.23	0.41	3.32	0.85	3.39	0.73
	g_s	47.76	5.65	27.68	6.67	25.28	2.85
	E	0.98	0.1	0.58	0.12	0.43	0.09
	WUE	3.28	0.21	5.85	0.75	8.32	1.04
	C_i/C_a	0.64	0.02	0.57	0.05	0.52	0.04
113	A	3.16	0.73	3.45	0.76	5.7	1.11
	g_s	49.91	9.88	24.02	4.77	34.63	8.58
	E	1	0.17	0.52	0.1	0.72	0.16
	WUE	3.03	0.46	6.79	0.74	8.47	1.17
	C_i/C_a	0.67	0.04	0.5	0.05	0.54	0.05
121	A	3.03	0.38	4.55	1.09	5.8	0.88
	g_s	36.79	4.74	31.25	5.72	31.87	5.16
	E	0.77	0.08	0.68	0.12	0.69	0.11
	WUE	3.9	0.09	6.01	1	8.55	0.54
	C_i/C_a	0.57	0.01	0.55	0.06	0.53	0.03
124	A	2.83	0.35	4.19	0.81	3.9	1.02
	g_s	33.98	5.42	36.04	5.07	21.76	3.25
	E	0.71	0.09	0.78	0.11	0.47	0.07
	WUE	4.02	0.24	6.03	1.4	7.85	1.01
	C_i/C_a	0.56	0.03	0.58	0.07	0.57	0.05

APPENDIX V

Table A.5. Net CO_2 assimilation (A)(mmol CO_2 m^{-2} s^{-1}), stomatal conductance (g_s)(mmol H_2O m^{-2} s^{-1}), intercellular to surface CO_2 ratio, leaf transpiration rate (mmol H_20 m^{-2} s^{-1}), and water-use efficiency (WUE) (mmol CO_2/H_2O) means and standard deviations for ambientand elevated CO_2 treatments after 90 days of treatment.

Provenance		Ambient CO2		530 ppm CO2		700 ppm CO2	
		Mean	SE	Mean	SE	Mean	SE
Overall	A	2.05	0.15	4.94	0.27	6.62	0.35
	g_s	54.65	2.88	51.83	2.27	54.33	2.2
	E	1.03	0.05	1.04	0.05	1.12	0.04
	WUE	2.06	0.12	5.04	0.28	6.09	0.33
	C_i/C_a	0.85	0.04	0.72	0.02	0.69	0.01
1	A	2.28	0.54	3.65	0.92	7.43	1.36
	g_s	46.97	8.1	69.93	7.83	70.72	7.4
	E	0.91	0.15	1.39	0.17	1.48	0.14
	WUE	2.45	0.28	2.76	0.6	4.91	0.59
	C_i/C_a	0.75	0.03	0.83	0.04	0.73	0.03
17	A	1.7	0.29	6.57	1.49	6.95	0.72
	g_s	42.32	5.16	45.97	4.45	59.67	4.89
	E	0.84	0.11	0.94	0.07	1.29	0.12
	WUE	2.3	0.47	7.19	1.75	5.63	0.82
	C_i/C_a	0.77	0.03	0.64	0.06	0.67	0.04
58	A	2.67	0.42	5.55	0.98	7.72	1.28
	g_s	66.43	8.44	48.35	6.72	45.1	3.87
	E	1.33	0.14	0.95	0.09	0.9	0.05
	WUE	2.1	0.33	5.75	0.72	4.74	0.8
	C_i/C_a	0.77	0.04	0.85	0.16	0.73	0.04
78	A	2.17	0.23	5.97	0.69	7.72	1.28
	g_s	41.52	4.76	47.9	6.5	60.58	7.61
	E	0.82	0.1	0.95	0.12	1.29	0.18
	WUE	2.94	0.58	6.33	0.17	5.98	0.46
	C_i/C_a	0.7	0.02	0.66	0.07	0.67	0.02
81	A	2.13	0.49	4.88	0.49	5.48	0.93
	g_s	58.15	9.85	60.88	5.06	45.5	4.7
	E	1.08	0.17	1.19	0.1	0.98	0.08
	WUE	2.15	0.41	4.17	0.46	6.02	1.44
	C_i/C_a	0.77	0.04	0.7	0.03	0.73	0.03
86	A	1.9	0.38	4.8	0.52	7.05	1.52
	g_s	68.17	6.67	45.27	5.05	57.93	7.32
	E	1.26	0.12	0.89	0.09	1.15	0.11
	WUE	1.55	0.29	5.48	0.28	6.04	1.23
	C_i/C_a	0.96	0.26	0.62	0.02	0.73	0.01

96	A	2.08	0.68	3.82	0.7	6.53	1.14
	g_s	54.82	16.47	45.9	8.15	41.18	8.43
	E	0.98	0.24	0.99	0.24	0.87	0.16
	WUE	2.05	0.33	4.19	0.69	7.99	1.08
	C_i/C_a	.78.	0.03	0.7	0.04	0.6	0.05
112	A	1.15	0.51	4.7	3.11	6.78	3.42
	g_s	53.9	16.38	41.12	17.2	55.18	21.8
	E	1.01	0.29	0.97	0.59	1.1	0.38
	WUE	1.2	0.61	5.07	2.98	6.76	4.9
	C_i/C_a	0.8	0.16	0.78	0.16	0.72	0.06
113	A	1.77	0.46	3.95	0.66	8	1.01
	g_s	46.53	9.88	50.37	9.23	48.18	5.91
	E	0.86	0.17	1.04	0.18	0.99	0.11
	WUE	2.12	0.41	3.92	0.4	8.25	1
	C_i/C_a	0.78	0.05	0.71	0.03	0.65	0.02
121	A	2.82	0.47	4	0.73	6.67	1.31
	g_s	70.6	8.56	58.45	12.15	59.17	8.41
	E	1.24	0.14	1.05	0.16	1.2	0.15
	WUE	2.2	0.22	4.33	0.8	5.36	0.55
	C_i/C_a	0.77	0.02	0.69	0.05	0.67	0.04
124	A	1.9	0.93	6.45	0.53	5.98	1.1
	g_s	51.8	12.83	56.03	4.47	54.42	7.63
	E	0.98	0.23	1.06	0.07	1.09	0.14
	WUE	1.58	0.42	6.3	0.81	5.33	0.8
	C_i/C_a	0.98	0.16	0.74	0.06	0.7	0.02

APPENDIX VI

Table A.6. Seedling shoot mass (g), root mass (g), total mass (g), and shoot to root ratios (means + SE) for ambient and elevated CO_2 treatments after 60 and 90 days of treatment.

Provenance		Ambient CO_2		530 ppm CO_2		700 ppm CO_2	
		Mean	SE	Mean	SE	Mean	SE
Overall	Shoot Mass						
	Day 60	2.45	0.11	2.65	0.11	2.73	0.1
	Day 90	4.73	0.18	5.1	0.2	4.9	0.18
	Root Mass						
	Day 60	0.67	0.04	0.69	0.04	0.76	0.03
	Day 90	1.48	0.07	1.46	0.07	1.36	0.06
	Total Mass						
	Day 60	3.12	0.14	3.34	0.13	3.49	0.12
	Day 90	6.21	0.24	6.56	0.26	6.27	0.23
	Shoot:Root						
	Day 60	4.08	0.19	4.32	0.27	3.77	0.22
	Day 90	3.44	0.15	3.81	0.19	3.76	0.11
1	Shoot Mass						
	Day 60	2.58	0.28	2.62	0.51	2.52	0.47
	Day 90	6.04	0.49	5.55	0.57	5.14	0.46
	Root Mass						
	Day 60	0.62	0.09	0.72	0.16	0.71	0.12
	Day 90	1.65	0.25	1.33	0.16	1.2	0.16
	Total Mass						
	Day 60	3.2	0.35	3.34	0.66	3.23	0.59
	Day 90	7.7	0.71	6.89	0.7	6.33	0.6
	Shoot:Root						
	Day 60	4.35	0.38	3.85	0.36	3.48	0.14
	Day 90	3.9	0.39	4.27	0.35	4.46	0.3
17	Shoot Mass						
	Day 60	1.72	0.28	3.37	0.28	3.15	0.29
	Day 90	4.96	0.33	5.69	0.7	6.21	0.42
	Root Mass						
	Day 60	0.44	0.06	0.81	0.09	0.95	0.14
	Day 90	1.37	0.06	1.7	0.37	1.54	0.16
	Total Mass						
	Day 60	2.16	0.33	4.18	0.33	4.1	0.42
	Day 90	3.63	0.2	3.83	0.46	4.14	0.28
	Shoot:Root						
	Day 60	4.84	0.6	5.48	1.8	3.28	0.05
	Day 90	3.63	0.2	3.83	0.46	4.14	0.28

58	Shoot Mass						
	Day 60	1.74	0.32	2.2	0.31	2.8	0.12
	Day 90	3.54	0.38	5.38	0.39	3.81	0.43
	Root Mass						
	Day 60	0.37	0.05	0.58	0.17	0.85	0.02
	Day 90	1.16	0.19	1.63	0.19	1.18	0.19
	Total Mass						
	Day 60	2.11	0.36	2.77	0.39	3.65	0.14
	Day 90	4.71	0.55	7.01	0.48	4.99	0.56
	Shoot:Root						
	Day 60	4.84	0.6	5.48	1.8	3.28	0.05
	Day 90	3.19	0.25	3.5	0.41	3.48	0.46
78	Shoot Mass						
	Day 60	2.98	0.28	2.44	0.45	2.58	0.18
	Day 90	6.15	0.44	4.95	0.83	5.12	0.51
	Root Mass						
	Day 60	0.77	0.17	0.63	0.15	0.77	0.08
	Day 90	1.78	0.24	1.35	0.24	1.46	0.16
	Total Mass						
	Day 60	3.74	0.42	3.06	0.54	3.34	0.23
	Day 90	7.92	0.67	6.3	1.04	6.58	0.64
	Shoot:Root						
	Day 60	5.31	1.38	4.41	0.69	3.46	0.27
	Day 90	3.62	0.24	3.9	0.53	3.6	0.27
81	Shoot Mass						
	Day 60	2.78	0.31	2.79	0.24	3.36	0.55
	Day 90	4.48	0.32	5.01	0.62	5.74	0.42
	Root Mass						
	Day 60	0.81	0.06	0.85	0.1	0.76	0.06
	Day 90	1.42	0.18	1.46	0.16	1.53	0.28
	Total Mass						
	Day 60	3.6	0.35	3.64	0.33	4.11	0.61
	Day 90	5.91	0.46	6.47	0.74	7.27	0.64
	Shoot:Root						
	Day 60	3.46	0.34	3.43	0.31	4.33	0.37
	Day 90	3.28	0.28	3.53	0.36	4.13	0.54
86	Shoot Mass						
	Day 60	3.34	0.26	2.56	0.23	2.26	0.35
	Day 90	6.4	0.2	6.05	0.78	5.08	0.64
	Root Mass						
	Day 60	0.91	0.09	0.79	0.09	0.63	0.08
	Day 90	1.59	0.07	1.62	0.39	1.28	0.18

	Total Mass						
	Day 60	4.25	0.33	3.34	0.27	2.9	0.42
	Day 90	7.99	0.26	7.67	1.08	6.35	0.79
	Shoot:Root						
	Day 60	3.74	0.22	3.38	0.4	3.63	0.44
	Day 90	4.03	0.08	4.97	1.56	4.09	0.38
96	Shoot Mass						
	Day 60	2.35	0.42	2.32	0.35	2.73	0.35
	Day 90	4.23	0.78	4.29	0.91	5.17	0.64
	Root Mass						
	Day 60	0.72	0.19	0.63	0.09	0.66	0.1
	Day 90	1.71	0.33	1.26	0.27	1.53	0.15
	Total Mass						
	Day 60	3.07	0.6	2.96	0.44	3.39	0.34
	Day 90	5.94	1.06	5.55	1.13	6.69	0.79
	Shoot:Root						
	Day 60	3.57	0.25	3.73	0.16	5.61	2.32
	Day 90	2.64	0.34	3.75	0.49	3.33	0.12
112	Shoot Mass						
	Day 60	3.18	0.33	3.04	0.48	2.65	0.25
	Day 90	4.63	0.47	5.23	0.56	4.49	0.4
	Root Mass						
	Day 60	0.94	0.08	0.71	0.16	0.79	0.07
	Day 90	1.54	0.33	1.44	0.11	1.43	0.15
	Total Mass						
	Day 60	4.12	0.39	3.75	0.63	3.44	0.32
	Day 90	6.17	0.76	6.67	0.56	5.92	0.54
	Shoot:Root						
	Day 60	3.39	0.23	4.87	0.68	3.35	0.07
	Day 90	3.33	0.33	3.74	0.49	3.2	0.18
113	Shoot Mass						
	Day 60	2.36	0.28	2.16	0.19	2.76	0.33
	Day 90	2.9	0.25	4.64	0.77	3.82	0.78
	Root Mass						
	Day 60	0.66	0.1	0.62	0.04	0.85	0.11
	Day 90	1.03	0.17	1.23	0.34	1.26	0.23
	Total Mass						
	Day 60	3.02	0.37	2.77	0.22	3.61	0.4
	Day 90	3.93	0.42	5.87	1.1	5.08	0.96
	Shoot:Root						
	Day 60	3.69	0.2	3.51	0.27	3.37	0.35
	Day 90	3.05	0.3	4.43	0.58	3.24	0.4

121	Shoot Mass						
	Day 60	1.82	0.21	2.54	0.25	2.68	0.28
	Day 90	4.03	0.65	5.16	0.78	4.99	0.81
	Root Mass						
	Day 60	0.47	0.13	0.68	0.08	0.75	0.05
	Day 90	1.39	0.43	1.56	0.16	1.24	0.11
	Total Mass						
	Day 60	2.29	0.33	3.23	0.32	3.43	0.32
	Day 90	5.41	0.96	6.72	0.92	6.23	0.89
	Shoot:Root						
	Day 60	4.69	0.91	3.88	0.43	3.58	0.25
	Day 90	4.44	1.3	3.25	0.3	3.97	0.41
124	Shoot Mass						
	Day 60	2.15	0.35	3.1	0.38	2.57	0.27
	Day 90	4.63	0.43	4.14	0.54	4.41	0.62
	Root Mass						
	Day 60	0.65	0.16	0.61	0.13	0.67	0.07
	Day 90	1.69	0.11	1.52	0.07	1.35	0.34
	Total Mass						
	Day 60	2.79	0.43	3.71	0.4	3.24	0.34
	Day 90	6.32	0.54	5.66	0.58	5.76	0.92
	Shoot:Root						
	Day 60	3.93	0.63	6.66	1.92	3.87	0.05
	Day 90	2.71	0.13	2.72	0.34	3.75	0.46

APPENDIX VII

Table A.7. Seedling height (cm) and root collar diameter (mm) (means ± SE) for ambient and elevated CO_2 treatments after 0, 30, 60, and 90 days of treatment.

Provenance		Ambient CO2		530 ppm CO2		700 ppm CO2	
		Mean	SE	Mean	SE	Mean	SE
Overall	Height (cm)						
	Day 0	13.85	0.27	13.59	0.26	14.06	0.25
	Day 30	17.24	0.31	17.49	0.29	17.78	0.28
	Day 60	22.84	0.43	23.92	0.4	23.93	0.36
	Day 90	28.34	0.63	28.87	0.7	31.1	0.67
	RCD (mm)						
	Day 0	1.8	0.04	2.03	0.05	2.01	0.04
	Day 30	2.52	0.04	2.72	0.05	2.73	0.04
	Day 60	3.55	0.05	3.67	0.06	3.72	0.04
	Day 90	5.02	0.09	5.07	0.1	5.06	0.07
1	Height (cm)						
	Day 0	13.68	0.7	12.09	0.46	14.26	0.45
	Day 30	17.56	0.74	16.09	0.53	16.79	0.93
	Day 60	24.05	1.13	22.97	1.09	23.96	1.53
	Day 90	30.95	1.27	29.12	1.48	30.42	1.8
	RCD (mm)						
	Day 0	1.92	0.12	2.13	0.14	2.08	0.17
	Day 30	2.75	0.11	2.77	0.13	2.77	0.17
	Day 60	3.91	0.14	3.63	0.2	3.73	0.2
	Day 90	5.36	0.22	4.73	0.25	5.19	0.23
17	Height (cm)						
	Day 0	12.23	0.93	12.9	0.46	14.26	0.45
	Day 30	14.62	1.02	17.04	0.68	18.6	0.53
	Day 60	18.56	1.23	23.91	1.13	25.75	0.71
	Day 90	26.18	2.93	31.43	1.62	32.25	1.26
	RCD (mm)						
	Day 0	1.55	0.11	2.23	0.17	2.25	0.14
	Day 30	2.23	0.11	2.93	0.16	3.01	0.12
	Day 60	3.22	0.17	3.88	0.16	4.02	0.13
	Day 90	4.95	0.31	5.27	0.32	5.36	0.08
58	Height (cm)						
	Day 0	10.89	0.44	13.03	0.64	14.18	0.68
	Day 30	13.58	0.41	16.68	0.75	17.87	0.76
	Day 60	18	0.51	22.67	1.01	23.86	1
	Day 90	26.1	1.93	30.55	2.24	29.37	2.1
	RCD (mm)						
	Day 0	1.5	0.09	1.93	0.18	1.88	0.1
	Day 30	2.19	0.1	2.63	0.14	2.61	0.09
	Day 60	3.17	0.13	3.6	0.16	3.59	0.08
	Day 90	4.71	0.11	5.16	0.27	4.72	0.13
78	Height (cm)						
	Day 0	13.4	0.41	12.13	0.59	11.87	0.52
	Day 30	16.42	0.53	15.22	0.87	15.59	0.55
	Day 60	21.43	0.91	20.31	1.44	21.79	0.75
	Day 90	29.92	2.09	26.65	3.31	28.1	1.99

	RCD (mm)						
	Day 0	2.26	0.11	2.36	0.15	2.46	0.13
	Day 30	2.99	0.12	3.03	0.17	3.15	0.13
	Day 60	3.95	0.21	3.87	0.23	4.04	0.14
	Day 90	5.85	0.31	5.03	0.52	5.17	0.28
81	Height (cm)						
	Day 0	15.68	0.77	15.63	0.86	16.28	1.13
	Day 30	19.42	0.82	19.62	0.97	19.7	1.19
	Day 60	25.57	1.05	26.12	1.18	25.32	1.36
	Day 90	30.18	1.03	28.55	1.71	34.05	1.3
	RCD (mm)						
	Day 0	1.73	0.1	1.64	0.13	2	0.17
	Day 30	2.49	0.11	2.39	0.11	2.76	0.14
	Day 60	3.58	0.12	3.47	0.1	3.83	0.14
	Day 90	4.97	0.23	4.8	0.19	5.32	0.21
86	Height (cm)						
	Day 0	15.73	0.64	14.78	0.85	14.4	0.79
	Day 30	20.26	0.66	19.21	0.92	17.88	0.91
	Day 60	27.71	0.84	26.59	1.17	23.6	1.22
	Day 90	32	0.98	31.25	2.16	32.18	2.03
	RCD (mm)						
	Day 0	2.13	0.09	1.98	0.19	2	0.13
	Day 30	2.89	0.08	2.79	0.19	2.65	0.12
	Day 60	3.89	0.11	3.96	0.17	3.83	0.14
	Day 90	5.27	0.22	5.62	0.26	4.99	0.36
96	Height (cm)						
	Day 0	13.08	0.99	11.41	0.98	12.1	0.85
	Day 30	17.13	1.28	15.58	1.12	16.05	0.98
	Day 60	23.82	1.78	22.14	1.63	22.68	1.35
	Day 90	28.33	2.79	25.53	2.58	30.42	2.43
	RCD (mm)						
	Day 0	1.68	0.18	1.65	0.17	1.59	0.1
	Day 30	2.41	0.18	2.34	0.17	2.4	0.1
	Day 60	3.52	0.21	3.29	0.22	3.54	0.18
	Day 90	5.07	0.46	4.65	0.41	5.08	0.3
112	Height (cm)						
	Day 0	16.76	0.77	16.69	0.72	16.28	0.66
	Day 30	20.85	0.92	20.06	0.93	20.32	0.81
	Day 60	27.53	1.21	25.64	1.36	26.95	1.22
	Day 90	30.77	1.65	30.57	2.6	37.28	2.89
	RCD (mm)						
	Day 0	1.98	0.13	2.21	0.11	2.03	0.08
	Day 30	2.71	0.09	2.85	0.12	2.8	0.06
	Day 60	3.73	0.08	3.71	0.18	3.62	0.14
	Day 90	4.98	0.21	5.21	0.23	5.19	0.18
113	Height (cm)						
	Day 0	13.07	0.95	14.47	0.95	14.22	0.79
	Day 30	16.28	1.08	18.48	0.92	17.49	1.04
	Day 60	21.53	1.33	25.38	1.37	22.82	1.43
	Day 90	22.9	3.19	28.4	6.43	28.77	7.26

	RCD (mm)						
	Day 0	1.36	0.11	1.98	0.16	1.81	0.13
	Day 30	2.05	0.12	2.62	0.12	2.5	0.13
	Day 60	3.08	0.14	3.51	0.17	3.45	0.15
	Day 90	4.07	0.74	5.16	0.8	4.54	0.86
121	Height (cm)						
	Day 0	12.93	0.61	13.09	0.98	14.73	0.86
	Day 30	15.47	0.62	17.33	0.98	17.69	0.84
	Day 60	19.72	0.86	24.23	1.35	22.6	0.92
	Day 90	24.8	1.88	27.53	2.69	28.38	1.9
	RCD (mm)						
	Day 0	1.71	0.09	2.24	0.14	1.95	0.11
	Day 30	2.39	0.12	2.96	0.14	2.66	0.09
	Day 60	3.31	0.18	3.91	0.22	3.64	0.14
	Day 90	4.68	0.35	5.26	0.54	4.87	0.13
124	Height (cm)						
	Day 0	14.9	1.02	13.25	0.7	13.75	0.66
	Day 30	18.08	0.98	16.94	0.75	17.57	0.79
	Day 60	23.34	1.01	23.12	1.05	23.91	1.15
	Day 90	29.63	1.96	27.98	2.6	30.93	1.7
	RCD (mm)						
	Day 0	1.96	0.19	1.93	0.14	2.02	0.14
	Day 30	2.66	0.14	2.6	0.12	2.7	0.12
	Day 60	3.66	0.14	3.54	0.13	3.64	0.14
	Day 90	5.34	0.15	4.83	0.28	5.23	0.21