

**DROUGHT ADAPTATIONS OF HYBRID POPLAR CLONES COMMONLY
GROWN ON THE CANADIAN PRAIRIES**

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ABSTRACT

As a result of predicted climate change, environmental conditions may make woody plant species such as poplars (*Populus* spp.) vulnerable unless they are sufficiently adaptable to the new environment. This greenhouse study examined the responses of Hill, Northwest, Okanese and Walker hybrid poplar clones to drought, a potential outcome of a changing climate. Plants were grown from cuttings and subjected to two soil moisture treatments; a well-watered treatment and a 'drought' conditioning treatment in which plants were subjected to cycles of soil moisture deficit. The first study examined growth and gas exchange following treatments, while the second study examined concurrent changes in leaf water potential and gas exchange during a period of increasing soil moisture deficit, following treatments.

Hill and Okanese plants had reduced shoot:root ratios, possibly leading to more positive plant moisture balances compared to Northwest and Walker plants. Stomatal characteristics related to steady state gas exchange with Okanese plants having stomata predominantly on lower leaf surfaces, and lower stomatal conductance and Northwest plants having relatively large stomata and increased stomatal conductance. Hill and Okanese plants had the most responsive stomata, which began to close at much higher levels of leaf water potential (Ψ_{lf} of -0.45 and -0.54 MPa) than Northwest or Walker plants (Ψ_{lf} of -1.03 and -0.88 MPa); however, closure was more gradual in Okanese plants. Drought preconditioning resulted in stomatal closure occurring at higher leaf water potentials in droughted Northwest and Walker plants compared to well-watered plants. Regardless of soil moisture treatment, WUE was highest in Okanese and Walker plants. The drought treatment did however lead to increased WUE in Hill and Northwest plants.

Overall, Okanese plants appear to be the best adapted to conditions of reduced soil moisture based on growth and physiological traits, while Northwest and Hill seem better suited to areas where moisture deficits are likely to be less frequent or less severe. Results indicate that variability exists in adaptability of hybrid poplar clones to drought, suggesting that there may also be other hybrid clones that are adaptable to reduced soil moisture conditions, which may merit further investigation.

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ABBREVIATIONS

Water-use efficiency.....	WUE
Net photosynthesis ($\mu\text{mol CO}_2 \text{ m}^{-2} \text{ s}^{-1}$).....	A
Stomatal conductance ($\text{mol H}_2\text{O m}^{-2} \text{ s}^{-1}$).....	g_s
Intrinsic water-use efficiency.....	A/g_s
Stable carbon isotope composition.....	$\delta^{13}\text{C}$
Soil water potential.....	Ψ_{soil}
Leaf water potential.....	Ψ_{lf}
Threshold leaf water potential.....	$\Psi_{\text{lf}<75\%}$
Water potential at which g_s or A was considered halfway shut down.....	$\Psi_{\text{lf}<40\%}$
Water potential at which g_s or A were considered shut down.....	$\Psi_{\text{lf}<15\%}$
Vapour pressure deficit.....	VPD
Photosynthetic photon flux density.....	PPFD

1. INTRODUCTION

Plant species composition, distribution and productivity in different environments are determined by their adaptation to the prevailing environmental conditions. Scientific evidence indicates that the climate of earth is changing, becoming warmer and drier as mean annual temperatures increase (increasing evaporative demand) and in some areas, annual precipitation decreases (IPCC, 2001; Christensen et al., 2007). As a result of these expected changes, many plant species or communities could be faced with new environmental conditions to which they may not be well adapted. It is likely that some of these species or communities may undergo growth reductions or even high rates of mortality, in favour of others more adapted to the new environment. To minimize the impacts of climate change, knowledge of the extent or nature of adaptability of plant species or communities growing in ecosystems believed to be most vulnerable to the expected new environmental conditions is essential.

The Canadian Prairies, mainly due to its central location in North America and with the Rocky Mountains to the west blocking moist air from the Pacific Ocean, has a subhumid to semiarid climate (CCEA, 2005). Precipitation ranges from 250 mm in the arid grasslands of southeast Alberta and southwest Saskatchewan to slightly less than 700 mm in the Lake Manitoba Plain (CCEA, 2005). In addition to the generally low precipitation, strong dry winds predominate, leading to high evaporative demands, and as a result, soil moisture deficits are relatively common in this region (CCEA, 2005). In addition, periods without appreciable precipitation (drought conditions) also occur periodically. Because of these environmental patterns, grassland is the predominant vegetation type in the region. Moving northwards and eastwards, precipitation increases, resulting in a transition to a mosaic of fescue grasslands and woodland groves, and eventually boreal woodlands (CCEA, 2005).

The northern plains of North America, of which the Canadian Prairies are a part, are expected to be particularly vulnerable under a climatic change scenario (Hengeveld, 2000). Climate change models predict that average temperatures in the Canadian Prairies will increase by up to 3.5-5° C over the next century (IPCC, 2001 and Christensen et al., 2007). Increasing temperatures will cause increases in saturation vapour pressure, and thus greater evaporation and greater atmospheric moisture. While this will result in greater precipitation in much of North America, high levels of evaporation will cause net surface drying in other areas (Christensen et al., 2007). It is also predicted that the frequency and severity of some extreme weather events

such as drought will increase, making some ecosystems, including the Prairies, particularly vulnerable (Christensen et al., 2007). Although there have been other episodes of increasing temperatures in the Earth's history, the rapid rate of temperature increase predicted over the next century will not allow many plant species opportunities for adaptive responses to the change (Bawa and Dayanandan, 1998). Long-lived species such as woody plants may be particularly vulnerable unless they are sufficiently adaptable to the new environment. In the Canadian Prairies, response to drought therefore is going to be a major factor in the establishment, growth and survival of many woody species.

Native and cultivated woody plant species are important components of the Canadian prairie landscape for biodiversity, environmental protection, and fibre production. While grass species dominate, several woody species (trembling aspen, *Populus tremuloides* and balsam poplar, *Populus balsamifera*) form groves along the northern edge of the Prairie Ecozone, one of the three main Ecozones of the Canadian Prairies (Wiken, 2005a). In the Boreal Plains and Boreal Shield, the other two main Ecozones of the Prairies, about 85% of the land area is covered by native deciduous and coniferous woody plant species (Wiken, 2005b, 2005c). Planted woody species have also become more prominent due to efforts of land owners and several government organizations (i.e., Agriculture and Agri-Food Canada Shelterbelt Centre, SaskPower Shand Greenhouse). The Shelterbelt Centre, for example, has shipped over 650,000,000 woody seedlings to areas across the Prairies since 1901, causing a significant change in the prairie landscape (Wark, 2006). Many of the native and planted species/communities such as poplar (*Populus* spp.), however, are not generally drought tolerant and will be particularly vulnerable with increasing drought frequency and severity.

Poplars are valuable woody plant species throughout the world due to their ease of propagation and hybridization, and inherently fast rate of growth. On the Canadian Prairies, poplar clones are widely planted for protection of agricultural lands (riparian areas and shelterbelts) and farmsteads, ornamental purposes and afforestation. Many poplar species are native to areas where there is high soil moisture, such as along riverbanks or in depressions where water tends to collect, thus are believed to be relatively intolerant of soil moisture deficits (Demeritt, 1990). However, hybrid clones are being used in many areas of the Prairies where soil moisture may be limiting and evaporative demands high. As a result, there is increasing

concern whether currently used poplar clones will be able to withstand the increasingly severe and more frequent drought periods predicted with climatic change.

A number of studies have looked at the response of species or hybrids of select species of poplar including *Populus alba* (Blake et al., 1984), *Populus balsamifera* (Rhodenbaugh and Pallardy, 1993; Liu and Dickmann, 1996; Brignolas et al., 2000; Voltas et al., 2006), *Populus deltoides* (Bassman and Zwier, 1991; Rhodenbaugh and Pallardy, 1993; Gebre et al., 1994, 1997, 1998; Tschaplinski et al., 1994, 1998; Liu and Dickmann, 1996; Souch and Stephens, 1998; Marron et al., 2003), *Populus fremontii* (Leffler and Evans, 2001), *Populus kangdingensis* (Yin et al., 2005), *Populus maximowiczii* (Blake et al., 1984), *Populus nigra* (Blake et al., 1984; Gebre et al., 1998; Tschaplinski et al., 1998; Liu and Dickmann, 1996; Souch and Stephens, 1998; Brignolas et al., 2000; Marron et al., 2003; Voltas et al., 2006), *Populus trichocarpa* (Bassman and Zwier, 1991; Gebre et al., 1998; Tschaplinski et al., 1994, 1998; Souch and Stephens, 1998; Bond and Kavanagh, 1999), and *Populus tristis* (Liu and Dickmann, 1996) to soil moisture deficit or drought conditions. These studies have indicated that these *Populus* spp. are generally not very drought tolerant, with reduced gas exchange and limited growth of plants under soil moisture deficit conditions compared to plants growing under well-watered conditions. Although these studies are important in understanding the responses of *Populus* spp. to moisture deficit, information from these studies has limited application to the Canadian prairie environment. The vast majority of these studies were conducted with clones that are grown in areas with much higher soil moisture availability, lower vapour pressure deficits, and where drought events are not predicted to occur to the same extent as in the Prairies. Furthermore, a majority of the clones examined are not suitable for the low winter temperatures of the Canadian Prairies.

Many of these studies also did not examine poplar's ability to adjust their growth responses or physiology following pre-exposure to soil moisture deficits. Responses to moisture stress are often quite different depending on whether plants have been pre-exposed to moisture stress. In areas where moisture is lacking at some point during the year, most plants will be subjected to several water stress or drought events during a season, allowing for adjustments in growth and physiology (acclimation), resulting in plants that are more adapted to moisture deficit conditions. The negative effects of the moisture stress are often lessened in these acclimated plants compared to plants that have never been exposed to the stress (Sullivan and Eastin, 1974).

Examples of acclimation responses include enhanced root growth, increased stomatal responsiveness to declining soil (leaf) moisture, increased photosynthetic efficiency, and increased water-use efficiency (WUE). Because a majority of the water stress studies with poplar have either looked at plant responses during or after a single water stress event, it is unknown to what extent poplar are able to acclimate to moisture stress, and how this will affect their growth and physiology under subsequent moisture stress events.

There has also been no detailed physiological characterization of poplar clones commonly used on the Canadian Prairies. It is not known whether clones commonly grown here will survive or continue to grow well when soil moisture deficits become more common and more severe. Clones commonly grown on the Prairies include hybrids of *P. deltoides* var. *occidentalis* (Plains cottonwood), *P. balsamifera* (balsam poplar), *P. laurifolia* (laurel-leaf poplar), and *P. nigra* (black poplar), many of which are species native to cold and dry regions. The clones with the best ideotype will be the ones that have continued productivity despite limiting soil moisture. This could be a result of changes in soil moisture sensitivity, WUE or gas exchange characteristics among others. Evaluating the growth, gas exchange and WUE under soil moisture deficit conditions will help identify the clones with the best ideotype, as well as determine the reasons for their continued productivity under limiting soil moisture. Examining the relative WUE and the basis of changes in WUE of clones can also help identify clones and lines that could be used more extensively in breeding or genome work.

The overall objective of this thesis, therefore, was to examine the growth characteristics, gas exchange, and WUE of several hybrid poplar clones commonly grown on the prairies following a growing period under either well-watered or soil moisture deficit (droughted) conditions. A review of pertinent literature can be found in chapter two. The first study, reported in chapter three, examines the growth, steady state gas exchange and WUE of hybrid poplar clones after a growing period under either well-watered or droughted conditions. The second study, reported in chapter four, examines dynamic changes in gas exchange with changes in leaf water potential as a result of decreasing soil moisture. Chapter five outlines the overall conclusions and how the results from this thesis can allow for the selection of superior hybrid poplar clones that will be best adapted to current and future climate conditions.

2. LITERATURE REVIEW

2.1 Plant Responses to Moisture Deficits

Water is one of the most important environmental factors limiting plant distribution and productivity. Water limitations can occur either as soil moisture deficits or high vapour pressure deficits, although both often occur simultaneously. Soil moisture deficit occurs when the amount of water required for optimum growth is greater than water available by either precipitation or soil storage. A moisture deficit caused by high vapour pressure deficit occurs when plants are losing water from their leaves faster than it can be supplied from the roots, resulting in a moisture deficiency in the leaves. Regardless of the cause, when plants are exposed to moisture deficits, biomass production and leaf area expansion is reduced, biomass allocation often shifts towards increased root growth, stomata close to prevent further water loss causing reduced photosynthesis, and under severe deficits, plant mortality may result. Plants that can adapt to moisture deficits will undergo numerous morphological, physiological, biochemical and molecular changes in an attempt to balance water lost through transpiration and water taken up by the root system while still remaining relatively productive. These changes are typically species or clonal specific and depend on the degree of acclimation possible, which in turn depends on the phenotypic plasticity that plants possess. Because plasticity is a heritable characteristic, similar responses would be expected in clonal material. Non-adaptive species are generally less plastic and will typically be unable to adjust their biochemistry, physiology, or morphology in response to limiting soil moisture. This makes them less able to maintain productivity and may even undergo plant mortality under future soil moisture deficit conditions.

2.1.1 Morphological responses

When plants grow under conditions in which soil moisture is limiting to growth or under conditions of high vapour pressure deficit (VPD), many morphological changes take place. Some of these changes are a growth effect of a limiting resource or stress condition not directly related to a better functioning, while others are adjustments meant to help the plant function better (e.g., better resource capture, reduced water loss) under moisture deficit conditions. As long as moisture deficits are not too severe, drought adapted species can adjust their morphology and acclimate to lower moisture levels. Typically, total biomass is reduced under soil moisture deficit conditions due to limited root expansion and shoot growth, and leaf and branch abscission.

Limiting moisture can also cause changes in leaf characteristics such as reduced leaf area, increased leaf thickness, and an increased number of smaller stomata per leaf area. There may also be shifts in carbon allocation to roots over shoots compared to plants that have grown in well-watered conditions. Acclimation responses include decreased sensitivity of cells to lower leaf moisture, increasing leaf cuticle thickness, and increased carbon allocation towards root growth. Many of these acclimation responses will allow drought adapted species to not only resist the moisture stress, but also continue growing even when soil moisture is below optimum levels.

When soil moisture is limiting, biomass production is reduced, resulting in plants that are shorter with less total biomass compared to plants growing under well-watered conditions (Rhodenbaugh and Pallardy, 1993; Tschaplinski et al., 1994; Souch and Stephens, 1998; Guarnaschelli et al., 2003). The main cause of reduced biomass accumulation under moisture deficit conditions is reduced carbon gain (Farquhar and Sharkey, 1982; Jones, 1985; Sharkey, 1985; Chaves, 1991). When soil moisture is below optimum, stomatal closure acts to limit moisture loss from the plant, but will also limit leaf level gas exchange, restricting gain of CO₂, thus restricting carbon gain within the plant (Farquhar and Sharkey, 1982; Sharkey, 1985).

Growth reductions under moisture deficit condition can also be attributed to changes in cell turgor (osmotic pressure within cells) or cell wall extensibility, which limit cell expansion as cell water contents decrease (Hsiao, 1973; Turner, 1974 cited in Turner, 1986; Turner, 1986; Roden et al., 1990; Spollen et al., 1993; Carpita and McCann, 2000). Cell growth (thus plant growth) is caused by turgor pressure exerted on the cell wall coupled with the loosening of cell wall structures (Hsiao and Acevedo, 1974; Carpita and McCann, 2000). This pressure provides the necessary physical force from within to drive cell expansion and cause cell walls to stretch (Hsiao, 1973; Spollen et al., 1993). Cell turgor is related to the water content of the cells, and as soil moisture declines, the cell water content often decreases, reducing cell turgor (Spollen et al., 1993). Once cell turgor declines below some critical threshold, growth is halted (Hsiao and Acevedo, 1974). At the same time, cell walls become less flexible and thus less able to expand following exposure to limiting soil moisture (Hsiao, 1973; Nonami and Boyer, 1990a, b; Spollen et al., 1993). Because cell wall extensibility or loosening is a primary determinant in the rate of cell expansion (Carpita and McCann, 2000), declines in this extensibility can also restrict cell, thus plant growth as well.

While overall biomass production is generally lower in plants growing under soil moisture deficit conditions compared to well-watered plants, there is generally a greater impact on shoot growth than root growth. Reduced shoot biomass under soil moisture deficit conditions results mainly from the sensitivity of leaf growth and expansion to limiting moisture. Because of this, moisture limited plants have smaller leaves than plants growing with adequate moisture (Braatne et al., 1992; Rhodenbaugh and Pallardy, 1993; Souch and Stephens, 1998; Centritto et al., 2002). Growth differences between well-watered plants and those growing under moisture deficit conditions can also result because of total leaf area differences between plants from the different moisture regimes, thus, different photosynthetic surface areas (Turner and Begg, 1981).

Because roots are generally less sensitive to moisture deficit conditions, root expansion will often continue below soil moisture levels that inhibit shoot expansion (Westgate and Boyer, 1985; Sharp et al., 1988; Spollen et al., 1993; Tschaplinski et al., 1998; Brignolas et al., 2000; Chaves et al., 2002). This is likely an adaptive mechanism to allow plants to reach deeper water supplies when soil moisture is limited in upper soil horizons (Tschaplinski et al., 1994; Tschaplinski et al., 1998; Chaves et al., 2002). Differences in soil moisture sensitivity between shoots and roots are believed to be due to elevated solute accumulation in roots when moisture levels drop below optimum (Hsiao and Acevedo, 1974). This solute accumulation (osmoregulation) decreases the water potential of root cells resulting in a net flow of water into the cells allowing for maintenance of cell turgor under decreasing soil moisture. While some solute accumulation occurs in the shoots, it is typically not to the same extent as in the roots, thus shoot growth tends to be affected by loss of turgor sooner when moisture is limiting (Hsiao and Acevedo, 1974; Morgan, 1984; Sharp et al., 1988).

Apart from differing moisture sensitivities, differences in growth between shoots and roots under limiting moisture may also be partially a result of preferential allocation of carbon to root growth. Hsiao and Acevedo (1974) reported that in some cases root growth was higher in plants growing under soil moisture deficit conditions compared to plants from well-watered conditions. This is likely to occur when soil moisture levels are low enough to inhibit shoot expansion, but not so low as inhibit photosynthesis (Turner and Begg, 1981). Regardless of the mechanism driving differences in carbon allocation between above and below ground plant parts, the shoot:root ratio is often lower in plants growing under a soil moisture deficit compared to plants from well-watered conditions (Westgate and Boyer, 1985; Rhodenbaugh and Pallardy,

1993; Kramer and Boyer, 1995; Bray, 1997; Tschaplinski et al., 1998). Lower shoot:root ratios may create a more positive plant water status, thus higher water-use efficiency, and result in a plant more likely to remain productive when soil moisture levels decline.

In addition to differences in overall shoot and root growth, plants growing under soil moisture deficit conditions may also have changes in leaf structure and stomata characteristics. Leaves tend to be relatively thicker in plants grown under limiting moisture as expansion of leaves is restricted and the cuticle layer becomes thicker (Ceulemans et al., 1984). The leaf cuticle is important for limiting diffusion of leaf water from mesophyll cells, and increased thickness further limits water loss from the leaves. Leaves formed under drought conditions also tend to have a higher density of smaller stomata, and fewer stomata on the adaxial (upper) leaf surface relative to the abaxial (lower) surface compared to leaves formed when moisture is abundant (Weyers and Meidner, 1990; Willmer and Fricker, 1996). This usually allows for better control of moisture loss from the leaves. Because stomata are the primary sites of gas exchange, any changes in stomata characteristics will then have a direct effect on gas exchange, water-use efficiency and ultimately growth.

2.1.2 Physiological responses

Changes in plant morphology under soil moisture deficit will be a result of physiological responses to decreasing soil, and thus plant moisture. Physiological adjustments usually permit continued CO₂ assimilation while minimizing water loss and avoiding cellular damage. Similar to growth acclimation responses, physiological acclimation can also occur in plants exposed to moisture deficit conditions, increasing the likelihood of survival and potentially allowing plants to remain productive under future moisture limitations. Physiological acclimation responses include increased stomata responsiveness and photosynthetic efficiency or improved water-use efficiency (Schulte and Hinckley, 1987; Jones, 1993; Earl, 2000; Silim et al., 2001). Increased stomatal responsiveness results in more rapid stomatal closure once the leaf water potentials fall below a certain level. A more efficient photosynthetic system is able to function on lower concentrations of intercellular CO₂ (a consequence of stomatal closure), and is able to deal with excess energy without essential proteins or enzymes being damaged when photosynthesis is not functioning at its maximum.

2.1.2.1 Stomatal conductance

The main function of stomata is to control the rate of transpiration while at the same time control the CO₂ partial pressure at the sites of carboxylation inside the leaf (Farquhar and Sharkey, 1982). Stomatal movements prevent desiccation of plant tissue, with the magnitude of aperture changing to maximize the ratio of assimilation to evaporation (Cowan, 1977). With this ratio maximized, the carbon gain per unit of water lost will also be maximized (increased water-use efficiency). Changes in stomatal conductance, a measure of stomatal opening, will affect both plant water loss and plant carbon gain, thus stomata effectively modulate leaf and canopy temperatures, carbon assimilation (thus plant productivity), and plant water-use efficiency (Farquhar and Sharkey, 1982).

Stomatal closure prevents further water loss when soil or atmospheric moisture deficits are high, and is a critical step in minimizing water stress within the tissues (Mansfield and Davies, 1981, 1985; Rhodenbaugh and Pallardy, 1993; Tschaplinski et al., 1994; Marron et al., 2003). Stomatal closure during periods of moisture deficit are mainly a response to turgor changes in the guard cells (Boyer and Potter, 1973 cited in Farquhar and Sharkey, 1982; Turner, 1986; Raschke, 1975), but may also be partially due to a biochemical or hormonal signal such as abscisic acid released by the roots in response to declining soil moisture (Shulze, 1986). Responsiveness of stomata to declining plant water as well as the overall rate of stomatal conductance will reflect either the species specific adaptability to reduced moisture or the environmental conditions that plants have been pre-exposed to and the ability to acclimate to reduced moisture. For example, there tends to be differences in both stomatal conductance and stomatal responsiveness between plants grown under well-watered conditions relative to those that have been exposed to soil moisture deficit conditions. Because stomata provide an essential role for plant water regulation, in order to understand the impact that reduced soil moisture can have on productivity and WUE and whether plants can acclimate to moisture deficits, it is important to compare stomatal function in plants that have grown under differing soil moisture conditions.

Stomatal conductance is a function of the density and size of stomata on leaf surfaces as well as the magnitude of stomatal aperture. Stomata density and size are species specific and can be affected by the environment during development, but in general plants with more and larger stomata typically have higher rates of stomatal conductance, while plants with fewer and smaller

stomata typically have lower rates of stomatal conductance. Regardless of stomata size and density, plants will adjust the magnitude of the stomatal apertures to maintain a particular CO₂ concentration within leaf tissues based on the capacity for CO₂ assimilation, as well as to maintain a particular plant water balance (Willmer and Fricker, 1996; Buckley, 2005). Changes in stomatal aperture are achieved through changes in turgor pressure of adjacent guard cells (related to both the osmotic pressure and water potentials of those cells), usually through active changes in osmotic pressures by plants (Buckley, 2005). Guard cell turgor pressures may also be regulated by changes in turgor pressures of the surrounding epidermal cells (Franks et al., 1995, 1998). Turgor pressure is a function of leaf water potential and osmotic potential, and because epidermal osmotic potential tends to be relatively constant diurnally, epidermal turgor pressure is mainly a function of the water potentials of the epidermal cells (Willmer and Fricker, 2005).

Plants generally have two water potential strategies, categorized as anisohydric or isohydric depending on whether the water potentials of their leaves fluctuate with changing vapour pressure deficit (VPD) or soil moisture. In anisohydric plants, leaf water potentials will fluctuate throughout the day depending on the soil water supply and evaporative demand (Bonal and Guehl, 2001). Specifically, as the vapour pressure deficit increases or soil moisture decreases, leaf water potentials will decrease (and vice versa) in plants displaying anisohydric behaviours. Leaf water potentials of the plants can decrease to very low levels, making anisohydric plants quite drought tolerant. In contrast, isohydric plants will maintain constant leaf water potentials, regardless of the soil moisture or atmospheric vapour pressure, almost to death in some cases (Tardieu and Simonneau, 1998; Bonal and Guehl, 2001; Buckley, 2005). As a result, most isohydric plants are generally not considered to be drought tolerant.

Regardless of leaf water potential strategy, once a critical level of plant moisture is reached, called the critical threshold, stomata will begin to close, limiting transpiration (Hsiao, 1973; Cowan, 1977). The critical threshold tends to be species dependent but can differ between leaves of different ages and those formed during differing growth conditions. Not surprisingly, the critical leaf water potential thresholds tend to be higher in plants with anisohydric strategies (Bonal and Guehl, 2001). It is generally believed that the threshold leaf water potential is related to the critical xylem water potential (the water potential that will cause 100% cavitation in xylem vessels), which will vary widely depending on the relative cavitation susceptibility of the plant species (Jones and Sutherland, 1991; Tyree et al., 1992; Sperry, 2000; Cochard et al., 2002). In

more drought resistant species, plants will tolerate lower plant moisture, thus have lower threshold water potentials, or be more responsive to declining leaf water potential, thus have higher threshold water potentials, but with gradual stomatal closure as moisture levels decrease (Ni and Pallardy, 1991; Willmer and Fricker, 1996). This is the case with sugar maple, *Acer saccharum* (Ellsworth and Reich, 1992) and olive, *Olea europaea* (Giorio et al., 1999), two relatively drought tolerant species that have gradual stomata closure coinciding with declining soil moisture. Other less drought tolerant species, such as several *Populus* spp. or black walnut (*Juglans nigra*), will have stomata that shut almost immediately once the threshold leaf water potential is reached (Schulte et al., 1987; Ceulemans et al., 1988; Furukawa et al., 1990; Parker and Pallardy, 1991). Because drought intolerant species such as these are not adapted to reduced plant moisture, the threshold leaf water potentials will also be relatively high; however, stomatal closure will be immediate at these thresholds (contrast to gradual closure in adapted species).

Regardless of how and when stomata normally respond to moisture fluctuations, exposure to a period of soil moisture deficit is important for stomata preconditioning. Changes that occur to stomatal aperture, thus stomatal conductance, will have a large impact on plant survival and productivity during subsequent soil moisture limitations. In general, plants that are able to adapt will have adjustments in stomatal conductance so that steady state conductance values are lower in plants pre-exposed to moisture deficit events compared to plants that have grown with ample soil moisture. In non-adapted species, stomatal conductance will not be adjusted, and will remain the same regardless of the environment that plants had grown under (Tschapinski et al., 1994; Olivás-García et al., 2000; Aasamaa and Söber, 2001; Silim et al., 2001; Aspelmeier and Leuschner, 2004; Monclus et al., 2006).

Stomatal responsiveness will also be affected by the previous moisture conditions that plants were exposed to, with changes reflecting adaptedness as well. In some species, including *Populus trichocarpa*, *Acer platanoides*, and *Tilia cordata*, the stomata tend to become more responsive (closing at higher water potentials) to moisture deficits following pre-exposure to water stress (Schulte and Hinckley, 1987; Aasamaa and Söber, 2001). Others, such as *Olea europaea*, have similar stomata responses to leaf water potential regardless of the water regime they have been exposed (Giorio et al., 1999). Other species may have stomata that become less responsive to decreasing leaf water potential following exposure to moisture deficit conditions. For example, Ackerson (1980) found that when cotton plants were pre-exposed to an increasing

number of stress cycles, the threshold leaf water potential decreased (i.e. leaves were drier) with each subsequent cycle that plants were exposed to.

Because of the essential role that stomata play in water conservation and carbon assimilation, understanding how plants respond, survive and grow under limiting soil moisture requires that we understand how stomatal conductance is affected by limiting moisture. This includes examining how stomatal conductance changes during and after soil moisture stress events, and understanding the strategy that plants are using in terms of water conservation during these events. Because of the direct effect that stomata will have on carbon assimilation (photosynthesis), any impact that soil moisture conditions will have on stomatal conductance will also affect photosynthesis.

2.1.2.2 Photosynthesis

The growth or biomass accumulation by plants is dependent on the rate, patterns and duration of carbon assimilation (photosynthesis). Any factor, internal to the plant or external to the plant (environmental conditions), which affects CO₂ assimilation, will therefore affect plant biomass accumulation. Under soil moisture deficit conditions, CO₂ assimilation is mainly affected by changes in substrate (CO₂) availability, but may also be affected by metabolic changes (i.e. altered capacity to regenerate ribulose biphosphate) or more indirectly by other secondary effects such as oxidative stress (Chaves et al., 2008). The main restriction on CO₂ supply under water stress occurs as a result of stomatal closure, but changes in mesophyll conductance will also reduce CO₂ availability at the sites of carboxylation. Regardless of the cause, the impact that restricted photosynthesis will have depends on the plant species, as well as the inherent adaptability and/or acclimation capabilities of different plant species (Aasamaa and Söber, 2001; Chaves et al., 2008).

The diffusion of CO₂ from the atmosphere to the sites of carboxylation is a series of resistances, including the resistance imposed by the degree of stomata aperture (Farquhar and Sharkey, 1982; Sharkey, 1985) and those imposed by the mesophyll (Chaves et al., 2008; Flexas et al., 2008). Stomatal limitation has been found to be the dominant restriction on photosynthesis during mild to moderate moisture deficit conditions, caused by either low soil water or high vapour pressure deficit (Sharkey, 1990; Chaves, 1991; Chaves et al., 2002; Chaves and Oliveira, 2004; Grassi and Magnani, 2005). More recently, however, a number of studies have reported that changes in mesophyll conductance under moisture deficit can also impact CO₂ availability.

Significant reductions in mesophyll conductance under water stress have been found to contribute to reduced C assimilation, particularly under moderate to severe soil moisture deficits (Grassi and Magnini, 2005; Flexas et al., 2004, 2006; Diaz-Espejo et al., 2007; Galmés et al., 2007).

In addition to direct restrictions on CO₂ availability, stomatal and mesophyll diffusive restrictions will also cause changes to the ratio of carbon dioxide and oxygen at the sites of carboxylation. Rubisco (Ribulose biphosphate carboxylase/oxygenase), the first enzyme responsible for carbon fixation in C3 plants, has an active site that binds both CO₂ and O₂ (Farquhar et al., 1980). Because of this, both substrates are in competition for the active site and net CO₂ assimilation of C3 plants depends on the relative concentration of the two gases (Farquhar et al., 1980). When the ratio of CO₂ to O₂ decreases, there is typically a shift in electron flux to reduction of O₂, via photorespiration (Baker, 1993). While photorespiration acts as a sink for excess energy in the photosynthetic apparatus, this shift in activity of Rubisco may also impact the rate of photosynthesis beyond C-limitations (Baker, 1993).

When stomata close, transpirational water loss (which also acts as a cooling mechanism) is also restricted, causing leaf and canopy temperatures to rise. Elevated leaf tissue temperatures can cause damage to enzymes or proteins, including nitrate reductase and sucrose phosphatase (Farquhar and Sharkey, 1982; Sharkey, 1990). There may also be reduced capacity for ribulose biphosphate regeneration (Farquhar and Sharkey, 1982). This is because regeneration of ribulose biphosphate, the substrate that CO₂ is first bound to in C3 plants, depends on the capacity for electron transport, which may become reduced or affected during water stress (Farquhar and Sharkey, 1982). Differences in electron transport capacity are often a result of light induced damage to the photosynthetic reaction centers, particularly photosystem II (PSII) (Powles, 1984; Maxwell and Johnson, 2000). While damage to photosystem complexes often occurs under normal growth conditions, inactive complexes do not tend to accumulate because the repair rate will keep pace with the rate of damage (Baker, 1993). When carbon assimilation is inhibited (such as in water stress conditions), the capacity for repair of proteins in the reaction centers is often exceeded by the rate of photodamage, which can result in photoinhibition (Baker, 1993). The impact that photoinhibitory damage will have on assimilation activity after the stress is relieved (i.e., water is resupplied) will depend on the degree of damage to the proteins within

these complexes and the ability to degrade and resynthesize new proteins into PSII complexes (Baker, 1993).

Regardless of the origin of the limitation, net photosynthesis (A) will decrease as leaf water potentials decrease, but usually not until leaf water potentials are slightly lower than those prompting stomatal closure (Ellsworth and Reich, 1992). Patterns of photosynthetic response will be similar to the patterns of stomatal closure and will depend on the species adaptations to soil moisture limitations. In drought tolerant species such as oak or maple, there will be a gradual decline in A coinciding with decreasing leaf water potential (Ni and Pallardy, 1991; Epron and Dreyer, 1993). Drought intolerant species such as walnut or poplar will have photosynthetic activity that will continue until leaf water potentials are lower, at which point there will be an immediate decrease in photosynthetic rate (Regehr et al., 1975; McGee et al., 1981; Bassman and Zwier, 1991; Ni and Pallardy, 1991; Parker and Pallardy, 1991). Both photosynthetic rates as well as the pattern of photosynthetic changes during declining soil moisture are often different in plants exposed to moisture deficit conditions compared to those that have grown under well-watered conditions. However, the impact that reduced soil moisture will have on overall photosynthetic rate will depend on the ability of plants to acclimate to soil moisture deficit conditions. For example, Bassman and Zwier (1990) found that *Populus trichocarpa* clones adapted to areas of higher soil moisture had photosynthetic rates that remained high until leaves were wilted past the point of recovery. In the same study, plants from a typically drier area (thus likely pre-exposed to moisture deficit conditions) had gradual declines in photosynthetic activity that began when stem xylem potentials were still relatively high.

2.1.2.3 Water-use efficiency

In order for plant populations to remain productive or even survive when a growth resource, such as water, is limited, it is important that plants use that resource with as much efficiency as possible. Water-use efficiency (WUE), an important measure of plant productivity in ecology and agronomy, is the ratio of biomass, yield or carbon gain per unit of water used by a plant. Examining WUE allows for the comparison of the relative productivity per unit of water used or lost between plant or crop species, populations or cultivars. In general, plant species or populations with higher WUE will be more successful when water supply is limited compared to plants with less WUE.

Traditionally, the WUE of individual plants or plant populations has been determined gravimetrically by measuring the actual amount of water used by the plant(s) over the growing season and comparing it to the total amount of biomass produced during the same period (Kramer and Boyer, 1995). This measure of yield or biomass production at the whole plant level is termed the *transpiration efficiency* (Ehleringer et al., 1993). While this method of determining WUE allows for comparison of assimilation with water lost through transpiration over long time periods, it can be slow, tedious, complicated and result in errors.

More recently, short-term carbon gain and water loss at the leaf level has been determined by examining the ratios of net photosynthetic rates (A) to either transpiration rate (E) or stomatal conductance (g_s) (Farquhar et al., 1989). Whereas the ratio of net photosynthesis to transpiration (A/E), termed instantaneous WUE, may be a preferred method of WUE determination because it gives the actual amount of water loss (transpired), the underlying assumption requires vapour pressure deficits (VPD) to be constant between measurements. This is because changes in VPD (which in turn are affected by changes in temperature and relative humidity) can have a huge impact on rates of transpiration (Ehleringer et al., 1993). Maintaining a constant VPD is not always easy, however, because of the constantly changing environmental conditions in the field.

In contrast, WUE determined from photosynthesis (A) and stomatal conductance (g_s) measurements (calculated as A/g_s), termed intrinsic WUE, does not require that VPD be constant between measurements. This is because measurement of plant stomatal conductance, in contrast to plant transpiration, does not include vapour pressure in its calculation. Therefore, intrinsic WUE includes only the biological components that determine the carbon gain and water loss through the stomata in plants which allows for direct comparisons of the contribution of physiological components to WUE (Ehleringer et al., 1993). Both methods of determining WUE (A/E or A/g_s) generally have a strong correspondence with overall plant WUE (Osório and Pereira, 1994). However, they may not necessarily reflect the WUE for the entire life of a plant but rather only the time of measurements and conditions prevailing at that time.

Another method of WUE determination can be obtained by measuring stable carbon isotope composition ($\delta^{13}C$) of plant tissues. Numerous studies have found a strong correlation between the stable carbon isotope composition and gravimetric, instantaneous or intrinsic WUE (Farquhar and Richards, 1984; Hubick et al., 1986; Condon et al., 1987; Ehleringer et al., 1988;

Wright et al., 1988; Farquhar et al., 1989; Ismail and Hall, 1992; Silim et al., 2001). $\delta^{13}\text{C}$ is easier to determine than either instantaneous WUE or gravimetric WUE. It allows for measurement of a large numbers of plants and integrates the conditions over the whole growth period or life of a plant rather than a single point of time as is the case with gas exchange measurements (Farquhar and Richards, 1984; Hubick et al., 1986; Wright et al., 1988; Ehleringer et al., 1988; Ismail and Hall; 1992; Kramer and Boyer, 1995).

There are two measures of tissue carbon isotope content typically used in WUE determination; stable carbon *composition* ($\delta^{13}\text{C}$) and stable carbon isotope *discrimination* ($\Delta^{13}\text{C}$) referred to as integrative WUE. Stable carbon isotope composition ($\delta^{13}\text{C}$), is a measure of the $^{12}\text{C}:^{13}\text{C}$ content relative to $^{12}\text{C}:^{13}\text{C}$ of a carbonate standard. This standard is a fossil belemnite from the Pee Dee Formation in South Carolina, termed Pee Dee Belemnite carbonate (O'Leary, 1981). A measurement of stable carbon isotope discrimination ($\Delta^{13}\text{C}$) compares the $\delta^{13}\text{C}$ of the tissue to the $\delta^{13}\text{C}$ of the source air, providing a positive value of absolute discrimination against ^{13}C . Discrimination can be calculated as:

$$\Delta^{13}\text{C} = (\delta^{13}\text{C}_{\text{air}} - \delta^{13}\text{C}_{\text{plant}}) / (1 + \delta^{13}\text{C}_{\text{plant}}/1000), \quad [2.1]$$

with a typical C3 plant having a discrimination value of around 20‰ or parts per mil (Farquhar et al., 1989).

Differences in $\delta^{13}\text{C}$ between plant species, populations or environments reflect differences in the fractionation of ^{12}C and ^{13}C during photosynthesis (O'Leary, 1988, 1993). Atmospheric values of $^{13}\text{C}:^{12}\text{C}$ are approximately 1:89 (Stuiver, 1982 cited in Farquhar et al., 1989), representing a carbon isotope composition ($\delta^{13}\text{C}$) of about -8 ‰, while typical C3 plant material is around 1:91, which is -27.6‰ (Farquhar et al., 1989). In C3 plants, the majority of this fractionation is a result of the low reactivity that ribulose 1,5-bisphosphate carboxylase/oxygenase (Rubisco), the carboxylating enzyme in C3 plants, has with ^{13}C relative to ^{12}C (Whelan et al., 1973; Melander and Saunders, 1979 cited in Farquhar et al., 1982; Roeske and O'Leary, 1984; Guy et al., 1987 cited in Farquhar et al., 1989; Farquhar et al., 1989). A small amount of fractionation may also occur during diffusion of CO_2 through the air and the leaf boundary layer (Craig, 1954 cited in Farquhar et al., 1989; O'Leary, 1981; Farquhar, 1983) and/or dissolution in water of mesophyll cells (O'Leary, 1984).

For plants with C3 metabolism, discrimination against ^{13}C is dependent on the relative concentration of intercellular CO_2 to atmospheric CO_2 (c_i/c_a) (Evans et al., 1986; Farquhar et al.,

1988; Donovan and Ehleringer, 1994; Silim et al., 2001). Less discrimination occurs when c_i/c_a is low compared to when c_i/c_a is high, which results in $\delta^{13}\text{C}$ values that are generally higher (or less negative) under reduced c_i/c_a . Because phosphoenolpyruvate carboxylase, the first enzyme in CO_2 fixation in C4 plants, does not discriminate against ^{13}C as much as Rubisco, this relationship does not apply for plants with C4 metabolism (Evans et al., 1986; Farquhar et al., 1988; O'Leary, 1988).

Because both the photosynthetic rate (A) and stomatal conductance (g_s) will affect WUE as determined by $\delta^{13}\text{C}$, any factor including water stress, affecting A or g_s or both, will affect $\delta^{13}\text{C}$ (Farquhar and Sharkey, 1982; Sharkey, 1985; O'Leary, 1988). Generally, stomatal conductance (g_s) tends to be more affected than photosynthesis by soil moisture deficit conditions, resulting in lower values of c_i in plants that have grown under moisture deficit conditions compared to plants grown with abundant moisture (Schulze et al., 1989; Ismail and Hall, 1992; Silim et al., 2001). Because $\delta^{13}\text{C}$ tends to be higher or less negative when c_i is low, WUE is also higher in plants grown under soil moisture deficit conditions. As a result, numerous studies have found that $\delta^{13}\text{C}$ and WUE are higher in plants that had grown under moisture deficit conditions compared to those under well-watered conditions (Farquhar and Richards, 1984; Hubick et al., 1986; Donovan and Ehleringer, 1994; Wright et al., 1994; Silim et al., 2001).

Under reduced soil moisture, $\delta^{13}\text{C}$ or WUE tends to be higher which can result from either an increased rate of photosynthesis (A), decreased stomatal conductance (g_s), or both. It is important to understand the underlying cause of the observed increase in WUE as indicated by $\delta^{13}\text{C}$ (Hubick et al., 1986; Condon et al., 1987; Ehleringer, 1990; Wright et al., 1994). If changes in A are driving changes in WUE (or $\delta^{13}\text{C}$), there tends to be a linear positive correlation between WUE and biomass production, but not when g_s is responsible for changes in WUE (Hubick et al., 1986; Condon et al., 1987; Wright et al., 1988; Ehleringer, 1990; Silim et al., 2001). In some plant species, such as soybean (Earl, 2002) and wheat (Condon et al., 1987), differences in WUE have been attributed to differences in stomatal regulation, but in others including *Picea sitchensis* (Silim et al., 2001) and peanut (Wright et al., 1988, 1994), differences in WUE appear to be a result of differences in A efficiency and not g_s .

If WUE is to be a useful indicator of performance under drought conditions, it is preferable to have a plant that has increased WUE due to decreased g_s but minimally affected A . In contrast, if a plant has increased WUE as a result of increased A but unchanged g_s , water use

will not change, which may be detrimental under water limiting conditions and productivity will inevitably be lower in these plants.

2.1.3 Biochemical and molecular responses

Changes in physiology and morphology in response to soil moisture deficits are first manifested in molecular and biochemical responses. Many of these changes in biochemistry allow for cellular protection or maintenance of positive turgor pressure as plant moisture declines. Specific examples include osmotic adjustment, accumulation of antioxidants, and changes in hormonal balances of major plant tissues (Morgan, 1984; Bray, 1997; Ramanjulu and Bartels, 2002). Other responses include increased production of aquaporins, a family of transmembrane channel proteins that facilitate water transport along water potential gradients (Maurel and Chrispeels, 2001; Ramanjulu and Bartels, 2002), or accumulation of LEA (late embryogenesis abundant) proteins believed to help maintain protein or membrane structure, sequester ions, bind water and act as molecular chaperones (Bray, 1997; Ramanjulu and Bartels, 2002).

2.2 The Role of Phenotypic Plasticity in Drought Adaptation and Acclimation

Phenotypic plasticity, the production of alternative phenotypes from a single genotype, is an important characteristic for adaptation in heterogeneous environments or where environmental changes are faster than the generation times of the population (Bradshaw, 1965; Bazzaz, 1991; Berrigan and Scheiner, 2004). Adapted phenotypic expression in plants will depend on the degree of plasticity or ability to have altered physiology in response to changes in environmental conditions (Bradshaw, 1965, 1973; Rehfeldt et al., 2001). Because of immobility and long-lived nature, woody plants often experience a wide range of environments throughout their lifetimes, leading to the development of phenotypic plasticity in many plants (Bradshaw, 1965, 1972; Scheiner 1993). Because of this phenotypic plasticity, plants with identical genetic makeup (clones) of a given individual can differ in morphology and physiology depending on the prevailing environmental conditions during their development (Bazzaz, 1991). Acclimation, a shift in a plant's response pattern following exposure to an environmental condition, is related to the degree of plasticity that the plant possesses (Bazzaz, 1991). During acclimation, the growth and physiology of adaptable plants adjust to the prevailing environment, often increasing their success when those conditions are again encountered relative to plants that have never been exposed to those same conditions. Plasticity and acclimation lessen the need that plants be

grown in precise habitats and will be important for the survival of woody plant species with changing climatic conditions.

Plants may respond in several ways to changes in their environment predicted under global climate change: (1) They may be plastic enough in their phenotypic expressions so as to be able to withstand change, (2) they may adapt through adaptive evolution, (3) they may migrate via dispersal to optimum environments or (4) they will be unable to sufficiently respond and will not survive a rapidly changing climate (Bawa and Dayanandan, 1998). Because of their long-lived nature, the persistence and performance of current agroforestry species, populations or clones under changed climatic conditions will primarily depend on their ability to adapt phenotypic expressions to the new ecological regimes.

Plants with high degrees of plasticity will be able to adapt, while those with low plasticity may not meet the challenges of predicted climate change. In the case of hybrid poplar, clones that will be adaptable and thus suitable under more frequent and severe drought conditions will be those that are highly plastic, thus able to acclimate to reduced soil moisture, allowing for minimally impacted growth when soil moisture deficits are encountered.

2.3 Poplar Biology

The genus *Populus* (poplars) is a group of native and hybrid (natural and artificial) woody plants belonging to the family *Salicaceae*. The genus is comprised of five sections, four of which are native to North America including the sections *Aigeiros* (cottonwoods and black poplars), *Leucoides* (swamp poplars and bigleaf poplars), *Populus* (aspens and white poplars), and *Tacamahaca* (balsam poplars) (Demeritt, 1990). The fifth section, *Turanga* (subtropical poplars), are native to east Africa and southwest Asia. Most *Populus* species are native to riparian areas or upland sites with good moisture holding capacity, and a pH of 6.0 to 7.0 (Demeritt, 1990). Because of their native habitat, it is assumed that poplars are not adapted to moisture deficit conditions, thus not drought tolerant (Demeritt, 1990). In fact, it is not uncommon to observe top dieback in several species including *Populus tremuloides* (aspen) and *Populus deltoides* (cottonwood) as a result of drought or soil moisture deficit or lowered water table (Tyree et al., 1992; Rood et al., 2000). Poplar hybrids have however been found to show variation in tolerance and adaptedness to adverse site conditions such as reduced soil moisture (Demeritt, 1990). This variation may indicate a potential for selection of more drought tolerant hybrid poplar clones.

3. GROWTH, GAS EXCHANGE AND WATER-USE EFFICIENCY OF HYBRID POPLAR UNDER TWO CONTRASTING SOIL MOISTURE REGIMES IN A GREENHOUSE

3.1 Introduction

A warming climate coupled with variable precipitation patterns, are likely to make soil moisture a limiting factor in the survival and growth of many plant species (Christensen et al., 2007). Because species composition and productivity is determined by their adaptation to the prevailing environmental conditions, knowledge of the adaptability of plant species growing in ecosystems believed to be most vulnerable to changes in soil moisture under a changing climate is essential. In the Canadian Prairies, response to drought is going to be a major factor in the establishment, growth and survival of many woody species under a changing climate. The impact that soil moisture deficit will have on these species will ultimately depend on their adaptability to reduced soil moisture.

Poplars are valuable trees, widely planted for the protection of agricultural lands (riparian areas and shelterbelts) and farmsteads, ornamental purposes and afforestation. Although many poplar species are native to areas where there is high soil moisture (Burns and Honkala, 1990), hybrid clones are being used in many areas where soil moisture may be limiting. As a result, there is increasing concern whether currently used hybrid poplar clones will be able to withstand the increasingly severe and more frequent drought periods predicted with climatic change.

A number of studies have looked at the response of species or hybrids of select species of poplar to soil moisture deficit or drought conditions (Blake et al., 1984; Bassman and Zwier, 1991; Rhodenbaugh and Pallardy, 1993; Gebre et al., 1994, 1997, 1998; Tschaplinski and Tuskan, 1994; Tschaplinski et al., 1994, 1998; Liu and Dickmann, 1996; Souch and Stephens, 1998; Bond and Kavanagh, 1999; Brignolas et al., 2000; Leffler and Evans, 2001; Marron et al., 2003; Yin et al., 2005; Voltas et al., 2006). Most have indicated that these *Populus* spp. are not very drought tolerant. When poplars are growing where soil moisture is reduced, leaf area production is restricted and overall growth is reduced (Rhodenbaugh and Pallardy, 1993; Tschaplinski et al., 1994, 1998; Souch and Stephens, 1998; Brignolas et al., 2000; Marron et al., 2003). In some cases, drought conditions have resulted in severe xylem cavitation in poplars leading to branch abscission or top dieback or even mortality (Tyree and Sperry, 1988; Tyree et

al., 1994; Rood et al., 2000). However, studies (e.g., Blake et al., 1984; Bassman and Zwier, 1991; Gebre and Kuhns, 1991; Brignolas et al., 2000) and observations of poplar hybrid growth in the Canadian Prairies indicate that there may be large variability in drought responses between species or hybrids of poplar.

Plant responses to moisture stress will vary depending on whether they have been pre-exposed to that stress. In dry areas, plants will be subjected to several water stress or drought events during a season, allowing for adjustments in growth and physiology (acclimation) and better adaptation to moisture deficit conditions. The negative effects of the moisture stress are often then lessened in these acclimated plants compared to plants that have never been exposed to the stress (Sullivan and Eastin, 1974). A majority of the studies examining drought tolerance in hybrid poplar did not evaluate drought acclimation responses after exposure to soil moisture deficit conditions. In the ones that did examine acclimation responses, a preferential allocation of carbon to root growth was observed in poplar plants exposed to moisture deficit conditions (Tschaplinski et al., 1994; Ibrahim et al., 1998; Tschaplinski et al., 1998; Chaves et al., 2002; Monlus et al., 2005). In addition, both stomatal conductance and photosynthesis were generally reduced and water-use efficiency (WUE) was often higher in poplar clones that have been pre-exposed to moisture deficit conditions (Bassman and Zwier, 1991; Tschaplinski et al., 1994; Liu and Dickmann, 1996; Monclus et al., 2006).

Although these studies are important in understanding the responses of *Populus* spp. to moisture deficit, information from these studies has limited direct application to the Canadian Prairie environment. The vast majority of these studies were conducted with clones that are grown in areas with much higher soil moisture availability and lower vapour pressure deficits and where drought is not as frequent as in the Prairies. There has also been limited physiological characterization of poplar clones commonly used in the Prairies.

It is not known whether clones commonly grown here will continue to grow well or even survive when soil moisture deficits become more common and more severe. Clones commonly grown on the Prairies include hybrids of *P. deltoides* var. *occidentalis* (plains cottonwood), *P. balsamifera* (balsam poplar), *P. laurifolia* (laurel-leaf poplar), and *P. nigra* (black poplar), many of which are species native to cold and dry regions. Unpublished work indicates that some hybrid poplar clones grown on the Prairies perform (or survive) better than others under drought conditions (S. Silim, personal communication). Thus, evidence seems to indicate that there are

poplar clones more suited to drought conditions than generally recognized and it is therefore necessary to identify those clones.

The clones with the best ideotype will be the ones that have continued productivity despite limiting soil moisture. This could be a result of changes in drought tolerance, WUE or gas-exchange characteristics among others. Evaluating growth, gas exchange and WUE under soil moisture deficit conditions will help identify the clones with the best ideotype and understand the underlying mechanisms behind their continued productivity under limiting soil moisture. Examining the relative WUE and the basis of changes in WUE of clones can also help identify clones and lines that could be used more extensively in breeding or genome work. This study examines the responses of several clones of hybrid poplar commonly grown on the Prairies to soil moisture deficit. Results from this study will allow for selection of superior hybrid poplar clones that will be best adapted to current and future climate conditions.

The main objective of this part of the study was to examine the adaptability of four hybrid poplar clones (Hill, Northwest, Okanese, and Walker) commonly grown in the Canadian Prairies to soil moisture deficits and identify the physiological basis for differences in adaptability.

3.2 Objectives

The specific objectives of the study were to examine:

1. growth responses, including plant height, stem diameter, biomass production, leaf area, leaf numbers, and stomata density and size,
2. gas exchange responses, including net photosynthesis, stomatal conductance, and intrinsic WUE, and
3. integrative WUE determined by carbon isotope composition,

of four hybrid poplar clones to soil moisture deficit conditions. The four clones (Hill, Northwest, Okanese, and Walker) were chosen based on their observed contrasting responses to soil moisture deficit conditions in a preliminary (unpublished) study.

3.3 Materials and Methods

3.3.1 Plant material

Four hybrid poplar (*Populus* spp.) clones were used in this study. The clones are hybrids of *Populus deltoides* var. *occidentalis* and *P. balsamifera* or *P. x petrowskyana* and are currently grown on the Canadian Prairies. They are:

1. *Populus* x cv. ‘**Walker**’ (*P. deltoides* W. Bartr. ex Marsh. var. *occidentalis* Rydb. x *P. x petrowskyana* Schneid. (*P. laurifolia* Ledeb. x *P. nigra* L. var. *italica* DuRoi)),
2. *Populus* x cv. ‘**Hill**’ (*P. deltoides* var. *occidentalis* x *P. petrowskyana*),
3. *Populus* x *jackii* Sarg. cv. ‘**Northwest**’ (*P. balsamifera* L. x *P. deltoides* var. *occidentalis*), and
4. *Populus* x ‘**Okanese**’ (formerly ‘**WP69**’, *P.* ‘Walker’ x *P. petrowskyana*).

Seven cm long cuttings were made from whips collected during the previous winter from stooling beds located at the Agriculture and Agri-Food Canada (AAFC) Shelterbelt Centre in Indian Head, Saskatchewan. To ensure physiological uniformity, cuttings were taken from the midpoint of the whip and had two buds and a diameter of about 7 mm.

3.3.2 Growth environment

Cuttings were planted in 1.65 L TreepotsTM (Stuewe and Sons, Inc., Corvallis, OR) in a media containing peat moss, perlite, and dolomite lime with a pH of about 6.5. Plants were grown in a greenhouse (AAFC Shelterbelt Centre, Indian Head, Saskatchewan) at 24°/18° C day/night temperatures under natural light (800 to 1200 $\mu\text{mol m}^{-2} \text{s}^{-1}$ PPFD (photosynthetic photon flux density)) extended to 18 h daylength with CeramaluxTM high pressure sodium lights (Philips Electronics Ltd., Markham, ON) and ambient relative humidity (25 to 30%). The growing media was kept moist until bud break (emergence of the first leaf tip above the soil surface), which was uniform and took about 10 days. The soil moisture treatments (see below) were initiated 2.5 wks (+/- 4 d) after bud break and continued for 5.5 wks (+/- 2 d) (study duration of about 8 wks). Beginning 1 wk after bud break and continuing throughout the study, plants were fertilized with a modified Hoagland’s solution (Hoagland and Arnon, 1950) containing 10 mM N, 0.4 mM P, 3.52 mM K, 1.35 mM Ca, 0.5 mM Mg, 0.53 mM S, 20 μM Fe, 0.2 μM Cu, 0.1 μM Mo, 6 μM Mn, 0.4 μM Zn, 24 μM B with every watering (once or twice per week depending on the treatment). Plants in the well-watered treatment were given half-strength

nutrients approximately twice per week, while plants in the drought treatment were given full strength nutrients about once per week.

3.3.3 Experimental design

The experiment was set up as a completely randomized design with the four hybrid poplar clones being randomly assigned and subjected to either a well-watered or droughted soil moisture treatment. Plants were kept in trays so that droughted plants were kept together for ease of treatment and tracking. Weekly, each plant was rotated within the tray and each tray of plants was rotated throughout the greenhouse at random.

3.3.4 Soil moisture treatments

A soil moisture retention curve, giving the relationship between ψ_{soil} and soil moisture content (% g g^{-1}) was constructed to give the soil weight that corresponded to about -1.2 MPa ψ_{soil} (Appendix A). ψ_{soil} was measured using a Psypro psychrometer (Wescor Inc., Logan, UT). Two and a half wks after bud break, forty plants per clone were subjected to either a well-watered treatment or a soil moisture deficit treatment (hereafter referred to as the drought treatment). Plants in the well-watered treatment were watered regularly to maintain the soil moisture level at or near field capacity of the pots for the duration of the study. Plants in the drought treatment underwent six cycles of soil drying (lasting about 1 wk per cycle) achieved by allowing the soil to dry gradually through evapotranspiration until the soil water potential (ψ_{soil}) had reached about -1.20 MPa, determined gravimetrically. At the start of each cycle, pots were weighed after watering to field capacity. For the duration of each cycle, soil moisture loss (from field capacity weight) was determined twice daily (gravimetrically) to determine the soil water content and thus soil moisture content. Using water loss from field capacity to determine soil water content minimized the effect that increasing plant weight had on soil water determination. Once the equivalent weight loss corresponding to a soil water potential of about -1.20 MPa was reached, the pots were re-watered to field capacity. This was an equivalent loss of about 850g of the water from the pots.

3.3.5 Growth measurements

After eight weeks of growth, five plants per clone per treatment were harvested for growth assessments. The following variables were determined: stem diameter, height, number of leaves, leaf area, and biomass of roots, shoots, stem and leaves, and total plant biomass. Stem diameter was the diameter of the main stem measured at soil level. Plant height was measured

on the main leader from the soil to the tip of the youngest leaf. Number of leaves included only the fully expanded leaves. Leaf area was determined using a LI-3100 leaf area meter (LiCor Inc., Lincoln, Nebraska). After stem diameter, height, leaf numbers and leaf areas were measured, plants were separated into shoots and roots. Roots were gently washed to remove the growth media. All plant components were dried in an oven at 60 °C until they reached a constant weight (about 48 hours), after which the dry weights of leaves, stems, cuttings and roots were determined. Leaf area per gram of root dry weight ($\text{cm}^2 \text{g}^{-1}$) was calculated to estimate transpirational surface area per mass of roots.

3.3.6 Determination of stomatal density, size and distribution

Stomata density, size and distribution were determined by examining impressions that were made on mature, uniformly aged leaf surfaces prior to harvest. For each of the five plants per clone per treatment harvested, one impression was made of an adaxial leaf surface and one impression was made of the abaxial leaf surface (on the same leaf). Impressions were taken on a 2.25 cm^2 interveinal area by applying a fast drying nail polish. Impressions were removed when dry and secured on microscope slides with a cover slip. For each impression, stomatal and cell density was determined by counting either the number of stomata or number of cells in three randomly selected areas per impression at 200x magnification. Lengths of five random open and five random closed stomata were also measured at 200X magnification to give stomatal size. Open and closed stomatal lengths were determined because there was considerable variation in stomatal length depending on whether stomata were open (considered open if aperture was $>0.005 \mu\text{m}$ wide) or closed. Because there were no open stomata observed on Okanese leaves, statistical comparisons of open stomata sizes were only done between Hill, Northwest and Walker plants. Stomatal index, the ratio of stomata to epidermal cells, was calculated as (Willmer and Fricker, 1996):

$$\text{SI} = \frac{\text{stomata density}}{\text{cell density} + \text{stomata density}} \quad [3.1]$$

3.3.7 Gas exchange measurements

Steady state gas exchange was measured one day after all the plants from both moisture treatments were watered to field capacity (i.e., plants from both moisture treatments were under well-watered conditions). The measurements were on mature leaves of similar age, once when plants were five weeks old and again when they were six weeks old. Plants from the drought treatment had undergone at least three cycles of soil moisture deficit (of about 5 days in duration)

before measurements were made. Measurements were made between 9:00 and 13:00 hours using an ADC LCPro gas exchange system (ADC Bioscientific, UK) at 1056 $\mu\text{mol m}^{-2} \text{s}^{-1}$ PPFD, 26 °C, about 35% RH and about 370 ppm CO_2 . Net photosynthetic rate (A , $\mu\text{mol CO}_2 \text{ m}^{-2} \text{ s}^{-1}$), stomatal conductance to water vapour (g_s , $\text{mol H}_2\text{O m}^{-2} \text{ s}^{-1}$), intercellular CO_2 concentration (c_i), and atmospheric CO_2 concentration (c_a) were read directly from the LCPro. Intrinsic WUE efficiency was calculated as A/g_s ($\mu\text{mol CO}_2 \text{ m}^{-2} \text{ s}^{-1} / \text{mol H}_2\text{O m}^{-2} \text{ s}^{-1}$). Plants that were used for measurements were the same that were subsequently harvested for growth and carbon isotope composition ($\delta^{13}\text{C}$) determination.

3.3.8 Determination of carbon isotope composition

Carbon isotope composition ($\delta^{13}\text{C}$), used as a measure of integrative WUE was determined from subsamples of ground leaf tissue. Dried leaf tissue was ground to powder in a pulverizer mill and 1 mg subsamples were analyzed by Isotope Ratio Mass Spectrometry at the University of Saskatchewan (Europa Tracer 20-20, Europa Scientific, Crewe, UK). $\delta^{13}\text{C}$ of leaf tissue was determined using the formula

$$\delta^{13}\text{C} = [R/(R_s - 1)] \quad [3.2]$$

and expressed as values per mil relative to values of Pee Dee Belemnite (PDB) carbonate where R is the abundance ratio ($^{13}\text{C}/^{12}\text{C}$) of plant material and R_s is that of a standard (PDB carbonate) (Farquhar et al., 1989).

3.3.9 Statistical analyses

The general linear model for the two factorial treatment is fixed and is represented by:

$$y_{ijk} = \mu + A_i + B_j + (AB)_{ij} + \varepsilon_{ijk}, \quad [3.3]$$

where i corresponds to the soil moisture treatments and j corresponds to the clones, ij is the interactions, k is the number of plants, and ε_{ijk} is the random error. The clones and the moisture treatments are fixed.

The null hypothesis was that growth, gas exchange and WUE will be the same for all clones regardless of treatment. The type I error rate (α) was set at 0.05 for all tests. Analysis of variance were computed for: plant height, stem diameter, total biomass, shoot biomass, root biomass, leaf numbers, total leaf area, area per leaf, shoot:root ratio, leaf area per root dry weight, total biomass per leaf area, specific leaf weight, leaf area ratio, stomatal density, stomatal length, stomatal distribution, stomatal index and $\delta^{13}\text{C}$. Analysis of variance were also computed for net photosynthesis (A), stomatal conductance (g_s), A/c_i and A/g_s . Because gas exchange was

measured twice, analyses of variance were also used to test for the significance of date effects on gas exchange. Differences between means were tested using Tukey's test. Correlation analyses were performed between $\delta^{13}\text{C}$ and A/g_s , $\delta^{13}\text{C}$ and A , $\delta^{13}\text{C}$ and g_s , $\delta^{13}\text{C}$ and c_i/c_a , $\delta^{13}\text{C}$ and total biomass and A/g_s and total biomass. All analyses were performed using Minitab ver. 13 (Minitab Inc., State College, PA, USA).

3.4 Results

3.4.1 Growth responses

3.4.1.1 Height, stem diameter and biomass

After 8 wks of growth, there were significant differences in growth between clones. Regardless of soil moisture treatment, Okanese and Walker plants were taller (Figure 3.1a), had larger stem diameters (Figure 3.2a) and more shoot and total biomass than either Hill or Northwest plants (Figure 3.3a). Okanese and Walker plants were about 20% taller than Northwest plants and about 40% taller than Hill plants with 30% more shoot and 20% more total biomass than either Hill or Northwest plants. Okanese plants also had more root biomass (30-60% more) than the other three clones (Figure 3.3a).

Regardless of clone, growth was reduced in plants that had grown under the drought treatment. There were no significant ($p \leq 0.05$) clone \times treatment interactions for height, stem diameter, shoot, root, total or shoot:root biomass, indicating that all clones were similarly impacted by the drought treatment. Droughted plants were about 20% shorter (Figure 3.1b), had smaller stem diameters (Figure 3.2b), and had 30% less shoot biomass and total biomass (Figure 3.3b) than well-watered plants. Overall, root biomass was about 30% lower in droughted plants. However, only Okanese plants had differences in root biomass production between well-watered and droughted plants (Figure 3.3b).

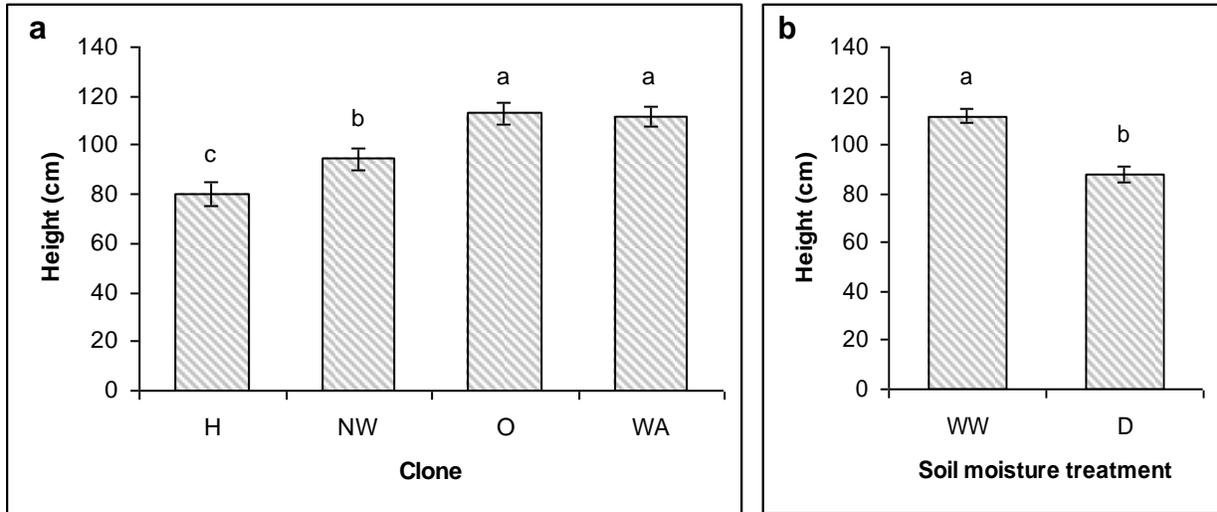


Figure 3.1 Mean height of (a) Hill (H), Northwest (NW), Okanese (O) and Walker (WA) hybrid poplar plants or of (b) plants from either the well-watered (WW) or droughted (D) soil moisture treatments after 8 wks of growth. Error bars represent standard error of the mean, for (a) $n=5$ and (b) $n=10$. Between clones or soil moisture treatments, bars followed by the same letter are not significantly different according to Tukey's test at $p<0.05$.

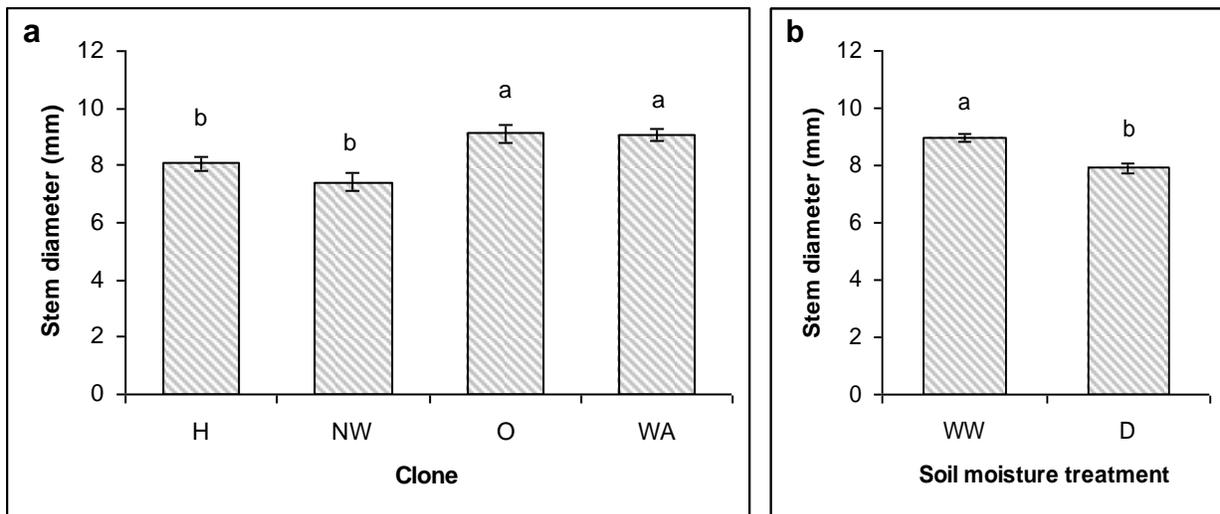


Figure 3.2 Stem diameters of (a) Hill (H), Northwest (NW), Okanese (O) and Walker (WA) hybrid poplar plants or of (b) plants from either the well-watered (WW) or droughted (D) soil moisture treatments after 8 wks of growth. Error bars represent standard errors of the means, (a) $n=5$ and (b) $n=10$. Between clones or soil moisture treatments, bars followed by the same letter are not significantly different according to Tukey's test at $p\leq 0.05$.

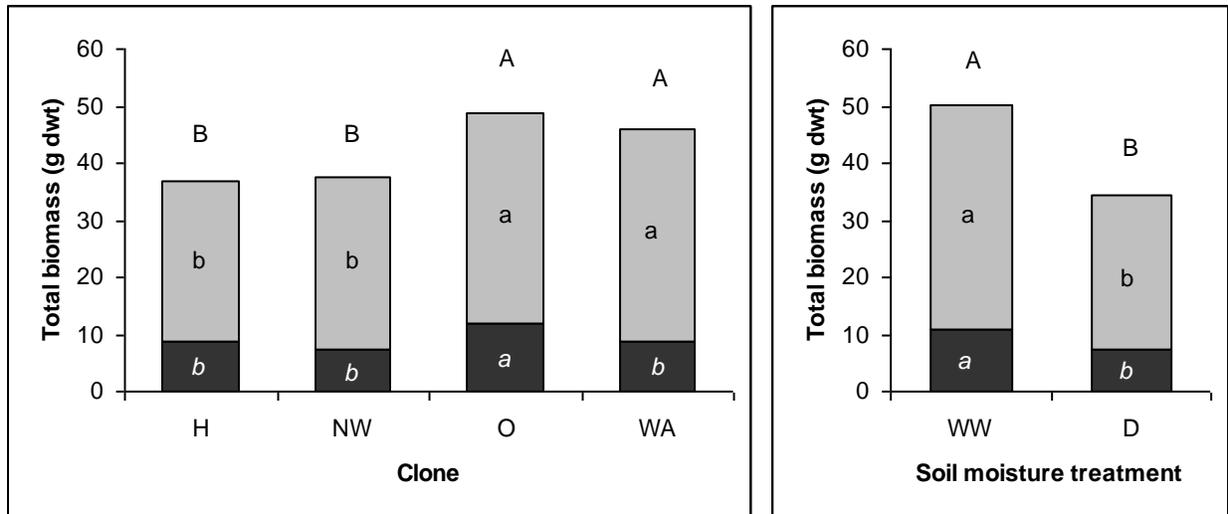


Figure 3.3 Total biomass including shoot (light shading) and root (dark shading) biomass of (a) Hill (H), Northwest (NW), Okanese (O) and Walker (WA) hybrid poplar plants, $n=5$, or of (b) plants from either the well-watered (WW) or droughted (D) soil moisture treatments, $n=10$. Between clones or soil moisture treatments, bars followed by the same letter are not significantly different according to Tukey's test at $p \leq 0.05$.

3.4.1.2 Leaf number and size

Regardless of soil moisture treatment, Walker plants had the highest number of leaves (46) and Northwest plants had the fewest (28) number of leaves (Figure 3.4a), despite droughted plants having about 10% fewer leaves than well-watered plants (Figure 3.4b). The average area per leaf also differed between clones, although differences depended on the soil moisture treatment that plants had grown under (Table 3.1). Among well-watered plants, Hill and Walker had the smallest leaves and Northwest plants had the largest leaves, while among droughted plants, differences in area per leaf were less distinct because the drought treatment affected leaf expansion of Northwest plants more than the other clones (40% vs. 25% in the other clones). This resulted in droughted Hill, Northwest and Okanese plants having similarly sized leaves. Regardless of differences in leaf numbers or sizes, total leaf area per plant was highest in Okanese and Walker plants, regardless of soil moisture treatment plants had grown under (Figure 3.5a). Total leaf area was similarly restricted by the drought in all clones (Figure 3.5b).

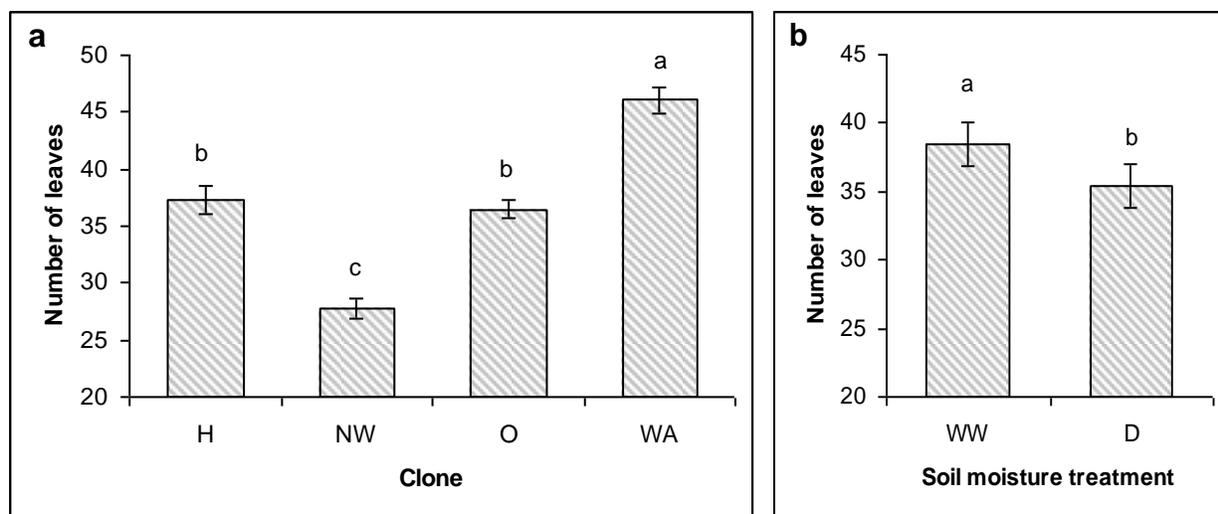


Figure 3.4 Number of leaves of (a) Hill (H), Northwest (NW), Okanese (O) and Walker (WA) hybrid poplar plants or of (b) plants from either the well-watered (WW) or droughted (D) soil moisture treatments after 8 wks of growth. Error bars represent standard errors of the means, (a) $n=5$ and (b) $n=10$. Between clones or soil moisture treatments, bars followed by the same letter are not significantly different according to Tukey's test at $p \leq 0.05$.

Table 3.1 Area per leaf of Hill, Northwest, Okanese and Walker hybrid poplar clones after 8 wks under either well-watered or droughted soil moisture conditions.

	Area per leaf (cm ²)	
	Well-watered	Droughted
Hill	68.47c [†]	51.83ab
Northwest	93.14a	56.85ab
Okanese	81.78b	61.09a
Walker	66.54c	49.40b

[†] Within a column, values followed by the same letter are not significantly different according to Tukey's test at $p \leq 0.05$.

3.4.1.3 Carbon allocation patterns

Although Okanese plants were the largest and Hill plants the smallest, the shoot:root ratio was similarly low in Okanese and Hill plants (3:1) compared to Northwest and Walker plants (4:1, Figure 3.6). Clones also differed in the ratio of leaf area to root biomass, with Okanese plants having about 30% lower leaf area:root biomass than Walker and Northwest plants (Figure 3.7). There were no differences in leaf area:root biomass between Okanese and Hill clones, however. Because both shoot and root growth were similarly reduced overall by the drought

treatment, the ratios of shoots:roots nor leaf area:root biomass were not significantly different between droughted and well-watered plants however (data not shown).

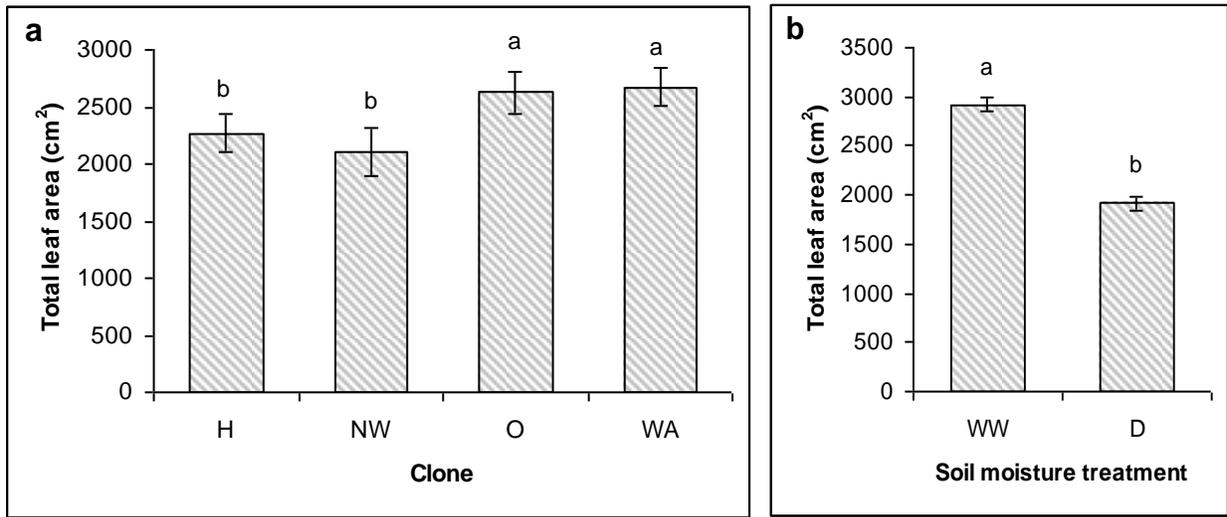


Figure 3.5 Total plant leaf area of (a) Hill (H), Northwest (NW), Okanese (O) and Walker (WA) hybrid poplar plants or of (b) plants from either the well-watered (WW) or droughted (D) soil moisture treatments after 8 wks of growth. Error bars represent standard errors of the means, (a) n =5 and (b) n=10. Within a clone or soil moisture treatment, bars followed by the same letter are not significantly different according to Tukey's test at $p \leq 0.05$.

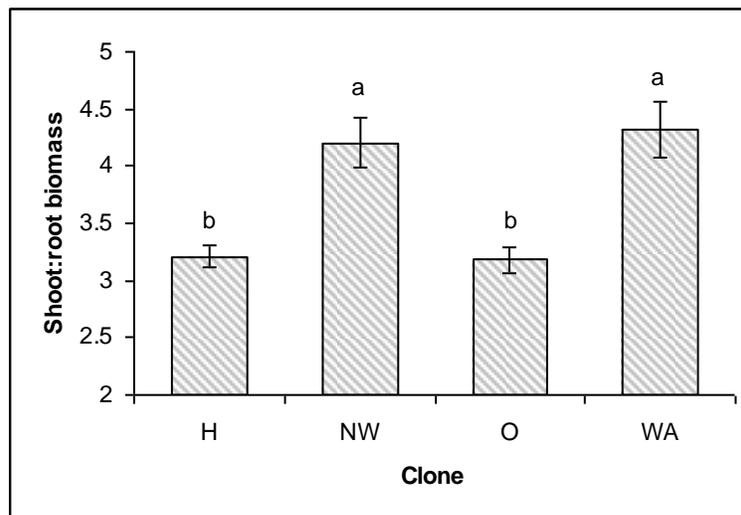


Figure 3.6 Mean shoot:root ratio of Hill (H), Northwest (NW), Okanese (O) and Walker (WA) hybrid poplar plants after 8 wks of growth under well-watered and droughted soil moisture treatments. Error bars represent standard errors of the means, n =5. Bars followed by the same letter are not significantly different according to Tukey's test at $p \leq 0.05$.

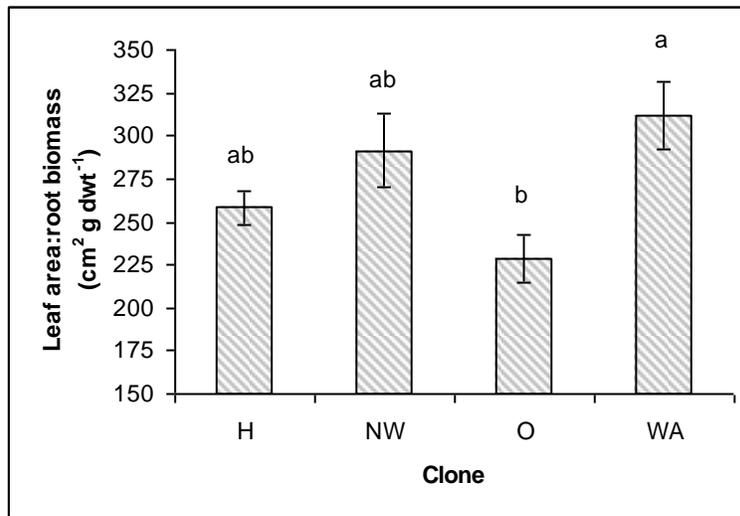


Figure 3.7 Leaf area: root biomass ($\text{cm}^2 \text{g}^{-1}$) of Hill (H), Northwest (NW), Okanese (O) and Walker (WA) hybrid poplar plants after 8 wks of growth under well-watered and droughted soil moisture treatments. Error bars represent standard errors of the means, $n = 5$. Bars followed by the same letter are not significantly different according to Tukey's test at $p \leq 0.05$.

3.4.1.4 Stomata density, distribution and size

Among well-watered plants, leaves of Okanese plants had a total stomatal density that was about 50% lower than that of the other clones, while among droughted plants Okanese stomatal densities were only about 30% lower than that of the other clones (Table 3.2). This was a result of the total stomatal density being affected by soil moisture treatment in only Okanese plants; with droughted plants having higher stomata densities compared to well-watered treatment plants. For plants from both treatments, clonal differences in overall stomatal density were mainly a result of differences in stomatal densities on adaxial (upper) leaf surfaces, rather than abaxial (lower) leaf surfaces (Table 3.2). Because stomatal densities on adaxial leaf surfaces were not significantly affected by the soil moisture treatment, Hill, Northwest and Walker plants had 5 to 6 times the adaxial stomatal density as Okanese plants, regardless of soil moisture treatment. Clonal differences in stomatal densities on abaxial leaf surfaces were affected by soil moisture treatment that plants had grown under. Among well-watered plants, Okanese plants had the lowest abaxial stomata densities, while among droughted plants, Okanese, Hill and Northwest plants had lower abaxial stomata densities than Walker plants (Table 3.2). Because of the large contrasts in density between leaf surfaces, the ratio of abaxial:adaxial stomata also differed in Okanese plants compared to that of the other three

clones. Okanese plants had an abaxial:adaxial ratio of over 6:1 compared to a ratio of only about 1.5:1 in Hill, Northwest and Walker plants (Figure 3.8).

As with stomatal densities, there were also differences in stomatal lengths between clones and soil moisture treatments, at least on adaxial leaf surfaces. These clonal and soil moisture treatment effects on stomatal size were different depending on whether stomata were closed or open, however. Walker plants had smaller closed adaxial stomata than Hill, Northwest or Okanese plants (Table 3.3), while both Hill and Walker plants had smaller open adaxial stomata than Northwest plants (open stomata of Okanese plants could not be compared because there were no open stomata observed). The soil moisture treatment had an effect on adaxial stomatal length in closed stomata of Okanese and open stomata of Northwest plants only, with droughted plants of these two clones having smaller stomata than their well-watered counterparts (Table 3.3).

Table 3.2 Adaxial, abaxial and total (adaxial + abaxial) stomatal densities of Hill, Northwest, Okanese and Walker hybrid poplar clones after 8 wks of growth under either well-watered (WW) or droughted (D) soil moisture conditions.

Clone	Adaxial (upper) stomatal density (no. mm ⁻²)		Abaxial (lower) stomatal density (no. mm ⁻²)		Total stomatal density (no. mm ⁻²)	
	WW	D	WW	D	WW	D
Hill	94.34a [†]	93.75a	103.23b	106.19b	197.57a	199.94a
Northwest	78.34a	82.22a	105.36b	104.15b	183.70a	186.37a
Okanese	12.44c*	18.25c	86.52a	128.00a	98.96b*	133.93b
Walker	73.13b	77.39b	113.30b	117.21b	186.43a	205.39a

[†]Within each column, values followed by the same letter are not significantly different according to Tukey's test at $p \leq 0.05$.

*Indicates significant differences between soil moisture treatments according to Tukey's test at $p \leq 0.05$.

Table 3.3 Size (length of stomatal pore) of closed and open adaxial stomata on Hill, Northwest, Okanese and Walker hybrid poplar plants after 8 wks of growth under either well-watered (WW) or droughted (D) soil moisture conditions.

Clone	Size of closed adaxial (upper) stomata (μm)		Size of open adaxial (lower) stomata (μm)	
	WW	D	WW	D
Hill	5.85 [†]	5.90a	5.91b	5.77ab
Northwest	6.02ab	5.97a	6.29a*	5.84a
Okanese	6.27a*	5.86a	-	-
Walker	5.25c	5.36b	5.53c	5.39b

[†]Within each column, values followed by the same letter are not significantly different according to Tukey's test at $p \leq 0.05$.

*Indicates significant differences between soil moisture treatments according to Tukey's test at $p \leq 0.05$.

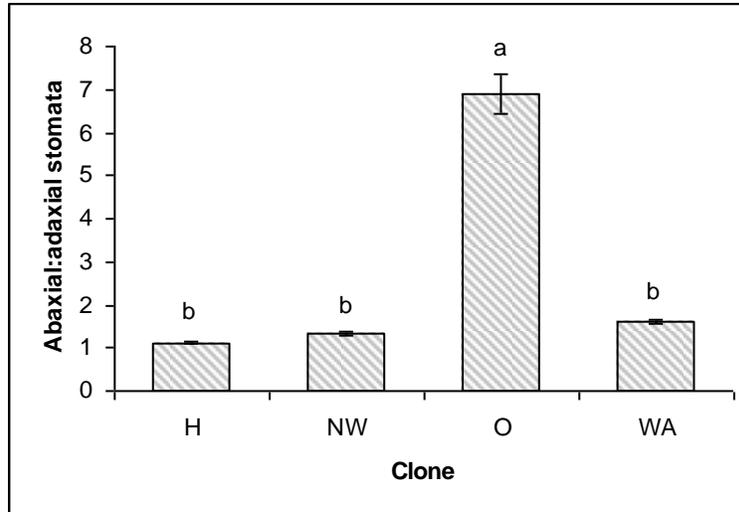


Figure 3.8 Ratio of abaxial:adaxial stomatal density of Hill (H), Northwest (NW), Okanese (O) and Walker (WA) hybrid poplar plants after 8 wks of growth. Error bars represent standard errors of the means, $n = 5$. Bars followed by the same letter are not significantly different according to Tukey's test at $p \leq 0.05$.

3.4.2 Physiological responses

3.4.2.1 Gas exchange responses

Both stomatal conductance (g_s) and net photosynthesis (A) differed between clones; with differences depending on the soil moisture treatment. Among well-watered plants, Okanese plants had lower stomatal conductance than Hill, Northwest or Walker plants (Table 3.4). However, among droughted plants, Hill, Okanese and Walker plants all had similarly low values of g_s compared to Northwest plants, due to droughted Hill and Walker plants having reduced g_s compared to well-watered plants. Among well-watered plants, net A was highest in Northwest plants, while among droughted plants Northwest as well as Okanese plants had higher net A than the other clones (Table 3.4). Net A of only Hill plants was significantly affected by the drought treatment, however (Table 3.4).

Table 3.4 Net photosynthesis (A) and stomatal conductance (g_s) of Hill, Northwest, Okanese and Walker hybrid poplar clones after 8 wks of growth under either well-watered (WW) or droughted (D) soil moisture conditions.

Clone	A ($\mu\text{mol CO}_2 \text{ m}^{-2} \text{ s}^{-1}$)		g_s ($\text{mol H}_2\text{O m}^{-2} \text{ s}^{-1}$)	
	WW	D	WW	D
Hill	18.22ab [†]	15.50b	0.57a	0.47b
Northwest	19.32a	18.39a	0.62a	0.55a
Okanese	17.60b	17.62a	0.48b	0.46b
Walker	17.74ab	16.85ab	0.63a	0.49ab

[†]Within each column, values followed by the same letter are not significantly different according to Tukey's test at $p \leq 0.05$.

*Indicates significant differences between soil moisture treatments according to Tukey's test at $p \leq 0.05$.

3.4.2.2 Water-use efficiency and carbon isotope composition

Regardless of treatment, Okanese plants had higher intrinsic WUE (A/g_s , Figure 3.9a) and carbon isotope compositions ($\delta^{13}\text{C}$) than the other clones (Figure 3.10b), while Hill and Northwest plants had the lowest values of $\delta^{13}\text{C}$. The soil moisture treatment did affect the values of A/g_s and $\delta^{13}\text{C}$ however, with droughted plants having higher A/g_s (Figure 3.9b) and $\delta^{13}\text{C}$ (Figure 3.10b) than well-watered plants.

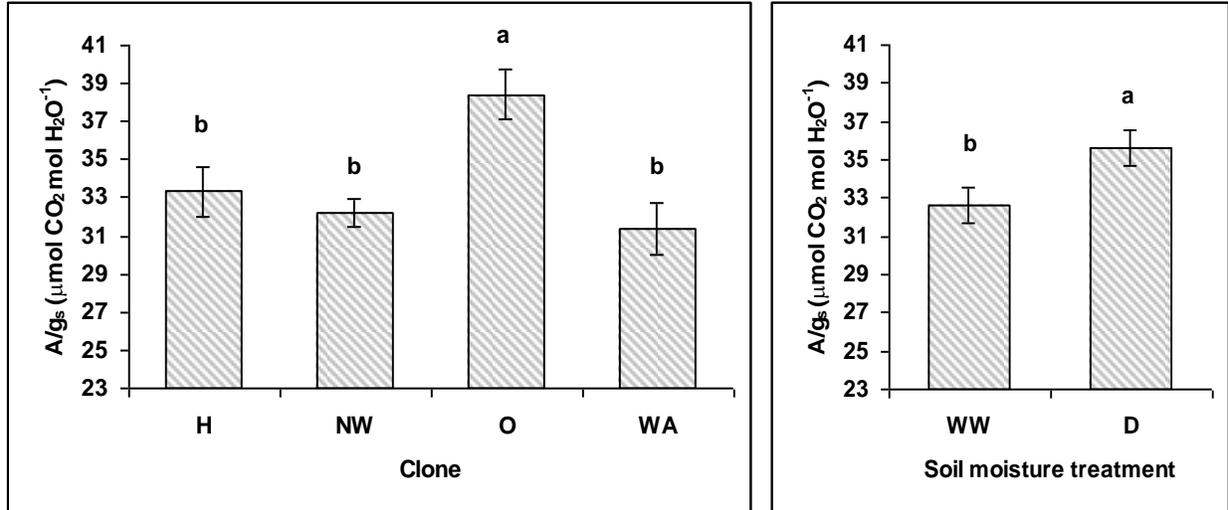


Figure 3.9 Intrinsic WUE (A/g_s , $\mu\text{mol CO}_2 \text{ mol H}_2\text{O}^{-1}$) of (a) Hill (H), Northwest (NW), Okanese (O) and Walker (WA) hybrid poplar plants or of (b) plants from either the well-watered (WW) or droughted (D) soil moisture treatments after 8 wks of growth. Error bars represent standard errors of the means, (a) $n = 5$ and (b) $n = 10$. Between clones or soil moisture treatments, bars followed by the same letter are not significantly different according to Tukey's test at $p \leq 0.05$.

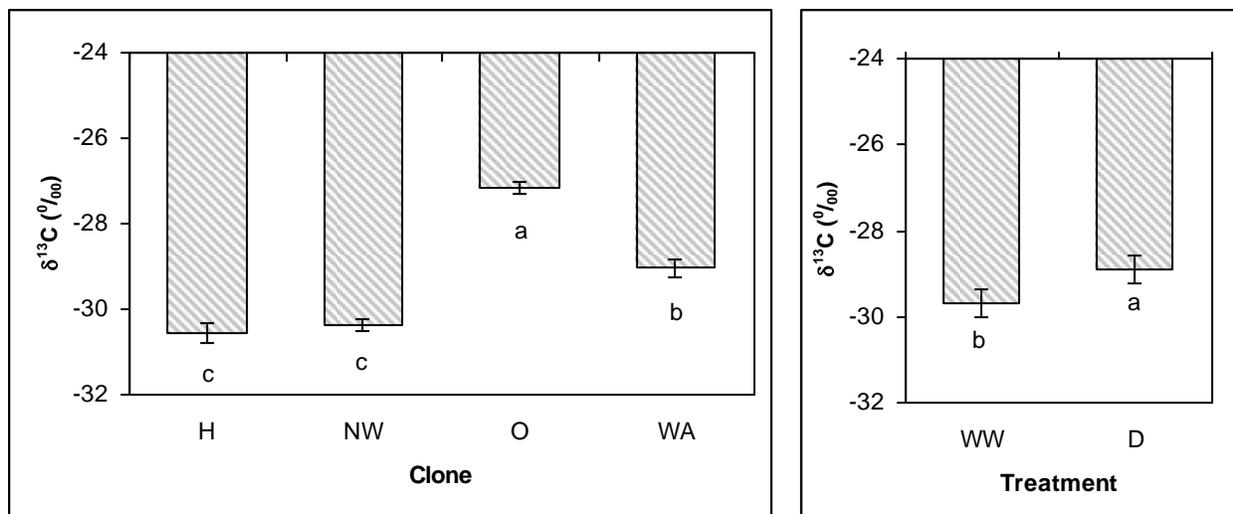


Figure 3.10 Carbon isotope compositions ($\delta^{13}\text{C}$) of (a) Hill (H), Northwest (NW), Okanese (O) and Walker (WA) hybrid poplar plants or of (b) plants from either the well-watered (WW) or droughted (D) soil moisture treatments after 8 wks of growth. Error bars represent standard errors of the means, (a) $n=5$ and (b) $n=10$. Within a clone or soil moisture treatment, bars followed by the same letter are not significantly different according to Tukey's test at $p \leq 0.05$.

3.4.3 Relationship between growth, gas exchange and water-use efficiency

There was a significant positive linear relationship between $\delta^{13}\text{C}$ and A/g_s for Walker plants (Table 3.5) but not for Hill, Northwest or Okanese plants (Table 3.5). There was also a significant negative linear relationship between $\delta^{13}\text{C}$ and A for Hill plants (Table 3.5), and between $\delta^{13}\text{C}$ and g_s for both Hill and Walker plants (Table 3.5). The relationship between total plant biomass and $\delta^{13}\text{C}$ was significant and negative for Hill and Okanese plants (Table 3.5), but not for either Northwest or Walker plants (Table 3.5).

Table 3.5 Relationship between stomatal conductance (g_s), total plant biomass, net photosynthesis (A), intrinsic WUE (A/g_s), and carbon isotope composition ($\delta^{13}C$) for Hill, Northwest, Okanese and Walker hybrid poplar clones.

	g_s	Total biomass	$\delta^{13}C$	A
Hill				
Total biomass	-0.47 [†] (0.04 [§])			
$\delta^{13}C$	-0.67 (0.00)	-0.56 (0.01)		
A	0.36 (0.13)	0.73 (0.00)	-0.66 (0.00)	
A/g_s	-0.83 (0.00)	-0.04 (0.88)	0.29 (0.22)	0.21 (0.38)
Northwest				
Total biomass	0.62 [†] (0.01 [§])			
$\delta^{13}C$	-0.06 (0.83)	-0.31 (0.23)		
A	0.43 (0.08)	0.28 (0.27)	-0.05 (0.86)	
A/g_s	-0.68 (0.00)	-0.44 (0.08)	0.02 (0.93)	0.35 (0.16)
Okanese				
Total biomass	0.09 [†] (0.68 [§])			
$\delta^{13}C$	-0.07 (0.76)	-0.81 (0.00)		
A	0.51 (0.02)	0.04 (0.88)	-0.06 (0.79)	
A/g_s	-0.94 (0.00)	-0.11 (0.66)	0.08 (0.73)	-0.19 (0.42)
Walker				
Total biomass	0.47 [†] (0.06 [§])			
$\delta^{13}C$	-0.63 (0.01)	-0.44 (0.08)		
A	0.54 (0.03)	0.20 (0.44)	-0.44 (0.08)	
A/g_s	-0.91 (0.00)	-0.44 (0.08)	0.55 (0.02)	-0.21 (0.43)

[†] Correlation Coefficient, r

[§] P-Value, p

3.5 Discussion

The overall objective of this study was to determine whether four hybrid poplar clones (Hill, Northwest, Okanese and Walker) possess either an inherent adaptability to moisture deficit conditions (or drought), or whether they can be pre-conditioned to limiting soil moisture after being pre-exposed to mild soil moisture deficits. While poplar are not considered to be drought tolerant in general (Tyree and Sperry, 1988; Rhodenbaugh and Pallardy, 1993; Tyree et al., 1994; Tschaplinski et al., 1994, 1998; Souch and Stephens, 1998; Brignolas et al., 2000; Rood et al., 2000; Marron et al., 2003), the ability to acclimate to reduced soil moisture during drought episodes would allow hybrid poplar to not only survive, but continue growing despite reduced soil moisture. The specific objectives were to examine the growth, gas exchange and WUE responses of four hybrid poplar clones to soil moisture deficit conditions.

In order to remain productive, or even survive in environments where moisture deficits are common, plants typically possess morphological and physiological adaptations allowing increased uptake (or storage) and/or reduced loss of water. These adaptations include deep, multi-branched root systems, stomata only on abaxial (lower) leaf surfaces, the ability to change leaf orientations, reduced stomatal conductance, and increased WUE (Kramer, 1983; Blake et al., 1984). After growing for eight weeks in the greenhouse there were significant differences in biomass allocation, stomatal densities and distributions, gas exchange and WUE between the hybrid poplar clones that could be expected to result in differences in relative adaptability of the clones to reduced soil moisture conditions.

Plants that produced less shoot growth or leaf area relative to root biomass may be better able to maintain a positive water balance within plant tissues when soil moisture is reduced compared to plants that have an elevated ratio of shoot biomass or leaf area to root biomass. For example, varieties of wheat with deeper rooting were found to produce higher yields under drought conditions compared to shallower rooting varieties (Hurd, 1968). Among the hybrid poplar clones, Okanese had relatively more root biomass, and along with Hill had lower shoot biomass to root biomass and leaf area to root biomass ratios than Northwest and Walker. This morphological balance may partially lead to Okanese and Hill plants being able to survive or possibly grow in areas where moisture deficits occur more frequently compared to Northwest and Walker plants.

While stomatal densities, distribution and size tend to vary between poplar species (ie//*P. trichocarpa*, *P. deltoides*, *P. nigra*), ecotypes from more xeric environments (and thus considered more drought tolerant in general) have either a higher density of smaller stomata or an increased density of stomata on abaxial leaf surfaces compared to ecotypes from more mesic environments (Pallardy and Kozlowski, 1979; Ceulemans et al., 1984; Ceulemans et al., 1987; Pearce et al., 2005). In the clones examined here, Okanese had significantly lower stomatal densities (per unit leaf area) than Hill, Northwest and Walker. More importantly, most of Okanese plants stomata were on the abaxial leaf surfaces, while in the other clones, adaxial and abaxial stomatal densities were similar. In general, stomata sizes were fairly similar except for Northwest plants that had significantly larger open stomata on adaxial leaf surfaces. Interestingly, there were no open stomata observed on adaxial leaf surfaces in Okanese plants. With most stomata on abaxial leaf surfaces and no stomata open on adaxial leaf surfaces, it seems that

Okanese plants have stomatal characteristics more similar to plants from xeric environments (Pearce et al., 2005). Not surprisingly, based on the relatively low density of stomata per unit leaf surface, g_s (also measured by unit of leaf surface) was lower in Okanese plants compared to the other clones. In contrast, Northwest plants had significantly higher rates of g_s and A , likely the result of bigger stomatal aperture, as evidenced by significantly larger open stomata, at least on adaxial leaf surfaces.

Increased values of A/g_s and $\delta^{13}C$ in Okanese plants and $\delta^{13}C$ in Walker plants indicates that these clones have increased WUE compared to the other two clones. In the case of Okanese plants, A/g_s and $\delta^{13}C$ are likely higher as a result of lower values of g_s , since A was similar to the other clones. In the case of Walker plants, the lack of relationship between $\delta^{13}C$ and A/g_s makes it difficult to determine the cause for increased $\delta^{13}C$ in this clone. Point in time gas exchange measurements do not necessarily reflect the gas exchange over the life of a plant, rather just under the exact time and conditions of the measurements. Regardless, higher values of A/g_s or $\delta^{13}C$ indicate that WUE was highest in Okanese and to a lesser extent, Walker plants, compared to Hill and Northwest plants. In general, plant species or populations with higher WUE will be more successful when water supply is limited compared to plants with lower WUE, thus physiologically, Okanese and Walker would likely have an advantage in terms of water conservation compared to Hill and Northwest plants.

Despite growth similarities in carbon allocation between Hill and Okanese plants, differences in stomatal and gas exchange characteristics suggest that Okanese plants would do much better under reduced soil moisture compared to Hill plants. Northwest and Walker plants drought suitability falls between the two. Comparisons of well-watered and droughted plants suggest, however, that the suitability of Hill, Northwest or Walker plants to environments of reduced moisture may be increased following pre-exposure to slight moisture stress.

Not surprisingly, most growth characteristics determined in this study (plant height, total, shoot and root biomass, number of leaves and leaf area) were lower in plants that had grown under droughted conditions compared to those grown under well-watered conditions. This is consistent with numerous studies finding reductions in biomass production in poplar plants grown under reduced soil moisture compared to plants growing with abundant soil moisture (Rhodenbaugh and Pallardy, 1993; Tschaplinski et al., 1994; Souch and Stephens, 1998; Guarnaschelli et al., 2003). However, there were some differences in the impact of reduced soil

moisture on the four clones suggesting differences in adaptability among the clones, which may be important when these plants are growing under reduced soil moisture in the field. This is similar to other studies where the impact of the soil moisture deficit treatments varied, often depending on the individual, species, population and ecotype (Rhodenbaugh and Pallardy, 1993; Tschaplinski et al., 1994; Souch and Stephens, 1998; Guarnaschelli et al., 2003).

Shoot biomass production of all clones was about 30% lower in droughted plants compared to well-watered plants, but there were no significant soil moisture treatment differences in root biomass production in Hill, Northwest and Walker plants. This is consistent with others reporting a greater impact of soil moisture deficits on shoot growth compared to root growth (Westgate and Boyer, 1985; Sharp et al., 1988; Spollen et al., 1993; Tschaplinski et al., 1998; Brignolas et al., 2000; Chaves et al., 2002). In Okanese plants, however, both shoot and root growth was significantly reduced in droughted plants compared to well-watered plants. It is likely that root production of well-watered Okanese plants was more rapid than that of the other clones (reflected by root biomass of up to 90% higher than the other clones), but under droughted conditions, without sufficient soil moisture, Okanese plant growth, including root growth is lessened based on soil moisture limitation.

In addition to affecting plant growth, the soil moisture regime also significantly affected stomata densities and sizes at least in Northwest and Okanese plants. Droughted Okanese plants had a higher density of stomata, but smaller (closed) adaxial stomata compared to well-watered Okanese plants, while droughted Northwest plants had smaller (open) stomata compared to well-watered plants. Others have found similar results with plants grown under drought conditions, having a higher density of smaller stomata and fewer stomata on adaxial leaf surfaces compared to leaves formed when moisture is abundant (reviewed by Weyers and Meidner, 1990; Willmer and Fricker, 1996).

Despite non-significant soil moisture treatment differences in stomatal densities or sizes, droughted Hill and Walker plants had significantly lower g_s than well-watered plants. Droughted Northwest plants also had reduced g_s compared to well-watered plants, although differences were not statistically significant. These findings are consistent with other studies observing lower g_s in plants pre-exposed to soil moisture deficit conditions (i.e. Silim et al., 2001). In the case of Okanese plants, there were no significant soil moisture treatment effects on g_s , suggesting that the g_s rates remain stable in this clone, regardless of soil moisture conditions.

Photosynthesis was significantly affected by soil moisture treatment only in Hill plants. Even though g_s was also reduced in Hill plants, it is not likely that A was being limited by reduced CO_2 as a result of increased stomatal resistance (i.e., stomatal closure) because c_i of plants from both soil moisture treatments was similar during the measurements (Table B.5). Rather, the soil moisture treatment seems to have affected the photosynthetic apparatus in Hill, preventing recovery to full photosynthetic capacity in plants during the course of this study. This is likely a result of changes in mesophyll resistance to CO_2 , which has been found to be significantly increased in plants grown under soil moisture limiting conditions (Chaves et al., 2008; Flexas et al., 2008). This is supported by lower values of A/c_i in droughted Hill plants as well. There were no significant soil moisture treatment effects on net photosynthesis in plants of the other three clones, suggesting that while photosynthesis did likely decline during the drought cycles, full recovery was possible once water was re-supplied.

Even though both photosynthesis and stomatal conductance were reduced overall in droughted plants compared to well-watered plants, A/g_s was significantly ($p \leq 0.05$) higher in droughted plants, although this was only significant for Walker plants. In this clone, g_s was reduced proportionally more than A by the drought treatment, likely due to increased sensitivity of g_s to reduced moisture (Schulze et al., 1989). A/g_s was not significantly different between treatments for each of the other clones, because g_s and A were similarly reduced by the soil moisture treatment.

While all plants from the soil moisture deficit treatment tended to have slightly higher $\delta^{13}C$ compared to well-watered plants, this effect was only significant in Hill and Walker plants. Since changes in $\delta^{13}C$ can result from either an increased rate of photosynthesis (A), decreased stomatal conductance (g_s), or both, it is important to understand the underlying cause of the observed increase in $\delta^{13}C$ (Hubick et al., 1986; Condon et al., 1987; Ehleringer, 1990; Wright et al., 1994). Because g_s was reduced in droughted Hill and Walker plants compared to well-watered plants, reductions in g_s are likely driving changes in $\delta^{13}C$. Further supporting this is that there was not a positive linear relationship between $\delta^{13}C$ and biomass production for either clone. If changes in A are driving changes in WUE ($\delta^{13}C$ or otherwise), then there would be a linear positive relationship between WUE and biomass production, but not when changes in g_s are responsible for changes in WUE (Hubick et al., 1986; Condon et al., 1987; Wright et al., 1988; Ehleringer, 1990; Silim et al., 2001).

3.6 Conclusions

Under the conditions of this greenhouse study, clones differed in some key morphological and physiological characteristics that may contribute or detract from their success during reduced moisture in the field. Most of the factors, whether morphological or physiological, involve continual maintenance of a water balance within plant tissues, whether by increased uptake, and/or minimized water loss. In areas where plants are exposed to reoccurring episodes of reduced soil moisture, the ability to acclimate to reduced soil moisture may also be important.

A desirable hybrid poplar clone for production under drought conditions will still have a relatively high rate of (shoot) growth, balanced by relatively high root growth, and have stomata characteristics and gas exchange that results in increased water conservation. Regardless of the soil moisture treatment, both Hill and Okanese plants had much lower shoot:root ratios compared to Northwest and Walker plants, suggesting they may be better able to balance water uptake with water loss. In terms of stomatal characteristics, Okanese plants had a majority of stomata on abaxial leaf surfaces. Gas exchange differences as a result of differences in stomata characteristics among the clones led to Okanese plants generally having the highest WUE. In contrast, Hill plants had a similar number of stomata on both adaxial and abaxial leaf surfaces, leading to increased g_s (but not A) and subsequently much lower WUE than Okanese plants.

Despite the lack of inherent adaptabilities to reduced soil moisture compared to Okanese plants, a few differences observed here suggest that the suitability of Hill, Northwest or Walker plants to environments of reduced moisture may be increased following pre-exposure to slight moisture stress. For example, droughted Walker plants had significantly lower rates of g_s , which resulted in elevated WUE compared to well-watered plants. This effect was also observed in Hill plants, although to a lesser extent. Overall, Northwest plants do not appear suitable to areas expected to have drought events of any duration or severity as they show little adaptation. The general growth habit (reduced number of very large leaves compared to the other clones) of this clone further supports this conclusion.

Overall, after examining many morphological and physiological characteristics of Hill, Northwest, Okanese and Walker hybrid poplar, it appears that of the four, Okanese plants are the most suitable for areas expected to experience more frequent or severe drought events.

Northwest, on the other hand, should likely only be grown where soil moisture will remain available for most of the season.

4. STOMATAL RESPONSE DURING A PROGRESSIVE SOIL MOISTURE DEFICIT IN FOUR HYBRID POPLAR CLONES FOLLOWING GROWTH UNDER TWO SOIL MOISTURE REGIMES

4.1 Introduction

Stomatal closure prevents further water loss when soil or atmospheric moisture deficits are high, and is a critical step in minimizing the development of water deficit stress within plant tissues (Mansfield and Davies, 1981; Mansfield and Davies, 1985; Rhodenbaugh and Pallardy, 1993; Tschaplinski et al., 1994; Marron et al., 2003). Once a level of leaf water potential is reached, called the critical threshold, stomata begin to close, limiting leaf conductance (Hsiao, 1973; Cowan, 1977). Although the critical threshold differs between leaves of different ages and those formed during differing growth conditions, it is species dependent. In more drought resistant species, plants may be able to tolerate lower levels of plant moisture, thus have lower threshold water potentials, or alternatively, may have stomata that will respond immediately as plant moisture levels decline, but closure will be gradual as leaf water potentials decrease (Ni and Pallardy, 1991; Willmer and Fricker, 1996). Other less drought tolerant species, cannot tolerate very low levels of plant moisture, thus have stomata that shut at relatively high thresholds with almost immediate closure once the threshold leaf water potential is reached (Schulte et al., 1987; Ceulemans et al., 1988; Furukawa et al., 1990; Parker and Pallardy, 1991). While responsiveness of stomata to declining leaf water potentials generally reflect species adaptability to reduced moisture, in species with the ability to acclimate to reduced moisture, pre-exposure to soil moisture limitations can pre-condition plants to become more responsive when similar conditions are again encountered. Patterns of photosynthetic response are also similar to the patterns of stomatal closure inherent adaptability and/or acclimation capabilities of plants (Aasamaa and Söber, 2001).

Many studies have reported rapid declines in g_s and A with increasing soil water deficit in poplars (Regher et al., 1975; McGee et al., 1981; Schulte et al., 1987; Ceulemans et al., 1988; Bassman and Zwier, 1991; Ni and Pallardy, 1991). Differences in gas exchange responses which appear to be related to drought tolerance have also been reported among and within species and among clones (Regher et al., 1975; Schulte et al., 1987; Ceulemans et al., 1988; Rood et al., 2003). A detailed examination of the dynamic responses of leaf gas exchange to water deficit in

relation to drought tolerance in poplar clones grown in semi-arid environments such as the Canadian Prairies has not been done, however. Clones suitable for this dry, continental environment should have leaf gas exchange characteristics which maximize C gain while minimizing water loss, and avoiding potentially damaging effects of drought-induced stress.

The study reported in Chapter 3 found clonal and soil moisture treatment differences in gas exchange and WUE, however differences in WUE based on steady state gas exchange measurements were not necessarily consistent with measurements of (integrative) WUE based on $\delta^{13}\text{C}$ measurements. Because stomata play an essential role in plant water regulation, examining stomatal responses of plants that have grown under differing soil moisture conditions during declining plant moisture is important for understanding how reduced soil moisture will impact productivity and WUE. Drought tolerant clones are expected to have not only lower steady state g_s as examined in chapter three, but also higher g_s and A at lower leaf water potentials and more gradual reduction in leaf gas exchange in response to a progressive drought than moderately tolerant and drought sensitive clones. Drought preconditioning will improve the leaf water potential and gas exchange responses to a progressive drought in drought tolerant clones more than in drought sensitive and moderately tolerant clones. A greater understanding of the variation in stomatal responses to drought in poplar clones will help identify tolerant genotypes and physiological ideotypes suitable for this region or similar dry environments. It should also help to predict the long-term performance of poplar biomass plantations in this region.

4.2 Objectives

The main objective of this chapter was to further examine the physiological basis for differences in biomass accumulation, gas exchange and WUE found in the study reported in chapter 3. Specifically, the objectives were to characterize the dynamic responses of Ψ_{leaf} and leaf gas exchange to a progressive drought in four poplar clones with contrasting drought tolerance, and examine the effects of drought preconditioning on these parameters.

4.3 Materials and Methods

4.3.1 Plant material

The same four hybrid poplar clones used in study one were used for this study ('Walker', 'Hill', 'Northwest', and 'Okanese'). As with study one, 7 cm long cuttings were made from whips collected during the previous winter from stooling beds located at the Agriculture and

Agri-Food Canada (AAFC) Shelterbelt Centre in Indian Head, Saskatchewan. Cuttings were taken from the midpoint of the whip and had two buds and a diameter of about 7 mm.

4.3.2 Growth environment

Cuttings were planted in 1.65 L TreepotsTM (Stuewe and Sons, Inc., Corvallis, OR) in a media containing peat moss and sandy:loam soil (3:1 peat:soil) with a pH of about 7. Plants were grown in a greenhouse (AAFC Shelterbelt Centre, Indian Head, Saskatchewan) with 24°/18° C day/night temperatures under natural light (800 to 1200 $\mu\text{mol m}^{-2} \text{s}^{-1}$ PAR) extended to 18 h daylength with CeramaluxTM high pressure sodium lights (Philips Electronics Ltd., Markham, ON) and ambient relative humidity (25 to 30%). The growing media was kept moist until bud break (emergence of the first leaf tip above the soil surface), which was uniform and took about 10 d. Plants were given a nutrient solution about once per week, beginning one week after bud break, consisting of 5mM N ($\text{NH}_4^+:\text{NO}_3^-$) and a modified Hoagland's solution (Hoagland and Arnon, 1950) containing 0.4 mM P, 3.52 mM K, 1.35 mM Ca, 0.5 mM Mg, 0.53 mM S, 20 μM Fe, 0.2 μM Cu, 0.1 μM Mo, 6 μM Mn, 0.4 μM Zn, 24 μM B with every watering (once or twice per week depending on the treatment). Plants in the well-watered treatment were given half-strength nutrients approximately twice per week, while plants in the drought treatment were given full strength nutrients about once per week.

4.3.3 Experimental design

The experiment was set up as a completely randomized design with plants from each clone randomly assigned to either a well-watered (control) treatment or the drought pre-conditioning treatment. Plants were kept in trays so that droughted plants were kept together for ease of treatment and tracking. Weekly, each plant was rotated within the tray and each tray of plants was rotated throughout the greenhouse at random.

4.3.4 Soil moisture treatments and drought pre-conditioning

Drought pre-conditioning began when plants were about 3 wks old. Fourty uniformly sized plants from each clone were selected and divided into two groups. One group was well-watered (control treatment) and the other group underwent successive periods of a drought pre-conditioning treatment. The soil in plants from the well-watered treatment was maintained at field capacity of the pots at all times by regularly watering to saturation and allowing the excess water to drain. The soil in plants from the drought preconditioning treatment was allowed to dry following each watering until the soil water content reached a corresponding soil water potential

of -1.0 MPa (+/- 0.2 MPa), determined gravimetrically on a subset of plants from each clone. This point was determined based on a soil moisture retention curve constructed prior to the beginning of this study, giving the relationship between ψ_{soil} and soil moisture content (% g g⁻¹) (Appendix A, Figure A.3 and A.4), where ψ_{soil} was measured using a Psyspro psychrometer (Wescor Inc., Logan, UT). The pots were then re-watered to field capacity of the pots. The plants in the drought preconditioning treatment went through three cycles of drought over a period of 6 wks.

4.3.5 Response measurements

Dynamic changes in leaf gas exchange and water potential (Ψ_{lf}) to a progressive soil drought (dry-down) was examined at the end of the drought preconditioning treatment. Plants from both the control (well-watered) and drought preconditioning treatments were watered to field capacity of the pots and the soil allowed to progressively dry-down through evapo-transpiration. This corresponded to the fourth drying cycle for preconditioned plants. Measurements began 48 h after all plants were re-watered to ensure that plants from both moisture treatments were under well-watered conditions and that preconditioned plants were no longer under moisture stress. To examine the relationships between Ψ_{soil} , Ψ_{leaf} and leaf gas exchange, soil moisture content, gas exchange and Ψ_{lf} were determined continuously on 10-12 plants per clone per treatment throughout the progressive dry-down until stomata had completely closed ($g_s < 0.05 \text{ mol H}_2\text{O m}^{-2} \text{ s}^{-1}$) and plants were beginning to wilt. The dry-down period took between 4 and 6 d, depending on the clone (fastest in Northwest plants and slowest in Okanese plants).

4.3.5.1 Soil moisture and soil water potential

Concurrent with gas exchange and leaf water potential measurements, soil moisture content (% moisture, g g⁻¹) was determined gravimetrically during the progressive dry-down. At the beginning of the dry-down, pots, including the plant and soil were weighed to determine the weight at field capacity of the pots. Immediately prior to each gas exchange measurements, pots were reweighed to determine weight lost from pot capacity weight, corresponding to the amount of water lost between measurements. Using calibrated curves determined prior to the beginning of this study relating soil moisture contents and water loss from field weight, soil moisture content was then calculated. Soil water potentials were then determined using the equation:

$$y = y_o + a * (1 - \exp(-b * x)), \quad [4.1]$$

where: $y = \Psi_{\text{soil}}$, $x = \text{soil water content (\%)}$, $y_0 = -999.67$, $a = 999.59$, $b = 0.3740$ (Appendix A).

4.3.5.2 Gas exchange responses to decreasing soil moisture

From each plant, two to four mature leaves of similar age from the mid-height of the plant were used to examine the relationship between leaf gas exchange and Ψ_{lf} . The steady state gas exchange was based on the first 5 measurements made at the beginning of the dry-down when the soil water content was at pot capacity. Measurements were made between 9:00 and 17:00 hours using an ADC LCPro+ gas exchange system (ADC Bioscientific, UK) at $1056 \mu\text{mol m}^{-2} \text{s}^{-1}$ PAR, $26 \text{ }^\circ\text{C}$, about 35% ambient RH and about 380 ppm CO_2 . Photosynthetic rate (A , $\mu\text{mol CO}_2 \text{ m}^{-2} \text{ s}^{-1}$), stomatal conductance to water vapour (g_s , $\text{mol H}_2\text{O m}^{-2} \text{ s}^{-1}$), and intercellular CO_2 concentration (c_i), and atmospheric CO_2 concentration (c_a) were read directly from the gas exchange system. Water-use efficiency (WUE) was calculated as A/g_s (intrinsic WUE, $\mu\text{mol CO}_2 \text{ m}^{-2} \text{ s}^{-1}/\text{mol H}_2\text{O m}^{-2} \text{ s}^{-1}$).

4.3.5.3 Leaf water potential

Following each gas exchange measurement, the leaf was excised and the corresponding Ψ_{lf} determined immediately. The petiole was cut straight across using a sharp razor and Ψ_{lf} were measured using a pressure bomb (PMS Instrument Co., Albany, OR). The minimum Ψ_{lf} achieved by the plants was determined using the three lowest Ψ_{lf} values observed per clone per treatment.

4.3.5.4 Stomata and photosynthetic responsiveness

Stomatal responsiveness to decreasing Ψ_{lf} was examined by comparing clonal Ψ_{lf} means for both well-watered and drought preconditioned plants when stomatal conductance (g_s) values had decreased to 75%, 40% and below 15% of steady state conductance values. Stomatal conductance was considered to be at 75% when g_s was between 70 and 80% of steady state values, at 40% when g_s was between 25 and 45% of steady state values and below 15% when g_s was less than 15% of steady state values (Table 4.1). The mean Ψ_{lf} related to when g_s values had decreased to 75% of steady state values was considered to be the threshold Ψ_{lf} ($\Psi_{\text{lf} \leq 75\%}$), while the mean Ψ_{lf} when g_s values were 40% was the Ψ_{lf} at which stomata were considered to be over half shut down ($\Psi_{\text{lf} \leq 40\%}$), and the Ψ_{lf} at g_s values that were less than 15% of steady state was the Ψ_{lf} ($\Psi_{\text{lf} \leq 15\%}$) where stomata were considered to be closed. Photosynthetic responsiveness was

examined in a similar manner as g_s responsiveness, except that all data was grouped based on A measurements (Table 4.2).

Table 4.1 Stomatal conductance (g_s) values relative to steady state g_s of Hill (H), Northwest (NW), Okanese (O) and Walker (WA) hybrid poplar clones after 6 wks of growth under either a well-watered or drought pre-conditioning treatment.

Clone	g_s (mol H ₂ O m ⁻² s ⁻¹)						
	Steady state [§]	80%	70%	45%	25%	15%	
		$\Psi_{If<75\%}$		$\Psi_{If<40\%}$		$\Psi_{If<15\%}$	
		Well-watered					
H	0.66	0.53	0.46	0.30	0.16	0.10	
NW	0.66	0.52	0.46	0.30	0.16	0.10	
O	0.56	0.45	0.39	0.25	0.14	0.08	
WA	0.68	0.54	0.48	0.31	0.17	0.10	
		Droughted					
H	0.52	0.42	0.37	0.23	0.13	0.08	
NW	0.63	0.51	0.44	0.29	0.16	0.10	
O	0.52	0.42	0.37	0.23	0.13	0.08	
WA	0.68	0.54	0.47	0.31	0.17	0.10	

[§] Values in the 80, 70, 45, 25 and 15% columns are relative to steady state g_s means and were used to categorize measurements into categories termed $\Psi_{If\leq 75\%}$, $\Psi_{If\leq 40\%}$ and $\Psi_{If\leq 15\%}$.

Table 4.2 Photosynthesis (A) values relative to steady state A of Hill (H), Northwest (NW), Okanese (O) and Walker (WA) hybrid poplar clones after 6 wks of growth under either a well-watered or drought pre-conditioning treatment.

Clone	A (μmol CO ₂ m ⁻² s ⁻¹)						
	Steady state [§]	80%	70%	45%	25%	15%	
		$\Psi_{If<75\%}$		$\Psi_{If<40\%}$		$\Psi_{If<15\%}$	
		Well-watered					
H	19.15	15.32	13.41	8.62	4.79	2.87	
NW	20.92	16.73	14.64	9.41	5.23	3.14	
O	19.72	15.77	13.80	8.87	4.93	2.96	
WA	20.30	16.24	14.21	9.13	5.07	3.04	
		Droughted					
H	18.37	14.69	12.86	8.27	4.59	2.76	
NW	19.12	15.29	13.38	8.60	4.78	2.87	
O	19.64	15.71	13.75	8.84	4.91	2.95	
WA	20.01	16.01	14.01	9.01	5.00	3.00	

[§] Values are relative to steady state A means[§] and were used to categorize measurements into categories termed $\Psi_{If\leq 75\%}$, $\Psi_{If\leq 40\%}$ and $\Psi_{If\leq 15\%}$.

4.3.6 Statistical analyses

The general linear model for the two factorial treatments is fixed and is represented by:

$$y_{ijk} = \mu + A_i + B_j + (AB)_{ij} + \varepsilon_{ijk}, \quad [4.2]$$

where i corresponds to the soil moisture treatments and j corresponds to the clones, ij is the

interactions, k is the number of plants, and ε_{ijk} is the random error. The clones and the moisture treatments are fixed.

The null hypothesis was that gas exchange, minimum Ψ_{lf} , $\Psi_{lf \leq 75\%}$, $\Psi_{lf \leq 40\%}$, and $\Psi_{lf \leq 15\%}$ will be the same for all clones regardless of treatment. The type I error rate (α) was set at 0.05 for all tests. Analyses of variance were computed for: steady state gas exchange, minimum Ψ_{lf} , $\Psi_{lf \leq 75\%}$, $\Psi_{lf \leq 40\%}$, and $\Psi_{lf \leq 15\%}$. Differences between means were tested using Tukey’s test. All analyses were performed using Minitab ver. 13 (Minitab Inc., State College, PA, USA).]

4.4 Results

4.4.1 Relationship between soil and leaf water potentials

At the beginning of the dry-down, when plants were well watered and soil water potentials (Ψ_s) were close to zero, the leaf water potential (Ψ_{lf}) of all clones were similar (about -0.45 MPa), regardless of the soil moisture regime that plants had previously been grown under (Figure 4.1). As expected, when the soil dried and soil water potentials decreased, leaf water potentials also decreased (Figure 4.1). The dry-down continued until soil water potentials had declined to between -1.00 and -1.30 MPa, and despite similar soil water potentials (Table C.1), the clones differed in the leaf water potential minimums that had been reached (Table 4.3). Okanese plants had the lowest minimum Ψ_{lf} at -1.50 MPa, while Hill, Northwest and Walker plants reached Ψ_{lf} s of about -1.30 MPa. Pre-conditioning to drought conditions did not significantly change the minimum Ψ_{lf} reached by any of the clones however.

Table 4.3 Minimum leaf water potentials (Ψ_{lf}) at the end a soil dry-down of Hill, Northwest, Okanese and Walker hybrid poplar clones previously grown under either a well-watered (WW) or a drought pre-conditioning (D) treatment.

Clone	Minimum Ψ_{lf} (MPa)	
	WW	D
Hill	$-1.28ab^\dagger$	$-1.25ab$
Northwest	$-1.18a$	$-1.40bc$
Okanese	$-1.48b$	$-1.50c$
Walker	$-1.30ab$	$-1.18a$

[†] Within each column, values followed by the same letter are not significantly different according to Tukey’s test at $p \leq 0.05$.

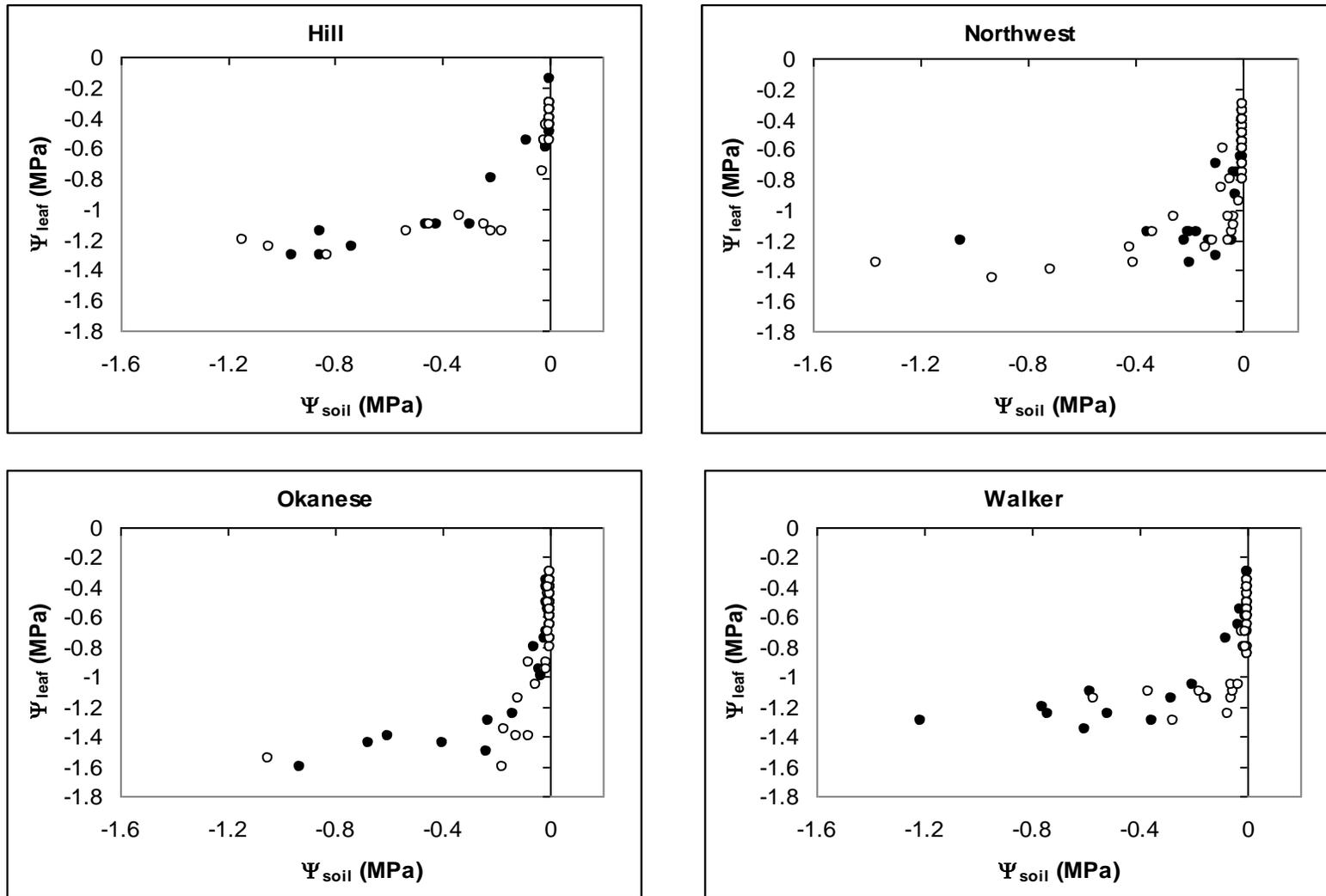


Figure 4.1 Changes in leaf water potential (Ψ_{leaf}) with decreasing soil water potential (Ψ_{soil}) of Hill, Northwest, Okanese, and Walker hybrid poplar clones previously grown under either a well-watered (closed circles) or drought preconditioning (open circles) soil moisture treatment. Each point represents one measurement on one plant.

4.4.2 Stomatal responses to decreasing leaf water potentials

As the soil dried and Ψ_{lf} values began to decrease, stomata began to close. The threshold Ψ_{lf} ($\Psi_{lf < 75\%}$) where stomata began to close (considered as the point when g_s values were 75% of the steady state values) and the Ψ_{lf} at which stomata were considered closed ($\Psi_{lf < 15\%}$, the point when g_s values were less than 15% of steady state values) varied between clones (Table 4.4). The threshold Ψ_{lf} was highest in Hill and Okanese plants (-0.45 and -0.54 MPa, respectively), lowest in Northwest plants (-1.03 MPa), and intermediate in Walker plants (-0.88 MPa). Stomata of Okanese plants did not close until Ψ_{lfs} were much lower (-1.52 MPa) compared to the Ψ_{lfs} causing stomatal closure in the other clones (about -1.25 MPa).

In Northwest and Walker plants, stomatal closure appeared more rapid once the threshold Ψ_{lf} had been reached, compared to more gradual closure in Okanese and Hill plants (Figure 4.2). Stomatal conductance of Northwest and Walker plants declined to 40% of steady state g_s within about 0.2 MPa of the threshold Ψ_{lf} and had almost complete stomatal closure within 0.23 and 0.4 MPa of the threshold Ψ_{lf} . In contrast, the g_s of Okanese plants decreased to 40% of steady state values within about 0.60 MPa from the threshold Ψ_{lf} , and stomatal closure did not occur until Ψ_{lfs} were almost 1.00 MPa less than the threshold Ψ_{lf} .

Table 4.4 Leaf water potentials (MPa) when stomatal conductance (g_s) of Hill (H), Northwest (NW), Okanese (O) and Walker (WA) plants previously grown under well-watered (WW) or droughted (D) conditions were 75% ($\Psi_{lf < 75\%}$, threshold Ψ_{lf}), 40% ($\Psi_{lf < 40\%}$) or 15% ($\Psi_{lf < 15\%}$) of steady state values. Means represent the mean of between 3 and 10 measurements.

Clone	$\Psi_{lf < 75\%}$		$\Psi_{lf < 40\%}$		$\Psi_{lf < 15\%}$	
	WW	D	WW	D	WW	D
Hill	-0.45a [†]	-0.54	-1.0	-1.15	-1.25a	-1.23
Northwest	-1.03b*	-0.53	-1.18	-1.25	-1.25a	-1.32
Okanese	-0.54a	-0.59	-1.13	-1.11	-1.52b	-1.46
Walker	-0.88ab*	-0.55	-1.13	-1.08	-1.29a	-1.23

[†] Within each column, values followed by the same letter are not significantly different according to Tukey's test at $p \leq 0.05$.

* Within each clone, means followed by * indicate significant soil moisture treatment differences.

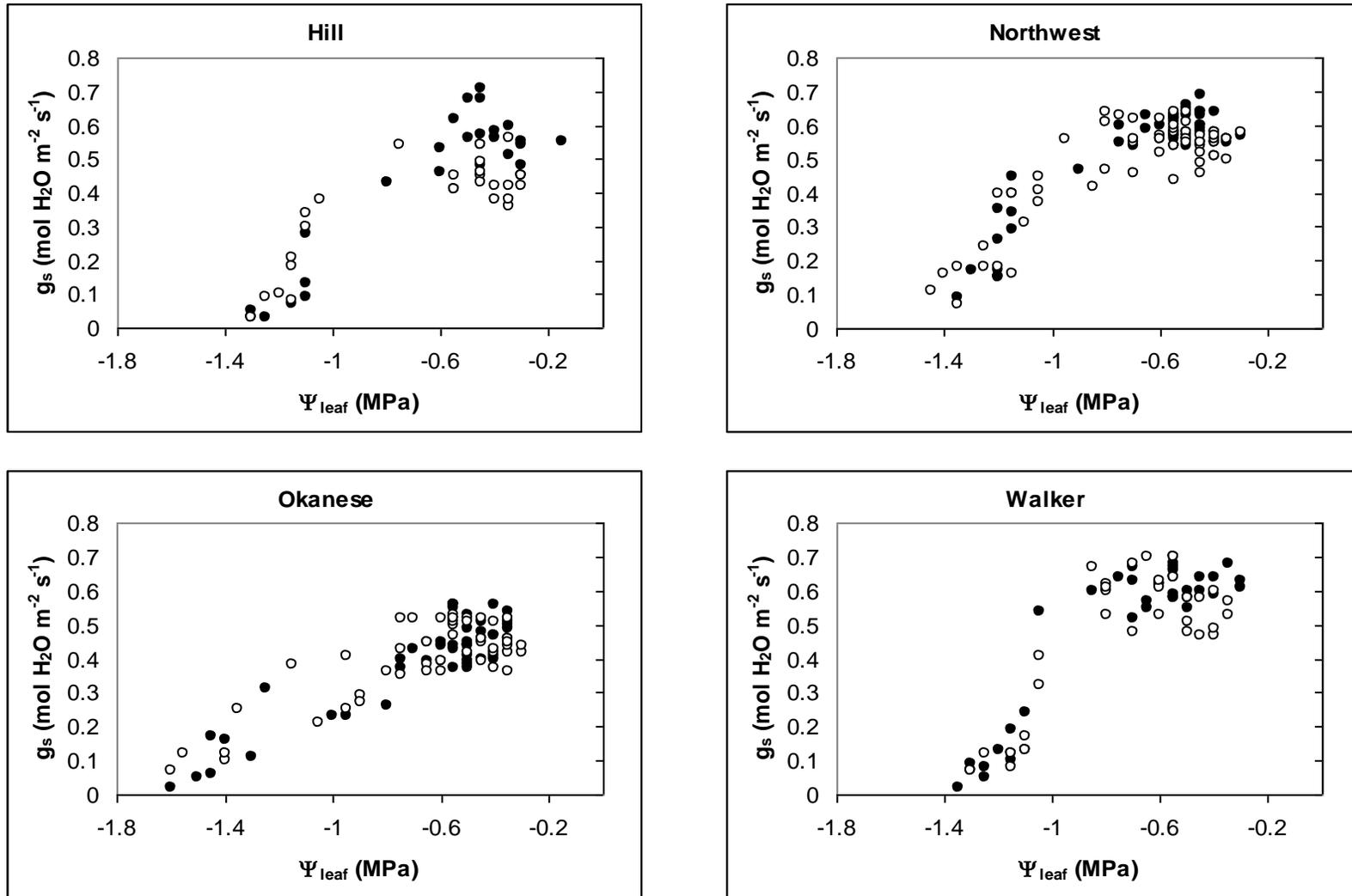


Figure 4.2 Changes in stomatal conductance with decreasing leaf water potentials during a soil drying cycle in Hill (H) (a), Northwest (NW) (b), Okanese (O) (c) and Walker (WA) (d) hybrid poplar clones following growth under well-watered (closed circles) or droughted (open circles) conditions. Each data point represents one measurement on one plant.

Pre-exposure to drought conditions generally resulted in increased threshold Ψ_{ifs} and more gradual rates of stomatal closure, at least in Northwest and Walker plants. The threshold Ψ_{if} of previously droughted Northwest and Walker plants was about 0.50 MPa and 0.30 MPa higher, respectively, than the threshold Ψ_{ifs} of well-watered plants of each clone (Table 4.1). Pre-exposure to drought conditions did not significantly affect the threshold Ψ_{ifs} for Hill or Okanese plants. Pre-exposure to drought conditions also caused the rate of stomatal closure to be much more gradual in Northwest and to some extent Walker plants compared to well-watered plants (Figure 4.2). Stomatal conductance values of droughted Northwest and Walker plants had decreased to about 40% within about 0.72 MPa and 0.53 MPa, respectively of the threshold Ψ_{if} , with stomatal closure occurring within about 0.77 MPa of threshold Ψ_{if} . In well-watered Northwest and Walker plants, g_s decreased to 40% within only about 0.15 and 0.25 MPa, respectively of the threshold Ψ_{if} , with stomatal closure within only 0.23 and 0.42 MPa, respectively, of the threshold Ψ_{if} .

4.4.3 Photosynthetic responses to decreasing leaf water potentials

Similar to changes in stomatal conductance, as soil dried and Ψ_{if} values began to decrease, photosynthesis (A) also began to decline, although at slightly lower values of Ψ_{if} than g_s (Table 4.4 and 4.5). There were no significant clonal differences in the threshold Ψ_{if} for A, but the Ψ_{if} at which photosynthesis was considered shut down ($\Psi_{\text{if} < 15\%}$) did vary slightly between clones (Table 4.5). Similar to g_s , A of Okanese plants did not shut down until a much lower Ψ_{if} (-1.55 MPa) than Hill and Walker plants (-1.30 MPa). Because A of Northwest plants remained at least 40% of steady state A, the shut down Ψ_{if} could not be compared to that of the other clones. In general, photosynthesis appeared to decline more gradually in Okanese and Walker plants, decreasing to 40% of steady state A within 0.50 MPa of the threshold Ψ_{if} , while A of Hill and Northwest plants decreased 40% of steady state A within less than 0.30 MPa (Figure 4.3).

Depending on the clone, pre-exposure to drought conditions had an affect on the rate at which A decreased and/or the Ψ_{if} at which A shut down, but not the threshold Ψ_{if} . Photosynthesis of droughted Hill and Walker plants decreased to 40% of steady state A within only 0.08 and 0.15 MPa, respectively of the threshold $\Psi_{\text{if} < 75\%}$, while in well-watered Hill and Walker plants, A decreased to 40% within 0.29 and 0.55 MPa, respectively, of the threshold Ψ_{if}

(Figure 4.3). Pre-exposure to drought conditions resulted in continued A in Okanese and Walker plants to much lower Ψ_{lf} compared to well-watered plants, with droughted plants having rates of A that were still at least 40% of steady state A at Ψ_{lfs} of -1.55 and -1.25 MPa, respectively, compared to well-watered plants in which A had shut down at similar values of Ψ_{lf} .

Table 4.5 Leaf water potentials when photosynthesis (A) of Hill (H), Northwest (NW), Okanese (O) and Walker (WA) plants previously grown under well-watered (WW) or droughted (D) conditions were 75% ($\Psi_{lf < 75\%$, threshold Ψ_{lf}), 40% ($\Psi_{lf < 40\%}$) or 15% ($\Psi_{lf < 15\%}$) of steady state values. (Means represent the mean of between 2 and 7 measurements.)

Clone	$\Psi_{lf < 75\%}$				$\Psi_{lf < 40\%}$				$\Psi_{lf < 15\%}$			
	WW		D		WW		D		WW		D	
	μ	SE	μ	SE	μ	SE	μ	SE	μ	SE	μ	SE
H	-0.83 [†]	0.267	-1.1	0.05	-1.13	0.025	-1.18a	0.025	-1.28a	0.025	-1.3	0
NW	-0.98	0.101	-1.08	0.025	-1.28	0.075	-1.4ab	0.05	- [§]	-	-	-
O	-1.0	0.132	-1.0	0.05	-1.4	0.05	-1.5b	0.1	-1.55b	0.05	-	-
WA	-0.68	0.232	-1.075	0.025	-1.23	0.044	-1.23ab	0.075	-1.3a	0.05	-	-

[†] Means within a column followed by the same letter are not significantly different at $p \leq 0.05$, columns with no letters have no significant differences.

[§]Note: Because photosynthesis of droughted NW, O and WA did not decrease below 15% of maximum rates, no Ψ_{lf} means can be reported.

4.4.4 Changes in stomatal conductance and photosynthesis

As Ψ_{lf} declined, and both g_s and A declined, Okanese plants had slightly higher rates of A for a given g_s , while Hill plants had slightly lower A for a given g_s (Figure 4.4). For example, at a calculated g_s (using the equations determined using best fit lines on raw g_s and A data) of about $0.30 \text{ mol H}_2\text{O m}^{-2} \text{ s}^{-1}$, rates of A were still around $16.0 \text{ } \mu\text{mol CO}_2 \text{ m}^{-2} \text{ s}^{-1}$, while rates of A in Hill plants were only about $14.0 \text{ } \mu\text{mol CO}_2 \text{ m}^{-2} \text{ s}^{-1}$ (Table 4.6). The relationship between A and g_s was similar regardless of soil moisture treatment (data not shown).

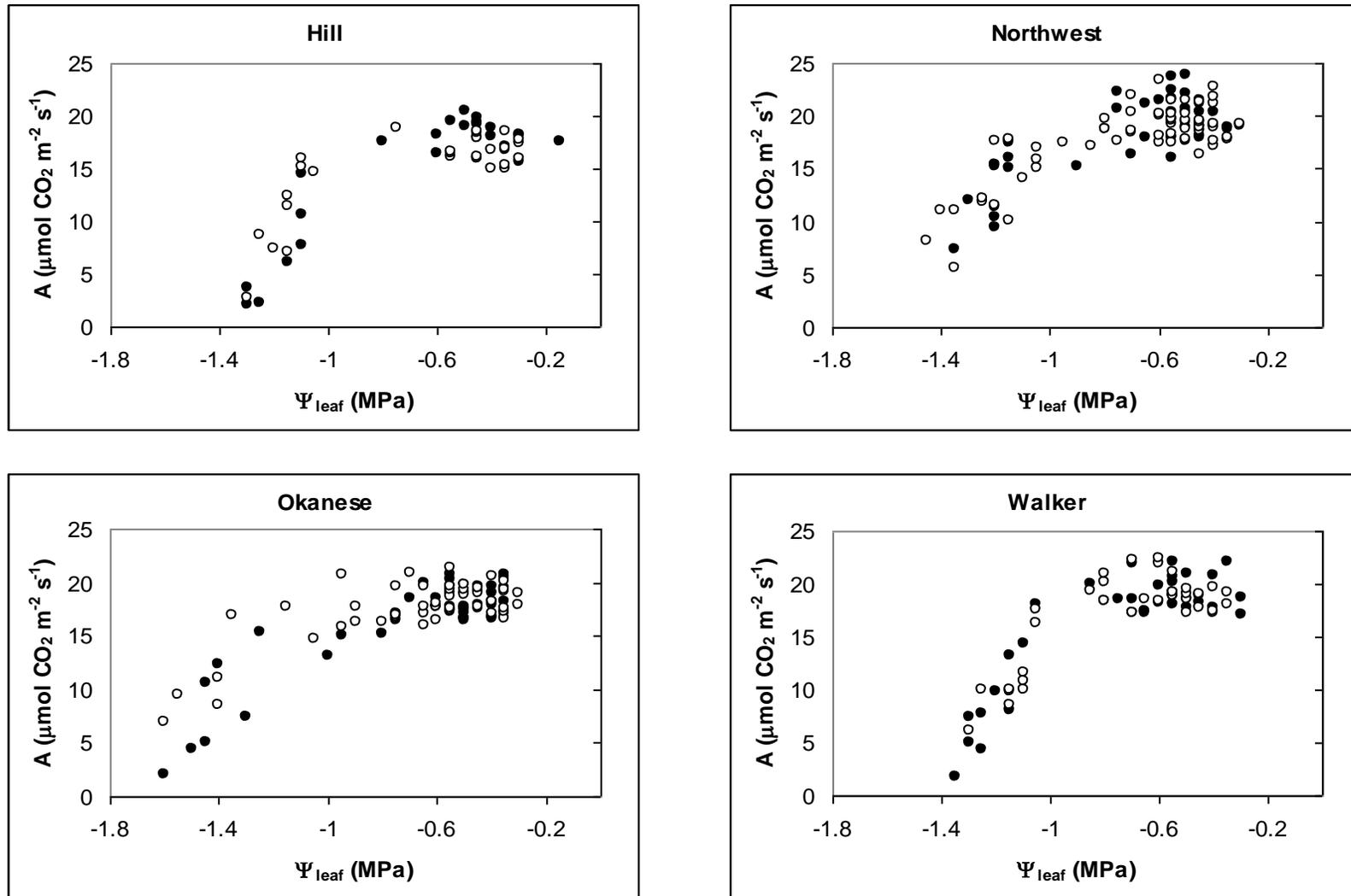


Figure 4.3 Changes in photosynthesis (A) with decreasing leaf water potentials (Ψ_{lf}) during a soil drying cycle in Hill (H) (a), Northwest (NW) (b), Okanese (O) (c) and Walker (WA) (d) hybrid poplar clones following growth under well-watered (closed circles) or droughted (open circles) conditions. (Each data point represents one measurement on one plant.)

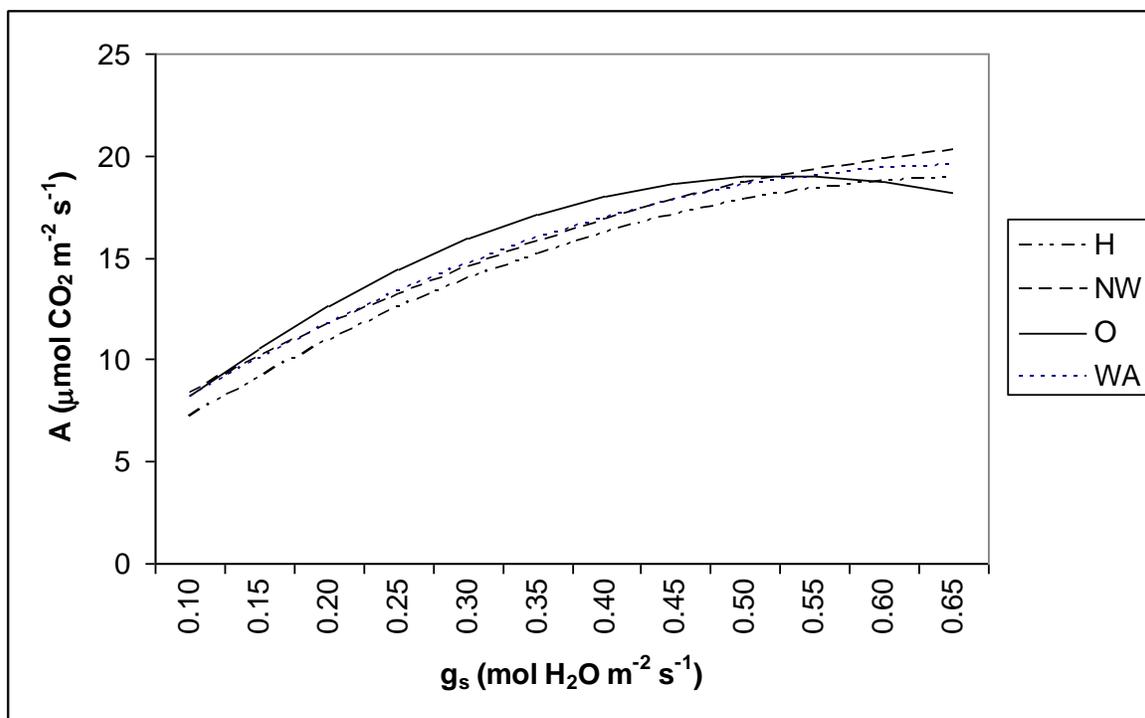


Figure 4.4 Changes in photosynthesis (A) and stomatal conductance (g_s) during a soil drying cycle in H (Hill), NW (Northwest), O (Okanese) and WA (Walker) hybrid poplar clones following growth under well-watered conditions. Lines drawn using trend-line equations for each clone.

Table 4.6 Calculated photosynthesis (A) for a given stomatal conductance (g_s) for Hill, Northwest, Okanese and Walker hybrid poplar clones. Equations are taken from best fit lines drawn on raw data.

g_s	A-Hill	A-Northwest	A-Okanese	A-Walker
	$A = -35.829(g_s^2) + 48.194(g_s) + 2.7051$ ($r^2: 0.96$)	$A = -26.849(g_s^2) + 41.81(g_s) + 4.4229$ ($r^2: 0.82$)	$A = -58.46(g_s^2) + 62.002(g_s) + 2.5886$ ($r^2: 0.88$)	$A = -34.351(g_s^2) + 46.525(g_s) + 3.8425$ ($r^2: 0.93$)
0.10	7.17	8.33	8.20	8.15
0.20	10.91	11.71	12.65	11.77
0.30	13.94	14.55	15.93	14.71
0.40	16.25	16.85	18.04	16.96
0.50	17.84	18.62	18.97	18.52
0.60	18.72	19.84	18.74	19.39

4.5 Discussion

Observations during a previous study (reported in Chapter 3) indicated that there were clonal differences in biomass accumulation and allocation, gas exchange and WUE, which varied

slightly depending on the soil moisture regimes that plants had been grown under. The clones differed in their ability to regulate Ψ_{lf} with declining soil moisture, as well as their stomatal and photosynthetic responses to declining Ψ_{lf} . In some cases, pre-exposure to soil drought did affect these responses.

As soil moisture declined, there were clonal differences in leaf water potentials, particularly once Ψ_{soil} decreased below about -0.80 MPa. Below this soil moisture level, leaf water potentials were lowest in Okanese plants, resulting in the biggest difference between Ψ_{soil} and Ψ_{lf} in this clone. Differences in water potential between the soil and plants will depend on the evaporative demand and soil moisture availability as well as the levels of solute accumulation in plant tissues (Turner and Begg, 1981). Solute accumulation under periods of water stress, termed osmotic adjustment allows plants to maintain positive turgor even under developing water deficit (Hsiao et al., 1975 and Turner and Jones, 1980). Plants that have higher levels of solutes can maintain positive turgor even under soil moisture deficits, which allows for continued leaf expansion and photosynthesis (Hsiao et al., 1976; Sharp and Davies, 1979; Turner and Jones, 1980). This also enables depletion of soil water to lower levels, possibly allowing for continued root growth, thus increased soil exploration.

When plants are pre-exposed to soil moisture deficit conditions, there are often changes in the osmotic potentials of plant cells as a result of increased solute accumulation (termed osmotic adjustment). This usually leads to pre-exposed plants having lower Ψ_{lf} than non-exposed plants and Ψ_{lf} responses to changes in Ψ_{soil} being different than that of plants not exposed to moisture deficits (Gebre et al., 1994; 1998). In the clones examined here, there were neither depressions in the levels of Ψ_{lf} reached with declining Ψ_{soil} , nor differences in Ψ_{lf} responses between droughted and well-watered plants. Because Ψ_{lf} was not that different between plants from the two soil moisture treatments, it is probable that solute accumulation was also not that different (although not measured in this study). It is possible that osmotic adjustment does not occur to a significant extent in the clones examined here or the growing conditions that plants were subjected to did not allow plants to adjust osmotically. Turner and Begg (1980) reported that osmotic adjustment tends to be small in plants that have either undergone rapid declines in soil moisture or relatively small degrees of soil moisture stress. It is therefore possible that the lack of differences in Ψ_{lf} response could have occurred because either

the rate of stress was too rapid during the cycles of soil drying or that the degree of stress was not severe enough to allow the plants to adjust osmotically to reduced soil moisture.

Similar to clonal differences in Ψ_{lf} responses to declining soil moisture, there were clonal differences in both the threshold Ψ_{lf} that prompted stomatal closure as well as the rate of stomatal closure. This fact is consistent with other studies examining stomatal responses of *Populus* spp. to decreasing plant moisture (McGee et al., 1981; Ceulemans et al., 1988; Mazzoleni and Dickman, 1988; Bassman and Zwier, 1991; Rood et al., 2003). Although the threshold Ψ_{lf} at which stomata began to close was similarly high in Hill and Okanese plants, the rate of stomatal closure was much more gradual in Okanese plants. Northwest plants also had slightly more gradual stomatal closure compared to Hill and Walker plants. Relatively high threshold Ψ_{lf} and more gradual pattern of stomatal closure has been associated with plants that have a more conservative water use strategy and are thus considered more drought tolerant than those exhibiting rapid stomatal closure (Bassman and Zwier, 1991).

Because pre-exposure to drought is important for stomata preconditioning and impacts subsequent drought responses in plants, the threshold Ψ_{lf} is often altered when plants are pre-exposed to drought conditions (Schulte and Hinckley, 1987; Aasamaa and Sober, 2001; Monclus et al., 2006). Pre-exposure to drought resulted in an increase in the threshold leaf potential of Northwest and Walker plants as well as a more gradual stomatal closure than well-watered plants of these two clones. It is generally believed that the threshold leaf water potential is related to the critical xylem water potential (the water potential that will cause 100% cavitation in xylem vessels), which will vary widely depending on the relative cavitation susceptibility of the plant species (Jones and Sutherland, 1991; Tyree et al., 1992; Sperry, 2000; Cochard et al., 2002). Stomatal closure often acts to protect plants against xylem cavitation and thus, so it is possible that this adjustment in the threshold leaf potential in Northwest and Walker plants could be a protective mechanism preventing xylem cavitation.

Similar to the patterns of stomata closure, patterns of photosynthetic response are dependant on the species adaptations to moisture limitations, with more drought tolerant species generally having a higher threshold Ψ_{lf} and/or maintaining photosynthetic activity for a longer period and to a lower leaf water potential than less drought tolerant species (Ni and Pallardy, 1991; Epron and Dryer, 1993). While there were no clonal differences in the threshold Ψ_{lf} , there was a difference in the rate that photosynthesis decreased as the leaf water potentials decreased.

Okanese and Walker plants had a more gradual decline in photosynthetic activity with decreasing leaf water potentials compared to Hill and Northwest plants. They also had continued A at lower levels of Ψ_{lf} than the other two clones, with Northwest plants having rates of A that did not ever decrease below about 40% of maximum values, despite plants that were visibly wilted (at which point measurements were halted to ensure plant survival). Under conditions of limited soil moisture, it could be expected that both Okanese and Northwest would continue photosynthetic activity (thus plant growth) despite reduced moisture, which would give them a productive advantage over Hill and Walker plants in the same conditions. However, continued A despite lack of sufficient water in leaf tissues could also be fatal in the case of Northwest plants.

Pre-exposure to drought conditions did not significantly alter the threshold Ψ_{lf} for A of any of the clones. However, the rate of decline was affected. Once the threshold Ψ_{lf} was reached, A declined much more rapidly in Hill and Walker plants that had been pre-exposed to drought compared to plants that had been well-watered. In addition, pre-exposure resulted in Okanese and Walker plants having continued A to much lower levels of Ψ_{lf} compared to well-watered plants.

As g_s and A decreased with declining Ψ_{lf} , Okanese plants had a higher A for a given g_s than the other clones. Differences in CO_2 assimilation are likely due to differences in substrate (CO_2) availability, mainly due to restrictions on CO_2 supply caused by stomatal closure and reductions in mesophyll conductance (Sharkey, 1990; Chaves, 1991; Chaves et al., 2002; Chaves and Oliveira, 2004; Flexas et al., 2004; Grassi and Magnini, 2005; Diaz-Espejo et al., 2007; Galmes et al., 2007). Because comparisons were for the same values of g_s , differences in A were likely caused by differences in mesophyll conductance during the soil drying cycle. While CO_2 assimilation can also be affected by metabolism changes or secondary effects such as oxidative stress (Chaves et al., 2008), it is unlikely that the soil water stress in this study was severe enough to induce these conditions in Hill, Northwest or Walker plants.

4.6 Conclusion

Results from this study indicate that there are differences in the ability of these four hybrid poplar clones to regulate leaf water potentials water under increasing soil moisture deficit. There were also differences in the timing and rapidity of stomatal and photosynthetic responses to these changes in Ψ_{lf} . In the case of Okanese and Hill to a lesser extent, stomata began to close at relatively higher levels of Ψ_{lf} than Walker and Northwest plants; however, the rate of stomatal

closure was much more gradual in Okanese plants than the other three clones. This relatively conservative pattern of g_s response displayed in Okanese plants is a pattern similar to that found in more drought tolerant species (examples sugar maple and olive) and may likely confer an adaptive advantage in areas where soil moisture deficits are more likely to occur. In terms of photosynthetic activity continuing during declining soil moisture, Okanese and Northwest plants are likely to have a productive advantage over the other clones because A decreases at a much slower rate with declining Ψ_{lf} and continued until much lower levels of Ψ_{lf} compared to Hill and Walker plants. However, this will only be beneficial if soil moisture levels do not decrease below permanent wilting point, for Northwest in particular, which was observed to continue A almost to the point of death in this study. In terms of acclimation abilities, pre-exposure to drought conditions resulted in stomata of Northwest and Walker plants becoming more responsive and the rate of closure to become more gradual compared to well-watered plants, which may permit better control of water loss following pre-exposure to drought conditions.

5. GENERAL DISCUSSION

On the Canadian Prairies, poplar clones are widely planted for protection of agricultural lands (riparian areas and shelterbelts) and farmsteads, ornamental purposes and afforestation. Many poplar species are native to areas where there is high soil moisture; however, hybrid poplar clones are being used in many areas of the Prairies where soil moisture may be limiting and evaporative demands high. In addition, climate change models predict that average temperatures in the Canadian Prairies will increase by up to 3.5-5° C over the next century, causing increases in saturation vapour pressure and thus greater evaporation (IPCC, 2001; Christensen et al., 2007). It is also predicted that the frequency and severity of some extreme weather events such as drought will increase, making some ecosystems, including the Prairies, particularly vulnerable (Christensen et al., 2007). Long-lived species, including woody plants such as poplars may be particularly vulnerable unless they are sufficiently adaptable to the new environment. In the Canadian Prairies, response to drought is therefore going to be a major factor in poplar establishment, growth and survival.

Because of immobility and long-lived nature, woody plants often experience a wide range of environments throughout their lifetimes, leading to the development of phenotypic plasticity in many plants (Bradshaw, 1965, 1972; Scheiner, 1993). Acclimation, a shift in a plant's response pattern following exposure to an environmental condition, is related to the degree of plasticity that the plant possesses (Bazzaz, 1991). During acclimation, the growth and physiology of adaptable plants adjust to the prevailing environment, often increasing their success when those conditions are again encountered relative to plants that have never been exposed to those same conditions. Plasticity and acclimation lessen the need that plants (poplars in particular) be grown in precise habitats and will be important for the survival of woody plant species with changing climatic conditions.

The overall objective of this thesis was to examine the growth characteristics, gas exchange, and WUE of several hybrid poplar clones commonly grown on the Prairies following a growing period under either well-watered or soil moisture deficit (droughted) conditions. Evaluating the growth, gas exchange and WUE under soil moisture deficit conditions will help identify the clones with the best ideotype, as well as determine the reasons for their continued productivity under limiting soil moisture. The hybrid poplar clones with the best ideotype will be the ones that have continued productivity despite limiting soil moisture.

Plants that produce less shoot growth or leaf area relative to root biomass may be better able to maintain a positive water balance within plant tissues when soil moisture is reduced compared to plants that have an elevated ratio of shoot biomass or leaf area to root biomass. Okanese and Hill plants had a similarly low ratio of shoot biomass to root biomass and leaf area to root biomass than Northwest and Walker plants. This morphological balance may partially lead to Okanese and Hill plants being able to survive or possibly grow in areas where moisture deficits occur more frequently compared to Northwest and Walker plants.

While stomatal densities, distribution and size tend to vary between poplar species, ecotypes from more xeric environments (and thus considered more drought tolerant in general) have been found to have either a higher density of smaller stomata or an increased density of stomata on abaxial leaf surfaces compared to ecotypes from more mesic environments (Pallardy and Kozlowski, 1979; Ceulemans et al., 1984; 1987; Pearce et al., 2005). Although Okanese plants had significantly lower stomatal densities per unit leaf area than Hill, Northwest and Walker plants, leaf area was also relatively high in Okanese plants; therefore, overall stomata densities were not that different. More importantly however, was that most of the stomata on Okanese plants were on the abaxial leaf surfaces, compared to a fairly even adaxial:abaxial distribution for the other clones. As a result of the lower stomata density per leaf area and possibly few stomata on adaxial leaf surfaces, g_s was lower in Okanese plants compared to the other clones, but interestingly A was not correspondingly low. In contrast, Northwest plants had significantly higher rates of g_s and A , likely the result of bigger stomatal aperture, as evidenced by significantly larger adaxial stomata. Higher values of A/g_s and $\delta^{13}C$ indicate that WUE was highest in Okanese and Walker plants. In general, plant species or populations with higher WUEs will be more successful when water supply is limited compared to plants with lower WUEs, so physiologically Okanese and Walker would likely have an advantage in terms of water conservation compared to Hill and Northwest plants.

To further examine the four hybrid poplar clones inherent adaptability, a second study (Chapter 4) examined concurrent changes in plant moisture contents and gas exchange during a cycle of increasing soil moisture deficit. It was hoped that examining gas exchange throughout the entire soil drying cycle would help explain clonal differences in growth, productivity and water-use efficiency found in study one.

As soil moisture declined, there were clonal differences in the level of moisture that was maintained in plant tissues, as well as the responsiveness of stomatal conductance and photosynthesis to the changing plant moisture levels. At relatively low levels of soil moisture, the Ψ_{lf} of Okanese plants had decreased to much lower levels than that of the other clones, indicating that solute concentrations were likely higher in this clone; however, exact details of this were not measured. Because plants that have higher levels of solutes can deplete soil water to lower levels, positive turgor can be maintained even under soil moisture deficits. This allows for continued root growth (thus increased soil exploration), leaf expansion and photosynthesis to lower levels of soil moisture (Hsiao et al., 1976; Sharp and Davies, 1979; Turner and Jones, 1980).

As plant moisture levels declined, stomata of Hill and Okanese plants appeared to be similarly responsive, closing at higher threshold Ψ_{lf} than Northwest or Walker plants. Once stomata began to close, the rate of stomatal closure was much more gradual in Okanese plants, and to a certain extent, Northwest plants. Similarly, the decline in photosynthetic activity with decreasing leaf water potentials was also more gradual in Northwest and Okanese plants. In addition, A of Northwest and Okanese plants continued to much lower levels of Ψ_{lf} than the other two clones. In fact, Northwest plants maintained rates of A that were at least 40% of maximum values, despite plants that were visibly wilted (at which point measurements were halted to ensure plant survival).

Under conditions of limited moisture, continued photosynthetic activity (thus plant growth) would likely give Okanese and Northwest a productive advantage over Hill and Walker plants under the same conditions. However, if stomata remain open to lower levels of plant moisture as was the case with Northwest plants, tissues may dry to the point of irreversible damage. Relatively high threshold Ψ_{lf} and a more gradual pattern of stomatal closure has been associated with plants that have a more conservative water use strategy and are thus considered more drought tolerant than those exhibiting rapid stomatal closure (Bassman and Zwier, 1991).

In terms of morphological characteristics, Hill and Okanese plants appeared to have an advantage with a relatively low ratio of shoot to root biomass production. Okanese plants also have stomatal characteristics (increased density overall but lower density on adaxial leaf surfaces) that are likely to minimize water loss, while increasing their growth and productivity under reduced soil moisture. In terms of physiological adaptations to drought, Okanese, and

Walker plants to a certain extent, appear to be better adapted to reduced soil moisture through increased WUE. Okanese plants appear to be better adapted to conditions of reduced soil moisture with relatively low rates of g_s , relatively high threshold Ψ_{lf} , a gradual pattern of stomata closure and continued photosynthesis to much lower levels of plant moisture.

In the two studies reported in this thesis, plants were either grown under well-watered conditions for the duration of the study or subjected to a number of soil moisture deficit (or ‘drought’) cycles. The drought cycles were used to examine whether the hybrid poplar clones studied here have the ability to adjust morphologically or physiologically (acclimate) to reduced soil moisture, allowing for continued productivity despite lower levels of soil moisture.

Not surprisingly, growth of all four clones was reduced among plants from the drought treatment compared to plants from the well-watered treatment. The effects of the drought treatment were similar for all clones for most growth measurements. The exception was reductions in area per leaf, where area per leaf was reduced by almost 40% in Northwest plants, but only about 25% in the other three clones. Northwest plants typically have fewer, but very large leaves. Obviously the drought treatment had a major impact on leaf expansion in this clone, indicating that productivity could be limited in Northwest plants growing in areas where soil moisture deficits are likely to occur.

Only Okanese and Northwest plants exhibited adjustments in stomata characteristics; however, these did not necessarily relate to soil moisture treatment differences in steady state gas exchange measurements. Okanese plants from the drought treatment had an increased density, but smaller stomata than plants from the well-watered treatment. Droughted Northwest plants had smaller stomata than well-watered plants. Neither stomatal conductance, nor photosynthesis, was significantly reduced in droughted plants of either clone. In contrast, despite insignificant soil moisture treatment effects on stomatal densities or size, Hill and Walker plants from the drought treatment had significantly lower g_s than well-watered plants. In the case of Hill plants, photosynthesis was also significantly lower in droughted than well-watered plants. As a result of reduced g_s in droughted Hill and Walker plants, WUE was also higher in droughted than well-watered plants of these two clones.

In the clones examined here, there were neither depressions in the levels of Ψ_{lf} reached with declining Ψ_{soil} , nor differences in Ψ_{lf} responses between droughted and well-watered plants. It is likely that solute accumulation was also not that different (although not measured in this

study). While it is possible that the clones examined here are unable to adjust osmotically following exposure to soil moisture stress, it is likely that the lack of differences in Ψ_{lf} response were because either the rate of stress was too rapid during the cycles of soil drying or that the degree of stress was not severe enough to allow the plants to allow for osmotic adjustment. It is therefore possible that under field conditions, some slight differences may occur not only in the degree of osmotic adjustment but also in some of the growth and physiology responses during episodes of reduced soil moisture.

Pre-exposure to episodes of soil moisture deficit caused stomata of Northwest and Walker plants to become more responsive, beginning to close at higher levels of plant moistures (threshold Ψ_{lf}), as well as having a more gradual pattern of stomatal closure than well-watered plants. Because stomatal closure often acts to protect plants against xylem cavitation, it is possible that this adjustment in the threshold leaf potential in Northwest and Walker plants could be a protective mechanism reducing the likelihood of xylem cavitation.

Overall, the clones examined here did not exhibit significant growth acclimation to soil moisture deficit under the conditions of this study; however, some slight indications of physiological acclimation were evident. Because of the significant effect that the drought treatment had on leaf expansion in Northwest plants, productivity may be limited, especially in areas with reduced soil moisture. While soil moisture treatment differences in stomatal characteristics did not generally relate to differences in steady state gas exchange, a decrease in stomatal size in Northwest plant may have contributed to increased stomatal responsiveness to declining plant moisture in plants pre-exposed to soil moisture deficit conditions.

Despite the fact that both of the studies reported in this thesis were relatively short greenhouse studies, observations indicate variation in drought responses among the clones which merits further examination of field drought responses of these (and other) hybrid poplar clones. In terms of general adaptiveness, Okanese plants appear to be the best adapted to conditions of reduced soil moisture. Of the four clones examined here, this clone is more likely to not only survive, but continue growing in more drought prone regions of the Prairies. In contrast, growth and physiological traits of Northwest, and Hill plants to a certain extent, make them most suitable to areas where moisture deficits are rare. Despite being one of the most widely planted clones on the Canadian Prairies, results from this study indicate that Walker may not be successful in areas where drought events are expected to occur.

Admittedly, this study only examined the responses of four hybrid poplar clones, while there are many others currently grown or being considered for use on the Canadian Prairies. The four clones examined here were chosen based on contrasting responses to reduced soil moisture during a preliminary study. The variation in drought responses observed here suggest that there may be other hybrid clones that possess the inherent adaptability or ability to acclimate to reduced soil moisture conditions. From the studies reported here, we found that the hybrid poplar with the highest WUE did in fact have a number of morphological and physiological traits that contributed to them being more tolerant of reduced soil moisture conditions.

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APPENDIX A.
SOIL DRYING CALIBRATION CURVES

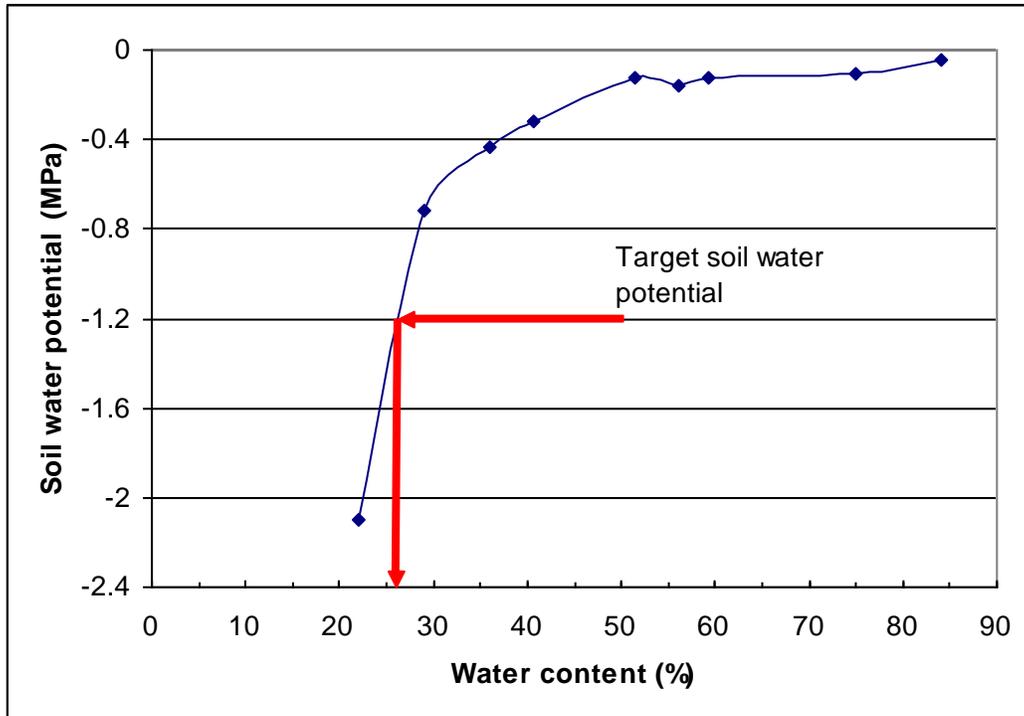


Figure A.1 Soil saturation curve relating the soil water content (%) to soil water potential from fully saturated peat-based soil of around 85% soil moisture.

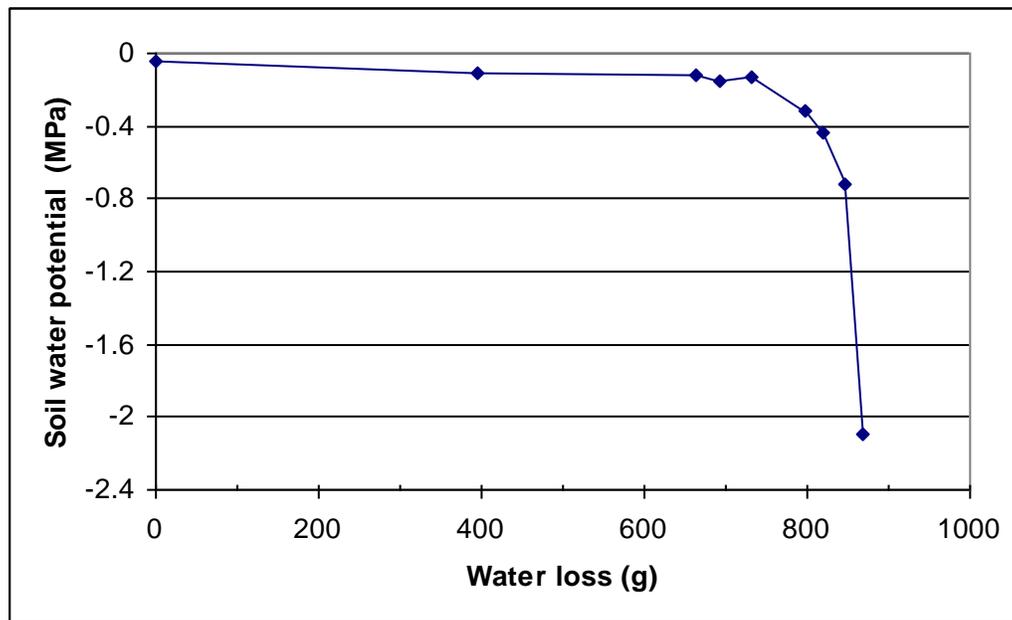


Figure A.2 Relationship between soil water potential and the amount of water loss from a peat based soil.

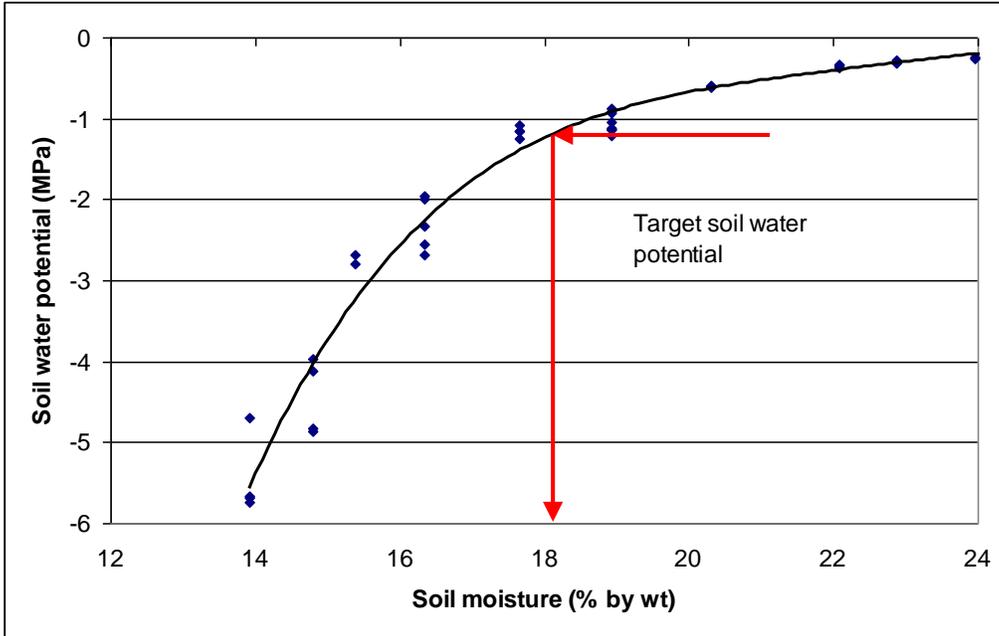


Figure A.3 Relationship between soil moisture and soil water potential of a soil that was 3 parts peat and 1 part loam soil.

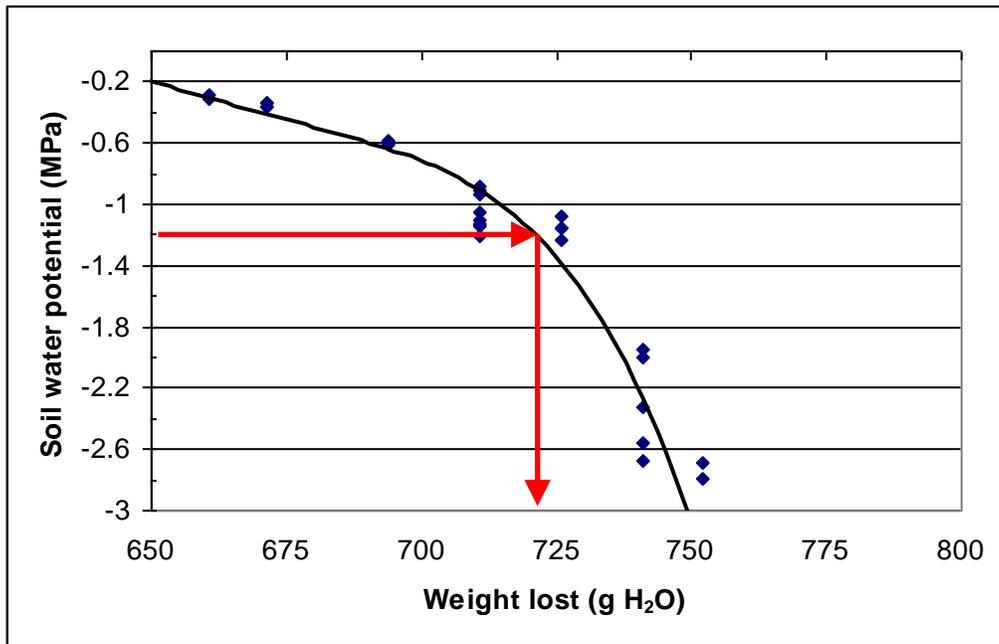


Figure A.4 Relationship between soil water potential and the amount of water loss from a soil that was 3 parts peat and 1 part loam soil.

APPENDIX B
ANOVA AND CORRELATION ANALYSIS TABLES

Table B.1 ANOVA table for plant height of four hybrid poplar clones (Clone) grown under two soil moisture treatments (Trt). (Data shown in Figure 3.1)

Source	DF [†]	Seq SS	Adj SS	Adj MS	F ratio	P value
Trt	1	5880.6	5880.6	5880.6	213.70	0.000
Clone	3	7291.7	7291.7	2430.6	88.32	0.000
Trt * Clone	3	21.1	21.1	7.0	0.26	0.857
Error	32	880.6	880.6	27.5		
Total	39	14074.0				

[†] DF (degrees of freedom)

Table B.2 ANOVA table for stem diameter of four hybrid poplar clones (Clone) grown under two soil moisture treatments (Trt). (Data shown in Figure 3.2)

Source	DF [†]	Seq SS	Adj SS	Adj MS	F ratio	P value
Trt	1	20.463	20.463	20.463	119.54	0.000
Clone	3	9.094	9.094	3.031	17.71	0.000
Trt * Clone	3	1.176	1.176	0.392	2.29	0.097
Error	32	5.478	5.478	0.171		
Total	39	36.211				

[†] DF (degrees of freedom)

Table B.3 ANOVA table for total biomass of four hybrid poplar clones (Clone) grown under two soil moisture treatments (Trt). (Data shown in Figure 3.3)

Source	DF [†]	Seq SS	Adj SS	Adj MS	F ratio	P value
Trt	1	2524.92	2524.92	2524.92	124.70	0.000
Clone	2	1071.31	1071.31	357.10	17.64	0.000
Trt * Clone	2	61.95	61.95	20.65	1.02	0.397
Error	32	647.92	647.92	20.25		
Total	39	4306.11				

[†] DF (degrees of freedom)

Table B.4 ANOVA table for root biomass for four hybrid poplar clones (Clone) grown under two soil moisture treatments (Trt). (Data shown in Figure 3.3)

Source	DF [†]	Seq SS	Adj SS	Adj MS	F ratio	P value
Trt	1	117.924	117.924	117.924	33.89	0.000
Clone	3	107.78	107.78	35.927	10.33	0.000
Trt * Clone	3	14.484	14.484	4.828	1.39	0.264
Error	32	111.336	111.336	3.479		
Total	39	351.523				

[†] DF (degrees of freedom)

Table B.5 ANOVA table for shoot biomass of four hybrid poplar clones (Clone) grown under two soil moisture treatments (Trt). (Data shown in Figure 3.3)

Source	DF [†]	Seq SS	Adj SS	Adj MS	F ratio	P value
Trt	1	1551.52	1551.52	1551.52	161.59	0.000
Clone	3	636.52	636.52	212.18	22.10	0.000
Trt * Clone	3	26.29	26.29	8.76	0.91	0.446
Error	32	307.25	307.25	9.60		
Total	39	2521.61				

[†] DF (degrees of freedom)

Table B.6 ANOVA table for stem biomass of four hybrid poplar clones (Clone) grown under two soil moisture treatments (Trt). (Data not shown)

Source	DF [†]	Seq SS	Adj SS	Adj MS	F ratio	P value
Trt	1	293.439	293.439	293.439	193.46	0.000
Clone	3	284.331	284.331	94.777	62.49	0.000
Trt * Clone	3	7.730	7.730	2.577	1.70	0.187
Error	32	48.536	48.536	1.517		
Total	39	634.037				

[†] DF (degrees of freedom)

Table B.7 ANOVA table for leaf biomass of four hybrid poplar clones (Clone) grown under two soil moisture treatments (Trt). (Data not shown)

Source	DF [†]	Seq SS	Adj SS	Adj MS	F ratio	P value
Trt	1	495.475	495.475	495.475	114.47	0.000
Clone	3	112.468	112.468	37.489	8.66	0.000
Trt * Clone	3	9.899	9.899	3.300	0.76	0.524
Error	32	138.515	138.515	4.329		
Total	39	756.357				

[†] DF (degrees of freedom)

Table B.8 ANOVA table for number of leaves of four hybrid poplar clones (Clone) grown under two soil moisture treatments (Trt). (Data shown in Figure 3.4)

Source	DF [†]	Seq SS	Adj SS	Adj MS	F ratio	P value
Trt	1	99.23	99.23	99.23	13.14	0.001
Clone	3	1677.68	1677.68	559.23	74.07	0.000
Trt * Clone	3	38.27	38.27	12.76	1.69	0.189
Error	32	241.60	241.60	7.55		
Total	39	2056.78				

[†] DF (degrees of freedom)

Table B.9 ANOVA table for area per leaf of four hybrid poplar clones (Clone) grown under two soil moisture treatments (Trt). (Data shown in Table 3.1)

Source	DF [†]	Seq SS	Adj SS	Adj MS	F ratio	P value
Trt	1	5149.3	5149.3	5149.3	194.74	0.000
Clone	3	2090.2	2090.2	2090.2	26.35	0.000
Trt * Clone	3	640.8	640.8	640.8	8.08	0.000
Error	32	846.1	846.1	846.1		
Total	39	8726.4				

[†] DF (degrees of freedom)

Table B.10 ANOVA table for total plant leaf area of four hybrid poplar clones (Clone) grown under two soil moisture treatments (Trt). (Data shown in Figure 3.5)

Source	DF [†]	Seq SS	Adj SS	Adj MS	F ratio	P value
Trt	1	10051586	10051586	10051586	213.90	0.000
Clone	3	2323124	232 3124	774375	16.48	0.000
Trt * Clone	3	124846	124846	41615	0.89	0.459
Error	32	1503768	1503768	46993		
Total	39	14003324				

[†] DF (degrees of freedom)

Table B.11 ANOVA table for shoot:root ratio of four hybrid poplar clones (Clone) grown under two soil moisture treatments (Trt). (Data shown in Figure 3.6)

Source	DF [†]	Seq SS	Adj SS	Adj MS	F ratio	P value
Trt	1	0.1232	0.1232	0.1232	0.36	0.551
Clone	3	11.4425	11.4425	3.8142	11.23	0.000
Trt * Clone	3	0.5655	0.5655	0.1885	0.55	0.649
Error	32	10.8696	10.8696	0.3397		
Total	39	23.008				

[†] DF (degrees of freedom)

Table B.12 ANOVA table for leaf area:root weight of four hybrid poplar clones (Clone) grown under two soil moisture treatments (Trt). (Data shown in Figure 3.7)

Source	DF [†]	Seq SS	Adj SS	Adj MS	F ratio	P value
Trt	1	4344	4344	4344	1.53	0.225
Clone	3	40546	40546	13515	4.77	0.007
Trt * Clone	3	8141	8141	2714	0.96	0.425
Error	32	90674	90674	2834		
Total	39	143705				

[†] DF (degrees of freedom)

Table B.13 ANOVA table for total stomata density of four hybrid poplar clones (Clone) grown under two soil moisture treatments (Trt). (Data shown in Table 3.2)

Source	DF [†]	Seq SS	Adj SS	Adj MS	F ratio	P value
Trt	1	2287.9	2287.9	2287.9	12.62	0.001
Clone	3	44391.0	44391.0	14797.0	81.61	0.000
Trt * Clone	3	1974.2	1974.2	658.1	3.63	0.023
Error	32	5802.3	5802.3	181.3		
Total	39	54455.4				

[†] DF (degrees of freedom)

Table B.14 ANOVA table for abaxial stomata density (mm⁻²) of four hybrid poplar clones (Clone) grown under two soil moisture treatments (Trt). (Data shown in Table 3.2)

Source	DF [†]	Seq SS	Adj SS	Adj MS	F ratio	P value
Trt	1	1388.91	1388.91	1388.91	21.13	0.000
Clone	3	2190.30	2190.30	730.10	11.11	0.000
Trt * Clone	3	1532.34	1532.34	510.78	7.77	0.000
Error	32	2103.05	2103.05	65.72		
Total	39	7214.59				

[†] DF (degrees of freedom)

Table B.15 ANOVA table for adaxial stomata density (mm⁻²) of four hybrid poplar clones (Clone) grown under two soil moisture treatments (Trt). (Data shown in Table 3.2)

Source	DF [†]	Seq SS	Adj SS	Adj MS	F ratio	P value
Trt	1	111.6	111.6	111.6	1.75	0.195
Clone	3	36415.8	36415.8	12138.6	190.37	0.000
Trt * Clone	3	56.8	56.8	18.9	0.827	0.827
Error	32	2040.4	2040.4	63.8		
Total	39	38624.5				

[†] DF (degrees of freedom)

Table B.16 ANOVA table for abaxial:adaxial stomata density ratio of four hybrid poplar clones (Clone) grown under two soil moisture treatments (Trt). (Data shown in Figure 3.8)

Source	DF [†]	Seq SS	Adj SS	Adj MS	F ratio	P value
Trt	1	0.191	0.191	0.191	0.34	0.566
Clone	3	231.459	231.459	77.153	135.40	0.000
Trt * Clone	3	0.778	0.778	0.259	0.46	0.716
Error	32	18.234	18.234	0.570		
Total	39	250.662				

[†] DF (degrees of freedom)

Table B.17 ANOVA table for abaxial stomatal index of four hybrid poplar clones (Clone) grown under two soil moisture treatments (Trt). (Data not shown)

Source	DF [†]	Seq SS	Adj SS	Adj MS	F ratio	P value
Trt	1	3.88	3.88	3.88	2.18	0.143
Clone	3	1087.38	1087.38	362.46	203.30	0.000
Trt * Clone	3	79.64	79.64	26.55	14.89	0.000
Error	32	199.69	199.69	1.78		
Total	39	1370.59				

[†] DF (degrees of freedom)

Table B.18 ANOVA table for adaxial stomatal index of four hybrid poplar clones (Clone) grown under two soil moisture treatments (Trt). (Data not shown)

Source	DF [†]	Seq SS	Adj SS	Adj MS	F ratio	P value
Trt	1	0.795	0.795	0.795	0.99	0.322
Clone	3	431.350	431.350	143.783	178.94	0.000
Trt * Clone	3	8.134	8.134	2.711	3.37	0.021
Error	32	89.993	89.993	0.804		
Total	39	530.273				

[†] DF (degrees of freedom)

Table B.19 ANOVA table for length of closed adaxial stomata (μm) of four hybrid poplar clones (Clone) grown under two soil moisture treatments (Trt). (Data shown in Table 3.3)

Source	DF [†]	Seq SS	Adj SS	Adj MS	F ratio	P value
Trt	1	0.0000273	0.0000273	0.0000273	1.45	0.230
Clone	3	0.0017996	0.0017996	0.0005999	31.96	0.000
Trt * Clone	3	0.0002028	0.0002028	0.0000676	3.60	0.015
Error	192	0.0036038	0.0036038	0.0000188		
Total	199	0.0056334				

[†] DF (degrees of freedom)

Table B.20 ANOVA table for length of closed abaxial stomata (μm) of four hybrid poplar clones (Clone) grown under two soil moisture treatments (Trt). (Data not shown)

Source	DF [†]	Seq SS	Adj SS	Adj MS	F ratio	P value
Trt	1	0.002119	0.002119	0.002119	1.92	0.167
Clone	3	0.000817	0.000817	0.000272	0.25	0.863
Trt * Clone	3	0.002654	0.002654	0.000885	0.80	0.494
Error	192	0.211521	0.211521	0.001102		
Total	199	0.217112				

[†] DF (degrees of freedom)

Table B.21 ANOVA table for length of open adaxial stomata (μm) of four hybrid poplar clones (Clone) grown under two soil moisture treatments (Trt). (Data shown in Table 3.3)

Source	DF [†]	Seq SS	Adj SS	Adj MS	F ratio	P value
Trt	1	0.00019	0.000216	0.000216	10.73	0.001
Clone	2	0.000902	0.000885	0.000442	21.97	0.000
Trt * Clone	2	0.000075	0.000075	0.000037	1.86	0.159
Error	140	0.002819	0.002819	0.0000201		
Total	145	0.003986				

[†] DF (degrees of freedom)

Table B.22 ANOVA table for length of open abaxial stomata (μm) of four hybrid poplar clones (Clone) grown under two soil moisture treatments (Trt). (Data not shown)

Source	DF [†]	Seq SS	Adj SS	Adj MS	F ratio	P value
Trt	1	0.001268	0.001268	0.001268	0.68	0.409
Clone	3	0.007672	0.007672	0.002557	1.38	0.250
Trt * Clone	3	0.011351	0.011351	0.003784	2.04	0.109
Error	192	0.355331	0.355331	0.001851		
Total	199	0.375621				

[†] DF (degrees of freedom)

Table B.23 ANOVA table for stomatal conductance (g_s) of four hybrid poplar clones (Clone) grown under two soil moisture treatments (Trt) measured on two dates (Dt). (Data shown in Table 3.4)

Source	DF [†]	Seq SS	Adj SS	Adj MS	F ratio	P value
Dt	1	0.207888	0.181065	0.181065	76.41	0.000
Trt	1	0.110125	0.101063	0.101063	42.65	0.000
Clone	3	0.162949	0.14645	0.048817	20.6	0.000
Dt*Trt	1	0.008215	0.008903	0.008903	3.76	0.058
Dt*Clone	3	0.021609	0.017476	0.005825	2.46	0.072
Dt*Trt*Clone	3	0.00745	0.007997	0.002666	1.12	0.347
Trt*Clone	3	0.028455	0.028455	0.009485	4.0	0.012
Error	57	0.135075	0.135075	0.00237		
Total	72	0.681767				

[†] DF (degrees of freedom)

Table B.24 ANOVA table for net photosynthesis (A) of four hybrid poplar clones (Clone) grown under two soil moisture treatments (Trt) measured on two dates (Dt). (Data shown in Table 3.4)

Source	DF [†]	Seq SS	Adj SS	Adj MS	F ratio	P value
Dt	1	0.07	0.041	0.041	0.03	0.866
Trt	1	25.850	22.517	22.517	15.80	0.000
Clone	3	36.708	34.113	11.371	7.98	0.000
Dt*Trt	1	0.001	0.047	0.047	0.03	0.857
Dt*Clone	3	8.966	9.537	3.179	2.23	0.094
Dt*Trt*Clone	3	2.911	2.298	0.766	0.54	0.659
Trt*Clone	3	19.293	19.293	6.431	4.51	0.007
Error	57	81.245	81.245	1.425		
Total	72	175.043				

[†] DF (degrees of freedom)

Table B.25 ANOVA table for intrinsic WUE A/g_s of four hybrid poplar clones (Clone) grown under two soil moisture treatments (Trt) measured on two dates (Dt). (Data shown in Figure 3.9)

Source	DF [†]	Seq SS	Adj SS	Adj MS	F ratio	P value
Dt	1	853.46	760.81	760.81	69.01	0.000
Trt	1	116.17	110.05	110.05	9.98	0.003
Clone	3	580.84	548.74	182.91	16.59	0.000
Dt*Trt	1	4.42	5.06	5.06	0.46	0.501
Dt*Clone	3	105.56	87.37	29.12	2.64	0.058
Dt*Trt*Clone	3	57.84	57.88	19.29	1.75	0.167
Trt*Clone	3	53.99	53.99	18.00	1.63	0.192
Error	57	628.44	628.44	11.03		
Total	72	2400.71				

[†] DF (degrees of freedom)

Table B.26 ANOVA table for stable carbon isotope composition ($\delta^{13}\text{C}$) of four hybrid poplar clones (Clone) grown under two soil moisture treatments (Trt). (Data shown in Figure 3.10)

Source	DF [†]	Seq SS	Adj SS	Adj MS	F ratio	P value
Trt	1	6.357	6.357	6.357	44.32	0.000
Clone	3	74.599	74.599	24.866	173.36	0.000
Trt * Clone	3	1.063	1.063	0.354	2.47	0.080
Error	32	4.590	4.590	0.143		
Total	39	86.609				

[†] DF (degrees of freedom)

Table B.27 ANOVA table for net photosynthesis per unit of water transpired A/E of four hybrid poplar clones (Clone) grown under two soil moisture treatments (Trt) measured on two dates (Dt). (Data not shown)

Source	DF [†]	Seq SS	Adj SS	Adj MS	F ratio	P value
Dt	1	0.03297	0.032168	0.03168	0.93	0.340
Trt	1	0.01488	0.01526	0.01526	0.45	0.507
Clone	3	0.9777	0.96153	0.32051	9.39	0.000
Dt*Trt	1	0.00098	0.00013	0.00013	0.00	0.952
Dt*Clone	3	0.08193	0.05543	0.01848	0.54	0.656
Dt*Trt*Clone	3	0.21568	0.21001	0.0700	2.05	0.117
Trt*Clone	3	0.17443	0.17443	0.05814	1.70	0.177
Error	57	1.94881	1.94661	0.03415		
Total	72	3.44516				

[†] DF (degrees of freedom)

Table B.28 ANOVA table for net photosynthesis per unit of intercellular CO₂ A/c_i of four hybrid poplar clones (Clone) grown under two soil moisture treatments (Trt) measured on two dates (Dt). (Data not shown)

Source	DF [†]	Seq SS	Adj SS	Adj MS	F ratio	P value
Dt	1	0.0000787	0.0000772	0.0000772	2.15	0.148
Trt	1	0.0003123	0.0002720	0.000272	7.59	0.008
Clone	3	0.0007158	0.0006831	0.0002277	6.36	0.001
Dt*Trt	1	0.0000011	0.0000038	0.0000038	0.11	0.747
Dt*Clone	3	0.0001256	0.0001326	0.0000442	1.23	0.306
Dt*Trt*Clone	3	0.0001433	0.0001253	0.0000418	1.17	0.331
Trt*Clone	3	0.0004307	0.0004307	0.0001436	4.01	0.012
Error	57	0.0020415	0.0020415	0.0000358		
Total	72	0.0038491				

[†] DF (degrees of freedom)

Table B.29 ANOVA table for intercellular CO₂ c_i of four hybrid poplar clones (Clone) grown under two soil moisture treatments (Trt) measured on two dates (Dt). (Data not shown)

Source	DF [†]	Seq SS	Adj SS	Adj MS	F ratio	P value
Dt	1	1245.81	1166.62	1166.62	24.07	0.000
Trt	1	53.67	48.53	48.53	1.00	0.321
Clone	3	1512.61	1500.69	500.23	10.32	0.000
Dt*Trt	1	15.94	21.41	21.41	0.44	0.509
Dt*Clone	3	163.37	120.81	40.27	0.83	0.482
Dt*Trt*Clone	3	337.14	330.73	110.24	2.27	0.090
Trt*Clone	3	342.92	342.92	114.31	2.36	0.081
Error	57	2762.50	2762.50	48.46		
Total	72	6433.97				

[†] DF (degrees of freedom)

Table B.30 Summary of correlation analysis of g_s (stomatal conductance), total plant biomass, A (photosynthesis), A/ g_s (photosynthesis/stomatal conductance), c_i/c_a (intercellular CO₂/atmospheric CO₂) and $\delta^{13}C$ (carbon isotope composition) for data of all clones combined. (data not shown)

	g_s	Total biomass	$\delta^{13}C$	A	A/ g_s
Total biomass	$r^*=-0.163$ $P^{**}=0.169$				
$\delta^{13}C$	-0.468 0.000	0.261 0.026			
A	0.450 0.000	0.215 0.067	-0.188 0.112		
A/ g_s	-	-0.024 0.837	0.454 0.000	-0.002 0.987	
c_i/c_a	0.685 0.000	-0.207 0.396	-0.354 0.002	-0.316 0.007	-0.941 0.000

Cell contents: *Correlation Coefficient (r)

**P-Value (note: bolded values indicate statistical significance at $P \leq 0.05$)

Table B.31 Summary of correlation analysis of g_s (stomatal conductance), total plant biomass, A (photosynthesis), A/ g_s (photosynthesis/stomatal conductance), c_i/c_a (intercellular CO₂/atmospheric CO₂) and $\delta^{13}C$ (carbon isotope composition) for Hill plants (replicated in Table 3.5).

	g_s	Total biomass	$\delta^{13}C$	A	A/ g_s
Total biomass	$r^*=-0.469$ $P^{**}=\mathbf{0.043}$				
$\delta^{13}C$	-0.667 0.002	-0.560 0.013			
A	0.360 0.130	0.730 0.000	-0.658 0.002		
A/ g_s	-0.829 0.000	-0.036 0.882	0.297 0.217	0.213 0.381	
c_i/c_a	0.609 0.006	-0.207 0.396	-0.051 0.835	-0.517 0.023	-0.943 0.000

Cell contents: *Correlation Coefficient (r)

**P-Value (note: bolded values indicate statistical significance at $P \leq 0.05$)

Table B.32 Summary of correlation analysis of g_s (stomatal conductance), total plant biomass, A (photosynthesis), A/ g_s (photosynthesis/stomatal conductance), c_i/c_a (intercellular CO₂/atmospheric CO₂) and $\delta^{13}C$ (carbon isotope composition) for Northwest plants (replicated in Table 3.5).

	g_s	Total biomass	$\delta^{13}C$	A	A/ g_s
Total biomass	$r^*=-0.624$ P**=0.007				
$\delta^{13}C$	-0.056 0.830	-0.308 0.228			
A	0.432 0.083	0.283 0.272	-0.046 0.859		
A/ g_s	-0.682 0.003	-0.435 0.081	0.024 0.928	0.354 0.163	
c_i/c_a	0.384 0.128	0.222 0.393	-0.010 0.969	-0.654 0.004	-0.927 0.000

Cell contents: *Correlation Coefficient (r)

** P-Value (note: bolded values indicate statistical significance at $P \leq 0.05$)

Table B.33 Summary of correlation analysis of g_s (stomatal conductance), total plant biomass, A (photosynthesis), A/ g_s (photosynthesis/stomatal conductance), c_i/c_a (intercellular CO₂/atmospheric CO₂) and $\delta^{13}C$ (carbon isotope composition) for Okanese plants (replicated in Table 3.5).

	g_s	Total biomass	$\delta^{13}C$	A	A/ g_s
Total biomass	$r^*=-0.098$ P**=0.680				
$\delta^{13}C$	-0.072 0.762	-0.806 0.000			
A	0.507 0.022	0.037 0.877	-0.061 0.797		
A/ g_s	-0.939 0.000	-0.106 0.656	0.084 0.725	-0.191 0.419	
c_i/c_a	0.835 0.000	0.107 0.653	-0.055 0.816	-0.030 0.900	-0.967 0.000

Cell contents: *Correlation Coefficient (r)

**P-Value (note: bolded values indicate statistical significance at $P \leq 0.05$)

Table B.34 Summary of correlation analysis of g_s (stomatal conductance), total plant biomass, A (photosynthesis), A/ g_s (photosynthesis/stomatal conductance), c_i/c_a (intercellular CO₂/atmospheric CO₂) and $\delta^{13}C$ (carbon isotope composition) for Walker plants (replicated in Table 3.5).

	g_s	Total biomass	$\delta^{13}C$	A	A/ g_s
Total biomass	r=0.470 P=0.057				
$\delta^{13}C$	-0.631 0.007	-0.436 0.080			
A	0.540 0.025	0.203 0.435	-0.436 0.080		
A/ g_s	-0.913 0.000	-0.443 0.075	0.547 0.023	-0.206 0.427	
c_i/c_a	0.736 0.001	0.362 0.154	-0.436 0.080	-0.130 0.619	-0.936 0.000

Cell contents: *Correlation Coefficient (r)

**P-Value (note: bolded values indicate statistical significance at $P \leq 0.05$)

APPENDIX C. ANOVA TABLES

Table C.1 ANOVA table for minimum soil water potentials ($\min \Psi_{\text{soil}}$) for four hybrid poplar clones (Clone) grown under two soil moisture treatments (Trt). (Data not shown)

Source	DF [†]	Seq SS	Adj SS	Adj MS	F ratio	P value
Trt	1	0.0038	0.0038	0.0038	0.04	0.923
Clone	3	0.4141	0.4141	0.1380	1.46	0.290
Trt * Clone	3	0.7679	0.7679	0.2559	2.71	0.071
Error	38	1.5135	1.5135	0.0946		
Total	45	2.6994				

[†] DF (degrees of freedom)

Table C.2 ANOVA table for minimum leaf water potentials ($\min \Psi_{\text{lf}}$) for four hybrid poplar clones (Clone) grown under two soil moisture treatments (Trt). (Data shown in Table 4.3)

Source	DF [†]	Seq SS	Adj SS	Adj MS	F ratio	P value
Trt	1	0.0026	0.0026	0.0026	0.43	0.904
Clone	3	0.2353	0.2353	0.0784	12.98	0.000
Trt * Clone	3	0.0903	0.0903	0.0301	4.98	0.079
Error	16	0.0967	0.0967	0.0060		
Total	23	0.4249				

[†] DF (degrees of freedom)

Table C.3 ANOVA table for threshold leaf water potential ($\Psi_{\text{lf} < 75\%}$) for stomatal conductance (g_s) for four hybrid poplar clones (Clone) grown under two soil moisture treatments (Trt). (Data shown in Table 4.3)

Source	DF	Seq SS	Adj SS	Adj MS	F ratio	P value
Trt	1	0.00017	0.1235	0.1235	2.94	0.094
Clone	3	0.2479	0.4685	0.1562	3.72	0.019
Trt * Clone	3	0.5027	0.5027	0.1676	3.99	0.014
Error	38	1.5939	1.5939	0.0419		
Total	45	2.3448				

[†] DF (degrees of freedom)

Table C.4 ANOVA table for leaf water potential ($\Psi_{\text{lf} < 40\%}$) at which stomatal conductance g_s was less than 40% of steady state values for four hybrid poplar clones (Clone) grown under two soil moisture treatments (Trt). (Data shown in Table 4.3)

Source	DF	Seq SS	Adj SS	Adj MS	F ratio	P value
Trt	1	0.0244	0.0089	0.00898	0.37	0.553
Clone	3	0.0770	0.0601	0.02003	0.83	0.501
Trt * Clone	3	0.0304	0.0304	0.01012	0.42	0.742
Error	13	0.3139	0.3139	0.02415		
Total	20	0.4457				

[†] DF (degrees of freedom)

Table C.5 ANOVA table for leaf water potential ($\Psi_{lf <15\%}$) at which stomatal conductance (g_s) was considered shut down for four hybrid poplar clones (Clone) grown under two soil moisture treatments (Trt). (Data shown in Table 4.3)

Source	DF	Seq SS	Adj SS	Adj MS	F ratio	P value
Trt	1	0.00002	0.0016	0.00162	0.2	0.660
Clone	3	0.2443	0.2489	0.08299	10.31	0.000
Trt * Clone	3	0.0154	0.0154	0.00514	0.6	0.600
Error	18	0.1449	0.1449	0.00805		
Total	25	0.4047				

† DF (degrees of freedom)

Table C.6 ANOVA table for threshold leaf water potential ($\Psi_{lf <75\%}$) for photosynthesis (A) for four hybrid poplar clones (Clone) grown under two soil moisture treatments (Trt). (Data shown in Table 4.4)

Source	DF	Seq SS	Adj SS	Adj MS	F ratio	P value
Trt	1	0.1408	0.1813	0.1813	2.25	0.153
Clone	3	0.1261	0.0639	0.0213	0.26	0.850
Trt * Clone	3	0.1125	0.1125	0.0375	0.47	0.711
Error	16	1.2901	1.2901	0.0806		
Total	23	1.6696	1.6696			

† DF (degrees of freedom)

Table C.7 ANOVA table for threshold leaf water potential ($\Psi_{lf <40\%}$) at which photosynthesis (A) was less than 40% of steady state values for four hybrid poplar clones (Clone) grown under two soil moisture treatments (Trt). (Data shown in Table 4.4)

Source	DF	Seq SS	Adj SS	Adj MS	F ratio	P value
Trt	1	0.0134	0.0194	0.0194	2.53	0.143
Clone	3	0.2226	0.2274	0.0758	9.89	0.002
Trt * Clone	3	0.0117	0.0117	0.0039	0.51	0.684
Error	10	0.0767	0.0767	0.0077		
Total	17	0.3244	0.3244			

† DF (degrees of freedom)

Note: ANOVA could not be run for the leaf water potential at which photosynthesis (A) was considered shut down ($\Psi_{lf <15\%}$) because A of Northwest plants and well-watered Okanese plants did not decrease below 15% of steady state values during the measurement period.

APPENDIX D. RECOVERY DATA

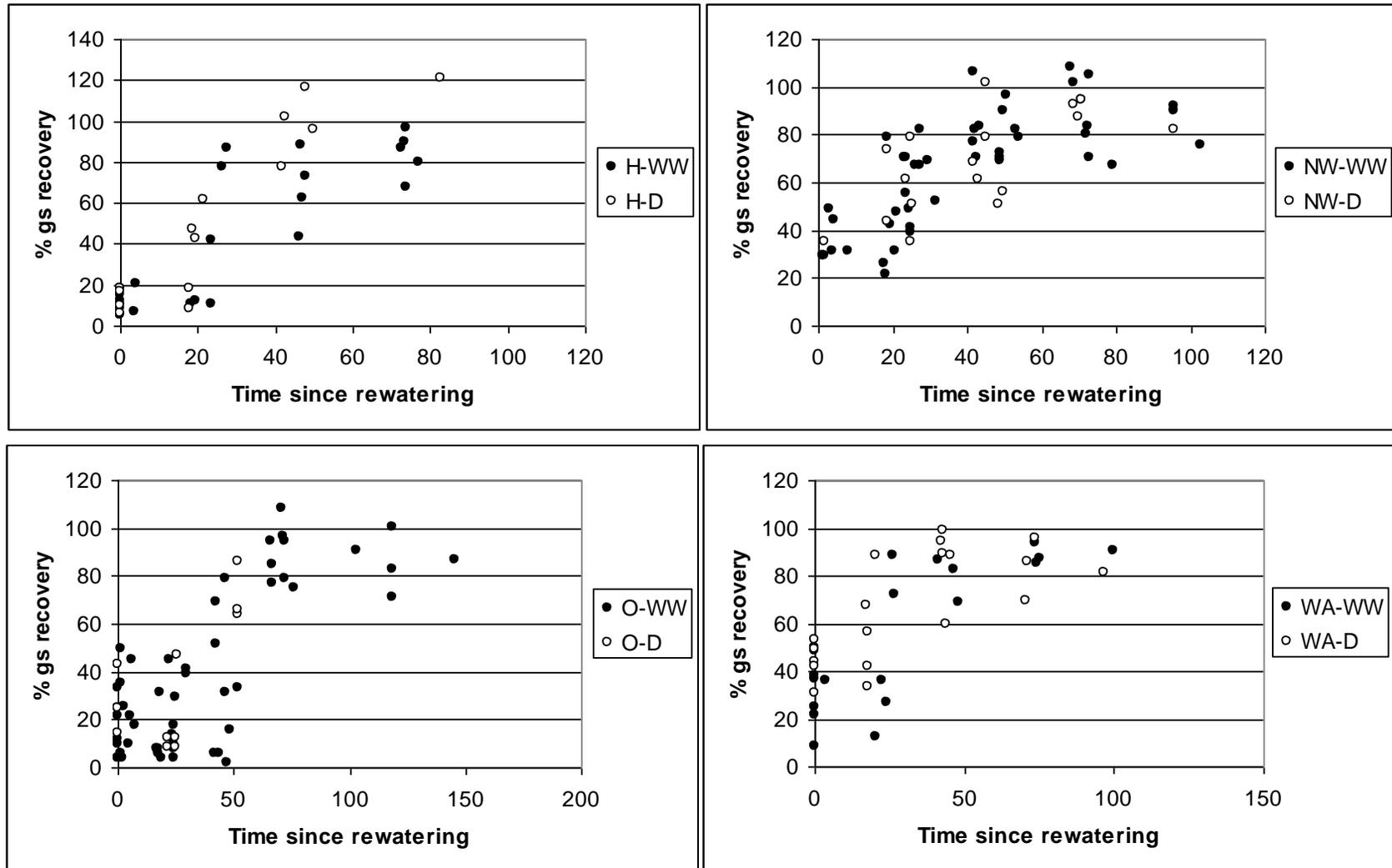


Figure D.1 Recovery of stomata conductance (g_s) following a cycle of soil of soil moisture deficit and rewatering of Hill (H), Northwest (NW), Okanese (O), and Walker (WA) hybrid poplar previously grown under well-watered or droughted conditions.

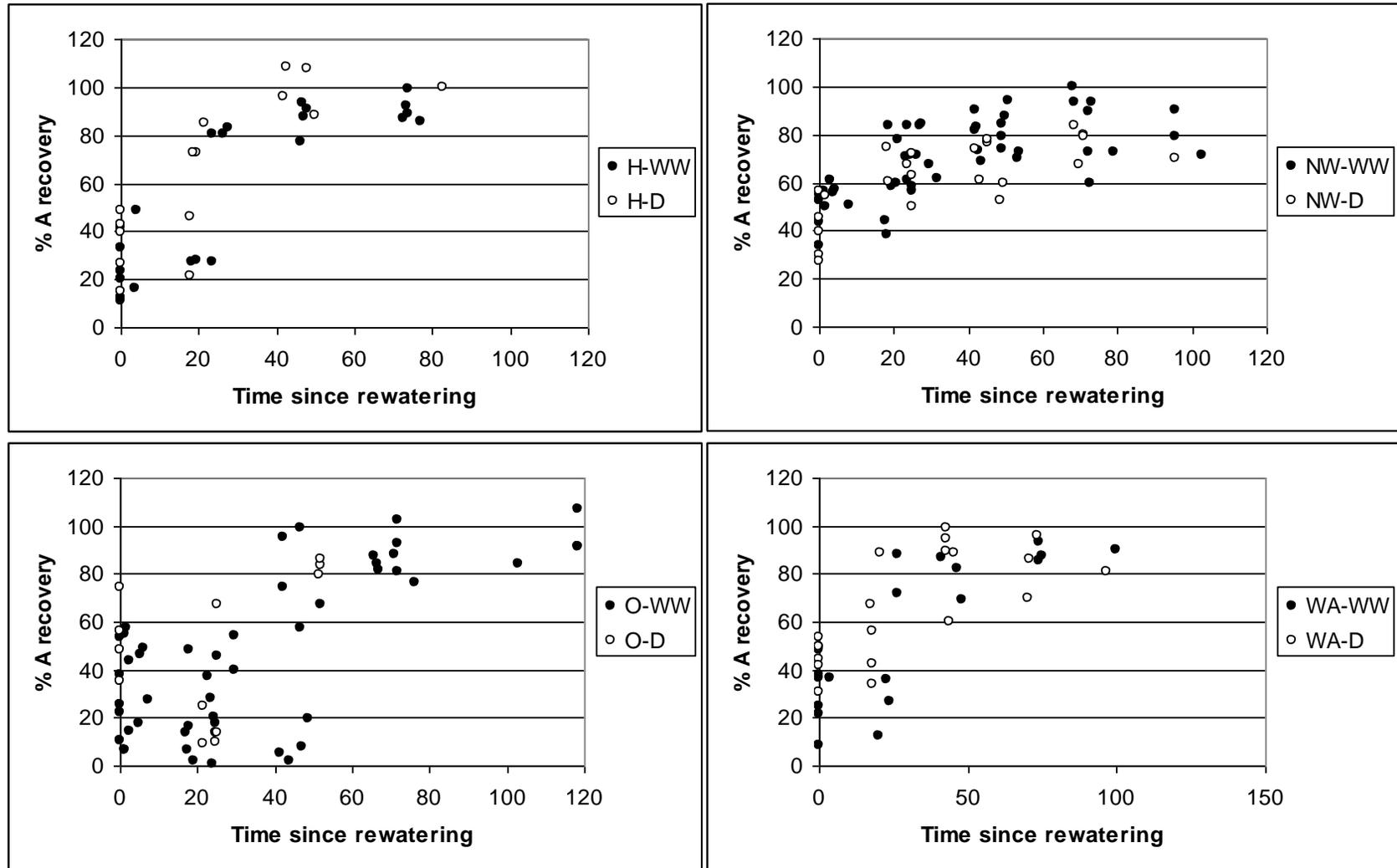


Figure D.2 Recovery of photosynthesis following a cycle of soil of soil moisture deficit and rewatering of Hill (H), Northwest (NW), Okanese (O), and Walker (WA) hybrid poplar previously grown under well-watered or droughted conditions.