University of Alberta

Response of trembling aspen (*Populus tremuloides* Michaux) and hybrid poplar (*Populus* spp.) to increased CO_2 in the greenhouse

by

Kendall Anne Tupker 

A thesis submitted to the Faculty of Graduate Studies and Research in partial fulfillment of the requirements for the degree of Master of Science

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The undersigned certify that they have read, and recommend to the Faculty of Graduate Studies and Research for acceptance, a thesis entitled Response of trembling aspen (*Populus tremuloides* Michaux) and hybrid poplar (*Populus* spp.) to increased CO_2 in the greenhouse submitted by Kendall Anne Tupker in partial fulfillment of the requirements for the degree of Master of Science in Forest Biology and Management.

Dr. S. Ellen Macdonald

Dr. Barbara Thomas

Dr. Janusz Zwiazek

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Sept 27/01

DEDICATION

I would like to dedicate this thesis to my parents, Krys and Jules Tupker for their love, encouragement and their unwavering support in everything I do. This is also dedicated to Chrenan for his love and his firm belief that I would finish this thesis.

ABSTRACT

I examined growth, morphological and gas exchange responses of thirty-four clones of *Populus tremuloides* Michaux from three regions in Alberta and eight hybrid poplar (*Populus* spp.) clones to ambient (350 ppm) or twice ambient (800 ppm) CO_2 in the greenhouse. All clones were propagated in greenhouse chambers under the two CO_2 levels before the experiment.

Aspen grown under high CO_2 had greater height (14.2%) and caliper growth, larger specific leaf area, and a lower shoot-to-root ratio compared to aspen grown under ambient CO_2. Net assimilation and water use efficiency were also greater by about 50% in aspen grown under elevated CO_2. Hybrid poplar had a similar response to elevated CO_2 with an increase in stem height (8.7%), caliper, net assimilation (45.2%) and water use efficiency (33.5%). Twelve aspen clones, identified by industry as 'superior', were found to have greater height and stem growth, irrespective of CO_2 conditions compared to unselected aspen. No significant differences due to gender (male and female) were found for aspen. There were no significant differences among the three provenances in growth or gas exchange under both CO_2 levels, however, there were high levels of variation among clones within provenance for aspen. The P38 P38 and Green Giant hybrid poplar clones grew the best and Sargentii the poorest under both CO_2 levels.

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gas exchange measurements go more quickly and still be enjoyable.

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NA= net assimilation	$\mu mol\ CO_2\ m^{-2}\ s^{-1}$
WUE= water use efficiency	$\mu mol\ CO_2\ m^{-2}\ s^{-1}/mmol\ H_2O$ transpired
Gs= stomatal conductance to H_20	$mmol\ H_20\ m^{-2}\ s^{-1}$
R= leaf dark respiration	$\mu mol\ CO_2\ m^{-2}\ s^{-1}$

CHAPTER ONE

GENERAL INTRODUCTION

1.1. Exposure to elevated CO_2 in the greenhouse

Forests store 90% of the earth's terrestrial organic carbon and this accumulation of forest biomass is a major regulator of atmospheric CO_2, thus, forest trees represent a potential sink for increasing atmospheric CO_2 (Strain and Bazzaz 1983, Mousseau and Saugier 1992, Bazzaz and Miao 1993, Ceulemans and Mousseau 1994). Forests are composed largely of plants with a C_3 photosynthetic pathway and increased atmospheric CO_2 causes an increase in natural photosynthetic rates of most forest trees (Bazzaz and Miao 1993, Ward and Strain 1999). Since CO_2 is a limiting substrate for photosynthesis in C_3 species, an increase in CO_2 concentration in the atmosphere accelerates the rate of photosynthesis (Mousseau and Saugier 1992). Therefore, there is a possibility that increases in atmospheric CO_2 would be accompanied by increased photosynthetic rates by individual tree species which could buffer rises in atmospheric CO_2.

Carbon dioxide enrichment has long been recognized as increasing horticultural crop yield in greenhouses (Mortensen 1987, Mousseau and Saugier 1992). Elevated atmospheric CO_2 concentrations in the greenhouse have been shown to affect photosynthesis, respiration, growth and development for a variety of plants (Bazzaz 1990, Ceulemans and Mousseau 1994, Curtis and Wang 1998, Ward and Strain 1999). Tree seedlings grown in the greenhouse under high CO_2 levels have exhibited increased growth rates and increased rates of photosynthesis (NA) and water use efficiency (WUE) (Körner 1993, Idso and Idso 1994, Ceulemans and Mousseau 1994). Kimball and Idso (1983), Eamus and Jarvis (1989), Bazzaz (1990) and others have conducted experiments

detailing plant responses to increased CO_2 in the greenhouse. They have shown that elevated atmospheric CO_2 can result in: increased photosynthesis (NA) and water use efficiency (WUE) in C_3 plants; increased biomass yield; increased content of nonstructural carbohydrates; increased dry matter allocation to root biomass; and reduced stomatal conductance and transpiration levels. Use of elevated CO_2 in the greenhouse could allow for modifications in biomass accumulation to produce larger tree seedlings, in a shorter period of time or tree seedlings with a relatively larger root biomass. However, some studies have indicated that higher photosynthetic rates are not maintained over long periods under elevated CO_2 (months and years) (Curtis and Wang 1998, Tjoelker et al. 1998, Tissue et al. 1999, Jach and Ceulemans 1999). After long periods of exposure to elevated CO_2, plants return to photosynthetic rates similar to that found under ambient CO_2 conditions. Therefore, one must be cautious in interpreting short-term responses to elevated CO_2, which may overestimate the increase in carbon accumulation of plants over long term exposure (Ward and Strain 1999).

Elevated CO_2 stimulates biomass production of C_3 plants, such as poplars, which are expected to respond vigorously to increased levels of CO_2 in the greenhouse (Ceulemans et al. 1996). Furthermore, when nutrients and water are not limiting, plants grown under elevated CO_2 have a higher growth response compared to those grown under limiting conditions (Curtis and Wang 1998). Therefore, poplars grown in elevated CO_2 under greenhouse conditions, should show a positive response with an increase in stem biomass.

1.2. Effect of elevated CO_2 on net photosynthetic carbon fixation

Photosynthesis is the main plant process directly affected by increases in

atmospheric CO_2 concentration. However, C_3 and C_4 plant species differ in their response to increased CO_2 because of differences in photosynthetic pathways. Photosynthesis is limited by the concentration of CO_2 at the site of fixation, and therefore, the rate of diffusion of CO_2 through the stomates and into the plant is a critical factor (Barbour et al. 1987). In a photosynthesizing cell CO_2 concentrations at the chloroplast are low relative to CO_2 concentrations in the outside air (340 ppm), so a gradient exists which dictates the rate of CO_2 diffusion (Barbour et al. 1987). Also, in the presence of atmospheric levels of oxygen (21%), the enzyme responsible for CO_2 fixation (RuBP carboxylase/oxygenase) may fix oxygen instead of CO_2 (Barbour et al. 1987). Thus, O_2 and CO_2 compete for the same enzyme, which reduces the amount of carbon fixed.

In the most common plant photosynthetic pathway, C_3, carbon dioxide is first bound to an acceptor molecule ribulose-1,5-bisphosphate (RuBP); this reaction is catalyzed by the enzyme Rubisco (RuBP-carboxylase/oxygenase) to produce two molecules of 3C intermediate phosphoglycerate acid (PGA) (Larcher 1995). Then PGA is reduced to glyceraldehyde-3-phosphate (GAP) which enters a pool of carbohydrates and provides material to produce sugars and starches and to resynthesize ribulose-1,5-bisphosphate (RuBP), the primary acceptor of CO_2 (Long et al. 1996). In the alternate photosynthetic pathway, C_4, CO_2 is bound by the primary acceptor PEP (phosphenol-pyruvate) to form oxaloacetate. Oxaloacetate is then converted into malate and asparate, which are then broken down into pyruvate and CO_2. The CO_2 is captured by RuBP, which concentrates CO_2 at the site of RuBP carboxylase reducing RuBP oxygenase activity (Larcher 1995). This C_4 photosynthetic system acts as a CO_2 pump,

which allows photosynthesis to function efficiently at current atmospheric levels of CO_2 (~350 ppm).

At ambient CO_2 levels, atmospheric carbon dioxide levels limit photosynthesis in plants with a C_3 pathway. In the short term, an increase in CO_2 concentration will cause an increase in net leaf photosynthetic uptake by increasing the rate of carboxylation of Rubisco and decreasing the rate of oxygenation (Brown and Higgenbotham 1986, Ceulemans and Mousseau 1994, Atkinson et al. 1997, Drake and Gonzàlez-Meler 1997, Ward and Strain 1999). When the CO_2 content of air is artificially raised, C_3 plants can bind up to three times as much CO_2 as under ambient CO_2 levels (Larcher 1995). Drake and Gonzàlez-Meler (1997) found that in a survey of 60 experiments, exposure to elevated CO_2 increased photosynthesis 58 % compared with plants grown at ambient CO_2. Another survey of 39 tree species found an average increase in photosynthetic rates of 44% under double CO_2 concentrations (Gunderson and Wullschleger 1994). Wang et al. (2000) also found that for six genotypes of aspen grown at elevated CO_2 (~700-750 ppm), net assimilation was 51 to 40% higher compared to aspen grown under ambient conditions.

Increased levels of CO_2 have also been shown to affect the photosynthetic apparatus. *Desmodium paniculatum* (a C_3 plant) was found to have lower chlorophyll content after growth under increased CO_2 levels, but photosynthetic rates on a per chlorophyll basis were still higher for plants grown under high CO_2 compared to ambient due to an increase in the size or number of photosynthetic components (Wulff and Strain 1982). This increase allows for more photochemical reactions which causes the leaves of plants grown under high CO_2 to be more efficient in capturing or using light in

photosynthesis (Wulff and Strain 1982).

Under high CO_2, the quantum yield (photosynthetic carbon gain per photons absorbed) at low light is enhanced allowing leaves to utilize low light environments more efficiently (Wullschleger et al. 1992, Long and Drake 1991). Also, exposure to high CO_2 conditions, which can increase the size of the electron transport system, increases the amount of photochemical energy the plant is able to absorb (Stitt 1986). Both yellow poplar (*Liriodendron tulipifera*) and white oak (*Quercus alba*), grown under high CO_2, were found to have an increase in open or oxidized PSII (photosystem II) reaction centers available for photosynthesis, which allows for an increase in available light use (Wullschleger et al. 1992). Leaf anatomical changes under high CO_2 may also help to improve the plants' ability to utilize light and consequently increase the photosynthetic potential. Increased leaf thickness, larger mesophyll cells which increases the internal surface area and increased chloroplast density under high CO_2 could ultimately increase the photosynthetic potential of leaves (Radoglou and Jarvis 1990a,b).

1.3. Effect of elevated CO_2 on respiration

Respiration is the process whereby energy captured during photosynthesis is used in growth (synthesis of new biomass) and maintenance (maintenance of existing plant material) (Kimmins 1987). Under increased atmospheric CO_2 levels both an increase and a decrease in leaf dark respiration rates have been found (Amthor 1991, Bunce 1994, Amthor 2000).

Evidence derived from experiments suggests that leaf dark respiration may increase in response to elevated CO_2 concentrations (Poorter et al. 1992). This increase has been attributed to the fact that the resulting increase in plant size, due to an increase

in NA, should in turn stimulate whole plant maintenance respiration. As well, elevated CO_2 often results in an increased carbohydrate supply, which then leads to an increase in the supply of substrate for respiration (Hrubrec et al. 1985, Wullschleger et al. 1994, Gonzàlez-Meler and Siedow 1999, Amthor 2000). Azcón-Bieto et al. (1994) found that when concentrations of sugars, such as fructose and glucose, were high, the supply of substrate to the mitochondria was increased and foliar dark respiration rates increased.

Other studies, however, show an inhibitory effect of elevated CO_2 concentrations on dark respiration for whole plants (Bunce 1990), seedlings (Reuveni and Gale 1985), roots (Reuveni and Gale 1985, Rogers et al. 1994) and leaves (Amthor 1991, Bunce 1990, Bunce 1994, Curtis and Wang 1998), with an average 20% reduction in respiration for a doubling of atmospheric CO_2 (Drake and Gonzàlez-Meler 1997). Reduction in dark respiration may be linked to the inhibition of enzymes (cytochrome c oxidase (Cytox) and succinate dehydrogenase) of the mitochondrial electron transport system (Gonzàlez-Meler and Siedow 1999, Drake and Gonzàlez-Meler 1997) and reduced activity of other enzymes in response to dissolved inorganic carbon (Amthor 1991, Amthor 2000). In a survey of 41 different tree species, including aspen, Curtis and Wang (1998) found that leaf dark respiration decreased significantly under CO_2 enrichment. However, potential effects on respiration remain a puzzling topic and additional experiments are needed to establish the mechanism for changes in respiration due to increased CO_2.

1.4. Effect of elevated CO_2 on stomata and resulting water use efficiency

Most often stomatal aperture, conductance and transpiration per unit leaf area decrease with increasing CO_2 concentrations, which contributes to an increase in water use efficiency (Mott 1990, Rogers et al. 1994). Woodward (1987) found a 40% decrease

in stomatal density in leaves of herbarium specimens over the past 200 years, which is thought to be a response to an increase in atmospheric CO_2. Changes in stomatal size or number or even a change in the sensitivity to atmospheric CO_2 could be an adaptive response to elevated CO_2, since CO_2 diffusion into the leaf will be less of a limitation to photosynthesis as CO_2 concentration rises (Long et al. 1996). Contrary to this, Radoglou and Jarvis (1990a,b) found that for *Populus* spp. stomatal density and pore length remained unaffected by CO_2 enrichment and a decrease in stomatal conductance was due to an effect of increased CO_2 on stomatal openings.

The reduction of stomatal conductance of C_3 plants in response to increased CO_2 levels, results in decreased transpiration rates and increased conservation of water (Ward and Strain 1999). Since photosynthetic rates increase with increasing CO_2 levels, there will also be a corresponding increase in instantaneous water use efficiency (WUE) (ratio of the rate of CO_2 -uptake to rate of water loss) which might lead to increased yields with no additional loss in water consumption (Salisbury and Ross 1992, Ward and Strain 1999). Most studies show an enhancement of WUE, and Field et al. (1995) summarized results from 23 tree species, which showed that transpiration would be lowered by an average of 23%, which, coupled with yield enhancement suggests a doubling of WUE with increasing NA. Morison (1985) also reported an increase in WUE in the range of 60-160%, with a doubling of CO_2. Wang et al. (2000) found decreased stomatal conductance (by almost 50% in the second year of growth) for aspen grown under elevated CO_2 conditions. Increased WUE under elevated CO_2 concentrations may then lead to improved plant growth in regions that experience drought by limiting water losses at the leaf level (Tolley and Strain 1985, Idso and Idso 1994).

1.5. Effect of elevated CO_2 on morphology

Increased levels of atmospheric CO_2 also influence plant growth and morphology. The rate of height growth and branching increase in some tree species grown under increased CO_2 (Eamus and Jarvis 1989, Curtis et al. 1995, Curtis and Wang 1998). Experiments have shown that at elevated CO_2 (650 ppm) the number of branches doubled and total leaf weight increased 79% for sour orange trees (Idso et al. 1991), whereas a 50% increase in total leaf weight was observed for loblolly pine compared to ambient CO_2 (Kozlowski et al. 1991). Ceulemans et al. (1996) found elevated CO_2 increased stem biomass and the number of branches for two *Populus* hybrids (Beaupré (*Populus trichocarpa* Torr. and Gray x *P. deltoides* Bartr. *ex* Marsh) and Robusta (*P. deltoides* Bartr. *ex* Marsh. x *P. nigra* L.)). As well, increased branching is related to increased leaf number, which can increase the photosynthetic rate of each leaf and result in higher total carbon assimilation for the plant.

Elevated CO_2 is typically associated with a decrease in the shoot to root ratio (shoot biomass/ root biomass). Elevated CO_2 allows plants to allocate more biomass to roots to increase the uptake of limiting soil resources, since plants are no longer CO_2 limited aboveground (Rogers et al. 1994, Ward and Strain 1999). An experiment by Rogers et al. (1992) looking at soybean root growth under 350 ppm and 700 ppm, found that roots exhibited a greater growth enhancement than shoots under high CO_2 concentrations which may enable trees to exploit a greater soil volume. Higher CO_2 concentrations have also been shown to increase the production of storage organs such as roots, bulbs, corms and tubers in a variety of species (Bowes 1993, Rogers et al. 1994).

Leaf number, leaf area and leaf weight per plant and leaf thickness increase in

response to increasing levels of carbon dioxide (Eamus and Jarvis 1989, Dippery et al. 1995). Generally under high CO_2 there is an increase in total leaf area due to larger leaves or a greater number of leaves. Individual leaf area tends to increase due to an increase in the number of cells or to a greater rate of cell expansion through changes in the cell wall properties (Ceulemans and Mousseau 1994). The dry weight of the unit leaf area increased under elevated CO_2 due to an increased starch content or additional cell layers, which can lead to a decrease in specific leaf area (SLA) (amount of leaf area per gram of leaf dry weight) (Eamus and Jarvis 1989, Kozlowski and Pallardy 1997). In *Populus* spp. increased total leaf thickness at higher CO_2 concentrations was found to be due to an increase in the spongy parenchyma layer (Radoglou and Jarvis 1990a). As well, increasing CO_2 was also found to increase the size of mesophyll cells and intracellular spaces, which may affect the absorption of CO_2 (Radoglou and Jarvis 1990a,b).

1.6. Acclimation to elevated CO_2 levels

Strong initial photosynthetic responses to experimentally increased CO_2 can be diminished by various negative feedback processes that result from long term exposure to increased CO_2 levels (Körner 1993). This reduction in photosynthetic capacity, referred to as acclimation, is the down-regulation of photosynthetic rates to those found at ambient CO_2 levels (Rey and Jarvis 1998, Tjoelker et al. 1998). In the long term, various biochemical and molecular mechanisms are thought to control the down-regulation of photosynthesis to increased levels of CO_2. This acclimation is characterized by reduced chlorophyll content, reduced Rubisco content and activity, limitations in RuBP and P_i (inorganic phosphorous) regeneration and decreased leaf nitrogen content on a leaf mass

basis (Sage 1994, Wullschleger et al. 1994, Tissue et al. 1995, Ward and Strain 1999). One idea for the down-regulation of photosynthesis is related to an imbalance in source-sink relations, caused by an increase in leaf carbohydrate accumulation (Tissue et al. 1999). Higher photosynthetic rates increase the amount of carbohydrates within the plant under high CO_2 levels, which causes a feedback mechanism that subsequently reduces photosynthetic capacity. The plant may be unable to use all the additional carbohydrate produced under high CO_2, which causes a decrease in source activity (Drake and Gonzàlez-Meler 1997, Tissue et al. 1999). Starch accumulation in the chloroplast is cited as an obvious symptom of the source-sink imbalance (Acock and Allen 1985). In extreme cases of starch accumulation, starch grains may disrupt the grana of the chloroplast. As well, large starch grains have been found to reduce the amount of light able to reach the chloroplast (Acock and Allen 1985). The increased carbon accumulation of carbohydrates can result in feedback inhibition of photosynthesis at a molecular level. Glucose and other sugars are known to suppress the transcription of photosynthetic genes, which cause a decrease in photosynthesis (Drake et al. 1997, Tissue et al. 1999).

Acclimation over the long term has also been linked to below-ground limitations related to inadequate pot size. Downward acclimation of photosynthesis capacity is correlated with a small pot size which causes acclimation of photosynthesis in elevated CO_2 due to space and nutrient limitations (Arp 1991). However, concentration of nutrients, not pot size is a more important regulator of root growth and acclimation (Arp 1991). Trees grown in pots supplemented with nutrients, as well as trees grown in open top chambers show little photosynthetic acclimation (Curtis and Wang 1998, Gunderson

et al. 1993).

1.7. Selection and genetic variation under elevated CO_2

Tree improvement programs determine performance of trees through the assessment of variables such as stem height and volume, biomass accumulation and photosynthesis and WUE rates (Williams et al. 1987, Cantin et al. 1997). Evaluation of genetic superiority through early growth in the greenhouse would allow families or individuals with less desirable genetic traits (eg. slow stem growth) to be removed from a tree improvement program earlier. As well, if future field performance could be predicted from early greenhouse performance, it might shorten the time required for evaluation of families or individuals, with regard to inherently superior growth (Williams et al. 1987). Various studies have reported a correlation between juvenile growth under controlled environment conditions and future field performance (Ceulemans and Impens 1980, Nelson and Ehlers 1984, Williams et al. 1987, Carter et al. 1990, Wu et al. 1997, Cantin et al. 1997). For black spruce (*Picea mariana* (Mill)) individuals, strong correlations were found between early greenhouse growth measurements and field performance at age 13, which indicates that the poorest performing individuals could be identified during juvenile growth in a greenhouse (Williams et al. 1987).

Early selection of superior clones under present conditions is important, however it is also important to gain an understanding of how future field conditions (eg, increased atmospheric CO_2 levels) will affect growth. A species specific response to growth and biomass allocation in response to elevated CO_2 has been demonstrated for a variety of tree species (Brown 1991, Wang et al. 1994, Wang et al. 1995, Thomas 1996, Cantin et al. 1997, Wang et al. 2000). Wang et al. (1994, 1995) found that although 16 different

black spruce families all displayed some positive response to increased CO_2 for a variety of traits (biomass allocation, NA), but the magnitude of response varied among families. This genetic variation of trees in response to increased CO_2 will be important in the selection of superior trees for growth and reforestation under future climatic conditions. A similar phenomenon was found in aspen, where although all six genotypes responded positively to elevated CO_2, there was a difference in the magnitude of response between genotypes (Wang et al. 2000). These genetically mediated differences in growth and photosynthetic ability of individuals and families under high CO_2 conditions, may allow tree improvement programs to select individuals that can take advantage of future CO_2 environments (Ceulemans and Impens 1983, Jelinski 1993, Ceulemans and Isebrands 1996, Thomas 1996). Therefore, in order to understand and predict the possible effect that future CO_2 levels will have on productivity, the economic value of timber species and how selection criteria may change, it is necessary to evaluate the long term impact of elevated CO_2 on a variety of tree species.

1.8. Gender-specific response to elevated CO_2

Male and female individuals of dioecious species often differ physiologically and ecologically from one another. Grant and Mitton (1979) found an ecological difference between the habitats of male and female aspen in the Colorado Rockies where a greater number of female aspen was found at lower elevations, compared to a greater number of male aspen found at higher elevations. Male and female species of arctic willow (*Salix arctica*) and boxelder (*Acer negundo*) have also developed sex-specific physiological traits that allow males and females to occupy different habitats where more females are found on mesic and high nutrient sites compared to males, which are more abundant on

dry sites (Dawson and Bliss 1993, Dawson and Ehleringer 1993, Jones et al. 1999). Although the mechanism is not known, this difference is thought to be due to different reproductive costs, with female trees requiring a larger amount of resources to be allocated for reproduction (Sakai and Burris 1985, Dawson and Ehleringer 1993).

One theory put forward to explain physiological differences between male and female plants is a difference in carbon assimilation, however if this carbon assimilation is differentially affected by CO_2 enrichment, it may alter productivity and population structure under future elevated CO_2 conditions. Jones et al. (1999) found for arctic willow, under ambient CO_2, females had greater NA than males, but under increasing levels of CO_2, male arctic willows had higher NA rates. Wang and Curtis (2001) found that male aspen trees had higher net NA than females, at both ambient and elevated CO_2. This differing response between male and female trees to increased levels of CO_2 should be considered when forecasting the response of male and female individuals to future CO_2 environments.

1.9. The study species: aspen and hybrid poplar

Poplar is the general term for trees in the genus *Populus*, a member of the willow family (Salicaceae). Species of the genus *Populus* are all single-trunked, deciduous, dioecious trees that usually spread clonally through vegetative root sucker shoots and are among the fastest growing native temperate trees (Peterson and Peterson 1992, Eckenwalder 1996). The genus contains approximately 29 different species, not including the large number of hybrids, which include aspens (eg. *P. tremuloides, P. tremula*), balsam poplar (eg. *P. balsamifera*) and cottonwoods (eg. *P. deltoides, P. trichocarpa*) (Peterson and Peterson 1992, Eckenwalder 1996). Trembling aspen

(*Populus tremuloides*) is the most widely distributed tree in North America and is found in the boreal forest. It grows on a variety of soil types and quickly pioneers sites after disturbance through root suckering. Although dioecious, aspen spreads mainly through vegetative reproduction, which can lead to the development of large clones over time. Aspen also has a high degree of genetic diversity among clones for morphology, growth and gas exchange, which allows for the selection of clones with superior growth characteristics (Jelinski and Cheliak 1992, Thomas et al. 1997a,b). Recently aspen has become an economically important species used for lumber and due to its low lignin, and high carbohydrate content, for pulp products (Peterson and Peterson 1992).

In the genus *Populus*, there are high levels of cross-fertility among species (Eckenwalder 1996, Wu and Stettler 1998). Hybrids are produced when plants of different species are cross fertilized, which can occur naturally or through plant breeding techniques (Bisoffi and Gullberg 1996). One of the approaches used in poplar breeding is to hybridize parents from species that differ in several components of productivity and to examine their progenies in the F_1 generation (Wu and Stettler 1998). The resulting hybrid poplar species are planted in a wide range of habitats as high productivity, short-rotation tree crops (Radoglou and Jarvis 1990a) and they have shown a large positive response to short term CO_2 enrichment (Radoglou and Jarvis 1990a,b) under controlled experimental conditions.

Poplars, including both aspen and hybrid poplars, are often planted in forestry operations because of their rapid growth (20-26 year rotation for hybrid poplars, 40-50 year rotation for aspen), high biomass production, ease of propagation and use in a wide variety of wood and fiber products (Ceulemans et al. 1987, Zsuffa et al. 1996, Scarascia-

Munozza et al. 1997). In addition to biomass production, poplars are also used in riparian zone conservation and rehabilitation, for windbreaks, and more recently, for reclamation and phytoremediation of contaminated soils and groundwater (Peterson and Peterson 1992, Zsuffa et al. 1996). Planting poplars as windbreaks (or shelterbelts) protects from loss of soil in farm areas due to wind, and using poplars along riparian zones helps to protect banks from erosion and can help improve water quality (Zsuffa et al. 1996). After disturbance from mining, poplars help in long-term stabilization of the mine site due to their fast growth and large root system; in addition, the trees can eventually be harvested and the reforestation aids in developing wildlife habitat (Zeleznik and Skousen 1996). As well, large clonal differences in growth and biomass allocation allow poplar clones with particular growth patterns to be selected for certain environments or purposes.

1.10. Objectives

The objectives of this study were to:

1. assess the impact of CO_2 enhancement during growth under greenhouse conditions on the morphology, physiology and growth of a variety of genotypes of aspen (*Populus tremuloides* Michaux) and hybrid poplar (*Populus* spp.).
2. determine if growth under high CO_2 in the greenhouse could be used to modify the growth and physiology of individual trees for specific environmental conditions or purposes (eg. reclamation and reforestation).
3. determine whether early growth in the greenhouse indicates if there are any morphological and physiological differences between aspen clones identified as showing superior growth in the field versus randomly selected clones and whether growth under elevated CO_2 influences this.

4. determine whether early growth in the greenhouse indicates if there are any morphological and physiological differences between male and female aspen clones and whether growth under elevated CO_2 influences this.
5. determine whether early growth in the greenhouse indicates if there are any morphological and physiological differences between provenances (Athabasca, Peace River and Ft. McMurray) and whether growth under elevated CO_2 influences this.

CHAPTER TWO

EXPERIMENTAL APPROACH

2.1. Materials

Root collections were made from twelve clones (gender unknown) of phenotypically 'superior' *Populus tremuloides* (hereafter referred to as superior aspen), from the Forest Management Agreement areas of Daishowa-Marubeni International Ltd. (DMI) and Alberta-Pacific Forest Industries Inc. (Al-Pac) during the fall of 1997 and the spring of 1998. Identification of aspen material considered 'superior' was based on a set of characteristics determined by the forest companies (eg. large stem diameter for age, little lateral branching along stem, greater height, free from obvious insect and disease damage). Root material was also collected from twenty-two randomly selected male and female aspen (11 of each gender) identified based on flowering, (hereafter referred to as 'unselected') from areas around Peace River (56-57°N, 118-117°W in Northwestern Alberta), Fort McMurray (56-57° N, 112-111°E in Northeastern Alberta) and Athabasca (55-54°N, 112-113°E in North-central Alberta) (see Table 2-1). A distance of at least 1 km was maintained between each aspen clone collected to avoid selecting the same clone twice.

Six different hybrid poplar clones (in the form of 30 cm stem cuttings) were provided by the Prairie Farm Rehabilitation Administration (PFRA) Shelterbelt Center (Indian Head, Saskatchewan) (Walker, Assiniboine, Manitou, Sargentii, Northwest and CanAm). Syncrude Industries and Al-Pac provided two additional poplar clones (P38 P38 and Green Giant) (see Table 2-1).

2.2. Collection and Propagation

2.2.1. Aspen

Aspen clones were collected in fall 1997 and spring 1998. Gender was determined on the unselected aspen during flowering (in March/April) through the examination of flowering catkins. To obtain a large number of cuttings per clone, approximately 25-32 lateral root sections were collected from each tree. Each root section was 20-30 cm long, with a diameter ranging from 0.3-2.0 cm. Once collected, the root sections were placed into plastic bags and kept cool during transport until storage. Before storage, the root material was thoroughly cleaned in water. The roots were then placed in a perforated plastic bag to prevent either drying out or excessive moisture collecting and stored at 2°C.

Material collected in the fall and the spring were dusted with Wilson Bulb and Soil Dust (Carbaryl 5%, Captan 5% Wilson Laboratories Inc., Dundas ON) to prevent any mildew or fungus formation during storage. These roots were stored in paper bags and placed in plastic containers at 2 °C. Roots were monitored weekly for excess moisture.

In June 1998, half of the root sections from each clone were placed in a greenhouse at ambient CO_2 levels (~350-400 ppm) and the other half were placed in another greenhouse maintained at elevated CO_2 levels by CO_2 gas cylinders (~800-850ppm). Each room received natural light, supplemented by sodium and mercury vapour (400 watts) lights, with a constant 16 hour photoperiod during the propagation phase of the experiment. Light levels, on a clear day, with shade screens down, ranged from 600 to 900 μmol photons m^{-2} s^{-1}. Temperature and humidity were monitored in both greenhouses, but not controlled. Also, shade cloth and ceiling fans were used to

control the temperature inside the rooms and to facilitate air circulation. Roots (approximately 5-7 root sections/ tray) were planted into trays half filled with horticultural grade vermiculite, then covered with additional vermiculite. Once covered, the trays were treated with a fungicide drench (Benlate, 1 g/ liter, DuPont Agricultural Products) to prevent damping off of new suckers. The trays (approximately 1 tray/ clone) were placed on benches within each room (24 trays/ room) and randomly moved three times during the propagation phase of the experiment. Once sprouting commenced, cuttings were taken over a three month period (from June 22- August 16, 1998). Suckers of approximately 4 cm in height were cut off, dipped into Wilson Stim Root #2 (semi-hardwood) Rooting Powder (0.4% indole-3-butyric acid, Plant Products Co. Ltd., Brampton, ON) and placed in a Spencer-Lemaire tray (4 cells per row, 20 cm deep) filled with Benlate treated Metromix (Metromix coarse 290, Terra-Lite 2000, Grace Horticultural Products, W.R. Grace & Co. of Canada Ltd.) with a 1 cm layer of sand on top (Masterfeeds, Edmonton, grit #2). If necessary, each cutting had some leaves removed to reduce transpiration. Between each cutting, the scalpel used was sterilized in a 10% bleach solution and rinsed thoroughly in distilled water to minimize the spread of disease. Spencer-Lemaire trays were placed in a modified misting bench made from PVC piping and polyethylene plastic to maintain high humidity and controlled light. Foliar 20-20-20 (5 g/ 4 L) fertilizer was applied approximately six times during the rooting phase (from June-October 1998). Once the sucker cuttings had been in the misting tent for approximately two weeks, they were placed back on the greenhouse bench under natural light levels.

Aspen suckers in high CO_2 showed reduced rooting ability compared to the

ambient rooms. Therefore, two weeks prior to all aspen material entering dormancy the level of carbon dioxide in the high CO_2 greenhouse was reduced to ambient levels to promote rooting.

The stecklings (rooted cuttings) remained in the greenhouse until the first week of September when they were placed outside to induce bud set, senescence and dormancy. They were covered by a shade screen for the first week, to prevent shock due to exposure to full sunlight.

2.2.2. Poplar

Poplar stem material was obtained in March 1998. Long stem pieces of each clone were then cut into 15-20 cm sections (with at least three buds per section) and placed in perforated plastic bags (approximately 30 stem sections per bag) containing moistened Metromix soil. All the bags were then placed in plastic containers and stored in the cold room at 2°C. All poplar material was planted into Metromix filled Spencer-Lemaire trays in July of 1998.

Prior to planting, whips were soaked in water with a 1% liquid root stimulate (Wilson Root Stimulator #2, 0.4% indole-3-butyric acid, Plant Products Co. Ltd., Brampton, ON) for approximately one week and the water was changed daily. Whips were planted in Spencer -Lemaire trays (four cells per row, 20 cm deep) containing Metromix and a 50:50 mixture of 14-14-14 fertilizer (Nutricoate slow release) and Super Phosphate (0-20-0). Before planting, the whips were dipped in a root hormone (Wilson Stim Root #2), and then pushed firmly into the soil, leaving a single bud above the soil surface. A 1 cm layer of sand (Masterfeeds, Edmonton, grit #2) was placed on top. Once planted, the whips were randomly divided between the two greenhouse

environments, with equal numbers of each hybrid poplar in each greenhouse and maintained on the open bench to promote rooting. Additional micro- and macro-nutrients were added 2-3 times during the growing period by foliar drench (5 g 20-8-20/ 2.5 g Fe/ 1 L water).

The hybrid poplar whips were allowed to grow in the Spencer-Lemaire trays in each greenhouse for four weeks before being placed outside (by August 15, 1998) under shade screens for the first week, until October to induce bud set, senescence and dormancy, along with the aspen material. Two weeks prior to cold storage, the trees and stecklings underwent slight water stress to help induce dormancy.

Once both the aspen stecklings and rooted poplar whips became dormant (by October 1998), they were placed into walk-in cold rooms at 2°C with a three hour photoperiod and watered weekly for six weeks. All plant material was placed in cold storage to allow both whips and stecklings to reach a common physiological condition and to fulfill any chilling requirements before starting the measurement phase of the experiment. After the period of cold storage, the material was repotted in preparation for the greenhouse experiment. All of the aspen material was placed into 12 cm plastic pots (Kord Standard 5") and all of the poplar material was repotted into 1 litre plastic pots (Listo Pots, Vancouver, B.C.). Metromix (coarse 290) and Nutricoate slow release fertilizer (14-14-14) (Chisso-Asahi Fertilizer Co. Ltd., Tokyo, Japan) was added to all the pots (6.6 g Nutricoate/ 12 cm pot, 10.6 g Nutricoate/1 L pot) at the time of repotting. As well, all pots where topped with 1 cm of sand to reduce water loss. After 30 days, a supplemental foliar fertilizer (1 g/ L MgSO4, 0.05 g/ L Fe, 0.5 g/ L 20-8-20) was applied

with a power sprayer (Dosmatic Fertilizer Injector, The Plus, Carrollton, TX) every two weeks until harvest.

Biological control, in the form of pirate bugs and *Amblyseius cucumeris*, was used to control a small thrip population. In addition, both Insecticidal soap (Safer's Soap, 50.5% Potassium Salts/ Fatty Acids, 30ml/ 1L water) and Ortho dormant oil (Chevron, 97% mineral oil) were used to control aphids. Dormant oil was applied to the foliage (in all rooms) using a power sprayer (Dramm Portable Electric Power Sprayer, Model CS-5AGS, Manitowoc, WI). All of these methods were employed from one to three times throughout the duration of this experiment.

2.3. Experiment

During the rooting phase, all clonal material was randomly divided equally between the two treatment environments, high CO_2 (~800-850 ppm) and ambient CO_2 (~350-400 ppm). After the dormancy period all material that was initially suckered under high CO_2 was then randomly divided into two greenhouse chambers under high CO_2 conditions. All material that was initially suckered under ambient CO_2 conditions was also randomly divided into two greenhouse chambers under ambient CO_2 conditions. A General Purpose Infrared Analyzer (Model #865, Beckman Instrument Inc. USA) monitored elevated CO_2 levels in the greenhouse. When CO_2 levels fell beneath 800 ppm, CO_2 was then added from gas cylinders containing pure CO_2 to maintain elevated CO_2 levels in both greenhouses. Artificial lights (sodium and magnesium vapour, 400 watts) were used to extend the natural photoperiod. In addition, the photoperiod was adjusted every two weeks to simulate the changing spring conditions (see Table 2-2). Shade screens and fans were used in each room to moderate daytime temperatures.

Light levels in the greenhouses on a clear day, with shade screens down, ranged from 600 to 900 μmol photons m^{-2} s^{-1}. Temperature, humidity and ambient CO_2 levels were monitored throughout the experiment, but not controlled in each greenhouse. Pots were placed randomly within the greenhouse rooms and re-randomized three times (after each measurement date) (Lee and Rawlings 1982). The greenhouse experiment ran for approximately 14 weeks starting in mid January 1999 with photoperiod conditions set to coincide with spring conditions from April to August based on average photoperiod between Calgary (south) and Meander River (north) (see Table 2-2).

Each greenhouse chamber had three replicates from each clone (3 replicates per clone/ greenhouse chamber/ treatment). Height (cm) was measured four times (Day 0, 39, 60 and 95) and caliper (stem diameter at the root collar) was measured at the start and end of the experiment (Day 0 and Day 95). Due to time constraints and equipment problems, only replicates 1 and 2 were measured at Day 60 for all morphological and gas exchange variables. Bud burst was evaluated at week 1 and week 2 of growth in order to capture the time period when approximately half of the plants had started to flush. Bud burst was evaluated using a quantitative scale of 0-5, which ranged from no development to full flush (see Table 2-3). The shape of preformed leaves was measured approximately four weeks into the greenhouse experiment after full flush of all clones. Leaf shape was measured on two leaves of every plant (replicates 1-3); width was measured at the widest part of the leaf, and length was measured on the diagonal from the middle of the lobe to the tip of the leaf. Leaf shape was then calculated by dividing leaf length by leaf width with an average for each replicate being calculated (the two leaves on each plant were averaged) (Thomas 1996).

Gas exchange was measured three times during the experiment (Day 39, 60 and 95) (see Table 2-4). At Day 60, only replicates 1 and 2 in all greenhouse chambers were measured. Plants were removed from the greenhouses for measurement purposes to maintain CO_2 levels in the greenhouse. To prevent any diurnal biases in gas exchange measures, plants from greenhouses at ambient CO_2 were alternated with those from high CO_2 greenhouses during each set of gas exchange measurements. An infra red gas analyzer (LCA-3 Analytical Development Corp., Hoddesdon, U.K.) equipped with a broad leaf cuvette (PCL(B)) was used to measure gas exchange. Gas exchange of the plants was measured at the CO_2 level they were grown in and to maintain desired gas levels, pure tanks of the prescribed CO_2 level (800 ppm and 350 ppm) during measurements. A slide projector with a halogen bulb provided saturating light levels (approximately 1200 μmol photons m^{-2} s^{-1}). Leaves usually filled the entire cuvette (6.25 cm^2), however, any smaller leaves used for gas exchange were measured for leaf area separately (Li-Cor 3100, Lambda Instruments Corporation, Lincoln Nebraska). Gas exchange was measured on the first fully expanded leaf counting down from the top of the plant. Typically, leaf 5-8 on the terminal shoot was used (Thomas 1996). Leaf dark respiration rates (μmol CO_2 m^{-2} s^{-1}) were obtained by placing an envelope over the same leaf used for photosynthesis measurement, and leaving the leaf covered for approximately 1-2 hours. Leaf dark respiration rates on Days 60 and 95 were measured on only replicates 1 and 2 in all greenhouse chambers due to time constraints. Net CO_2 exchange was then measured in the dark in the same way as photosynthesis. Net assimilation of CO_2 (NA, μmol of CO_2 m^{-2} s^{-1}), stomatal conductance to water vapour (Gs, mmol m^{-2} s^{-1}) and water use efficiency (WUE, μmol CO_2 absorbed/ mmol H_2O transpired) were

calculated using standard equations (von Caemmerer and Farquhar 1981).

At the end of the experiment, replicates 1-3 of each clone in each greenhouse chamber were destructively harvested and the leaves, stems and roots were removed and placed in paper bags. However, in order to have sufficient number of plants for outplanting, not all replicates of each clone were destructively harvested. Leaf shape was measured on indeterminate shoots at harvest and total specific leaf area was calculated by dividing the total leaf area (cm^2) by the dry weight of the leaves (g) (Thomas 1996). Roots were washed and then placed in paper bags. All material was dried at 68°C to a constant weight and weighed to the nearest third decimal (g) on a Mettler (AJ150) digital scale. Shoot to root ratios were then calculated based on dry weights (g) (Thomas 1996). The remaining plants were placed back into cold storage prior to outplanting.

After the greenhouse experiment, those plants designated for outplanting were placed in a cold room to induce dormancy and outplanted after approximately six weeks. These trees will be monitored in the field for a further five years.

Due to poor suckering of certain aspen clones (6 superior and 3 unselected aspen clones) under high CO_2, clones with excess suckers grown under ambient CO_2 were moved and subsequently grown under high CO_2 conditions. These plants were not included in the analysis of the main experiment, but were analyzed separately to determine any CO_2 pre-treatment effect. This allowed for analysis of high CO_2 and ambient CO_2 pre-treatment affect on later performance for growth, morphological and gas exchange variables.

2.4. Statistical model and Data Analysis

2.4.1. Aspen

Analysis of variance using SAS (version 8.0) (Littell et al. 1996) PROC MIXED was used to test for mean differences in performance of trembling aspen as related to provenance, sex, treatment, phenotype (superior versus unselected) and genotype (clone). A mixed model is a generalization of the standard linear model used in GLM procedure, the generalization being that data can be analyzed with several sources of variation instead of just one (Littell et al. 1996). PROC MIXED obtains a (restricted) maximum likelihood estimated (REML) of the variance components. A p value less than 0.05 was used to determine significance in the ANOVA.

Data from superior and unselected aspen material suckered under both high and ambient CO_2 and subsequently grown under high CO_2 only were analyzed for differences due to pre-treatment according to the following model:

[1] $Y_{ijk} = \mu + O_i + Cl_j + O_iCl_j + e_{k(ij)}$

where Y_{ijk} is an observation on the kth tree from the jth clone of the ith pre-treatment, μ is the overall mean, O_i is the effect of the ith pre-treatment (i=1,2), Cl_j is the effect of the jth clone (j= 1,..,9), O_iCl_j is the interaction of the ith pre-treatment and the jth clone, and $e_{k(ij)}$ is the random (residual) error. Pre-treatment was considered to be a fixed effect while all other terms were considered random.

Data collected from all 34 aspen clones was analyzed for differences due to phenotype ('superior' versus 'unselected' for all three provenances), were examined using the follow model:

[2] $Y_{ijklm} = \mu + T_i + G_{j(i)} + F_k + Cl_{l(k)} + T_iF_k + G_{j(i)}F_k + T_iCl_{l(k)} + G_{j(i)}Cl_{l(k)} + e_{m(ijkl)}$

where Y_{ijklm} is an observation on the mth tree from the lth clone in the kth phenotype in the jth greenhouse of the ith treatment, μ is the overall mean, T_i is the effect of the ith treatment (i=1,2), $G_{j(i)}$ is the effect due to the jth greenhouse nested in the ith treatment (j=1,2), F_k is the effect of the kth phenotype (k=1,2), $Cl_{l(k)}$ is the effect of the lth clone nested in the kth phenotype (l=1,..,14), T_iF_k is the interaction of the ith treatment and the kth phenotype, $G_{j(i)}F_k$ is the interaction of the jth greenhouse nested in the ith treatment and the kth phenotype, $T_iCl_{l(k)}$ is the interaction of the ith treatment and the lth clone nested in the kth phenotype, $G_{j(i)}Cl_{l(k)}$ is the interaction of the jth greenhouse nested in the ith treatment and the lth clone nested in the kth phenotype and $e_{m(ijkl)}$ is the random error. Treatment and phenotype were considered fixed effects while all other terms were considered random effects.

Data from all unselected aspen (from all three provenances) were analyzed for differences due to sex using the following model:

[3] $Y_{ijklm} = \mu + T_i + G_{j(i)} + S_k + Cl_{l(k)} + T_iS_k + G_{j(i)}S_k + T_iCl_{l(k)} + G_{j(i)}Cl_{l(k)} + e_{m(ijkl)}$

where Y_{ijklm} is an observation on the mth tree from the lth clone in the kth sex in the jth greenhouse of the ith treatment, μ is the overall mean, T_i is the effect of the ith treatment (i=1,2), $G_{j(i)}$ is the effect due to the jth greenhouse nested in the ith treatment (j=1,2), S_k is the effect of the kth sex (k=1,2), $Cl_{l(k)}$ is the effect of the lth clone nested in the kth sex (l=1,..,8), T_iS_k is the interaction of the ith treatment and the kth sex, $G_{j(i)}S_k$ is the interaction of the jth greenhouse nested in the ith treatment and the kth sex, $T_iCl_{l(k)}$ is the interaction of the ith treatment and the lth clone nested in the kth sex, $G_{j(i)}Cl_{l(k)}$ is the interaction of the jth greenhouse nested in the ith treatment and the lth clone nested in the kth sex and $e_{m(ijkl)}$ is the random error. Treatment and sex were considered to be fixed

effects while all other terms were considered random effects.

Data from all unselected aspen (from all three provenances) was analyzed for differences due to provenance using analysis of variance according to the following model:

[4] $Y_{ijklm} = \mu + T_i + G_{j(i)} + P_k + Cl_{l(k)} + T_iP_k + G_{j(i)}P_k + T_iCl_{l(k)} + G_{j(i)}Cl_{l(k)} + e_{m(ijkl)}$

where Y_{ijklm} is an observation on the mth tree from the lth clone in the kth provenance in the jth greenhouse of the ith treatment, μ is the overall mean, T_i is the effect of the ith treatment (i=1,2), $G_{j(i)}$ is the effect due to the jth greenhouse nested in the ith treatment (j=1,2), P_k is the effect of the kth provenance (k=1, , 3), $Cl_{l(k)}$ is the effect of the lth clone nested in the kth provenance (l=1,..,8), T_iP_k is the interaction of the ith treatment and the kth provenance, $G_{j(i)}P_k$ is the interaction of the jth greenhouse nested in the ith treatment and the kth provenance, $T_iCl_{l(k)}$ is the interaction of the ith treatment and the lth clone nested in the kth provenance, $G_{j(i)}Cl_{l(k)}$ is the interaction of the jth greenhouse chamber nested in the ith treatment and the lth clone nested in the kth provenance and $e_{m(ijkl)}$ is the random (residual) error. Treatment and provenance were considered to be a fixed effect while all other terms were considered random.

2.4.2. Poplar

The poplar data were analyzed separately. Provenance and gender were not considered in this analysis. For treatment effects on poplars the following model was used:

[5] $Y_{ijkl} = \mu + T_i + G_{j(i)} + Cl_k + T_iCl_k + G_{j(i)}Cl_k + e_{l(ijk)}$

where Y_{ijkl} is an observation on the lth tree from the kth clone in the jth greenhouse of the ith treatment, μ is the overall mean, T_i is the effect of the ith treatment

(i=1,2), $G_{j(i)}$ is the effect due to the jth greenhouse in the ith treatment (j=1,2), Cl_k is the effect of the kth clone (k=1,..,8), T_iCl_k is the interaction of the ith treatment and the lth clone, $G_{j(i)}Cl_k$ is the interaction of the jth greenhouse nested in the ith treatment and the kth clone and $e_{l(ijk)}$ is the random error. Treatment and clone were considered fixed effects while all other terms were considered random effects.

Table 2-1. a) Summary of the location and the total number of aspen clones used in the experiment. b) Summary of poplar clones used and parentage.

a)

	Provenance Collection (Region)		
	Peace River 56-57°N,112-111°W	**Athabasca 55-54°N,112-113°W**	**Ft. McMurray 56-57°N,112-111°W**
Male Unselected	4	4	3
Female Unselected	4	4	3
Superior	6	6	N/A

b)

Hybrid Poplar	
Assiniboine *O.P *P. deltoides* cv. Assiniboine	**P 38 P 38** *P. balsamifera x P. simonii* c.v. P38 P38
CanAm O.P. *P. deltoids* c.v. CanAm	**Walker** O.P. *P. deltoides* x (*P. laurifolia x P. nigra*) c.v. Walker
Green Giant O.P. *P. deltoids* c.v. Green Giant	**Sargentii** *P. deltoides* c.v. Sargentii
Manitou O. P. *P. deltoids* c.v. Walker c.v. Manitou	**Northwest** *P. deltoides* x *P. balsamifera* = *P. jackii* c.v. Northwest

*O.P.= Open Pollinated *P. deltoides*

Table 2-2. Photoperiod programming for the greenhouse using Calgary (south) and Meander River (North) average* daylight hours (Jan 15- Apr 29, 1999).

Week	Period of Supplemental light
Week 1-2 (Jan 15- Jan 29)	15 hr 50 min
Week 3-4 (Jan 30- Feb 13)	16 hr 40 min
Week 5-6 (Feb 14- Feb 28)	17 hr 14 min
Week 7-8 (Mar 1- Mar 15)	17 hr 27 min
Week 9-10 (Mar 16- Mar 30)	17 hr 15 min
Week 11-12 (Mar 31- Apr 14)	16 hr 42 min
Week 13-14 (Apr 15- Apr 29)	16 hr 42 min

* average daylight based on adding daylight hours (spring conditions) from Calgary and Meander River and averaging the two

Table 2-3. Quantitative scale used to assess bud burst development.

0 = no bud burst
1 = buds swelling, some green visible
2 = lateral buds open
3 = leaves slightly emerged from terminal
4 = leaves emerged, but erect
5 = leafed out (buds fully flushed)

Table 2-4. a) Summary of phenotype and number of clones used in each analysis of variance. b) Numbers of replicates/ clone/ greenhouse chamber/ treatment (R) used at each measurement date.

a)

Effect Tested in ANOVA	Number of Clones	Clones Used
ASPEN		
Pre-Treatment	n=9	superior, unselected
Effect of CO_2	n=34	superior, unselected
Phenotype	n=34	superior, unselected
Sex	n=22	unselected only
Provenance	n=22	unselected only
HYBRID POPLAR		
Effect of CO_2	n=8	hybrid poplar
Clone	n=8	hybrid poplar

b)

Morphological Variables (Height, Caliper, Destructive Harvesting)				Gas Exchange (Gs, NA, WUE)			Respiration		
Day0	**Day39**	**Day60**	**Day95**	**Day39**	**Day60**	**Day95**	**Day39**	**Day60**	**Day95**
R 1-3	R 1-3	R 1-2	R 1-3	R 1-3	R 1-2	R 1-3	N/A	R 1-2	R 1-2

CHAPTER THREE

RESULTS

3.1. Greenhouse Chambers

For the experiment, four greenhouse chambers were used with two at high CO_2 and two at ambient CO_2. Carbon dioxide levels in all four greenhouse chambers were recorded at various times during the experiment (n=20) (Figure 3-3). Mean CO_2 levels in greenhouse chambers 1 and 2 (high CO_2) were 868.3±8.6 ppm and 837.5±12.5 ppm. Mean ambient CO_2 levels in greenhouse chambers 3 and 4 were 372.8±3.1 ppm and 368.3±4.7 ppm respectively. Both temperature and humidity were also recorded for each of the four greenhouse chambers over the course of the experiment (Figure 3-1, 3-2). Chamber 1 (High CO_2) had a mean minimum temperature of 17.0± 0.2°C and a mean maximum temperature of 29.8±0.4°C. Chamber 2 (High CO_2) had similar minimum and maximum temperatures (17.7±0.3°C and 30.2±0.5°C respectively). The two greenhouse chambers used for ambient CO_2 levels had similar maximum temperatures as did the two greenhouse chambers used for high CO_2, but slightly lower minimum temperatures. Chamber 3 (Ambient CO_2) had mean minimum temperatures of 14.5±0.4 °C and mean maximum temperatures of 28.8±0.5°C. Chamber 4 (Ambient CO_2) had minimum and maximum temperatures of 15.8±0.3°C and 29.1±0.5°C respectively.

The difference in mean humidity values for all four greenhouse chambers was relatively small. For chambers 1 and 2 (High CO_2) the mean minimum and maximum humidity values were 31.6±1.1%RH and 78.9±1.7%RH, and 32.1±0.9%RH and 83.6±1.7 %RH respectively. Greenhouse chamber 3 (Ambient CO_2) had a mean minimum and maximum humidity of 27.9±0.8 %RH and 76.7±3.1 %RH. Greenhouse chamber 4

(Ambient CO_2) had a mean minimum and maximum humidity of 29.7±1.4 %RH and 77.6±1.7%RH.

3.2. Aspen

3.2.1. Pre-treatment (high and ambient CO_2) effects on subsequent growth under high CO_2

Propagation of aspen under increased levels of CO_2 and ambient levels of CO_2 and subsequently all grown under high CO_2 had no significant effect on morphological variables except for specific leaf area (SLA) after 95 days of growth under elevated CO_2 (Table 3-1a). A significant effect for CO_2 treatment (high and ambient CO_2) during propagation and subsequent growth under high CO_2 was found on gas exchange variables at Day 39 and 60 (Table 3-1b). At Day 39, both stomatal conductance (Gs) and water use efficiency (WUE) showed a significant effect for pre-treatment. However, by Day 60, there was only a significant effect on WUE.

There was no significant difference in height between aspen propagated in high versus ambient CO_2 and subsequently grown under high CO_2 (Figure 3-4). Aspen propagated under ambient CO_2 appeared to have a slightly higher mean growth rate compared to aspen propagated under high CO_2 from Day 39 to 60 (Figure 3-4) but by Day 95, aspen from high CO_2 was slightly taller (13%). Specific leaf area (SLA) on Day 95 of the greenhouse experiment was found to be 20 % lower for plants propagated under high CO_2 compared to ambient CO_2 (Figure 3-5).

Aspen propagated under high CO_2 had lower Gs and higher rates of NA and WUE for both Day 39 and Day 60, compared to aspen propagated under ambient CO_2 (Figure 3-6). By Day 95, Gs and WUE rates were similar for both the two pre-treatments (high versus ambient CO_2), however, NA (11%) and WUE (9%) rates for

aspen propagated under high CO_2 were higher compared to aspen propagated under ambient CO_2.

3.2.2. Effects of CO_2 treatment on growth and physiology of aspen

To determine the effect of CO_2 on the morphology and physiology of aspen the analysis of variance (ANOVA) testing for the effect of phenotype were used (Table 2-4). Therefore, this analysis included all 34 aspen clones (superior and unselected) used in the experiment (Table 3-2a). In the statistical analysis, low power for treatment due to having only two replications of each CO_2 treatment (degree of freedom= 2) may have had an effect upon the test results. A significant effect of CO_2 treatment was found for the following morphological variables: height, caliper, root dry weight and shoot-to-root ratios.

Aspen grown under high CO_2 had a significantly greater mean height at both Day 39 and 60 compared to aspen grown under ambient CO_2 (Figure 3-7). By Day 95 mean height of aspen grown in high CO_2 was 14.2% greater than those in ambient CO_2, although this difference was not statistically significant. Initial caliper was similar for both treatments (high and ambient CO_2), however, the final caliper of aspen grown under high CO_2 was significantly larger (16.3%) than that of ambient CO_2 (Figure 3-8).

Growth under high CO_2 was found to have an effect on only two gas exchange variables, NA and WUE (Table 3-2b). Aspen grown under high CO_2 had significantly greater NA rates throughout the experiment (Day 39, 60 and 95) compared to aspen grown under ambient CO_2 (Figure 3-9). Likewise, WUE was significantly greater on both Days 39 and 95 for aspen grown under high CO_2 (Figure 3-9).

Aspen grown under high CO_2 had a greater total leaf dry weight, total stem dry

weight and significantly higher root dry weight compared to aspen grown under ambient CO_2 (Figure 3-10). Aspen grown under high CO_2 conditions had greater leaf and stem dry weights, although not significant, and a significantly greater root dry weight and lower shoot-to-root ratio (Figure 3-10).

3.2.3. Variation in growth and physiology of superior and unselected aspen under high and ambient CO_2

No significant phenotype or phenotype by treatment interactions were found for either morphological or physiological variables, except for bud burst at Day 7 (Table 3-2a,b). Superior aspen broke bud earlier compared to unselected aspen by Day 7 under both CO_2 treatments (Figure 3-11). Bud burst measurements were also taken at Day 14, however, all the clones had fully opened leaves in both CO_2 treatments.

Superior aspen also showed a consistently greater, although not significant, mean height throughout the experiment under both ambient and high CO_2 treatments (Figure 3-12). By Day 95, superior aspen was taller than unselected aspen by 13.3% and 10.8% under high and ambient CO_2 treatments, respectively. Superior aspen also had a larger (11.4%) final caliper (Figure 3-13) as well as greater (17.5%) total leaf area compared to unselected aspen (Figure 3-14) (not significant).

3.2.4. Variation in growth and physiology of female and male aspen under high and ambient CO_2 conditions

There were no significant effects of gender or gender by treatment interactions on any morphological or gas exchange traits (Table 3-3a,b). Despite the lack of statistical significance, at the end of the experiment, male aspen were 8.7% taller than female aspen under ambient CO_2 conditions, while under high CO_2 conditions females were 8.3% taller than males (Figure 3-15). Under ambient CO_2 conditions, both male and female aspen

had similar final caliper measurements. However, under high CO_2 female aspen had a larger final caliper (7.5%) than male aspen (Figure 3-16).

3.2.5. Variation among provenances (Peace River, Athabasca, Ft McMurray) and clones in growth and physiology of aspen under high and ambient CO_2 conditions

There were no significant provenance or treatment by provenance interactions for any morphological or physiological variables (Table 3-4a,b). Under both high and ambient CO_2 conditions, clones from Athabasca were consistently taller, while those from Ft. McMurray were the smallest (Figure 3-17). There was more genetic variation at the level of clone within provenance. Clone within provenance (Athabasca, Peace River and Fort McMurray) was significant for both pre-formed leaf shape, leaf shape of mature leaves, and total leaf area at Day 95 (Figures 3-18 and 3-19). Significant genetic variation at the clone within provenance level was also found for Gs at Day 39 and 95 (Figure 3-20).

3.3. Hybrid Poplar

3.3.1. Effect of CO_2 treatment on growth and physiology of hybrid poplar

There were no significant effects of CO_2 treatment on morphological variables, except for bud burst, for the eight hybrid poplar clones (Table 3-5a). Poplars grown under high CO_2 had a greater mean height (8.7%) and final caliper (8%) compared with poplars grown under ambient CO_2, but this effect was not significant (Figure 3-21, 3-22). Poplars grown under high CO_2 had a significantly higher NA rate on Days 60 and 95 compared to poplars grown under ambient CO_2 (Figure 3-23). Also, at Days 39 and 95, hybrid poplar grown under high CO_2 had significantly higher WUE compared to those grown under ambient CO_2 (Figure 3-23).

3.3.2. Hybrid poplar clonal differences

Only one variable, bud burst after seven days, showed a significant treatment by clone interaction (Table 3-5a,b). Under high CO_2, six poplar clones (Assiniboine, CanAm, Green Giant, P38 P38, Walker and Northwest) had started to break bud by Day 7, compared to four clones (Assiniboine, Green Giant, P38 P38 and Northwest) in ambient CO_2 (Figure 3-24). Under both CO_2 treatments, P38 P38 was the first clone to break bud, however, bud burst was more advanced under high compared to ambient CO_2.

Significant differences were found among hybrid poplars for most of the morphological variables, except for final caliper, specific leaf area and shoot-to-root ratios (Table 3-5a,b). P38 P38 and Green Giant clones were the tallest, while Sargentii was the poorest performer (shortest) at the end of the experiment, irrespective of CO_2 treatment (Figure 3-25). As well, P38 P38, followed by Northwest, had the largest caliper whereas, Sargentii, had the smallest caliper by Day 95, in both high and ambient CO_2 treatments (Figure 3-26).

Significant clonal differences were found for leaf, stem and root dry weight (Figure 3-27). Under both CO_2 treatments, P38 P38, followed by Green Giant and Manitou had the highest mean shoot and root dry weights and at the same time, a lower shoot-to-root ratio due to a proportionally greater root dry weight. Both Assiniboine and CanAm had lower root dry weights compared to other clones, which caused a corresponding increase in shoot-to-root ratio in both CO_2 treatments.

Although not significant, there was considerable variation in NA and WUE among the hybrid poplar clones (Figure 3-28). By Day 95, Sargentii had the highest NA and Walker had the lowest (Figure 3-28). Sargentii also had the highest WUE with

Northwest having the lowest by the end of the experiment. There was significant clonal variation in respiration on Day 95. Respiration rates increased by Day 95 compared to Day 60, with P38 P38 having the highest rates (Figure 3-29). However, respiration rates for both Walker and Sargentii decreased by Day 95 compared to Day 60.

Table 3-1. Results of analysis of variance (p values) testing for the effect of CO_2 pre-treatment (high and ambient CO_2) conditions during propagation during subsequent growth under high CO_2 a) morphological and b) gas exchange variables for trembling aspen clones (6 superior and 3 unselected clones). Significant p values (<0.05) are indicated in bold.

a)

Source*	Intht	Hgt2[1]	Hgt3	Fnlht	Intcal	Fnlcal	BB1	LA[2]	LDW	SDW	RDW	SLA	shtrt	ALW
PT***	0.617	0.060	0.066	0.336	0.804	0.657	0.254	0.937	0.143	0.486	0.348	**0.025**	0.701	0.935
Clone	0.052	0.317	0.125	0.070	0.085	0.052	0.077	0.302	0.086	0.052	0.143	0.371	0.439	0.798
PT*Clone	NS**	0.278	NS	0.253	NS	NS	0.252	NS	NS	NS	NS	NS	NS	0.284

b)

	Day 39 Gas Exchange			Day 60 Gas Exchange				Day 95 Gas Exchange			
Source*	Gs	NA	WUE	Gs	NA	WUE	Resp	Gs	NA	WUE	Resp
PT***	**0.017**	0.611	**<0.001**	0.092	0.055	**0.009**	0.297	0.432	0.173	0.291	0.259
Clone	0.373	NS	NS	0.297	0.339	0.219	0.298	0.067	0.191	0.262	0.158
PT*Clone	NS	0.486	NS	0.308	NS	NS	NS	NS	NS	0.254	0.246

where Intht= height measured at Day 0, Hgt2= height measured at Day 39, Hgt3= height measured at Day 60, Fnlht= height measured at Day 95, Intcal= caliper measured at Day 0, Fnlcal= caliper measured at Day 95, BB1= bud burst measured at Day 7, ALW= preformed leaf shape, LA= total leaf area per plant, LDW= leaf dry weight, SDW= stem dry weight, RDW= root dry weight, SLA= specific leaf area, shtrt= shoot-to-root dry weight ratio, Gs= stomatal conductance, NA= net assimilation, WUE= water use efficiency and Resp= leaf dark respiration.

*Source effects where PT is CO_2 pre-treatment conditions (high and ambient CO_2), Clone is superior and unselected aspen clones and PT*Clone is the interaction of CO_2 pre-treatment and clone.

**NS is not significant p values. SAS PROC MIXED does not provide p values since the variance is inestimably small.

***PROC MIXED only gives degrees of freedom (df) for fixed effects (in this case pre-treatment). For all growth and gas exchange variables PT, Clone and PT*Clone had a numerator df= 1 and a denominator df= 8. For all other variables (from destructive harvest) PT, Clone and PT*Clone had a numerator df= 1 and a denominator df= 6 (see Ch. 2, pg 26, Model 1).

[1] Initial height (Intht) used as covariate in PROC MIXED

[2] Final height (Fnlht) used as covariate in PROC MIXED

Table 3-2. Results of analysis of variance (p values) testing for the effects of CO_2 treatment (high and ambient CO_2) conditions and phenotype (superior and unselected) a) morphological and b) gas exchange variables for trembling aspen clones (12 superior and 22 unselected clones). Significant p values (<0.05) are indicated in bold.

a)

Source*	Intht	Hgt2[1]	Hgt3[1]	Fnlht[1]	Intcal	Fnlcal[2]	BB1	LA[3]	LDW	SDW	RDW	SLA	shtrt	ALW	A2LW
T	NT	**0.019**	**0.030**	0.093	NS	**0.035**	**<0.01**	0.870	0.069	0.053	**0.026**	0.144	**0.039**	0.357	0.542
Ch(T)	NS	0.479	NS	0.429	NS	0.371	NS	0.208	0.451	0.374	0.394	0.225	NS	NS	NS
Ph	0.496	0.232	0.245	0.128	0.054	0.084	**0.017**	0.225	0.181	0.345	0.927	0.728	0.928	0.873	0.213
Cl(Ph)	0.125	0.065	0.274	0.292	NS	0.168	0.255	0.055	NS	0.428	0.479	0.394	0.052	0.117	0.125
T*Ph	0.781	0.936	0.908	0.638	0.303	0.528	0.154	0.162	0.357	0.938	0.359	0.941	0.319	0.266	0.489
T*Cl(Ph)	0.239	NS	0.278	0.194	0.176	NS	0.157	NS	0.204	0.130	0.319	NS	NS	0.260	0.244
Ch(T)*Ph	NS	0.249	0.293	NS	NS	0.396	NS	0.44	0.455	NS	NS	NS	NS	NS	NS
Ch(T)*Cl(Ph)	NS	0.350	0.432	NS	NS	NS	NS	0.151	NS	NS	NS	NS	0.191	0.459	NS

b)

	Day 39 Gas Exchange			Day 60 Gas Exchange				Day 95 Gas Exchange			
Source	Gs	NA	WUE	Gs	NA	WUE	Resp	Gs	NA	WUE	Resp
T	0.253	**0.003**	**0.024**	0.168	**0.038**	0.399	0.355	0.352	**0.020**	**0.005**	0.229
Ch(T)	0.265	NS	0.195	0.165	0.197	0.166	0.195	0.205	0.187	0.201	0.381
Ph	0.361	0.346	0.799	0.732	0.646	0.574	0.405	0.443	0.356	0.119	0.125
Cl(Ph)	0.171	0.425	0.168	0.248	0.458	NS	NS	0.288	0.416	NS	NS
T*Ph	0.289	0.238	0.581	0.467	0.353	0.934	0.415	0.188	0.734	0.514	0.841
T*Cl(Ph)	NS	0.466	NS	NS	NS	0.210	NS	NS	0.335	NS	NS
Ch(T)*Ph	NS	NS	NS	NS	0.452	NS	0.2923	NS	NS	NS	NS
Ch(T)*Cl(Ph)	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS

Refer to Table 3-1 for definition of variables

*Source effects where T is CO_2 treatment conditions (high and ambient CO_2), Ch(T) is greenhouse chamber nested in treatment, Ph is phenotype (superior and unselected), Cl(Ph) is clone nested in phenotype, T*Ph is the interaction of treatment and phenotype, T*Cl(Ph) is the interaction of treatment and clone nested within phenotype, Ch(T)*Ph is the interaction of chamber nested within treatment and phenotype and Ch(T)*Cl(Ph)Clone is the interaction of chamber nested within treatment and clone nested within

phenotype.
** NT is not testable due to lack of treatment replication.
***NS is not significant p values. SAS PROC MIXED does not provide p values since the variance is inestimably small.
****PROC MIXED only gives numerator and denominator degrees of freedom (df) for fixed effects. For Intht and Intcal, the Ph and T*Ph interaction had a numerator df= 1 and a denominator df= 12. For BB1, the T, Ph and T*Ph interaction had a numerator df= 1 and denominator df= 12. For all other morphological and gas exchange variables, T, Ph and T*Ph had a numerator df= 1 and a denominator df= 2 (see Ch. 2, pg 26, Model 2).
[1] Initial height (Intht) used as covariate in PROC MIXED.
[2] Initial caliper (Intcal) used as covariate in PROC MIXED.
[3] Final height (Fnlht) used as covariate in PROC MIXED.

Table 3-3. Results of analysis of variance (p values) testing for the effects of CO_2 treatment (high and ambient CO_2) conditions and gender (female and male) a) morphological and b) gas exchange variables for trembling aspen clones (22 unselected clones). Significant p values (<0.05) are indicated in bold.

a)

Source*	Intht	Hgt2[1]	Hgt3[1]	Fnlht[1]	Intcal	Fnlcal[2]	BB1	LA[3]	LDW	SDW	RDW	SLA	shtrt	ALW	A2LW
T****	NT**	**0.009**	0.053	0.170	NT	0.070	**0.004**	0.394	0.056	0.064	**0.023**	0.158	**0.037**	0.175	0.991
Ch(T)	NS***	NS	0.283	0.419	NS	0.257	NS	0.405	NS	NS	NS	0.280	NS	NS	NS
S****	0.894	0.857	0.934	0.926	0.516	0.319	0.331	0.185	0.338	0.569	0.393	0.448	0.706	0.394	0.426
Cl(S)	0.143	0.106	0.124	0.231	NS	0.255	0.107	0.184	NS	NS	NS	0.483	0.111	0.121	0.241
T*S****	0.732	0.937	0.399	0.286	0.722	0.344	0.243	0.469	0.299	0.459	0.761	0.881	0.612	0.495	0.549
T*Cl(S)	0.294	0.388	NS	0.224	0.157	NS	0.474	NS	0.328	0.119	0.381	0.364	NS	0.377	0.235
Ch(T)*S	NS	NS	NS	NS	NS	NS	NS	0.244	0.281	0.404	NS	NS	NS	NS	0.433
Ch(T)*Cl(S)	NS	0.494	0.404	NS	NS	NS	NS	0.257	NS	NS	0.374	NS	0.314	0.310	NS

b)

	Day 39 Gas Exchange			Day 60 Gas Exchange				Day 95 Gas Exchange			
Source	Gs	NA	WUE	Gs	NA	WUE	Resp	Gs	NA	WUE	Resp
T	0.562	**0.006**	**0.028**	0.182	**0.038**	0.366	0.566	0.209	**0.021**	**0.008**	0.227
Ch(T)	0.259	NS	0.209	0.171	0.289	0.179	0.171	0.215	0.199	NS	NS
S	0.315	0.787	0.186	0.440	0.399	0.483	0.682	0.133	0.213	0.745	0.338
Cl(S)	0.144	0.316	0.274	0.261	NS	NS	0.421	NS	NS	NS	0.364
T*S	0.207	0.979	0.230	0.668	0.424	0.874	0.467	0.675	0.395	0.801	0.699
T*Cl(S)	NS	0.372	NS	NS	NS	0.297	NS	NS	NS	NS	NS
Ch(T)*S	NS	NS	NS	NS	NS	0.444	NS	NS	NS	0.282	NS
Ch(T)*Cl(S)	NS	NS	NS	0.467	NS	0.475	NS	NS	NS	NS	NS

Refer to Table 3-1 for definition of variables

*Source effects where T is CO_2 treatment conditions (high and ambient CO_2), Ch(T) is greenhouse chamber nested in treatment, S is gender (female and male), Cl(S) is clone nested in gender, T*S is the interaction of treatment and gender, T*Cl(S) is the interaction of treatment and clone nested within gender, Ch(T)*S is the interaction of chamber nested within treatment and gender and Ch(T)*Cl(S)

is the interaction of chamber nested within treatment and clone nested within gender.
** NT is not testable due to lack of treatment replication.
***NS is not significant p values. SAS PROC MIXED does not provide p values since the variance is inestimably small.
****PROC MIXED only gives numerator and denominator degrees of freedom (df) for fixed effects. For Intht and Intcal, the S and T*S interaction had a numerator df= 1 and a denominator df= 7. For BB1, the T, S and T*S interaction had a numerator df= 1 and a denominator df= 7. For all other morphological and gas exchange variables, T, S and T*S had a numerator df= 1 and a denominator df= 2 (see Ch. 2, pg 27, Model 3).
[1] Initial height (Intht) used as covariate in PROC MIXED.
[2] Initial caliper (Intcal) used as covariate in PROC MIXED.
[3] Final height (Fnlht) used as covariate in PROC MIXED.

Table 3-4. Results of analysis of variance (p values) testing for the effects of CO_2 treatment (high and ambient CO_2) conditions and provenance (Peace River, Athabasca and Ft. McMurray) a) morphological and b) gas exchange variables for trembling aspen clones (22 unselected clones). Significant p values (<0.05) are indicated in bold.

a)

Source*	Intht	Hgt2[1]	Hgt3[1]	Fnlht	Intcal	Fnlcal[2]	BB1	LA[3]	LDW	SDW	RDW	SLA	shtrt	ALW	A2LW
T****	NT**	**0.049**	0.112	0.246	NT	0.083	**0.003**	0.426	0.087	0.106	**0.023**	0.147	0.054	0.223	0.515
Ch(T)	NS***	NS	NS	NS	NS	0.412	NS	0.207	NS	NS	NS	0.285	NS	NS	NS
P****	0.513	0.769	0.935	0.664	0.159	0.943	0.397	0.121	0.700	0.529	0.263	0.761	0.960	0.203	0.177
Cl(P)	0.104	0.176	0.089	0.156	NS	0.084	0.232	**0.023**	0.350	0.369	0.677	NS	**0.044**	**0.012**	**0.023**
T*P****	0.857	0.936	0.986	0.932	0.587	0.920	0.421	0.107	0.260	0.677	0.827	0.576	0.575	0.196	0.495
T*Cl(P)	0.059	0.195	NS	**0.048**	0.059	0.127	0.076	NS	0.129	0.052	0.211	NS	NS	NS	0.400
Ch(T)*P	NS	0.077	0.081	0.152	NS	0.211	NS	NS	0.193	0.127	NS	NS	0.301	NS	NS
Ch(T)*Cl(P)	NS	0.074	0.157	NS	NS	NS	NS	NS	NS	NS	0.371	0.414	NS	NS	0.237

b)

	Day 39 Gas Exchange			Day 60 Gas Exchange				Day 95 Gas Exchange			
Source	Gs	NA	WUE	Gs	NA	WUE	Resp	Gs	NA	WUE	Resp
T	0.488	**0.005**	**0.029**	0.187	**0.038**	0.341	0.537	0.205	**0.022**	**0.007**	0.378
Ch(T)	0.304	NS	0.223	0.184	0.229	0.175	0.171	0.231	0.232	0.368	NS
P	0.865	0.806	0.569	0.062	0.099	0.636	0.405	0.131	0.887	0.193	0.934
Cl(P)	**0.027**	0.447	0.187	0.104	NS	NS	0.304	**0.016**	0.127	0.436	0.228
T*P	0.466	0.703	0.952	0.609	0.097	0.546	0.225	0.584	0.963	0.552	0.182
T*Cl(P)	0.398	NS	NS	NS	NS	0.273	NS	NS	0.462	0.292	0.305
Ch(T)*P	0.316	NS	0.375	0.189	0.486	NS	NS	0.326	0.273	NS	NS
Ch(T)*Cl(P)	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS

Refer to Table 3-1 for definition of variables

*Source effects where T is CO_2 treatment conditions (high and ambient CO_2), Ch(T) is greenhouse chamber nested in treatment, P is provenance (Peace River, Athabasca and Ft. McMurray), Cl(P) is clone nested in gender, T*P is the interaction of treatment and gender, T*Cl(P) is the interaction of treatment and clone nested within gender, Ch(T)*P is the interaction of chamber nested within

treatment and gender and Ch(T)*Cl(P) is the interaction of chamber nested within treatment and clone nested within gender.
** NT is not testable due to lack of treatment replication.
***NS is not significant p values. SAS PROC MIXED does not provide p values since the variance is inestimably small.
****PROC MIXED only gives numerator and denominator degrees of freedom (df) for fixed effects. For Intht and Intcal, P and T*P interaction had a numerator df= 2 and a denominator df= 18. For BB1, T had a numerator df= 1 and a denominator df= 18, and P and T*P interaction had a numerator df= 2 and a denominator df= 18. For all other morphological and gas exchange variables, T, had a numerator df= 1 and a denominator df= 2, P and T*P had a numerator df= 2 and a denominator df= 4 (see Ch. 2, pg 27, Model 4).
[1] Initial height (Intht) used as covariate in PROC MIXED.
[2] Initial caliper (Intcal) used as covariate in PROC MIXED.
[3] Final height (Fnlht) used as covariate in PROC MIXED.

Table 3-5. Results of analysis of variance (p values) testing for the effects of CO_2 treatment (high and ambient CO_2) conditions and clone a) morphological and b) gas exchange variables for hybrid poplar clones (Assiniboine, CanAm, Green Giant, Manitou, P38 P38, Walker, Sargentii and Northwest) clones. Significant p values (<0.05) are indicated in bold.

a)

Source*	Intht	Hgt2[1]	Hgt3[1]	Fnlht[1]	Intcal	Fnlcal[2]	BB1	LA[3]	LDW	SDW	RDW	SLA	shtrt
T****	NT	0.779	0.982	0.854	NT	0.173	**0.001**	0.507	0.364	0.139	0.419	0.501	0.675
Ch(T)	NS	0.211	0.211	0.261	NS	0.453	NS	0.249	NS	NS	0.423	0.477	0.309
Cl****	**<0.001**	**0.005**	**0.012**	**0.001**	**<0.001**	0.489	**<0.001**	**0.002**	**0.001**	**0.003**	**0.005**	0.488	0.063
T*Cl****	0.213	0.154	0.871	0.460	0.207	0.668	**0.030**	0.051	0.162	0.144	0.649	0.698	0.847
Ch(T)*Cl	NS	NS	0.372	NS	NS	0.148	NS	NS	NS	NS	NS	0.265	0.452

b)

	Day 39 Gas Exchange			Day 60 Gas Exchange				Day 95 Gas Exchange			
Source	Gs	NA	WUE	Gs	NA	WUE	Resp	Gs	NA	WUE	Resp
T	0.105	0.148	**0.042**	0.264	**0.039**	0.475	0.654	0.110	**0.043**	**0.040**	0.369
Ch(T)	0.378	NS	0.295	0.272	NS	0.232	0.211	0.346	0.284	0.372	NS
Cl	0.148	0.269	0.759	0.226	0.133	0.498	0.783	0.058	0.159	0.074	**0.049**
T*Cl	0.549	0.338	0.674	0.941	0.890	0.885	0.569	0.762	0.084	0.066	0.488
Ch(T)*Cl	0.413	NS	0.500	NS	0.408	NS	NS	NS	NS	NS	NS

Refer to Table 3-1 for definition of variables.

*Source effects where T is CO_2 treatment conditions (high and ambient CO_2), Ch(T) is greenhouse chamber nested in treatment, Cl is hybrid poplar clones, T*Cl is the interaction of treatment and hybrid poplar clones, Ch(T)*Cl is the interaction of chamber nested within treatment and hybrid poplar clones.

** NT is not testable due to lack of treatment replication.

***NS is not significant p values. SAS PROC MIXED does not provide p values since the variance is inestimably small.

****PROC MIXED only gives numerator and denominator degrees of freedom (df) for fixed effects. For Intht and Intcal, Cl and T*Cl interaction had a numerator df= 7 and a denominator df= 80. For BB1, the T had a numerator df= 1 and a denominator df= 80, Cl and T*Cl interaction had a numerator df= 7 and a denominator df= 80. For all growth and gas exchange variables, T had a numerator df= 1 and a denominator df= 2, Cl and T*Cl had a numerator df= 7 and a denominator df= 14. For all other variables (from

destructive harvest) T had a numerator df= 1 and a denominator df= 2 and Cl had a numerator df= 6 and a denominator df= 11. and T*Cl had a numerator df= 5 and a denominator df= 11(see Ch. 2, pg 28, Model 5).

[1] Initial height (Intht) used as covariate in PROC MIXED.

[2] Initial caliper (Intcal) used as covariate in PROC MIXED.

[3] Final height (Fnlht) used as covariate in PROC MIXED.

Figure 3-1. Maximum and minimum temperatures (°C) measured throughout the experiment in each of the four greenhouse chambers representing the two treatment levels (high and ambient CO_2) (n=80).

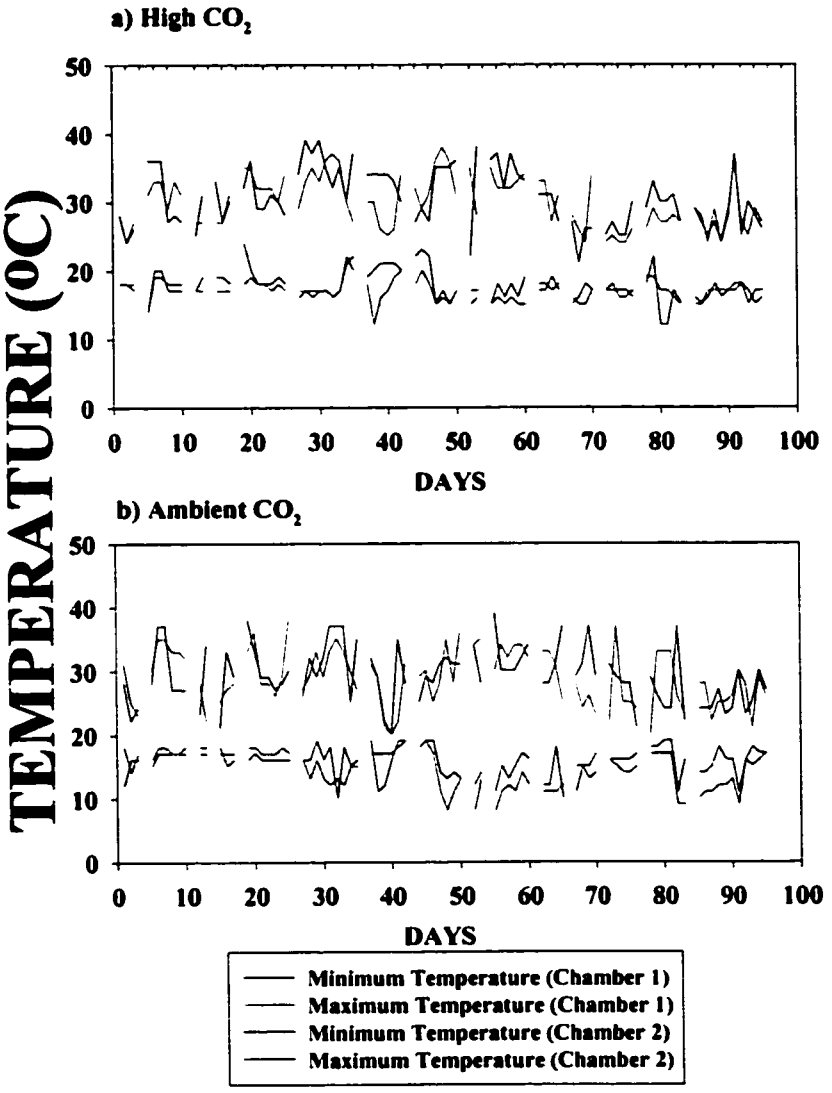

Figure 3-2. Maximum and minimum relative humidity levels (%RH) measured throughout the experiment in each of the four greenhouse chambers representing the two treatment levels (high and ambient CO_2) (n=80).

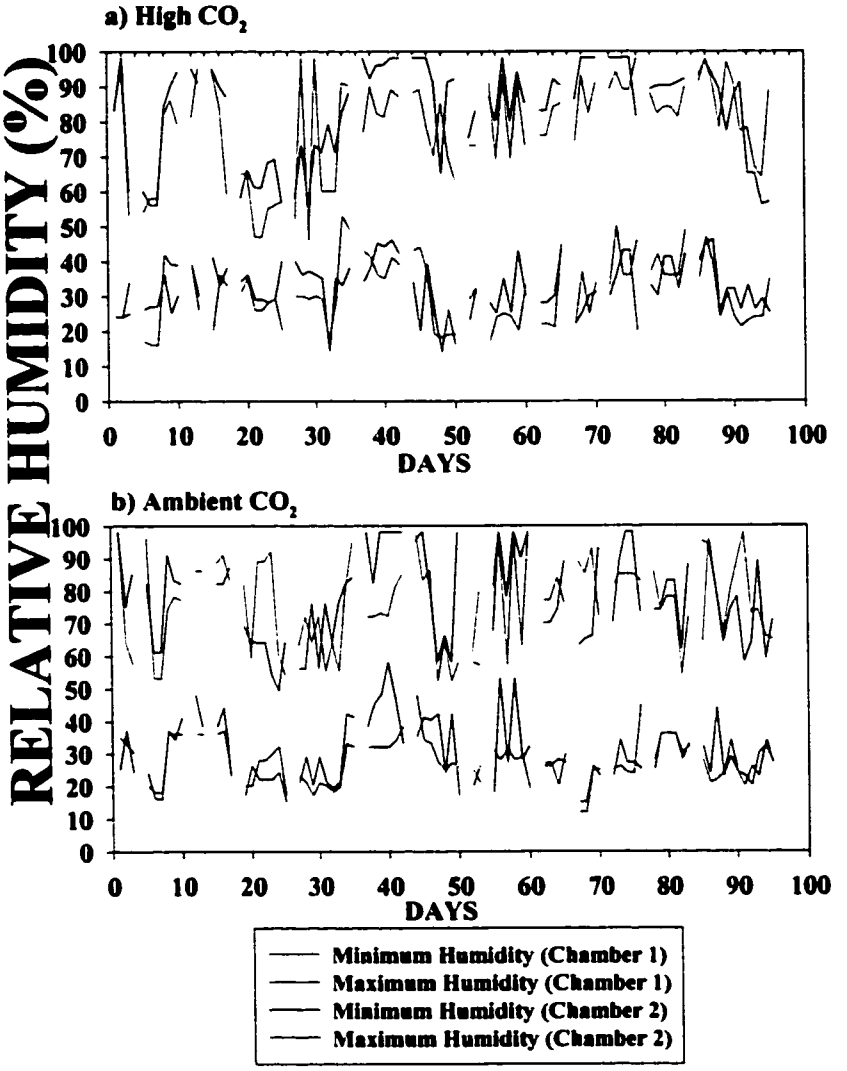

Figure 3-3. CO_2 (ppm) levels measured throughout the experiment in each of the four greenhouse chambers representing the two treatment levels (high and ambient CO_2) (n=10).

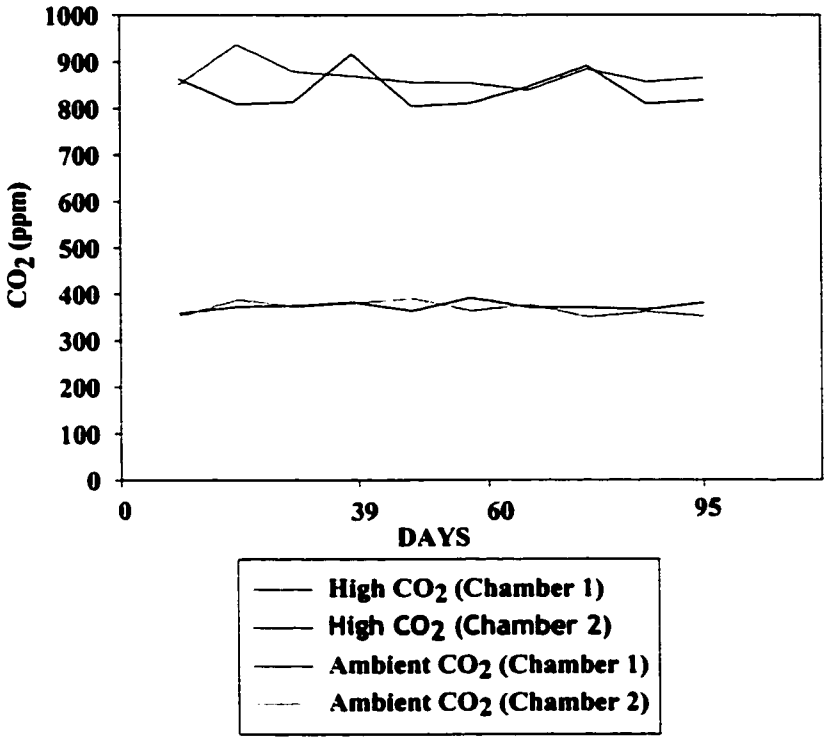

Figure 3-4. Mean height (cm) (±s.e.) for nine aspen clones (both superior and unselected) propagated under both pre-treatment levels (high and ambient CO_2) and subsequently grown under high CO_2 for 95 days. Only replicates 1-2 were measured on Day 60 (not significant) (see Table 3-1a).

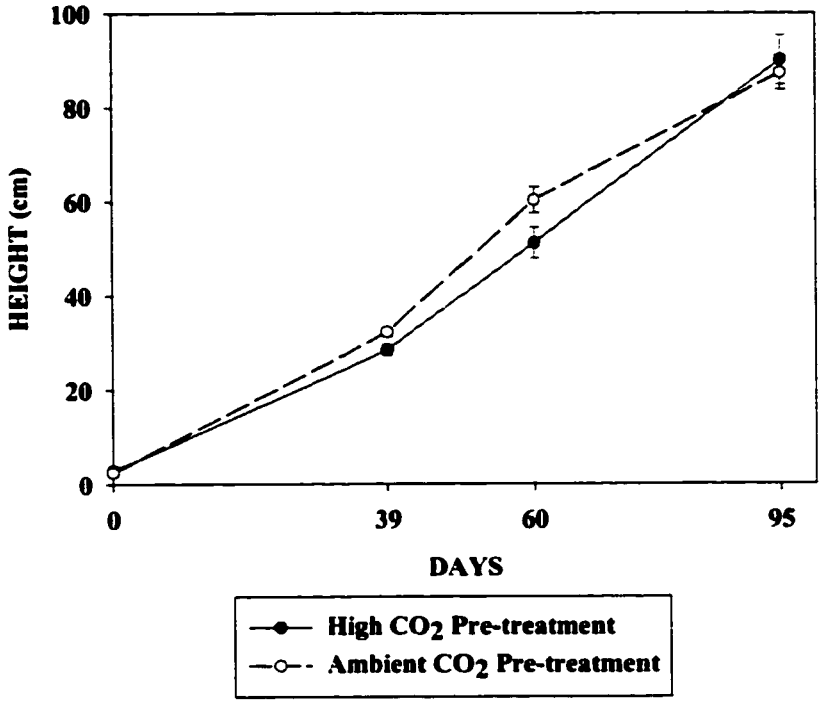

Figure 3-5. Mean specific leaf area (cm^2/g) (+s.e.) for nine aspen clones (both superior and unselected) propagated under both pre-treatment levels (high and ambient CO_2) and subsequently grown under high CO_2 for 95 days (where p_{pt} is the p value for the pre-treatment effect (pt)) (see Table 3-1a).

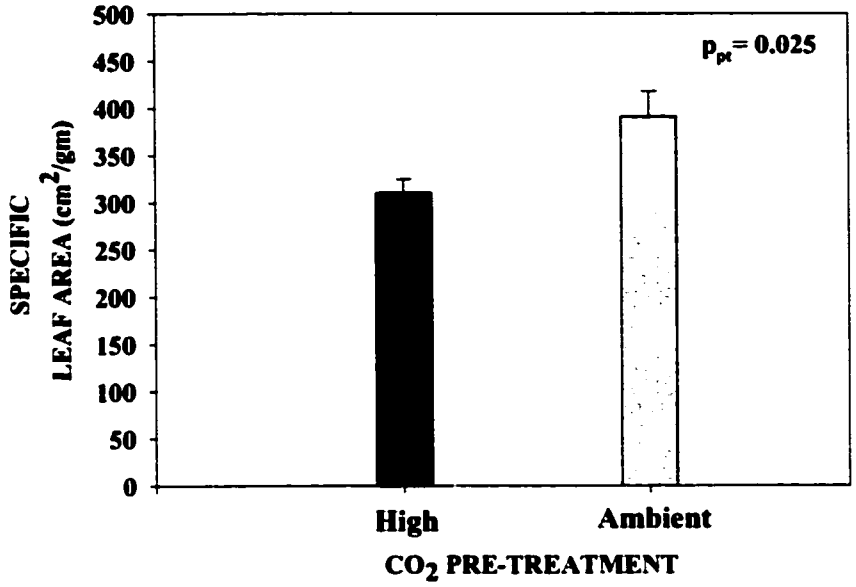

Figure 3-6. Mean (±s.e.) stomatal conductance (mmol H_2O m^{-2} s^{-1}), net assimilation (μmol CO_2 m^{-2} s^{-1}) and water use efficiency (μmol CO_2/mmol H_2O) for nine aspen clones (both superior and unselected) propagated under both pre-treatment levels (high and ambient CO_2) and subsequently grown under high CO_2 for 95 days. Only replicates 1-2 were measured on Day 60 (where p_{pt} is the p value for the pre-treatment effect (pt)) (see Table 3-1b).

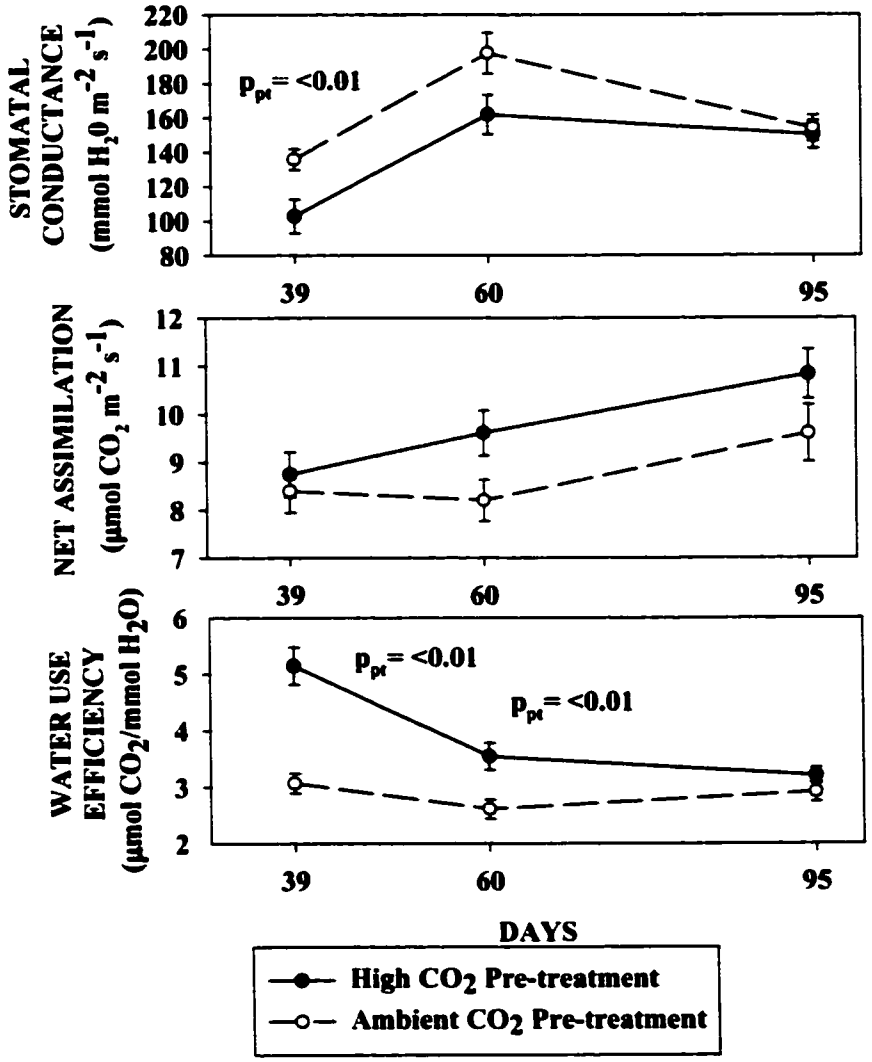

Figure 3-7. Mean height (cm) (±s.e.) including all 34 aspen clones (both superior and unselected) grown under both treatment levels (high and ambient CO_2) for 95 days . Only replicates 1-2 were measured on Day 60 (where p_t is the p value for treatment effect) (see Table 3-2a).

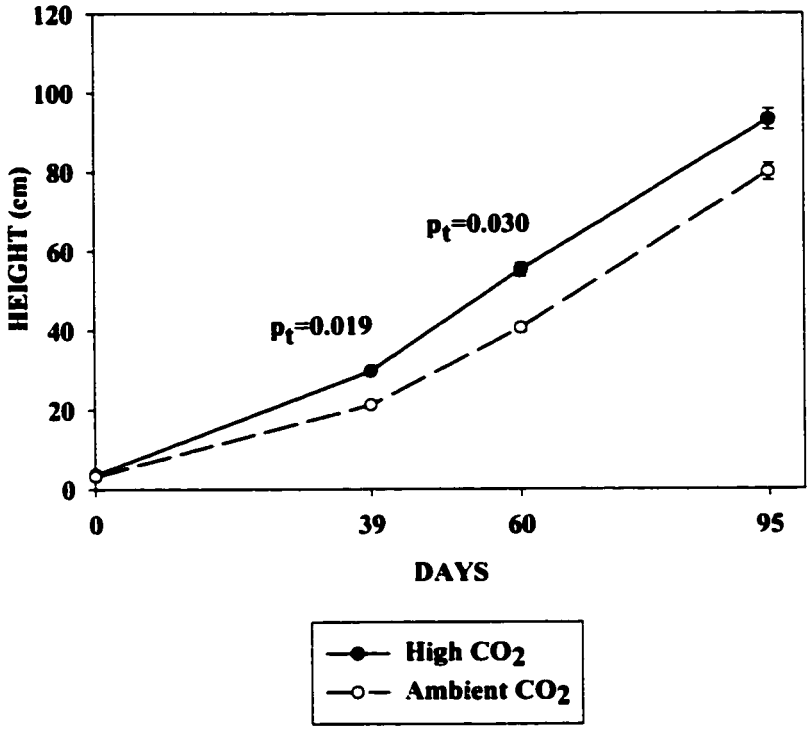

Figure 3-8. Mean initial and final caliper (cm) (+s.e.) including all 34 aspen clones (both superior and unselected) grown under both treatment levels (high and ambient CO_2) for 95 days (where p_t is the p value for treatment effect) (see Table 3-2a).

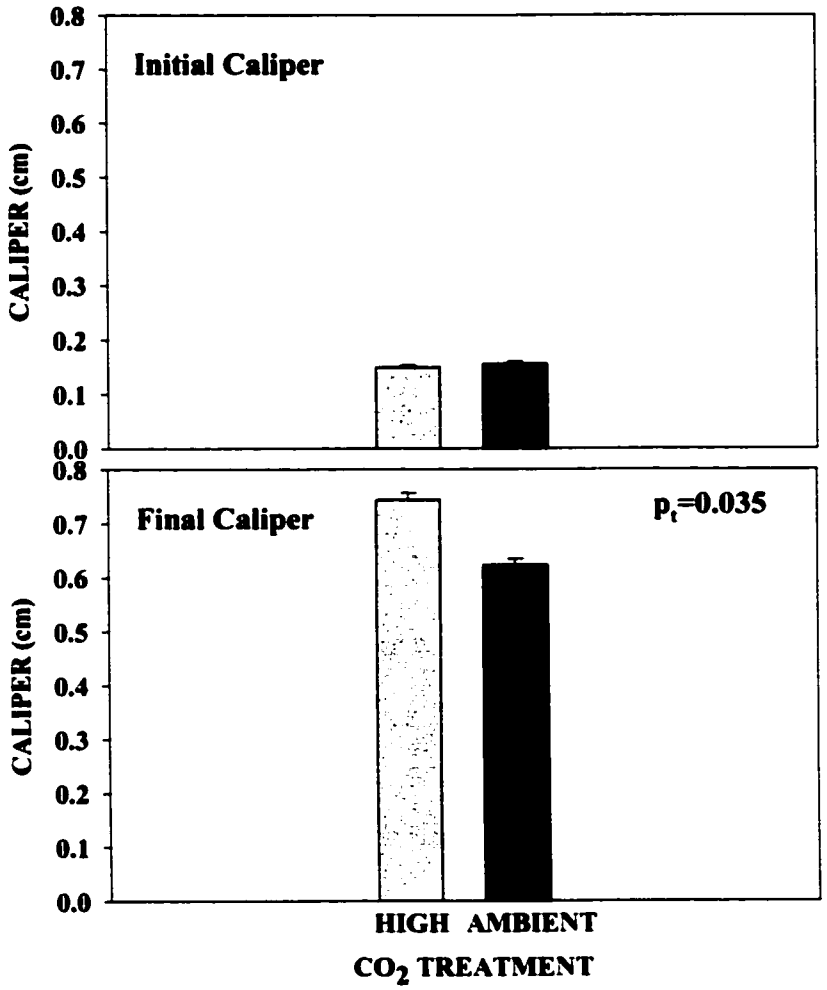

Figure 3-9. Mean (±s.e.) net assimilation (μmol CO_2 m^{-2} s^{-1}) and water use efficiency (μmol CO_2/mmol H_2O) including all 34 aspen clones (both superior and unselected) grown under both treatment levels (high and ambient CO_2) for 95 days. Only replicates 1-2 were measured on Day 60 (where p_t is the p value for treatment effect) (see Table 3-2b).

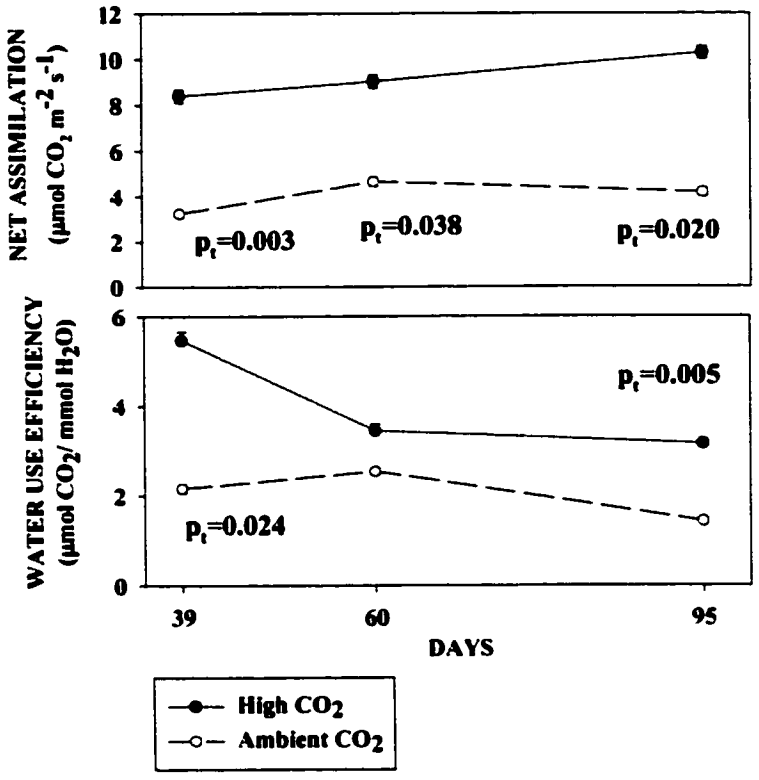

Figure 3-10. Mean leaf, stem and root dry weight (g) (+s.e.) and shoot-to-root ratio including all 34 aspen clones (both superior and unselected) grown under both treatment levels (high and ambient CO_2) for 95 days (where p_t is the p value for treatment effect) (see Table 3-2a).

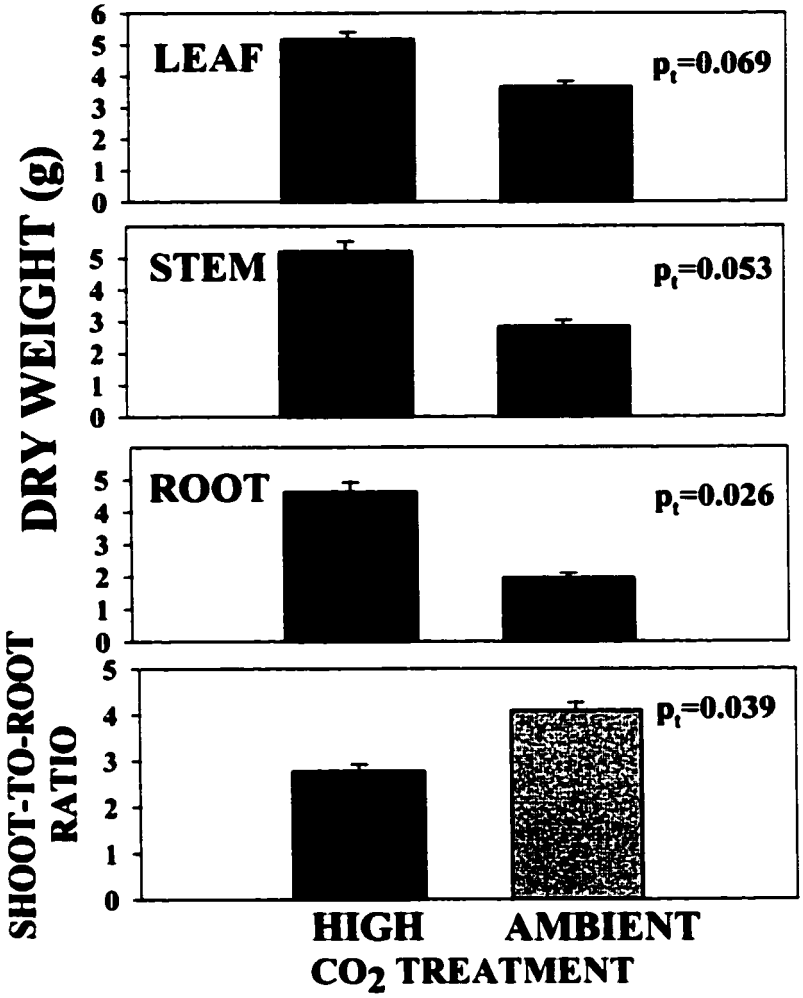

Figure 3-11. Mean score for bud burst (+s.e.) including all 34 aspen clones (both superior and unselected) across all treatment levels (high and ambient CO_2) after seven days where 1= tip of bud opening and 5= full bud flush (see Table 2-3) (where p_{ph} is the p value for difference due to phenotype) (see Table 3-2a).

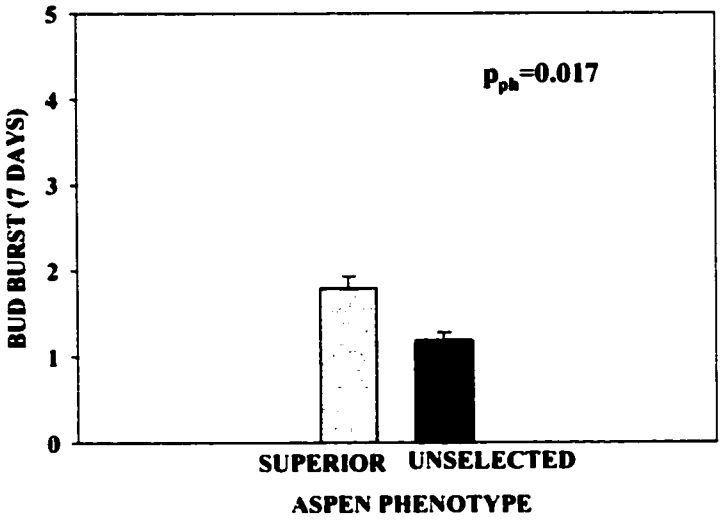

Figure 3-12. Mean height (cm) (±s.e.) including all 34 aspen clones (superior and unselected) grown under both treatment levels (high and ambient CO_2) for 95 days (where p_t is the p value for treatment effect). Only replicates 1-2 were measured at Day 60 (not significant) (where p_t is the p value for treatment effect) (see Table 3-2a).

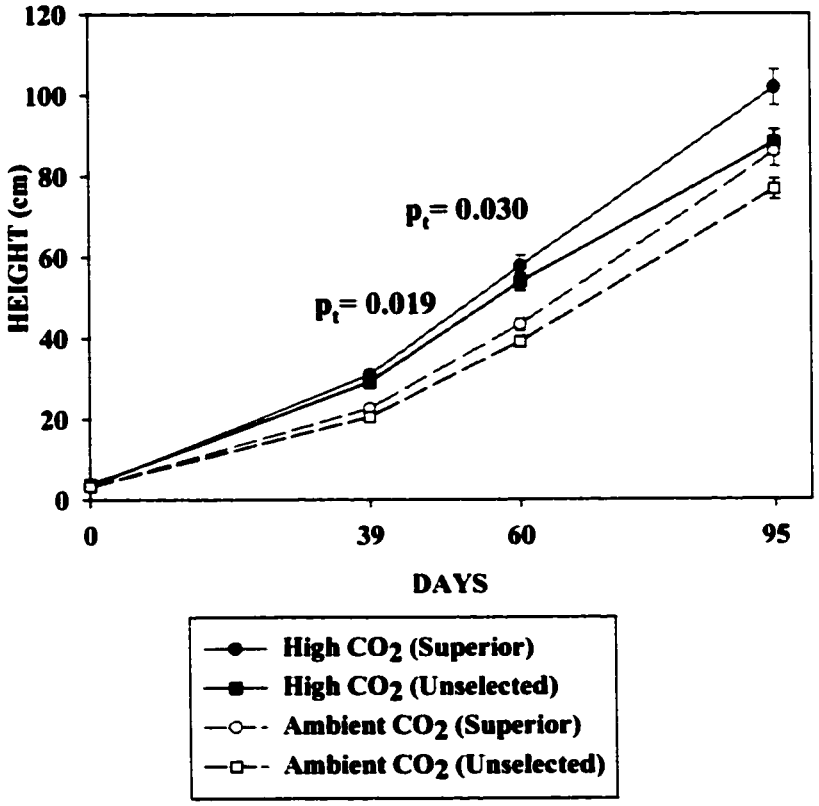

Figure 3-13. Mean initial (Day 0) and final caliper (Day 95) (cm) (+s.e) including all 34 aspen clones (superior and unselected) grown under both treatment levels (high and ambient CO_2) for 95 days (where p_{ph} is the p value for difference due to phenotype) (not significant) (see Table 3-2a).

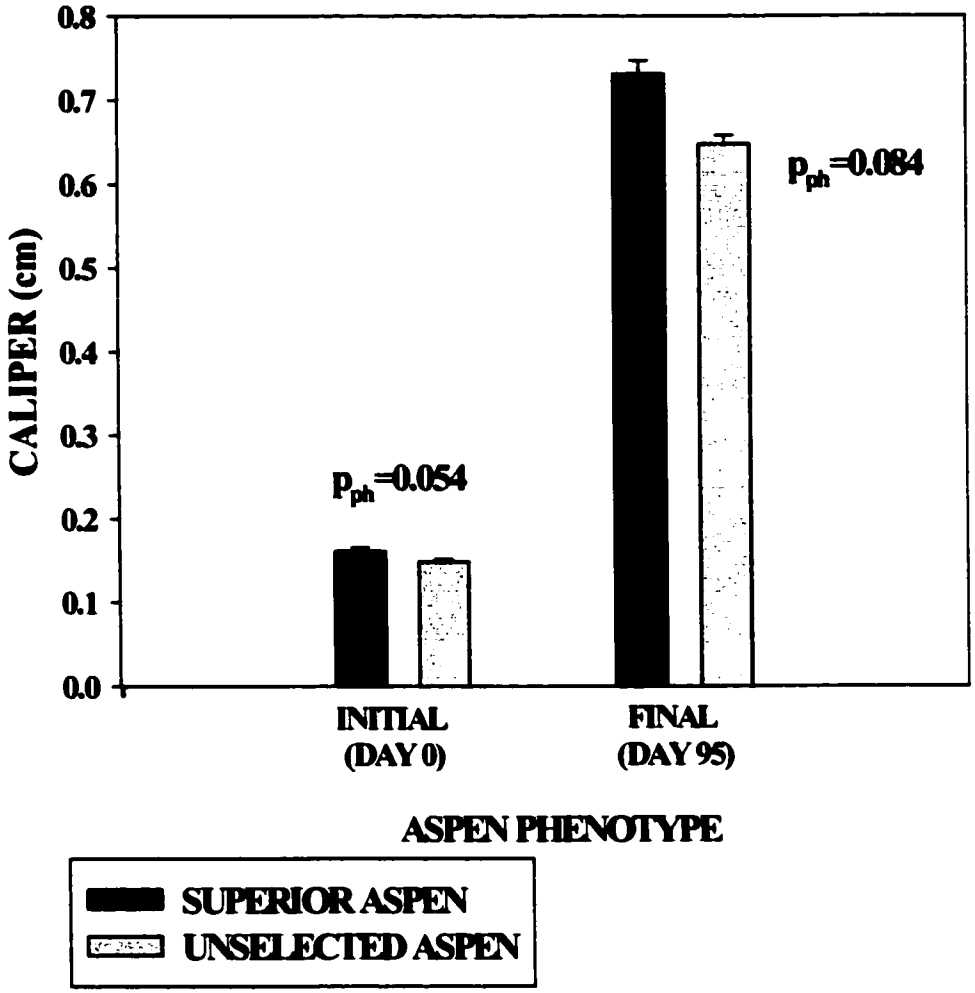

Figure 3-14. Mean total leaf area (cm^2) (+s.e.) including all 34 aspen clones (superior and unselected) grown under both treatment levels (high and ambient CO_2) for 95 days (not significant) (see Table 3-2a).

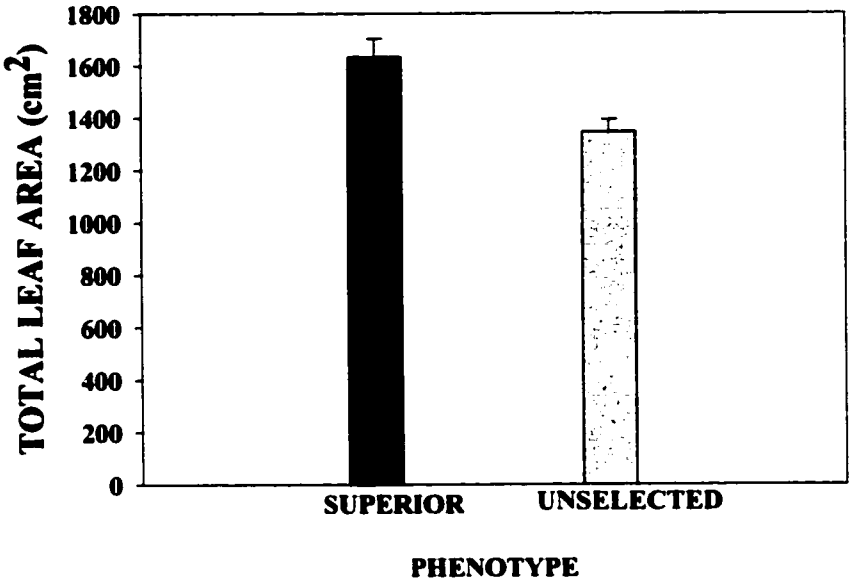

Figure 3-15. Mean height (cm) (±s.e.) including all 22 aspen clones (female and male) grown under both treatment levels (high and ambient CO_2) for 95 days. Only replicates 1-2 were measured on Day 60 (not significant) (see Table 3-3a).

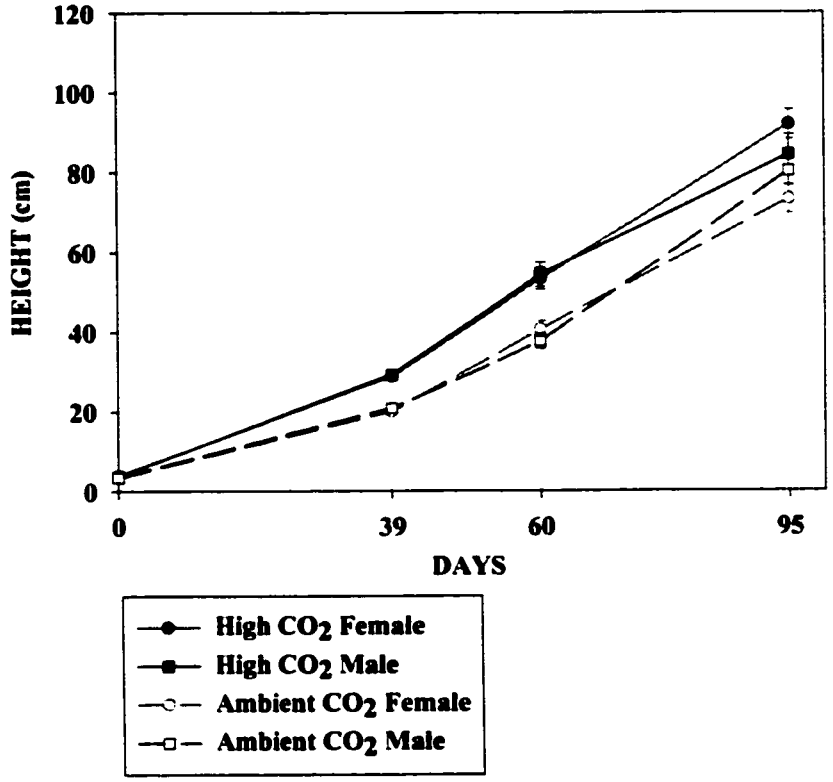

Figure 3-16. Mean final caliper (Day 95) (cm) (+s.e.) including all 22 aspen clones (female and male) grown under both treatment levels (high and ambient CO_2) for 95 days (not significant) (see Table 3-3a).

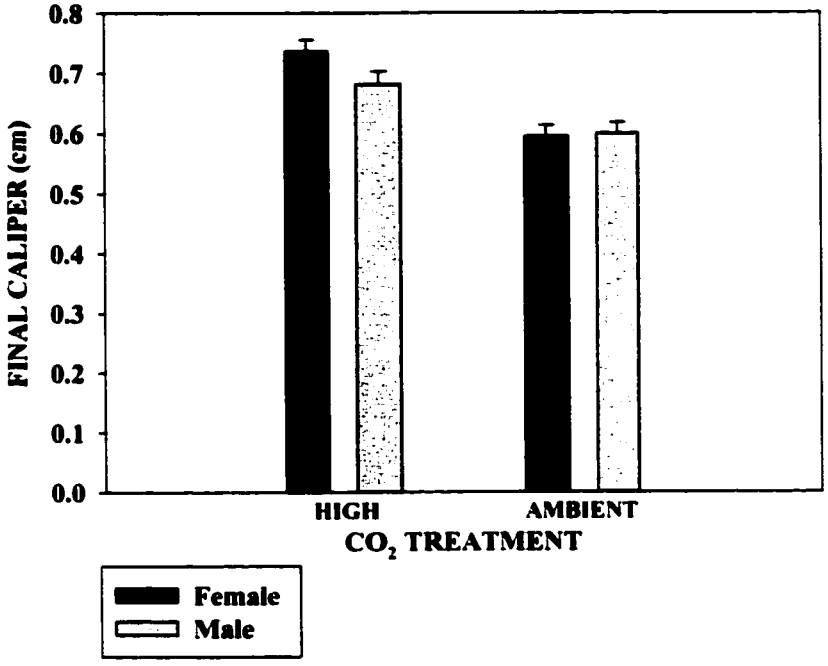

Figure 3-17. Mean height (cm) (±s.e.) including all 22 unselected aspen clones from three provenances (Peace River, Athabasca and Ft. McMurray) grown under both treatment levels (high and ambient CO_2) for 95 days. Only replicates 1-2 were measured on Day 60 (not significant) (see Table 3-4a).

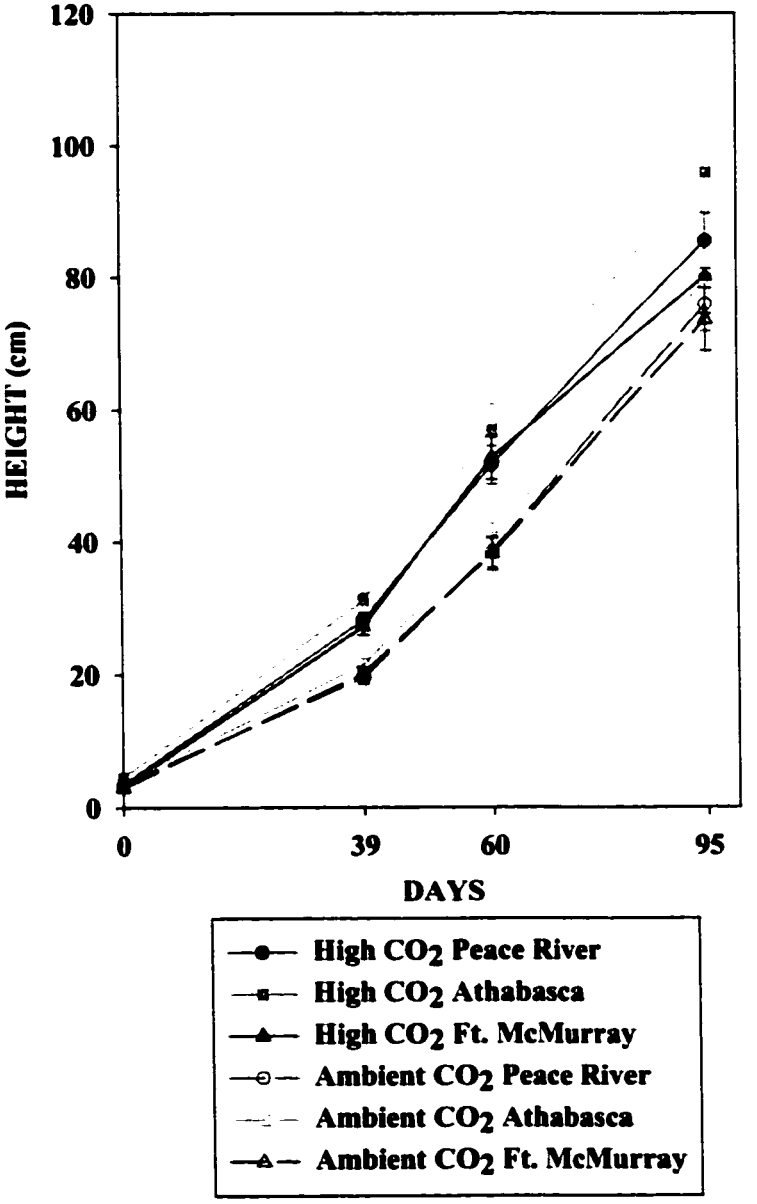

Figure 3-18. Mean preformed and mature leaf shape (cm) (+s.e.) for 22 unselected aspen clones from three provenances (Peace River, Athabasca and Ft. McMurray) grown under both treatment levels (high and ambient CO_2) for 95 days where clones 1-4 are unselected females and clones 5-8 are unselected males from both Peace River and Athabasca and clones 1-3 are unselected female and clones 5-7 are unselected males from Ft. McMurray (where $p_{cl(p)}$ is the p values for differences due to clone within provenance) (see Table3-4a).

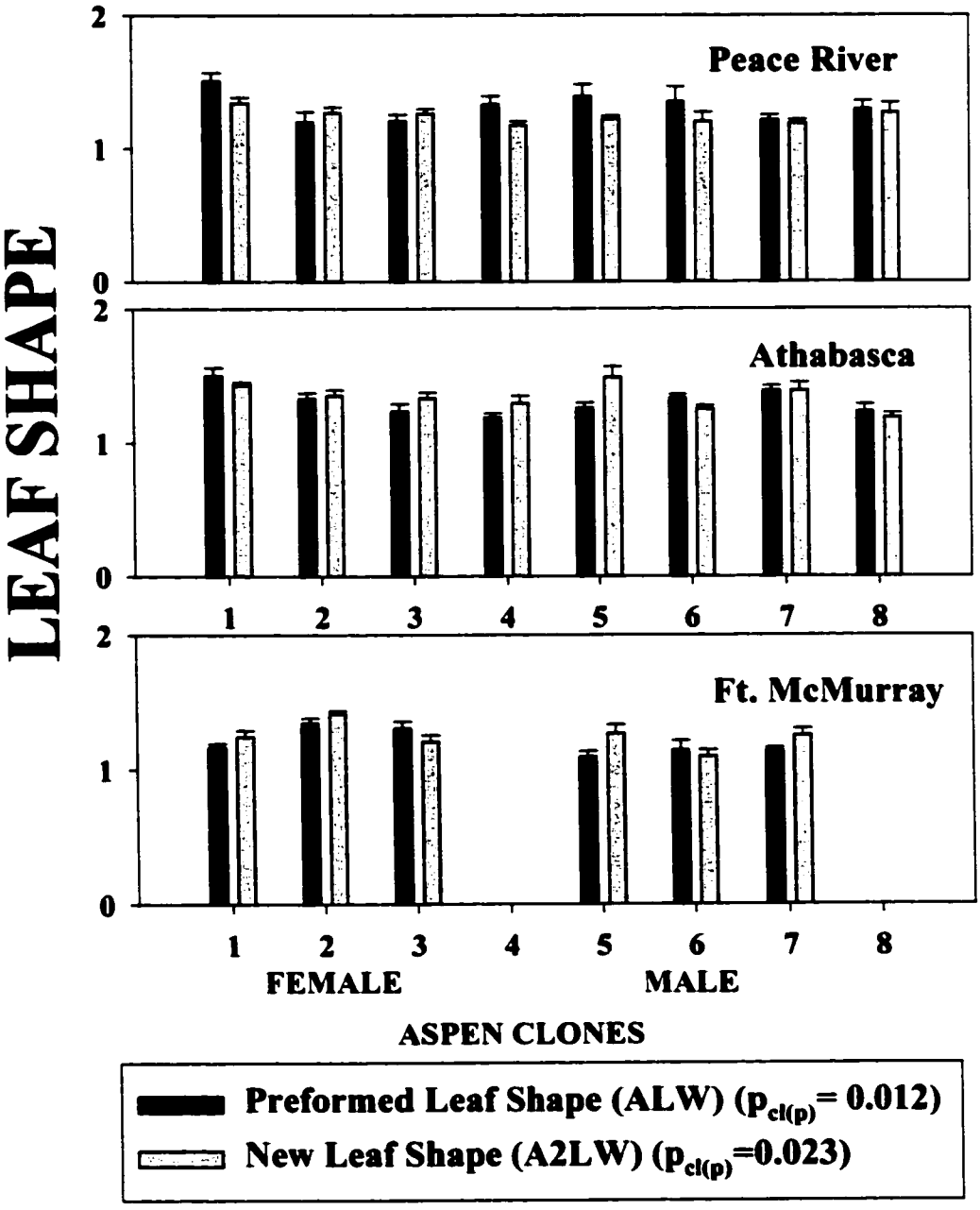

Figure 3-19. Mean total leaf area (cm^2) (+s.e.) for 22 unselected aspen clones from three provenances (Peace River, Athabasca and Ft. McMurray) grown under both treatment levels (high and ambient CO_2) for 95 days where clones 1-4 are unselected female and clones 5-8 are unselected male from both Peace River and Athabasca and clones 1-3 are unselected female and clones 5-7 are unselected males from Ft. McMurray (where $p_{cl(p)}$ is the p value for differences due to clone within provenance) (see Table3-4a).

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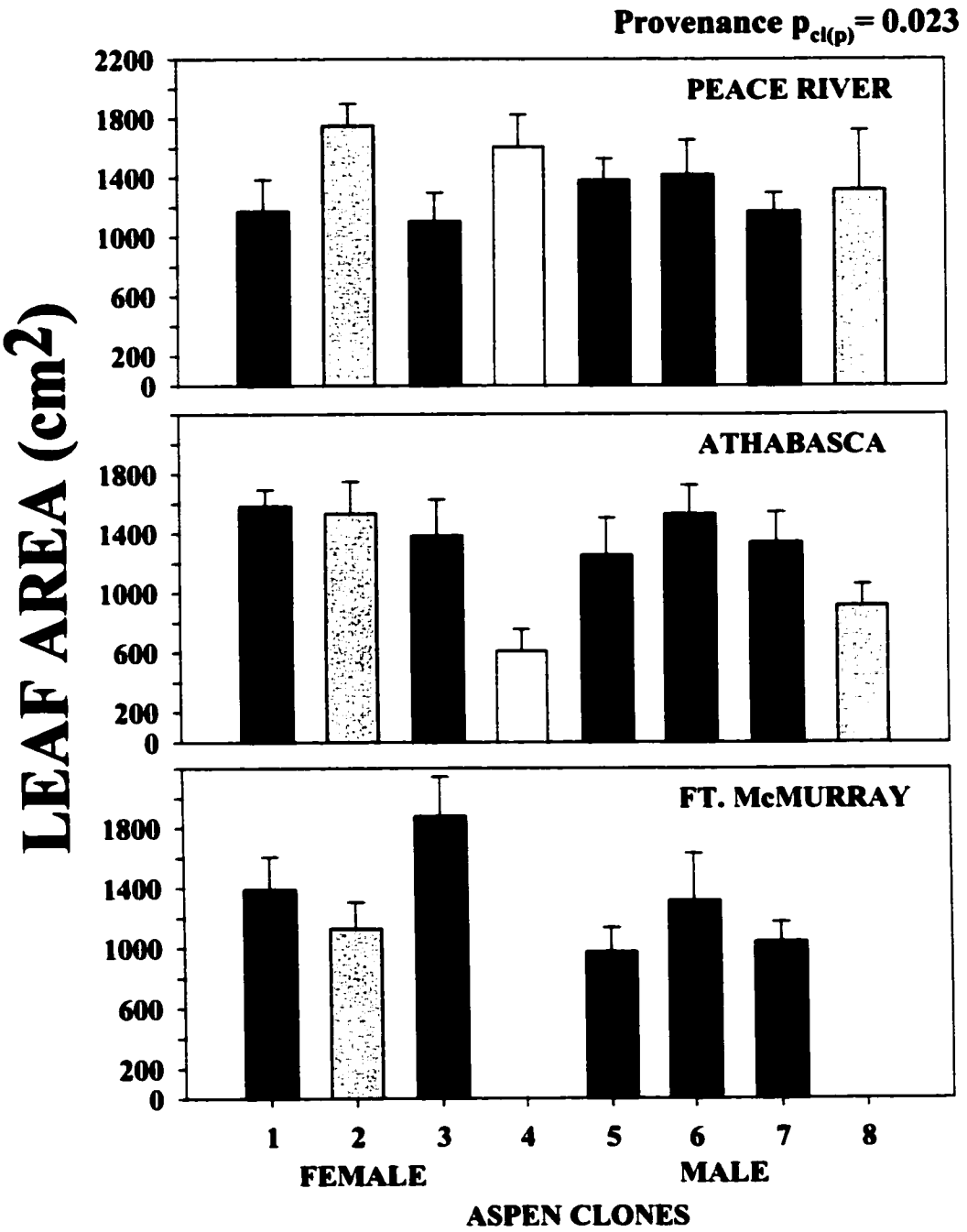


Figure 3-20. Mean stomatal conductance (mmol H_20 m^{-2} s^{-1}) (±s.e.) for 22 unselected aspen clones from three provenances (Peace River, Athabasca and Ft. McMurray) grown under both treatment levels (high and ambient CO_2) for 95 days where clones 1-4 are unselected females and clones 5-8 are unselected males from both Peace River and Athabasca and clones 1-3 are unselected females and clones 4-6 are unselected males from Ft. McMurray. Only reps 1-2 were measured at Day 60 (where $p_{cl(p)}$ is the p values for differences due to clone within provenance) (see Table3-4b).

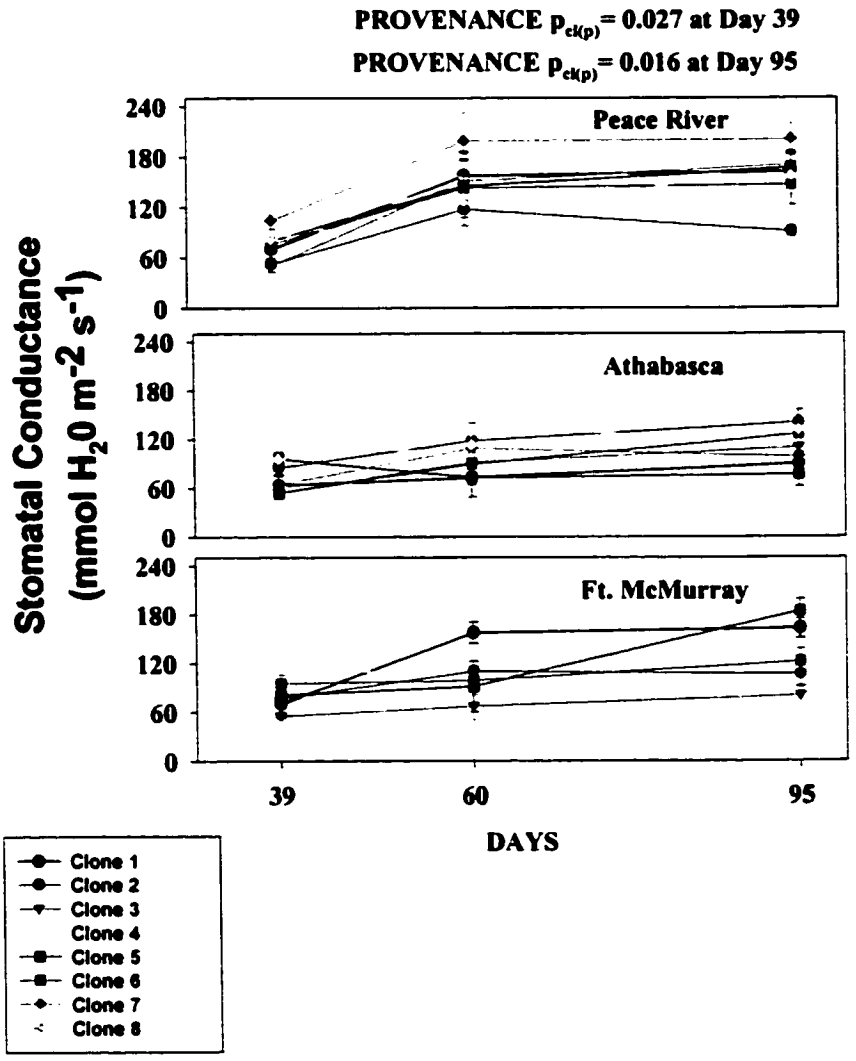


Figure 3-21. Mean height (cm) (±s.e.) for eight hybrid poplars (Assiniboine, CanAm, Green Giant, Manitou, P38 P38, Walker, Sargentii and Northwest) grown under both treatment levels (high and ambient CO_2) for 95 days. Only replicates 1-2 were measured on Day 60 (not significant) (see Table 3-5a).

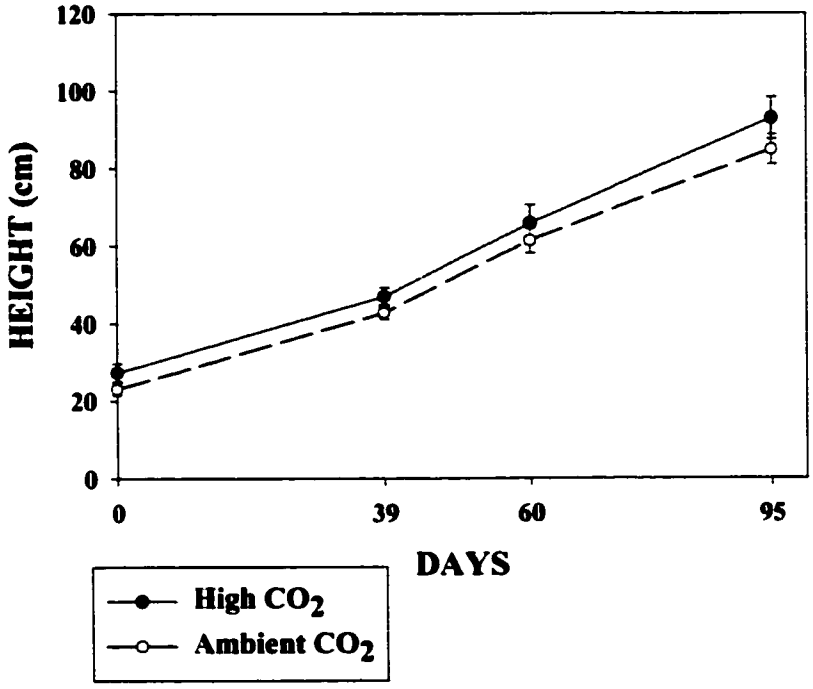


Figure 3-22. Mean final caliper (Day 95) (cm) (+s.e.) for eight hybrid poplars (Assiniboine, CanAm, Green Giant, Manitou, P38 P38, Walker, Sargentii and Northwest) grown under both treatment levels (high and ambient CO_2) (not significant) (see Table 3-5a).

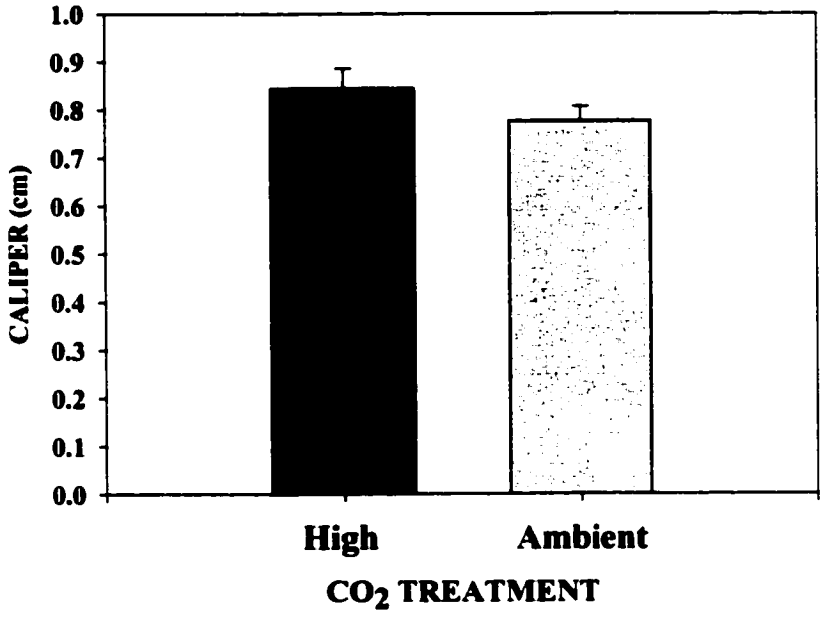


Figure 3-23. Mean (±s.e.) net assimilation (μmol CO_2 m^{-2} s^{-1}) and water use efficiency (μmol CO_2/mmol H_2O) for eight hybrid poplars (Assiniboine, CanAm, Green Giant, Manitou, P38 P38, Walker, Sargentii and Northwest) grown under both treatment levels (high and ambient CO_2) for 95 days. Only reps 1-2 measured on Day 60 (where p_t is the significant p values for treatment effect) (see Table 3-5b).

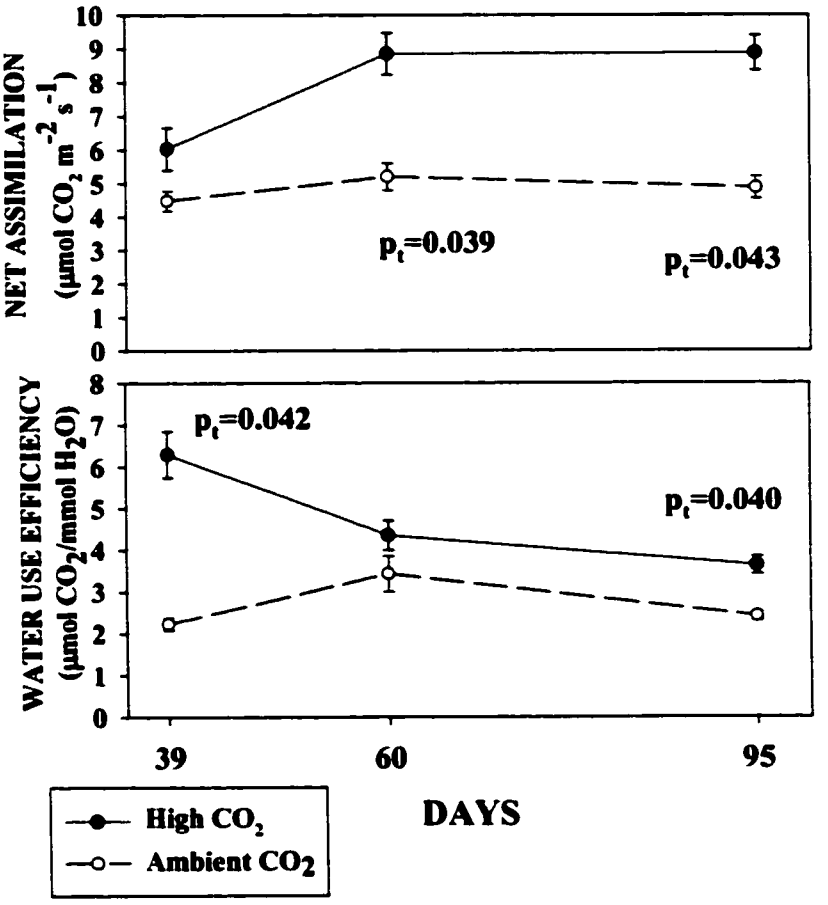


Figure 3-24. Mean score for bud burst (+s.e.) for eight hybrid poplar clones (A=Assiniboine, C= CanAm, GG= Green Giant, M= Manitou, P38= P38 P38, W= Walker, S= Sargentii and N= Northwest) under both treatment levels (high and ambient CO_2) on day seven (where p_{txcl} is the p value for the treatment by clone interaction) (see Table 3-5a).

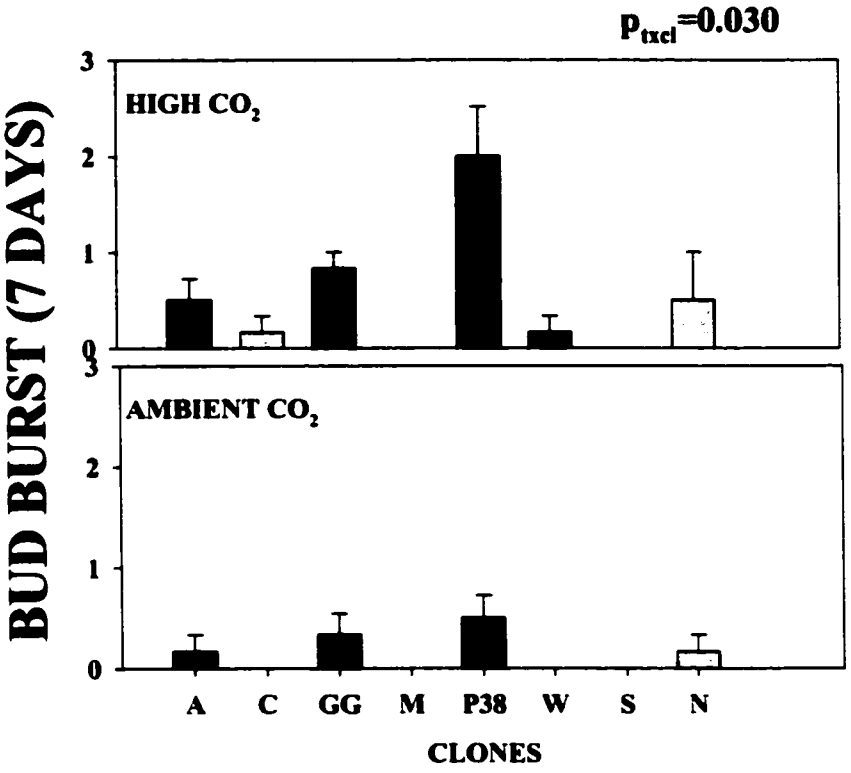


Figure 3-25. Mean height (cm) (±s.e.) for eight hybrid poplars (Assiniboine, CanAm, Green Giant, Manitou, P38 P38, Walker, Sargentii and Northwest) under both treatment levels (high and ambient CO_2) for 95 days. Only replicates 1-2 were measured on Day 60. (p_{cl} is the p values due to clonal variation) (see Table 3-5a).

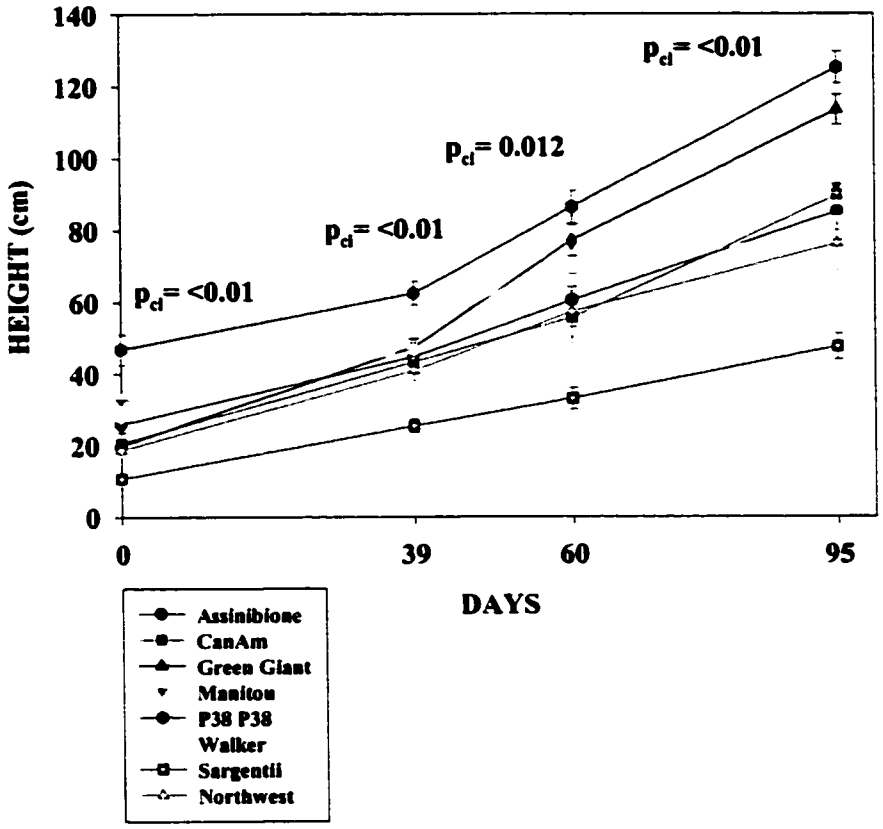


Figure 3-26. Mean initial (Day 0) and final (Day 95) caliper (cm) (+s.e.) for eight hybrid poplar clones (P38= P38 P38, N= Northwest A=Assiniboine, GG= Green Giant, M= Manitou, W= Walker, CA= CanAm and S= Sargentii) under both treatment levels (high and ambient CO_2) (where p_{cl} is the p values due to clonal variation) (see Table 3-5a).

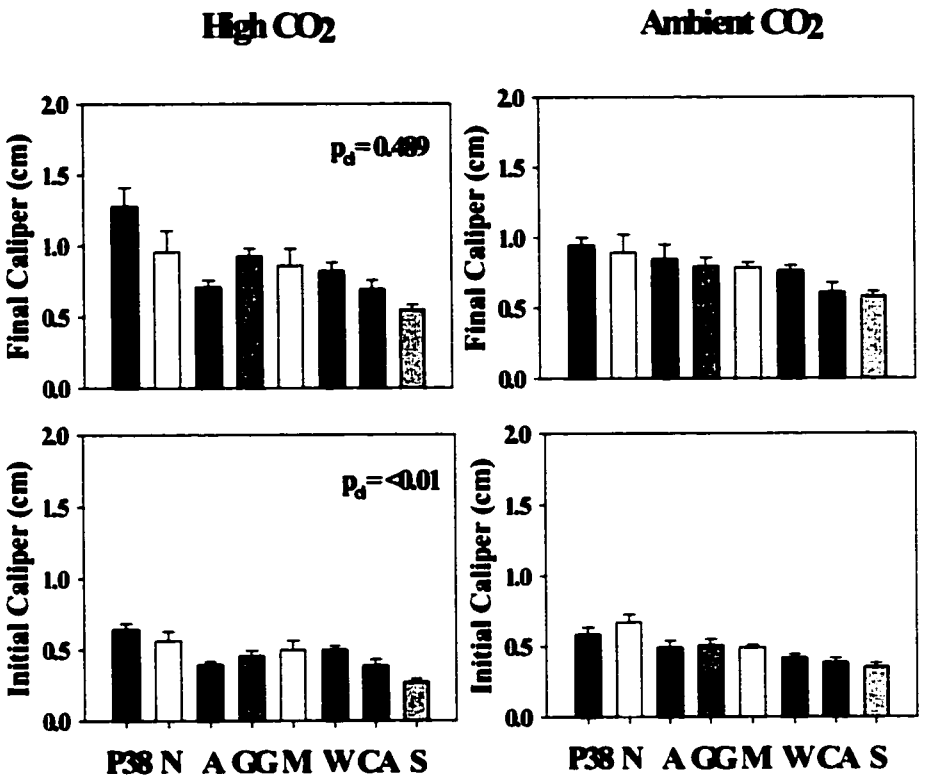


Figure 3-27. Mean leaf, stem and root dry weight (g) (+s.e.) and shoot-to-root ratio (+s.e.) for eight hybrid poplar clones (A=Assiniboine, C= CanAm, GG= Green Giant, M= Manitou, P38= P38 P38, W= Walker, S= Sargentii and N= Northwest) under both treatment levels (high and ambient CO_2) at Day 95 (p_{cl} is the p value due to clonal variation) (see Table 3-5a).

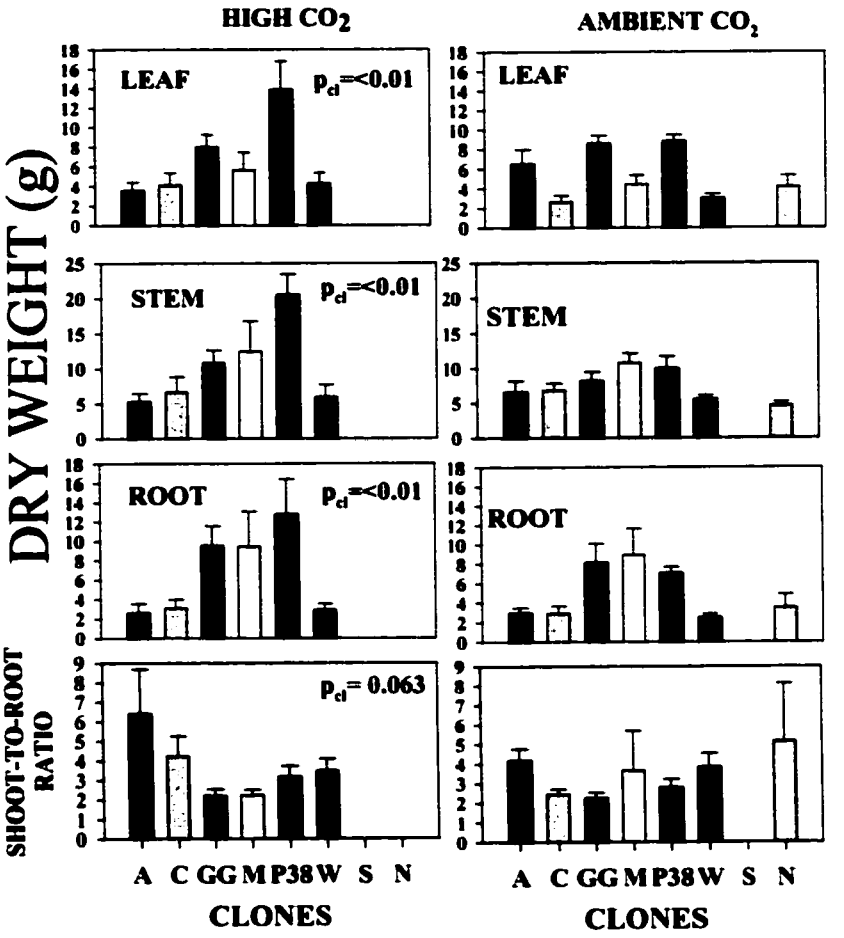


Figure 3-28. Mean (±s.e.) net assimilation (μmol CO_2 m^{-2} s^{-1}) and water use efficiency (μmol CO_2/mmol H_2O) for eight hybrid poplars (Assiniboine, CanAm, Green Giant, Manitou, P38 P38, Walker, Sargentii and Northwest) averaged over both treatment levels (high and ambient CO_2) for 95 days. Only replicates 1-2 were measured on Day 60 (not significant) (see Table 3-5b).

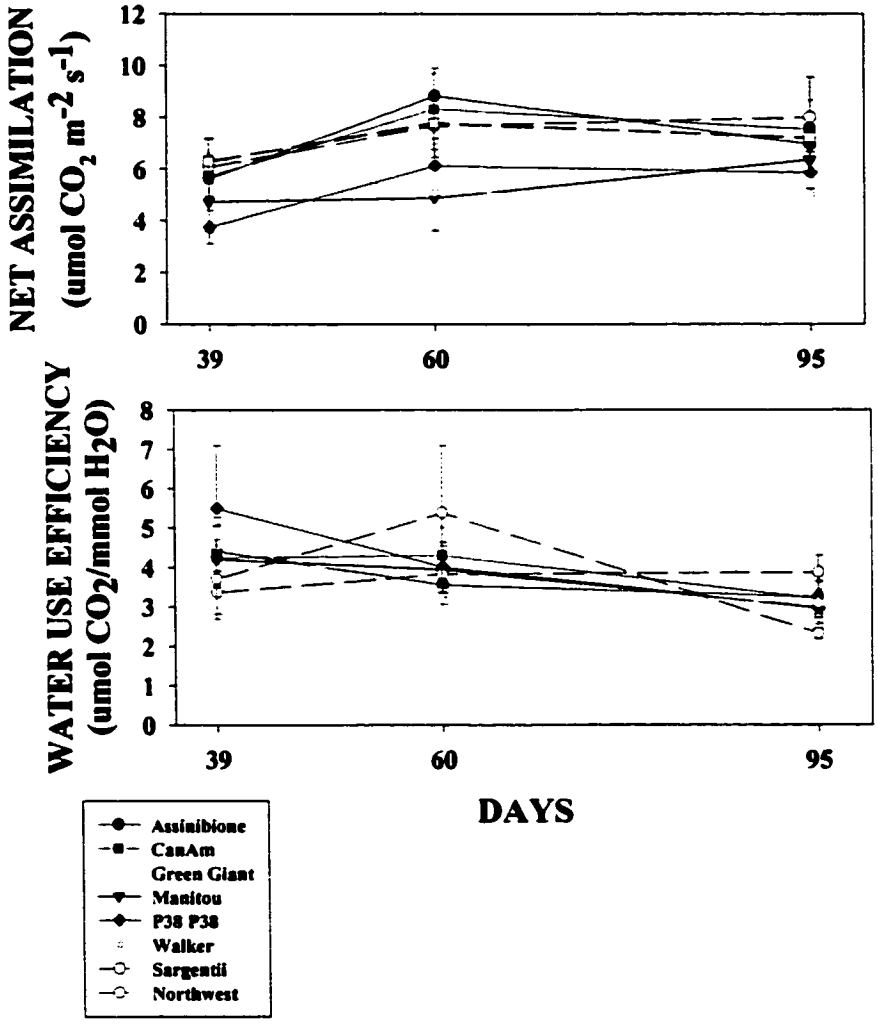
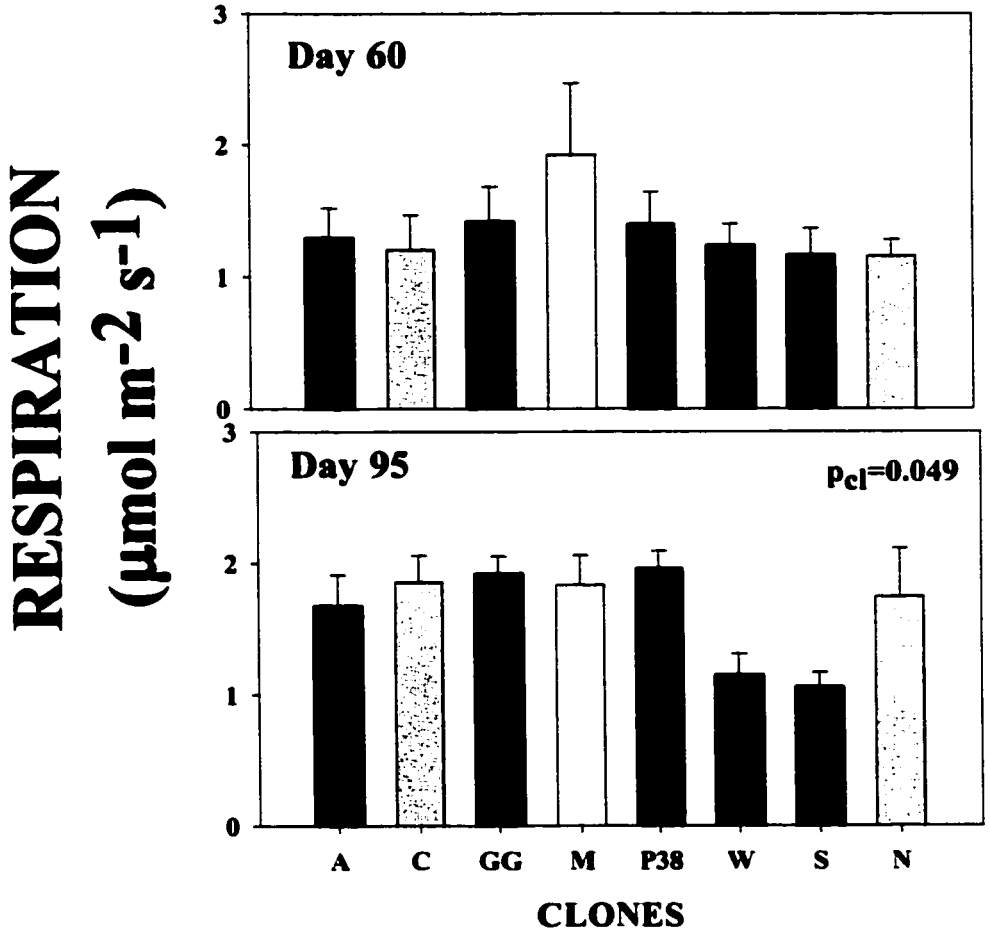


Figure 3-29. Mean respiration (μmol CO_2 m^{-2} s^{-1}) (+s.e) for eight hybrid poplar clones (A= Assiniboine, C= CanAm, GG= Green Giant, M= Manitou, P38= P38 P38, W= Walker, S= Sargentii and N= Northwest) under both treatment levels (high and ambient CO_2) at Day 60 and Day 95. Only replicates 1-2 were measured at both dates (p_{cl} is the p value due to clonal variation) (see Table 3-5b).



CHAPTER FOUR

DISCUSSION

4.1. Summary of Results

This study showed that increasing CO_2 levels in the greenhouse had a significant effect upon the physiology and morphology of both aspen and hybrid poplar, as has been found previously (Eamus and Jarvis 1989, Radoglou and Jarvis 1990a,b, Ceulemans and Mousseau 1994, Ceulemans et al. 1996, Curtis and Wang 1998, Ward and Strain 1999, Wang et al. 2000). Aspen grown under high CO_2 broke bud slightly earlier than aspen grown under ambient CO_2, which may lead to a longer growing season under high CO_2 conditions. Higher net assimilation (NA) and water use efficiency (WUE) for aspen grown under elevated CO_2 was accompanied by increased stem height and caliper, greater leaf and stem dry weights and a significantly larger root dry weight and lower shoot-to-root ratio compared to aspen grown under ambient CO_2. A similar trend was seen for hybrid poplar, which had a slight increase in stem growth (not significant) and a significant increase in NA and WUE when grown under elevated CO_2. Growth under high CO_2 conditions has been shown to increase photosynthesis in both aspen and hybrid poplar, which can allow for an increase in growth compared to trees grown under ambient CO_2 (Eamus and Jarvis 1989, Ceulemans and Mousseau 1994, Wang and Curtis 1998, Ward and Strain 1999).

Aspen classified as 'superior' in the field (larger stem height and caliper, little lateral branching, lack of visible insect or disease damage) had a larger stem height and caliper compared to unselected aspen (random males and females) after a short period of greenhouse growth, irrespective of CO_2 treatment. This could be due to earlier bud break

or to a larger total leaf area (LA), which may have increased whole plant net photosynthesis for superior aspen compared to unselected aspen. No significant morphological or physiological differences due to gender (female and male aspen) were found in this study. As well, no significant morphological or physiological differences were found among the three provenances (Athabasca, Peace River and Ft. McMurray), however there was a large amount of variation for aspen at the clone-within-provenance level, as has been found previously (Grant and Mitton 1979, Thomas 1996, Thomas et al. 1997a).

Physiological and morphological variables for the eight hybrid poplars, showed a high degree of clonal variation in both CO_2 treatments. Significant clonal differences were seen for caliper, bud burst, shoot-to-root ratios, NA, and WUE for hybrid poplar in both CO_2 treatments.

4.2. Aspen

4.2.1. Pre-treatment (ambient and high CO_2) effect on subsequent growth under elevated CO_2

In this study aspen material was initially propagated under either high CO_2 or ambient CO_2 conditions to pre-condition (or acclimate) the aspen to the different CO_2 environments (elevated and ambient CO_2) before the start of the main experiment. A number of the aspen clones (both superior and unselected) propagated under either elevated or ambient CO_2 were subsequently grown under high CO_2 conditions. Subsequent performance was then assessed to determine if propagation under elevated CO_2 levels gave any morphological or physiological advantage for growth under elevated CO_2, compared to propagation under ambient CO_2.

In the literature, most experiments initially grow or propagate all plant material

under ambient CO_2 levels and then subsequently divide the plants into the two CO_2 treatment levels for future growth. In this experiment, I anticipated that propagation under the two treatment levels (high and ambient CO_2) would enable the aspen to pre-acclimate to the experimental conditions. In particular, propagation under elevated CO_2 might result in morphological or physiological differences (eg. decreased stomatal density, increased leaf area (LA) or net photosynthesis (NA)) enabling aspen pre-acclimated to a high CO_2 environment to photosynthesize more efficiently under elevated CO_2, compared to aspen propagated under ambient CO_2. It has been shown that growth under elevated CO_2 often results in an increase in leaf number, leaf area and leaf weight per plant (Mousseau and Saugier 1992, Ceulemans and Mousseau 1994). Both Tolley and Strain (1984) and Radoglou and Jarvis (1990 a, b) found that under increased CO_2, total leaf area increased in *Populus* spp. due to a greater number of leaves. As well, increased CO_2 levels caused the formation of thicker, heavier leaves resulting from an increase in the number and size of mesophyll cells and intercellular spaces (Radoglou and Jarvis 1990a) or from the accumulation of carbohydrates in leaves formed under high CO_2 (Ceulemans et al. 1995). An increase in mesophyll cell size could increase the surface area available for CO_2 absorption, which may increase the rate of photosynthesis for aspen propagated under high CO_2 (Radoglou and Jarvis 1990a). Therefore, I thought that propagation and initial growth under high CO_2 could cause an increase in carbohydrate reserves or an increase in leaf cell size in pre-formed, as well as in neo-formed leaves. As well, growth under elevated CO_2, has been found to cause a reduction in stomatal density in young expanding poplar leaves, which indicates that increased CO_2 might have an effect on the initiation of the number of stomata during leaf formation

(Ceulemans et al. 1995). A decrease in the number of stomata will lead to a decrease in stomatal conductance and a corresponding increase in WUE for aspen propagated under high CO_2. All of these changes in aspen morphology during development under high CO_2 could produce larger stecklings with large carbohydrate reserves and with higher NA and WUE rates. Consequently, aspen propagated under high CO_2 could then grow faster when subsequently grown under high CO_2 due to morphological changes in response to propagation conditions. However, aspen initially propagated under ambient CO_2 would not develop morphological changes that would pre-acclimate them to high CO_2, and thus, might not grow or perform as well when subsequently grown under elevated CO_2, as compared to aspen propagated under high CO_2.

As seen in previous experiments (Eamus and Jarvis 1989, Ceulemans and Mousseau 1994, Curtis and Wang 1998, Ward and Strain 1999, Wang et al. 2000) I found that aspen responded strongly to increased CO_2 levels during subsequent growth, regardless of pretreatment propagation conditions. However, contrary to my prediction, aspen propagated under ambient CO_2 had greater height increases on Day 39 (12%) and 60 (15%) compared to aspen propagated under high CO_2. Although aspen from both propagation treatments (high and ambient CO_2) were no longer CO_2 limited (for photosynthesis) during the final growth period, the height difference could be due to a difference in the biomass allocation between aspen from the two propagation treatments. I believe that aspen propagated under high CO_2 may have initially allocated more carbohydrates into root biomass during the propagation phase (aspen propagated under high CO_2 were 13% taller at Day 0), since they may have been more belowground limited, whereas aspen propagated under ambient CO_2 may have initially allocated

relatively more carbohydrates towards stem growth. However, by Day 95, aspen propagated under high CO_2, also subsequently grown under high CO_2, had a greater final height (13%) compared to the aspen propagated under ambient CO_2. This increase in stem height for aspen propagated under high CO_2 may also be due to a difference in biomass allocation, where aspen propagated under high CO_2 had developed adequate root resources and started to allocate more carbohydrates into stem growth near the end of the experiment. By Day 95, aspen propagated under high CO_2 had a larger leaf dry weight, a larger root mass, a smaller specific leaf area (SLA) (surface area/ leaf mass) and higher NA and WUE, suggesting that aspen propagated under high CO_2 was ultimately better able to utilize high CO_2 conditions more efficiently in the long term compared to aspen propagated under ambient CO_2.

Aspen propagated under high CO_2 had consistently higher NA (11%), WUE (9%) and lower Gs (stomatal conductance) (3%) over all three measurement dates compared to aspen propagated under ambient CO_2, when grown under high CO_2. A decrease in Gs, possibly caused by a decrease in stomatal density (Ceulemans et al. 1995) or stomatal aperture (Radoglou and Jarvis 1990a), resulting from high internal CO_2 levels will result in both increased NA and WUE (ratio between the instantaneous rates of photosynthesis and transpiration) for plants propagated under high CO_2 (Ward and Strain 1999, Drake and Gonzàlez-Meler 1997). Even though aspen propagated under high CO_2 had higher NA and WUE over all measurement dates, those plants had a smaller height at Day 39 and 60, compared to aspen propagated under high CO_2. This difference could be due to a difference in biomass allocation, with aspen propagated under high CO_2 allocating more carbohydrates to root growth (by Day 95, aspen propagated under high CO_2 had a 7%

greater root dry weight), or it could be due to a difference in leaf dark respiration rates. At Day 60, aspen propagated under high CO_2 had higher leaf dark respiration rates (on a leaf area basis), which might offset the increase in NA, but by Day 95, aspen propagated under ambient CO_2, had higher respiration rates.

Under high CO_2, plants are less aboveground CO_2 limited, which allows for an increase in carbohydrate allocation to root growth in order to increase the uptake of limiting materials such as water and nutrients (Rogers et al. 1994, Ward and Strain 1999). Thus, I thought that aspen suckers propagated under high CO_2 would root faster, compared to aspen suckers propagated under ambient CO_2. Contrary to my hypothesis I found that, although aspen showed prolific root suckering under both high and ambient CO_2, subsequent rooting under high CO_2 was poor; a substantial number of suckers failed to root (personal observation). Stanton and Viller (1996) state that during the initial growth stage, *Populus* seedlings may be susceptible to damping-off fungi, and in my experiment stecklings produced under high CO_2 seemed to be more susceptible to fungal infection (damping off) compared to those in the ambient CO_2 greenhouse. Based on my observations, propagation under high CO_2 is not recommended. Further study should be undertaken to determine the effect of elevated CO_2 levels on both aspen sucker production and steckling rooting, as well as its influence on fungal infections. As well, the ability of CO_2 pretreatment conditions to cause a plant to acclimate to future experimental conditions must be examined further. A follow-up study involving a larger number of aspen clones propagated under different CO_2 treatment conditions and then grown under high CO_2 for a longer period of time might indicate any benefit due to pre-treatment conditions.

4.2.2. Effects of CO_2 treatment on growth and physiology of aspen

The effect of elevated CO_2 on the timing of bud burst remains unclear. In my experiment, aspen grown under high CO_2 broke bud slightly earlier than aspen grown under ambient CO_2. A similar situation was seen for Scots pine, where earlier bud burst under elevated CO_2 was seen for two growing seasons (Jach and Ceulemans 1999). This contrasts, however, with findings from Guak et al. (1998) who found that bud burst was delayed (approx 10 days) by exposure to elevated CO_2 in Douglas-fir (*Pseudotsuga menziesii*). For poplars, Ceulemans et al. (1995) also found that exposure to increased CO_2 (~700 ppm) delayed bud burst by 2 to 5 days in the poplar hybrid clone Beaupré, but had no effect on the poplar hybrid clone Robusta, however, Thomas (1996) found that aspen broke bud earlier under increased CO_2 (with an increase in temperature). For my experiment, under controlled greenhouse conditions, an earlier bud burst could account for an extended growing season, which causes a potential increase in biomass accumulation (Beuker 1994).

In the field, an earlier bud burst may increase the risk of damage from early spring frosts, but it has been speculated that growth under high CO_2 may increase the frost hardiness of buds due to an increased cellular content of sugars which act in cellular protection against frost damage, allowing buds to open earlier in the spring (Guak et al. 1998). Elevated CO_2 may also affect bud phenology directly through physiological changes (hormonal concentrations), which can alter the timing of growth and dormancy periods (Jach and Ceulemans 1999). It is important to note, that a longer growing season due to earlier spring bud break, can be offset by an earlier bud set which might shorten the growing season. Ceulemans et al. (1995a) found that poplar clones Robusta and

Beaupré broke bud later and set bud up to 20 days earlier under high CO_2 compared to ambient CO_2. Findings from Curtis and Teeri (1992), however, show delayed senescence which allowed for continued late season CO_2 assimilation for *Populus grandidentata* (bigtooth aspen) under elevated CO_2, compared to ambient CO_2 grown plants which had begun to decline in NA and begun to enter dormancy. Therefore, the effects of elevated CO_2 on bud phenology may alter carbon assimilation throughout the growing season, which in turn, will affect growth.

In response to elevated CO_2, aspen grew taller and produced larger diameter stems, as compared to aspen grown under ambient conditions, as has been reported for poplars (Ceulemans and Mousseau 1994, Ceulemans et al. 1996, Wang and Curtis 2000, Wang and Curtis 2001). I observed a 14% increase in stem height and a 16% increase in final caliper in response to elevated CO_2 compared to aspen grown under ambient CO_2, which agrees with finding from Wang and Curtis (2001) who found that aspen grown under double CO_2 concentration (high nitrogen levels) had significantly greater height (19%) compared to aspen grown under ambient CO_2. Ceulemans et al. (1996) also found that poplar clones Beaupré and Robusta were 7 and 18% taller, respectively, and had an increase in stem volume (43 and 58% respectively) after two years growth under high CO_2 (~700 ppm).

In agreement with other published reports (Ceulemans and Mousseau 1994, Curtis et al. 1995, Curtis and Wang 1998, Ceulemans et al. 1996, Wang and Curtis 2000) I observed a tendency towards greater total leaf area (LA) (18%) in response to elevated CO_2. Tolley and Strain (1984) and Radoglou and Jarvis (1990a) reported that an observed increase in leaf area was due to an increase in the number of leaves, rather than

a change in leaf size. Similar increases in LA were found by Zak et al. (2000) and Curtis et al. (2000) who found that LA increased by 28% under elevated CO_2 for aspen. After 2.5 growing seasons, Curtis et al. (2000) found that elevated CO_2 caused an increase in LA of aspen in the upper, middle and lower portions of the crown. This increase in the canopy could possibly lead to greater self shading of the leaves which would limit the potential for increased growth under high CO_2 (Jach and Ceulemans 1999, Curtis et al. 2000). Thus, under elevated CO_2, a larger leaf area would increase the number of leaves photosynthesizing, however, this increase could result in self-shading within the canopy, such that there was relatively little additional growth resulting from an increased leaf area. The increase in SLA (leaf area/ leaf weight) that I observed under elevated CO_2 has been shown to be due to increased leaf thickness caused by an increase in cell size or numbers (Radoglou and Jarvis 1990a) or increased carbohydrate accumulations under elevated CO_2 (Ceulemans et al. 1996). An increase in leaf cell numbers (mesophyll) may help to increase photosynthetic capacity per unit leaf area (Curtis et al. 2000). Thus, an increase in total leaf area and mesophyll cell numbers, in addition to increased levels of atmospheric CO_2, could contribute to the increase in total NA for aspen grown under high CO_2.

An increase in biomass accumulation of aspen after 95 days exposure to elevated CO_2 was similar to the average response of 21 other woody plant species to similar treatments, as summarized by Curtis and Wang (1998). Past studies have shown a 30% increase in leaf dry weight (Zak et al. 2000) and 34% increase in stem dry weight of aspen (Curtis et al. 2000, Wang et al. 2000) under high CO_2, I observed a 30% increase in leaf and a 45% increase in stem dry weights under high versus ambient CO_2. In addition

to increases in stem and leaf biomass, under elevated CO_2 more biomass accumulation occurs below ground with a significant increase in carbon allocation to roots, especially fine roots (Pregitzer et al. 1995, Kubiske et al. 1997). Zak et al. (2000) found a 52% increase in root biomass, which is comparable to the 58% increase in root biomass found in my experiment. I found a disproportionate increase in below ground dry weight, leading to a significant decrease in the shoot-to-root ratio (32% lower for high CO_2) for aspen grown under elevated CO_2, which is consistent with other studies (Pregitzer et al. 1995). Zak et al. (2000), however, found that elevated CO_2 did not cause disproportionate allocation of biomass to roots.

Elevated CO_2 causes a decrease in the shoot-to-root ratio because there is an increase in carbon allocation to roots as belowground resources (water and nutrients) become the more limiting factor (Rogers et al. 1994, Dickson et al. 1998). Thus, a larger LA or leaf number and a corresponding higher NA of the crown, may allow for more carbon to be allocated to the roots. The larger and more productive root system, resulting from growth under high CO_2, could lead to better acquisition of water and nutrients during establishment after outplanting and even during drought conditions. Therefore, the response of plant biomass allocation, especially to roots, has important implications for the future performance of greenhouse produced trees used in reforestation and reclamation.

The effect of elevated CO_2 on NA in my experiment followed a pattern typical of many woody species, with an increase in NA (Radoglou and Jarvis 1990a, Ceulemans et al. 1995, 1996, Kalina and Ceulemans 1997, Curtis et al. 2000). Wang et al. (2000) found that for six genotypes of aspen grown at elevated CO_2 (~700-750 ppm), net CO_2

assimilation was 51 to 40% higher than aspen grown under ambient conditions, which compares to results from my experiment where aspen grown under ambient CO_2, had a 60% greater NA (by Day 95) when grown under elevated CO_2. An increase in total NA for poplar under elevated CO_2 has been attributed to an increase in total leaf area (Radoglou and Jarvis 1990a) together with an increase in the number of reaction centers or an improved turnover rate of the redox status (increased Rubisco activity) of the photosynthetic apparatus (Ceulemans et al. 1995). Thus, increases in atmospheric CO_2 and changes in leaf structures, which allow for more efficient CO_2 fixation, and a larger total leaf area all contribute to greater total plant NA under high CO_2. I found no significant effect of elevated CO_2 on Gs, which agrees with the conclusions reached by Curtis and Wang (1998) who found that generally elevated CO_2 had no effect upon tree Gs. These findings contrast with recent research by Thomas (1996) and Ceulemans et al. (1996) who found a decrease in Gs at elevated CO_2, likely due to changes in stomatal aperture. In my experiment, even though no significant difference in Gs between the two treatments (high and ambient CO_2) was seen, aspen grown under elevated CO_2 had both higher NA and WUE compared to aspen grown under ambient CO_2. Wang et al. (2000) found a similar situation among six genotypes of aspen, where some genotypes showed increased NA under high CO_2, but Gs remained unaffected. Under high CO_2, other factors such as changes in leaf structure and increased atmospheric CO_2, instead of stomatal conductance, might determine rate of CO_2 fixation (Field et al. 1995, Norby et al. 1996, Wang et al. 2000).

Many studies report that elevated levels of NA are not maintained during long term exposure to increased CO_2, and that reductions in photosynthesis (referred to as

acclimation or down regulation of photosynthesis) may occur (Gunderson and Wullschleger 1994, Tjoelker et al. 1998, Curtis and Wang 1998, Li et al. 1999, Tissue et al. 1999). Wang and Curtis (2001) found a significant negative adjustment (14-16% decrease), or acclimation of NA, for aspen grown for five months at elevated CO_2 (~700 ppm). However, at the end of my experiment, no acclimation of NA over time with growth under elevated CO_2 was seen, which agrees with other studies (Gunderson et al. 93, Sage 1994, Drake and Gonzàlez-Meler 1997, Liu and Teskey 1995). For aspen, Curtis et al. (2000) found no down regulation of NA in aspen grown under high CO_2 after 2.5 growing seasons, which is similar to results seen in hybrid poplars after 1 to 2.5 growing seasons (Curtis et al. 1995, Kalina and Ceulemans 1997). The decrease of NA under high CO_2 reported in many studies, might be due to reductions in carbohydrate sinks (eg. roots) caused by restricted root growth, which cause feedback inhibition of NA through starch accumulation (Arp 1991, Sage 1994). Therefore, plants grown in large pots, as was the case in this experiment, or in the ground may continue to respond strongly to increased CO_2 over longer time periods. Therefore, processes such as carbohydrate and nutrient allocation within the plant need to be examined further to understand the long term consequences of rising CO_2 on photosynthesis in aspen.

In summary, increased CO_2 had a positive effect upon growth, above and below ground biomass accumulation, WUE and NA in *P. tremuloides*. In addition, there was a relatively greater increase in stem biomass, which translated into taller and larger diameter stems for aspen grown under high CO_2. Allocation of carbohydrates to belowground limited structures, such as root biomass was also increased under high CO_2. Both total plant NA and WUE increased in response to elevated CO_2, due to a number of

factors, which allowed for an increase in growth for aspen.

4.2.3. Variation in growth and physiology of superior and unselected aspen under two CO_2 treatments

Tree improvement programs attempt to determine genetic superiority for some economically important traits, such as increased stem volume and height by early testing at a juvenile age under greenhouse conditions (Williams et al. 1987, Zsuffa 1992). This early performance under controlled conditions may be reflective of later field performance (Wu et al. 1997). If performance of clones could be determined through assessment of morphological traits during juvenile growth in a controlled environment, it might allow insight into future genetic performance of older clones under field or plantation conditions. Studies have shown a significant correlation between early growth in the greenhouse and later field performance (Ceulemans et al. 1987, Wu et al. 1997, Cantin et al. 1997). For jack pine, Carter et al. (1990) found a correlation between height after 16 months and height after seven years in the field. For black spruce (*Picea mariana* (Mill)), strong correlations were found between early greenhouse growth measurements and field performance by age 13 (Williams et al. 1987). In addition to assessing morphological traits, many researchers have tried to determine a correlation between yield and net photosynthesis (Ceulemans and Impens 1983, Ceulemans et al. 1987, Nelson 1988). Clonal selection for photosynthetic characteristics (increased NA, WUE) in the greenhouse, may have a potential for predicting biomass production in the field, however there are few reports of a correlation between early physiological performance and later growth for most poplars and other species (Nelson and Ehlers 1984, Isebrands et al. 1988, Thomas 1996). Correlations between early growth in a controlled environment and future performance in field conditions for morphological and

physiological traits would allow for early identification of clones with less desirable genetic traits (slow stem growth, increased lateral branching, susceptibility to insects and disease) so that they could be removed from the breeding program earlier and not used in future intensive silvicultural systems.

Currently, *Populus* spp. (including aspen and hybrid poplars) are used in forestry due to their rapid growth, ease of vegetative propagation (hybrid poplars only) and ability to grow on a variety of sites (Gordon and Promnitz 1976). These characteristics allow for poplars to be used in increasing wood production for forestry, and more recently, in the reclamation (revegetation) of land disturbed by mining operations. Thus, it is important to determine if early growth under controlled greenhouse conditions could provide an opportunity for the selection of 'superior' aspen clones with desirable traits, such as increased biomass production (accelerated stem growth) for forestry plantations or a larger root system to aid in reclamation of oil and gas sites.

Since it is predicted that our global environment will change, due to an 1.8% per annum increase in atmospheric CO_2 (Eamus 1996), the selection of genetically superior clones should consider how trees will respond to that future environment (Wang et al. 1994, Cantin et al. 1997, Wang et al. 2000). Studies indicate that for jack pine, families selected for rapid growth under present ambient CO_2 conditions will continue to be fast-growing with a doubling of atmospheric CO_2 (~700 ppm) (Cantin et al. 1997). Wang et al. (2000) found differential growth and biomass allocation responses to increased CO_2 for six different genotypes of aspen, and genetic variation in response to increased CO_2 had also been seen for families of black spruce (Wang et al 1994). Genetic variation among genotypes in response to increased CO_2 suggests that clones selected for

'superior' traits at current CO_2 levels, may not be the best performers under future conditions. Understanding any genetic variability in response to increased CO_2, may allow tree breeders to select clones that might be better able to take advantage of future high CO_2 environments.

In this experiment, aspen clones deemed to be phenotypically 'superior' (determined by forestry guidelines) in the field were tested under controlled conditions to determine if early greenhouse performance of superior clones differed when from that of randomly selected male and female aspen clones. Under greenhouse conditions, superior aspen was found to break bud earlier compared to unselected aspen, irrespective of CO_2 treatment levels. This early bud burst may result in the early onset of growth, which can extend the growing season and allow for a greater increase in biomass production (Beuker 1994). It is interesting to note, that by the end of the experiment, superior aspen showed a slight decline (4%) in NA compared to unselected aspen (data not shown). This decline in NA might indicate superior aspen was beginning to enter dormancy and bud set before unselected aspen. Earlier bud burst and bud set, compared to unselected aspen, suggest that it was not a longer growing season, but other traits such as more efficient CO_2 fixation by leaves or larger total leaf area, that may account for the greater stem growth demonstrated by superior aspen. Superior aspen were taller than unselected aspen under both high CO_2 (13%) and ambient CO_2 (11%). Superior aspen also had a greater initial and final caliper (8% and 11% respectively) compared to unselected aspen under both treatment levels.

Superior aspen had a greater total leaf area (18%) and slightly smaller specific leaf area (2%) compared to unselected aspen. An increase in total LA might indicate an

increase in the number of leaves of superior aspen, whereas a decrease in SLA may indicate an increase in leaf cell numbers and size, which may lead to a corresponding increase in total plant NA. Therefore, aspen trees selected for superior growth characteristics in the field continued to display superiority under controlled growth in the greenhouse irrespective of CO_2 treatment. The genetic superiority of the selected aspen clones can be attributed to a possible longer growing season, greater total leaf area and higher NA and WUE throughout the first part of the experiment (data not shown). Thus, the results from this experiment suggest that trees selected as superior in the field were genetically superior and that those superior traits were detectable during a short period of greenhouse growth. Growth under elevated CO_2 did not change the trend of superior aspen clones out-performing unselected aspen, although both superior and unselected aspen responded positively to elevated CO_2. For example, compared to growth of superior clones under ambient CO_2, height and caliper by Day 95 increased 16% and 17% respectively, with a corresponding decrease in the shoot-to-root ratio, under high CO_2. Therefore, growth in the greenhouse under elevated CO_2 may indicate that aspen selected for superiority under current ambient CO_2 may also display superior characteristics under future, elevated CO_2 environments.

4.2.4. Variation in growth and physiology of male and female aspen under two CO_2 treatments

Past studies have shown that male and female individuals of dioecious species often differ physiologically and ecologically from one another (Dawson and Bliss 1989a, b, Dawson and Ehleringer 1993, Delph and Meagher 1995, Laporte and Delph 1996, Wang and Curtis 2001) For example, male and female species of arctic willow (*Salix arctica*) have sex-specific physiological traits and occupy different habitats (more

females on mesic and high nutrient sites compared to males) (Dawson and Bliss 1993, Jones et al. 1999). Grant and Mitton (1979) found an ecological difference between the habitats of male and female aspen in the Colorado Rockies. A greater number of female aspen was found at lower elevations, compared to more males found at higher elevations. Although the mechanism is not known, this difference is thought to be due to differences in biomass allocation, with female trees allocating a larger amount of resources into reproduction (Boudreau 1958, Grant and Mitton 1979, Dawson and Ehleringer 1993). Thus, the resources needed for reproduction may restrict females to more favourable habitats where they can best capture resources required for photosynthesis, and ultimately, reproduction.

Under ambient CO_2 I found that male aspen were taller (9% by Day 95, but not significant) than female aspen, but had a similar caliper, which is similar to findings by Bourdeau (1958) who found that female aspen had a smaller stem height and caliper compared to male aspen. Wang and Curtis (2001), however, only found significant gender differences in low-nitrogen soils, where female aspen were taller (12.8% taller under ambient CO_2 and 11.5% taller under elevated CO_2) than males at both elevated and ambient CO_2. In the same experiment, under high-nitrogen soils, nutrient conditions similar to my experiment, male aspen was taller (11.9% taller under ambient CO_2 and 5.4% taller under elevated CO_2) than female aspen under both CO_2 treatments (but not significant). Under ambient CO_2, the height difference might be due to males having a slightly higher NA (6% greater by Day 95) and a lower SLA compared to females (not significant) (data not shown). Higher NA rates in male plants has been attributed to females allocating more nitrogen to reproductive structures, causing a decrease in

nitrogen available for photosynthesis (Laporte and Delph 1996).

However, under elevated CO_2, the average height growth for both males and females increased, but female aspen were taller (8%) with a larger caliper (8%) compared to male aspen by the end of the experiment. Under high CO_2, female aspen was taller than male aspen possibly due to: earlier bud break, which extended the growing season, and a larger total LA (26%, not significant), which increased total carbon assimilation. The greater size of female aspen versus male aspen under high CO_2 was not linked to a greater NA; in fact, males had greater NA than females (9% larger NA for males by Day 95) under high CO_2. Laporte and Delph (1996) found that male *Silene latifolia* had smaller growth, but higher NA compared to females. As well, Wang and Curtis (2001) found that male aspen trees had higher NA than female aspen at both ambient and elevated CO_2. However, males had a lower biomass compared to females under high CO_2, similar to findings in this experiment. This discrepancy between NA and biomass response to increased CO_2 may be due to higher respiration rates in male trees which might have offset the higher NA and resulted in lower biomass accumulation (Wang and Curtis 2001).

In this experiment, gender differences (although not significant) between male and female aspen occurred in non-reproductive plants, suggesting that different reproductive costs of males and females may not be the only reason for ecological and physiological differences between genders. Males and females may also diverge physiologically and morphologically, not because of differences in the absolute cost of reproduction, but due to requirements for different resources needed for both reproduction and growth (Dawson and Geber 1999). Results from this experiment also

indicate there is the possibility that exposure to increased levels of high CO_2 and the differing response between male and female trees could alter the productivity and distribution of *Populus tremuloides* if atmospheric CO_2 concentrations rise over the next decade. However, more research is needed into the possible underlying mechanisms for differences in NA and biomass accumulation between male and female aspen at both high and ambient CO_2.

4.2.5. Variation among provenances in growth and physiology of aspen under two CO_2 treatments

Between the three different provenances (Peace River, Athabasca and Ft. McMurray), more variation in morphological and gas exchange traits were found at the clone within provenance level, compared to the provenance level. Such high levels of variation among genotypes within provenance in both morphological and gas exchange traits have been seen in Sitka spruce (Centritto et al. 1999, Centritto and Jarvis 1999) as well as aspen (Cheliak and Dancik 1982, Jelinski and Cheliak 1992, Thomas et al. 1997a,b, Wang et al. 2000). As seen in my experiment, Thomas et al. (1996, 1997a,b) found more genetic variation at the level of the clone within provenance rather than among provenances for aspen. Clonal variability within provenances was significant for pre-formed leaf shape and for mature leaf shape (neo-formed growth), as well, as for leaf area and stomatal conductance (only at Days 39 and 95). Lack of significant provenance variation in this experiment may indicate that for aspen, populations are not specialized for certain environments and the high degree of genetic variation among clones within provenances would provide opportunity for future adaptations of populations to other environments (Thomas 1996). Although variation at the provenance level was not seen in the greenhouse, provenance performance must be assessed in the field at a number of

sites to determine if provenance has an effect on aspen performance.

All clones from the different provenances displayed an increase in stem height and caliper when grown under elevated CO_2 as compared to growth under ambient CO_2. In both CO_2 treatments, there is a clear trend with the more northerly clones (Ft. McMurray) always being the shortest, while those from the more southern provenance (Athabasca), were always the tallest. Aspen from Athabasca may have outperformed clones from the other provenances under elevated CO_2 since they broke bud slightly earlier, which could extend the growing season, and they had higher NA and WUE throughout most of the experiment (not significant, data not shown). There does not seem to be any obvious reason for the poor performance of clones from the Fort McMurray region, although the clones tended to have slightly lower shoot-to-root ratio which may indicate more carbohydrate allocation towards root growth compared to stem growth (data not shown). Clone response to photoperiod programming for the greenhouse chambers may also have had an effect on performance, where the daylength used in the greenhouse mimicked growth conditions of the more southern regions (average of spring conditions for Meander River and Calgary). The natural growing season for more northern regions would have a longer daylength and colder temperatures compared to growing seasons in more southern areas. The photoperiod used in the experiment stimulated natural conditions found in southern areas, which could possibly affect the timing of bud burst and growth of clones acclimated to northern photoperiod and conditions. If photoperiod programming was the cause of relatively lower growth for clones from more northern regions, it further stresses the need to test for provenance differences in the field, as well as clone within provenance variation.

Despite the evidence of extensive variation among aspen clones found in previous studies, there was little evidence of significant differential response to elevated CO_2 found in this study (only a significant treatment by clone within provenance interaction for height at Day 95). In a study of hybrid polar clones Robusta, Beaupré, Columbia River and Raspalje all responded positively to increased CO_2, however, their response ranged from a 22% increase in dry mass for Columbia River to a 90% increase in dry mass for Robusta (Radoglou and Jarvis 1990a, Ceulemans et al. 1995a). As well, Wang et al. (2000) found variation in the response of aspen genotypes to CO_2 enrichment for both biomass and NA. This variation in growth response to elevated CO_2 may indicate a shift in performance of aspen clones under a future, elevated CO_2 environment.

4.3. Hybrid Poplar

4.3.1. Effect of CO_2 treatment on growth and physiology of hybrid poplar

Since fast growing poplar hybrid clones are seen as providing an opportunity to increase biomass production for wood, as well as for reclamation and reforestation, it is important to examine how these plants might benefit from greenhouse growth under elevated CO_2 (Ceulemans et al. 1987). As discussed for aspen, elevated CO_2 has been shown to cause an increase in total tree productivity (approximately 30% in deciduous trees) as a result of increasing NA under elevated CO_2 (Eamus and Jarvis 1989, Mousseau and Saugier 1992, Ceulemans and Mousseau 1994, Ceulemans et al. 1995a,b, 1996, Curtis and Wang 1998, Ward and Strain 1999). I found that hybrid poplar showed only modest increases in height (9%) and stem caliper (8%) as a result of exposure to elevated CO_2, but, the magnitude of the response of hybrid poplar in this experiment is lower than what has been seen in past studies (Radoglou and Jarvis 1990 a,b, Will and

Ceulemans 1997, Dickson et al. 1998). Results from Ceulemans et al. (1995 a,b) found that two poplar clones (Beaupré and Robusta) both responded positively to elevated CO_2 (~700 ppm) with increased stem height (14 and 17% respectively) and above ground biomass production after one growing season.

Studies have shown that, on average, photosynthetic rates for most tree species were 40-50% greater at higher CO_2 concentrations compared to ambient CO_2 concentrations (Gunderson and Wullschleger 1994, Ceulemans and Mousseau 1994, Ward and Strain 1999). For hybrid poplars, Ceulemans et al. (1995a) found a stimulation of NA under elevated CO_2 for poplar clones Robusta and Beaupré in the order of 70%. I found slightly lower results, where NA (45%) and WUE (34%) for hybrid poplar clones grown under high CO_2 were significantly higher, by the end of the experiment, compared to hybrid poplar clones grown under ambient CO_2. This increase in NA under high CO_2, may allow the poplars to allocate an increasing amount of resources into stem growth. Under high CO_2, a 30% and 16% increase respectively in stem and leaf dry weights, indicates that a large proportion of carbohydrate due to an increase in NA is allocated to the stem under high CO_2 (not significant, data not shown).

As was seen in aspen, NA was significantly higher throughout the experiment for hybrid poplars grown under elevated versus ambient CO_2, and there was no evidence of down regulation of NA by the end of the experiment, which agrees with some past studies (Gunderson et al. 1993, Sage 1994, Drake and Gonzàlez-Meler 1997, Liu and Teskey 1995). Under elevated CO_2, Gunderson et al. (1993) found no NA down regulation (acclimation) for both yellow poplar (*Liriodenderon tulipifera*) and white oak (*Quercus alba*) after three years. For poplars, Kalina and Ceulemans (1997) observed no

acclimation of NA for the hybrid poplar clone Beaupré, however, Robusta did show acclimation of NA after two years. The lack of NA acclimation seen is this experiment may be due to unlimited rooting volume (small rooting volume leads to sink limitations) (Gunderson et al. 1993) or due to the experiment only covering one growing season.

In this experiment, there was a large amount of clonal variation for a number of morphological and physiological traits, including bud burst. A significant clone by treatment interaction for bud burst was seen in the greenhouse under high CO_2, which advanced bud burst to a different degree for each clone. In contrast, Ceulemans et al. (1995) found that exposure to increased CO_2 (~700 ppm) delayed bud burst by 2 to 5 days in the poplar hybrid clone Beaupré, but had no effect on the poplar hybrid clone Robusta. In my experiment, six clones broke bud earlier under high CO_2 compared to ambient CO_2; only four of those, Green Giant, P38 P38, Northwest and CanAm showed a positive growth response to high CO_2 (15%, 7%, 24% and 22% increase in growth respectively under high CO_2). The earlier bud burst under high CO_2 may have allowed for a longer growing season, ultimately leading to greater growth. Although both Assiniboine, and Walker also broke bud earlier under high CO_2, they had greater growth under ambient CO_2 (10% and 5% respectively). These differences show that performance and response of clones relative to each other can vary with CO_2 treatment levels.

There is considerable genetic variation in growth responses of poplar to increased CO_2 (Radoglou and Jarvis 1990a, Ceulemans et al. 1995, 1996, Kalina and Ceulemans 1997, Dickson et al. 1998, Wang and Curtis 2000). Most of the hybrid poplar genotypes I tested exhibited a varied response to increased CO_2, but clones varied significantly in

the extent of response for: height, biomass accumulation, LA, shoot-to-root ratios, bud burst and respiration. Irrespective of treatment, P38 P38 consistently had the largest stem height and caliper and the largest above ground biomass. Although NA rates did increase under high CO_2, the large increase in growth is likely also due to this clone having the greatest total LA under both treatments, which could increase total plant NA (data not shown).

A different growth strategy was seen for the clone Green Giant, which had the second largest stem height with a small caliper. Instead of allocating resources into stem growth, Green Giant was seen to allocate more resources into root growth, as seen in the low shoot-to-root ratio under both treatment levels. Similar growth patterns are seen with Tristis hybrid, which favours early root growth and develops a larger root system compared to Eugenei, which allocates more carbon towards leaf production and height growth under high CO_2 (Michael et al. 1988, Dickson et al. 1998). Sargentii consistently had the smallest stem height and caliper under both CO_2 treatments, as well as one of the highest NA rates by the end of the experiment. Unfortunately, there were not enough Sargentii clones to destructively harvest, and I can only hypothesize that Sargentii was disproportionately allocating a larger proportion of photoassimilates into root biomass compared to stem biomass. For many of the other clones, there was a change in rank depending upon the CO_2 level. Thus, these genetically controlled growth responses are significant factors in the individual clonal response to elevated CO_2.

In this experiment, the genetic variation in growth response among the clones saw a 7% and 12% increase in height and caliper for Green Giant to a 24% and 7% increase in height and caliper for Northwest under high CO_2. It is interesting to note that both

Assiniboine (10% and 16.3% larger height and caliper under ambient CO_2) and Sargentii (13.1% and 6% larger height and caliper under ambient CO_2), were actually larger under ambient CO_2 suggesting that some hybrid poplar clones are poor performers under high CO_2. Radoglou and Jarvis (1990a), found total dry mass of four clonal poplars was significantly increased by 45% on average after growth under high CO_2 (~700 ppm), however the increase in growth response varied among the clones (eg. 22% for Columbia River clone and 90% for a Robusta clone) as seen in this experiment. As well, genetic variation in the magnitude of response of NA to elevated CO_2 ranged from a 69% increase for Sargentii to only a 3% increase for Manitou. This agrees with Kalina and Ceulemans (1997) who found large differences in the response of NA to elevated CO_2 in two hybrid poplar genotypes (228% versus 80% increase). Thus, tree breeders interested in biomass production or biomass allocation might be able to take advantage of this variability in response to high CO_2, to modify particular clones for growth in certain environments or specific purposes. For example, Green Giant clones that favoured root growth and had an increase in root growth under high CO_2 might be more drought tolerant and better able to gather water and nutrients in harsher environments.

Respiration in response to elevated CO_2, has been found to increase, decrease or remain unchanged (Wullschleger et al. 1994, Curtis and Wang 1998, Amthor 1991, 2000, Wang and Curtis 2001). Respiration rates in the hybrid poplars were seen to increase for six of the hybrid poplar clones in response to elevated CO_2 by Day 95. Amthor (2000) suggested that leaf dark respiration might increase due to higher carbohydrate concentrations, which may then lead to increased phloem loading and translocation. A decrease in respiration rates, by the end of the experiment was seen for Sargentii and

Walker. A summary of 41 species by Curtis and Wang (1998) found that leaf dark respiration was significantly reduced under elevated CO_2, possibly due to lower leaf nitrogen or protein content under high CO_2.

The eight hybrid poplar clones I studied showed considerable variation in growth strategies and carbon allocation, which may allow clones with different growth strategies to be selected for particular environments. As well, although only a few clones showed consistent performance across both treatment levels, most of the hybrid poplar clones changed rank for variables such as height and caliper under elevated versus ambient CO_2. Changes in growth patterns and biomass allocation among different poplar hybrids under high CO_2 allow select clones to be modified by growth under high CO_2 for different environmental conditions. For example, a further decrease in the shoot-to-root ratio for Green Giant is seen under high versus ambient CO_2, which indicates greater allocation of resources into root growth under high CO_2. A large root mass would be beneficial to hybrid poplar clones used in reclamation and soil recovery. Conversely, fast growing clones such as P38 P38, which allocates more resources into stem growth would be important in clonal forestry plantations where water and nutrients are less limited.

4.4 Conclusions

4.4.1. *Populus* spp. and greenhouse growth under high CO_2

Exposure to increased levels of CO_2 in the greenhouse has been correlated with increases in growth of both aspen and hybrid poplar. This appeared to be due to a combination of lengthened growing season, greater total LA and greater NA per unit of leaf area under high CO_2. In addition, greenhouse CO_2 treatments can effectively modify carbon allocation, resulting in a greater proportional allocation to root biomass for aspen

and hybrid poplar clones. Physiologically, increased CO_2 resulted in increased NA and WUE rates without a corresponding decrease in Gs. Thus, greenhouse growth under high CO_2 treatment could be used to produce a taller tree with a proportionally larger root biomass, which may help in tree establishment in the field. Exposure to increased CO_2 and a differing response between male and female trees could alter the productivity and distribution of aspen under future, high CO_2 environments.

Recent studies indicate that temperate forests are a globally important sink for atmospheric CO_2. Under experimental conditions, elevated CO_2 can substantially increase carbon assimilation and growth in many tree species, including aspen, which presents the possibility that increased tree growth under high CO_2, due to increased NA, could further increase carbon storage in forests. Based on results from this study, the increase in NA seen in aspen grown under high CO_2, may indicate that aspen stands may increase global carbon storage. However, since individual species respond differently to increased CO_2, competitive shifts might lead to an alteration in the composition, structure and function of natural plant communities. More research is needed to examine the effect of increased CO_2 in forests and the effect it will have on the entire ecosystem.

An unexpected effect of increased CO_2 was seen when, despite prolific aspen root suckering under both CO_2 treatments, fewer suckers rooted under high CO_2. Based on these observations, aspen propagation under high CO_2 in the greenhouse is not recommended.

4.4.2. Application of results to forestry and reclamation

The data presented in this thesis were based on experiments involving 34 clones of trembling aspen and 8 hybrid poplar clones. The experiments were designed to

address questions about variation in growth, morphology and gas exchange traits of aspen due to phenotypic, gender and provenance differences, as well as the response to increased atmospheric CO_2 in the greenhouse.

In the greenhouse, production of taller trees due to increased CO_2 would be useful for the establishment of plantation trees, whereas, trees with a larger root biomass would be useful on postmining reclamation sites where a large root system would help in both establishment and the absorption and removal of toxins. Growth under high CO_2 in the greenhouse may help to produce larger trees, where faster growth due to increased CO_2 might decrease rotation time in the greenhouse.

In this experiment, superior aspen clones selected from the field also showed superior growth in the greenhouse compared to unselected aspen. This suggests that early screening through greenhouse trials might allow for the rapid selection of superior clones. Early growth in the greenhouse might also indicate poplar clones with growth characteristics suitable for different environments and purposes; for example, clones exhibiting rapid height growth and increased caliper growth might be of interest in plantations. In the greenhouse, clonal variation was expressed among both aspen and hybrid poplar clones. This extensive genetic variability in poplars suggests an opportunity for tree breeders to select genotypes for qualities such as biomass production or carbon allocation within the tree. Poplars with increased stem height and caliper might be selected for wood biomass purposes, while poplars with an increased root system might be selected for reclamation purposes. The large genetic variation inherent in aspen might also suggest that clonal selections for tree breeding programs can be made without considering a clone's provenance.

As with past studies, both morphological and physiological differences among genotypes and individuals of poplar were found in response to increased CO_2 in the greenhouse. Evidence of extensive genetic variation in response to increased CO_2 suggests that it is important to determine tree species response to elevated CO_2 to determine whether the superior clones selected to grow under ambient CO_2, will still perform in a future, increasing CO_2 environment. Intraspecific variation in growth responses to elevated CO_2 could possibly effect the fitness of populations and have significant impacts on the structure and productivity of plant communities in a future, high CO_2, world.

Tree response to increased CO_2 is a complicated subject and this experiment only examined a small aspect of that response. Further examination is needed to determine the effect elevated CO_2 has upon sucker production and sucker rooting ability, and if there is any interaction between aspen susceptibility to fungal infection and increasing levels of CO_2. As well, there is little research into gender-specific responses of tree species to both ambient and elevated CO_2, and the reasons males and females differ physiologically and ecologically. The relationship between nitrogen levels, NA and the ability for NA to acclimate to increased CO_2 is also something that should be examined further.

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